Chapter 1. Introduction

Research and ecological context

Importance of the mutualistic symbiosis

Truffle-like fungi are an important component in many terrestrial ecosystems. They provide a food resource for mycophagous ('fungus-eating') animals and are an essential symbiont for many plant species (Claridge 2002; Brundrett 2009). Most truffle-like fungi are considered ectomycorrhizal (EcM) in which the fungus hyphae penetrate the plant root structure and form a sheath ('Hartig Net') around plant root tips. This enables the exchange of water, minerals, and metabolites between fungus and plant (Nehls *et al.* 2010). The fungus can form extensive networks of mycelium in the soil extending the effective surface of the plant-host root system. EcM fungi can also confer resistance to parasites, predators, pathogens, and heavy-metal pollution, as well as the breakdown of inorganic substrates (Claridge 2002; Gadd 2007; Bonfante & Genre 2010). EcM fungi can reproduce through mycelia (vegetative) growth or through spores. Truffle-like EcM fungi form reproductive below-ground (hypogeous) fruit-bodies (sporocarps) in which spores are entirely or partly enclosed within fungal tissue of the sporocarp ('sequestrate'), and often referred to as 'truffles'. The sporocarps of these fungi ('truffles') generally require excavation and consumption by mycophagous mammals for spores to be liberated and dispersed to new locations (Johnson 1996; Bougher & Lebel 2001). In contrast, epigeous or 'mushroom-like' fungi produce stipitate above-ground sporocarps in which spores are not enclosed within fungal tissue and are actively or passively discharged into the surrounding environment. Consequently, truffle-like taxa are considered to have evolved to be reliant on mycophagous animals for their dispersal.

The interaction between truffle-like fungi, their plant-hosts and mycophagous animals has been considered a tripartite mutualistic symbiosis that confers benefits to all three groups of organisms (Johnson 1996). In some cases it is considered an obligate mutualism, in which each is dependent on the other organisms for survival (Claridge & Trappe 2005; Schickmann *et al.* 2012). Mutualisms are common in nature and underpin many ecosystem processes, such as seed dispersal and pollination, which are essential to healthy ecosystem function. Mutualistic networks are often complex and involve many participating species (Jordano *et al.* 2003; Montoya *et al.* 2006). Understanding how mutualists cooperate provides insights into processes essential for ecosystem function and the persistence of both individual species and natural communities. Although the majority of truffle-like taxa form EcM, there are a few species for which ecological associations or phylogenetic placement suggest a saprotrophic or a root biotroph (i.e. obligate parasites) nutritional

mode (Lebel & Catcheside 2009; Tedersoo *et al.* 2010; Ge & Smith 2012; Lebel & Vellinga 2012). Little is known of these taxa although differences in nutritional modes among taxa suggest that truffle-like fungi may provide a number of ecosystem services at several trophic levels.

EcM fungi and ecosystem function

The worldwide diversity of EcM fungi is high with 162 fungal genera exhibiting an EcM lifestyle and an additional 52 genera likely to be EcM based on their phylogenetic position within EcM-forming lineages (Tedersoo *et al.* 2010). In at least 16 orders, the EcM lifestyle has independently evolved multiple times from wood or humus saprotrophic ancestors (Bruns & Shefferson 2004; Tedersoo *et al.* 2010). Similarly, the sequestrate and hypogeous fruiting form has also been found to have evolved multiple times in an example of convergent evolution (Peintner *et al.* 2001; Hosaka *et al.* 2006; Albee-Scott 2007). It is estimated that between 86% to 94% of plants are mycorrhizal, using their fungal symbionts to acquire mineral nutrients in the soil, particularly phosphorous (Brundrett 2009; Lambers *et al.* 2010). An estimated 94% of angiosperm species and all gymnosperms are mycorrhizal, with most of the latter considered obligately mycorrhizal (Wang & Qiu 2006; Brundrett 2009). The EcM symbiosis is less common among vascular land plants (12%) than arbuscular mycorrhizal (AM) formation (67%) but is the most common type of mycorrhizal formation among macrofungi, representing a large proportion of global macrofungal diversity (Brundrett 2002). The AM symbiosis extends back at least 400 million years while the EcM symbiosis is relatively more recent, with the first fossil evidence dating to the middle Eocene at 50 million b.p. (LePage *et al.* 1997; Wang & Qiu 2006). EcM forming plant species are typically associated with nutrient-poor environments, a potential evolutionary strategy in response to increasing global aridity and climactic variability. In comparison to AM fungi species, which are widely distributed and form associations with a wide number of plant-hosts, species of EcM-forming ascomycete and basidiomycete fungi generally associate with fewer plant-host species i.e. they exhibit greater host-specificity.

Plant families in which the EcM symbiosis dominates include Asteropeiaceae, Betulaceae, Casuarinaceae, Cistaceae, Dipterocarpaceae, Ericaceae, Fabaceae, Fagaceae, Myrtaceae, Nothofagaceae, Pinaceae, Rhamnaceae, Salicaceae, Sapotaceae, and Sarcolaenaceae (Wang & Qiu 2006; Brundrett 2009). Of these families, Myrtaceae, Fabaceae, Fagaceae, and Dipterocarpaceae have the highest plant species richness. Most of the estimated 6000 EcM plant species are shrubs or trees (Brundrett 2009). A large majority of dominant tree and shrub species of Australian woodlands and forests belong to genera considered to be EcM-forming species including *Eucalyptus* (Myrtaceae), *Allocasuarina*, (Casuarinaceae), *Acacia* (Mimosaceae) and *Nothofagus* (Nothofagaceae)(Bougher 1995). Consequently, the EcM symbiosis is an important process in ecosystem function within these habitats. Australia supports a high diversity of truffle-like fungi

characterised by high species (95%) and genus (35%) endemism (Bougher & Lebel 2001; Glen *et al.* 2008). Brundrett (1995) estimated that Australia may support up to 6000 species of EcM fungi, while Bougher & Lebel (2001) estimated 1278–2450 species of truffle-like fungi for Australia. As most truffle-like taxa are considered EcM, their symbiosis with dominant tree and shrub species is likely to be an important process for vegetation dynamics and plant species distributions in Australia.

Distribution patterns and dispersal

Knowledge of natural factors driving the distribution of truffle-like fungi is limited and largely based on observations in the northern hemisphere (Bougher & Lebel 2001). Mechanisms determining EcM fungal diversity and composition include soil type, soil fertility, plant stress, host-plant identity, disturbance history, temperature, rainfall and soil moisture (Bruns 1995; Swaty *et al.* 2004; Tedersoo *et al.* 2006; Abell-Davis 2008; Peay *et al.* 2010b). In temperate south-eastern Australia, the climactic factors of temperature and moisture were the best predictors for some truffle-like fungi distributions at a landscape scale (Claridge *et al.* 2000a). In the northern hemisphere, host specificity has also been identified as an important factor in shaping distribution patterns (Bougher & Lebel 2001). Australian taxa are thought to associate with multiple host-plant species, although there is little known of species-specific truffle-plant associations and little evidence for exclusive host-plant associations. However, some species have exhibited an increased likelihood of occurrence in the presence of particular tree species due to either shared habitat preferences or host-specificity on the part of the fungus. More common species have broad host ranges but may share habitat preferences with the tree species they are associated with (Jumpponen *et al.* 2004). Habitat preferences may also be exhibited at the genus-level, as suggested by positive association among three *Arcangeliella* species in south-eastern Australia (Jumpponen *et al.* 2004) and genus-level habitat specificity in tropical northern Australia (Abell-Davis *et al.* 2012). Vascular plant species commonly co-occur to form natural communities due to shared habitat preferences and in some cases, mutualistic interactions. The cryptic nature of fungi, particularly truffle-forming taxa, is a challenge to detecting similar relationships and spatial patterns. There has been evidence from Australia that some truffle-like taxa co-occur due to shared climactic, host-plant or habitat preferences. Negative associations have also been observed, suggesting differences in habitat preference (Jumpponen *et al.* 2004).

Few studies have investigated spatial patterns in species richness and composition for truffle-like fungi. An understanding of where and why species or assemblages are distributed across a landscape and within their range is imperative for informing conservation management actions and mitigating anthropogenic impacts on natural values. The spatial distribution of taxa also has important implications for the dispersal ecology of truffle-like taxa and those of their host-plants.

Dispersal is an important biogeographic and demographic process influential in population dynamics, genetic structuring of populations, patterns of species richness, and the (re-)colonisation of unoccupied habitat (Morales & Carlo 2006; Douhan *et al.* 2011; Peay *et al.* 2012). Habitat associations of species and assemblages can inform our understanding of dispersal dynamics and gene flow in macrofungi: where species have no specific habitat associations at a local scale and are distributed uniformly across a landscape, dispersal of spores may be less important as (re)-colonisation of habitats, gene flow, and range expansions can largely occur through mycelia growth. However, if species and assemblages are associated with habitat types (e.g. host-plants or vegetation types) patchily distributed within a landscape, than spore dispersal is essential for these processes that maintain species and assemblage distributions (Peay *et al.* 2007). In addition, spore dispersal dynamics can inform our understanding of past biogeographic events and predictions of future events (e.g. climate change). Although dispersal is considered important in fungal ecology, little is known of how it influences populations, communities, or spatial patterns. Underlying ecological information such as spore bank longevity or spore dispersal distances that would allow linkages to be made between dispersal and ecological processes, such as succession, are largely absent (Peay *et al.* 2008; Bruns *et al.* 2009; Molina *et al.* 2011). Such knowledge can also inform our management of mycophagous mammals, truffle-like fungi, and EcM host-plant species and associated floristic communities.

There is recent evidence that dispersal may be an important and limiting process in shaping EcM fungal community assemblage structure (Peay *et al.* 2007, 2012; Geml *et al.* 2011), geographic genetic differentiation within EcM species (Carriconde *et al.* 2008; Douhan *et al.* 2011; Vincenot *et al.* 2012) and truffle-like taxa (Murat *et al.* 2004; Grubisha *et al.* 2007), and succession dynamics (Blaalid *et al.* 2011). The ingestion and dispersal of EcM fungal spores by mammals (endozoochory) may also influence patterns of recruitment in host-plant species through the conferred benefits or obligate requirements of mycorrhizal colonisation (Tedersoo *et al.* 2009b; Peay *et al.* 2010b; Schickmann *et al.* 2012). Dispersal by mycophagous mammals facilitates the deposition of spores into soil spore banks, allowing propagules of species to persist in the soil between successional stages or disturbance events (Ashkannejhad & Horton 2006; Rusca *et al.* 2006; Bruns *et al.* 2009). Mycophagous mammal dispersers have been shown to maintain arbuscular mycorrhizal (AM) soil inoculum potential and subsequent mycorrhizal colonisation of host plants (Gehring *et al.* 2002) and to facilitate the establishment of EcM trees beyond the parent plant and its associated mycorrhizal network (Frank *et al.* 2009). Through the same process, spore dispersal may be an important process in the (re-)colonisation of unoccupied post-disturbance habitat by EcM forming plant taxa, such as after fire (Claridge *et al.* 2001; Bent *et al.* 2011; Kipfer *et al.* 2011), glaciation (Cázares & Trappe 1994), or volcanic eruption (Nara 2006). Dispersal of spores by mammals across habitat boundaries may also facilitate vegetation shifts in response to

fire regimes or climactic cycles (Vernes & Dunn 2009). Consequently, the dispersal of spores by mycophagous mammals may play an important role in forest regeneration, resilience, and succession (Izzo *et al.* 2005b; Schickmann *et al.* 2012).

Implications of truffle-mammal interactions in space

The abundance of sporocarps may vary across a landscape and this has implications for dispersal. Sporocarp production has recently been linked to dispersal potential in epigeous EcM species (Peay *et al.* 2007). It may also influence the mycophagous habits of mammalian dispersers (Johnson 1994b; Cázares *et al.* 1999; Meyer 2005). As truffles comprise an important food resource for mycophagous mammals, spatial variation has implications for the conservation management of mycophagous mammal populations (Claridge & May 1994; North *et al.* 1997; Abell-Davis 2008; Frank *et al.* 2009). The distribution of food resources can influence animal foraging behaviour (McIntyre & Wiens 1999; Vernes & Haydon 2001) and habitat preferences (Vernes 2003; Abell-Davis 2008). Mycophagous mammals may contribute differentially to truffle-like fungi dispersal through varying in the quantity, diversity and composition of fungal spores they ingest (Schickmann *et al.* 2012). The relative importance of dispersers may vary according to different spatial scales. Some mammal species may disperse few spores but disperse these long distances, facilitating biogeographic range expansions or spore dispersal and gene exchange among populations or discreet patches of habitat. The importance of long-distance dispersal (LDD) events are disproportionate to their frequency, being the major driving factor in shaping biogeographic patterns in species distributions and gene flow at larger spatial scales (Nathan *et al.* 2008). Other mammals may disperse large numbers of spores of many taxa shorter distances, thereby maintaining local distributions of truffle-like taxa and possibly those of their host-plants. Dispersal distances facilitated by animal vectors are largely a product of their movement behaviour and the length of time they retain spores in their guts (i.e. ‘gut-retention time’). Consequently, the foraging range of mycophagous mammals has recently been associated with genetic spatial structuring and genetic differentiation (or lack thereof) in fungal populations (Kretzer *et al.* 2005; Grubisha *et al.* 2007). Some animal vectors may also provide higher quality dispersal by dispersing spores to habitats or microsites more amenable to establishment (i.e. ‘directed dispersal’; Wenny 2001).

A large number of animal vectors are implicated in fungal spore dispersal including insects (Fogel & Peck 1975), birds (Simpson 2000), and mammals (Claridge & May 1994) although the latter group are considered the most important vector for truffle-like fungi. Mycophagous mammals vary in their reliance on fungal sporocarps as a food resource, ranging from opportunistic consumption to obligate reliance within or across seasons. For some mammals, truffles are a main dietary item, such as members of the Australian Potoroidae (*Potorous* and *Bettongia* species) and the North American northern flying squirrel *Glaucomys sabrinus* (Meyer 2005). Research to date has focused

mainly on the mycophagous habits of small to medium-sized mammal species, largely neglecting the role that less frequent fungal consumption by larger ranging mammals may play in the dispersal ecology of truffle-like fungi (but see Launchbaugh & Urness 1992; Claridge *et al.* 2001; Ashkannejhad & Horton 2006; Vernes 2010). Specialist mycophagous mammals in Australia all fall within the 'critical weight range' group of medium-sized mammals which have experienced severe declines in distribution and abundance since European settlement. Predation by exotic predators is largely implicated in these declines although habitat loss and fragmentation are also considered major contributing and interacting factors (Johnson & Isaac 2009; Bilney *et al.* 2010). When the heavily mycophagous habits of all *Potorous* and some *Bettongia* species is considered with their relatively large home range size and high mobility, their potential importance as spore vectors for a wide range of truffle-like taxa may be considerable. The loss or reduced abundance of these dispersers across whole landscapes may be having a considerable impact and places a greater importance on the potential dispersal services provided by larger-ranging macropods (Vernes 2010) and ubiquitous native or exotic small mammals (Vernes & McGrath 2009).

Sampling techniques

A number of studies have taken advantage of the mycophagous habits of mammals by sampling their scats for fungal spores. Most have been concerned with simply establishing whether species consume and potentially act as vectors for fungal spore dispersal. Some studies have gone further to establish the composition and abundance of fungal species in a species diet and furthermore, fungal resource partitioning between species (Orrock *et al.* 2003). A few studies have sampled mammal diets for comparison to sporocarp collections (Johnson 1994b; Carey *et al.* 2002; Meyer 2005; Izzo *et al.* 2005b; Frank *et al.* 2006). Such studies provide insights into the mycophagous habits of mammals, resource-partitioning or competition among species, differential contribution to dispersal, preferential consumption or avoidance of truffles produced by different fungal taxa, and responses of animals to truffle availability and taxon richness. A common finding is that mycophagous mammals are exceptionally good at sampling species richness of truffles within a locality, with many additional taxa uncovered by simultaneously sampling mammal diets with other techniques (Izzo *et al.* 2005b). Some studies have gone further to infer spatial trends in fungal composition from mammalian diets and link these to ecological processes (Vernes & Dunn 2009; Frank *et al.* 2009). Consequently, sampling diets represents a potential technique to infer spatial trends in truffle-like fungal richness and composition but also represents a comparative method to test the conclusions of sporocarp surveys. As discussed above, a good understanding of spatial trends in richness and composition are of vital importance in making sound decisions in the conservation management of natural environments (Molina *et al.* 2011). A knowledge of spatial trends and dispersal dynamics in fungi can inform how we manage individual species, natural

assemblages, woodland and forested ecosystems, and truffle food resources for mycophagous mammals, many of which are threatened with extinction in their natural habitats.

Objectives and thesis structure

The overall aim of this thesis is to investigate mutualistic interactions between truffle-like fungi and mammals, with a particular focus on spatial trends and dispersal ecology. A secondary aim is to determine whether mammal diets can be used to detect spatial trends in fungal richness and composition or provide information additional to sporocarp surveys. Specific research objectives are to:

- determine whether the richness, abundance, and composition of truffle-like fungi varies spatially by broad habitat type (Chapter 2);
- examine how habitats may differ in the (truffle-like) fungal food resources available to mycophagous mammals (Chapter 2);
- investigate whether the diets of some key mycophagous mammals (bush rat *Rattus fuscipes* and swamp wallaby *Wallabia bicolor*) reflect spatial trends in truffle abundance, richness, and composition across three broad habitat types or alternatively, suggest different mycophagous habits in response to broad habitat type (Chapters 3 and 4);
- examine whether mammal species differ in their contribution to spore dispersal for truffle-like fungi (Chapters 3-6);
- estimate spore dispersal distances, spatial patterns of deposition, and the frequency and extent of LDD events facilitated by a key mycophagous macropod (swamp wallaby) within the model system (Chapter 5); and
- explore the potential role of a nonstandard mammalian dispersal vector (microbats) in facilitating LDD events for fungi and report on the discovery of a fungus in a novel environment (Chapter 6).

The primary area and model system studied in this thesis is detailed in Chapter 1. Further descriptions are provided sequentially in each Chapter as relevant to the topic. Methodologies are described in each Chapter and reference made to previous ones wherever possible to avoid unnecessary reiteration. For terms used in the thesis relating to dispersal ecology see a Glossary provided after Chapter 7.

Chapter 2 investigates richness and assemblage structure in truffle-like fungi among four broad habitat types within the study area. I was specifically interested in whether different habitats supported distinct taxon assemblages both at the species and genus taxonomic levels. Trends across

habitats in the abundance, size, and diversity of truffles available for mycophagous mammals to consume and disperse were also investigated.

Chapter 3 investigates the mutualistic interactions between mammals and EcM fungi across three broad habitat types with a focus on their role as dispersal vectors. I tested whether small mammal species differ as spore dispersal vectors in the abundance, diversity and composition of fungal taxa ingested. Comparisons were made with the results of Chapter 2 to gain additional ecological insights and for comparing the relative efficacy of sampling sporocarps versus small mammal diets in detecting spatial trends in truffle-like fungal richness and assemblage structure.

Chapter 4 examines spatial trends in the mycophagous habits and spore dispersal services of a specific mycophagous mammal, the swamp wallaby. Comparisons were made in the diversity and composition of truffle-like taxa found in swamp wallaby diets to trends across habitat types exhibited in small mammal diets and in the soil. These were undertaken to investigate differential contribution to spore dispersal among mammal species within the study area but also to elucidate the efficiency of these different sampling techniques to detect spatial trends in fungal diversity and composition according to different habitat types. Through Chapters 2 to 4, a number of analyses examine responses to habitat type at both the species- (morphospecies) and genus-level. This was undertaken to gain insights into habitat responses at a higher taxonomic level but also to facilitate comparison between sporocarp survey and dietary results.

The following Chapter (5) explores spatial patterns of fungal spore deposition by the swamp wallaby through the estimation of dispersal kernels using mechanistic models. Associations between predicted patterns of spore deposition and space use were investigated, along with the overall influence of movement behaviour on dispersal patterns. The potential role of swamp wallabies in facilitating long-distance dispersal (LDD) events, spore dispersal and gene exchange among discrete populations and habitats, and facilitating vegetation shifts is discussed in relation to findings.

Chapter 6 reports the discovery of a secondary, non-standard mammalian vector (microbats) for macrofungi along with the first account of a truffle-like fungus in a cave environment. This Chapter assesses the role microbats may play in fungal spore dispersal and explores potential implications for biogeographic events and dispersal dynamics. The final Chapter (7) provides a summary and synthesis of the thesis results.

Terminology

For the sake of simplicity, when referring specifically to the sporocarps (fruit-bodies) of truffle-like fungi, the term “truffles” is used hereafter wherever this distinction is required. It is acknowledged that the term ‘truffle’ strictly speaking applies to members of the genus ‘Tuber’ otherwise referred

to as the 'true truffles' (Bonito *et al.* 2010; Murat *et al.* 2013). Fungal taxa forming ectomycorrhizal associations with plant hosts are also referred to by the abbreviated term 'EcM taxa' after the first instance. The reader is also referred to a glossary of terms located after the thesis synthesis in Chapter 7.

The study area and model system

A general description of the study area is given in this section, with further relevant details given in each subsequent Chapter. The main study area offered a unique opportunity to study truffle-like fungi and mutualistic interactions with mammals within a large and un-fragmented forested wilderness area in south-east Australia. The area supports a diverse array of plant communities differing greatly in structural characteristics and floristic composition (Hunter & Sheringham 2008). Mammalian diversity is also very high and community composition varied (Vernes *et al.* 2006). This locality also offered the opportunity to build on previous research within the area, particularly on mycophagy but also on vegetation dynamics (Clarke & Myerscough 2006; Williams & Clarke 2006; Hunter & Bell 2007; Kumar *et al.* 2007; Vernes & Dunn 2009).

The study area is located on the New England Tablelands of northern New South Wales, Australia, on the eastern edge of high plateau (900-1200m) and is characterised by rugged country of high ridges and steep valleys. Encompassed within the study area are three National Parks: Barool, Gibraltar Range, and Washpool. Gibraltar Range NP consists of an undulating granite plateau in its northern section and steep valleys along the escarpment boundary. Nutrient deficient soils of coarse-grained sands and leucogranite outcrops characterise much of the park, although gullies support more mesic tall wet forest and open sedge swamps are scattered across the central and northern sections of the park. Extensive areas of warm-temperate rainforest are supported within Gibraltar Range and Washpool National Parks, largely along the escarpment edge although more restricted occurrences are present in Barool National Park.

Broad vegetation classes include temperate rainforest, subtropical rainforest, wet sclerophyll forest, heathy dry forest, dry open forest ('grassy forest'), heathy woodland, and montane sedge bogs (Figure 1.1). Each broad vegetation class is similarly marked by a rich variety of plant communities. For example, Hunter & Sheringham (2008) designated 8 different plant communities within the broad vegetation class of 'Northern Escarpment Dry Sclerophyll Forest' defined by Keith (2004). Gibraltar Range is characterised by a high diversity of markedly different habitat types and floristic communities. For example, it is one of the few places in Australia where Warm Temperate Rainforest is juxtaposed to a large area of shrubby dry forest (Keith 2004).

The high species diversity in the area can be partly attributed to its position at the boundary between subtropical and temperate faunas, being at the limit of many species ranges. The diverse

range of habitats supported within the park also plays an important role. Gibraltar Range NP supports a high diversity of macropod species (Vernes *et al.* 2006) along with populations of rare mycophagous macropods or 'critical-weight range' (CRW) mammals including the parma wallaby *Macropus parma*, long-nosed bandicoot *Perameles nasuta*, long-nosed potoroo *Potorous tridactylus*, and rufous bettong *Aepyprymnus rufescens*. However, the few records made for the latter three species from recent surveys suggest they presently occur at low abundance in correspondence with declines elsewhere in Australia (Vernes *et al.* 2006; Johnson & Isaac 2009). Otherwise, mammalian communities are largely intact including those of native carnivorous mammals (e.g. dingo *Canis lupus dingo* and tiger quoll *Dasyurus maculatus*). The mycophagous habits of several mammal species have previously been assessed within the park at a small spatial scale (Vernes & Dunn 2009) with numerous species along a steep vegetation gradient found to consume truffles. Based on previous and concurrent research (Hollis *et al.* 1986; Claridge *et al.* 2001; Vernes 2010; Danks 2012), it was considered that the swamp wallaby was likely to be a key dispersal vector for truffle-like fungi within the study area. The species broad habitat breadth (Southwell *et al.* 1999; Menkhorst & Knight 2001), established mycophagous habits, and large home range made it an ideal candidate for assessing longer-distance fungal spore dispersal.

The low level of anthropogenic disturbance and modification was an important consideration for the selection of the area for my study. This was of particular importance for the estimation of spore dispersal by the swamp wallaby. Fragmentation, associated edge effects, anthropogenic barriers, and logging can influence the movement behaviour of animals (Di Stefano *et al.* 2007) and consequently for patterns of spore deposition, as has been shown for plant-frugivore dispersal systems (Carlo & Morales 2008; Herrera & García 2010).

The extent of sampling for research findings presented in Chapters 2-4 is defined by the main study area, a 21 km (north-south) by 19 km (east-west) rectangle, measuring 392 km^2 in area, encompassing three National Parks, and bounded by the longitudes 152°10'7" E to 152°21'54" E and latitudes -29°27'20.34" S to -29°38'38" S (Figure 3.1). The greatest distance between sample points was approximately 24 kilometers. Research presented in Chapter 5 was focused on a smaller area within Gibraltar Range National Park and was selected based on the area supporting a mosaic of the main habitat types within the study area along with considerable distance (≈3 kilometres) from any main road or human disturbance. Research presented in Chapter 6 incorporated sampling sites over a larger scale (>100 km) than previous Chapters. All dietary sampling was undertaken within the New England Tableland bioregion but largely represented collection sites at a smaller spatial scale within a fragmented pastoral landscape north of Armidale township.

Figure 1.1 Examples of habitat types within the study area including (from left to right) heathy woodland (top left), wet sclerophyll forest, warm temperate rainforest, montane sedge swamp ('montane bogs'), and rocky granite (leucogranite) outcrops (bottom left). An example of the high ridges and steep valleys (bottom right) that characterise the escarpment edge within the study area.

Chapter 2. Composition, richness, and sporocarp production in truffle-like fungi among contrasting habitats and implications for mycophagous mammals

Introduction

Australia supports a high regional diversity of hypogeous sequestrate (below ground, truffle-like) fungi and is characterised by a high level of species (95%) and genus (35%) endemism (Bougher & Lebel 2001; Glen *et al.* 2008). With nearly 300 species and 75 genera, the number of species described from Australia far exceeds those from North America or Europe (Claridge 2002). In 2001, Bougher & Lebel (2001) estimated that between only 12 to 23% of Australia's truffle-like fungi had been discovered. A single landscape-scale survey across forest and woodland habitats of south-eastern Australia discovered 56 new species, representing 27% of all species collected, and 16 new genera (Claridge *et al.* 2000b). Such studies illustrate our limited knowledge of the fungal diversity in Australia. Truffle-like taxa in Australia belong to one of three major classes of fungi: Ascomycetes, Zygomycetes, and Agaricomycetes, with the majority of Australian species belonging to the latter (Claridge & May 1994; Bougher & Lebel 2001; Claridge 2002).

Truffle-like fungi are largely ectomycorrhizal (EcM), forming an obligate mutualism with host plant species (Luoma *et al.* 1991). Inoculation of Australian plants with EcM fungi has been shown to improve nutrient uptake and growth, while fungus-plant associations can increase plant resistance to pathogens, drought, heavy metals and salinity (Reddell *et al.* 1999; Bougher & Lebel 2001). Inoculation with EcM fungi has also been shown to increase growth rates and nutrient uptake in tree seedlings elsewhere (Turjaman *et al.* 2006). Mycelia networks can greatly increase the effective area of plant root systems, improving root uptake of water and nutrients (Dahlberg 2001). They may also form conduits for organic and inorganic exchange among many different plants (Bougher & Lebel 2001; Dahlberg 2001; Kennedy *et al.* 2003). Some fungi may also play a pivotal role in vegetation post-fire recovery through soil stabilization (Claridge *et al.* 2009a). Through these mechanisms EcM fungi are thought to play significant roles in ecosystem function.

The Australia landscape is characterised by aridity, nutrient-poor soils, and frequent fire events, with a distinctive flora adapted to these conditions (Fox 1999). Factors driving the evolution of sequestrate fruiting structures in fungal taxa are thought to include low soil moisture, whereby spore-bearing tissue is protected from desiccation by an enclosing peridium and the below-ground habitat (Johnson 1996). Consequently, many mycorrhizal associations between fungi and Australian plant species are thought to be driven by nutrient poor soils and dry environmental

conditions (Bougher 1995; Claridge 2002).

Factors considered important for determining the distribution of truffle-like fungi in Australia are largely derived from studies in the northern hemisphere (Bougher & Lebel 2001) although there has been some progress towards a greater understanding of the Australian context (Claridge *et al.* 1993b, 2000a; b, 2009b; Jumpponen *et al.* 2004; Abell-Davis 2008; Danks *et al.* 2012; Abell-Davis *et al.* 2012). Natural mechanisms driving EcM fungal diversity and community composition are poorly understood although soil type (Gehring *et al.* 1998), plant stress (Swaty *et al.* 2004) and host plants are thought to be important (Bruns 1995; Tedersoo *et al.* 2006). In Australia, moisture availability has been identified as a major factor in explaining patterns of EcM species richness both at the landscape and local scale (Claridge *et al.* 1993b, 2000a). For individual taxa, moisture availability and minimum temperature appear to be important factors in the landscape-scale distribution of some species. These responses have been found to be either negative or positive. At the local scale, diversity has been found to be influenced by substrate type (e.g. soils), topography (Scotts & Seebeck 1989; Claridge *et al.* 1993b), and the number of host eucalypt species (Claridge *et al.* 2000a).

There has been limited evidence for host specificity in truffle-like fungi. Most Australian EcM species are thought to be host generalists, associating with multiple host plant species ranging from herbs (Warcup 1980; Kope & Warcup 1986) to trees (Reddell *et al.* 1999; but see Tedersoo *et al.* 2008). The wide host range includes structurally dominant trees and shrubs belonging to the genera *Eucalyptus*, *Leptospermum*, *Allocasuarina*, *Acacia*, *Nothofagus*, and *Melaleuca* (Bougher 1995; Bougher & Lebel 2001). A strong potential for mycelia networks linking plants has been inferred from evidence for wide host range in EcM fungi (Leake *et al.* 2004).

Little is known on the ecology of truffle-like fungi and community structure (Claridge *et al.* 2000b). Research into EcM fungal communities has focused on host-specificity (Bougher & Lebel 2001; Tedersoo *et al.* 2008), and the impacts of forestry (Dunham *et al.* 2007) and fire (Meyer *et al.* 2005a; Trappe *et al.* 2009a). There has been some evidence for EcM community composition differing by vegetation type (Nantel & Neumann 1992; Tedersoo *et al.* 2008) although studies to date have compared relatively similar vegetation types. At a smaller scale, differences in EcM fungal communities have been found for two co-occurring oak species (*Quercus* spp.) in the northern hemisphere (Morris *et al.* 2008). In the Australian context, there has been some evidence for fungal taxon composition varying according to rainfall (Glen *et al.* 2008), diversity of forb species (Barrett *et al.* 2009), and host species (Tedersoo *et al.* 2008). Dietary studies of mycophagous mammals also provide some evidence for potential differences in EcM species composition and abundance in response to vegetation type (Reddell *et al.* 1997; Vernes & Dunn 2009).

Understanding the spatial distribution, richness, and rarity of truffle-like fungi is critical for informing sound conservation planning and management actions (Bougher & Lebel 2001). Information on how richness and rarity varies across the landscape can be used to prioritise habitat protection and habitat management. If suites of species commonly co-occur (i.e. distinct communities), then management actions can be simplified through a 'habitat-based conservation approach', as opposed to a single-species approach to conservation planning (Claridge *et al.* 2000b). Species richness and rarity are also important factors in characterising assemblages of organisms and their distribution in space (Magurran 2004).

The tripartite relationship among truffle-like fungi, plant host species, and mycophagous mammals is well established (Johnson 1996; Bougher & Lebel 2001) and underlines the importance of this fungal group at multiple trophic levels. Truffles are an important food resource for many Australian mammals (Claridge & May 1994; Reddell *et al.* 1997; Claridge *et al.* 2000a; Vernes *et al.* 2001; Vernes & Dunn 2009), particularly critical weight range (35g-5500g) species that have undergone the greatest declines in distribution post-European settlement (Johnson & Isaac 2009; Bilney *et al.* 2010). Differences in the diversity, abundance, composition, and spatial arrangement of truffles among habitat types may have important implications for mycophagous mammals (Izzo *et al.* 2005a; b) such as range restriction, home range, and foraging behaviour (Claridge *et al.* 1993a; Claridge & May 1994; Vernes & Haydon 2001; Vernes *et al.* 2004b). By dispersing fungal spores, mycophagous mammals are implicated in maintaining mycorrhizal fungal diversity and community structure (Gehring *et al.* 2002; Vernes & McGrath 2009) and recolonisation of sites after disturbance events or through natural successional stages (Ashkannejhad & Horton 2006). Through consuming different fungal taxa and varying in home range and habitat use, the composition and health of a mycophagous mammal community can also impact on truffle-like fungi (Izzo *et al.* 2005a). Despite its apparent importance, this inter-dependent, community-level interaction between truffle-like fungi and mammals remains poorly understood.

The primary aims of this study were to i) increase our understanding of local scale distribution and taxon richness of truffle-like fungi in response to vegetation type, ii) establish whether distinct communities of fungal taxa exist, iii) investigate the sufficiency of sampling intensity in estimating species richness, and iv) to identify whether differences existed among broad vegetation types in the availability of truffles for mycophagous mammals.

To achieve these aims, truffles (sporocarps) were sampled at 59 plots stratified by broad habitat type (4 types). Habitat types were investigated for differences in truffle-like taxon richness, assemblage composition, and also sporocarp ('truffle') numbers, dry weight, diversity, and dominance. Sampling completeness and estimated total species richness (accounting for unseen species) was investigated using various techniques including species accumulation and rarefaction

curves. Comparisons among habitats in taxon richness and composition were undertaken using frequency or presence-absence of species among plots. Application of abundance data is problematic for fungi due to the difficulty with defining an 'individual' in a fungal community (Zak & Willig 2004) and the potential for sporocarp numbers (counts) to vary from biomass (Castellano *et al.* 2004) and also from mycorrhiza abundance - to the extent that it can show an opposite pattern (Dahlberg 2001). Truffle abundance, biomass, and evenness among vegetation types were investigated to explore patterns in the diversity and abundance of truffles available for mycophagous mammals.

Truffle-like fungi were compared across contrasting vegetation types based on the hypothesis that large differences in habitat features (host plant species, soil type, water availability and soil moisture) would provide the greatest contrast in truffle-like taxon richness and composition. Such an approach should include vegetation types expected to be less favourable habitat for truffle-like EcM fungi and with a greater dominance of arbuscular mycorrhizal (AM) fungi, such as rainforest or grass-dominated plant communities (Brundrett *et al.* 1995; Reddell *et al.* 1997; Zhao *et al.* 2001; McGuire 2008). The spatial extent of sampling was restricted to an area small enough (≈10-km X 10-km) that geographic barriers or dispersal limitation were unlikely to be significant factors in restricting species occurrence in any of the vegetation types sampled. In addition, large differences in climatic conditions or events may obscure patterns in taxon richness through influencing fruiting events (Abell-Davis 2008).

Methods

Study area

The study area was located across several national parks on Gibraltar Range in the New England Tablelands of north-eastern New South Wales, Australia. Truffle plots were distributed across three contiguous national parks: Barool National Park, Washpool National Park, and Gibraltar Range National Park. The greatest distance between two plots was ≈10 km. Based on the wide distribution of many truffle-like taxa (Claridge 2002), the spatial scale of this investigation could be considered 'local scale'. The study area is ideal for undertaking an investigation of local species diversity and composition as it supports a diverse array of vegetation types and abiotic conditions (Figure 1.1). It is the only known location in Australia where a large continuous expanse of temperate rainforest and dry heathy vegetation adjoin one another (Keith 2004).

