# Tropical Hypogeous Fungal Sporocarp Distribution in Time and Space.

## Implications for an Endangered Specialist Mycophagous Marsupial, *Bettongia tropica.*

Thesis submitted by

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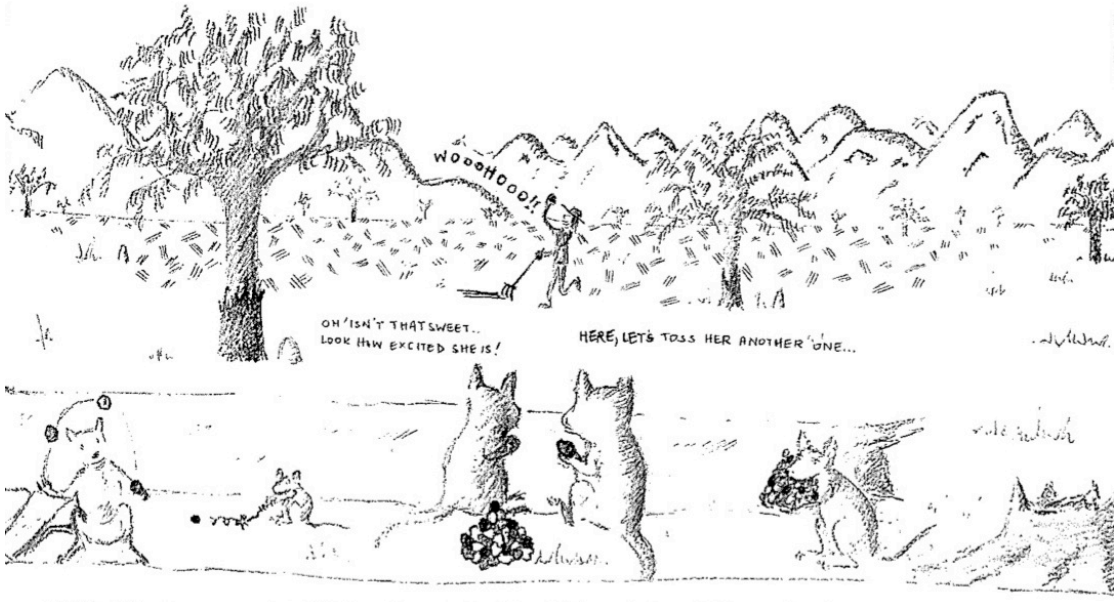


THE BETTONGS WATCHED AS SANDRA SEARCHED AND SEARCHED FOR TRUFFLES...

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Mathams, S., **Abell, S.E.**, Latch, P. and Winter, J., 2009. Potential reintroduction sites for the endangered northern bettong, *Bettongia tropica*, determined by fungal and plant resource availability. Wildlife Research. manuscript in final preparation.

# THESIS ABSTRACT

This thesis examines the relative importance of abiotic and biotic factors in determining the temporal and spatial distribution of tropical hypogeous fungal sporocarps along an altitude gradient of ecotonal sclerophyll forest. The implications of the fungal distribution on the habitat restriction within this ecotonal forest for an endangered specialist mycophagous marsupial, *Bettongia tropica* Wakefield, were subsequently investigated.

Fungal availability was quantified in the Early-Wet, Late-Wet, Early-Dry and Late-dry seasons within known *B. tropica* habitat in seven surveys over a period of two years. A total of eighteen sites within three vegetation types, including wet sclerophyll forest, *Allocasuarina* forest and *Eucalyptus* woodland, were examined using the time standardised raking method, in Far North Queensland, Australia.

*Bettongia tropica* is thought to be restricted to habitats where seasonal availability of hypogeous fungi, their principal food resource, remains high. In the first year of sampling a relationship was found between precipitation and fungal availability. The abundance of hypogeous fungi was significantly lower in the late dry season. Fungal availability correlated strongly with the seasonal rainfall pattern determined from 74-year monthly means. *Alloteropsis semialata* R.Br. (cockatoo grass) use by bettongs increased significantly during the period of low fungal availability. *Bettongia tropica* appear to be restricted to habitats where seasonal availability of hypogeous fungi, in synchrony with the availability of critical grass resources, remains high.

Temporal fruiting and availability of ectomycorrhizal hypogeous fungal species has previously been linked with both temperature and moisture (season) in temperate northern and southern hemisphere ecosystems. In contrast, the first year of data suggested that precipitation may be the sole factor influencing fruiting and diversity in tropical ecosystems. This hypothesis was tested using the data from all seven surveys. Season or temperature did not appear to be associated with fruiting of tropical hypogeous fungi. Instead, highly significant correlations between precipitation and abundance as well as biomass of sporocarps were detected. Precipitation also correlated with species richness and significantly influenced hypogeous fungal taxonomic assemblage. Availability of moisture appears to be the sole factor influencing the temporal distribution of fruiting and diversity of hypogeous fungi in this tropical ecosystem.

Spatial patterns of animal and plant diversity are used to infer mechanisms underlying community composition. Species distributions, associated with latitude and altitude gradients, are often positively correlated with moisture availability. Fungal taxonomic assemblage did not differ between the three contiguous vegetation types. Counter-intuitively, less fungal productivity occurred in one of the wettest forest types; wet sclerophyll forest. Higher sporocarp abundance and biomass were found in the *Allocasuarina* forest that had equivalent soil moisture levels. Fungal abundance and biomass appeared to be reduced by high inorganic nitrogen and phosphorous in the wet sclerophyll forest, while the effect of high nitrogen was counteracted by low phosphorous in the *Allocasuarina* forest.

The effects of soil phosphorous as well as other environmental variables on hypogeous fungal availability and richness were examined further at the micro-spatial scale of site. As predicted, negative correlations between phosphorous and fungal availability were also observed at this finer scale. However, phosphorous levels could not entirely explain the spatial patterns of fungal richness observed. Positive correlations were found for the mean number of *Allocasuarina* stems with the number of fungal genera as well as species. The most plausible explanation for the mean number of fungal species was an interactive effect between the number of *Allocasuarina* stems and a positive correlation with altitude. As well as strengthening the evidence that phosphorous levels affect the availability of hypogeous fungi, analysis at the micro-spatial scale allowed new information about fungal richness to be uncovered. An increase in host monodominance appears to increase fungal richness within this ecotonal sclerophyll forest.

These findings help to explain the habitat restriction of *B. tropica* within wetter sclerophyll habitats, increasing the temporal availability of their principal resource hypogeous fungi. The spatial distribution of fungal, as well as other important food resources, also explains *B. tropica* spatial habitat restriction within *Allocasuarina* forest and *Eucalyptus* woodland. The habitat restriction of the endangered *B. tropica* within a narrow band of ecotonal sclerophyll vegetation along the western margin of Wet Tropical rainforests in North Queensland Australia, can be attributed to the availability of their critical food resource hypogeous fungi, in both time and space.

# Chapter One: Thesis Rationale

## 1.1 Ecological context

Mutualisms, cooperative interactions between species, are the rule rather than the exception in nature (Herre *et al.* 1999). This is true from the cellular to the ecosystem level (Herre *et al.* 1999). What we know of as a single organism cannot exist without the complex interaction of many other species exploiting each other to the net benefit of all species within the association (Doebeli and Knowlton 1998; Leigh and Rowell 1995; Maynard Smith and Szathmary 1995; Nowak *et al.* 1994). This is also what defines a healthy ecosystem; a complex interaction of many species exploiting each other to the net benefit of all species.

Understanding how mutualists cooperate not only provides insight into individual species ecology, but also clarifies how ecosystems function in general. Unravelling mutualistic relationships is complex, especially when multiple species are interacting, but being able to do so becomes vitally important when endangered species are involved. This thesis examines the tripartite mutualism between an endangered marsupial, *Bettongia tropica* Wakefield, the fungi that these small mammals feed upon and the plant hosts of those fungi.

Northern bettongs, *B. tropica*, are principally mycophagous (fungus eating) marsupials of the family Potoroidae (Seebeck and Rose 1989). They are listed as endangered at the Australian State (*Nature Conservation Wildlife Regulation 2006)* and Federal government levels (*Commonwealth Environment Protection and Biodiversity Conservation Act 1999)*.

*Bettongia tropica* also have an international endangered status under the following IUCN criteria: extent of occurrence estimated to be less than 5000 $km^2$, severely fragmented or known to exist at no more than five locations, and a decline in area, extent and/or quality of habitat (IUCN Species Survival Commission 2007).

Current populations of *B. tropica* persist in a narrow band (<10km) of wet to moist sclerophyll forest on the western margin of Wet Tropics rainforest in Far North Queensland, Australia (Vernes and Pope 2001). Disjunct populations occur on the Lamb and Coane Ranges, as well as on the Mt Windsor and Carbine Tablelands. Populations at Mt Spurgeon on the Carbine Tablelands (Grant and Naylor 1993), Mt Windsor (Laurance 1997) and on the Coane Range are very small. A recent study in 2007 failed to trap even one *B. tropica* individual from the Coane Range population (Brook Bateman, unpublished data). The Lamb Range is the last stronghold of *B. tropica* (Vernes and Pope 2006) with one of the most stable populations on the Lamb Range at Davies Creek (Vernes and Pope 2006); the location of this study. The reasons for both historic and current population declines of *B. tropica* are currently unknown.