Survey sites were stratified by four major habitat types of warm temperate rainforest (hereafter 'rainforest'), wet sclerophyll forest (hereafter 'wet sclerophyll'), dry open forest with a grassy understorey (hereafter 'dry forest'), and heathy woodland. These habitat types do not follow formal local scale vegetation classification, but rather a broader grouping based on marked differences in both plant species and structural composition. Consequently, these vegetation formations are referred to as 'habitat types'. Habitat types were chosen for representing the greatest contrast in soil moisture, plant species composition, differences in likely host-plant cover, and habitat structure (e.g. structural strata, leaf litter, and logs). These factors are potentially important in determining the distribution of truffle-like fungi based on previous studies (Scotts & Seebeck 1989; Claridge *et al.* 1993b, 2000a; b). Plots were positioned in areas without recent evidence of logging or other anthropogenic disturbance.

Rainforest habitat supported a closed canopy of *Ceratopetalum apetalum* and *Caldcluvia paniculosa*, an open shrub understorey consisting of *Pittosporum multiflorum* and *Tasmannia insipida*, and various vine, fern, and sedge species forming a sparse understorey. Heathy woodland was characterised by an open canopy layer dominated by *Eucalyptus olida*, *Eucalyptus ligustrina* and *Eucalyptus cameronii*, a low and uniform mid-storey dominated by heath-like species (*Leptospermum* spp., *Leucopogon melaleucoides*, *Monotoca scoparia*), *Acacia* species, and *Xanthorrhoea johnsonii*, and a sparse groundcover of grasses, prostrate shrubs, and bare ground. This habitat occurred on shallow to skeletal sandy granitic soils and supported a sparse litter cover. Dry open forest habitats were dominated by *Eucalyptus campanulata*, with clumps of various shrubs species including *Acacia* spp., *Leucopogon* spp. *Banksia* spp. forming a low middle strata, and a groundstorey dominated by grasses (*Entolasia stricta, Poa sieberiana*, *Microlaena stipoides*), sedges (*Dianella caerulea*, *Lomandra* spp.) and herbs. Wet sclerophyll was characterised by a tall

canopy variously composed of *Eucalyptus obliqua, Eucalyptus campanulata, Eucalyptus brunnea, Eucalyptus laevopinea*, and *Eucalyptus saligna*, a tall mesic middle layer of shrubs and commonly tree ferns (*Cyathea australis*), and a groundcover consisting of ferns, vines, sedges, herbs, abundant logs, and deep litter.

Data collection

The survey was undertaken during winter as previous research suggested truffle production may be greatest between late-autumn to early-winter in south-eastern Australia (Claridge *et al.* 1993b) and also a peak period of sporocarp consumption by mycophagous mammals within the local area (K. Vernes pers. comm.). As sporocarp production is likely to be influenced by rainfall (Claridge *et al.* 2000b; Abell-Davis 2008), winter was the most reasonable time of year to sample to ensure that each habitat type was experiencing the same rainfall pattern. Winter rainfall is markedly less variable than summer rainfall within the study area (Australian Government Bureau of Meteorology 2012). Rainfall peaks at the escarpment edge and decreases inland on the tablelands, a pattern pronounced during summer but not during winter.

The survey was undertaken within a three week period in June 2006 within habitat types randomly assigned for surveys each day. The sampling period was therefore short enough to reduce the potential for weather to influence fruiting production between habitat types. A minimum of 14 plots per habitat type were sampled with 16 plots surveyed in wet sclerophyll. Adequate distances were required between plots to ensure independence. Eucalypt root systems have been shown to extend 1.5 to 2.5 times tree height (Sudmeyer *et al.* 2004) and EcM rhizomorphs may extend these further. Maximum tree height across plots generally ranged between 15 m (heathy woodland) and 60 m (wet sclerophyll forest), equating to root systems extending 38 m to 150 m outwards from the tree base. Consequently, attempts were made to position plots >200m from one another with the exception of pairs of plots associated with mammal trapping grids (Figure 3.1; Chapter 3). The mean distance between all plots and their nearest neighbour was 359.2 ± 38.3 m. The mean distance to the nearest neighbouring plot for non-paired plots (n=37) was 490.6 ± 49.6 m (range: 115-1184 m). To reduce influencing effects from adjoining habitats, care was also taken to avoid plots near habitat boundaries. Resulting plots had a mean distance to the nearest neighbouring habitat boundary of 158.0 ± 18.5 m.

In total, fifty-nine plots across four different habitat types were sampled for truffles using the time-standardised technique (Claridge *et al.* 2000b). A pilot study using a species-time relationship was used to determine whether 100 person minutes was adequate to provide a reliable sample of species diversity within a 50 m x 20 m plot (Claridge *et al.* 2000b). In 9 of the 10 sites sampled no additional species were recovered after 50 minutes. One hundred person-minutes was therefore

deemed sufficient to sample species diversity within the defined area.

Corner boundaries of 50 m x 20 m (1000 m^2) plots were marked prior to surveys commencing. Several workers were involved in plot surveys, each instructed in the sampling method and truffle identification prior to surveys commencing. Plots were sampled by raking litter and soil up to a depth of 10 cm with four-tined cultivars, sampling a variety of microhabitats within the 100 person-minute time period. When found, sporocarps were placed in brown paper bags for processing at a later stage, using a new bag in each additional instance of finding sporocarps, regardless of species. Each of these samples represented a "collection", a sample of sporocarps constrained to a single location within the plot. Descriptions of fresh sporocarps were made at the end of each day and collections identified to genus-level where possible following methods given by (Claridge *et al.* 2000b). Sporocarps were cut in half and air dried with a portable food dehydrator at 45 ^{C} for 8-10 hours followed by placement in air-tight sealable plastic bags.

In the laboratory, thin sections of sporocarps were placed on 3 slides with the addition of Melzer's reagent, 5% potassium hydroxide (KOH), and water to one of each slide. A coverslip was then dropped onto the slide and the material examined using light microscopy. Features of sporocarp structure, spore features, and reactions to reagents were recorded. Specimens were identified to species wherever possible, with the use of published and unpublished keys and numerous publications providing taxonomic descriptions of truffle-like taxa.

Although total sporocarp counts may correlate with biomass (Claridge *et al.* 2000b) there was concern that differences in the dominance of some species between habitats and marked differences between these species in dry weight (g) could result in inaccurate estimates of biomass if based purely on abundance. Specifically, large numbers of light-weight *Cortinarius globuliformis* sporocarps were collected in some habitats while others had a high frequency of heavy-bodied *Mesophellia* sporocarps (e.g. *Mesophellia* spp.). Consequently, habitats were compared both on numbers of sporocarps (i.e. abundance) and biomass (i.e. dry weight). Sporocarps in each collection were counted and collections weighed to the nearest 0.001 g. Samples of *Mesophellia* spp., *Hysterangium aggregatum*, and *Gummiglobus joyceae* were treated differently (Appendix A) to reduce the potential bias these species may introduce into estimates of truffle biomass available for mycophagous mammals to consume. It should be noted that the time-constrained sampling method provides only a crude measure of true sporocarp dry weight and abundance (Claridge *et al.* 2000b) and does not provide an area-density ratio (e.g. per hectare dry weight). Rather, this method provides an approximate measure of relative dry weight and abundance among habitat types and plots.

Analysis

General

Where Kolmogorov-Smirnov normality test or Levene's test for homoscedasticity were significant, transformations of increasing severity were applied to data in attempts to improve normality and homoscedasticity. Where data met assumptions of normality and equality of variance, Analysis of Variance (ANOVA) in MYSTAT ver. 12 (SYSTAT Software Inc. 2011) was used to test for significant differences among habitat types. Where transformations failed, data were square-root transformed and the nonparametric Kruskal–Wallis test applied. Extreme outliers were removed prior to testing. Where data met assumptions of normality and equality of variance, Tukey's HSD (honestly significant difference) was used to test for significant differences among pairs of habitats. The non-parametric Mann-Whitney U-test (Bonferroni corrected) was used for pair-wise comparisons where data did not meet these assumptions. Both pair-wise tests were performed in the freeware statistical software PAST ver. 2.13 (Hammer *et al.* 2001).

Truffle abundance: counts and dry weight

As rainforest was hypothesized to support few truffle-like taxa and was likely to bias among-habitat comparisons, tests of significant differences were also undertaken with rainforest excluded. When rainforest was excluded, sporocarp dry weight and counts (square-root transformed) conformed to expectations of normality (Shapiro-Wilk test, statistic=0.986, P=0.873/P>0.15; statistic=0.973, P=0.401/P>0.05 respectively) and homoscedasticity (Levene's equality of variances: $F_{2,39}$=0.517, P=0.600/P>0.05; $F_{2,39}$=2.143, P=0.131/P>0.05 respectively). Consequently, tests were undertaken using parametric ANOVA to further investigate whether significant differences exist among groups, and between pairs of habitats through Tukey's HSD post hoc pair-wise tests.

In testing for differences between burnt and unburnt plots in species richness and truffle abundance, Trappe *et al.* (2006) excluded *Cortinarius globuliformis* due to the species potential dominance and ability to obscure patterns among other species. Consequently, additional comparisons were made among habitat types in truffle abundance and dry weight with this species excluded.

Species richness and species richness estimators

As there are significant issues with defining an 'individual' unit in fungal communities, the presence-absence (frequency) of species within samples (plots) was used following previous recommendations (Zak & Willig 2004). To estimate the total species richness of truffle-like fungi within habitat types and the sufficiency of sampling, accumulation curves were calculated. Non-

parametric methods were chosen as they are more useful in producing higher values for assemblages with higher number of rare species (e.g. 'uniques') and are less negatively biased than observed richness (Melo 2004).

No consensus has been reached as to which estimator is most well-suited to varying circumstances (Walther & Moore 2005; Soberón *et al.* 2007). Choice of a species richness estimator for accumulation curves followed decision guidelines of Brose *et al.* (2003) for sessile organisms and incidence data, but also the results of previous research. The Jackknife2 estimator has also been recommended where a calculated average sample completeness falls within of range of 50-74 % (Brose, Martinez, & Williams 2003; Mertl, Ryder Wilkie, & Traniello 2009; see calculation below) as was found in the current study (Table 2.1). Jackknife estimators have been found to be the best performing for incidence data and large-grain sampling methods (Brose *et al.* 2003; Hortal *et al.* 2006; Williams *et al.* 2007) while others have found these estimators to perform well where there are many rare taxa (Colwell & Coddington 1994) and for species rich communities (Chiarucci *et al.* 2003), a common occurrence with fungi (O'Dell *et al.* 2004). For these reasons, Jackknife2 species richness estimates and accumulation curves are presented.

To satisfy requirements for comparison of area-based accumulation curves, calculations were made on the same number of sampling events for each habitat type (n=14). The same sampling method and effort was undertaken for each habitat type. For each community, 100 randomizations (Kaeser & Kirkman 2009) of successively pooled randomly selected samples, with *no* replacement, were performed to calculate each species richness estimator. For estimators where no analytical variance is calculated (i.e. Jackknife2, ICE, and Bootstrap), sampling was undertaken *with* replacement to provide meaningful variance (among randomisations) on the right hand end of accumulation curves and allow comparison among datasets and estimators in variance (Gotelli & Colwell 2001).

Sample-based rarefaction curves (Mao Tau estimator) with 95% confidence intervals were also calculated for each habitat type to assess sample coverage and to allow a more rigorous assessment for difference among habitat types in species richness (Colwell *et al.* 2004). In contrast to accumulation curves, rarefaction curves allow direct comparison of taxon richness among factors (i.e. habitat type) at the same sample size. Significant difference among habitat types in fungal diversity were determined by visual assessment of whether 95% confidence intervals of sample-based rarefaction curves implemented in EstimateS v8.2.0 (Colwell 2006) overlapped with one another (Gotelli & Colwell 2001; Gardner *et al.* 2008; Tedersoo *et al.* 2008).

Uniques and duplicates

EstimateS was used to calculate the mean number of uniques (where a species is observed in only one sample) and duplicates (species observed twice; Longino, Coddington, & Colwell 2002;

Kaeser & Kirkman 2009) among runs. This information was used as another measure of the completeness of sampling for each habitat type (Mertl *et al.* 2009). The unique and duplicate curves should decline where sampling has been sufficiently comprehensive (Longino *et al.* 2002). The number of uniques and its proportion to total species richness within a habitat type respectively provide estimates of rare species numbers (Longino *et al.* 2002) and a measure of species rarity within assemblages (King & Porter 2005).

EstimateS v8.2.0 was used to calculate all species richness estimates, accumulation curves, rarefaction curves, diversity indices (see below), and comparisons of similarity based on presence-absence data.

Sampling completeness/coverage

Sample completeness is the proportion of taxa collected by an inventory (i.e. survey) of species within an area relative to the total true richness. In the absence of a complete inventory of species for a given area, this may be assessed by examining cumulative species-abundance curves (Palmer 1991) or by dividing the observed richness by the estimated total richness (Watson 2003). Following the recommended procedure of Brose, Martinez, & Williams (2003) and Brose & Martinez (2004) for selecting an 'optimal' nonparametric species richness estimator, the average of a range of estimators (ICE, Chao2, Jackknife 1, Jackknife2, Bootstrap) was calculated and used in the equation below to assess sample completeness:

$$\left(\frac{number\ of\ species\ observed}{average\ of\ all\ species\ richness\ estimators}\right) \times 100$$

This calculation provides an evaluation of the percentage of species richness which has been sampled (observed number of species) based on predicted total species richness by the chosen nonparametric estimators (Brose, Martinez, & Williams 2003). The average sample completeness was also used in the selection of an optimal species richness estimator to compare habitat types using accumulation curves (i.e. Jackknife 2; see Methods above).

Sporocarp species diversity and dominance

Diversity and evenness indices were calculated using sporocarp numbers (i.e. counts) to test whether habitats differed in truffle resources available to mycophagous mammals. The Shannon index (H') was calculated as a common measure of diversity. Fisher's log-series alpha was also calculated due to its greater capacity to discriminate among communities, and relative insensitivity to sample size, abundances of dominant species, and by the evenness of individuals among component species within communities (Zak & Willig 2004). The latter was a particularly desirable trait, as the distribution of 'individuals' (e.g. sporocarps) were likely to be highly variable among

samples. Community evenness was calculated by Pielou's Index (*J'*) using both sporocarp counts and dry weight data, with the latter calculated to account for habitat types where taxa producing large sporocarps were frequently encountered (e.g. *Mesophellia* spp.).

Dominance plots and species-abundance distribution models were calculated to explore differences in species-sporocarp traits among habitat types and as an additional comparative measure of truffle diversity and composition (Magurran 2004; Maciá-Vicente *et al.* 2008; Karpati 2010). Rank-abundance curves (i.e. *K*-dominance, Whittaker, and partial dominance curves) were calculated in the PRIMER v6 software package (PRIMER-E Limited; Clarke & Gorley 2006) for pooled samples within each habitat type (Zhou *et al.* 2007). Partial dominance curves, a variant of the more commonly used *K*-dominance, were used to view differences among habitats with reduced influence of the most abundant (sporocarp counts) species (Clarke & Gorley 2006). The relative diversity among habitat types is indicated by the relative elevation of cumulative *K*-dominance curves, with a lower elevation indicating higher diversity. Comparisons among cumulative rank-abundance plots were implemented in the DOMDIS routine in PRIMER by calculating the Manhattan distance (using log-weighting of species ranks to reduce the influence of lower ranked species) in 9999 permutations between all pairs of samples. An analysis of similarity (ANOSIM) was then used on the resulting dissimilarity matrix to test for significant differences among habitat types (Clarke & Gorley 2006).

To test for differences among habitat types in the dominance of species with small or large-bodied sporocarps, abundance (sporocarp counts) and biomass data were entered into PRIMER and the dominance plot routine used to generate ABC plots for each sample. Resulting *W* statistics, measuring the extent that the biomass curve lies above or below the abundance (counts) curve, were exported to PAST and univariate analysis of variance (ANOVA) used to compare values across habitat types and Tukey's HSD employed for further post hoc pair-wise significance tests among habitat types. Outliers were removed prior to analysis and data transformed to positive values before a square-root transformation to meet expectations and homogeneity and homoscedasticity. As for rank-abundance analysis, ABC plots for averaged data for each habitat type were also inspected visually (Clarke & Gorley 2006).

Taxon composition

Taxon composition among habitat types was first investigated using the number of shared species (observed) and several common indices of similarity. Sørensen's index of similarity (I_s) was calculated in the software PAST to compare patterns in beta diversity among habitat types as recommended by Magurran (2004). The number of observed shared species, and the incidence-based similarity indexes, Chao-Jaccard and Chao-Sorensen, were calculated using EstimateS v8.2.0

software (Colwell 2006). The latter two indices allow comparisons among groups using incidence frequency data and provide more accurate estimates than classic Sorenson or Jaccard indices by accounting for the number of unseen shared species among groups (Chao *et al.* 2005). They have also been shown to be insensitive to under-sampling, unequal sample size, and robust in comparing different data sets. The values for these indices vary between 0 and 1, with 1 indicating that the two communities have identical species composition (Cardoso *et al.* 2009).

Further trends in truffle-like fungal composition were investigated using Nonmetric Multidimensional Scaling (NMDS) ordinations and hierarchical cluster analyses. NMDS ordinations were calculated using presence-absence data, Bray-Curtis similarity matrices, and a minimum stress value of 0.01. Resultant extreme outliers (n=3) in both analyses were removed and the procedure repeated. The non-parametric Analysis of Similarity (ANOSIM) was used to test whether there were significant differences among habitat types in taxon composition, both at the species- and genus-level.

Similarity Percentage (SIMPER) was estimated to determine indicator species within habitat types and within-group similarity in species composition. PRIMER vector overlay plots (species and genus) were used as an additional test to explore monotonic relationships between habitat type and taxa (Trebitz *et al.* 2009). This statistical routine tests for correlations between species presence and the results of an NMDS ordination. Pearson rank correlation was used as the statistic of association, using 0.05 as a conservative cut-off for reporting species associations with any of the two NMDS axes (Laliberté *et al.* 2010). The strength and sign of the correlation with the two NMDS axes is represented by the length and direction of the lines (vectors) respectively. These analyses were all undertaken in PRIMER 6 software.

A recent critique of multivariate analysis methods (e.g. MDS, PCA, PERMANOVA, ANOSIM, SIMPER) using distance-based metrics (e.g. Bray-Curtis) found they can have low power to detect taxon composition differences unless the mean-variance ratio is constant across taxa or driven by high-variance taxa (Warton *et al.* 2012). Bray-Curtis was the best-performing of distance metrics but can still confound location effects for location/dispersion effects in MDS ordinations. Also, the SIMPER test may overweight the explanatory importance of high-variance taxa and underestimate that of taxa with low-variance but high between-group effects. Consequently, a conservative approach was taken in the interpretation of dispersion effects, taxon effects (i.e. discriminatory taxa), and non-significant ANOSIM results unless supported by other analyses.

Results

General survey results

Fifty-nine plots were surveyed for the truffles, with at least one collection made from all plots surveyed. In total, 342 collections of fungal sporocarps were made, the majority (321) of which were of truffle-like taxa, representing 1672 individual truffles (Table 2.1). Across all habitat types, a high percentage (93.3-100%) of plots supported truffles, although this proportion was slightly lower (91.5%) when *C. globuliformis* was removed from the analysis and was lowest in dry forest (73.3%). Eighty-five species and 25 genera of truffle-like fungi were collected across the study sites. Of these species, 30 (≈35%) are undescribed, one was placed in a new genus, and one is a new sequestrate representative (i.e. *Lepiota geogenia*; Lebel & Vellinga 2012) in a genus comprised largely of agaricoid taxa (Ge & Smith 2012). The most species-rich genera were *Arcangeliella* (13 species), *Descomyces* (13 species), *Cystangium* (9 species), *Cortinarius* (7 species) and *Hysterangium* (7 species). These same genera also supported the greatest number of undescribed species. Close to half (52%) of other genera recorded were represented by only a single species.

Truffle abundance

The number of collections per plot were significantly different among habitat types (ANOVA: F=25.96, df=3, 54, P<0.0001; Table 2.1) with pair-wise comparisons revealing that both wet sclerophyll and rainforest were significantly different from all other habitat types (Tukey's HSD P<0.001 and P<0.001 respectively; Table 2.1). Over three times (3.2) more collections were made in wet sclerophyll than in any other habitat type. This figure represents more than half (58%) of all collections and over a third (38%) of all sporocarps collected. Wet sclerophyll was also characterised by a smaller number of sporocarps per collection than other habitat types, suggesting other habitats supported species producing many sporocarps in one location or sporocarps overall having a more 'clumped' distribution. The lowest numbers of collections were made in rainforest plots, approximately half the number of the next most abundant habitat type (dry forest). Similar total and mean number of collections were found in heathy woodland and dry forest habitats.

Wet sclerophyll and heathy woodland had the highest total and mean truffle abundance (sporocarp counts). While heathy woodland supported the highest mean and total number of sporocarps, rainforest supported the lowest. The total number of sporocarps in heathy woodland and wet sclerophyll was ≈1.9 times the number collected in dry forest and ≈8.6 times the number collected in rainforest. Likewise, mean truffle abundance was similar for wet sclerophyll and heathy

woodland (38.9g and 36.0g respectively) and was ≈1.4 times greater than in dry forest and 6 times greater than in rainforest. Mean sporocarp numbers across plots (log10 transformed data) was significantly different among habitat types (ANOVA: F =23.970, df=3, 54, P<0.001). Pair-wise comparisons, however, revealed that only rainforest was significantly different from other habitat types (Tukey's HSD P<0.001; Table 2.1).

Total biomass (dry weight) of all collections was 227.4g, and 134.5 g after weights (of *Mesophellia* spp., *Gummiglobus joycea*, and *Hysterangium aggregatum*) were corrected for estimated non-fungal material. One species, *C. globuliformis*, accounted for 17.13% of total biomass and 36.8% of all sporocarps collected. The dominance of *C. globuliformis* was most notable across heathy woodland plots where the species accounted for 64.2% of all sporocarps collected.

Reflecting patterns in truffle abundance, total and mean dry weight was highest from wet sclerophyll (54.07g, 3.97±3.2 SD) and lowest from rainforest (3.38g; 0.24±0.4 SD). Mean dry weight (with correction) was similar among dry forest, wet sclerophyll, and heathy woodland (2.47-3.38g). Rainforest total and mean dry weight was less than a tenth that of the next lowest habitat type. Although dry weight was significantly different among habitat types (Kruskal-Wallis: χ^2_3=26.072; P=0.0001), pair-wise tests revealed only rainforest was significantly different from all other habitat types (Mann-Whitney P<0.001; Table 2.1).

When *C. globuliformis* was excluded from the analysis, there was a significant difference among habitat types in truffle abundance (Kruskal-Wallis: χ^2_3=23.58; P<0.0001) and dry weight (Kruskal-Wallis: $\chi^2_{3=}$19.94, P=0.00017). In post-hoc tests, only wet sclerophyll was significantly different in truffle abundance from all other habitat types (Mann-Whitney P<0.01; Table 2.1) and only rainforest in dry weight (Mann-Whitney P<0.05). Wet sclerophyll supported a greater number of sporocarps (2.4 times) and mean dry weight (1.7 times) than any other habitat type. Exclusion of *C. globuliformis* revealed this species as abundant in heathy woodland and dry forest habitats and relatively rare in wet sclerophyll and rainforest.

Table 2.1 Summarised survey results for truffle-like fungi across four habitat type; mean ± SD. Asterix following variable name denotes a statistical significant difference among habitats (one-way ANOVA or Kruskal-Wallace test, *P<0.01; ** P<0.001). Different letters denote significant (Tukey's HSD or Mann-Whitney test, P<0.01) pairwise difference between habitat types. Variables without letters did not differ significantly among habitats.

	Habitat				All Sites
	wet sclerophyll	heathy woodland	dry open forest	rainforest	
Full dataset	N = 16	N = 14	N = 14	N = 14	N = 58
% plots with truffle-like taxa	100	100	93.3	100	98.3
Total # collections	186	59	50	26	321
# collections**	12.38 ± 5.21 [a]	6.00 ± 2.94 [b]	5.29 ± 2.64 [b]	2.21 ± 1.12 [c]	6.67 ± 5.06
# sporocarps in collections**	3.54 ± 5.58 [a]	10.71 ± 18.0 [b]	6.60 ± 9.97 [b]	2.85 ± 2.95 [c]	5.28 ± 10.03
Total # of sporocarps	656	634	330	74	1694
# sporocarps (plots)**	41.0 ± 24.6 [a]	45.3 ± 34.4 [a]	23.6 ± 13.1 [a]	5.3 ± 4.7 [b]	29.1 ± 26.9
Dry weight (g)**	3.38 ± 2.54 [a]	3.04 ± 2.08 [a]	2.47 ± 2.23 [a]	0.24 ± 0.41 [b]	2.32 ± 2.32
Excluding *C. globuliformis*					
% plots with truffle-like taxa	100	92.9	73.3	100	91.5
Total # of sporocarps	623	227	147	74	1071
# of sporocarps (plots)**	38.9 ± 20.1 [a]	16.2 ± 28.6 [b]	10.5 ± 12.3 [b]	5.3 ± 4.7 [b]	18.5 ± 22.6
Dry weight (g)**	3.32 ± 2.44 [a]	1.96 ± 1.89 [a]	1.97 ± 2.38 [a]	0.24 ± 0.41 [b]	1.92 ± 2.22

Taxon richness among habitat types

Observed species and genus richness differed significantly (Kruskal-Wallis: χ^2_3=37.856, P<0.0001; χ^2_3=32.303, P<0.0001 respectively) among habitat types (Table 2.2). Wet sclerophyll habitat supported the greatest mean and total number of species and genera. The mean number of species per plot in wet sclerophyll was more than double (2.3 times) than in any other habitat. In addition, wet sclerophyll had the highest minimum number of species (4) and genera (3) per plot. While dry forest and heathy woodland supported similar species and genus richness, rainforest supported the lowest mean and total number of species and genera. Pair-wise comparisons (Bonferroni corrected Mann-Whitney U-tests; Table 2.2) revealed that both wet sclerophyll (P<0.05) and rainforest

(P<0.01) were significantly different in mean species richness to all other habitat types. Wet sclerophyll had a significantly greater species richness that any other habitat type, while rainforest had significantly lower species richness than any other habitat type. Dry forest and heathy woodland had very similar levels of species richness (P=1.0) with the latter supporting a slightly higher mean and total species and genus richness. Although differences in genus richness were less pronounced, the general pattern was similar, with wet sclerophyll supporting a significantly (Mann-Whitney P<0.01) greater number of genera and rainforest supporting a significantly (Mann-Whitney P<0.01) lower number of genera. Although the mean number of 'uniques' (a species detected only once) calculated in EstimateS was greatest in wet sclerophyll, the proportion (%) of uniques to total observed species was relatively high across all habitat types (50-71%).

Table 2.2 Summary data for truffle-like taxon richness, rarity (uniques), species richness estimates, and sample completeness. Asterix following variable name denotes a statistical significant difference among habitats (one-way ANOVA or Kruskal-Wallace test, *P<0.01; ** P<0.001). Different superscript letter denotes a significant (Tukey's HSD or Mann-Whitney test, P<0.01) pairwise difference between habitat types. Values are shown with ± standard deviation.

	Habitat			
Observed	wet sclerophyll	heathy woodland	dry open forest	rainforest
Species (observed)	64	27	21	7
Genera (observed)	21	17	15	5
Species/plot**	9.31 ± 3.38 [a]	4.00 ± 2.18 [b]	3.29 ± 1.77 [b]	1.43±0.65 [c]
Genera/plot**	5.94±1.69 [a]	3.57±1.99 [b]	3.21±1.67 [b]	1.36±0.63 [c]
Uniques mean	32 (50%)	18 (67%)	12 (57%)	5 (71%)
Estimates				
Jackknife2	105.02	56.77	38.47	15.93
Estimator range[‡]	67.6 - 119.3	33.8 - 81.0	25.9 - 38.5	8.78 - 27.6
Jackknife2 sample completeness (%)	60.9	47.6	54.6	43.9
Average sample completeness[‡] (%)	66.37	49.01	63.09	47.04
Sobs (Mao Tau) ± SD[†]	55 ± 5.2	27 ± 4.2	21 ± 2.9	7 ± 2.0
Sobs 95% CI	(44.8, 65.2)	(18.8, 35.2)	(15.4, 26.6)	(3.1, 10.9)

*P<0.05; ** P<0.001; [†] based on an even sample size (n=14), [‡] across all estimators (ICE, Chao2, Jackknife1, Jackknife2 and Bootstrap)

All richness estimators predicted wet sclerophyll to support the greatest number of species, reflecting patterns among habitats in the observed number. The order of species richness among habitat types was wet sclerophyll>heathy woodland>dry forest>rainforest for all estimators. There were considerable differences in total richness predicted by the five estimators. The Chao2 estimator consistently predicted the highest richness and the Bootstrap estimator the lowest,

although all estimators predicted greater species richness than observed. Estimated average sample completeness was relatively low (47-66%) for all habitats, although wet sclerophyll was predicted to be best sampled. Both Jackknife2 species accumulation curves and the relationship between uniques and duplicate curves suggested a need for greater sampling effort with the possible exception of rainforest (Appendix B).

Visual inspection of overlaps in 95% confidence intervals (CIs) of rarefaction curves showed a significant difference in species richness among all habitat types except heathy woodland and dry forest (Figure 2.1: A-B). The greatest distance between CIs was between rainforest and wet sclerophyll even at the lowest sample size, whilst dry forest and heathy woodland were indistinguishable with increasing sample size. Asymptotes for species accumulation were not reached in any habitat, strongly suggesting that sampling was not sufficient and that all habitats likely support greater species richness than that observed.

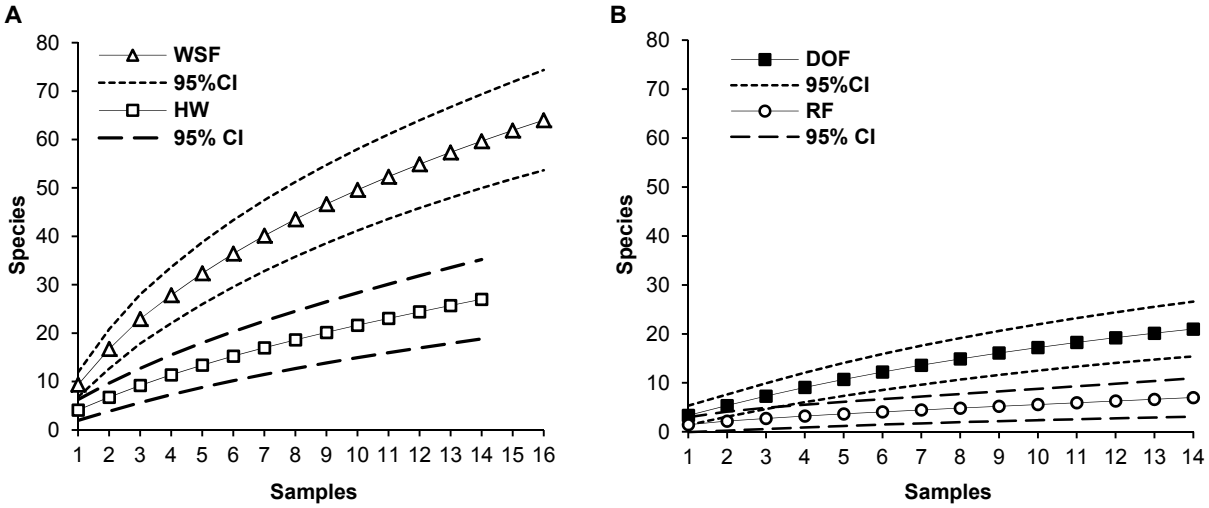

Figure 2.1 Comparison of truffle-like fungi rarefaction curves (Moa Tau) calculated for A) wet sclerophyll ('WSF'; *n*=16) and heathy woodland ('HW': *n*=14) and for B) dry forest ('DOF'; *n*=14) and rainforest ('RF'; *n*=14). Rarefaction curves are shown with lower and upper bound 95% confidence intervals. Note that confidence intervals for heathy woodland and dry forest overlap.

Truffle (sporocarp) species diversity and dominance

Indices calculated for truffle diversity followed patterns in species richness with wet sclerophyll supporting the greatest diversity (two to three times more diverse), dry forest and heathy woodland plots lower and similar diversity, and rainforest plots the lowest (Table 2.3). Fisher's alpha index estimated greater relative differences in diversity among habitat types compared to the Shannon-Wiener (H') index. Species evenness (variation among species in sporocarp numbers) was highest in wet sclerophyll and lowest in dry forest and heathy woodland, with the latter two habitats supporting a similarly low evenness. Species evenness in biomass (dry weight) showed a similar

pattern due to a strong correlation between biomass and truffle numbers. However, there was some difference between evenness indices based on sporocarp numbers and dry weight calculated for heathy woodland and dry forest, suggesting species were more uneven in sporocarp numbers than in dry weight.

Table 2.3 Diversity and evenness indices for truffles among habitat types.

	Habitat			
	wet sclerophyll	heathy woodland	dry open forest	rainforest
Shannon-Wiener (H')	3.43	1.53	1.67	1.35
Fisher's alpha	17.54	5.72	4.99	1.9
J' Evenness (Pielou)-abundance	0.82	0.46	0.55	0.69
J' Evenness (Pielou)-dry weight	0.82	0.66	0.72	0.75

A general pattern of truffle diversity and abundance (sporocarp counts) was attained through comparing cumulative and partial *k*-dominance curves (Figure 2.2B) derived from pooled samples for each habitat type. Visual inspection of cumulative *k*-dominance curves suggested rainforest supported the lowest diversity of sporocarps and wet sclerophyll the highest based on having the least and most elevated curves respectively (Magurran 2004). Following patterns in species richness, truffle diversity among habitats was ordered wet sclerophyll>dry forest/heathy woodland >rainforest, with heathy woodland and dry forest exhibiting very similar diversity. Comparisons of cumulative *K*-dominance curves using Analysis of Similarity (ANOSIM) showed a significant (P=0.001) global difference among habitat types (Table 2.4). Wet sclerophyll supported a significantly higher diversity of sporocarps and rainforest habitat a significantly lower diversity than other habitat types. In contrast, non-significant results (P=0.546) and negative *R*-value (-0.014) for pair-wise comparisons of heathy woodland and dry forest suggested these habitats supported highly similar levels of truffle diversity.

Comparisons of partial *k*-dominance curves (Figure 2.2B) also revealed that rainforest, heathy woodland, and dry forest plots were strongly dominated by the sporocarps of one to five species. In contrast, wet sclerophyll was characterised by low species dominance and high evenness in truffle abundance as suggested by a comparatively shallow slope in partial *k*-dominance (Figure 2.2B) while also revealing wet sclerophyll as having a relatively high proportion of species with a low abundance of sporocarps. Truffle production in heathy woodland was also revealed as being dominated by a smaller (n=3) number of species than dry forest. Both dry forest and heathy woodland exhibited a high degree of species dominance among pooled samples compared to wet sclerophyll (Figure 2.2B).

There was a significant difference among habitat types in *W*-statistics (ANOVA F=3.899, df=3,39,

P=0.016), which measure the extent a biomass (dry weight) curve lies above or below the abundance (counts) curve and characterises the dominance of species with different sized sporocarps. However, pair-wise tests revealed only heathy woodland and dry forest were significantly different (Tukey's HSD Q=3.956, *P*=0.038; Figure 2.2A). Heathy woodland exhibited a negative *W*-statistic (w=0.141) with the remaining habitat types exhibiting biomass on average being close to or above abundance curves, while dry forest exhibited the most positive *W*-statistics. These results suggest that heathy woodland had a high preponderance of species with small-bodied sporocarps while dry forest had a greater dominance of species with larger-bodied sporocarps. In addition, a high standard error for *W*-statistics across rainforest samples suggests that sporocarp size was variable.

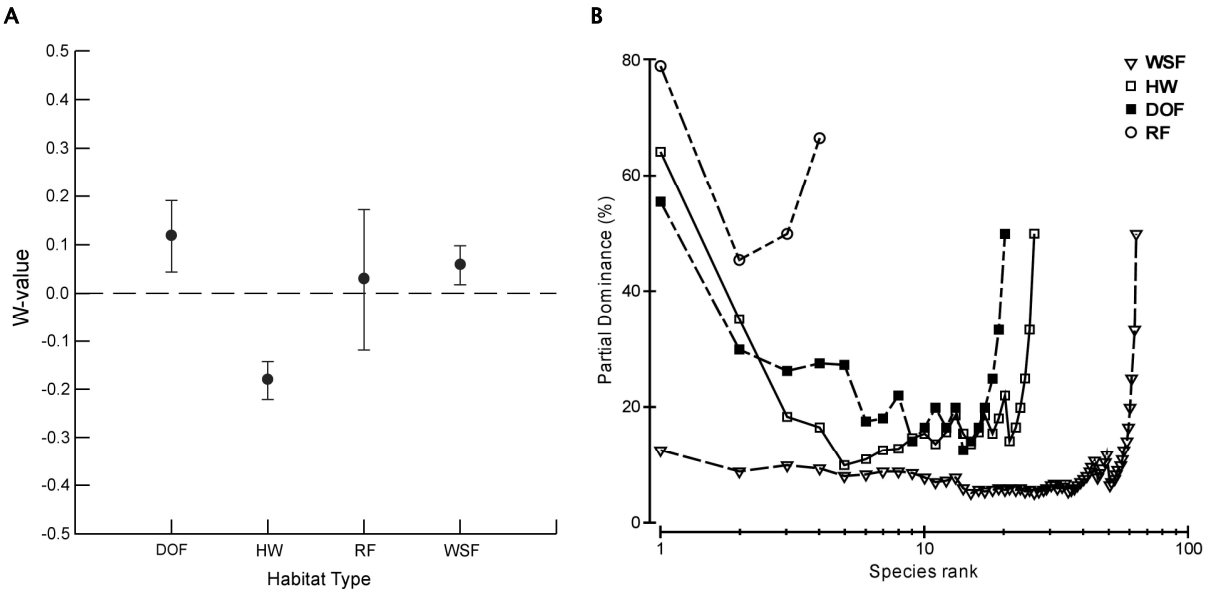

Figure 2.2 Species dominance patterns in truffle (sporocarp) biomass and counts among four habitat types, including A) *W*-statistics for sample ABC curves and B) partial *k*-dominance curves with log species rank for pooled samples among four habitat types. *W*-statistics for ABC curves (A) represents the degree to which the sporocarp biomass (dry weight) curve lies above the cumulative abundance (sporocarp counts) curve (mean ± standard error). Habitat abbreviations are: WSF=wet sclerophyll, HW=heathy woodland, DOF=dry forest, RF=rainforest.

Table 2.4 Global and pair-wise ANOSIM (two-way crossed) tests of *K*-dominance curves among habitat types as a resemblance matrix calculated using Manhattan distance with log-weighting of species ranks in the DOMDIS routine of PRIMER, showing the *R-value* and resulting significance value (*P*). WSF=wet sclerophyll forest, HW=heathy woodland, DOF=dry forest, RF=rainforest.

	Habitat	*R*	*P*
Global		0.268	0.001
Pair-wise	HW, WSF	0.441	0.001

	Habitat	*R*	*P*
Pair-wise	HW, DOF	-0.014	0.537
	HW, RF	0.189	0.002
	WSF, DOF	0.409	0.001
	WSF, RF	0.603	0.001
	DOF, RF	0.136	0.005

Truffle-like taxon composition

Only nine species were observed at five or more plots. These more commonly encountered taxa were *Cortinarius globuliformis*, *Descomyces varians*, *Endogone* sp. A, *Hydnangium carneum*, *Hysterangium* sp. nov. B, *Mesophellia glauca*, *Stephanospora flava*, *Arcangeliella* sp. nov. A, and *Arcangeliella microsporus*. Only three species, *C. globuliformis*, *Endogone* sp. A, *M. glauca*, and *S. flava*, were present at 10 or more plots. The most species-rich genera collected were *Descomyces* (13 species), *Arcangeliella* (13 species), *Cystangium* (9 species), and *Hysterangium* (7 species). Species belonging to these genera were most frequently encountered in wet sclerophyll. Only one genus, *Endogone*, was commonly collected in rainforest. *C. globuliformis* was abundant in both heathy woodland and dry forest habitats.

Estimated assemblage similarity at the species and genus-levels among habitat types was greatest between heathy woodland and dry forest (Chao-Sorenson: 0.78-0.98; Table 2.5). The level of similarity among dry forest, heathy woodland, and wet sclerophyll increased at the genus-level according to all measures of similarity. All indices indicate that wet sclerophyll and dry forest were the least similar at the species level among the three eucalypt dominated habitat types. Only rainforest was consistently dissimilar to all other habitat types at both the species (Chao-Sorenson: 0.07-0.11) and genus-levels (Chao-Sorenson: 0.20-0.33), while indices corrected for unseen taxa suggest there was little difference among all other habitats (Chao-Sorenson: 0.95-0.99) at the genus-level. The Chao incidence-based indices predict a higher similarity between heathy woodland and wet sclerophyll than Sorenson's Index at both the species and genus-level, suggesting a higher number of shared unseen taxa between these two habitat types than among others.

Table 2.5 Similarity comparisons among habitat types based on truffle-like taxa at the species- and genera-level, including observed shared taxa (Obs.) and three incidence-based indices accounting for 'unseen species'. Values are shown with ± standard deviation where applicable. WSF=wet sclerophyll forest, HW=heathy woodland, DOF=dry forest, RF=rainforest.

	Species				Genus			
	Obs.	Sorenson	Chao-Jaccard	Chao-Sorensen	Obs.	Sorenson	Chao-Jaccard	Chao-Sorensen
HW, WSF	14	0.31	0.54 ± 0.23	0.70 ± 0.20	13	0.72	0.91 ± 0.19	0.95 ± 0.12
HW, DOF	11	0.46	0.63 ± 0.13	0.78 ± 0.10	12	0.77	0.97 ± 0.08	0.98 ± 0.04
HW, RF	1	0.06	0.06 ± 0.06	0.11 ± 0.10	3	0.27	0.19 ± 0.19	0.33 ± 0.21
WSF, DOF	12	0.28	0.23 ± 0.14	0.37 ± 0.16	11	0.63	0.99 ± 0.20	0.99 ± 0.14
WSF, RF	3	0.09	0.05 ± 0.04	0.10 ± 0.07	3	0.23	0.19 ± 0.12	0.32 ± 0.17
DOF, RF	1	0.07	0.04 ± 0.04	0.07 ± 0.07	2	0.19	0.11 ± 0.14	0.20 ± 0.19

An NMDS ordination with a low two-dimensional stress level of 0.09 shows a clear distinction between wet sclerophyll, rainforest, and heathy woodland/dry forest habitats in truffle-like taxon composition (Figure 2.3A). There was a clear overlap in species composition among dry forest and heathy woodland sites, while there was a higher dispersion of wet sclerophyll plots, potentially reflecting greater variability in taxon composition.

Six species were significantly (r^2 >0.5) correlated with the NMDS ordination axes (Figure 2.3B; Trebitz *et al.* 2002). Four of these species, *Arcangeliella microsporus*, *Arcangeliella claridgei*, *Descomyces* sp. A, and *Hydnangium carneum* were associated with the NMDS 2 dimensional space dominated by wet sclerophyll samples, suggesting wet sclerophyll was characterised by a greater frequency of these species. Only *Cortinarius globuliformis* was associated with heathy woodland and dry forest habitats, suggesting this species was a primary factor in the overlap between these habitats in taxon composition. Similarly, only *Endogone* sp. A was correlated with the 2 dimensional NMDS space associated with rainforest.

Hierarchical cluster analysis shows similar patterns to the NMDS ordination, with three highly significant divisions (P=0.001) separating all rainforest plots from the majority of wet sclerophyll plots, and most dry forest and heathy woodland plots from all other clusters (Figure 2.3C). Six significant divisions were found overall. The majority of dry forest and heathy woodland plots were indistinguishable from one another based on species composition, forming a significant (P<0.05) grouping. The strongest groupings were for rainforest plots (P=0.005) with a low percentage similarity level (6%) and the majority of wet sclerophyll plots (P=0.001).

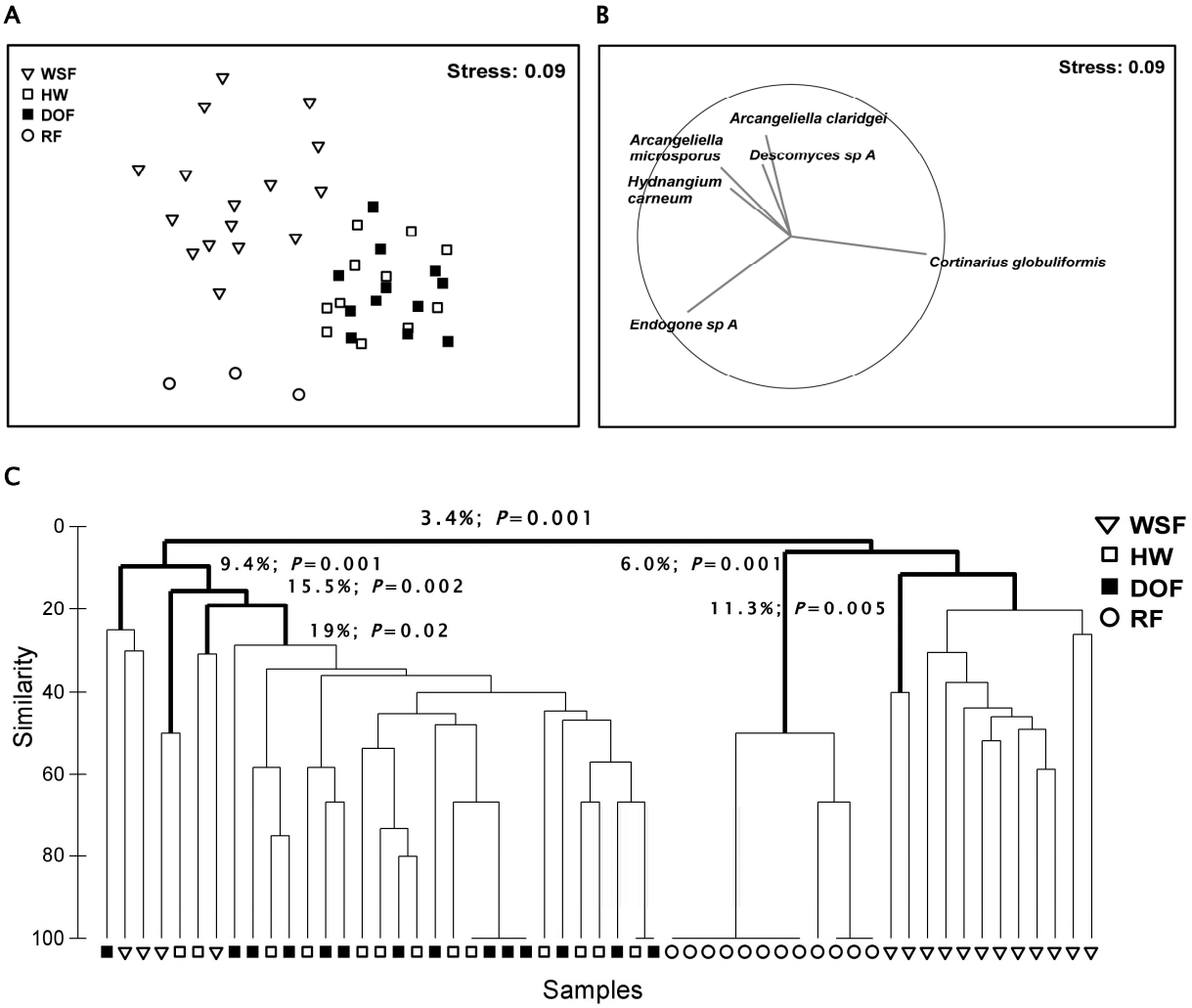

Figure 2.3 Composition of truffle-like species among four habitat types shown in a A) nonmetric multidimensional scaling (NMDS) plot of Bray-Curtis similarity coefficients, B) vector (species) overlay, and C) hierarchical cluster analysis for all plots (n=55) based on incidence data. The cluster analysis is shown with percentage similarity and *P*-values at each significant division (bold lines) as determined by a SIMPROF similarity profile test. The vector (species) overlay B) shows the sign (direction) and strength (line length) of each species correlation (where Pearson correlation r>0.5) with any of the two NMDS axes. The circle represents a 100% correlation (Anderson *et al.* 2010). WSF=wet sclerophyll forest, HW=heathy woodland, DOF=dry forest, RF=rainforest.

An NMDS ordination of truffle-like taxa composition among plots at the genus-level showed similar patterns to the species-level NMDS. Rainforest and wet sclerophyll were clearly distinguished from all other habitats while dry forest and heathy woodland overlapped in composition (Figure 2.4A). Rainforest was more distinctive while dry forest and heathy woodland plots were more dispersed than at the species level. Nine genera were significantly correlated (r^2 >0.5; explaining at least 50% of the variation) with the NMDS ordination axes. The vector correlation shows six genera associated with wet sclerophyll samples, one with rainforest, and two

with dry forest and heathy woodland (Figure 2.4B). The vector overlay suggests that *Austrogautieria* spp. may also contribute to differentiating heathy woodland and dry forest plots from other habitat assemblages in addition to *C. globuliformis*.

Groupings based on habitat type were strongly supported in a hierarchical cluster analysis for truffle-like fungi genera, reflecting the same patterns in the NMDS (Figure 2.4C). Two highly significant divisions (P=0.001) resulted in three distinct groups represented by all rainforest plots, all wet sclerophyll plots, and the great majority of dry forest/heathy woodland plots. Only a single plot was outside of these groupings based on habitat type. Rainforest plots were least similar (4%) to any other habitat type in genera. Additionally, non-significant divisions among samples within the three groupings (wet sclerophyll, rainforest, and heathy woodland-dry forest) are indicative of less variation among samples. Overall these results show that truffle-like fungi may form distinct communities among contrasting habitat types at the species level that are also reflected at the genus-level.

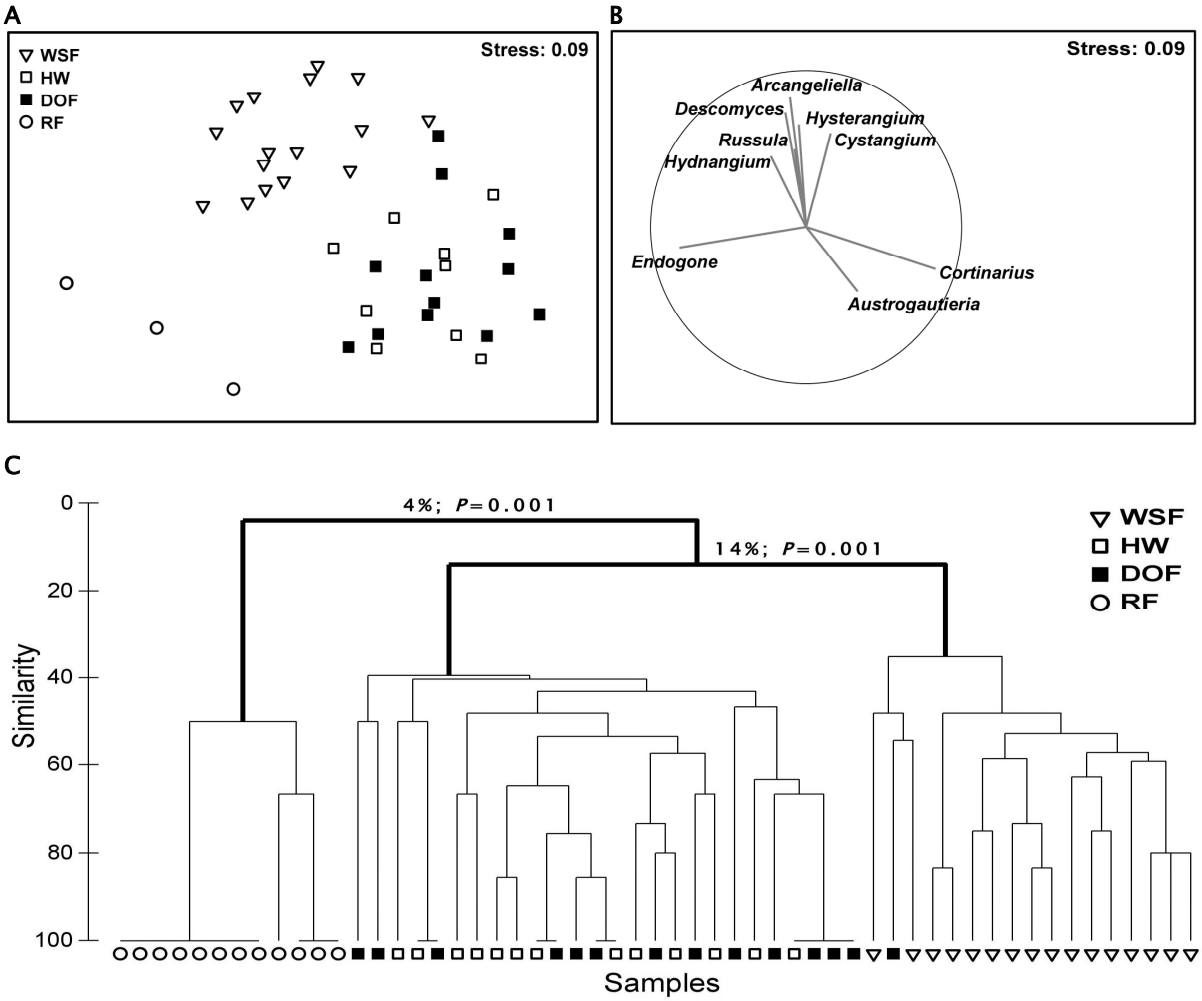

Figure 2.4 Composition of truffle-like fungi (genus-level) across all plots (n=55; three extreme outliers removed), as expressed in a A) nonmetric multidimensional scaling (NMDS) ordination plot, B) corresponding vector (genus) overlay plot, and C) hierarchical cluster analysis using presence-absence data. Symbols indicate habitat type. The vector plot shows all genera correlated (r>0.5) with any of the two NMDS plot axis. Line angle and length reflecting each taxon's correlation to the ordination axes. The circle represents a 100% correlation (Anderson *et al.* 2010). WSF=wet sclerophyll forest, HW=heathy woodland, DOF=dry forest, RF=rainforest.

A comparison of species composition among habitat types using Analysis of Similarity (ANOSIM) revealed an overall high Global *R*-value of 0.71 among habitat types (*R*=0.71; Table 2.6). Only heathy woodland and dry forest fungal assemblages were not significantly different at the 0.01% significance level. An ANOSIM test showed the two habitat types supported similar species, as suggested by a negative *R*-value (*R*=-0.019, Table 2.6). Wet sclerophyll supported a significantly different (*P*=0.01) assemblage of species, yielding high *R*-values (*R*=0.728-0.836). Similarly, rainforest supported a significantly dissimilar assemblage of taxa to all other habitat types (*R*=0.6-0.851; *P*=0.01). Significant differences among habitats in composition were also exhibited at the genus-level (Global *R*-value: 0.73; Table 2.6).

SIMPER tests also suggested high composition dissimilarity among all habitat pairs at both the species (61-99%) and genus (57-99%) level (Table 2.6) except between dry forest and heathy woodland. Several species (2-14) and genera (2-6) were important in discriminating habitat assemblages at a 50% cumulative dissimilarity cut-off and many (14-54) at a 90% cut-off (Table 2.6).

Table 2.6 Analysis of Similarity (ANOSIM) and SIMPER results comparing truffle-like taxon composition among four habitat types at both species and genus taxonomic levels: n.s.=not significant; *=significant at $P<0.05$. SIMPER no. of taxa relates to number of taxa contributing to differences between habitat types at cumulative 50% dissimilarity cut-off. WSF=wet sclerophyll forest, HW=heathy woodland, DOF=dry forest, RF=rainforest.

	Species				Genus			
Groups	*R*-value	*P*	SIMPER	Dissimilarity	*R*-value	*P*	SIMPER	Dissimilarity
HW, WSF*	0.8	0.001	15	95.96	0.749	0.01	6	86.63
HW, DOF n.s.	-0.019	0.61	8	61.24	-0.04	0.81	5	57.36
HW, RF*	0.9	0.001	3	97.75	0.831	0.01	3	97.52
WSF, DOF*	0.836	0.001	14	96.65	0.854	0.01	5	89.62
WSF, RF*	0.728	0.001	11	95.48	0.864	0.01	4	92.32
DOF, RF*	0.924	0.001	2	98.81	0.927	0.01	2	98.81

SIMPER tests revealed that only rainforest plots showed a relatively high species similarity (69%), with lower similarity among plots in all remaining habitats (21.09% to 39.92%). Low similarity among plots in wet sclerophyll suggested this habitat had the greatest variation in species composition. Four species contributed to >50% of similarity among wet sclerophyll plots: *Arcangeliella microsporus* (18.59%), *Hydnangium carneum* (16.78%), *Descomyces varians* (12.21%), and *Arcangeliella* sp. A (11.69%). Two species accounted for >50% of similarity among plots within both heathy woodland and dry forest habitats: *C. globuliformis* (HW: 76.7%; DOF: 89.1%) and *Mesophellia glauca* (HW: 11.22%; DOF: 3.85%). One species, *Endogone* sp.1, accounted for 96.6% of similarity among plots within rainforest. These results were in general agreement with those from the NMDS vector overlays (Figure 2.3B).

At the 50% cut-off for SIMPER tests, a single genus accounted for >50% similarity among plots within three of the habitat types (dry forest, heathy woodland, and rainforest; Table 2.6). *Cortinarius* accounted for 67.5% and 81.58% of similarity within heathy woodland and dry forest habitats respectively. *Endogone* accounted for 96.6% of similarity among sites within the rainforest habitat type. Within wet sclerophyll, the genera *Arcangeliella* and *Descomyces* accounted for 32.6 and 26.2% of similarity among plots respectively. Genera accounting for >10% of similarity were *Hysterangium* (14%) and *Hydnangium* (10.5%).

Discussion

Richness and rarity

In total, 85 species of truffle-like fungi were observed across four habitat types in this local scale 'snapshot' study of 59 plots. Truffles were frequently encountered, with nearly all plots containing at least one species and most supporting two or more. Observed species richness was higher than in a similar study in tropical north-eastern Australian forests and woodlands (49 species, 18 plots/72 samples; Abell-Davis 2008) but was equivalent to another landscape scale (≈32 281 km^2) study in temperate south-eastern Australia of primarily eucalypt dominated habitats (209 species, 136 plots/272 samples; Claridge *et al*. 2000a). Total observed genus richness (25 genera) was also similar to previous Australian local scale studies of varying sampling intensity (Trappe *et al.* 2006: 18 genera; Abell-Davis 2008: 25 genera; Trappe *et al.* 2008: 33 genera; Barrett *et al.* 2009: 12 genera). In comparison to overseas studies of truffle-like fungi, species richness was greater than a study of two contrasting (deciduous and evergreen) *Nothofagus* dominated forest types (27 species; Nouhra *et al.* 2011).

Within Gibraltar Range, Hunter & Sheringham (2008) estimated vascular plant species richness for a floristic community ('Community 1') nested within the 'heathy woodland' habitat type to be over 150 vascular species, and a community ('Community 4') nested within 'wet sclerophyll forest' to support more than 190 species. In comparison, this study predicted at least 60 truffle-like taxa within heathy woodland habitat and over 100 truffle-like taxa in wet sclerophyll. Consequently, for every two vascular plant species there may be at least one distinct truffle-like taxa within the study area. This is impressive, considering that only a certain proportion of vascular plants would form EcM fungal associations. The high plant species richness and endemism within the study area may have some part to play in supporting such a high richness of truffle-like taxa, along with the potential dominance of EcM plant species within some habitats.

In this local scale study, species and genus richness differed significantly among habitat types. In the taxonomic context, differences were exhibited at the genus-level and were not solely at the species level. Concordance between species- and genus-level patterns in richness provides further support for differences in taxonomic diversity among habitats. Similarly, differences in taxon composition across taxonomic levels may indicate greater taxonomic distinctiveness of assemblages within habitats. Differences in taxon richness among plots were most pronounced within wet sclerophyll which supported a significantly greater number of species and genera than any other habitat type. Mean and total observed richness was over twice that of the next nearest habitat type, heathy woodland. At both taxonomic levels, rainforest was found to be species and

genera poor. In contrast, dry forest and heathy woodland were very similar (non-significant) in richness at both the species- and genus-level.

Few other Australian studies have compared differences in taxon richness among heterogeneous habitat types. In contrast to this study, Abell-Davis (2008) found no truffle-like taxon richness responses to habitat type in tropical north-eastern Australia. Average and total taxon richness among habitat types did not differ markedly (26-29 species, 15-18 genera; Abell-Davis 2008). Although Claridge *et al.* (2000a) did not measure differences among habitats in species richness, greater numbers of taxa were found in more sheltered situations including gullies that would presumably support microhabitats with higher litter cover and in many cases vegetation broadly similar to the wet sclerophyll forest in the current study. With one exception (Abell-Davis 2008), previous comparative studies have looked at broadly similar habitat types, differing only in dominant tree species or elevation. At the landscape scale in Australia, species richness has been previously shown to vary spatially in response to topography, number of *Eucalyptus* species, and geology (Claridge *et al.* 2000a).

Species detected in only a single plot ('uniques') dominated collections across all habitats. This is not a surprising result for an EcM community which typically have a high frequency of rare species (Dunham *et al.* 2007; Tedersoo *et al.* 2008). However, the high number of uniques reported in the current study is likely to be strongly influenced by the relatively low sample coverage and the 'snapshot' nature of survey methods (i.e. a single sampling session restricted to one season). Nonetheless, other studies in Australia have found rare species to dominate truffle assemblages, despite repeat sampling or more comprehensive sampling techniques. For example, although Claridge *et al*. (2000a) sampled a larger number of plots (n=136) and across seasons (2), they found few species to occur at a sufficient number of sites (20%) to allow statistical predictions of distribution.

In contrast to the current study, Abell-Davis (2008) found no significant difference among vegetation types in the proportion of uniques to total richness. It is possible this contrasting result could be due to less marked differences among habitats in species composition in the previous study and also different treatment of data by the use of sporocarps as the sampling unit as opposed to presence-absence of taxa.

Fewer undescribed genera were encountered in this study (n=1) compared to those found by Claridge *et al*. (2000b; n=18) in a landscape scale survey. This result would be expected based on the smaller geographic scale of the current study. However, the number (30 species) and proportion (35%) of undescribed species discovered in this survey is very similar to that reported by Claridge *et al.* (2000b; 56 species; 27%) using similar sampling methods. The high proportion of new species in both studies underlines our limited knowledge of truffle-like fungi in Australia and adds

further evidence to Australia being a diversity hotspot (Bougher & Lebel 2001). The high proportion of taxa belonging to the sequestrate Basidiomycota follows trends elsewhere in Australia (Trappe *et al.* 2008).

As a comprehensive inventory of truffle-like fungi would take many years of repeat sampling to be fully realised (Castellano *et al.* 2004; Tedersoo *et al.* 2008), a much greater number of species are likely to occur within habitats than observed in this study. Species richness estimators and associated accumulation curves are a useful means of estimating species richness and assessing the completeness of a survey. In addition, quantitative rarefaction curves facilitate the direct comparison of fungal species richness among areas or habitats subject to different sampling effort (Zak and Willig 2004). A Jackknife2 estimate of species richness using all plot samples predicted a total of 143 species for the study area, 58 more species than observed (85 species; 59% sample completeness). Although high, this estimate may be a minimum one based on the 'uniques' curve reaching an asymptote but not declining (Longino *et al.* 2002) and that nonparametric indices commonly underestimate species richness (Mertl *et al.* 2009). EcM fungal communities are typically impressively diverse (Horton and Bruns 2001) and this characteristic was exhibited in this study with over a hundred truffle-like taxa predicted to occur within a single habitat type (wet sclerophyll, Jackknife2; Table 2.2).

Estimates of total species richness among habitat types produced the same ranking of habitats as observed richness: wet sclerophyll > heathy woodland > dry forest > rainforest. The difference between Jackknife2 predicted and observed number of species was in this same order, with wet sclerophyll estimated to support 41 additional undetected species and rainforest 8 additional unseen species.

Jackknife2 species accumulation curves did not reach an asymptote with increasing sample size in any habitat (Appendix B). The contrasting shallow slope of rainforest and steep slope of wet sclerophyll accumulation curves suggest that species richness was relatively well characterised in the former but not the latter. Trends in unique and duplicates curves (Appendix B), the high proportion of uniques, and estimated sample coverage (Table 2.2), all suggest that sample completeness was relatively low across all habitat types. Consequently, true species richness is likely to be substantially greater than that observed or predicted.

The slopes of Jackknife2 curves (Appendix B) within this study conform to expectations based on previous studies for EcM fungal assemblages where few inventories have reached an asymptote (Horton & Bruns 2001; Peay *et al.* 2010a). Comparison of sample-based Moa Tau rarefaction curves using 95% confidence intervals provided further evidence of a significant difference among most habitat types in the species richness of truffle-like fungi and similarity between dry forest and heathy woodland habitats. Both accumulation and rarefaction curves suggest the less species-rich

rainforest and dry forest habitats to be best sampled and wet sclerophyll the least well sampled. Similar trends in undetected species and high fungal richness were observed in sampling of EcM fungal communities on the root tips of mature *N. cunninghamii* trees in an Australian cool temperate forest (Tedersoo *et al.* 2009a). Sample-based rarefaction curves calculated for an assemblage of matt-forming EcM fungi in a North American Douglas-fir forest did not reach an asymptote and suggested a higher species richness than that observed (Dunham *et al.* 2007) as has another study of polyporoid and corticioid fungi (Lindner *et al.* 2006).

Our understanding of factors driving patterns in EcM and truffle-like species richness is limited. Claridge *et al.* (2000a) found higher species richness in sites with a greater number of eucalypt species and more sheltered topography. Although wet sclerophyll in the current study did not support a greater number of eucalypt tree species, this vegetation type commonly occurs in more sheltered situations within the study area while both heathy woodland and dry forest plots occurred on relatively flat exposed situations. Soil texture and nutrient levels are also linked to the occurrence and relative abundance of truffles (Claridge *et al.* 1993b, 2000a; Johnson 1994a). Low species richness has been found to be associated with coastal sediment geology (Quaternary sediments typified by nutrient poor, deep sands; Claridge *et al.* 2000a), potentially explaining the low species richness of heathy woodland habitat. Abell-Davis (2008) found precipitation (moisture), altitude, and number of *Allocasuarina* stems to be positively correlated to EcM species richness and dominant tree species (the ratio of *Eucalyptus* to *Allocasuarina* stems) to genus richness. Positive correlation between *Allocasuarina* and taxon richness was thought to be associated with a broader host range than co-occurring *Eucalyptus* species and that monodominance may increase taxon richness, a pattern found previously for EcM rainforest (Connell & Lowman 1989) but not for EcM sclerophyll forest. In contrast to findings of Johnson (1994a), Abell-Davis (2008) also found only a weak negative correlation between soil phosphorous concentration and truffle abundance (sporocarp counts and biomass) and taxon richness (species and genus).

Overall, previous research suggests species richness responds to complex gradients in biotic and abiotic conditions (Bougher & Lebel 2001). Of the many environmental variables measured in one landscape study (Claridge *et al.* 2000a), only a few (three) explained patterns in species richness. Individual species responses have been found to be associated with multiple environmental factors. Claridge *et al.* (2000a) found one to seven environmental variables to be significant in explaining the probability of occurrence in each of seven species. In a related study, at least three environmental variables were significantly associated with the probability of occurrence in five additional species (belonging to the genera *Castoreum*, *Mesophellia*, and *Nothocastoreum cretaceum*; Claridge *et al.* 2009a). Based on previous research and results of this chapter, a broad

range of interacting factors are likely involved in determining patterns of species richness in truffle-like fungi. Local scale richness may only differ significantly in response to large changes in abiotic and biotic conditions. Overall, the richness of truffle-like taxa mirrors that of other groups within the study area, including vascular plants (878 species; Hunter & Sheringham 2008) and non-flying mammals (28 species; Vernes *et al.* 2006). A great diversity of host-plant species and mammalian vectors may create an environment favourable to high species richness.

Assemblage composition

This study provides strong evidence for distinctive 'communities' of truffle-like fungi associated with broad habitat types at both the species- and genus-level. NMDS ordinations, cluster analysis, ANOSIM tests, and similarity indices (e.g. Sørensen) all suggested strong differences in the composition of taxa between most habitat types. Three communities of fungi were discriminated and associated respectively with wet sclerophyll, rainforest, and the drier heathy woodland-dry forest habitat types. In contrast to these results, Abell-Davis (2008) reported no differences in taxa composition among four vegetation types in tropical north-eastern Australia. However, Claridge *et al.* (1993b) found changes in truffle abundance (sporocarp counts) for some taxa along an ecotonal gradient from gully to ridge in south-eastern Australia. Significant associations between vegetation type and EcM species composition has also been observed in Tasmanian wet eucalypt forests (Gates *et al.* 2011). Elsewhere, Nouhra *et al.* (2011) found two *Nothofagus* forest types to differ in truffle-like taxon composition while Tedersoo *et al.* (2006) also found managed and forested parts of a wooded meadow to harbour different communities of EcM fungi. In more similar habitat type comparisons, Peay *et al.* (2010b) found a significant difference in tropical EcM communities based on soil types (clay and sand) which supported different vegetation types. Weak habitat specificity in EcM communities has also been shown for 6 plant communities in alpine tundra (Ryberg *et al.* 2011). In North America, distinct EcM community composition has also been observed between coniferous and deciduous forests of North America (Nantel & Neumann 1992), oak savanna and oak forests (Dickie *et al.* 2009), and between upland and wetland Black Spruce *Picea mariana* forest (Robertson *et al.* 2006).

NMDS ordinations, hierarchical cluster analysis, and ANOSIM tests all suggested a similar pattern at both the species- and genus-level in assemblage composition among habitats. Fewer significant divisions in cluster analysis indicated habitat groupings of fungi based on genera were more distinct than at the species-level. Although genus-level differences provide some further support for the taxonomic distinctiveness of assemblages among habitats, more extensive sampling of sporocarps could reduce these apparent differences. Consequently, a robust dataset than collected in the current study is likely required to confirm genus-level differences.

The composition of species and genera encountered in this study were more similar to those reported for temperate south-eastern Australia (Claridge *et al.* 2000b; Trappe *et al.* 2006, 2008; Barrett *et al.* 2009) than for tropical north-eastern Australia (Abell-Davis 2008). Temperate and tropical regions of the world have been shown to support similar assemblages of EcM fungi at the family level (Peay *et al.* 2010b) although few studies have investigated these patterns in relation to truffle-like fungi (Castellano *et al.* 2004). General patterns of species occurrence among habitat types were strong by visual inspection of data. Species responses to habitat type could be visually grouped by genus. Species within *Arcangeliella*, *Cystangium*, *Descomyces*, *Gymnomyces*, and *Hysterangium* were predominantly recorded in wet sclerophyll plots. Species within *Austrogautieria, Cortinarius,* and *Nothocastoreum* were most frequently encountered in dry forest and heathy woodland plots and *Endogone* in rainforest. These visual patterns were generally confirmed by vector overlay and SIMPER results. At the genus-level, *Hydnangium* was identified as an additional indicator species for wet sclerophyll, both in vector correlations with NMDS ordinations and SIMPER analyses. Only *Austrogautieria* and *Cortinarius* were associated with dry forest and heathy woodland plots in vector overlays and only the latter genus in SIMPER tests.

At the species level, a greater number of species (four) contributed to similarity amongst plot-level wet sclerophyll fungal assemblages compared to those in dry forest, heathy woodland, and rainforest. These were two *Arcangeliella* species, one *Descomyces* species and *Hydnangium carneum*; although the identity of two species in the former two genera differed in NMDS vector correlations and SIMPER results. In contrast, NMDS vector correlations and SIMPER results suggest that high within- and between-group similarity in dry forest and heathy woodland plots was largely due to the dominance of *C. globuliformis*. Similarly, a single genus (*Endogone*) and species (*Endogone* sp. A) were implicated in the similarity among rainforest plots. These results are not surprising considering wet sclerophyll was found to be species-rich and rainforest species-poor, while both heathy woodland and dry forest were of intermediate species-richness (Table 2.2 and Figure 2.1). Associations with habitat at the genus-level are an interesting result and has been suggested previously based on observations of co-occurrence in similar habitats of species within the same genus such as *Arcangeliella* (Claridge *et al.* 1993a). Similarly, Jumpponen *et al.* (2004) found some clustering of species occurrence by genus, with three *Descomyces* species and three *Arcangeliella* species significantly associated with one another by preferring similar habitats. The results presented here suggest that broad habitat trends could be generalised for the fruiting abundance of some genera.