Johnson and McIlwee (1997) studied northern bettongs along a wet to dry sclerophyll gradient at Davies Creek. They showed that although there was little difference in the abundance (trap success) of bettongs between wet to medium sclerophyll forest types, there was a significant decline (50%) in abundance between medium to dry sclerophyll sites (Johnson and McIlwee 1997). Body condition and the number of juvenile bettongs within the population remained constant. This suggests that the factor limiting bettong numbers and distribution is likely to be a consumptive resource (eg. food), with each population along the wet to dry gradient being at equilibrium with the availability of this resource (Caughley *et al.* 1988).

Because *B. tropica* is considered to be a fungal specialist (Seebeck and Rose 1989), ectomycorrhizal hypogeous (below-ground) fruiting fungi have been assumed to be this important food source (Johnson and McIlwee 1997).

Colonisation of the roots of plants by fungi results in the formation of mycorrhiza, literally "fungus roots". Under ideal environmental conditions this is an obligate mutualism. The fungus provides the host plant with an otherwise unavailable supply of nutrients, improves the uptake of water and provides protection from root pathogens. In return the plant host supplies a rich carbohydrate resource for the fungus. This mutualism may become a fungal parasitism in unfavourable environmental conditions, particularly when high nutrients are available (Johnson *et al.* 1997). Mycorrhizas are otherwise a relatively stable mutualism (Herre *et al.* 1999) that have persisted for as long as plants have inhabited land (Wang and Qiu 2006).

There are different types of mycorrhiza, including arbuscular mycorrhiza (AM) and ectomycorrhiza (ECM). Ectomycorrhiza appear to have evolved multiple times (Wang and Qiu 2006) and are characterised as producing a fungal sheath entirely surrounding the tips of the host root. Ectomycorrhizal fungi either produce epigeous (above-ground, e.g. mushrooms) or hypogeous (below-ground, e.g. truffles) macroscopic reproductive structures (sporocarps). While spores from epigeous sporocarps are generally wind dispersed, hypogeous fungi are mutualistic with mycophagous animals and so their spores are dispersed via these mammal vectors. A food reward is provided in exchange for the dispersal of spores that otherwise would remain enclosed within the sporocarp, buried below the surface of the ground or leaf litter (Claridge and May 1994).

Fungal species that produce hypogeous sporocarps are generally ectomycorrhizal (Luoma *et al.* 1991) and are polyphyletic, with representatives in four out of the five fungal divisions including Zygomycota, Glomeromycota, Ascomycota and Basidiomycota. Co-evolution of ectomycorrhizas has occurred in multiple plant and fungal families under the selection of fluctuating environmental conditions, including both water and nutrient availability (Wang and Qiu 2006).

Selection for ectomycorrhizas have been especially important in regions where soil fertility has been declining due to extended time periods since renewable disturbance events (Wang and Qiu 2006). These soil conditions are characteristic of the ancient weathered soils on the arid Australian continent, where phosphorous especially is limiting. Similarly, the primary selective pressure driving the evolution from the epigeous to the hypogeous sporocarp morphology is thought to be the conservation of water (Albee-Scott 2007; Binder and Bresinsky 2002; Bruns *et al.* 1989; Thiers 1984).

Worldwide there are an estimated 5400 species of ectomycorrhizal fungi, including epigeous and hypogeous fruiting taxa (Molina 1992). As a potential consequence of the arid and low nutrient environment, hypogeous ectomycorrhizal fungal species are well represented in Australia. The estimated diversity is high (2000 species), as is endemicity (35% genera; 95% species) (Bougher and Lebel 2001).

Current data on what determines the distribution and abundance of hypogeous fungi comes from northern and southern hemisphere temperate region studies. In these regions seasonal peaks in production generally occur in both autumn and spring and taxonomic assemblage may be distinct within seasons (Beaton *et al.* 1984; Claridge *et al.* 1993; Eveling *et al.* 1990; Fogel 1976; Harvey *et al.* 1978; Hunt and Trappe 1987; Johnson 1994a).

Therefore, temporal availability of hypogeous fungi is thought to be a function of season, relating to an interaction between the availability of moisture and critical temperature thresholds (Beaton *et al.* 1984; Claridge *et al.* 1993; Eveling *et al.* 1990; Fogel 1976; Harvey *et al.* 1978; Hunt and Trappe 1987; Johnson 1994a; North 2002). However, clear patterns have been difficult to distinguish and the precise environmental cues that initiate fruiting are not known in either temperate or tropical ecosystems.

The factors that affect the spatial distribution of hypogeous fungi are also not well understood. Studies in the northern hemisphere have found host specificity to be the main driver for hypogeous fungal spatial distribution patterns (Bougher and Lebel 2001). However, when host is excluded factors such as moisture (O'Dell *et al.* 1999) and nutrient levels (Trudell and Edmonds 2004) appear to be important for epigeous sporocarp distribution.

Despite their ecological importance, few studies to date have examined spatial patterns of fungal distribution along environmental gradients in undisturbed natural ecosystems (O'Dell *et al.* 1999). The limited nature of previous studies means there is no understanding in the tropics but it is clear that the driving factors are likely to be different for both time and space.

The fruiting bodies of hypogeous fungi are an essential resource for many native Australian mammals (Bougher and Lebel 2001; Vernes and Pope 2006) and many specialist mycophagous mammals in Australia are either endangered (IUCN Species Survival Commission 2007) or are already extinct (Short 1998). It is not known what effect the extinction of specialist mycophagous mammals has on the overall health of an ecosystem. Therefore, understanding more about this plant-fungus-animal tripartite mutualism is a critical task.

## 1.2 Aim and Structure of the Thesis

The aim of this thesis is to establish the relative importance of different biotic and abiotic factors in determining both **a)** the temporal and **b)** spatial distribution of tropical hypogeous fungal taxa. The implications of these findings for defining the habitat restriction of an endangered specialist mycophagous marsupial, *Bettongia tropica*, will subsequently be investigated.

Chapter one provides a general introduction to the thesis topic. Although the same set of sites were used to collect the data relevant methodology are described in each individual results chapter. As seasonality is considered a significant driver of fungal fruiting in temperate zones, chapter two will investigate changes in sporocarp abundance and biomass across four tropical seasons, Early-Dry, Late-Dry, Early-Wet and Late-Wet, corresponding to the temperate region equivalents of winter, spring, summer and autumn respectively. The availability of sporocarps within three forest types, including wet sclerophyll, *Allocasuarina* forest and *Eucalyptus* woodland and the implications of the potential seasonality of their main resource, hypogeous fungi, for *B. tropica* habitat preference and use will be examined.

After gaining an insight into whether seasonality significantly influences tropical fungal reproduction, chapter three will further explore the abiotic factors driving sporocarp production across four tropical seasons surveyed seven times over a period of two years. This chapter will examine the effect(s) of temperature, precipitation, soil moisture and other available environmental variables on sporocarp biomass, abundance, species richness and taxonomic assemblage at the temporal scale.

Chapter four will explore the effect of biotic factors, specifically host preference, and abiotic factors including moisture, temperature, light and nutrient levels, at the forest-stand spatial scale, on the distribution of hypogeous fungi across an ecotonal altitude gradient. The abundance, biomass, species richness and taxonomic assemblage will be surveyed in known bettong habitat within three vegetation types, wet sclerophyll forest, *Allocasuarina* forest and *Eucalyptus* woodland.

Chapter five will examine the spatial distribution of hypogeous fungi at a finer scale along the same environmental elevation gradient. Factors identified in chapter four as well as multiple microhabitat variables that were measured at the micro-spatial scale of site will be examined to determine the principal factors that drive the availability and richness of hypogeous fungi within tropical ecotonal sclerophyll forest. Chapter six will synthesise the findings of previous chapters, provide recommendations for *B. tropica* management and suggest directions for future research.

# Chapter Two: Seasonal Resource Availability And Use By An Endangered Tropical Mycophagous Marsupial

## 2.1 Introduction

Availability of resources can impact on the temporal and spatial distribution of a species (Rosenzweig 1981; Shenbrot and Krasnov 2000; Wisheu 1998). This is especially important for opportunistic specialists in contrast with generalists (Rosenzweig 1981) and may be critical for those that rely on a seasonal resource. To understand the ecology of a species it is vital to determine if resources are temporally and/or spatially defining habitat preference. This basic ecological question becomes fundamental knowledge when the species is endangered or threatened.

The endangered northern bettong, *Bettongia tropica* (Potoroidae), currently occurs in four disjunct populations within sclerophyll forest adjacent, and to the west of, the world heritage rainforest massif in tropical far north Queensland, Australia (Dennis 2002). Northern bettongs prefer *Eucalyptus* woodland with a grassy–understorey on infertile soil and are restricted to woodland in close proximity to rainforest (Laurance 1997).

This habitat preference is thought to be associated with dietary specialisation. *Bettongia tropica* is considered a specialist consumer of hypogeous (fruiting below-ground) fungi (Seebeck and Rose 1989) that also utilises lilies, grasses, and the roots and tubers of species such as *Alloteropsis semialata* R.Br. (cockatoo grass) only as secondary resources (Johnson and McIlwee 1997; Vernes *et al.* 2001).

Consequently, *B. tropica*'s unusual and restricted distribution is attributed to a requirement for areas of high mycorrhiza abundance that occur across the rainforest to dry sclerophyll ecotone.