Species encountered relatively equally in wet sclerophyll, dry forest, and heathy woodland were *Hydnoplicata whitei*, *Cortinarius levisporus*, and *Mesophellia* spp. (particularly *M. glauca* and *M. trabalis*). *Mesophellia* spp. were thought to be associated with moist, cool habitats (Claridge *et al.*

2000a; Trappe *et al.* 2006) but more recent habitat modelling suggest that species preferences within *Mesophellia* may be more varied (Claridge *et al.* 2009b). Interestingly, despite the low sampling completeness of this study, *M. glauca* and *M. trabalis* were both observed to be broadly adaptable to a range of habitats, corresponding to results of habitat modelling elsewhere (Claridge *et al.* 2009b). Similarly, no environmental variables could delimit the occurrence of *H. whitei* (Claridge *et al.* 2000a) and based on the findings of this study, this species could also have a broad niche range. The association between *Arcangeliella* and wet sclerophyll plots may be due to higher nitrogen levels or high litter in this habitat type. Soil nitrogen has been shown to influence the occurrence of one *Arcangeliella* species (Claridge *et al.* 2000a), while another found various *Arcangeliella* species more common in low-lying areas where nutrients accumulate (Claridge *et al.* 1993b).

Rainforest was dominated by sporocarps of *Endogone* species. This confirms earlier reports of Australian rainforests supporting predominantly arbuscular mycorrhizal (AM) species (Brundrett *et al.* 1995). However, further surveys are required to establish the generality of this, as the predominance of AM fungi in rainforest habitats has recently been found not to be exclusive, with rainforests capable of supporting a high diversity of EcM fungi similar in composition at the family level to their temperate counterparts (Peay *et al.* 2010b). The relative composition of EcM and AM fungi appears to be largely dependent on whether the rainforest is composed of a monodominant stand of tree species primarily forming EcM fungal associations or several AM fungal host tree species (McGuire 2007, 2008). The dominant tree host within the rainforest plots, Coachwood *Ceratopetalum apetalum*, is known to form associations with arbuscular mycorrhizal fungi (McGee 1990; McGee & Furby 1992) and this is likely the main reason few EcM truffle-like fungi were observed in rainforest habitat within the study area.

The most frequently encountered Australian truffle-like taxon, *C. globuliformis*, was strongly associated with dry forest and heathy woodland habitats, with only a single collection made outside of these habitats (in wet sclerophyll). Habitat models suggest the species has a decreasing probability of occurrence with increasing moisture and time since fire (Claridge *et al.* 2000a). This fits with the present findings, with *C. globuliformis* more frequently encountered in drier habitats with rocky skeletal soils and low litter levels. Time since fire, however, only partly corresponds to these results. The large majority of heathy woodland and wet sclerophyll plots were subject to high-intensity wildfire in 2002, 4 years prior to surveys, while there are no records of recent (<40 years) wildfire or prescribed burns for the majority of dry forest plots. The association between time since fire and *C. globuliformis* occurrence may represent other related abiotic factors, such as litter cover which is sparse after fire (Claridge *et al.* 2000a; Trappe *et al.* 2009a). Amaranthus *et al.* (1994) found a positive association between course woody debris and sporocarp production (both

numbers and dry weight) in Douglas-fir forests while the results of one Australian study indicated that a reduced litter cover after fire may contribute to lower soil moisture less conducive to truffle formation (Trappe *et al.* 2006). The higher frequency of this species in the two drier habitat types (dry forest and heathy woodland) is surprising, considering that the species' fleshy sporocarps do not appear to be particularly resistant to desiccation and consequently more adapted to dry environments over wet ones (Claridge *et al.* 2000b). However, it is possible that soil-microbe and insect activity is lower within these habitat types compared to wet sclerophyll, allowing the sporocarps to persist longer and increasing detectability. Few other species were sampled sufficiently across plots to make any additional observations on species-specific responses to habitat type.

Abundance and diversity of truffles in relation mycophagous mammals

The sampling method used in this study does not provide accurate measures of truffle abundance and dry weight as a function of area (i.e. 'sporocarp production'; North *et al.* 1997; Claridge *et al.* 2000a) but may provide a measure of general trends among habitat types. Habitats were characterised based on truffle abundance and dry weight to explore the relative availability, diversity, and abundance for consumption by mycophagous mammals. The average abundance and dry weight of truffles was greatest in wet sclerophyll and heathy woodland, and lowest in rainforest habitat. Only rainforest was significantly different from other habitats however. Abell-Davis (2008) also found no significant difference in truffle abundance among habitats within season, in a similar habitat type comparison (e.g. eucalypt forests and woodlands). In contrast to the results of this study, average truffle abundance and biomass was found by Abell-Davis (2008) to be lowest in wet sclerophyll forest and highest in dry *Allocasuarina* forest. However, Abell-Davis (2008) notes that the wet sclerophyll forest in the study area contained a high proportion of rainforest tree species. If these tree species formed predominantly AM fungal associations, it may explain these contrasting results.

Other Australian studies have found truffle abundance to differ at a smaller microhabitat scale in relation to topography and soil conditions. Claridge *et al.* (1993b) found gully environments to support higher truffle abundance compared to mid-slope and ridge sites while a later study (Claridge *et al.* 2000a) also found higher truffle abundance in more sheltered aspects. In both studies, these landscape positions are associated with higher soil moisture and litter levels. Also at the microhabitat scale, Johnson (1994a) found truffles to be more numerous in clayey sands than in loam soil types, and more abundant on relatively acid soil with low phosphorus content. Overseas, truffle biomass has been found to differ between sites in Douglas fir stands for some species (Cázares *et al.* 1999), between plantations of *Eucalyptus dunnii* and *Pinus taeda* (Giachini & Souza 2004), and at a small spatial scale (100 m distance) between upland and riparian areas in mixed-

conifer forests in north America (Meyer & North 2005). In the latter study, riparian sites had significantly greater soil moisture and abundance of truffles.

In contrast, this study found no significant difference in truffle abundance among eucalypt dominated habitats types, with similar mean abundance found in the wettest, most sheltered habitat type (i.e. wet sclerophyll) and in the driest, most exposed habitat type (i.e. heathy woodland). However, when the abundant *C. globuliformis* was excluded from comparisons, wet sclerophyll was found to support a significantly higher average number of truffles (at least 2.4 times more) than any other habitat. Dry forest was also discovered to have a lower frequency (73.3%) of plots supporting truffle-like fungi while *C. globuliformis* was found to be a major component of fungal assemblages in both heathy woodland and dry forest habitats. In contrast, exclusion of *C. globuliformis* had no significant bearing on differences in average truffle biomass among habitat types. Nonetheless, if the palatability of *C. globuliformis* to mycophagous mammals is found to be low, wet sclerophyll could support a higher availability of truffles for mammals to consume. However, it must also be reiterated that the current study uses a highly restricted dataset, representing a single sampling session in one season. There can be considerable variation between seasons and years in truffle production (Fogel 1976; Claridge *et al.* 1993b, 2000b; Johnson 1994a; Luoma *et al.* 2004) and additional sampling may have yielded different results.

Truffle abundance has been shown to respond to rainfall events, season, soil conditions (type and nutrient levels), and moisture levels (Johnson 1994a; Bougher & Lebel 2001; Abell-Davis 2008). Wet sclerophyll sites are assumed to have higher soil moisture, were positioned in sheltered aspects and at lower points in the landscape. These conditions have been found to favour higher truffle production at the microhabitat (Claridge *et al.* 1993b; Meyer & North 2005) and landscape scales (Claridge *et al.* 2000a). These results also have relevance to investigations into the frequency and taxon composition of fungal spores in the diets of mycophagous mammals. The relative abundance of spore types in scats may vary according to sporocarp availability (North *et al.* 1997; Bougher & Lebel 2001). Consequently, this information is useful to subsequent studies comparing the diets of mycophagous mammals among habitat types and assessing whether consumption reflects sporocarp availability.

Diversity indices and comparisons of *K*-dominance curves suggest that the diversity of species (as measured by truffle abundance) differs among habitat types. Truffle diversity followed the same ranking of habitats in species richness: wet sclerophyll>heathy woodland>dry forest>rainforest. In pair-wise comparisons of *K*-dominance curves, only wet sclerophyll was found to support a significantly higher diversity of sporocarps as suggested by diversity indices (two to three times more diverse). Evenness in abundance and biomass as estimated by *J'* did not differ greatly amongst habitat types although wet sclerophyll had the highest evenness. Comparison of partial *K*-

dominance curves, however, suggests the latter habitat type may have a significantly more even distribution of species amongst sporocarps compared to other habitat types.

Habitats also differed in the size of sporocarps (W-values; Figure 2.2A) although only heathy woodland was found to be significantly different from other habitat types in supporting a high predominance of species with small-bodied sporocarps. Indices of truffle evenness and also dominance curves suggest strong differences among habitats in the taxon composition of truffles available to mycophagous mammals. Both dry forest and heathy woodland were dominated by the sporocarps of a small number of species. In contrast, wet sclerophyll was characterised by an even distribution of species amongst sporocarps with only a small number of species producing more abundant sporocarps. Although data were not collected on the spatial arrangement of truffles, the higher number of sporocarps per collection and dominance of a few species among sporocarps in dry forest and heathy woodland is weakly indicative of greater clumping of sporocarps. Conversely, the more even distribution of species among sporocarps and lower number of sporocarps per collection, suggests less clumping of truffles in wet sclerophyll habitats. A clumped distribution in sporocarps has been reported previously for truffle-like (Fogel 1976; Taylor 1992; Claridge *et al.* 1993b) and EcM fungi (Horton & Bruns 2001; Taylor 2002), and may in part reflect the spatial distribution of EcM mats (Griffiths *et al.* 1996). As the spatial distribution of resources can influence movement foraging strategies in mycophagous mammals (Vernes & Haydon 2001), foraging strategies may differ among habitat types. Also, the diets of individual mycophagous species may vary among habitats based on their ability to exploit a patchy resource.

Variation in *W*-values among habitats suggests a weak possibility of differences in foraging efficiency for mycophagous mammals among habitat types. Greater concentrations of fungal food resources through the predominance of larger-bodied sporocarps (as inferred from dry weight), such as in dry forest, may make foraging more efficient. Alternatively, a varied and more evenly distributed food resource (i.e. lower clumping) may increase consumption and encounter rates by mycophagous mammals. For example, wet sclerophyll supported a high diversity of truffles that were more dispersed in space (as suggested by lower numbers per collection). There is evidence for truffle-like fungi having preferences at the micro-habitat scale (Johnson 1994a) and for producing sporocarps at different depths in the soil (e.g. members of Mesophelliaceae generally produce fruits at deeper depths than species belonging to other families; Vernes & Lebel 2011) and in the soil/litter interface (e.g. genera *Protoglossum* and *Cortinarius* produce sporocarps rising above or just below the leaf litter horizon)(Claridge *et al.* 1993b; Bougher & Lebel 2001; Peintner *et al.* 2002). Many mammal species show foraging preferences at the micro-habitat scale (Claridge *et al.* 1993a; Vernes 2003) and have different physical adaptations for exploiting food resources (e.g. olfactory and digging abilities). Habitats supporting a large array of truffle-like taxa with different

visual and olfactory detectability, microhabitat preferences, and vertical position in the soil-litter profile will presumably cater to a wider range of mycophagous mammal species. In additional, nutritional value may vary among truffle-like taxa (Claridge & May 1994; Wallis *et al.* 2012). Functional grouping of truffle-like fungi in relation to mycophagy (e.g. as a food resource) requires further investigation and may reveal patterns in the mycophagous habits of mammals and adaptive evolution in the truffle form.

Interestingly, patterns in sporocarp numbers (counts) and dry weight among habitat types mirror those in species richness and composition. Dry forest and heathy woodland habitats were relatively similar in all variables measured and showed similar composition. In contrast, wet sclerophyll supported the greatest taxon richness, sporocarp numbers and dry weight and was markedly different in taxon composition. Rainforest supported the fewest sporocarps, taxon richness and supported different taxon composition to all other habitat types. Overall, wet sclerophyll supported the greatest diversity of truffles and rainforest the lowest. Based on these results, habitats may provide different truffle food resources for mycophagous mammals. Also, if clumping of sporocarps confers greater foraging efficiency, mycophagous mammals may be able to forage more efficiently in drier habitat types than in wet sclerophyll.

The present study provides some evidence for high beta diversity at the local spatial scale suggesting that protection of a variety of habitat types would assist in conserving the greatest number of species. Similarly, reservation of a broad range of habitat types is also like to benefit mycophagous mammals through maintaining a high diversity of truffle food resources. This is particularly important considering the low evenness of truffles across plots in many habitats, variable phenology among species, and potentially patchy distribution in space.

Limitations

There were various limitations in this study regarding species richness estimations. As neither Jackknife2 accumulation curves nor Mao Tau rarefaction curves reached an asymptote in any habitat, the sampling effort may be too low to fully characterize species composition (Tedersoo *et al.* 2006). Results of my comparisons in taxon composition among habitat types must be viewed in this context. Jackknife2 incidence-based richness estimator commonly requires 50% of species sampled before producing stable estimate values (Colwell & Coddington 1994) while many estimators may require ≈50% of total sampling effort to produce accurate and unbiased estimates (Foggo *et al.* 2003). Estimated sampling completeness in this study, based on average species richness estimate, ranged from 47% to 67% among habitat types. Sampling of over half the species in a fungal community is rarely achievable due to the cryptic nature of fungi, poor resolution of sampling techniques and the high diversity of fungal communities (Bougher & Lebel 2001;

Tedersoo *et al.* 2009a). Jackknife2 estimates presented here are considered conservative lower-bound estimates of species richness which is often the most achievable result for species rich groups such as fungi (Magurran 2004; Zak & Willig 2004).

Variation among plots in taxon composition was high (SIMPER results) for all habitats except rainforest. Inter-plot variation was greatest in wet sclerophyll, supporting observations from NMDS ordinations and likely due to the large number of 'uniques' within this habitat type. High variation among plots and in cases high similarity in shared taxa, suggest that it would be unwise to suggest that this study has sufficiently characterised the communities occurring in each habitat type. Further sampling may have revealed differences between dry forest and heathy woodland assemblages. As discussed previously, distance-based multivariate methods may have low power to detect taxon composition differences, confound location effects for location/dispersion effects in ordinations, and misidentify high-variance taxa as important discriminatory taxa. It is possible that methods used here were unable to detect real differences between dry forest and heathy woodland and may have over-estimated dispersion in wet sclerophyll. However, important taxa identified with SIMPER analysis agreed well with observations of the raw data.

Discrepancies between sporocarp and mycorrhiza abundance have been noted in several studies (Gehring *et al.* 1998). These can be extreme to the extent that community structure based on sporocarp abundances can be the mirror opposite of the abundance of mycorrhiza on the roots of host plants (Dahlberg 2001). There can also be considerable variation among fungal taxa in sporocarp production. As a result, truffle abundances in this study are used only to investigate patterns in relative availability among habitat types. Considering the high consumption rates of truffles by mammals (North *et al.* 1997), and potential for preferential consumption of taxa by mammals, mycophagy may obscure patterns in the relative abundance of sporocarps among species. For example, the apparently high abundance of *C. globuliformis* sporocarps in some habitats may partly reflect a potentially low palatability to mycophagous mammals. Furthermore, differences among habitats in mammal community structure may influence the detectability of truffle-like taxa, as results suggested mycophagous mammals differed in the taxon composition of truffles consumed. However, this is considered unlikely to be a strong enough effect to determine the observed differences in fungal composition and richness among habitats. Further investigation is required to deduce the influence of mycophagy on the sporocarp sampling method incorporating new molecular techniques (Izzo *et al.* 2005b).

All habitat types are likely to have experienced similar rainfall (events) and temperatures prior to and during the sampling period. However, variation in fruiting patterns of species by habitat type may have influenced the detection of species and observed differences or similarities among habitat types. It is likely that truffle-like fungi fruit at different times according to microclimate conditions

within different habitat types. A long term, multi-seasonal survey would detect such variation. Nonetheless, Izzo *et al.* (2005a) found annual occurrence of dominant EcM taxa to be constant at larger spatial scales but vary more at a smaller spatial scale. In addition, the latter study found that EcM community composition within plots across years was more similar than to other plots within the same year, suggesting that yearly variation may not mask differences among areas and some consistency in composition year-to-year despite rapid and micro-scale (<20cm) turnover observed. Consequently, this 'snapshot' study may reflect differences among habitat types and is not likely to be overly biased by inter-year variation in fungal taxon composition.

Chapter 3. Mycophagous habits of small-mammals among contrasting habitats and comparisons to sporocarp collections

Introduction

Many native Australian mammals species are known to consume the fruit-bodies ('sporocarps') of macrofungi and in particular, those of truffle-like fungi that are often referred to as 'truffles' (Claridge & May 1994; Reddell *et al.* 1997; Claridge 2002). The symbiotic relationship between a large number of vascular plants and mycorrhizal fungi is well established. Hypogeous or 'truffle-like' fungi are those fungi which produce subterranean or partially emergent sporocarps containing basidiospores that are not forcibly discharged or are entirely enclosed by fungal tissue within the sporocarp (sequestrate). This fungal group does not form a monophyletic group; with the hypogeous-sequestrate sporocarp form having evolved independently several times among several fungal lineages (Peintner *et al.* 2001). They have evolved to use animals, including insects (Lilleskov & Bruns 2005), birds (Simpson 2000), and most commonly mammals (Claridge & May 1994), to disperse their spores in the absence of the ability to actively discharge their spores combined with passive mechanisms (e.g. wind and water) for dispersal. Mycophagous mammal consume the sporocarps containing spores and disperse the spores in their scats to new locations and host plants, and in some cases enhance the viability of spores during gut-passage (Colgan III & Claridge 2002). Consequently, animals play an essential role in dispersing spores away from 'parent' sources and to sites amenable for infection of host plant roots, thereby maintaining the symbiosis between ectomycorrhizal (EcM) forming plant hosts and truffle-like fungi and forming a tripartite association between all three life-forms (Bougher & Lebel 2001).

Species of small mammals may exhibit different habitat type and microhabitat preferences (Vernes 2003). In addition, feeding strategies and foraging behaviour may also influence the abundance and composition of fungi in their diets. Consequently, mammals may differ in their relative role in facilitating fungal spore dispersal, the maintenance of fungal communities, and the symbiotic relationship between truffle-like fungi and their EcM plant hosts. Few studies have compared co-existing mammal species in the abundance, diversity and composition of fungal spores in their diets (but see Vernes & Dunn 2009). Some previous studies have suggested considerable overlap in fungal taxa consumed by co-occurring small mammal species (Reddell *et al.* 1997; Tory *et al.* 1997; Vernes *et al.* 2004a) while others have suggested considerable differences can occur (Cázares *et al.* 1999; Pyare & Longland 2001; Frank *et al.* 2006).

Australia supports a high diversity of truffle-like taxa compared to many other regions of the world (Bougher & Lebel 2001). Some research has suggested that dry climates drive the evolution towards a sequestrate and truffle-like form (Thiers 1984; Johnson 1996; Claridge & Trappe 2005). As this life-mode is considered better adapted to dry conditions than an epigeous form, where sporocarps desiccate in a shorter period of time, it has been hypothesised that drier habitats, such as xeric vegetation types, may support a higher diversity and abundance of taxa (Izzo *et al.* 2005b). In addition, more stressful environments (e.g. nutrient poor soils and dry climates) have been associated with mycorrhizal associations in vascular plants, as plant hosts could derive greater comparative benefits from symbiosis with EcM fungi in these environments through increased water and nutrient uptake (Bougher 1995; Claridge 2002). The results of Chapter 2 suggest that different vegetation types may support distinct communities of truffle-like fungi. Contrary to the hypothesis that drier and more nutrient-poor habitats may drive mycorrhizal associations in plants, the mesic wet sclerophyll was found to support a much higher truffle-like taxon richness than more xeric habitats (e.g. heathy woodland). It is unknown whether these same patterns would be reflected in small mammal diets.

Our knowledge of spatial patterns in truffle-like fungal diversity is poor both in Australia and elsewhere. For example, it has been estimated that only 12-23% of sequestrate fungi species in Australia are known and of described species, habitat preferences and distributional ranges are known for very few (Bougher & Lebel 2001). Partly this is due to the cryptic nature of truffle-like fungi which are identified by their ephemeral and patchily distributed sporocarps, requiring time-consuming field survey methods. Also, the high proportion of undescribed taxa, lack of monographic treatments, and relatively few professional mycologists also limit efforts to describe Australian taxa. Consequently, despite their potential importance to ecosystem health and some threatened mammal species, EcM fungi are rarely considered in assessments of biodiversity or habitat loss in planning decisions or as part of wider conservation programs.

The relatively rapid time-standardised census technique has been employed in Australia to assess the distribution of truffle-like taxa at a large scale in south-eastern Australia (Claridge *et al.* 2000a; b). Although still time-intensive compared to assessing plant species or many fauna groups, such methods provide an achievable means to assess spatial patterns in the distribution of species (i.e. habitat preferences), species richness, and assemblages of species. Even when undertaken in rapid fashion through a single replicated ‘snapshot’ study, this technique can yield insights into spatial gradients in truffle-like taxon richness and assemblage composition (Chapter 2). An additional means of indirectly sampling the fungal diversity with equivalent effort, albeit at a coarser taxonomic level, may be through the diets of mycophagous mammals. Mycophagous small mammals are highly efficient at detecting truffles, as exhibited by the high diversity of taxa often shown to be present in bush rat *Rattus fuscipes* diets (Tory *et al.* 1997), despite animals sampling

from a relatively small home range. Their ability to detect and sample from the fungal community may exceed that of researchers (Johnson 1994b; Carey *et al.* 2002; Lehmkuhl *et al.* 2004) using sporocarp sampling techniques such as the timed-census raking technique reported in Chapter 2.

To provide further insights into differences among mammals in the consumption of fungal food resources and dispersal of fungal taxa spores, I investigated mycophagy in several co-occurring small mammal species. All species investigated inhabit a variety of habitat types including eucalypt forests and woodlands and have relatively large distributions in south-eastern Australia.

I also assessed whether differences among habitats in taxon richness and composition detected in sporocarp surveys (Chapter 2) would be reflected in small mammal diets. Based on the results of sporocarp surveys, I expected small mammal diets in wet sclerophyll habitats to support the greatest diversity of truffle-like taxa and dry forest and heathy woodland to support similar levels of taxon richness. In addition, I expected taxon composition of spores in small mammal diets would be similar between heathy woodland and dry forest habitats and different in wet sclerophyll. Comparisons were therefore made between the results of sporocarp surveys and small mammal dietary analysis in describing local scale truffle-like fungal richness and composition among three habitat types.

Methods

Study area

Study sites were located across several adjoining national parks on Gibraltar Range in the New England Tableland of north-east New South Wales, Australia (Figure 3.1). Much of the study area is registered as World Heritage wilderness and is relatively uninfluenced by the impacts of fragmentation, supporting diverse mammalian (Vernes *et al.* 2006), floristic (Hunter & Sheringham 2008), and macrofungal communities (Chapter 2). Changes in mammalian community composition across a sharp habitat gradient has been shown previously within the study area (Vernes & Dunn 2009), as have distinct changes in truffle-like taxon composition according to broad habitat categories (Chapter 2). Consequently, the study area offered a unique opportunity to sample a range of unique mammal and truffle-like fungal communities across several contrasting habitat types.

As previous research (Chapter 2) found rainforest to support a low abundance and diversity of truffles, it was excluded from further surveys. Contrasting habitat types were selected (see Chapter 2) based on observed differences in biotic and abiotic characteristics such as soil type, plant community composition, litter cover, and soil moisture. Differing assemblages of truffle-like fungi were shown to occur across these contrasting habitat types (Chapter 2).

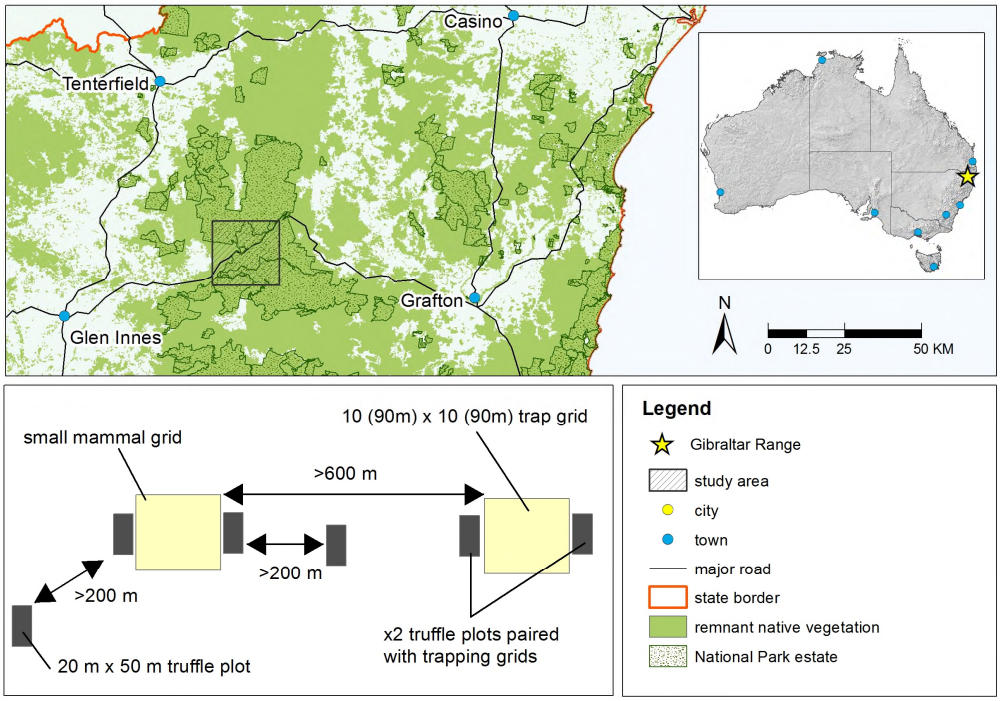

Figure 3.1 Location of study area in a heavily forested landscape of north-eastern New South Wales, Australia. Shown with sampling design diagram. Top right inset map shows location of Gibraltar Range on mainland Australia.

Sampling/trapping

Three habitat types were sampled based on previous work which found these supported a high diversity of truffle-like fungi (Chapter 2). Survey sites were stratified by the major vegetation types of wet sclerophyll, dry forest, and heathy woodland (see Chapter 2 for further details of habitats). Three grids for each of these three broad vegetation types were sampled for small mammal diets giving a total of nine survey grids. Within each habitat type, grids were positioned at even intervals from one another and in relation to the extent of sporocarp survey plots. At each site small mammals were sampled using a 10 x 10 trapping grid of Elliot traps (n=100), with trapping stations placed at 10 m intervals covering a total area of 8100 m^2 (0.81 ha). Each grid was trapped for four nights giving a total of 400 trap nights for each grid, 1200 for each vegetation type and was equivalent to 3600 trap nights in total.

Trapping was undertaken during winter over a 12 day period in early July 2006 after the completion of field surveys for truffles (Chapter 2). Sampling was designed to be short enough to minimize variations between grids and vegetation types due to changes in weather or other processes that may have influenced truffle fruiting or other food availability within the trapping period. Reducing the time period of trapping was also important for enabling a comparison of small mammal diets to truffles collected prior to trapping. Winter sampling of diets was chosen based on data showing fungal consumption to be highest during winter in small mammal and swamp

wallaby *Wallabia bicolor* diets within the study area (unpubl. data Vernes; Vernes 2010),and in bush rat diets elsewhere (Tory *et al.* 1997). Production of truffles has also been observed to peak between late-autumn to early-spring (May to September) in one south-eastern Australian study located within a similar climate to the study area (Claridge *et al.* 1993b).

Each animal was weighed, sexed, and marked (by clipping hair from above the base of the tail) before release. Faecal samples were collected from Elliot traps and handling bags and stored in vials containing 70% ethanol. Samples were collected only from new individuals so that the sampling unit was individual animals. This was to ensure that samples were not influenced by modifications to their normal foraging due to repeat captures in Elliot traps across nights.

Dietary samples

In the laboratory, a scat from each individual animal was lightly macerated in a mortar and then washed through a 100 µm sieve with distilled water and retained in a vial. The vial was then placed in a fridge overnight for the fine fraction to concentrate at the bottom. The supernatant was then decanted and 70% ethanol added to the vial which was shaken vigorously for one minute and left overnight for the fine fraction to settle at the bottom. These methods were likely to bias against the detection of Glomeromycota and Zygomycota taxa due to the small sieve size and large spore size (i.e. >100 µm) of many taxa within these phyla. However, I was primarily interested in truffle-like fungi and these are largely within the Basidiomycota.

A fresh glass pipette was used to extract the fine fraction to avoid contamination between samples. A single drop of this suspension was dropped onto a glass slide, stirred lightly to create an even smear and then left to dry until most ethanol had evaporated. A single drop of Melzer's Reagent was added and a glass coverslip dropped over the mixture. This process was repeated but with a drop of KOH added to the slide. For each sample 10 random fields of view were examined at 400x magnification for fungal spores. In each field of view, unique taxa were recorded and numbers of spores for each counted. Identification of spores was made to the highest taxonomic resolution possible using published descriptions, taxonomic keys, expert mycologists, and an extensive sporocarp field collection made at the study site within the same month (Chapter 2). In most cases spores could be assigned to a genus, although genera within some families (e.g. Russulaceae) cannot be identified based on spore morphology alone. An exception to this was for some taxa belonging to *Arcangeliella* with highly distinctive and unique spore ornamentation for which sporocarps had been previously collected from the study site. I retained this group as separate from other Russulaceae due to these taxa being frequently encountered in diets and/or in the soil. In addition, some genera cannot be distinguished from one another (i.e. *Andebbia* and *Gummiglobus*) and were grouped together.

Analysis

Comparisons among habitats and mammal species diets in fungal taxon richness were made using the nonparametric Kruskal-Wallace test. Mann-Whitney pair-wise tests were used to test for significant differences between pairs of habitats and small mammal diets in fungal taxon richness. Tests were performed in MYSTAT v12 (SYSTAT Software Inc. 2011). EstimateS v8.2.0 (Colwell 2006) was used to calculate accumulation curves and diversity indices based on presence/absence data.

Trends in fungal composition within small mammal diets were examined using multivariate analyses in the PRIMER v5.2.9 software package (PRIMER-E Limited; Clarke & Warwick 2001a). Individual Bray-Curtis similarity matrices were constructed using the presence-absence of fungal taxa in samples (animal faecal pellets) for each individual diet x fungal taxa combination. Separate matrices were constructed for three different versions of the same original dataset: i) all fungal taxa, ii) truffle-like taxa only, and iii) aggregation to the genus-level. Patterns in the composition of fungal taxa within the diets of mammals were examined using non-metric multi-dimensional scaling (MDS). Tests for significant differences among habitat types were made using non-parametric Analysis of Similarity (ANOSIM). A two-way crossed ANOSIM was used for comparisons with habitat type or mammal species as a varying block factor. This test was chosen to reduce the influence of habitat and species identity on detecting differences in fungal composition among species diets and habitat types respectively. For testing significant differences among habitat types in dietary composition of fungal taxa I used a one-way ANOSIM, both for bush rat and brown antechinus *Antechinus stuartii* diets. For comparisons between factors showing significant differences, SIMPER tests in PRIMER were used to identify fungal taxa most important in discriminating habitats in fungal composition. To quantify the respective variation (dispersion) among individual samples (animal diets) in each mammal species diet, I used the MVDISP function in PRIMER (Rabaut *et al.* 2007) and report the associated dispersion values for the samples of each species.

Dietary samples were pooled within each habitat type to compare to sporocarp sampling results. This was intended to provide a comparison between the truffle-like taxa identified through sporocarp surveys to occur within each habitat type with the composition of taxa being consumed and dispersed by mycophagous mammals. Both dietary and sporocarp survey datasets were aggregated to a higher taxonomic level (mostly genus-level) to allow comparison in fungal taxa frequency and community structure. Lower taxonomic comparison could have been made but not with consistency across both fungal taxonomic groups and datasets. Unique spore types for which no genus could be assigned were excluded from this analysis. Presence-absence data for each fungal genus x sample combination was converted to percentage occurrence across plots and individual diets in sporocarp surveys and dietary analysis respectively. Spearman rank correlation

tests and least-squares linear regression were used to test for similarities between dietary data and sporocarps found in the soil (sporocarp surveys) in estimating richness within genera and percentage occurrence of genera across samples.

Results

Trapping success across habitat types

Trapping resulted in 111 captures across all grids, comprised of 64 individual animals. Four species of small mammals were captured, bush rat (n=20), brown antechinus (n=33), fawn-footed melomys *Melomys cervinipes*, (n=6) and New Holland mouse *Pseudomys novaehollandiae* (n=6). The greatest numbers of individuals were trapped in dry forest and the least in heathy woodland. New Holland mouse was trapped only in Heathy Woodland and fawn-footed melomys was absent from wet sclerophyll. Relatively similar numbers of bush rats were captured across the three habitat types while a much greater number of brown antechinus captures were made in dry forest (Table 3.1). Trapping success (3.1%) and the number of dietary samples (n=64) obtained was very low, resulting in limitations to the level of analysis possible. Although the greatest number of samples were obtained for brown antechinus (n=33), sufficient sample sizes for comparing all three habitat types were obtained only for bush rats.

Small mammal mycophagy

Fungal spores were detected in all mammal species diets and across all habitat types suggesting all species consumed fungus to some degree (Table 3.1). A high proportion of scats contained fungal spores across all species and habitat types (47.6-100.0%). Truffle-like fungal spores were more frequently encountered than spores of epigeous fungi. Brown antechinus diets had the lowest percentage occurrence of fungal spores, while only bush rats had a consistently high percentage occurrence of fungal spores and spores belonging to truffle-like taxa across all three habitat types.

Table 3.1 Percentage occurrence of fungal spores in mammal species diets across three habitat types. Values in parenthesis are the percentage occurrence of faecal samples containing the spores of truffle-like and epigeous taxa respectively. The number (n=) of individual animals sampled within each habitat x mammal species combination is given below.

	Percentage occurrence of fungal spores in diets			
Habitat Type	brown antechinus	bush rat	fawn-footed melomys	New Holland mouse
heathy woodland	- n=1	100 (100, 33.3) n=6	100 (100, 33.3) n=3	100 (100, 0.0) n=6
dry open forest	47.6 (42.9, 9.5) n=21	100 (100, 100) n=6	- n=2	-
wet sclerophyll forest	63.6 (45.5, 36.4) n=11	100 (100, 100) n=8	-	-

Small mammal species differed markedly in the richness of fungal taxa spores represented in their diets, with bush rats consuming a much higher number than any other mammal species sampled (Figure 3.2). Of 61 fungal taxa identified across all samples, bush rat diets contained 59 (96.7%). In comparison, brown antechinus samples contained 28 (46%), fawn-footed melomys 10 and New Holland mouse 6 taxa. This pattern was found across all three habitat types bush rats were sampled in, with bush rats diets found to contain a significantly higher number of fungal taxa than other mammal species present in the same habitat type (Table 3.2). Similarly, bush rat diets contained a higher richness in spores of truffle-like taxa across all habitats and in epigeous taxa spores in two of the three habitats compared to all other small mammal species.

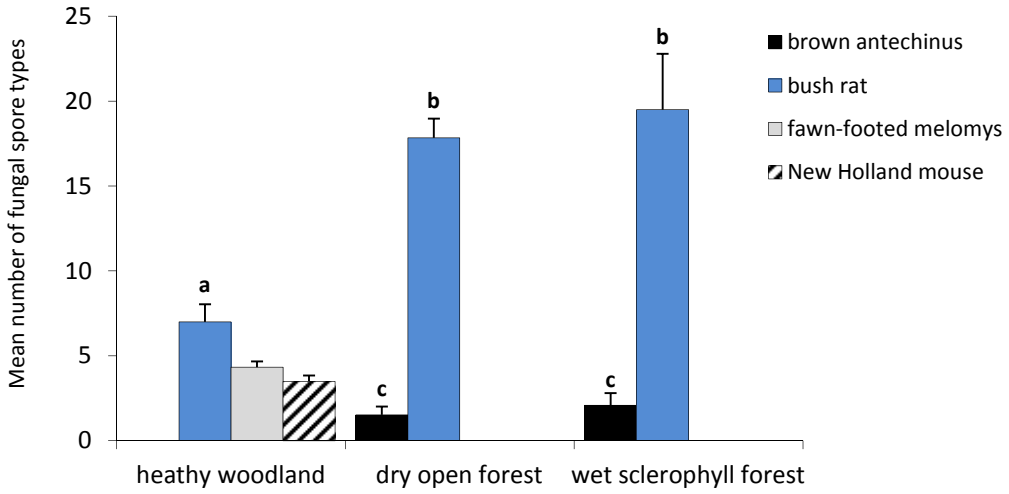

Figure 3.2 Mean number (±SE) of fungal taxa (spore types) per sample in the diets of five mammal species among three habitat types. Different letters above each bar denote significant (Mann-Whitney, $P<0.01$) pairwise differences between groups.

Although relatively moderate numbers of spores were observed in fawn-footed melomys and New Holland mouse diets, spore abundance in brown antechinus diets was <1% that found in bush rat diets, suggesting it may be indirectly ingesting fungal spores. The ratio of epigeous to truffle-like taxa was also highest in brown antechinus diets although the mean number of epigeous taxa was significantly greater in bush rat diets in two of the habitat types.

Only the diets of two species, bush rat and brown antechinus, were sampled in two or more habitat types and therefore available for comparing diets across habitats. The richness and abundance of fungal spores in brown antechinus diets was not significantly different between dry forest and wet sclerophyll (Table 3.2). Bush rat diets differed in measures of fungal spore richness among habitat types, with those sampled in open dry forest and wet sclerophyll having a significantly higher mean number of fungal (Figure 3.2) and truffle-like taxa compared to the driest habitat type, heathy woodland. A significantly (Kruskal-Wallis: $X^2_1=9.47$, $P=0.002$) higher mean diversity of epigeous

spores was also found in bush rat diets from wet sclerophyll compared to heathy woodland (Table 3.2). In both bush rat and brown antechinus diets, all measures of fungal spore diversity increased along a habitat gradient from heathy woodland to dry forest to wet sclerophyll, potentially reflecting a gradient from drier to more mesic habitat. In contrast, mean numbers of spores in bush rat diets exhibited the reverse gradient, although no significant differences were detected among habitat types.

Table 3.2 Total and mean measures of richness in fungal taxa (spore types) and spore abundance in mammal species diets across three habitat types. Sampling units were individual animals within each habitat type. Significant differences in richness means between mammal species within each habitat type are denoted by: *=P<0.01; **=P<0.001; ***=P<0.0001 (Kruskal-Wallace or Mann-Whitney for two-group tests). Different letters denote significant (Mann-Whitney, P<0.01) pairwise difference between habitat types within species. Means for fawn-footed melomys were not compared due to low sample size. Means are shown with ± standard error.

Habitat Type		brown antechinus	bush rat	fawn-footed melomys	New Holland mouse
heathy woodland	Total taxa	3	15	10	6
	Total epigeous : truffle-like taxa	0:3	3:12	1:9	0:6
	fungal taxa *	-	7.00 ± 1.03 [a]	4.33 ± 0.33	3.5 ± 0.34
	truffle-like taxa *	-	6.50 ± 0.76 [a]	4.00 ± 0.58	3.5 ± 0.34
	epigeous taxa	-	0.50 ± 0.34 [a]	0.33 ± 0.33	0.0
	spores per sample *	-	1204.7 ± 186.2 [a]	747.3 ± 480	307.7 ± 77.2
dry open forest	Total taxa	13	37	4	
	Total epigeous : truffle-like taxa	1:12	5:32	1:3	
	fungal taxa***	1.52 ± 0.49 [a]	17.83 ± 1.14 [b]	-	
	truffle-like taxa***	1.43 ± 0.47 [a]	15.83 ± 0.79 [b]	-	
	epigeous taxa***	0.10 ± 0.07 [a]	2.00 ± 0.52 [ab]	-	
	spores per sample***	3.1 ± 1.5 [a]	807.3 ± 81.8 [a]	-	
wet sclerophyll forest	Total taxa	17	51	-	
	Total epigeous : truffle-like taxa	5:12	9:42	-	
	fungal taxa**	2.09 ± 0.71 [a]	19.5 ± 3.28 [b]	-	
	truffle-like taxa**	1.55 ± 0.62 [a]	15.88 ± 2.74 [b]	-	
	epigeous taxa***	0.55 ± 0.25 [a]	3.63 ± 0.6 [b]	-	
	spores per sample*	3.1 ± 1.2 [a]	763.4± 162.4 [a]	-	

Taxon composition

NMDS ordination of the presence-absence of fungal taxa revealed differences between small mammal species and between habitats in the composition of diets (Figure 3.3). Diet samples from bush rats and New Holland mouse clustered comparatively closely within species (Figure 3.3). Brown antechinus dietary samples overlapped the diets of most other small mammal species in the 2D ordination. The PRIMER routine MVDISP showed brown antechinus diets to have a high dispersion (Dispersion Factor Value: 1.39) when compared to bush rats (Dispersion Factor Value: 0.744) and New Holland mouse (Dispersion Factor Value: 0.337). This high variability in brown antechinus diets resulted in a relatively low dissimilarity to other species diets. Analysis of Similarity (ANOSIM) tests revealed that small mammal diets were dissimilar (Global *R*-value=0.320) enough to be significantly different (*P*=0.001) (Table 3.3). High pair-wise *R*-values (0.649-0.794) and significant *P*-values (*P* <0.05) among bush rat, New Holland mouse, and fawn-footed melomys suggested that variation in fungal taxa consumption among these species accounted for the major differences in global comparisons. Brown antechinus diets, on the other hand, were relatively similar to all other small mammal species. When only truffle-like taxa were included, differences among species were overall greater (higher Global *R*-value=0.446). Several species (4-11) were variously identified as most important in distinguishing each pair of species in SIMPER tests. Common discriminating species belonged to the genera *Mesophellia*, *Hysterangium*, *Cortinarius*, *Rossbeevera*, *Arcangeliella*, *Austrogautieria*, and *Aroramyces*.

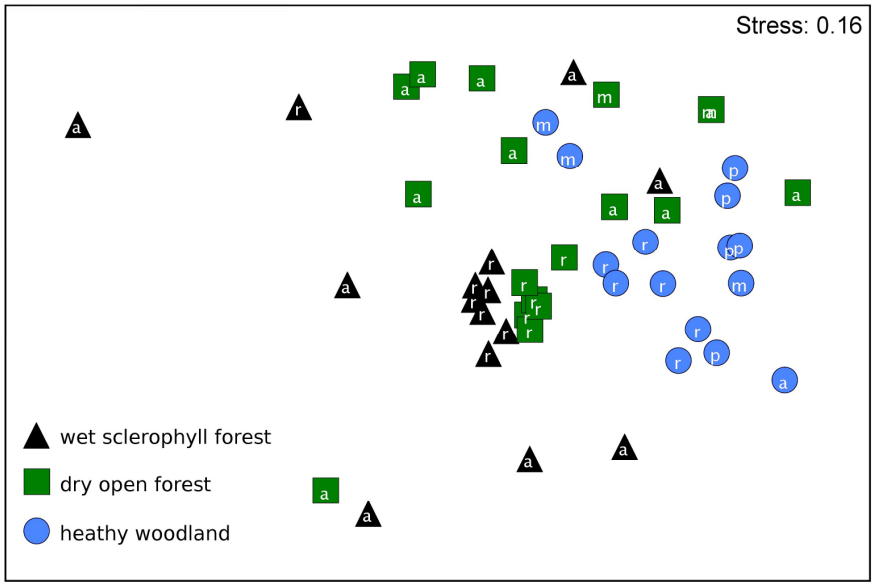

Figure 3.3 Nonmetric multidimensional scaling ordinations for the presence/absence of fungal taxa (spore types) in samples of small mammal species diets among three different habitat types. Letters represent mammal identity: bush rat = r; brown antechinus = a; fawn-footed melomys= m; New Holland mouse= p.

Table 3.3 Global and pair-wise Analysis of Similarity (two-way crossed ANOSIM with habitat as a block factor) results comparing small mammal species diets given with the associated *R*-value and significance level where $P<0.05$. Independent tests were performed for datasets including all fungal taxa and one with only truffle-like taxa.

Taxa	ANOSIM test	Species pairs	*R*-value	*P*
All fungal taxa	Global		0.320	0.001
	Pair-wise	brown antechinus - bush rat	0.251	0.006
		brown antechinus - fawn-footed melomys	0.060	n.s.
		brown antechinus – New Holland mouse	0.556	n.s.
		bush rat – fawn-footed melomys	0.794	0.001
		bush rat - New Holland mouse	0.649	0.004
		fawn-footed melomys - New Holland mouse	0.664	0.024
Truffle-like taxa	Global		0.446	0.001
	Pair-wise	brown antechinus - bush rat	0.423	0.001
		brown antechinus - fawn-footed melomys	0.081	n.s.
		brown antechinus – New Holland mouse	0.556	n.s.
		bush rat – fawn-footed melomys	0.790	0.001
		bush rat - New Holland mouse	0.656	0.004
		fawn-footed melomys - New Holland mouse	0.645	0.024

ANOSIM tests revealed highly significant ($P<0.01$) differences among all habitat types in the composition of fungal morphospecies observed in small mammal diets (Table 3.4). Pair-wise comparisons suggested that the greatest differences in small mammal community diets were between heathy woodland and the remaining habitat types (*R*-values=0.624-0.693), while wet sclerophyll and dry forest were the least dissimilar (*R*-value=0.279-0.359).

Comparisons among mammals using a dataset of all fungal taxa aggregated to the genus-level produced similar results (Global *R*-value:=0.324, $P=0.001$) as did comparisons among habitat types (Global *R*-value=0.370, $P=0.001$). When the dataset was reduce to only truffle-like taxa, significant differences among mammal species (Global *R*-value=0.407, $P=0.001$) and habitats (Global *R*-value=0.432, $P=0.001$) were maintained. These results suggest that distinct changes in genera occurred among small mammal species and between habitat types and that genus-level comparisons may produce similar results to those at the species-level.

Table 3.4 Global and pair-wise Analysis of Similarity (ANOSIM two-way crossed) results comparing habitat types in the taxon composition of fungal spores observed in small mammal species diets. The *R*-value and significance level (*P*) is given for all global and pair-wise tests. Independent tests were performed for datasets including all fungal taxa and one with only truffle-like taxa.

Taxa	ANOSIM test	Habitat	*R-value*	*P*
All fungal taxa	Global		0.451	0.001
	Pair-wise	wet sclerophyll forest – dry open forest	0.279	0.003
		wet sclerophyll forest – heathy woodland	0.624	0.001
		dry open forest – heathy woodland	0.628	0.001
Truffle-like taxa	Global		0.496	0.001
	Pair-wise	wet sclerophyll forest – dry open forest	0.359	0.001
		wet sclerophyll forest – heathy woodland	0.693	0.001
		dry open forest – heathy woodland	0.682	0.001

Species-specific changes in the dietary composition of spore types across habitat types could only be tested for brown antechinus and bush rat, and differences among all three habitat types for only the latter species. A low-stress NMDS ordination (Figure 3.4B) and ANOSIM results (*R*-value=0.158; Table 3.5) demonstrated that the composition of spores in brown antechinus diets were similar between wet sclerophyll and dry forest. In contrast, bush rat diets exhibited distinct clustering by habitat type in NMDS ordination (Figure 3.4A). Subsequent ANOSIM tests revealed diets were significantly different (Global *R*-value=0.644, *P*=0.001) between habitat types (Table 3.5), despite the small number of samples available for testing. Although bush rat diets were significantly different among all habitat pairs, heathy woodland was least similar to other habitats. These patterns and significant differences were maintained in an NMDS ordination and ANOSIM tests when only truffle-like taxa were considered (Global *R*-value: 0.663, *P*=0.001; Pairwise tests: *R*-values=0.510-0.856, *P*<0.01). Significant differences among habitat types in the composition of truffle-like taxa in bush rat diets were also maintained when aggregated to the genus-level (high Global *R*-value: *R*=0.581, *P*=0.001; results of pairwise comparisons: *R*=0.451-0.836; *P*<0.001). These changes accompanied distinct changes in fungal taxon richness among habitats, and similar pair-wise results, in bush rat diets presented earlier (Table 3.2).

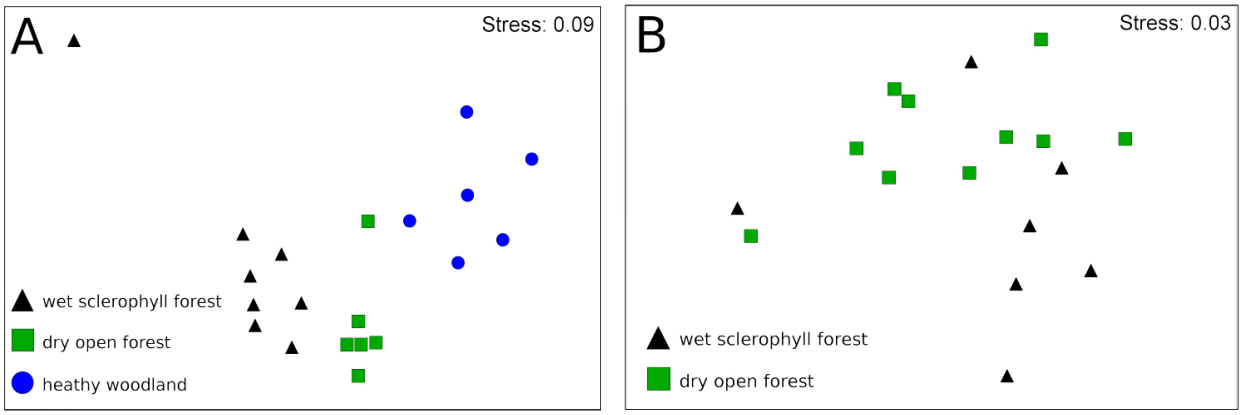

Figure 3.4 Comparison of fungal morphospecies composition among habitat types in the diets of individual bush rats (A) and brown antechinus (B) using nonmetric multidimensional scaling ordination and the presence/absence of fungal taxa.

Table 3.5 Global and pair-wise Analysis of Similarity (ANOSIM one-way) results comparing the composition of fungal morphospecies in the diets of bush rats and brown antechinus among habitat types with the associated *R*-value and significance level where $P<0.05$. Independent tests were performed for datasets including all fungal taxa and one only with truffle-like taxa. Tests were undertaken with presence-absence data.

		Habitat	R-value	*P*
bush rat	Global		0.644	0.001
	Pair-wise	wet sclerophyll forest - dry open forest	0.469	0.001
		wet sclerophyll forest – heathy woodland	0.721	0.001
		dry open forest – heathy woodland	0.844	0.002
brown antechinus	Global	wet sclerophyll forest - dry open forest	0.158	n.s.

Comparisons to sporocarp surveys

Direct comparison between the results of sporocarp surveys and small mammal dietary analysis is undertaken with caution as it is impossible to discriminate the comparative survey scale (e.g. total areas surveyed) and sampling intensity by the two techniques. The total number of truffle-like morphospecies detected in small mammal (50) and bush rat diets (48) was low compared to that detected through sporocarp surveys (85 species; Chapter 2). In contrast, the total number of genera was similar for both survey techniques: sporocarp surveys recorded 25 genera and small mammal dietary analysis 26 genera. Within habitat types, sporocarp surveys detected a much greater total number of species taxa in two habitat types, wet sclerophyll and heathy woodland (Table 3.6). Only in dry forest did the number of spore types detected in mammal diets exceed the number of species identified from sporocarp surveys. Conversely, only in heathy woodland did the total number of genera detected by sporocarp surveys exceed the number contained within small mammal diets. Largely, these results likely represent the greater discriminatory power of sporocarp surveys in

detecting taxa at the species level. The difficulty with distinguishing truffle-like Russuloid and Cortinariaceae taxa to genus-level based on spore morphology alone limits the ability of dietary analysis to estimate richness within these groups, particularly in habitats where these groups may be more diverse (e.g. wet sclerophyll).

In contrast to total richness, the mean number of species/spore types and genera among habitats exhibited remarkable similarity between sporocarp survey plots and individual small mammal diets. Both survey techniques estimated the same ranking of habitats in species and genus richness as one another: dry forest<heathy woodland<wet sclerophyll (Table 3.6). This result may be coincidental, however, as dry forest and heathy woodland did not differ greatly in mean richness values estimated by both survey techniques and bush rat diets (shown to be the dominant mycophagist and the only mammal sampled in all habitats) suggested a different trend across habitats. Nonetheless, totals, means, and Jackknife2 estimates of species richness for all three methods (sporocarp surveys, small mammal diets, and bush rat diets) predict wet sclerophyll to support the greatest richness in truffle-like taxa.

Table 3.6 Comparisons of truffle-like taxon richness estimates between truffle surveys, pooled small mammal diets and bush rat diets across three habitat types.

Habitat Type		sporocarp surveys	small mammal diets	bush rat diets
heathy woodland	Total species/spore types	27	16	12
	Total genera	17	9	8
	Mean no. of species	4.00 ± 0.58	4.69 ± 0.48	6.50 ± 0.76
	Mean no. of genera	3.57± 0.53	3.81 ± 0.36	5.33 ± 0.42
	Jackknife2 estimate	56.77	18.8	13.93
dry open forest	Total species/spore types	21	36	32
	Total genera	15	22	19
	Mean no. of species	3.29 ± 0.47	4.45 ± 1.16	15.83 ± 0.79
	Mean no. of genera	3.21 ± 0.45	3.72 ± 0.91	12.33 ± 0.68
	Jackknife2 estimate	38.47	55.4	47.4
wet sclerophyll forest	Total species/spore types	64	42	42
	Total genera	21	22	22
	Mean no. of species	9.31 ± 0.85	7.58 ± 2.03	15.88 ± 2.74
	Mean no. of genera	5.94 ± 0.42	4.95 ± 1.19	9.87 ± 1.43
	Jackknife2 estimate	105.02	58.24	62.21

Although the number of truffle-like taxa detected in bush rat diets within habitats was lower than those for all small mammal species, all genera (n=26) and most (96%) spore types in small mammal diets were also detected in bush rat diets. Due to this similarity and the relatively even

sample size among habitat types along with high percentage occurrence of truffle-like fungal spores, bush rat diets were used for comparisons in taxon composition to results from sporocarp surveys. Across all habitat types, sampling of bush rat diets detected a similar number of genera (n=26) to sporocarp surveys (n=25). Nine genera were unique to bush rat diet samples: *Amylascus*, *Dingleya*, *Elaphomyces*, *Glomus*, *Labyrinthomyces*, *Octaviania*, *Scleroderma*, *Sclerogaster*, and *Timgrovea* (Table 3.7). In comparison, six unique genera were detected from sporocarp surveys, including *Cystangium*, *Gymnomyces*, *Nothocastoreum*, *Russula*, *Protoglossum*, and *Quadrispora*. Although sampling of bush rat diets in heathy woodland yielded only a single additional genus (*Aroramyces*) to sporocarp surveys, sampling of diets in dry forest and wet sclerophyll detected an additional 10 and 8 genera respectively to those collected during sporocarp surveys. Consequently, these methods can be viewed as complimentary in describing communities of truffle-like fungi, particularly at the genus-level or higher.

The high diversity of Russulacea and *Descomyces* taxa uncovered by sporocarp surveys in wet sclerophyll were surprisingly reflected in bush rat diets. To a lesser degree species diversity within the genera *Hysterangium*, *Arcangeliella*, *Mesophellia*, and *Cortinarius* found in sporocarp surveys were also generally reflected in bush rat diets. A Spearman Rank tests between sporocarp surveys and bush rat diets in species counts within genera revealed a significant (r_s=0.623, P=0.0001) correlation between the results of the two survey methods (Table 3.7). Further testing revealed a significant positive linear correlation (r^2=0.397; P<0.001) in estimates of taxon richness within genera between sporocarp surveys and bush rats diets. This correspondence provides some evidence for similarity between the two methods in estimating taxon richness within genera across habitat types.

There was also some correspondence between survey methods in the taxa determined to be of greater influence in differentiating habitats from one another (Table 3.7). Both methods found taxa belonging to *Arcangeliella*, *Descomyces*, and the family Russulaceae were important in discriminating wet sclerophyll from other habitat types. Similarly, species within *Arcangeliella* and *Cortinarius* were found to be important by both methods in distinguishing dry forest from other habitats. Although there was no correspondence between methods in species differentiating heathy woodland, most of the genera detected in bush rat diets were also discovered in sporocarp surveys with species richness in some genera, such as *Hysterangium* and *Mesophellia*, reflected in bush rat diets.

Table 3.7 Comparative number of species (sporocarp surveys) or morphospecies (dietary analysis) within truffle-like genera (or lowest taxonomic grouping) across habitat types determined through both sporocarp surveys and bush rat dietary analysis. **Bold** type indicates genera found to have contributed most (average dissimilarity and higher average frequency) to discriminating each habitat type from another in the respective sampling method using a 50% cut-off in SIMPER tests.

		heathy woodland		dry open forest		wet sclerophyll forest	
	Genera	sporocarp surveys	bush rat diets	sporocarp surveys	bush rat diets	sporocarp surveys	bush rat diets
1	*Amylascus*	0	0	0	1	0	1
2	*Andebbia**	1	1	0	1	0	1
3	***Arcangeliella****	0	0	**1**	**2**	**3**	**3**
4	***Aroramyces***	0	**1**	0	**2**	1	2
5	***Austrogautieria***	**2**	1	2	**1**	0	1
6	***Chondrogaster***	1	**2**	0	1	1	2
7	***Cortinarius***	**5**	1	**4**	**2**	4	**3**
8	***Descomyces***	1	0	0	1	**12**	**5**
9	*Dingleya*	0	0	0	1	0	0
10	*Elaphomyces*	0	0	0	1	0	0
11	*Endogone*	0	0	0	1	1	1
12	*Glomus*	0	0	0	0	0	1
13	***Hydnangium***	0	0	0	0	**1**	1
14	***Hydnoplicata***	1	0	1	**1**	1	0
15	***Hysterangium***	**2**	3	1	3	**6**	3
16	*Labyrinthomyces*	0	0	0	1	0	0
17	***Leucogaster***	0	0	0	**1**	1	1
18	***Mesophellia***	**4**	2	2	2	**4**	2
19	***Nothocastoreum***	1	0	**1**	0	0	0
20	*Octaviania*	0	0	0	0	0	1
21	***Pogisperma***	1	0	0	0	1	**2**
22	***Protoglossum***	**2**	0	3	0	2	0
23	*Quadrispora*	0	0	1	0	0	0
24	*Rossbeevera*	1	1	1	1	1	1
25	**Russulaceae***	3	0	2	**5**	**23**	**5**
26	*Scleroderma*	0	0	0	0	0	2
27	*Sclerogaster*	0	0	0	0	0	1
28	***Stephanospora***	1	0	1	**1**	1	1
29	*Timgrovea*	0	0	0	0	0	2
	Total	14	8	12	19	16	22

* *Andebbia* also includes the genus *Gummiglobus* due to the inability to distinguish these genera on spore morphology alone. Russulacea includes hypogeous genera *Cystangium*, *Gymnomyces*, *Russula* and most species of *Arcangeliella* identified from sporocarp surveys. Only species within *Arcangeliella* that had highly distinctive spores were included within this genus in the summary table above. Both *Arcangeliella* and Russulaceae are in **bold** for wet sclerophyll to account for individual genera within Russulaceae identified in SIMPER tests as discriminating taxa for this habitat type.

Overall, the percentage occurrence of individual genera in small mammal and bush rat diets were markedly different from those obtained from sporocarp surveys across most habitat types (Figure 3.5 and Figure 3.6 respectively). Estimates from the two methods appeared to be most dissimilar in heathy woodland. Six of the fourteen genera detected in heathy woodland sporocarp survey plots were not found in small mammal diets, while one genus (*Aroramyces*) that was frequently encountered in diets was not detected across sporocarp plots. In contrast, a high proportion of genera detected in dry forest small mammal diets were not recorded by sporocarp surveys. An interesting contrast in results is the much higher dominance of *Cortinarius* estimated by sporocarp surveys in heathy woodland and dry forest compared to both pooled small mammal diets and bush rat diets. *Aroramyces* was also notable for being frequently encountered in diets across all habitat types but detected only infrequently across plots in wet sclerophyll by sporocarp surveys. This genus was also largely represented in diets by a taxon not detected in any plot by sporocarp surveys (*Aroramyces* sp. 2).

The apparent visual correspondence in wet sclerophyll between the percentage occurrence of some genera across mammal diets and sporocarp survey plots was supported by significant linear correlations between percentage occurrence estimates in both small mammal (r^2=0.433; P<0.010) and bush rat diets (r^2=0.356; P<0.024) (Figure 3.5-Figure 3.6: inset graphs). However, this test only included taxa detected by both survey methods and does not reflect correspondence in the entire dataset. Nonetheless, a small number of genera within wet sclerophyll exhibited close correspondence between percentage occurrence recorded in sporocarp surveys and small mammal diets (Figure 3.5), particularly in comparisons with bush rat diets (Figure 3.6). Percentage occurrence estimates for pooled small mammal diets differed from those calculated from bush rat diets, with the greatest difference being for dry forest. Nonetheless, although the relative dominance order varied, the same 5-7 genera had the highest percentage occurrence in both diet sets for each habitat type.

Despite the above discrepancies, some characteristics of truffle-like fungal communities among habitat types were similarly observed by both sampling methods. For example, heathy woodland is estimated by both sampling methods (diets and sporocarp surveys) to have a low evenness, being dominated by a few abundant species (Figure 3.5-Figure 3.6). Similarly, both methods are in agreement in suggesting wet sclerophyll to have low species dominance and high evenness in species occurrence.

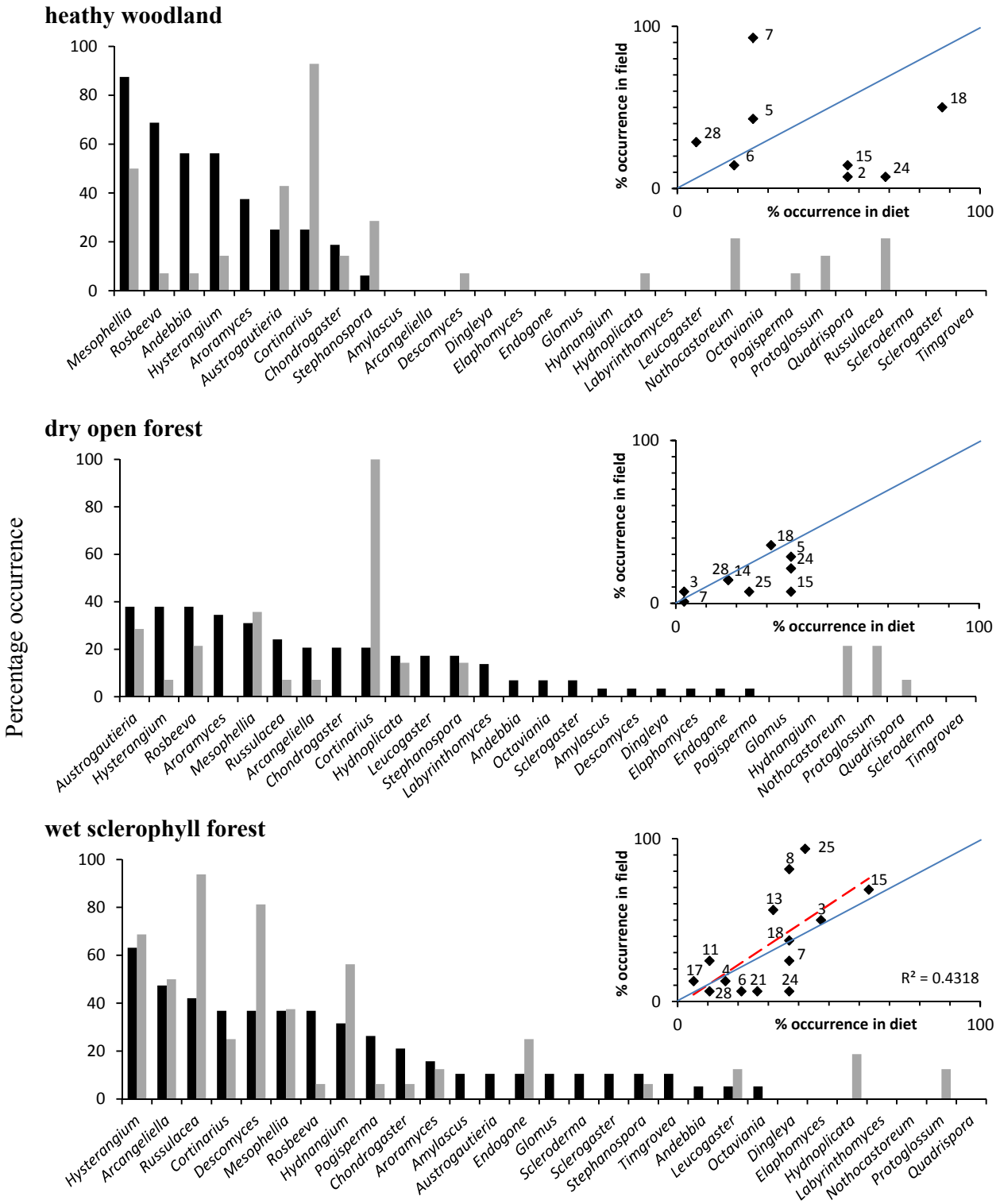

Figure 3.5 Percentage occurrence of truffle-like taxa (genus-level or higher) in small mammal diets (black bars) and sampled in sporocarp surveys (grey bars) among three habitat types. The genera *Andebbia* also incorporates spores potentially belonging to *Gummiglobus* species. Inset graphs show the relationship between % occurrence of taxa in diets and sporocarp surveys considering only taxa detected in both. Numbers correspond to genera listed in Table 3.7. The solid line represents complete correlation in genus composition and % occurrence in the field and diets. Wet sclerophyll is shown with a trend line (red dashed line) for a significant linear correlation (R^2= 0.43 d.f. 12, P=0.011).

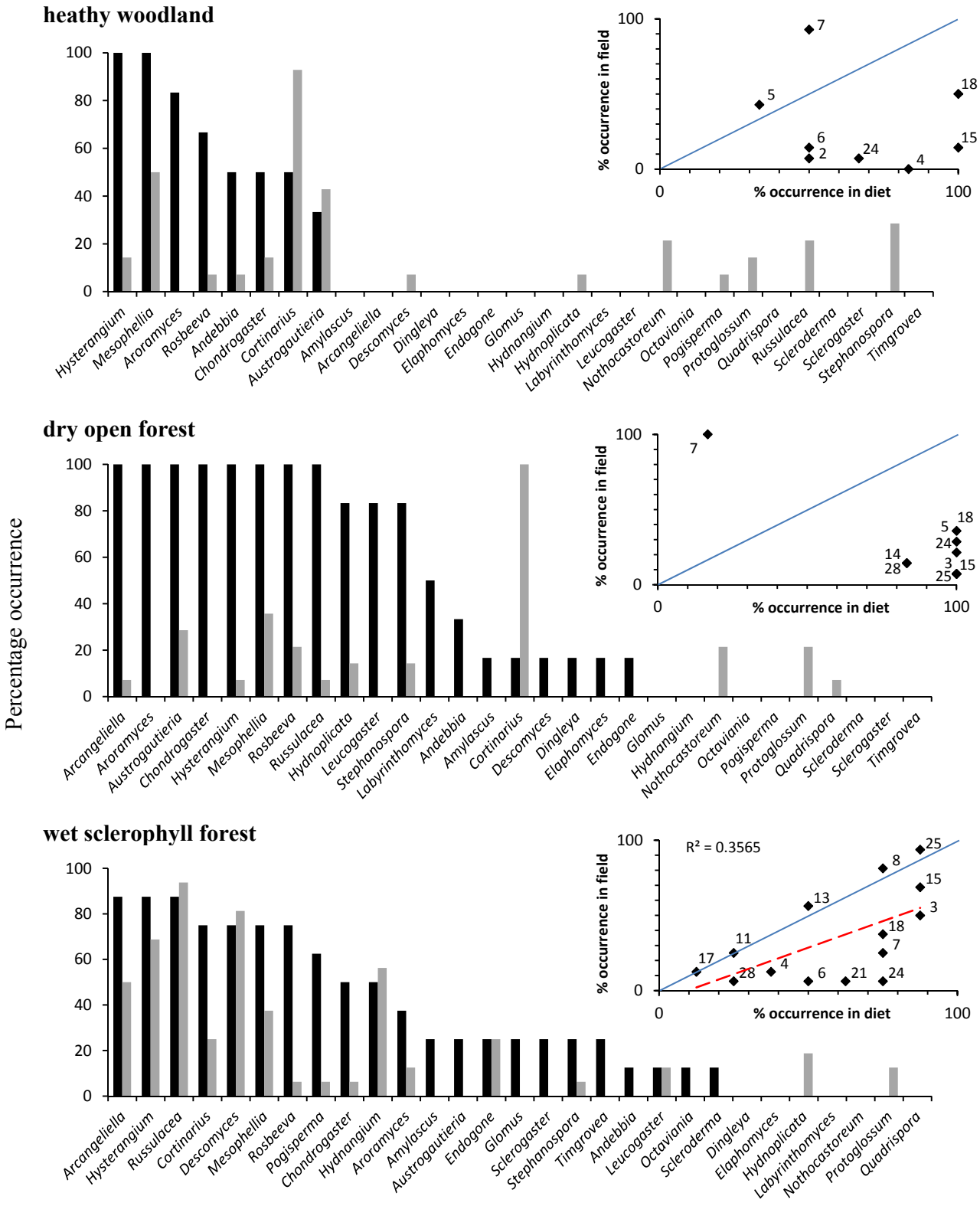

Figure 3.6 Percentage occurrence of truffle-like taxa in the diets of bush rats (black bars) and sampled in sporocarp surveys (grey bars) among three habitat types. See Figure 3.5 caption for further details. Inset graphs show the relationship between % occurrence of taxa in diets and sporocarp surveys considering only taxa detected in both.

Discussion

Frequency of fungi in small mammal diets

Co-occurring small mammal species have been shown to differ in the relative frequency of spores in their diets (Frank *et al.* 2006; Vernes & Dunn 2009) but have also been shown to be remarkably similar (Tory *et al.* 1997; Currah *et al.* 2000; Vernes *et al.* 2004a). Most of the small mammal species sampled in this study were similar in the frequency of fungal spores in their diets with the exception of brown antechinus in which diet frequency was approximately half that found in other small mammal species. Estimated frequencies of fungal spores in diets are consistent with an earlier study in the same area (Vernes & Dunn 2009) although estimates in this study were higher for fawn-footed melomys and New Holland mouse. This minor discrepancy between studies is likely due to Vernes & Dunn (2009) also sampling diets in rainforest habitats where few truffles were found and also that sampling in the current study was undertaken in a single trapping session during winter in which mycophagy in some species may be higher than in other seasons. For example, bush rat mycophagy in temperate south-west Victoria peaks in winter, which is thought to be in response to reduced availability of more nutritious food resources (Tory *et al.* 1997). Competition between co-occurring mycophagous mammal species and their relative abundance may also influence consumption of fungi (Orrock *et al.* 2003). Consequently, spatial and temporal changes in mycophagous habits of mammals may result in minor discrepancies between studies.

Considering the high percentage occurrence of fungi in their diets and relatively high abundance of spores, three of the species (bush rats, fawn-footed melomys, and New Holland mouse) sampled are likely to be directly consuming fungal sporocarps, predominated of truffle-like taxa. In contrast, the high variation in fungal taxon composition among individual brown antechinus and much lower comparative abundance of spores, suggests that the source of spores may be highly opportunistic or partly indirect through the consumption of mycophagous insects, as has been suggested for other observations of fungal spores in predatory dasyurid diets (Reddell *et al.* 1997). Based on the comparative diversity of taxa across mammal species diets, New Holland mouse, fawn-footed melomys, and brown antechinus are likely to be infrequently sampling from the fungal community. In contrast, the high diversity of taxa were observed in bush rat diets suggests they incorporate fungi as a major dietary component.

The percentage occurrence of fungi in brown antechinus and bush rat diets was relatively constant across habitats as were the mean number of spores per sample. However, the latter value is difficult to interpret due to different truffle-like taxa producing sporocarps with very different spore densities. As this study was not designed to directly assess response of mycophagous mammal consumption to the availability of sporocarps, only general trends can be suggested from the data.

In addition, the small sample size for bush rat limits the detection of differences among habitats in fungal consumption using percentage occurrence. Nonetheless, bush rat diets may have reflected the relatively constant dry weight of sporocarps among habitat types but not the significant differences among habitats in the number of sporocarps once a single dominant species was excluded (Chapter 2). This provides some evidence that bush rats do not increase consumption in response to higher availability. In contrast, Johnson (1994b) found a positive non-linear relationship between sporocarp production and the relative representation of fungus spores in Tasmanian bettong *Bettongia gaimardi* diets. Consequently, greater sample sizes may have revealed differences in bush rat consumption rates among habitat types.