Endomycorrhizal fungi of rainforest trees generally produce small sporocarps that bettongs cannot utilise (Johnson and McIlwee 1997). Ectomycorrhizal *Eucalyptus* and *Allocasuarina* species produce larger sporocarps of both epigeous and hypogeous fungi that mycophagous mammals including *B. tropica* are known to consume (Johnson and McIlwee 1997; Maser *et al.* 1978). While dietary specialisation is consistent with the absence of *B. tropica* in rainforest margins, it does not fully explain why higher population densities are observed in the *Eucalyptus* woodland relative to the ectomycorrhizal *Allocasuarina* forests (Laurance 1997; Vernes 2000), or the absence of populations in ectomycorrhizal wet sclerophyll forest that has been invaded by rainforest. Other inconsistencies associated with this hypothesis are also apparent.

In general, specialist frugivorous mammals are thought to limit reproduction during periods of seasonal fruit shortage and also experience a decline in body condition (Adler 1998; Dennis and Marsh 1997). These phenomena have also been observed for the specialist mycophagous Tasmanian bettong, *Bettongia gaimardi* Desmarest. Body condition of *B. gaimardi* improved when the proportion of fungus in the diet increased, and declined when secondary resources (fruit) were utilised (Johnson 1994b). In all previous studies of *B. tropica*, body condition and frequency of births remained uniform regardless of season (McIlwee and Johnson 1998; Vernes 2000; Vernes *et al.* 2001). Obtaining this result for a fungal specialist requires that both fungus availability and intake do not fluctuate enough to affect body condition or reproductive phenology.

This has led to a further extension of the habitat/diet specialisation hypothesis suggesting that *B. tropica* has a distinct preference for habitat that supports both a high abundance and low seasonality of hypogeous fungi (Dennis 2002; Johnson and McIlwee 1997). However, available data do not fully support this model.

Considerable variability has been observed in the proportion of fungi consumed by *B. tropica*. By use of faecal analysis the proportion of fungus in *B. tropica*'s diet was initially estimated at between 23% and 67% with some seasonal variation (Johnson and McIlwee 1997). More recently it has been estimated at 56% with no significant difference between seasons (Vernes *et al.* 2001). Unfortunately, no data are currently available on the distribution and abundance of hypogeous fungi independent of those eaten by *B. tropica*.

Extrapolation from temperate studies suggests that tropical hypogeous fungal abundance may not be strictly seasonal, but instead may track rainfall and moisture levels (Beaton *et al.* 1984; Claridge *et al.* 1993; Fogel 1976; Harvey *et al.* 1978; Hunt and Trappe 1987; Johnson 1994a). I hypothesise that **(a)** fungus availability will in general be seasonal in the tropics due to the highly seasonal rainfall, only rarely in years of unseasonably high rainfall will fungi be consistently available across seasons and **(b)** if tropical hypogeous fungi are seasonal then the observed maintenance of body condition and reproductive output by *B. tropica* across seasons requires the utilisation of a replacement resource of equivalent value during dry seasons, possibly *A. semialata* grass bases.

To test these hypotheses the seasonal abundance of hypogeous fungi was examined and the number of chewed and discarded (consumed) *A. semialata* bases was observed, along a gradient of wet to moist sclerophyll forest on the Lamb Range in north-eastern Australia. If correct, then fungal abundance should decline as rainfall decreases and use of *A. semialata* by bettongs should increase at the same time. Such an outcome would help to explain anomalies in the distribution and habitat associations of *B. tropica* and provide new information critical to the effective management of this endangered species.

## 2.2 Methods

### *2.2.1 Study sites*

The study site was located at Davies Creek on the Lamb Range in the Wet Tropics World Heritage Area of far North Queensland, Australia (145°35'E, 17°01'S). Fungal survey sites were positioned in three vegetation types across the gradient of wet to moist sclerophyll forest; (i) wet sclerophyll sites were located in tall open-forest on granite dominated by *Eucalyptus resinifera*, *Eucalyptus acmenioides*, *Eucalyptus intermedia*, *Eucalyptus cloeziana*, *Eucalyptus grandis* and *Syncarpia glomulifera* (Type 14b: Tracey 1982).

The understorey was predominantly sedge and in most sites rainforest tree seedlings and saplings, (ii) *Allocasuarina* forest sites were located in medium woodland on granite, with a dense canopy dominated by *Allocasuarina torulosa* and *Allocasuarina littoralis*, that may also have included *E. intermedia*, *Eucalyptus tereticornis*, *Tristania suaveolens*, *Acacia cincinnata*, *Acacia flavescens* and *Banksia compar* (Type 16e: Tracey 1982). Sedge and bracken fern (*Pteridium* spp.) dominated the understorey in almost all *Allocasuarina* sites. Lower altitude *Allocasuarina* sites (closer to the *Eucalyptus* woodland) contained a small proportion of grass in the understorey, (iii) *Eucalyptus* woodland sites were located in medium woodland on granite with a relatively open canopy that included *Eucalyptus acmenioides*, *E. tereticornis*, *E. intermedia*, *Syncarpia glomulifera*, *Tristania suaveolens*, *Melaleuca viridiflora*, *Acacia flavescens*, *Allocasuarina littoralis* and *Xanthorrhoea johnsonii* (Type 16m:Tracey 1982). The understorey was dominated by grass species including *Themeda triandra*, *Alloteropsis semialata* (cockatoo grass), *Heteropogon contortus* and *Imperata cylindrica*. The higher altitude sites (closer to the *Allocasuarina* forest) also contained a small proportion of sedge in the understorey.

### *2.2.2 Rainfall data*

Rainfall data for each site were not available. Therefore, rainfall data were obtained from the climatically closest weather station at Kairi (altitude 700m ASL), 22 km south of the study area. Rainfall data were also obtained from the geographically closest weather station at Mareeba, 10 km west of the study area.

The Australian Bureau of Meteorology provided monthly rainfall data from both stations for the 1995–1996 and 2004–2005 periods as well as the 74-year means (Figure 2-1).

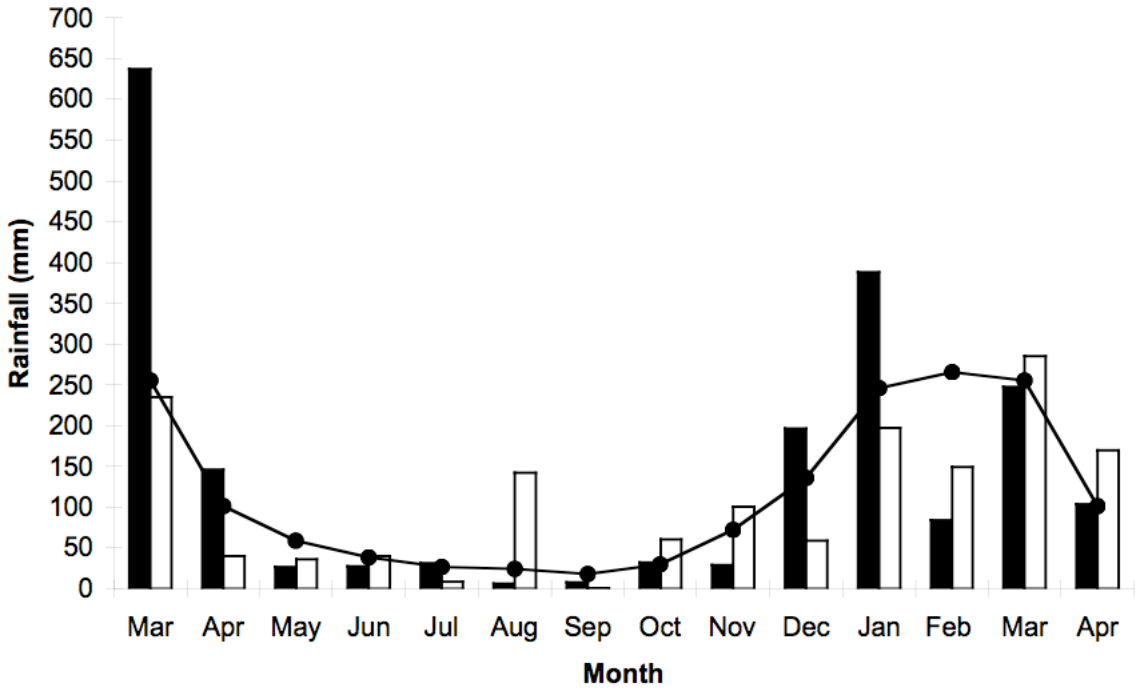


**Figure 2-1:** The monthly rainfall measured at Kairi weather station for two study periods, black columns 2004 to 2005 (this study), white columns 1995 to 1996 (Vernes et al., 2001) superimposed with the expected (●) seasonal pattern of rainfall (74-year monthly means).

### *2.2.3 Hypogeous fungi sampling*

Six sites within each of the *Eucalyptus* woodland, *Allocasuarina* forest and wet sclerophyll vegetation types were selected from a GIS vegetation map. All sites were 500 m apart, to ensure site independence and 500 m from well-used roads to minimise edge effects. Each site was located in the field by GPS. At each site four (one for each season), 50m by 20m quadrats were permanently marked with wooden stakes.

To minimise vegetation and environmental heterogeneity within sites the four season quadrats were positioned adjacent to each other with their long axes aligned with the same elevation contour. The four quadrats at each site were randomly assigned to a season prior to sampling.

Time-standardised census is a technique that has been used to assess habitat relationships of hypogeous fungi on a large scale in south-eastern Australia (Claridge *et al.* 2000b). This method was also used in the current study as it allowed a large number of sites to be sampled within a relatively short time, while minimising disturbance to the site (<20%) and allowing repeated sampling (Claridge *et al.* 2000b). A pilot study using species–time relationships was performed to determine the number of person–minutes required to sample 80%–100% of the morphologically different groups of hypogeous fungi present within the defined sampling area. Surveys were undertaken in June and September in 2004 as well as January and April in 2005. These sampling sessions were assigned to four seasons: Early-Dry (June), Late-Dry (September), Early-Wet (January) and Late-Wet (April).

Within a two and a half week period each season, one quadrat (1000$m^2$) at each site was raked with four-pronged truffling forks, for the standard time of 160 person–minutes. The number of hypogeous fungi sporocarps collected in a total of 18,000 $m^2$ was converted to a per hectare frequency of crop availability per season. All sporocarps were later dried then weighed to obtain the fungal dry weight (g) for both vegetation type and season. The number of sporocarps was used as an estimation of the quantity of sporocarps available as a resource for the bettongs and the dry weight of sporocarps relates to the biomass of that resource in the different vegetation types across seasons.

### *2.2.4 Cockatoo grass observations*

Cockatoo grass, *Alloteropsis semialata* (R.Br.), is a widespread perennial tropical to sub-tropical grass species that flowers early after the first rains of the wet season (Crowley and Garnett 2001). In the Davies Creek region *A. semialata* occurs predominantly in *Eucalyptus* woodland, and decreases in occurrence towards *Allocasuarina* forest (Vernes 2003). *B. tropica* favours the succulent stem base of the grass, a storage organ, that is located below the surface of the soil (Johnson and McIlwee 1997). As *B. tropica* require a low fibre, high quality diet, their behavioural strategy is to chew the base of *A. semialata* then disgorge the dry fibre pellet (Winter and Johnson 1995). Freshly disgorged fibre pellets appear to have been bleached and are highly visible in the field. From preliminary observations, the number of grass pellets appeared noticeably higher in the late dry season in comparison with the early dry season. Therefore, the abundance of grass pellets was quantified starting in the late dry season, continuing through the early and late wet seasons. As part of the routine environmental measurements taken at each site at the time of hypogeous fungi sampling, the number of *A. semialata* grass pellets per seasonal quadrat were counted and converted into a per hectare estimation of the frequency of cockatoo grass usage for each season.

### *2.2.5 Data analysis*

The data obtained for both sporocarp abundance and dry weight did not conform to expectations of normality due to large numbers of zero observations during particular seasons (Levene's equality of variances: $F_{3,62} = 9.069$; $P < 0.01$ and $F_{10,55} = 2.995$; $P < 0.01$).

Therefore, Kruskal–Wallis one-way non-parametric analysis of variance (ANOVA) was used to compare sporocarp abundance and dry weight across both vegetation type and season. Firstly, the effect of vegetation type was tested within each season separately. If vegetation type consistently had no significant effect on sporocarp abundance or dry weight then data from all vegetation types were pooled and a further Kruskal–Wallis ANOVA performed to test for between season effects. When data could not be pooled because of vegetation effects within seasons, separate tests of seasonal effects were performed for each vegetation type. When significant seasonal effects were obtained from any analysis, post-hoc pair-wise Mann–Whitney tests among seasonal categories were performed (with Bonferroni corrections) to identify which pairs of factors caused the significance. $\chi^2$ goodness of fit tests were used to compare sporocarp abundance and cockatoo grass use per hectare between seasons.

## 2.3 Results

### *2.3.1 Rainfall patterns*

Despite above average rainfall during both wet seasons, the overall seasonal pattern during 2004/2005 represents the typical rainfall pattern expected for this region (Figure 2-1). Rainfall was high in March, petered out between April and July 2004 and was almost absent in August and September (Figure 2-1). The wet season rains began in October 2004 and continued to April 2005 (Figure 2-1). The annual rainfall of 1861.5mm (March 2004–March 2005) was higher than the mean annual rainfall of 1274.3mm measured over a 74-year period. This above average rainfall was recorded during the wet season months of March 2004, December and January 2005 (Figure 2-1).

The geographically closest weather station Mareeba, although more arid than the study site, reported the same pattern of seasonal rainfall as the Kairi station. In contrast, during the study period of a previous project (Vernes *et al.* 2001) an aseasonal rainfall pattern was observed (Figure 2-1). Above average rainfall occurred during the dry season month of August 1995 while during both wet seasons, rainfall was below average (Figure 2-1).

### *2.3.2 Resource quantity*

No significant differences in sporocarp abundance were observed between vegetation types within seasons (Kruskal–Wallis: early wet $\chi^2_2$ = 0:480; P = 0.787; late wet $\chi^2_1$ = 1:265; P = 0.261; early dry $\chi^2_2$ = 1:473; P = 0.479; late dry $\chi^2_2$ = 2:224; P = 0.326). When vegetation types were pooled the abundance of sporocarps was significantly different between seasons (Kruskal–Wallis: $\chi^2_3$ = 30:5; P < 0.0001). The lowest ranking season was the late dry season. After this category was excluded there was no significant difference in sporocarp abundance between seasons (Kruskal–Wallis: $\chi^2_2$ = 2:229; P = 0.328). Posthoc pair-wise comparisons confirmed that the late dry season had a significantly lower number of sporocarps compared to all other seasons (Figure 2-2), while sporocarp numbers among the other three seasons did not differ significantly.

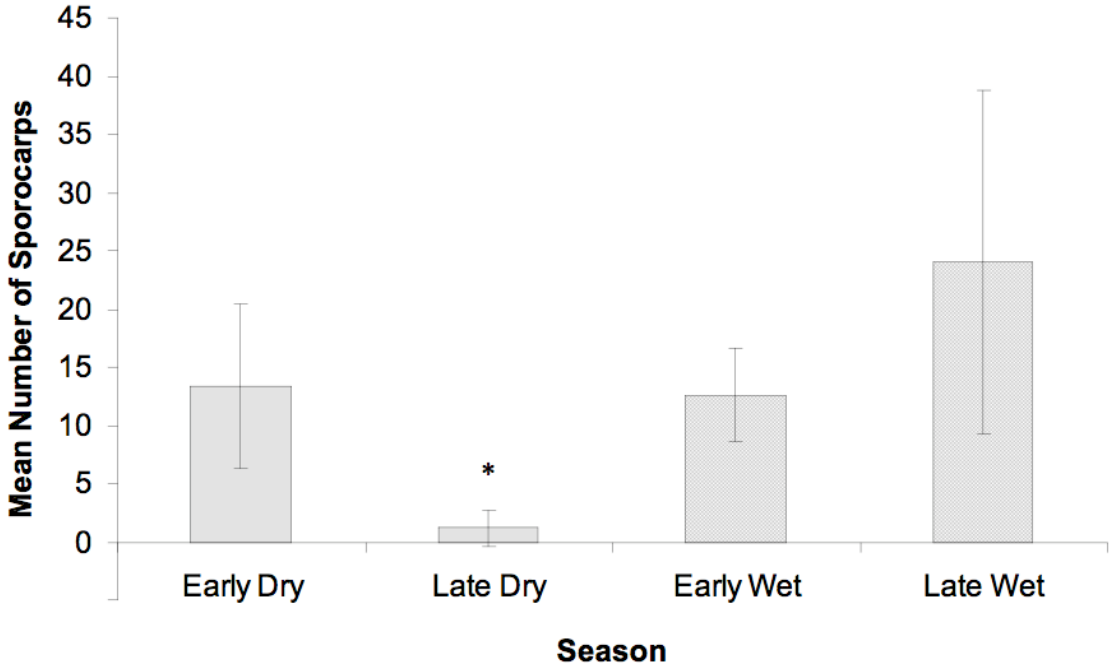


**Figure 2-2:** The mean (± 2 S.E.) number of sporocarps sampled in the early dry, late dry, early wet and late wet seasons 2004/5. * Significant difference

### *2.3.3 Resource biomass*

Significant differences in sporocarp biomass were observed between vegetation types within seasons. The biomass of sporocarps was significantly lower during the late dry season for the *Eucalyptus* woodland (Kruskal–Wallis: $\chi^2_3$ = 10:773; P = 0.013) and *Allocasuarina* forest (Kruskal–Wallis: $\chi^2_3$ = 14:505; P = 0.002) (Figure 2-3). Although the late dry season also ranked the lowest for the wet sclerophyll vegetation type, there was no significant difference in dry weight of sporocarps between seasons (Kruskal–Wallis: $\chi^2_3$ = 4:725; P = 0.094) (Figure 2-3).