Diversity and composition differences among mammal diets

Bush rat diets contained a much higher richness in fungal taxa that other sympatric small mammal species in each of the habitat types sampled. Bush rats also consumed an impressive proportion of the total taxa (96%) and genera (100%) detected across all small mammal diets. Not surprisingly, the greatest differences among species in dietary diversity were observed between bush rats and brown antechinus, with the latter species having the lowest diversity of fungal spores in their diets. Nonetheless, the scats of each mammal species contained the spores of at least several fungal taxa, most of which belonged to truffle-like fungi.

Few Australian studies have compared the diversity of fungal spores among co-occurring small mammal species diets. However, significant differences in dietary taxon richness have been found among co-occurring macropods (Vernes 2010) and some dissimilarity between swamp wallaby and introduced black rat *Rattus rattus* diets (Vernes & McGrath 2009) within the same general region as this study. In contrast, a study of the co-occurring bush rat and heavily mycophagous long-nosed potoroo *Potorous tridactylus* in south-western Victoria suggested that both species consumed a similar high diversity of truffle-like taxa (Tory *et al.* 1997). In North America, sympatric mycophagous small mammals have been shown to consume both similar (Carey *et al.* 1999; Vernes *et al.* 2004a) and dissimilar (Frank *et al.* 2006; Jacobs & Luoma 2008) diversity of taxa. Compared to North American mycophagist counterparts, the diversity of truffle-like taxa in Australian small mammal diets is considerably higher. For example, the mean number of fungal taxa in the heavily mycophagous northern flying squirrel *Glaucomys sabrinus* diet during the peak consumption period (summer) is reported as ≈2.0 species (Vernes *et al.* 2004a), an equivalent diversity to that found in insectivorous brown antechinus faecal pellets. In comparison, fungal taxon richness in bush rat faecal pellets varied between 7 and 19.5 among habitats, up to 9 times that found in northern flying squirrel pellets. Even at the genus-level, this high diversity is maintained with bush rat pellets containing on average between 5.3 and 12.3 truffle-like genera among habitats compared to 3.3 genera in northern flying squirrel pellets during peak (i.e. fall) truffle production (Jacobs &

Luoma 2008). As the large majority of spores observed across small mammal diets were those of truffle-like taxa, these results add further support to Australia having an extraordinary diversity of truffle-like fungi compared to other regions of the world (Bougher & Lebel 2001).

Marked differences in fungal taxon composition were also observed among mammal diets. Only the brown antechinus was not significantly different in pair-wise comparisons to other mammal diets, possibly due to the high variation among samples (but see Chapter 2:Methods and Warton, Wright, & Wang 2012), although species did differ when only truffle-like taxa when considered. Fungal dietary composition has shown to differ between the mycophagous swamp wallaby and introduced black rat (Vernes & McGrath 2009), among several macropod species (Vernes 2010), and between three co-occurring small mammal species in North America (Frank *et al.* 2006). The latter study suggested that diet differences in three mycophagous rodent species were driven by differences in two species of truffle-like taxa. In contrast, Tory *et al.* (1997) found little difference in the composition of taxa between long-nosed potoroo and bush rat diets. Similarly, Cázares *et al.* (1999) found no differences among three sympatric small mammal diets in dominant fungal taxa. Vernes *et al.* (2004a) also found that two co-occurring small mammals, the mycophagous northern flying squirrel and red squirrel *Tamiasciurus hudsonicus*, consumed a very similar composition of fungal taxa. The diversity and composition of taxa in each species diet and differences among them are likely driven by numerous factors including the i) the diversity of fungi available to consume among habitats, ii) animal foraging behaviour and iii) habitat utilisation, iv) interspecific competition for food resources, and the v) availability of other non-fungal food resources (Tory *et al.* 1997; Orrock *et al.* 2003; Vernes 2010).

Patterns in diversity and composition among habitats

Interestingly, the diversity of fungal and truffle-like taxa contained in bush rat diets varied across three habitat types, as did estimates based on a combined dataset using all small mammal species diets. In contrast, there was no corresponding difference in taxon richness in brown antechinus diets between dry forest and wet sclerophyll, perhaps reflecting incidental fungal spore ingestion.

Bush rat diets contained the greatest total and mean species richness in wet sclerophyll and the lowest in heathy woodland. Diets exhibited differences among habitats in fungal spore richness following a ranking across habitats of wet sclerophyll>dry forest>heathy woodland, although only the latter habitat type was significantly different from all others. The similarity between bush rat diets and sporocarp surveys in predicting differences between wet sclerophyll and heathy woodland suggests bush rat diets may be reflecting changes in the diversity of truffle-like taxa available to consume. This result corroborates earlier evidence that the mean fungal richness in bush rat diets can be significantly different among contrasting habitat types (Vernes & Dunn 2009), potentially

reflecting corresponding changes in the truffle-like community (Chapter 2).

Despite small sample sizes, I was also able to demonstrate that the composition of fungal (and truffle) taxa in small mammal diets differed among three contrasting habitats. This difference was best reflected in the diets of bush rats. As with some of the habitat differences indicated by sporocarp surveys, composition varied among habitats at both the morphospecies and genus-level. Surprisingly, these results reflect the same differentiation of wet sclerophyll from other habitat types in fungal taxon composition based on sporocarp collections (Chapter 2). Interestingly, morphospecies identified as most important in discriminating wet sclerophyll from other habitats in bush rat diets belonged to a number of the taxonomic groups (*Arcangeliella*, *Descomyces*, *Russulaceae*) also associated with differentiating this habitat in sporocarp survey analysis (Chapter 2). This agreement adds support to wet sclerophyll supporting a distinctive assemblage but also suggests bush rat diets may reflect spatial variation in taxon composition. Both the combined diets of all small mammal species sampled and bush rat diets independently suggested the least similar habitat types were the xeric heathy woodland and the mesic wet sclerophyll.

A contrasting result to sporocarp surveys was the differentiation of heathy woodland from dry forest in truffle-like fungal composition in bush rat diets. Two explanations are proposed: i) bush rats selected truffles of different taxa in the two habitat types or alternatively, ii) bush rats sampled widely from the truffle assemblage and fungal diets reflected differences in the true composition of truffle-like taxa between the two habitat types. The latter seems most plausible as within-habitat similarity in heathy woodland and dry forest communities estimated with sporocarp survey data was largely (77-89%) associated with the presence of just one species, *Cortinarius globuliformis* (Chapter 2). In addition, a large number (11) of fungal taxa from bush rat diets were associated with significant differences between heathy woodland and dry forest at the 50% cumulative similarity percentage cut-off in SIMPER tests.

Few other studies have investigated truffle-like fungi assemblages or species richness among highly contrasting habitat types to allow comparison. In North American forests, hypogeous fungal communities on oaks differ from those of conifers, with oak forests communities having a greater proportion of Ascomycota while conifer forests were dominated by Basidiomycota species (Frank *et al.* 2006). Lehmkuhl *et al.* (2004) found diets of northern flying squirrels to be similar in fungal taxon richness and composition among three different forest cover types (pine dominated, young-mixed conifer, mature mixed-conifer). However, the two least similar habitat types in structure were significantly different from one another in species richness with the more mesic habitat type supporting the higher richness. In addition, the forest cover type with the highest fungal richness estimated through sporocarp sampling also exhibited the highest diversity in mammal diets. Vernes & Dunn (2009) showed a significant difference in the composition of fungal taxa in the diets of

bush rats between rainforest and wet sclerophyll. This change coincided with an abrupt decline in EcM taxa relative to arbuscular mycorrhizal (AM) taxa in rainforest habitat. When considering the results of sporocarp surveys for rainforest and wet sclerophyll within the same area (Chapter 2), changes in bush rat diets convincingly followed a corresponding decline in EcM fungi and dominance of AM *Endogone* in rainforest habitat (Vernes & Dunn 2009). This comparison provides further evidence that bush rat diets may reflect large differences in truffle composition among contrasting habitats, in some cases driven by the dominance of EcM forming plant hosts. Elsewhere, Orrock *et al.* (2003) found no significant differences in the frequency of consumption of fungal spore types in woodland jumping mouse *Napeaozapus insignis* between eastern hemlock and mixed mesophytic forests. A potential explanation may be that the composition of taxa in diets are likely only to reflect significant differences in fungal community composition, in turn requiring marked changes in biotic (e.g. plant hosts) and abiotic (e.g. soil moisture, litter cover, and soil type) characteristics. Further investigations into patterns of species richness and composition in truffle-like fungi at varying spatial scales are required, particularly ones comparing contrasting habitats.

Community structure: truffle-like taxa among habitats

Truffle-like genera dominating the fungal spores observed in mammal diets included *Mesophellia*, *Rossbeevera*, *Hysterangium*, *Cortinarius*, *Descomyces*, *Austrogautieria*, *Aroramyces*, *Arcangeliella* and taxa within the family Russulaceae. All of these taxa have been previously reported in Australian mycophagist mammal diets and most have been reported as frequently encountered ones in dietary analysis (Claridge & May 1994; Johnson 1994b, 1997; Reddell *et al.* 1997; Vernes *et al.* 2001; Vernes & Trappe 2007). Within most habitat types, both bush rat diets and small mammal diets exhibited a gradient in the percentage occurrence of genera. Diets in heathy woodland were species poor and dominated by a small number of taxa. In contrast, percentage occurrences were more even among taxa in wet sclerophyll, with a distribution characterised by a long tail made up of rare taxa.

Previous research suggests truffle-like taxa may vary in their palatability to mycophagists, supported by feeding trials using northern flying squirrels (Waters *et al.* 2000), exclosure experiments where reductions in standing crop of taxa was measured (North *et al.* 1997), and differences among co-occurring mammal in the composition of fungal spores ingested (Jacobs & Luoma 2008). The reason for selection of one taxa over another remains unclear, although differences in nutritional value may play a role (Wallis *et al.* 2012). It is largely unknown whether Australian mycophagous mammals exhibit preferential consumption of taxa, although Johnson (1994b) showed that one abundant genus in the soil (*Elaphomyces*) was rarely sampled by Tasmanian bettongs. In North America, Cázares *et al.* (1999) demonstrated a correlation between the standing crop of the most frequently encountered truffle taxa and dietary frequency in each

mammal species diet. Carey *et al.* (2002) also found that rank abundances of fungi in northern flying squirrel and Townsend's chipmunk *Tanias townsendii* diets were strongly correlated with truffle abundances, with many species consumed according to their abundance. Similarly, (Johnson 1994b) found the Tasmanian bettongs to generally consume species in proportion to their abundance.

Although the methods used here to sample truffle-like fungi and small diets provides no direct information on preferential consumption of truffle-like taxa, some general trends were suggested by the data. Firstly, consumption (% occurrence in diets) was largely discordant with availability, as measured by the percentage occurrence of genera across plots, although wet sclerophyll showed weak positive correlation (Figure 3.5-Figure 3.6). Secondly, the majority of genera recorded across sporocarp survey plots were consumed by the small mammal community. Finally, a large proportion of dominant taxa in the soil were also the most frequently encountered in diets, providing some evidence that small mammal diets can indicate some of the more commonly occurring taxa fruiting in the soil.

Availability and consumption of truffles

Correspondence between available diversity of sporocarps to consume and that observed in bush rat diets among habitats suggests that bush rats may sample from a wide range of taxa available regardless of the habitat type, with the diet diversity increasing with corresponding increases in truffle diversity. Similarity between the diets of bush rats and long-nosed potoroo across seasons in both the variety and relative proportions of taxa consumed, even when fungi constituted only 5% of bush rat diets, has been inferred as reflecting each species consuming taxa relative to their availability (Tory *et al.* 1997). Consequently, bush rats may increase the diversity of taxa sampled in accordance with availability.

One obvious discrepancy between the results of diet and sporocarp sampling warrants comment: the much higher frequency of *Cortinarius* in sporocarp survey plots compared to small mammal diets. This taxon largely represents *Cortinarius globuliformis* in sporocarp surveys, a partially sequestrate species that often produces erumpent sporocarps (fruiting just above or at the soil surface) (Bougher & Trappe 2002; Jumpponen *et al.* 2004). This discrepancy suggests *C. globuliformis* could be avoided by mycophagous mammals. Interestingly, *Cortinarius* spore types were frequently observed in the fungal diet of brown antechinus, which is assumed to indirectly ingest spores when eating mycophagous insects. Conversely, both *Rossbeevera* and *Aroramyces* spores had a much higher frequency in diets than expected from sporocarp surveys, suggesting preferential consumption of these taxa. However, variable spore densities in sporocarps among taxa could influence relative representation and detection of taxa in diets (Tory *et al.* 1997) and could

account for some discrepancies between diets and sporocarp collections.

Overall importance as dispersal vectors

This study further illustrates that a number of small mammals are involved in fungal spore dispersal and that bush rats likely play a dominant role through consistently dispersing a much greater number of fungal taxa across a diverse range of habitat types supporting EcM forming plant species (Tory *et al.* 1997; Vernes & Dunn 2009). Through the dispersal of fungal spores, mammals play an important role in local scale maintenance of fungal communities (Gehring *et al.* 2002) and the recolonisation of early successional habitats by fungi and host plants (Cázares & Trappe 1994; Terwilliger & Pastor 1999). Passage through mammal guts may also act as a trigger for spore germination (Colgan III & Claridge 2002) and increase colonisation rates of host plants, which has been shown for arbuscular mycorrhizal fungi colonisation of a grass species host after spore passage through bush rat and fawn-footed melomys guts (Reddell *et al.* 1997).

Bush rats have previously been recorded consuming a diversity of truffle-like taxa, comparable to species such as the long-nosed potoroo that are considered specialist mycophagists (Bennett & Baxter 1989; Tory *et al.* 1997; Vernes & Dunn 2009). Potoroids, bettongs, and bandicoots are most well known for their mycophagous habits but have undergone severe declines since European settlement along with many other mammal species of similar weight range (i.e. Critical Weight Range), both in range and abundance (Johnson & Isaac 2009). There is evidence to suggest that a number of mycophagous species may have occupied a much wider range of habitats and landscapes in south-east Australia prior to European settlement (Bilney *et al.* 2010), suggesting a severe decline in the diversity and abundance of spore vectors for truffle-like fungi in many forested EcM fungi dominated systems. The bush rat in contrast remains widespread and common across south-eastern Australia, inhabiting a wide range of habitats (Menkhorst & Knight 2001). Bush rats may be a primary dispersal vector at a small spatial scale in the absence or reduced abundance of more heavily mycophagous species with wide-ranging habitats (Tory *et al.* 1997). Other mammals sampled also have wide distributional ranges across eastern Australia and may also play an important role in fungal spore dispersal in many other habitats. Interspecific variation in dietary fungal composition among small mammals suggests each may disperse different taxa to varying degrees. Consequently, a diverse assemblage of mammals may play an important role in ensuring dispersal services are provided to all taxa within a fungal community.

Although brown antechinus scats may contain a much lower spore abundance, even small numbers may be sufficient for effective dispersal to function. Spore deposition could be cumulative due to the potential for prolonged spore dormancy in some taxa (Cork & Kenagy 1989a; Colgan III & Claridge 2002; Rusca *et al.* 2006; Bruns *et al.* 2009), allowing spores to accumulative and remain

viable in the soil (i.e. in a spore bank). Based on a much higher proportional abundance of *Cortinarius* in brown antechinus diets relative to that found in other mammal species, this species may be an important vector for some truffle-like fungi, particularly for those potentially less palatable to more mycophagous mammals (i.e. *Cortinarius globuliformis*).

Each species importance as a spore vector may vary by habitat and its relative abundance in each habitat type. However, few species sampled here are constrained to foraging in one habitat type alone. Small mammal species sampled here also exhibit different habitat preferences and occur in different relative densities among habitat types, but are not constrained to a single habitat (Vernes *et al.* 2006; Vernes & Dunn 2009). As a result, each small mammal species may disperse spores across different habitat boundaries within a habitat mosaic. Habitat generalists such as bush rats may be more important than habitat specialists in dispersing spores across habitat boundaries. Habitat specificity of mycophagous mammals on the other hand, may play some role in the emergence among habitats of distinctive truffle-like assemblages over time. These results suggest that each of the small mammal species sampled is involved in facilitating spore dispersal for a fungal community, and that this process is not an ecological service undertaken by only a few specialist mycophagous species. Insects may also be important vectors for truffle-like fungi (Lilleskov & Bruns 2005), along with possums (Claridge & Lindenmayer 1998) and fungivorous reptiles (Jones *et al.* 2007; Cooper & Vernes 2011). Cervides in Europe and North America (Launchbaugh & Urness 1992; Cázares & Trappe 1994; Ashkannejhad & Horton 2006) and macropod species in Australia (Vernes 2010) may be particularly important for long-distance dispersal events. Also, not all mammal species present within each vegetation community were sampled during the current study. At least two additional small mammal species, the pale field rat *Rattus tunneyi* and Hastings River mouse *Pseudomys oralis*, were captured during a subsequent trapping session and found to consume truffle-like fungal spores (also see Vernes & Dunn 2009 for additional species).

Comparison and review of different sampling methods

Lehmkuhl *et al.* (2004) suggested that mycophagists may be better at sampling truffle-like fungi than humans, particularly in regards to taxa with highly clumped distributions. Johnson (1994) also reached the same conclusion when bettong diets were found to contain more taxa within a study area extensively sampled for truffles over two years. In support of these observation, even a ‘snapshot’ sampling of small mammal diets and particularly bush rat diets, provided information on differences in richness and composition of fungal taxa consumed by mammals across habitat types. Even with small sample sizes, differences could also be detected among habitats in richness and compositions.

Numerous additional genera were detected by examining small mammal diets. Similarly, Lehmkuhl *et al.* (2004) detected a greater number of truffle-like genera (19) by sampling northern flying squirrels diets compared to sporocarp collections (12) alone. Carey *et al.* (2002) also recorded additional genera (two) by sampling small mammal diets along with sporocarps. Collectively, the small mammal community diet contained a greater diversity of truffle-like fungal spores in each habitat than bush rat diets alone. Each species diet contributed at least one additional fungal taxa to those found in bush rat diets. In addition, I found some evidence for brown antechinus diets reflecting the frequency of one genus (*Cortinarius*) in dry forest to a greater degree than bush rat diets based on estimates derived from truffle surveys. Nonetheless, bush rat diets were found to contain the majority of taxa (≈96%) consumed by small mammals pooled across habitat types, and consistently contained a high diversity of morphospecies representing a large number of fungal genera. Differences in taxon composition among mammal diets, and different frequencies of taxa predicted by pooled small mammal diets compared to bush rats, show that different patterns in fungal community structure are suggested depending on the small mammal species sampled and whether samples are pooled or treated independently.

The sampling of small mammal diets provided a complimentary, and in some cases consistent, insight into truffle-like fungi diversity and composition to that provided by sporocarp surveys. Although small mammal diets mostly underestimated total species richness compared to sporocarp surveys due to poor taxonomic resolution, estimations of mean species and genus richness closely approximated those from sporocarp surveys. The higher total species richness detected by sporocarp surveys among habitat types largely reflected the greater ability to taxonomically differentiate species based on sporocarp and spore morphology compared to the latter alone, as is the case in dietary analysis. Similar differences among habitat types in taxon richness were also suggested by small mammal diets compared to those from sporocarp surveys. Diets indicated that wet sclerophyll had higher species richness compared to other habitats, corresponding to results from sporocarp surveys. There was some evidence that bush rat diets also provided an approximate estimate of relative species richness within some genera detected in both diets and sporocarp surveys (Table 3.7).

In Chapter 2, different habitat types were shown to support distinct communities of truffle-like fungi based on the results of sporocarp surveys (Chapter 2: Figure 2.3-Figure 2.4). Surprisingly, both bush rat and the pooled small mammal (to a lesser degree) diets also suggested marked differences in assemblage composition among habitat types (Figure 3.3-Figure 3.4). Both methods suggest wet sclerophyll supports a community distinct from the three other habitat types sampled. However, the most striking difference in comparing respective grouping by NMDS ordinations was the potential greater discriminatory power of bush rat diets in differentiating heathy woodland and

dry forest communities. These results suggest that large changes in fungal assemblage composition across space (e.g. habitats) may be detected by sampling small mammal diets, particularly more heavily mycophagist species such as bush rats. Faecal samples from each individual may represent a comparatively more intensive (temporally) and extensive (area) due to comparatively longer time spent sampling (i.e. long gut-retention time) than undertaken by human researchers using a time-constrained sampling method.

In concordance with sporocarp surveys (Chapter 2), there was high similarity between the results species- and genus-level analysis of bush rat diets in discriminating habitat types (both NMDS ordinations and ANOSIM tests). Therefore, local scale changes in taxon composition could potentially be detected by a coarser taxonomic resolution (i.e. genus) than the species/spore type level, although only between highly contrasting habitat types at a local scale. Hence, there may be some validity in undertaking assessments of spatial variation at the genus-level, and also potentially for richness where expertise in truffle-like fungi taxonomy or species-level knowledge of a particular area is low.

In contrast, dietary data was generally discordant with sporocarp surveys in estimations of assemblage structure (i.e. frequencies of taxa within habitats). Estimated frequencies of truffle-like genera differed greatly between diets and sporocarp surveys (Figure 3.5-Figure 3.6), although there was some correspondence in wet sclerophyll between the two methodologies. Consequently, these methods of sampling community structure will produce different results. However, this finding may be primarily due to inadequate sampling of communities by one or both methods as suggested by nearly all of the comparative species datasets not reaching an asymptote in species accumulation curves (Table 3.6). An important caveat to efficacy comparisons between diets and sporocarp surveys is that neither method can be assessed correctly without comparisons to a comprehensive assessment of fungal diversity and composition within the same location. Absent distribution data (particularly false negatives) are a major constraint for any short-term sampling technique applied to fungi (Tedersoo *et al.* 2010). Greater sampling intensity may improve correspondence between methods and would be required to adequately describe community structure within each habitat type. Nonetheless, many of the same dominant genera were detected by diet samples and sporocarp surveys while correspondence in wet sclerophyll suggest that insights can be attained on dominant taxa within habitats types, particularly in habitats with a higher abundance of sporocarps and greater diversity of truffle-like taxa (i.e. wet sclerophyll). Diets may also indicate where more cryptic taxa may have been under sampled or not detected by the time-standardised sporocarp survey technique (e.g. *Aroramyces* species).

Sporocarp sampling can also provide an inverse picture of species dominance within a given area compared to actual mycorrhizal dominance (Dahlberg 2001). Molecular techniques provide any

alternative method of estimating community structure although this method is more spatially restricted, may be biased by primer selection and specificity (Anderson & Cairney 2004) and by the host plant species selected for sampling, particularly where strong host preference is present (Tedersoo *et al.* 2008). The much smaller area sampled by molecular techniques in particularly restricts the discrimination of species-rich communities (Horton & Bruns 2001). Host sampling bias may be minor where a forested system is dominated by a small number of EcM plant hosts but highly problematic in the Australian context where a high proportion of plant species in sclerophyll woodlands, heaths, and forests may form associations with EcM fungi. Molecular techniques also require that molecular markers are available for species within a community (i.e. a high proportion of known species), a scenario unlikely to be achieved anywhere in the near future in the Australian context due to poor taxonomic knowledge and low proportion of known species. Conversely, the sampling of mammal diets may provide a relatively rapid method to coarsely assess large areas for patterns in truffle-like fungi diversity and assemblage composition, while incorporating undescribed taxa. Using novel molecular techniques (DNA sequencing) to identify truffle-like taxa in small mammal diets, Izzo *et al.* (2005) failed to detect many *Rhizopogon* species (four out of ten) collected in sporocarp surveys by another study (North 2002) in the same locality. However, mammal diets provided the next best sampling approach in detecting species present compared to sampling spore banks or roots using bioassay and molecular methods.

Conservation Implications

Considering the variation shown in fungal taxon composition and diversity among habitats, high habitat heterogeneity is likely to provide greater truffle food resources for mycophagous mammals. Consequently, the conservation of a variety of adjoining habitats in fragmented landscapes may improve food resources for mycophagous mammals. Retention and management of a diverse range of habitats is also likely to aid the conservation of truffle-like fungi and macrofungi in general (Grove & Meggs 2003; McMullan-Fisher *et al.* 2011). Additionally, conserving a diverse assemblage of mycophagous small mammals may be important for maintaining fungal communities and diversity, including recolonisation in post-fire environments and subsequent successional stages of EcM host plant dominated habitats. This is particularly important considering the reduced abundance or absence of many mycophagous mammals such as bandicoot, bettong, and potoroo species in fragmented landscapes and habitat types since European settlement (Bilney *et al.* 2010). Bush rats in particular may play a crucial role in the maintenance of fungal communities and health of host-plant communities at a small spatial scale in the absence or reduced abundance of other mycophagous mammals in south-eastern Australia.

Fungi and in particular, truffle-like taxa, are rarely considered when evaluating biodiversity in conservation and planning decisions. In part, this is due to the difficulty of detecting and

monitoring fungi compared to other lifeform groups but also the very few professional mycologists employed to survey for and describe fungi. A poor understanding of the taxonomic diversity and of the distributional ranges of most species within Australia also hampers assessments of rarity and identifying threatened species. It has been estimated that only 12-23% of Australian truffle-like species are known (Bougher & Lebel 2001). As a result, very few fungi are formally listed as threatened under state or commonwealth advisory lists or provisioned with additional protective measures under legislation (McMullan-Fisher *et al.* 2011). For instance, no species are listed as threatened under Commonwealth legislation (*Environmental Protection and Biodiversity Act 1999*), nine are listed as threatened in NSW, one species is protected under Victorian state legislation (effectively only on public land), while no species are recognized as threatened in the remaining six states. In the absence of detailed taxonomic knowledge, a rapid means of assessing the macrofungal diversity across landscapes and identifying hotspots of diversity may aid in focusing survey efforts and broad conservation measures for fungi. The sampling of mycophagous mammal diets, or rapid time-standardised sporocarp surveys, may provide an efficient means of coarsely describing truffle-like fungi diversity across habitats and landscapes. However, molecular techniques are rapidly advancing and are also promising in describing fungal diversity and spatial variation (Tedersoo *et al.* 2008) particularly where combined with sporocarp and dietary sampling (Izzo *et al.* 2005b).

Limitations

As discussed earlier, neither bush rat samples nor sporocarp surveys were shown to adequately describe the truffle-like fungal communities within each habitat. In addition, low numbers of dietary samples were obtained from mammals and comparisons are made to results of sporocarp surveys shown to have a relatively low sampling completeness (Chapter 2). More intensive sampling in both methods would likely have improved correspondence between datasets. For example, after two years of sampling diets and truffles in the soil, Johnson (1994) found the Tasmanian bettong consumed all (36 species) known to occur within a study site from sporocarp collections. Consequently, further evidence is required to establish whether patterns observed here extend to a wider spatial scale and across a greater range of habitats.

Sampling of small mammal diets was undertaken in winter due to bush rats reportedly consuming more fungi during winter (e.g. a seasonal mycophagist) than other seasons. Winter capture rates reported here were very low (2%) when compared to late summer capture rates experienced in heathy woodland and wet sclerophyll (20-30%) within the same study area. Nonetheless, considering the high numbers of genera (n=26) identified from only a small number of bush rat samples (n=20), higher replication would provide more meaningful insights into the spatial distribution of truffle-like taxa and truffle consumption by mycophagous mammals. In addition,

sampling of bush rat diets to estimate species richness may be more effective/efficient compared to sporocarp surveys where small mammal abundance is higher during winter than experienced within my study sites or where higher small mammal abundance coincides with peaks in fungal fruiting.

Chapter 4. Consumption and dispersal of truffle-like fungi by the swamp wallaby among contrasting habitats

Introduction

The consumption of fungus by mammals (mycophagy) is considered an important ecological process that contributes to maintaining ecosystem function (Johnson 1996). Mycophagy is particularly important for truffle-like fungi which produce hypogeous (below-ground) fruit-bodies ('sporocarps') and form ectomycorrhizal (EcM) associations with vascular plants. Most truffle-like fungi produce sporocarps ('truffles') that are entirely sequestrate, meaning that their spores lack a forcible discharge mechanism or are enclosed within the sporocarp fungal tissue. Consequently, consumption by mycophagous animals is required in most cases for spores to be released. Through sporocarp consumption, mycophagous mammals are largely implicated in dispersing truffle-like fungal spores (mycorrhizal inoculum) to new locations and host plants (Frank *et al.* 2009), facilitating the colonisation of plant root systems and thereby assisting in the maintenance of fungal populations (Claridge 2002). Primary spore dispersal by mycophagous mammals has been shown to be important in maintaining mycorrhizal fungal spore abundance and species richness in the soil and associated mycorrhizal colonisation of host plants (Gehring *et al.* 2002). The mutualistic relationship between EcM fungi and their plant hosts is thought to increase plant resilience to environmental stress through increasing water and nutrient uptake and disease (Bougher & Lebel 2001). For these reasons, mycophagous mammals are linked to successional processes (Cázares & Trappe 1994; Terwilliger & Pastor 1999) and the maintenance of EcM fungal communities and assemblages of EcM forming plant species, and overall forest health (Claridge *et al.* 1993b).

In Australia, numerous mammal species have been shown to consume the spore-bearing sporocarps of fungi and particularly those of truffle-like taxa (Claridge & May 1994; Reddell *et al.* 1997). Some Australian mammals, such as the potoroids (bettongs and potoroos) consume truffles across all seasons and sample from a high diversity of taxa (Bennett & Baxter 1989; Johnson 1994a; Green *et al.* 1999; Vernes *et al.* 2001; Bougher & Friend 2009). Other mycophagous mammals may consume smaller amounts of fungi and vary their intake of fungi seasonally, such as the mycophagous bush rat *Rattus fuscipes* and swamp wallaby *Wallabia bicolor* (Tory *et al.* 1997, Vernes 2010). Based on home range estimates (Fisher & Owens 2000; Sanecki *et al.* 2006), potoroos and bettongs may also have much greater movement capabilities than smaller-sized mycophagous mammals such as the bush rat. As a result, potoroids may be capable of dispersing spores longer distances and among contrasting topographies and habitats. Mycophagist dispersers

with greater movement capabilities may also be important for the re-colonisation of areas by truffle-like fungi after fire or other disturbance events (Cázares & Trappe 1994; Claridge *et al.* 2001; Vernes & Dunn 2009; McMullan-Fisher *et al.* 2011). However, most of the more heavily mycophagous species in Australia such as potoroos, bettongs, and bandicoots belong to the 'Critical Weight Range' (CRW) group of Australian mammals which have suffered severe declines in abundance and distribution since European settlement (Johnson & Isaac 2009; Bilney *et al.* 2010). Consequently, researchers have been interested in the role that more common mycophagous mammals may currently play in maintaining assemblages of truffle-like fungi, even introduced species of mammal (Vernes & McGrath 2009), where more heavily mycophagous mammals are no longer present or have drastically reduced abundance. A sound understanding of each mammal species role in dispersing truffle spores is essential to managing populations and communities of truffle-like fungi along with the long-term health of their plant hosts, particularly in forested ecosystems.

Several macropod species have been shown to consume the sporocarps of a diverse range of fungal taxa and are comparatively more common in distribution and abundance than potoroids (Claridge & May 1994; Vernes 2010). Compared to many small mammal species, they also have relatively large home ranges (Fisher & Owens 2000) and could also disperse fungal spores long distances among contrasting habitats and into successional environments post-disturbance (e.g. after fire). The swamp wallaby in particular is known to consume a considerable amount of fungus (Hollis *et al.* 1986) encompassing a large array of truffle-like fungi (Vernes 2010), and is a ubiquitous wallaby species inhabiting a wide range of habitats in both intact and fragmented landscapes of eastern Australia (Paull & Date 1999; Southwell *et al.* 1999; Menkhorst & Knight 2001; Paplinska *et al.* 2009). Mycophagy has also been shown to be a common feeding strategy among varying vegetation types (Claridge *et al.* 2001). However, the potential dispersal distances offered by swamp wallabies (Chapter 6), on account of a much larger home range size and greater movement capabilities (vagility), suggests the species may play a significant role in longer-distance dispersal of spores among distant populations of fungi and patches of amenable habitat within both intact vegetation mosaics and fragmented landscapes (Law & Dickman 1998).

The importance of a dispersal vector is determined by both the quantity (i.e. the number of spores and taxa dispersed) and quality of dispersal (e.g. gut-treatment and deposition patterns) provided (Spiegel & Nathan 2007; Schupp & Jordano 2010; Schickmann *et al.* 2012). Species of mycophagous mammal can vary greatly in the abundance of spores and diversity of taxa contained in their diets, consequently varying in their contribution to dispersal for assemblages of truffle-like fungi (Frank, Barry, & Southworth 2006; Vernes & Dunn 2009; Chapter 3). Mammals may also differ in the truffle-like taxa they consume (Frank, Barry, & Southworth 2006; Chapter 3),

collectively consuming and dispersing a greater diversity of taxa Comparisons between co-occurring species diets can reveal insights into their relative role in providing dispersal services to these assemblages.

The mycophagous habits of mammal species have been shown to vary according to habitat type or competition with other mycophagous species (Orrock *et al.* 2003; Schickmann *et al.* 2012). Swamp Wallaby consumption of plant food items has been observed to vary among habitats, driven by a mixed feeding strategy, where animals consume plant food items in inverse proportion to their availability (Di Stefano 2007). This strategy allows animals to counter plant toxins or optimize nutrient intake (Provenza *et al.* 2003; Marsh *et al.* 2006a; b; DeGabriel *et al.* 2009) which can result in a negative trend between food item (i.e. plant) availability and consumption (Di Stefano 2007). Different foraging behaviour in response to habitat type could influence the mycophagous habits of swamp wallabies and their relative role in fungal spore dispersal among varying habitat types.

The relative importance of truffle-like versus epigeous fungal taxa in swamp wallaby diets also requires further examination. The sporocarps of truffle-like fungi ('truffles') are often observed to be patchily distributed (Johnson 1994), clumped in space (Fogel 1976), and positioned below the soil surface, sometimes at significant depth (e.g. *Mesophellia*) (Dell *et al.* 1990; Vernes & Lebel 2011). These spatial traits require mycophagous mammals to use olfactory cues in locating truffles and physical adaptations to excavate them (Taylor 1992; Zabel & Waters 1997; Bougher & Lebel 2001; Vernes & Haydon 2001; Frank *et al.* 2006). Epigeous sporocarps may constitute a comparatively more accessible fungal food resources for swamp wallabies as they can be visually detected and require little energy expenditure to harvest. A comparison between the frequency of truffle-like and epigeous spores in wallaby diets would provide an insight into the comparative importance of truffles as a food resource. Additionally, such comparisons also reveal insights into the relative importance of swamp wallabies as dispersal vectors for truffle-like versus epigeous taxa.

As discussed in Chapter 3, the sampling of mammal diets may provide a course surrogate technique for detecting spatial trends in truffle-like fungal richness and composition (Chapter 2). Mycophagous mammals have exceptional abilities in detecting truffles through olfactory cues, are well-adapted to the excavation of sporocarps, and consume a high diversity of taxa (Johnson 1994b; Carey *et al.* 2002; Lehmkuhl *et al.* 2004). The frequency of some taxa in mammal diets may also approximate the true abundance of some taxa in the soil (Johnson 1994; Chapter 3). As sporocarps of some taxa are rarely collected in the field but their spores frequently encountered in mammal scats, sampling of mammal diets has been suggested as an additional method of detecting some species presence in an area (Claridge & May 1994). Consequently, the examination of

mammal scats may also reveal additional taxa to those detected in sporocarp surveys (Chapter 2), including those less abundant or more cryptic (Izzo *et al.* 2005b). The sampling of mammal diets could offer further insights into taxa most responsible for driving differences in assemblage composition among habitat types and also species characteristic of habitat types.

This chapter investigates A) the role of the swamp wallaby as a dispersal vector for epigeous and truffle-like fungi among three habitat types, and B) whether sampling of diets can be used as a surrogate technique in detecting differences among habitats in fungal richness and composition. Swamp Wallaby diets were sampled in wet sclerophyll, dry open forest, and heathy woodland habitats. Results are compared to trends among habitats through truffles collected from the soil (Chapter 2) and spores of truffle-like taxa observed in the fungal diets of co-occurring small mammals (Chapter 3).