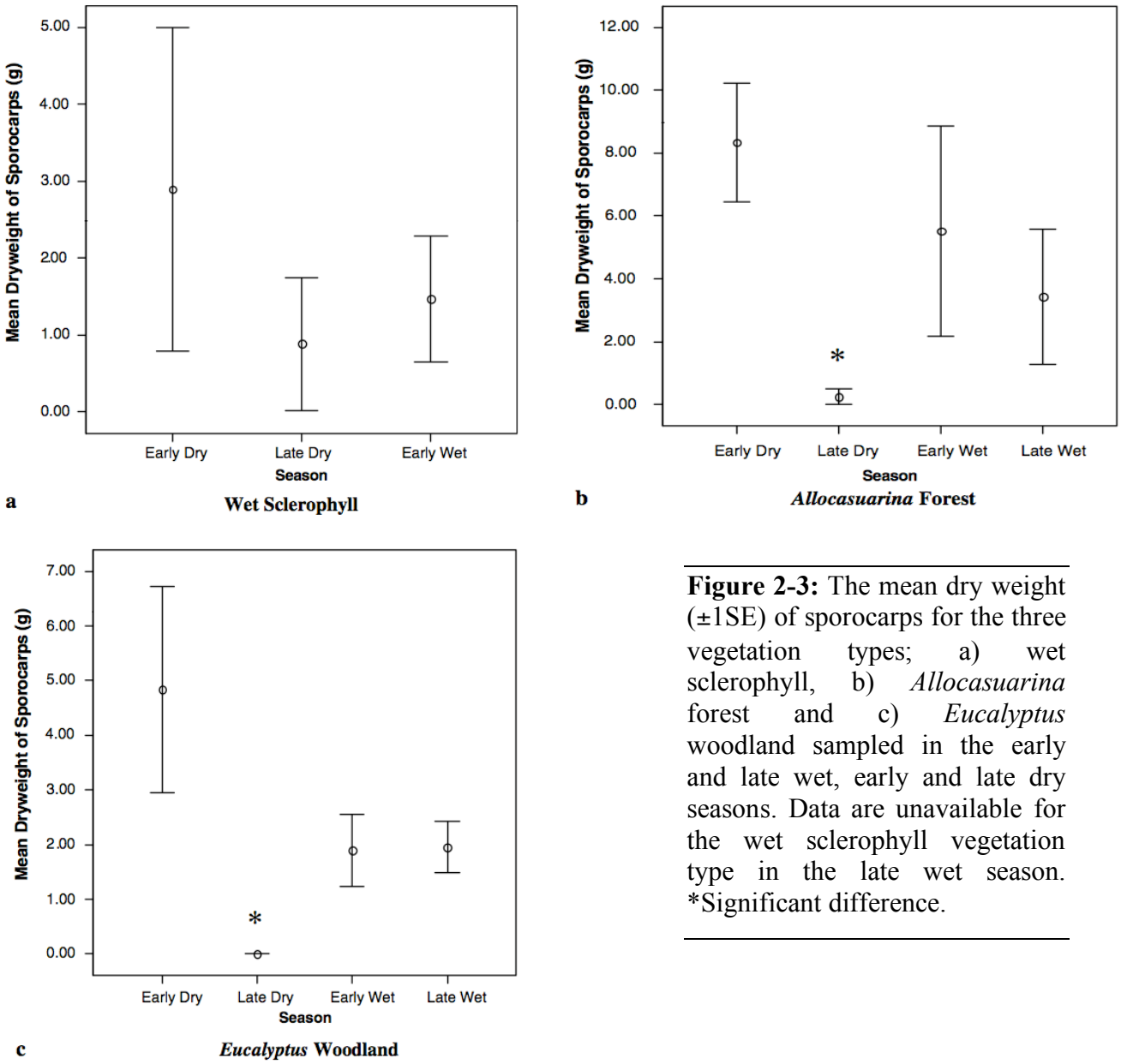


**Figure 2-3:** The mean dry weight (±1SE) of sporocarps for the three vegetation types; a) wet sclerophyll, b) *Allocasuarina* forest and c) *Eucalyptus* woodland sampled in the early and late wet, early and late dry seasons. Data are unavailable for the wet sclerophyll vegetation type in the late wet season. *Significant difference.

### *2.3.4 Resource availability and use*

There was also significant seasonality of total sporocarp numbers per hectare ($\chi^2$ = 207.298; df = 3; P < 0.001). The abundance of sporocarps per hectare was relatively stable during the early dry (145.83 $ha^{-1}$), early wet (137.5 $ha^{-1}$) and late wet (240 $ha^{-1}$) but dropped considerably to 6.67 $ha^{-1}$ in the late dry season (Figure 2-4).

This low abundance occurred after a three to four month period of minimal rain (May to August) that included a period of almost no rainfall one month prior to sampling (August) (Figure 2-1). The peak annual production of sporocarps (240 $ha^{-1}$) occurred in the late wet season (Figure 2-4) after extensive monsoonal rainfall (Figure 2-1). This contrasts with the peak use of *A. semialata* by *B. tropica*, which occurred in the late dry season (62.22 $ha^{-1}$) during the trough of fungal abundance (6.67 $ha^{-1}$) (Figure 2-4). The lowest use of *A. semialata* occurred during the early wet season when no chewed grass pellets were observed (Figure 2-4). *A. semialata* was used at a relatively low level (4.15 $ha^{-1}$) during the late wet season at the time of hypogeous fungi peak abundance (240 $ha^{-1}$) (Figure 2-4). Flowering culms of *A. semialata* were only observed during the early wet season sampling trip at a time when no grass pellets were present (Figure 2-4).

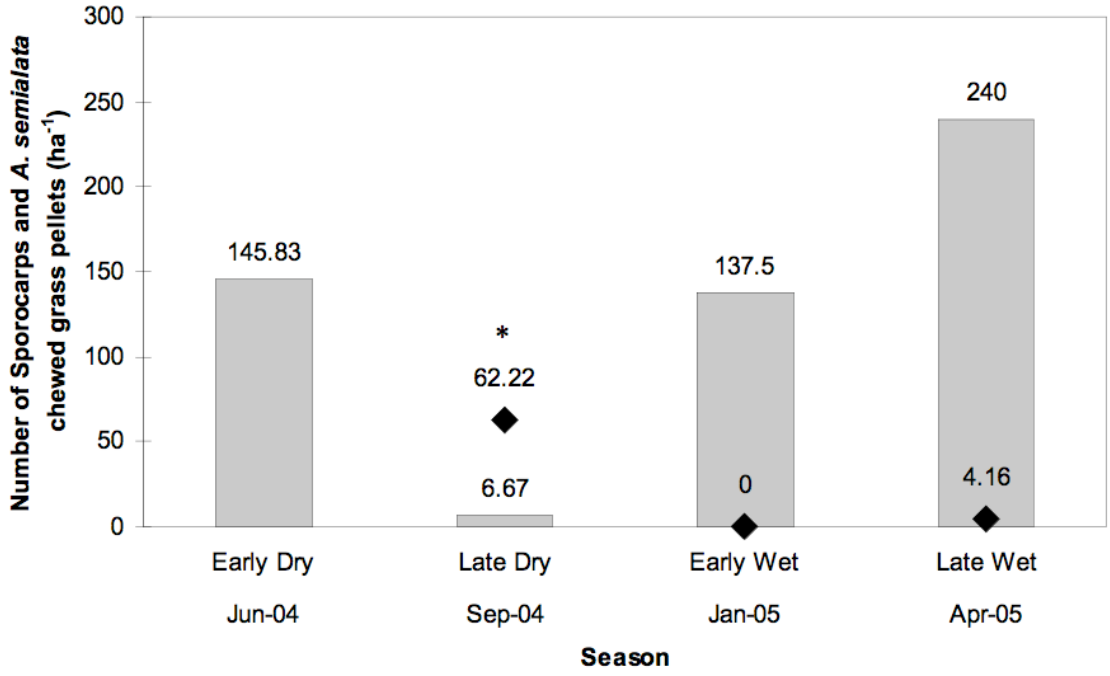


**Figure 2-4:** The number of sporocarps of hypogeous fungi (grey columns) and chewed grass pellets of *Alloteropsis semialata* (♦) sampled per hectare in four seasons, 2004/2005. Data are unavailable for *A. semialata* in the early dry season. *Significant difference.

Although not quantified, the number of grass pellets observed in the early dry season was clearly lower in comparison to the late dry season.

## 2.4 Discussion

### *2.4.1 Seasonality of tropical hypogeous fungi*

This is the first study to document an overall seasonal shortage of sporocarp production in Australian hypogeous fungi. Sporocarp abundance differed little between seasons except the late dry season when both the total and median abundance of sporocarps dropped to low levels. This appears to be directly correlated with the seasonal rainfall pattern experienced during the study period.

There have been two other published Australian surveys of hypogeous fungi at a seasonal scale, independent of those examining fungi consumed by mammals (Claridge *et al.* 1993; Johnson 1994a). Both studies were conducted in temperate ecosystems with relatively uniform annual rainfall. In those studies a positive relationship between sporocarp density and rainfall was documented for particular taxa including *Mesophellia* and *Hysterangium* (Johnson 1994a). However, the production of sporocarps was aseasonal, mirroring the aseasonal temperate rainfall (Claridge *et al.* 1993; Johnson 1994a). While different taxa may have particular soil-moisture preferences, it is possible that there is a lower threshold of soil moisture availability that limits the production of sporocarps. This appears to be the case for the hypogeous fungi observed. Fruiting intensity of hypogeous fungi is directly related to the supply of carbohydrates obtainable from the host (Maser *et al.* 1978; Vernes *et al.* 2001). Therefore, during the annual winter drought, production of sporocarps may be restricted because host plants have higher carbohydrate demands for their own growth and/or reproduction (Hatch 1937; Krueger and Trappe 1967).

The results of this chapter, suggesting that tropical sporocarp abundance tracks rainfall patterns, may explain disparities in the results obtained in two previous studies of *B. tropica*. The proportion of fungi in the diet of *B. tropica* as determined by Vernes (2001) did not vary with season while some seasonal variation was observed in the study of Johnson (1997). Record rainfall was experienced during the normally dry month of August in 1995 (Figure 2-1), suggesting relatively even precipitation across the seasons may have contributed to the lack of seasonal variation observed by Vernes in 1995 (Vernes *et al.* 2001).

In Tasmania, *B. gaimardi* body condition was correlated with the proportion of fungus in the diet (Johnson 1994a, b), despite the lack of an overall relationship between rainfall and sporocarp production. This is in contrast to the seasonally uniform body condition of *B. tropica* despite current and previous evidence of seasonality of resources (Johnson and McIlwee 1997). Our findings suggest that in most years *B. tropica* will be unable to rely on hypogeous fungi as their primary food resource for up to three months during the dry season. That *B. tropica* consistently maintain body condition year round means that individuals must access an alternative food source capable of replenishing body reserves during the dry period.

### *2.4.2 Substitutable resource*

Secondary resources of other potoroid species include fruits and seeds (*B. gaimardi*) or insects (*Potorous tridactylus*). Grasses and lilies are the second most abundant resource in the diet of *B. tropica* with the protein and carbohydrate rich shoot base of *A. semialata* thought to be an important component (Johnson and McIlwee 1997).

Our results further support these findings, suggesting that *B. tropica* seasonally switch to using *A. semialata* grass bases subject to hypogeous fungi availability (Figure 2-4).

*A. semialata* is a perennial C4 tropical to subtropical grass species that flowers almost immediately after the first rains of the wet season (Crowley and Garnett 2001). *Bettongia tropica* selectively feeds on the base of this grass, which is a storage organ. The function of the storage organ is to allow the plant to persist throughout the dry season and possibly, to allow it to flower early in the wet season, using stored nutrients and carbohydrates. This means that the nutritional status of *A. semialata* could be expected to change throughout the year, being low during the early wet season but peaking during the mid to late dry season. This is consistent with the pattern of *A. semialata* use by *B. tropica*. *Alloteropsis semialata* was not used during the early wet season even though hypogeous fungi were not at peak production. In contrast, when hypogeous fungi were at peak availability in the late wet season *A. semialata* was also used, although at low levels compared to use during the late dry season. This suggests that while *B. tropica* may preferentially use fungi, they do not ignore an available, potentially high quality, resource.

### *2.4.3 Habitat preference of B. tropica*

Previous authors have argued that *B. tropica* distribute themselves across the gradient of fungal abundance in accordance with an ‘‘ideal-free’’ theoretical model of distribution (Johnson and McIlwee 1997). This relationship has been used to explain their preference for *Eucalyptus* woodland as determined by Winter (1997) and Laurance (1997). In tropical ecosystems as aridity increases the seasonality of rainfall increases and the mean annual rainfall decreases.

Therefore, as aridity increases the time period that sporocarps are at critically low levels or unavailable to *B. tropica* would increase. Thus, the seasonality of hypogeous fungi could explain the habitat preference at the wetter end of the rainfall gradient and the restriction at the dryer end of this gradient. However, it does not entirely explain their habitat restriction at the wet end of the gradient.

The *Eucalyptus* woodland that is preferred by *B. tropica* does not lie on the very edge of the rainforest; at least two other vegetation types intervene, including ectomycorrhizal *Allocasuarina* and wet sclerophyll forest. There was no significant difference found in sporocarp abundance between these three different forest types in the first year of this study. If the northern bettong was purely restricted to habitat that supports a high abundance and low seasonality of fungus, all three forest types should support equivalent bettong populations. However, although the abundance of the fungal resource did not differ between the forest types the biomass of this resource did vary. The dry weight of sporocarps in the wet sclerophyll forest remained at low levels throughout the year and did not show the same seasonal increase during the wetter months (Figure 2-3). The consistent low biomass of this resource regardless of season may, in part, explain why wet sclerophyll is not utilised by *B. tropica*.

The wet sclerophyll and *Allocasuarina* forests also have a sedge (not grass), dominated understorey. *Alloteropsis semialata* is found in higher abundance within *Eucalyptus* woodland compared to *Allocasuarina* forest (Vernes 2003). This is possibly related to the increasing canopy coverage of *Allocasuarina* providing less light to support grass species in the understorey and/or allelopathy.

Therefore, *B. tropica's* apparent preference for *Eucalyptus* woodland over the other two forest types may also be explained, in part, by the distribution of *A. semialata*. This suggests that while decreasing hypogeous fungi abundance may restrict the distribution of *B. tropica* at the dry end of the rainfall gradient, the need to utilise *A. semialata* at specific times of the year may further restrict this species distribution at the wet end of this gradient. This identifies *B. tropica* as a true ecotonal specialist with a habitat preference for *Eucalyptus* woodland that is associated with rainforest margins and other transitional forest types between the rainforest to dry sclerophyll gradient.

# Chapter Three: Reproductive Cues And Strategies Of Tropical Hypogeous Fungi

## 3.1 Introduction

The nutrients and water made available by mycorrhizal fungi facilitate the acquisition of carbohydrates through photosynthesis by their plant hosts. In return sporocarp production is supported by a current photosynthate supply, between 30 and 60% of the net photosynthesis rate of their plant hosts (Norton *et al.* 1990; Simard *et al.* 1997a). Photosynthesis rate is determined by prevailing environmental conditions, including precipitation, temperature and sunlight availability (Clark 2004; Nara *et al.* 2003; Tester *et al.* 1986). Seasonal variability in rainfall is the most significant factor in determining primary productivity within tropical ecosystems (Schloss *et al.* 1999). The same environmental factors affecting mycorrhizal plant host productivity are expected to contribute to mycorrhizal fungal productivity, including reproduction.

Hypogeous (fruiting below-ground) fungi are representative of a diverse polyphyletic group within the kingdom fungi (Bougher and Lebel 2001). They directly contribute to all trophic levels, through their role in ectomycorrhizal nutrient cycling and availability as a resource to both generalist and often endangered specialist mycophagists. Fluctuations in richness, abundance and biomass for hypogeous fungi have been observed in many studies.

In temperate ecosystems, peaks and troughs in richness, abundance and biomass of hypogeous fungal sporocarps are thought to be a function of season, relating to an interaction between the availability of moisture and critical temperature thresholds (Beaton *et al.* 1984; Claridge *et al.* 1993; Fogel 1976; Harvey *et al.* 1978; Hunt and Trappe 1987; Johnson 1994a). Moisture may be the most important driver for reproduction of hypogeous fungi. In an Australian temperate region study, almost all variables explaining species diversity could be attributed to moisture availability (Claridge *et al.* 2000a).

At the landscape scale, diversity of hypogeous fungi was lower in sandy, high drainage soils compared to soils with more structure, less drainage and hence less moisture variability (Claridge *et al.* 2000a). At a more localized scale, diversity was higher in wetter sheltered slopes and gullies, while dryer exposed slopes and ridges reduced species diversity (Claridge *et al.* 2000a; Claridge *et al.* 1993). Individual hypogeous fungal taxa appear to respond either positively or negatively to increased moisture availability (Claridge *et al.* 1993; Johnson 1994a). These patterns are also found in temperate ecosystems on other continents (Fogel 1976; Harvey *et al.* 1978; Hunt and Trappe 1987). Despite the connection between plant and fungus productivity, to our knowledge, no study has shown a direct relationship at the community level between precipitation and species richness, abundance or biomass of hypogeous fungi.

All of the ecological studies on hypogeous fungi have been conducted in temperate ecosystems, where the effect of multiple environmental variables may interact with or mask direct correlations. Temperate region climate is generally characterized by low interannual fluctuations in precipitation and high fluctuations in temperature, relative to the tropical climate.

Therefore, the effect of precipitation on fungal productivity may not be as complicated by the time of year (season) in the tropics, brought about by an interaction with temperature.

The aim of this chapter is to determine the reproductive cues of ectomycorrhizal hypogeous fungi as well as the effect of season and/or climate on species richness and community assemblage within a tropical ecosystem. Due to previous evidence showing a lower threshold (correlated with low levels of precipitation) of hypogeous sporocarp production during the first year of sampling (Abell *et al.* 2006) I hypothesise that precipitation not temperature will be the main factor influencing fruiting. Increased rainfall in the second year will allow this hypothesis to be tested. If precipitation does have an effect on hypogeous fungal abundance regardless of the time of year, then fungal diversity may also increase with precipitation levels.

Trends in temperate studies suggest there may be distinct community assemblages attributed to the morphology of the fruiting body (Claridge *et al.* 2000b; Claridge *et al.* 1993; Johnson 1994a), or to the time of year (autumn or spring) (Claridge *et al.* 2000b; Fogel 1976; Johnson 1994a; Luoma *et al.* 1991). Therefore, the proportion of the community that responds to season and/or precipitation will also be closely examined.

## 3.2 Methods

### *3.2.1 Sample Design*

The study location was at Davies Creek on the Lamb Range in the Wet Tropics World Heritage Area of far North Queensland, Australia (145°35'E, 17°01'S).

Surveys were undertaken in June and September in 2004, January, April and September in 2005 as well as January and May 2006. These sampling sessions were assigned to the following four seasons: Early-Dry (June), Late-Dry (September), Early-Wet (January) and Late-Wet (April and May) (Figure 3-1). These tropical seasons correspond to the temperate region seasons of winter, spring, summer and autumn respectively. A description of the sample design including how the fungal survey sites were selected, detailed site vegetation descriptions as well as the hypogeous fungal sampling protocol are provided in a previous publication (Abell *et al.* 2006).