Methods

Study area

The study area was located on the New England Tablelands of north-eastern New South Wales incorporating several adjoining national parks constituting a large (150,620 hectares) contiguous area of wilderness. The study area falls within the Washpool/Gibraltar Range Group of reserves and includes one the largest areas on un-logged sclerophyll forest in New South Wales (Hunter & Sheringham 2008). The sampling area measured approximately 20 kilometres in diameter and supports a diverse array of floristic communities (Keith 2004; NPWS 2005; Hunter & Sheringham 2008) and a high diversity of mammal species (Vernes *et al.* 2006). The area offered a unique opportunity to sample swamp wallaby diets from contrasting habitat types over a large area relatively uninfluenced by the effects of fragmentation or human-disturbance.

Three habitat types were sampled within the study area, chosen for contrasting vegetation structure, plant species composition, soil type, and other environmental factors such as litter cover (see Chapter 2). These factors have been suggested by previous research to be important variables influencing the occurrence of some truffle-like taxa in Australian forests (Claridge *et al.* 2000a; Bougher & Lebel 2001; Danks *et al.* 2012). Large differences in plant lifeform cover (i.e. cover of grasses, shrubs, forbs, and ferns) also represented variation in the relative availability of different food items for swamp wallabies, which has been shown to influence their diets (Di Stefano 2007). They were also selected based on large continuous areas of each habitat being present within the study area, as opposed to patchily distributed vegetation types forming part of mosaics or sharp gradients. As swamp wallabies may forage over relatively large areas, they may sample fungi from multiple habitats where they are present within their home range, thereby obscuring any habitat

type influence on diets sampled within a defined area. Consequently, I attempted to minimize this influence by locating sampling sites within habitat types more often forming larger patches of homogenous vegetation within the study area.

The three contrasting habitat types sampled are described in detail in Chapter 2 although short descriptions are provided here. Heathy woodland was characterised by a low canopy of eucalypt species, a prominent heath-like shrub layer, and a sparse groundstorey of grasses, herbs, and large areas of bare ground and litter. Dry forest was dominated by widely-spaced tall canopy eucalypts with a sparse mid-storey of scattered shrubs and a groundstorey dominated mostly by grasses, sedges and herbs. Wet sclerophyll had a tall canopy with dense shrub cover and scattered tree ferns, and a groundstorey dominated by ferns, litter, herbaceous plants, sedges and grasses. Heathy woodland occurred on leucogranite outcrops with low-nutrient sandy granitic soils receiving high rainfall (2000 mm per year). Wet sclerophyll occurred on more fertile loam soils in sheltered gullies or easterly facing slopes, while dry forest occurred on flat terrain on granite soils (Keith 2004; Hunter & Sheringham 2008).

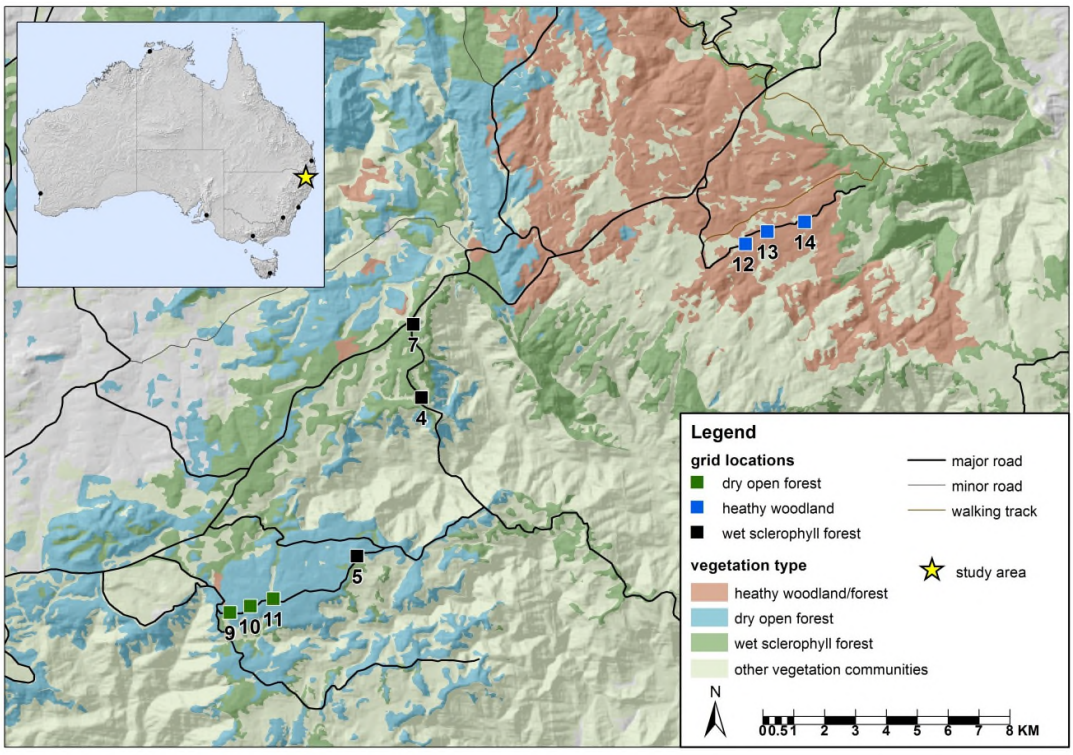

Figure 4.1 Location of study area and sampling grids at Barool and Gibraltar Range National Parks. The distribution of broad vegetation types (at a course spatial resolution) considered in the study is shown.

Sampling

Wallaby scats were sampled in nine grids established for small mammal trapping, with three grids in each habitat type (See Chapter 3). Grids were positioned at least 0.5 km distant from each other to ensure that each grid was an independent sampling unit. Movement distances of this magnitude are unlikely to be frequently traversed by swamp wallabies within the time period (4 days; see below) required to influence the independence of grids (Chapter 5).

Ten 90 m long transects were established at 10m intervals within each grid, covering a square area measuring 90 m x 90 m (0.81 ha). Along each transect, a 1m metre wide area was initially searched intensively for any macropod scats. All macropod scats found were removed from the site. Transects within grids were repeat surveyed for fresh swamp wallaby scats >4 days after the initial clearing of transect lines, equating to an area of 900 m^2 for each grid. Clearing of scats was performed to increase the temporal reliability of collected material, to ensure they were deposited by wallabies within the same general time-period as sampling of truffle surveys and small-mammal trapping. I examined each scat to ensure it was fresh material and was from a swamp wallaby. Collection of scats was undertaken by trained volunteers and myself.

Sampling of wallaby diets was undertaken during winter (July) within the same two-week period as sampling of small mammal diets (Chapter 3) and within two weeks of sampling truffles in the soil (Chapter 2). The occurrence and richness of truffle-like fungi in swamp wallaby diets has been shown previously to be highest during winter within the study area (Vernes 2010), potentially coinciding with peak consumption by bush rats based on one study in Victoria (Tory *et al.* 1997). Peaks in sporocarp production between late-autumn to early-spring (May to September) have also been observed elsewhere in south-eastern Australia (Claridge *et al.* 1993b). Consequently, winter was thought to be an ideal season to sample swamp wallaby diets, small mammal diets, and sporocarps in the soil for comparative purposes.

Dietary samples

In the laboratory, each wallaby scat was placed in a mortar and 5% potassium hydroxide (KOH) was added to break up binding mucus. The material was macerated and left to stand for 5 minutes. Material was then washed through a 100 μm sieve with distilled water. The resulting fine fraction was shaken vigorously for 1 min, transferred to a vial and centrifuged at 2500 r.p.m. for five minutes. Supernatant was decanted, replaced by distilled water and shaken vigorously to remove as much of the KOH as possible. Potassium hydroxide inflates fungal tissue and some spores but also has the undesired effect of blocking the ion channels in fungal spores and thereby inhibiting Melzer's reagent from entering spore membranes and any subsequent dextrinoid/amyloid reaction. This reaction is important in identifying taxa, particularly Russuloids. The fine-fraction containing

vial was subsequently placed in a fridge overnight for the fine fraction to settle at the bottom. After twenty-four hours, the vial was removed from the fridge and the distilled water supernatant decanted. Once a sample of the fine-fraction was taken for microscopic examination, 70% ethanol was added to the vial which was then vigorously shaken before storage. Methods for microscopic examination of the fine-fraction follow methods detailed in Chapter 3.

As abundance of spores in individual scats may unequally weight truffle-like taxa due to variation among species in spore production (Tory *et al.* 1997), spore counts for individual taxa in scats were converted to presence-absence. However, species-poor samples may also result in statistical artefacts (Clarke *et al.* 2006; Tedersoo *et al.* 2009a) and outliers in ordinations (Ejrnæs *et al.* 2002; O'Hara 2007). To resolve this issue and following Tedersoo *et al.* (2009a), scat samples with less than three morphospecies were randomly pooled within-grids.

Analysis

Diversity

The percentage (%) occurrence and average richness of fungal taxa detected in wallaby scats were calculated to provide a comparative measure among habitats and to trends among habitats found in small mammal diets (Chapter 3) and truffles in the soil (Chapter 2). Differences among habitats in the richness of fungal taxa contained in scats, both at morphospecies (spore type) and genus-levels were tested to determine whether the mycophagous habits and dispersal role of swamp wallabies differed among habitats and whether these were detectable at the genus-level. Averages of morphospecies and genus richness found in scats among habitats were statistically compared for spores of epigeous and truffle-like fungi separately to gain a better understanding of swamp wallaby mycophagy and role as a spore vector for assemblages of fungi among contrasting habitats.

Univariate tests for significant differences among habitat types were made using parametric Analysis of Variance (ANOVA) where data met assumptions of normality (Anderson-Darling Test) and equality of variance (Levene's Test). Transformations were applied (√- and log) in an attempt to normalise data and meet assumptions of homoscedasticity and homogeneity. Where assumptions of univariate tests were not met, the nonparametric Kruskal-Wallace test was applied to square-root transformed data. Pair-wise comparisons between habitats were made using Tukey's HSD test or Mann-Whitney U-tests for parametric and nonparametric tests respectively.

Total observed taxon richness was compared among habitats and to trends among habitats exhibited in bush rat and pooled small mammal diets using sample-based rarefaction curves. Sample-based rarefaction curves estimate expected richness at decreasing sample sizes (i.e. sub-samples of the pooled data) based on the observed richness, thereby allowing comparisons of

richness among factors at equivalent sample size (e.g. number of scats). Significant differences among rarefaction curves can be inferred through non-overlapping 95% confidence intervals (Colwell *et al.* 2012). Accumulation curves and estimates of total morphospecies and genus richness were calculated to test sampling adequacy and potential trends in total taxon richness among habitats accounting for unseen species. Estimates of total taxon richness were computed using the nonparametric Jackknife2 estimator, shown to be reliable with incidence data (Skov & Lawesson 2000; Chiarucci *et al.* 2003; Williams *et al.* 2007) and appropriate considering the estimated sample completeness among habitats (68-83%; (Brose *et al.* 2003). Sample completeness was estimated as (S_{obs}/S_{est}) x 100; where S_{est} =the mean of several species richness estimators (ICE, Chao2, Jackknife 1, Jackknife2, Bootstrap) and S_{obs} = total species richness observed (see Chapter 2). Rarefaction curves and species/genus richness estimates were all computed using presence-absence data (i.e. incidence) in the freeware EstimateS v8.2.0 software (Colwell 2006). Estimates were obtained by successively pooling randomly selected scat samples (without replacement) over 999 iterations. Prior to analysis, sample size for each habitat were standardised by randomly selecting samples to reach the lowest number of scats obtained from a single habitat (n=43).

The ratio of epigeous to truffle-like taxa in samples and the percentage each group comprised of total observed richness was examined to infer the relative importance of these different fungal food resources in swamp wallaby diets. The average abundance (counts) of epigeous and truffle-like fungal spores in scats was also calculated to investigate consumption patterns among habitats.

Comparisons with other datasets were made including results of surveys of truffles in the soil (Chapter 2) and sampling of small mammal diets sampled within the same grids (Chapter 3). I compared average total biomass (g) of truffles across plots, as a measure of food item availability, to the percentage occurrence of truffle-like fungal spores in swamp wallaby scats, as a measure of consumption. Such a comparison provides insights into general trends among habitats in swamp wallabies foraging habits. Trends exhibited in swamp wallaby diets in average and estimated total richness (Jackknife2) among habitats were compared to those estimated for small mammal diets, bush rat diets, and sporocarp surveys. Primarily this comparison was made to assess whether swamp wallaby diets reflected the available richness of truffles suggested by other sampling methods but also to explore the different trends among habitats suggested by sampling of mammal diets versus sporocarp surveys.

Composition

Comparisons among grids and habitats in the composition of truffle-like spores observed in diets were made using multivariate techniques (PRIMER 5.2.9, PRIMER-E Limited; Clarke & Warwick 2001a). Initially, the full dataset were explored by calculating a Bray-Curtis similarity matrix for

each scat sample x truffle-like taxa combination using presence-absence data. Non-parametric multidimensional scaling (NMDS) ordinations were used to visually explore trends among grids and the three habitats. Generally, 50 restarts were sufficient to ensure that a high proportion of iterations achieved the lowest stress level reported (Clarke and Warwick 2001). A two-way nested ANOSIM, with grids nested within habitats, was used to determine whether replicate samples (faecal scats) from grids could be pooled by habitat type and habitat differences tested using a one-way ANOSIM. A two-way nested ANOSIM tests first for differences among grid groups averaged across all habitat groups followed by differences among habitat groups using grids as samples (Clarke & Gorley 2006). Resulting significant differences among grids (*R*-value: 0.193, *P*=0.001; Table 4.3) were identified and consequently the more conservative significance values and associated *R*-values from the two-way nested ANOSIM are reported (Clarke & Warwick 2001b; Clarke & Gorley 2006). As pair-wise tests for habitat using grids as samples had no statistical power to detect differences (possible permutations=10), *R*-values are conservatively used as qualitative indicators of relative between-group differences (Clarke & Gorley 2006; Dethier & Schoch 2006; Vernes 2010). To identify which habitats exhibited differences among grids (e.g. all habitats or just one), one-way ANOSIM tests for grid differences were undertaken within each habitat. NMDS ordinations were also examined to visually assess in which habitat the greatest spatial variability (grid differences) was present (Appendix C).

Use of presence-absence data (as used in the two-way nested ANOSIM) in multivariate distance-metrics (i.e. Bray-Curtis) gives more weight to rare taxa at the expense of more common taxa (Clarke & Gorley 2006). Consequently, percentage (%) occurrence of taxa across scats within grids was used in further analysis to investigate habitat differences following previous research (Tory *et al.* 1997; Vernes & Dunn 2009; Vernes 2010). A square-root transformation was applied prior to analysis in order to balance the contribution of common and rarer species (Clarke & Warwick 2001b; Vernes & Dunn 2009). Due to the lower power of ANOSIM to detect a difference with a much reduced sample size (i.e. low number of possible permutations by using grids as replicates) a significance level of *P*=0.05 was used for global tests (Clarke & Gorley 2006). These analyses were undertaken at both a morphospecies and (mostly) genus-level to detect changes in assemblage composition at a higher taxonomic level.

Re-analysis using this approach allowed concurrent visualisation of differences and dispersion/location effects through NMDS ordination and also SIMPER estimation of important discriminatory taxa using the same dataset (Vernes 2010). The complete exclusion of 'uniques' was performed to examine their influence on structuring relationships among grids and habitats (Trebitz *et al.* 2009) but similar results were produced and subsequently are not reported. The MVDISP function (analysis of multivariate dispersion) using Bray-Curtis similarity was also used to

determine whether community structure was more variable (heterogeneous) in some habitats by quantifying the average dispersion of samples (grids) within habitats (Rees *et al.* 2005).

Results

Fungal occurrence and richness in diets

The percentage occurrence of fungal spores in swamp wallaby diets (scats) was high (98.1-100) among all habitat types (Table 4.1). Spores of truffle-like taxa were more frequently encountered in wallaby scats compared to those of epigeous taxa, with average counts of epigeous spores markedly lower than those of truffle-like taxa. Spores of all groups (all fungi, epigeous and truffle-like fungi) had the highest occurrence in diets sampled from dry forest and the lowest in wet sclerophyll. Significant differences among habitat types in the average spore abundance of fungal (Kruskal-Wallis: χ^2_3=11.66; P=0.0029) and truffle-like taxa (Kruskal-Wallis: χ^2_3=11.97; P=0.0025) were observed, driven largely by a significantly (Mann-Whitney P=0.002) higher abundance of truffle-like fungal spores in dry forest scats compared to those sampled from wet sclerophyll (Table 4.1).

Table 4.1 The percentage occurrence and mean number of fungal spores (±SE) observed in swamp wallaby *Wallabia bicolor* scats among three contrasting habitat types, including those of truffle-like and epigeous taxa. Total proportion was calculated as the percentage occurrence of all scats containing spores of the respective fungal groups. The number of individual scats sampled within each habitat is given (*n*) below. Significant differences among habitats denoted by * where P<0.01. Means not sharing a superscript letter are significantly different at P<0.01 (Kruskal-Wallace test).

	% occurrence			spore abundance		
Habitat Type	fungi	truffle-like	epigeous	fungi*	truffle-like*	epigeous
heathy woodland *n*=54	98.1	94.4	63.0	122.6 ± 20.3 ^{ab}	116.8 ± 20.2 ^{ab}	5.7 ± 1.5
dry open forest *n*=43	100.0	100.0	79.1	133.5 ± 39.3 ^{a}	122.4 ± 39.2 ^{a}	11.1 ± 2.5
wet sclerophyll *n*=54	94.4	83.3	51.9	106.7 ± 30.6 ^{b}	99.7 ± 29.5 ^{b}	7.0 ± 2.9

When compared to truffle-like taxa, few epigeous taxa were encountered in wallaby scats across all habitats. The total and average number of epigeous morphospecies observed in wallaby scats was also relatively similar among habitat types (Table 4.2). Nonetheless, some differences were detected among habitats, with the number of epigeous morphospecies in wallaby scats observed to be significantly different among habitat types, with a significant pair-wise difference detected between dry forest and wet sclerophyll (Tukey's HSD P<0.001) and weakly following the same

trend among habitats in taxon richness exhibited by truffle-like fungi (Figure 4.3A). The relative proportion of taxa that were epigeous or truffle-like did not differ greatly among habitats suggesting that the relative sampling of these fungi groups was a constant regardless of habitat type (Table 4.2).

Significant differences in fungal richness among habitats were observed, with habitats following an order of wet sclerophyll<heathy woodland<open dry forest, largely driven by differences in the average number of truffle-like taxa (Figure 4.3A; Table 4.2). This trend was supported by highly significant differences among habitats in the number of fungal (ANOVA: F=11.8, df=2, 35, P<0.001) and truffle-like morphospecies (Kruskal-Wallis: χ^2_3=10.43; P=0.005) contained in swamp wallaby scats. Pair-wise tests revealed that dry forest supported a significantly greater number of fungal (Tukey's HSD P<0.001) and truffle-like (Mann-Whitney P=0.001) morphospecies than wet sclerophyll. The same trends in taxon richness were exhibited at the genus-level, although pair-wise tests emphasised the significantly (Tukey's HSD P<0.01) lower number of genera in wet sclerophyll scats compared to all other habitats.

Table 4.2 Total and mean measures of fungal spore richness (spore type and genus) in swamp wallaby *Wallabia bicolor* diets (individual scats) across three habitat types. Significant differences in richness found in wallaby scats among habitats are denoted by: *=P< 0.01; **=P< 0.001. Different letters denote significant (Mann-Whitney, P<0.01) pairwise difference between habitat types. Jackknife2 estimate of total richness is for truffle-like taxa only. Means are shown with ±standard error.

		Habitat Type		
		heathy woodland (n=54)	dry open forest (n=43)	wet sclerophyll forest (n=53)
Species-level (spore type)	Total morphospecies (spore types)	49	63	55
	Ratio truffle-like:epigeous species	37:12	49:14	42:13
	Mean no. fungal spore types**	6.67 ± 0.52 **ab**	9.98 ± 0.87 **a**	5.09 ± 0.69 **b**
	Mean no. truffle-like spore types**	5.61 ± 0.50 **ab**	8.16 ± 0.81 **a**	4.17 ± 0.62 **b**
	Mean no. epigeous spore types*	1.07 ± 0.15 **ab**	1.72 ± 0.20 **a**	0.93 ± 0.17 **b**
	% truffle-like taxa	77.7 ± 3.5	78.9 ± 2.8	70.5 ± 5.0
	% epigeous taxa	20.4 ± 3.2	21.1 ± 2.8	23.9 ± 4.5
	Jackknife2 estimate	53.6	65.6	68.2
Genus-level	Total genera	24	36	28
	Ratio truffle-like:epigeous genera	19:5	28:8	22:6
	Mean no. fungal genera**	5.61 ± 0.42 **a**	7.72 ± 0.61 **a**	3.96 ± 0.46 **b**
	Mean no. truffle-like genera**	4.63 ± 0.38 **ab**	6.56 ± 0.56 **a**	3.37 ± 0.39 **b**
	Mean no. epigeous genera*	0.98 ± 0.13 **a**	1.16 ± 0.13 **a**	0.59 ± 0.13 **b**
	Jackknife2 estimate	30.7	32.9	26.9

The total observed number of taxa exhibited a slightly different trend among habitats, with heathy woodland supporting the lowest number of morphospecies (spore types) and genera, and dry forest the highest, for all taxa groups (i.e. epigeous, truffle-like, and all fungi). Rarefaction curves (Figure 4.2A) suggested a similar ranking of habitats at an even sample size of 43 scats and a higher total richness for dry forest even at very low sample size (<5), while heathy woodland and wet sclerophyll converged at low sample size (<10). Overlapping 95% confidence intervals of rarefaction curves for all three habitat types suggested that total observed species richness in diets was not significantly different among habitat types. Comparisons to observed rarefied total species richness estimates from bush rat and small mammal diets revealed swamp wallaby diets similarly predicted a similar lower richness for heathy woodland compared to other habitats. However, swamp wallaby diets had the opposite ranking of wet sclerophyll and dry forest in total species richness. At equivalent sample sizes, observed species richness in heathy woodland was higher in swamp wallaby diets than either small mammal or bush rat diets. In contrast, much higher richness was observed for wet sclerophyll in bush rat and small mammal diets compared to swamp wallaby diets.

Contrary to all other richness measures (i.e. means and total observed), the Jackknife2 estimator suggested wet sclerophyll diets were likely to contain the highest total species richness in truffle-like fungi, close to that predicted for dry forest (only <3 species higher) but much higher than heathy woodland (>15 species higher) (Table 4.2; Figure 4.2: B). Jackknife2 accumulation curves did not reach an asymptote for any habitat suggesting diets were not sufficiently sampled to estimate total richness. Interestingly, only at a larger sample size (36 samples) was wet sclerophyll predicted to have the highest total richness, indicating that greater sampling effort was required within this habitat type to reveal a potentially higher total species richness (Figure 4.2: B).

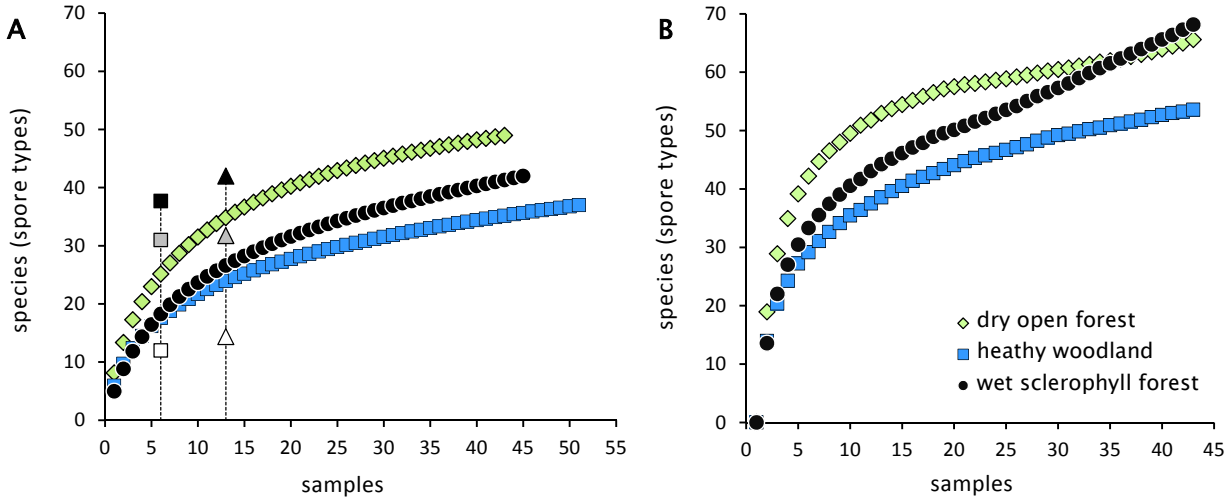

Figure 4.2 Comparisons of A) observed truffle-like morphospecies (spore type) richness in swamp wallaby *Wallabia bicolor* diets among three contrasting habitat types using sample-based rarefaction curves (Mao Tau estimator) and B) species accumulation curves using the Jackknife2 richness estimator for diets in each habitat type (n=43). Swamp wallaby rarefaction curves (A) were compared to rarefaction estimates from bush rat (squares symbols) and small mammal (rectangles) diets at sample sizes of 6 and 13 respectively among habitats (symbol fill coded by habitat: white = heathy woodland; grey = dry open forest; black = wet sclerophyll forest). Estimated 95% confidence intervals for all swamp wallaby habitat rarefaction curves (A) overlapped one another.

Comparisons to sporocarp surveys and small mammal diets

Comparisons were made between the results from sampling of swamp wallaby diets and those from sporocarp surveys (Chapter 2) and small mammal diets (Chapter 3), conservatively constrained to comparative trends among habitat types (Figure 4.3: B-D). The percentage occurrence of truffle-like fungal spores in swamp wallaby diets was found to follow an opposite trend among habitat types to sporocarp biomass (Figure 4.3: B). In contrast to small mammal diets and sporocarps (Chapters 2 and 3), observed fungal richness in swamp wallaby diets was highest in dry forest and lowest in wet sclerophyll (Figure 4.3: C). Swamp wallaby diets conflicted with sporocarp surveys and all other diets by having the lowest average richness in wet sclerophyll. Interestingly, sporocarp surveys and all diets agreed in the relative fungal richness of heathy woodland being lowest or intermediate when compared to the remaining habitats. Swamp wallaby scats also contained a much higher average richness in dry forest than those of small mammals.

In contrast to average taxon richness, estimates (Jackknife2) of total richness based on swamp wallaby diets, bush rat diets, and small mammal diets all exhibited identical trends among habitat types although they were less pronounced in swamp wallaby diets (Figure 4.3D). The trend in

richness observed across all diets was different to that predicted from sporocarp surveys: diets ranked dry forest as intermediate or equivalent morphospecies richness to wet sclerophyll as opposed to sporocarp surveys which ranked the habitat as supporting the lowest richness. This result suggested that either i) mammal diets did not consistently reflect the true total taxon richness among habitats or ii) sporocarp surveys underestimated richness in dry forest.

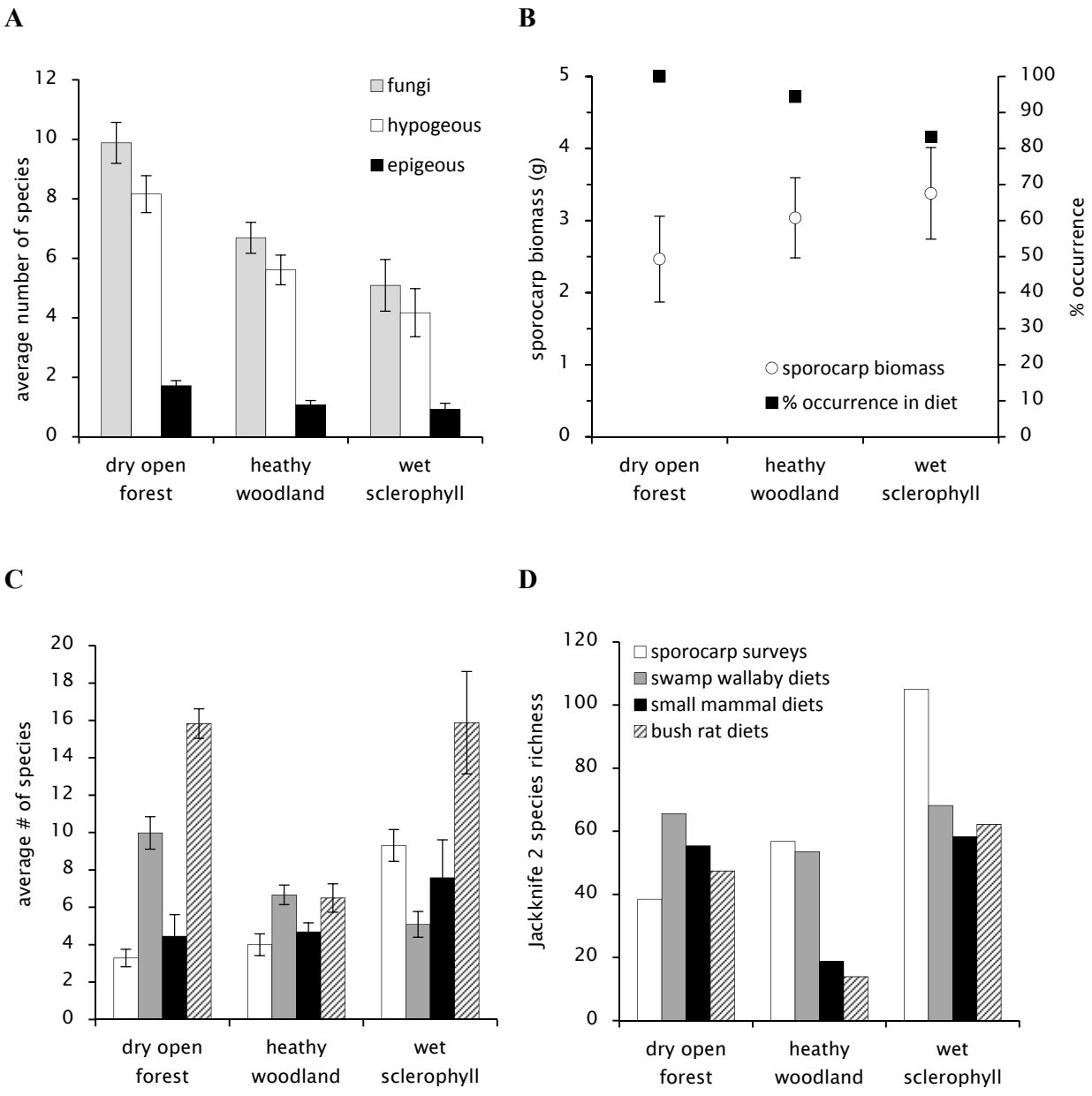

Figure 4.3 Trends in swamp wallaby *Wallabia bicolor* diets among three habitat types including: A) the average morphospecies richness of fungi, truffle-like and epigeous taxa detected in scats; B) a comparison between % occurrence of fungi in diets and sporocarp biomass (Chapter 2); and comparisons of swamp wallaby diets to sporocarp surveys and small mammal diets in C) average morphospecies richness in scats and D) estimated total richness (Jackknife2). Averages shown with ± standard error.

Composition of spores in diets

An NMDS ordination revealed differences between swamp wallaby diets in the composition of truffle-like taxa among some habitat types using presence-absence data and scats as replicate samples (Figure 4.4). Wet sclerophyll samples were separated from those of other habitats in truffle-like taxon composition, while there was substantial overlap of samples from heathy woodland and dry forests. Samples clustered mostly by grid although there was considerable dispersion and overlap, particularly across wet sclerophyll scats. Dispersion of samples was high in the NMDS ordination, possibly due to species-poor samples or a strong mean-variance relationship (Chapter 2: Methods; Warton, Wright, & Wang 2012).

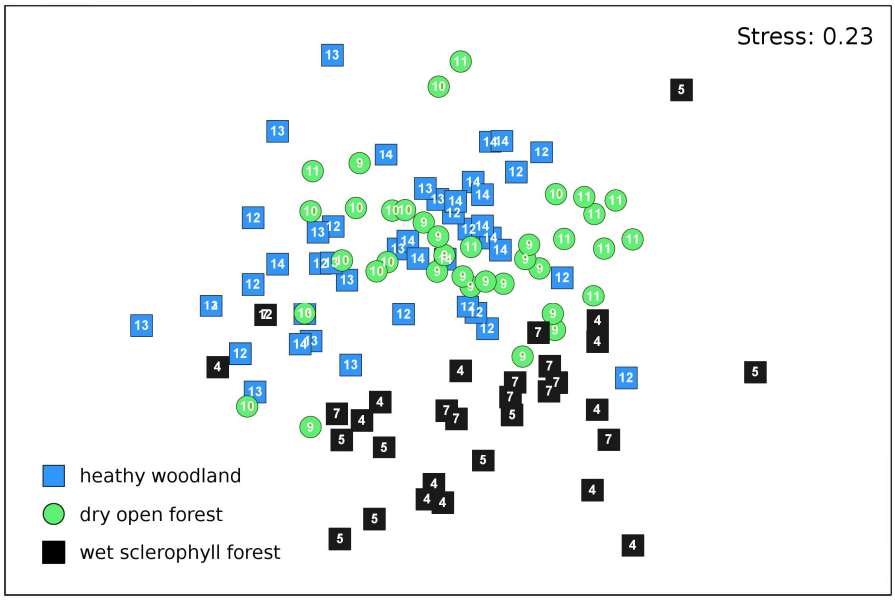

Figure 4.4 Nonmetric multidimensional scaling (NMDS) ordination for the presence/absence of truffle-like morphospecies in swamp wallaby *Wallabia bicolor* diets (scats as replicates) among three different habitat types. Numbers represent individual sampling grids.

A two-way nested ANOSIM test revealed significant (P=0.001) differences among grids in the composition of truffle-like taxa contained in replicate scat samples (Table 4.3). Pair-wise comparisons of grids within habitats using one-way ANOSIM tests revealed differences among grids to be present among all habitats although they were greatest in dry forest (Appendix C). Consequently, the null-hypothesis of no differences among grids within each habitat was rejected and habitats were compared using grid averages as samples. The subsequent ANOSIM test revealed a significant difference among habitats (R-value=0.391, P=0.014) although a qualitative evaluation of pair-wise R-values suggested that large differences in taxon composition were isolated to pair-wise comparisons with wet sclerophyll (Table 4.3) and a low dissimilarity between dry forest and heathy woodland (R-value=-0.037).

Table 4.3 Results of two-way nested ANOSIM testing for differences in truffle-like taxon composition among grids and among habitats in swamp wallaby *Wallabia bicolor* diets.

ANOSIM test		Habitat	*R-value*	*P*
Grid	Global		0.193	0.001
Habitat	Global		0.391	0.014
	Pair-wise	wet sclerophyll – dry open forest	0.519	-
		wet sclerophyll – heathy woodland	0.741	-
		dry open forest – heathy woodland	-0.037	-

NMDS ordinations of percentage occurrence of truffle-like taxa across scats within each grid (i.e. pooled average) revealed a separation of swamp wallaby diets by habitat type at both morphospecies (spore type) and genus-levels (Figure 4.5A-B). There was less overlap between heathy woodland and dry forest diets when samples were pooled by grids, likely due to lower variance among samples. Low stress levels (≤0.1) of ordinations suggested a low likelihood of misleading interpretation (Clarke & Warwick 2001b).

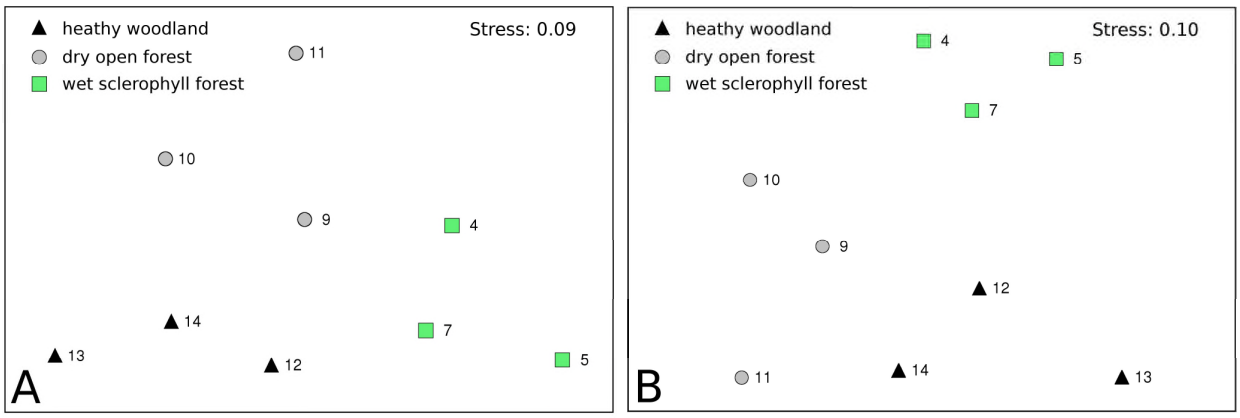

Figure 4.5 NMDS ordinations comparing truffle-like fungal composition in swamp wallaby *Wallabia bicolor* diets sampled from grids across three habitat types at different levels of taxonomic aggregation: A) morphospecies (spore types) and B) genera. Labels are grid numbers. Data used were the relative % occurrence of each truffle-like taxon (√- transformed) across scats collected from each grid.