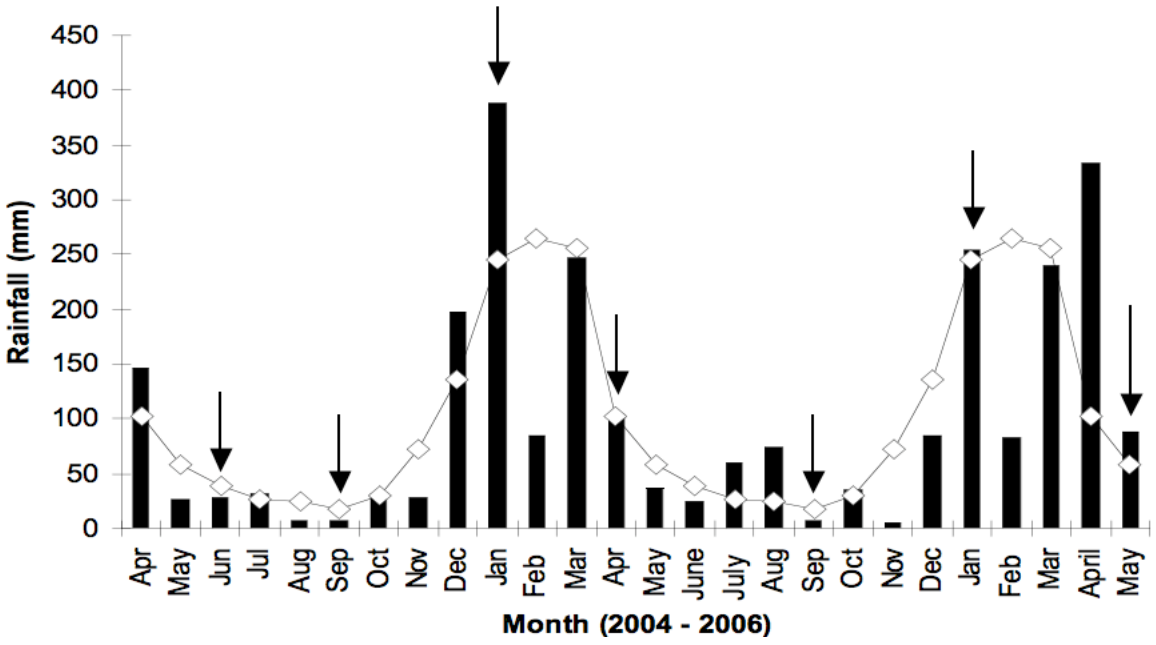


**Figure 3-1:** The monthly rainfall (black columns) measured at Kairi weather station for the study period (2004 – 2006), superimposed with the expected (◊) seasonal pattern of rainfall (74-year monthly means). Vertical arrows indicate collection dates.

### *3.2.2 Environmental data*

Climate data including precipitation, sunshine hours, cloud cover, maximum and minimum temperature were obtained for each survey date from the climatically closest weather station at Kairi (altitude 700m ASL), 22 km south of the study area. The Australian Bureau of Meteorology provided precipitation data for the 2004 – 2006 period as well as the 74-year means (Figure 3-1). Soil moisture was determined by collecting two sealed bags of topsoil 5m up-slope and 5m down-slope from the centre of each quadrat at the time of survey. A wet weight was obtained on return to the laboratory, the soil was dried and a dry weight was recorded. The difference between the wet weight and dry weight was calculated and converted to the mean soil moisture for each site in mL/g.

Two major climatic events occurred prior to the last survey (May 2006) with the passage of two severe tropical cyclones south (Cyclone Larry; Cat. 5; March 2006), then north (Cyclone Monica; Cat. 3; April 2006) of the study area. The extreme precipitation was more than three times the average for the month of April 2006 (Figure 1). Major structural damage to the vegetation was not observed within the survey sites. However, it is expected that a certain level of leaf loss of the host plants would have occurred in the high winds experienced during the cyclones.

### *3.2.3 Assessment of fungal diversity*

All sporocarps were photographed, measured and described in their fresh state prior to drying at low temperature overnight in a food dehydrator. Spores were also examined, photographed and included in a photographic reference library. Initially the reference library was used to group the collection into broad morphological groups.

Morphological groups were subsequently identified to genera (Appendix One) with the assistance of expert taxonomists Dr Teresa Lebel of the Royal Botanic Gardens Melbourne Australia, as well as Dr James Trappe of the Department of Forest Science Oregon State University Corvallis, USA.

Species identifications were carried out using current published keys as well as unpublished preliminary keys provided by and with the guidance, where possible, of both Dr Teresa Lebel and Dr James Trappe. Identification to species was deliberately conservative and performed first by using spore shape, size, ornamentation and reaction to chemicals (Meltzers reagent and KOH), while fresh and dried sporocarp characters were used only to add further weight to the groupings (Appendix Two). Identified species have been lodged in the Queensland Plant Pathology Herbarium (BRIP), Department of Primary Industries, with duplicates in the Australian Tropical Herbarium (ATH), James Cook University, Cairns. Undescribed/unknown species are currently awaiting description elsewhere and/or final identification, and will be deposited at the appropriate time.

### *3.2.4 Data Analysis*

The data obtained for mean sporocarp abundance, biomass and sporocarp size (biomass/abundance) as well as genera and species number did not conform to expectations of normality due to large numbers of zero observations during particular surveys (Levene's equality of variances: $F_{3, 116} = 4.060$; $P < 0.01$; $F_{3, 116} = 4.652$; $P < 0.01$; $F_{3, 110} = 4.197$; $P < 0.001$; $F_{1, 118} = 0.270$; $P < 0.01$ and $F_{1, 118} = 3{,}985$; $P < 0.05$ respectively).

Therefore, Kruskal–Wallis one-way non-parametric analysis of variance (ANOVA) was used to compare mean sporocarp abundance, biomass and sporocarp size across season. When comparing frequency of genera and species occurrence, Pearson one-way non-parametric chi square ($\chi^2$) analyses were used.

Available climate variables (sunshine hours, cloud cover, max/min and 9am temperature and precipitation) were examined initially using scatter-plots at different time scales (eg. 2 weeks, 1 month, 2 months) prior to and/or including the survey month. To correct for normality, data were transformed to means prior to regression analyses. The time scale with the largest and most significant linear regression $r^2$ values were used in subsequent regression analyses. Linear regression analyses were performed, for the climate variable (x) and mean sporocarp abundance (y), with the last survey outlier (May 2006) included then excluded for abundance and all surveys included for biomass and species number.

The species richness (S) was compared to the Shannon-Weiner Richness Index (*H'*) for the wet and dry seasons. Non-parametric diversity indices were also calculated using the EstimateS software (Version 7.5, R. K. Colwell, http://purl.oclc.org/estimates). Both the standard *Chao1* (Chao 1984) and also *ACE* indices were computed (Chao and Lee 1992; Chao *et al.* 1993). *ACE* provides a more conservative species diversity prediction in comparison to *Chao1* by taking into account the occurrence of singletons and doubletons (Chao 1984, 1987; Chao *et al.* 1992; Chao *et al.* 1993; Lee and Chao 1994). The following equation was used to determine the completeness of the sample: Estimated sampling completeness = observed species numbers x 100/ estimated species numbers (*Chao1* and *ACE*).

After detecting that abundance and biomass of sporocarps did not have the same pattern after extreme rainfall, two possible responses were predicted, either a) all species decreased in abundance but increased in biomass or b) a differential species response occurred. This was examined by comparing the change in biomass before and after the extreme rainfall event. Three potential species groups were predicted those that i) increased in abundance and biomass (increasers), ii) decreased in abundance and biomass (decreasers) and iii) did not change in biomass or abundance (stable). The data set was reduced to those species that were surveyed a minimum of three times including the extreme rainfall survey (14 species). To distinguish between the groups a standard K-means cluster analysis was performed. The parameters used to distinguish between groups were the change in biomass and abundance between "normal" precipitation surveys (under 400mm) and "extreme" rainfall surveys (above 400mm). A discriminant function analysis (DFA) was then performed to determine the validity of those groupings. The assumptions of DFA including a multivariate normal distribution and equal covariance are not fatal to the analysis as long as outliers are not observed in the data (Tabachnick and Fidell 1996). The data in this analysis although non-normal were skewed but did not contain outliers.

## 3.3 Results

### *3.3.1 Precipitation and abundance*

There was a significant effect of season on abundance (Kruskal-Wallis: $\chi^2_3 = 19.832;\ P < 0.001$). The lowest ranking season overall was the Late Dry Season (Figure 3-2). When this category was removed there was no significant effect of season found (Kruskal-Wallis: $\chi^2_2 = 3.719;\ P = 0.156$).

Post-hoc pairwise comparisons confirmed that the Late Dry Season had significantly less sporocarps compared to the other seasons (Figure 3-2).