A one-way ANOSIM test revealed a significant (*P*=0.004) difference among habitats in the composition of truffle-like taxa in swamp wallaby diets at both the morphospecies and genus-levels (Table 4.4). ANOSIM *R*-values suggested all habitat comparisons contributed to this difference. Wet sclerophyll consistently exhibited the highest differentiation from other habitats both in *R*-values and SIMPER dissimilarity. However, moderate average dissimilarity among habitats suggested that the relative % occurrence of many species did not vary greatly among habitats (overall dissimilarity=45.6-54.0%) and even more so at the genus-level (overall dissimilarity=34.9-

37.6%). In the full dimensional space, the PRIMER function MVDISP suggested that wet sclerophyll exhibited the greatest variability in sample composition (Dispersion Factor Value=1.20) and heathy woodland the least (Dispersion Factor Value=0.73), while dry forest was intermediate (Dispersion Factor Value=1.07).

Table 4.4 Global and pair-wise results of one-way ANOSIM tests for differences among habitats with grids as replicates using the relative % occurrence of each truffle-like taxon (√- transformed). Shown with ANOSIM R-values and significance level where P<0.05 and average dissimilarity from SIMPER tests.

	Habitat	*R-value*	*P*	Dissimilarity
Morphospecies (spore type)				
Global		0.745	0.004	
Pair-wise	wet sclerophyll – dry open forest	0.704	-	48.9
	wet sclerophyll – heathy woodland	0.852	-	54.0
	dry open forest – heathy woodland	0.704	-	45.6
Genera				
Global		0.844	0.004	
Pair-wise	wet sclerophyll – dry open forest	0.889	-	37.6
	wet sclerophyll – heathy woodland	0.889	-	37.5
	dry open forest – heathy woodland	0.741	-	34.9

SIMPER tests revealed that a large number of morphospecies (40-44 morphospecies) accounted for dissimilarity among habitats at the 90% cumulative contribution cut-off. The number of morphospecies contributing to 50% of the cumulative dissimilarity was lower (15-19 morphospecies) but still substantial (Table 4.5). Contributions by individual morphospecies were proportionally small (2.3-4.68%) in all pair-wise comparisons between habitats. Eight to nine morphospecies had a higher relative percentage occurrence in wet sclerophyll and were most important in discriminating the habitat from all others in assemblage composition. Important taxa included *Hysterangium* 2 and several Russulaceae taxa (*Arcangeliella* 2 and 3, Russulaceae 2-4). Dry open forest was most dissimilar from other habitats by a higher frequency of 10-12 morphospecies including *Mesophellia* 2, *Chondrogaster* 2, *Cortinarius* 2, *Cortinarius* 3, *Hydnoplicata convoluta, Aroramyces* 1-2, *Elaphomyces* 3, *Hysterangium* 2, and *Elaphomyces* 1. Heathy woodland diets were distinguished most by a higher percentage occurrence of 6-7 morphospecies including *Andebbia* Group, *Cortinarius* 2, *Cortinarius* 3, *Gautieria monospora, Labyrinthomyces* 2, *Protoglossum* 1, and *Pogisperma* 1.

Marked differences between habitats in the taxa contributing most to dissimilarities included the much higher average percentage occurrence of *Cortinarius* 2 and *Cortinarius* 3 in dry forest and

heathy woodland compared to wet sclerophyll and the higher occurrence of several Russulaceae taxa in the latter habitat compared to all others. Of those morphospecies most important in discriminating habitats from one another (Table 4.5), only a relatively small proportion (13-44%) were unique to one habitat in pair-wise comparisons. This figure mirrored the total proportion of truffle-like taxa unique to each habitat which was relatively similar across habitats (19.0-22.4%). Overall, a large proportion (40.9%) of truffle-like taxa were observed in only a single habitat, compared to 35% of taxa that were observed in all three habitats.

Table 4.5 Results of SIMPER analysis identifying truffle-like morphospecies contributing most (at a cumulative 50% cut-off) to dissimilarities among habitat groups in the composition of truffle-like taxa in swamp wallaby *Wallabia bicolor* diets. Data used were the relative percentage occurrence (√-transformed) of truffle-like fungi among scats within each grid. Shown with differences (< and >) in the average abundance (√-relative % occurrence) of taxa among habitats. Each morphospecies relative (%) contribution to the total dissimilarity among habitat types is shown, followed by the rank order of the ten highest contributing taxa (**bold** type).

	dry open forest – wet sclerophyll					heathy woodland – wet sclerophyll					dry open forest – heathy woodland				
Morphospecies*				%					%					%	
Andebbia Group	2.9	>	0.7	2.4		**6.5**	**>**	**0.7**	**4.1**	3	2.9	<	6.5	2.8	
Arcangeliella 1	-		-	-		2.0	<	5.8	2.7		-		-	-	
Arcangeliella 2	**0.0**	**<**	**2.6**	**2.6**	7	-		-	-		-		-	-	
Arcangeliella 3	**0.6**	**<**	**2.9**	**2.6**	10	0.3	<	2.9	2.6		-		-	-	
Aroramyces 1	-		-	-		1.8	<	2.8	2.7		**3.2**	**>**	**1.8**	**3.1**	7
Aroramyces 2	3.6	>	1.5	2.5		-		-	-		**3.6**	**>**	**0.6**	**3.1**	6
Austrogautieria 1	7.0	>	5.3	2.4		-		-	-		-		-	-	
Chondrogaster 2	2.5	>	0.4	2.4		-		-	-		**2.5**	**>**	**0.0**	**3.5**	2
Cortinarius 1	5.6	<	8.5	2.4		-		-	-		-		-	-	
Cortinarius 2	**7.4**	**>**	**2.3**	**3.4**	1	**12.2**	**>**	**2.3**	**4.7**	1	7.4	<	12.2	2.8	
Cortinarius 3	**7.0**	**>**	**1.4**	**3.3**	2	**9.6**	**>**	**1.4**	**4.3**	2	-		-	-	
Densospora 1	-		-	-		-		-	-		1.9	>	0.0	2.5	
Elaphomyces 1	**1.8**	**>**	**0.0**	**2.6**	9	-		-	-		**1.8**	**>**	**0.0**	**2.9**	10
Elaphomyces 3	**2.1**	**>**	**0.0**	**2.7**	6	-		-	-		**2.1**	**>**	**0.0**	**3.1**	8
Gautieria monospora	-		-	-		**4.1**	**>**	**0.0**	**4.0**	4	**0.9**	**<**	**4.1**	**3.4**	3
Hydnoplicata convoluta	-		-	-		-		-	-		**2.2**	**>**	**0.0**	**3.3**	4
Hysterangium 2	**4.0**	**<**	**6.4**	**2.6**	8	**1.7**	**<**	**6.4**	**3.0**	10	**4.0**	**>**	**1.7**	**2.9**	9
Hysterangium 5	4.2	>	1.5	2.4		5.0	>	1.5	3.0		4.2	<	5.0	2.7	
Labyrinthomyces 2	-		-	-		**4.9**	**>**	**0.4**	**3.7**	5	-		-	-	
Leucogaster meridionalis	-		-	-		-		-	-		1.0	>	0.0	2.2	

	dry open forest – wet sclerophyll					heathy woodland – wet sclerophyll					dry open forest – heathy woodland				
Morphospecies*				%					%					%	
Mesophellia 2	2.6	>	0.4	2.5		-		-	-		**2.6**	**>**	**0.0**	**3.6**	1
Octaviania 2	1.6	<	3.6	2.3		-		-	-		-		-	-	
Pogisperma 1	-		-	-		-		-	-		**0.2**	**<**	**3.0**	**3.2**	5
Pogisperma 2	-		-	-		**0.0**	**<**	**3.2**	**3.5**	6	1.0	>	0.0	2.2	
Protoglossum 1	-		-	-		**2.4**	**>**	**0.0**	**3.1**	8	-		-	-	
Quadrispora 1	-		-	-		-		-	-		1.6	>	1.4	2.3	
Russulaceae1	1.6	<	5.2	2.5		-		-	-		-		-	-	
Russulaceae2	**0.7**	**<**	**4.3**	**2.9**	5	**0.6**	**<**	**4.3**	**3.3**	7	-		-	-	
Russulaceae3	**0.7**	**<**	**5.1**	**3.3**	3	**0.9**	**<**	**5.1**	**3.0**	9	-		-	-	
Russulaceae4	**1.4**	**<**	**7.8**	**3.2**	4	2.9	<	7.8	2.8		1.4	<	2.9	2.3	

* *Andebbia* Group also includes the genus *Gummiglobus* due to the inability to distinguish these genera on spore morphology alone. 'Russulaceae' spores could not be assigned to specific genus but all were identified as belonging to one of the truffle-like fungi genera *Cystangium*, *Gymnomyces*, *Russulacea*, or *Arcangeliella*. Only *Arcangeliella* species with unique spore characteristics were differentiated.

SIMPER tests suggested the level of similarity among grids in spore composition was relatively constant across habitats (59.2%-65.4%; Table 4.6). A large number of morphospecies contributed to the 90% cumulative similarity between grids within habitats. The number of morphospecies ranged from 13 in heathy woodland, 20 in wet sclerophyll, to 23 morphospecies in dry forest. A smaller subset of morphospecies contributed to the 50% cumulative similarity within each habitat.

Morphospecies typical of a habitat (i.e. high average contribution) and unique to that habitat alone comprised a relatively small proportion of the most important distinguishing morphospecies in both dry forest and heathy woodland (44.4% and 20% respectively). In contrast, a greater proportion of higher contributing taxa were unique to wet sclerophyll (71.4%). For example, the taxa *Rossbeevera vittatispora* and *Cortinarius 1* had a high average contribution to similarities amongst grids across all habitats, while three morphospecies (*Cortinarius 2, Austrogautieria 1,* and *Cortinarius 3*) were important taxa shared amongst grids in both heathy woodland and dry forest. In addition, largely erumpent species within the family Cortinariaceae (three *Cortinarius* spp.) were typical of both dry forest and heathy woodland habitats, although the consistency of these morphospecies contribution was much higher in heathy woodland. Differences among habitats included two morphospecies of *Mesophellia* that were unique to dry forest in contributing most to similarities and one taxon (*Andebbia* Group) in heathy woodland. In contrast to other habitats, five of the seven morphospecies contributing most to similarities among wet sclerophyll grids were unique to that habitat, four of which belonged to the family Russulaceae. The consistency of

morphospecies contributions varied most in dry forest and wet sclerophyll and was markedly lower in heathy woodland, with a smaller group of taxa contributing relatively consistently to the similarity among grids.

Table 4.6 Results of SIMPER analysis identifying truffle-like morphospecies contributing most (at a cumulative 50% cut-off) to similarities in swamp wallaby *Wallabia bicolor* diets within habitat groups. The percentage contribution of each taxon to the cumulative 50% cut-off total is given along with the average contribution consistency (the ratio of the average contribution divided by the standard deviation across all pairs of samples) for each taxon. Data used was the relative % occurrence of taxa (√-transformed). The taxa '*Andebbia* Group 1' also incorporates spores potentially belonging to the genus *Gummiglobus*.

Habitat	Similarity	Morphospecies	Percentage contribution	Average similarity/SD
dry open forest	62.1	*Rossbeevera vittatispora*	9.72	14.03
		Austrogautieria 1	7.55	59.5
		Cortinarius 2	5.75	3.55
		Mesophellia 1	5.73	7.42
		Cortinarius 3	5.67	1.89
		Hysterangium 5	4.69	3
		Cortinarius 1	4.53	1.5
		Mesophellia 2	4.19	6.04
		Hydnoplicata convoluta	3.81	52.58
heathy woodland	65.42	*Rossbeevera vittatispora*	11.42	18.62
		Cortinarius 3	10.25	11.32
		Cortinarius 2	10.14	17.08
		Austrogautieria 1	8.38	48.25
		Andebbia Group 1	8.2	28.31
		Cortinarius 1	7.42	5.3
wet sclerophyll	59.2	*Rossbeevera vittatispora*	10.52	24.21
		Russulaceae 4	8.23	4.93
		Arcangeliella 1	8	9.41
		Cortinarius 1	7.19	6.1
		Russulaceae 3	7.15	5.58
		Hysterangium 2	6.66	2.86
		Russulaceae 2	6.5	26.43

Discussion

Mycophagous habits

A high proportion (94-100%) of swamp wallaby scats among contrasting habitats contained fungal spores, dominated by those of truffle-like fungi. These results are generally consistent with those reported from East Gippsland and south-eastern New South Wales (Claridge *et al.* 2001: 68%) and two studies on the New England Tablelands, one in a largely agricultural landscape (Vernes & McGrath 2009: 85%) and one in a wilderness area (Vernes 2010: 94.5% in winter). Vernes (2010) has shown that swamp wallabies within the study area consume fungi year-round with peaks in consumption over autumn and winter. Earlier studies have shown that fungal tissue comprises a significant proportion (15-20%) of dietary items consumed by the species (Hollis *et al.* 1986) and is likely to be an underestimate due to the high digestibility of fungus compared to plant food items (Hume 1999). Results of this study further confirm the potential importance of swamp wallabies as a dispersal vector for fungi, particularly truffle-like taxa.

The greater taxon richness and spore abundance of truffle-like fungi in diets suggest that the swamp wallaby preferential forage for these fungi over epigeous species, at least during winter. Considering the lower occurrence (52-79%), average abundance (5-9% that of truffle-like fungal spores), and taxon richness (12-14 epigeous versus 37-49 truffle-like) of epigeous spores in diets, swamp wallabies would appear to be consuming few epigeous sporocarps across all habitats and may play only a minor role as a dispersal vector. In addition, the consistency across habitats would suggest that consumption of epigeous taxa may be opportunistic. Other studies have also observed a greater dominance of truffle-like over epigeous taxa in Australian mycophagous mammal diets (Bennett & Baxter 1989; Claridge *et al.* 1993c; Tory *et al.* 1997; Claridge & Lindenmayer 1998) including swamp wallaby diets (Claridge *et al.* 2001; Vernes & McGrath 2009).

Contrary to these results, Vernes (2010) recorded a higher frequency of epigeous spores in swamp wallaby scats across all seasons (41-94%), with peaks in autumn (94%) and winter (93%). Epigeous spores had a higher occurrence than truffle-like spores, while the average number of epigeous taxa in scats was similar or higher than that of truffle-like taxa. Although the average richness of truffle-like taxa was similar to that recorded for epigeous taxa, Vernes (2010) detected only 8 epigeous taxa in swamp wallaby scats compared to 30 truffle-like taxa. This equates to a truffle-like to epigeous taxa ratio of 3.75:1, similar to that recorded here in this study (3.1:1 to 3.5:1). Moreover, the average number of epigeous taxa found in this study (0.93-1.72) per scat was also similar to the range across seasons (≈0.5-2.3) recorded by Vernes (2010). Spores of Agaricomycetes and Boletoid taxa were the most frequently encountered epigeous taxa in both

studies. It is possible that my sampling of diets coincided with a much lower abundance of epigeous sporocarps ('mushrooms') available for swamp wallabies to sample from. Alternatively, truffle abundance may have been high and consumed in inverse proportion to availability as part of a 'mixed feeding strategy' in wallabies (Di Stefano 2007). The first explanation seems most likely however, based on few observations of mushrooms during the course of surveys. Consequently, swamp wallabies may sample only a comparatively small number of taxa from the epigeous fungal community, frequently consumed when abundant. Differences between studies in epigeous taxa is not surprising, considering that standing crop of mushrooms may exhibit greater temporal variability than truffles (North *et al.* 1997). Combined with a substantially greater standing crop than epigeous counterparts and potentially greater palatability (North *et al.* 1997; Waters *et al.* 2000), truffles represent a potentially more stable and abundant food resource for mammals to exploit (Claridge *et al.* 1993b; North *et al.* 1997).

Abundance and richness - spatial trends

The average abundance of truffle-like fungal spores in scats differed among habitats and significantly between dry forest and wet sclerophyll. Average spore abundance exhibited the same trend among habitats as the % occurrence of truffle-like taxa in scats, providing some evidence for consumption rates by swamp wallabies being different among habitats. Although absolute differences were not great, differential consumption among habitats could influence the diversity of fungal taxa consumed and dispersed and consequently, the species importance as a spore dispersal vector.

Swamp wallabies consumed a high diversity of truffle-like fungi with the total number of taxa ranging from 49 to 63 among the three different habitat types. Vernes & McGrath (2009) recorded spores of 23 fungal taxa in only 34 swamp wallaby scats, consisting mostly of truffle-like taxa. In a study of macropod mycophagy on the New England Tablelands, Vernes (2010) similarly found truffle-like taxa comprised the majority of fungal richness found in swamp wallaby diets. Swamp wallaby diets were also predicted to contain a greater richness of fungal taxa compared to two other co-occurring macropods (parma wallaby *Macropus parma* and red-necked pademelons *Thylogale thetis*; Vernes 2010). Vernes (2010) recorded 37 fungal taxa (spore types) in swamp wallaby scats (n=199) collected mostly from heathy woodland habitat within the same study area but over a larger geographic area. Although direct comparison of total richness must be viewed with caution, at a sample size of 50 scats, a rarefied estimate of total species richness from the latter study was 27 fungal taxa, compared to a total of 48 fungal taxa estimated for heathy woodland in this study using the same software and estimation method (i.e. PRIMER). The higher estimate of total richness in this study is likely to be largely due to differences in the identification and categorisation of fungal spores, with the current investigation potentially benefiting from sporocarp

reference material. However, Vernes (2010) recorded a total of 5 epigeous and 19 truffle-like fungal genera, corresponding to the same number recorded within heathy woodland habitat by the current study. Only two genera recorded by Vernes (2010) were not recorded in this study (*Hymenogaster* and *Hysterogaster*). This similarity suggests that taxon richness measured at a higher taxonomic level from different studies can agree where undertaken in broadly the same habitat type and general locality and is useful to present for comparative purposes. Trends among habitats could also be compared, considering that examination at the genus-level reflected the same trends to those undertaken at the lowest taxonomic possible (i.e. morphospecies).

Differences between the observed and estimated species richness were considerable for all habitats, although they were most pronounced in wet sclerophyll. The Jackknife2 minimum estimate of total richness represented 62% more taxa than observed for wet sclerophyll compared to 44% for heathy woodland and 34% for dry forest. A comparatively steep accumulation curve and rising uniques curve similarly suggested a much greater richness of truffle-like taxa in wet sclerophyll than observed in diets. The large discrepancy in wet sclerophyll diets between observed species richness - both in average richness contained in scats and total observed species (Table 4.2; Figure 4.2A) - and Jackknife2 estimates of total species richness (Table 4.2; Figure 4.2B), could be explained by swamp wallabies consuming a low number and diversity of truffles from a species rich assemblage. Overall, results suggest richness was not fully described in any of the habitats sampled, and a much greater richness of taxa than observed is likely to occur in swamp wallaby diets. Considerably greater sampling effort would be required to accurately estimate total richness in swamp wallaby diets within individual habitat types.

No significant difference among habitats in total species or genus richness were suggested by 95% confidence intervals of rarefaction curves, suggesting the total number of truffle-like taxa dispersed by swamp wallabies did not differ significantly among habitats. However, there were more important and statistically significant differences among habitats in the average number of species and genera observed in swamp wallaby scats. The frequency, abundance, and taxon richness (both at species and genus-levels) of truffle-like fungal spores in swamp wallaby scats all exhibited the same ranking of habitats: dry forest>heathy woodland>wet sclerophyll. Accordingly, most significant differences were between opposite poles of this gradient: dry forest and wet sclerophyll. This result was surprising as it is the opposite ranking of habitats in fungal richness to that found by sporocarp surveys (Chapter 2) and in small mammal diets (Chapter 3). Comparisons with trends in the soil (Chapter 2) also show that average species richness observed in swamp wallaby diets was opposite to ranking of habitats in the diversity of sporocarps available.

Few studies have investigated the effect of habitat type on the diversity and composition of truffle-like fungal spores in the diets of mycophagous mammals. Previous research in North America has

found no significant effect of habitat type on the diversity of truffle-like taxa in diets (Lehmkuhl *et al.* 2004) or frequency of fungal spore type consumption (Orrock *et al.* 2003). However, Vernes & Dunn (2009) found bush rats diets to differ significantly in taxon richness between sclerophyll forest and rainforest habitats, reflecting marked changes in the truffle assemblage from which mammals could sample. More recently, Schickmann *et al.* (2012) found forest type to have a significant influence on EcM fungal spore type abundance and richness in some mycophagous mammal diets.

Abundance and richness – comparisons to sporocarp surveys and small mammal diets

Comparisons between the average richness of truffle-like fungi in swamp wallaby diets to the richness observed both in small mammal diets (Chapter 3) and sporocarp surveys (Chapter 2) suggested a potential negative trend between the taxon richness consumed and available in the soil. This trend may simply be the result of apparent differences in the amount of fungi consumed by swamp wallabies among habitats (i.e. consumption rates), as it follows the ranking of habitats in the frequency (i.e. percentage occurrence) and abundance of truffle-like fungal spores observed in scats. However, the higher richness in dry forest scats compared to heathy woodland reflects the same ranking of these habitats as observed in bush rat scats. Species richness estimates using Jackknife2 for all mammal diets also supported dry forest having a higher richness than predicted from sampling of sporocarps in the soil. The total number of truffle-like genera detected in swamp wallaby diets and in the soil was remarkably similar for heathy woodland (19 versus 17 respectively) and wet sclerophyll (22 versus 21), but differed greatly for dry forest (28 versus 15). Consequently, richness in this habitat type may have been underestimated by sporocarp surveys, an insight gained through simultaneous sampling of mammal diets.

Overall, these comparisons suggest that taxon richness in swamp wallaby diets do not consistently reflect the available diversity of truffle-like taxa across habitat types. In turn, swamp wallabies may be more important in some habitats than in others as a spore disperser, by dispersing a greater number of taxa. The species contribution to dispersing taxa within habitats relative to other mammal species may also differ. Rarefied to comparable sample sizes, the estimated total species richness contained within small mammal and bush rat diets in wet sclerophyll was much higher than that observed in swamp wallaby scats (Figure 4.2A). On the other hand, swamp wallaby diets contained a much higher total richness than other mammals sampled in heathy woodland. Swamp wallabies may play a relatively more important role as a dispersal vector in heathy woodland compared to wet sclerophyll, while all mammals were may be equally important in dry forest by dispersing a similar total number of taxa. However, although the average number of truffle-like

taxa in swamp wallaby scats in heathy woodland (5.6) was similar to that in bush rat diets (6.5), swamp wallaby scats contained a much lower average richness in the remaining habitats (4.2-8.2) compared to bush diets (15.8-15.9; Chapter 3). In addition, the average abundance of spores in swamp wallaby diets (100-117) was a fraction of that observed in bush rat scats (763-1205). Obviously, swamp wallabies are much less heavily mycophagous during winter than bush rats and may play a comparatively less important dispersal role at a small spatial scale. Nonetheless, a diverse community of mycophagous mammals would appear important in providing dispersal services for the fungal community.

Interestingly, the occurrence of truffle-like fungal spores in scats appeared to follow an opposite trend among habitats to the relative availability of sporocarps (biomass). This contrasts with previous suggestions that consumption by mycophagous mammals may increase with sporocarp availability (Claridge 2002) and a positive relationship found between sporocarp production and representation of fungus in Tasmanian bettong *Bettongia gaimardi* diets (Johnson 1994b). This response to habitat type could potentially be explained by truffle consumption being driven by a mixed feeding strategy (i.e. consuming food items in inverse proportion to their availability), as has been observed in swamp wallaby selection of plant food items among differing habitats (Di Stefano 2007). This strategy likely accounts for the observed trends in taxon richness among habitats (see above discussion), with swamp wallabies consuming a greater number of taxa in accordance with more frequent sampling of the fungal community. Alternatively, different protein (nitrogen) availability among habitats may have driven consumption pattern. Grassy habitats (i.e. dry forest) may support lower and less stable foliar nitrogen when compared to habitats dominated by shrubs and herbaceous plant species such as wet sclerophyll (Hume 1999; Di Stefano 2007; Skidmore & Ferwerda 2008; Jones 2011). Truffles may be high in nitrogen (Cork & Kenagy 1989a) and consequently a more important resource to exploit in grass-dominated dry forest compared to wet sclerophyll.

Numerous other factors may have accounted for these opposing trends between diets and sporocarps, including habitats varying in the palatability of truffles within the fungal assemblage present. Differences in the spatial arrangement of sporocarps (i.e. clumped or even distribution) and foraging strategies among habitats could also influence swamp wallaby consumption rates. Sampling of sporocarps in the soil (Chapter 2) suggested a more clumped distribution of sporocarps in dry forest and heathy woodland - suggested by a higher number of sporocarps per collection and greater dominance of species among sporocarps – compared to wet sclerophyll (Chapter 2). The spatial distribution of a resource can influence foraging strategies in mycophagous mammals (Vernes & Haydon 2001) and the ability to exploit a patchy resource may vary among individual species. It is possible that a more clumped distribution of large-bodied sporocarps suits

the foraging strategy of swamp wallabies, representing a food resource exploited with less foraging cost. Conversely, the much more even distribution and high diversity of sporocarps in wet sclerophyll may represent a costly resource to exploit. In addition, differential sporocarp distribution among habitats may be influential at the spatial scale that swamp wallabies forage at but not at the much smaller scale that small mammals forage. Such a relationship may partly account for the observed differences between swamp wallabies and small mammal in their responses to habitat type.

Composition– spatial trends

Distinct differences among habitats were also apparent in the composition of truffle-like taxa present in swamp wallaby diets. An NMDS ordination using scats as replicates (and presence-absence data) and a two-way nested ANOSIM test using grid averages as samples suggested that the composition of taxa in wet sclerophyll diets was different to that observed in the remaining habitats. Overlap in dietary composition between heathy woodland and dry forest reflected patterns found in the truffle-like fungal community observed through sampling sporocarps in the soil (Chapter 2). Similarly, the greatest difference in diets was between the mesic wet sclerophyll and the more xeric heathy woodland, again reflecting similar dissimilarities observed in small mammal diets and sampling of sporocarps in the soil. In contrast to sporocarp sampling, pooled small mammal and bush rat diets (Chapter 3) both suggested a significant difference among all habitats, including between heathy woodland and dry forest. High dispersion of samples, potentially driven by low numbers of taxa in individual scats, may have contributed to statistical similarity between heathy woodland and dry forest.

Pooling samples by grid offered an opportunity to counter potential misleading results due to species-poor samples and greater weighting to rare taxa in analysis using presence-absence data and individual scats as replicates. When grids were used as replicate samples and the contribution of taxa was expressed as relative % occurrence, a significant difference was found among habitats in the composition of truffle-like taxa in swamp wallaby diets at both the species and genus-level. *R*-values suggested all habitats contributed relatively equally to this difference. A large number of species accounted for differences among habitats in the composition of truffle-like fungi present in wallaby diets. This result suggests complex differences in dietary composition among habitats. The high percentage of taxa that were recorded from only one habitat (41%) further suggested diets in each habitat were unique. These results correspond with those of small mammal and bush rat diets (Chapter 3) although contrasts with the overlap predicted between heathy woodland and dry forest in assemblage composition suggested by sporocarp surveys (Chapter 2). Further research is required to clarify whether these two assemblages differ in composition or whether differentiation in diets was influenced by the mycophagous habits, such as preferential consumption of taxa, of the

sampled mammals.

Few studies have investigated the influence of habitat type on the composition of truffle-like taxa found in mammal diets. Research in tropical northern Australia found EcM fungal spores to be more common in diets sampled from eucalypt dominated sclerophyll forest compared to rainforest (Reddell 1997). More recently, significant compositional differences in bush rat diets were observed between sclerophyll forest and rainforest habitats which was considered to reflect changes in fungal taxon composition in the soil, shifting from EcM to AM fungal taxa (Vernes & Dunn 2009). A habitat effect on diets was also suggested for dissimilarities between southern brown bandicoot *Isoodon obesulus* and long-nosed bandicoot *Perameles nasuta* diets in the main types of fungi present (Claridge 2002). Comparisons to the results of Chapter 2 and 3 also suggest compositional dissimilarities in swamp wallaby diets among habitats may be similarly reflecting changes in truffle-like assemblages in the soil and could potentially be used as a surrogate technique for detecting large shifts in fungal taxon composition across space. The similarity in morphospecies and higher taxonomic level (mostly genus-level) patterns suggests higher-taxon surrogacy (Balmford 2000; Kallimanis *et al.* 2012) could be used to detect large changes in assemblage composition. This is particularly appealing for truffle-like fungi, a group in which many species are yet to be discovered or described and where identification to species level can be challenging to non-specialists and largely unattainable in examination of dietary samples from mycophagous mammals.

Clustering of samples by grid in NMDS ordinations and results of a two-way nested ANOSIM initially suggested spatial variation within habitats (i.e. grid differences) may have attributed to observed dispersion and overlap between heathy woodland and dry forest. However, subsequent tests revealed differences among grids were present within all three habitats sampled (Appendix C). Grid differences could reflect patchiness in fungal distributions (Griffiths *et al.* 1996; Bougher & Lebel 2001) or underlying variation in swamp wallaby diets due to animals foraging for truffles over large areas and in a variety of microhabitats before deposition of scats in grid plots. Sporocarps and mycelia mats of EcM fungi often exhibit an uneven spatial distribution and considerable variation in assemblage composition can occur over relatively small spatial scales (Dahlberg 2001; Peter *et al.* 2001; Tedersoo *et al.* 2003). In Chapter 2, the composition of truffle-like fungi based on sporocarp collections showed considerable variation amongst individual plots within habitats, suggesting considerable spatial heterogeneity. Simultaneous movement tracking and sampling of fungal spores in swamp wallaby diets and fungal taxa in the soil could reveal which of these explanations is correct, and should be a question addressed by future research.

Composition – comparisons to sporocarp surveys and small mammal diets

Although taxonomic groups differentiating diets among habitats were largely different to those from sporocarp surveys (Chapter 2), some notable similarities were apparent at the genus-level. Russulaceae and *Hysterangium* taxa were similarly important in differentiating wet sclerophyll, while *Cortinarius* taxa were important in differentiating dry forest and heathy woodland. There were also similarities between swamp wallaby and bush rats in taxa driving differences in diets among habitats. Russulaceae taxa were similarly important in discriminating wet sclerophyll in bush rat diets, as were *Aroramyces*, *Cortinarius*, and *Hydnoplicata* for dry forest. In contrast, there was no overlap in discriminating taxa between swamp wallaby and bush rat diets in heathy woodland, suggesting less dietary overlap. Nonetheless, correspondence between mammal diets suggest that compositional dissimilarity observed in diets among habitats may at least partly reflect different assemblages of truffle-like fungi in the soil, supporting previous inferences in the literature (Claridge 2002; Vernes & Dunn 2009). Greater similarity would be expected if the rank abundance of sporocarps in the soil was strongly correlated (positively) with abundance of spores in diets for most truffle-like taxa, as has been observed in a study of Tasmanian Bettong diets and the abundance of truffles at a single site (Johnson 1994a). However, such a relationship is unlikely to be observed in more opportunistic mycophagist species, such as the swamp wallaby. Large discrepancies between these two species diets (swamp wallaby and bush rat) and sporocarp surveys in important discriminating taxa suggest mycophagous species may sample different taxa or proportions of taxa from the assemblage available in each habitat. Differences in taxa consumed by co-occurring mycophagous mammals has been observed previously (Meyer *et al.* 2005; Frank *et al.* 2006; Chapter 3), suggesting that a more diverse assemblage of mammalian vectors may increase dispersal events for fungal communities.

The large number of taxa important for within-habitat similarity in diets also suggest complex differences among habitats in the assemblage from which they sampled. The much greater number of taxa unique to wet sclerophyll diets and much greater overlap in heathy woodland and dry forest taxa reflects trends in assemblage composition suggested by sporocarp surveys (Chapter 2), with wet sclerophyll possibly the more unique community. In comparing both swamp wallaby and bush rat diets to sporocarp surveys, a surprising result was the obvious importance of *Rossbeevera vittatispora* in diets despite being rarely detected in the soil. The species generally produces fruit close to the soil surface, meaning that it should be detected relatively easily by the methods employed in sporocarp surveys. One explanation for low representation in sporocarp surveys is that their sporocarps are highly palatable to mycophagous mammals and removed preferentially, eroding the sporocarp standing crop and reducing detectability. Spores of the genus *Rossbeevera* (syn. *Chamonixia*; Lebel *et al.* 2012a; b) have been previously recorded as common in swamp

wallaby diets (Claridge *et al.* 2001); (Claridge *et al.* 2001; Vernes 2010) and abundant in bush rat diets (Tory *et al.* 1997). Conversely, one genus of conspicuously lower frequency in swamp wallaby diets compared to bush rat diets and observed in the soil was *Descomyces*, suggesting low palatability to swamp wallabies. Considering the low diversity of truffle-like fungi in wet sclerophyll diets, it is possible that the sporocarps of many taxa more abundant in this habitat, such as *Descomyces* spp., were not palatable to swamp wallabies. Interestingly, previous investigations of swamp wallaby diets also found *Descomyces* to be absent (Vernes 2010) or infrequently encountered (Claridge *et al.* 2001).

These discrepancies also highlight the advantage of sampling mammal diets in revealing potential dominant species in the fungal community that would otherwise be missed using sporocarp surveys alone. Sampling of mammal diets represents a comparative method to test assumptions of changes in community structure based on sporocarp surveys. Johnson (1994) showed that abundance estimates for some truffle-like taxa in the soil may be biased by being subject to high consumption rates by mycophagous mammals. Patterns in relative abundance may also be obscured by preferential selection of some species sporocarps over others (Johnson 1994b; Zabel & Waters 1997). If consumption of truffles biases estimates of fungal richness and abundance based on sampling of sporocarps (Claridge *et al.* 1993), with the exception of exclosure methods (North *et al.* 1997), the simultaneous sampling of mycophagous mammal diets may be useful to ensure that incorrect conclusions are not made on the structure and richness of the fungal communities based on sporocarp sampling alone. In addition, numerous additional taxa can be revealed by sampling mammal diets (Izzo *et al.* 2005), ranging in this study from 9 to 15 genera per habitat type. In all cases, a greater number of genera were detected in swamp wallaby diets than in sporocarp surveys. However, results would suggest that swamp wallaby diets may be unreliable in estimating the true fungal richness among habitat types, although sampling can reveal weaker signals of composition dissimilarity among habitats.

Conservation implications

In the absence or much reduced abundance of many heavily mycophagous mammals that may have been common across the landscape in a wide variety of habitats pre-European settlement (Bilney *et al.* 2010), other more common mycophagous mammals may be playing greater relative roles in the maintenance of fungal communities (Vernes & McGrath 2009). Conversely, they may also be important in future conservation works in maintaining or re-establishing fungal food resources for threatened mycophagous species in critical areas of habitat and at a landscape scale (Bougher & Lebel 2001). Although the average richness and abundance observed in swamp wallaby diets was markedly lower than in bush rat diets, the total number of taxa consumed was large and possibly greater than bush rats in heathy woodland. Considering their large home ranges (10-60 ha; Stefano

2007), population persistence in fragmentised landscapes (Paplinska *et al.* 2009), and the wide array of truffle-like spores they disperse, swamp wallabies may be important in fragmented landscapes for maintaining functional connectivity among fungal populations. Similarly they are also likely to play an important role in re-establishment of assemblages in post-fire environments (Claridge *et al.* 2001). The replacement role that swamp wallabies can play in longer-distance spore dispersal in the absence of heavily mycophagous mammals, however, likely varies among habitat types and may be lower in more mesic habitats. It is possible that other macropodid species may fulfil a more important role in these habitats, such as mesic-dwelling *Thylogale* (pademelon) species (Vernes & Trappe 2007; Vernes 2010; Vernes & Lebel 2011).