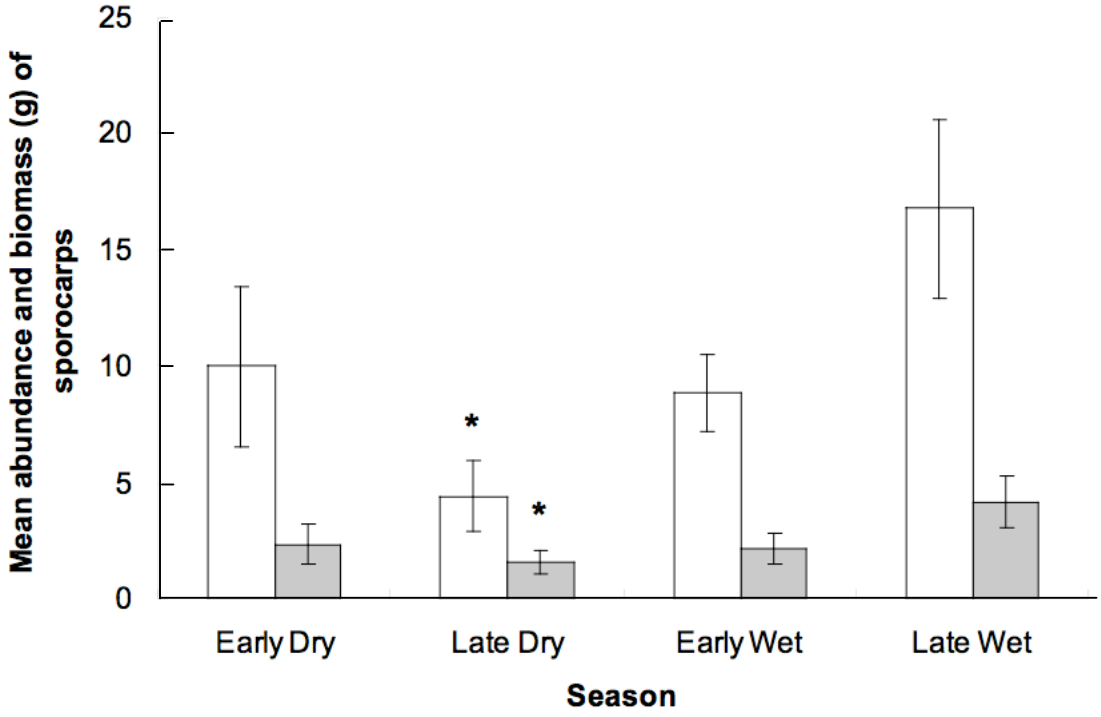


**Figure 3-2:** The mean (±1SE) sporocarp abundance (white columns) and biomass (g) (grey columns) surveyed in the Early-Wet, Late-Wet, Early-Dry and Late-Dry seasons 2004 to 2006. * Significant difference

There was no effect of soil moisture on the mean abundance of sporocarps *($r^2$ = 0.384; P = 0.137; linear regression)*. When the last survey was included, there were no significant linear correlations between the mean abundance of hypogeous sporocarps and precipitation (mm) two months prior *($r^2$ = 0.401; P = 0.127; linear regression),* at any other time scale, or with any other large-scale climate variable measured. When the last survey outlier (May 2006) was excluded from the analysis there was a highly significant positive linear correlation *(y = -2.051 + (0.072 * x), $r^2$ = 0.984; P = 0.001)* between the mean sporocarp abundance (y) and precipitation (x) two months prior (Figure 3-3). There were no significant linear correlations between any other large-scale climate variables or with soil moisture when the last survey was excluded.

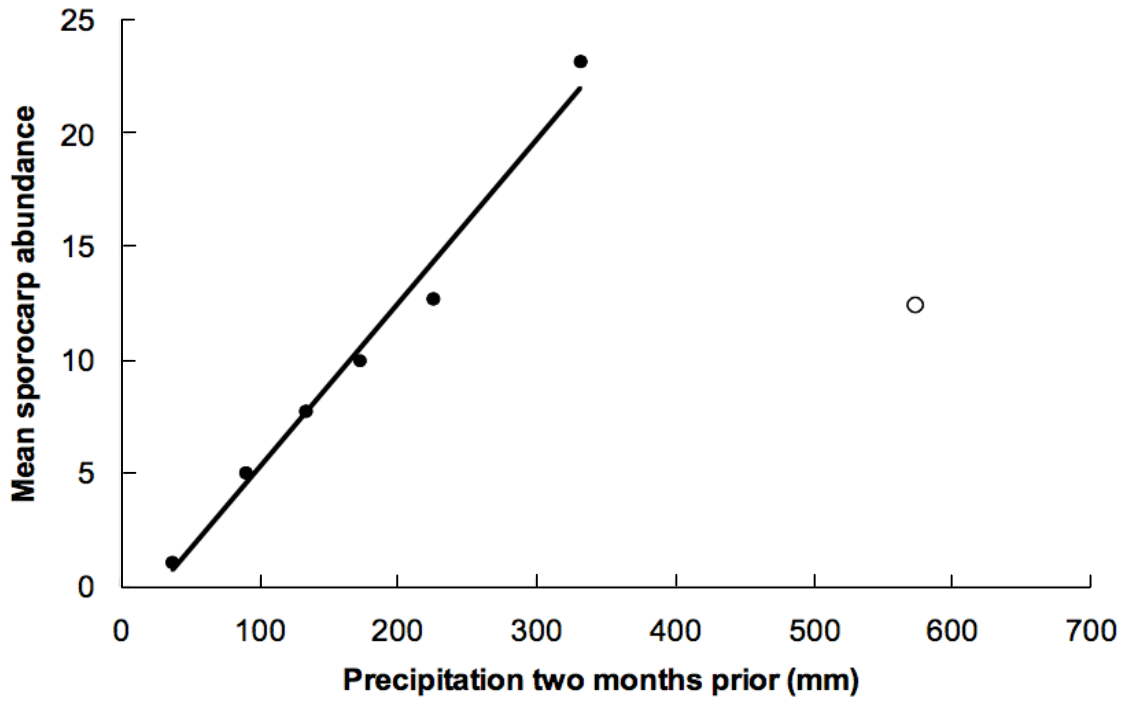


**Figure 3-3:** The positive linear relationship between the mean sporocarp abundance and precipitation two months prior to the survey with the May 2006 (empty circle) outlier excluded from the regression.

### *3.3.2 Precipitation and biomass*

There was a significant effect of season on biomass (Kruskal-Wallis: $\chi^2_3$ = 12.905; P < 0.01). The lowest ranking season was the Late Dry Season (Figure 3-2). When this category was removed there was no significant effect of season found (Kruskal-Wallis: $\chi^2_2$ = 1.975; P = 0.372). Post-hoc pairwise comparisons confirmed that the Late Dry Season had significantly less biomass compared to the other seasons (Figure 3-2).

There was no effect of soil moisture on the mean biomass of sporocarps ($r^2$ = 0.480; P = 0.084; linear regression). When all surveys were included, there was a highly significant positive linear correlation (y = 0.842 + (0.007 * x), $r^2$ = 0.842; P = 0.01) between the mean sporocarp biomass (y) and precipitation (x) two months prior (Figure 3-4). A number of positive increasing curve models were also found to significantly explain the relationship between these variables (Logarithmic, $r^2$ = 0.849, P < 0.01; Cubic, $r^2$ = 0.976, P < 0.01; S, $r^2$ = 0.948, P < 0.001).

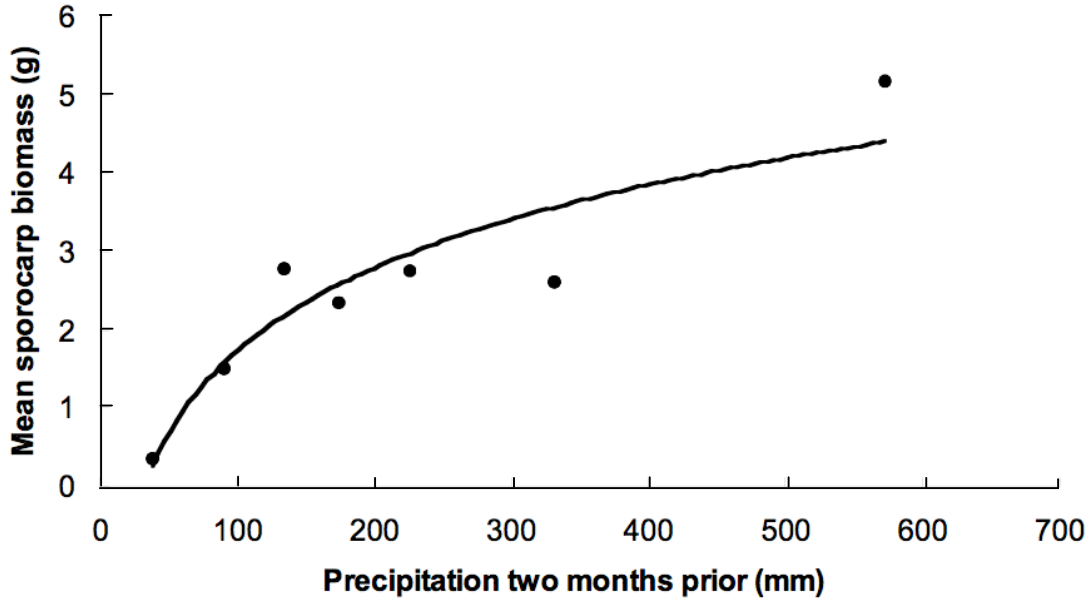


**Figure 3-4:** The positive logarithmic relationship between the mean sporocarp biomass and precipitation two months prior.

### *3.3.3 Precipitation and richness*

There was no effect of soil moisture on the number of fungal species *($r^2$ = 0.548; P = 0.057; linear regression)*. When all surveys were included, there were no significant positive linear correlations *($r^2$ = 0.399; P = 0.128: linear regression)* between the number of fungal species (y) and precipitation (x). Three positive increasing curve models were found to significantly explain the relationship between these variables (*Logarithmic, $r^2$ = 0.757, P < 0.05; Cubic, $r^2$ = 0.967, P < 0.05; S, $r^2$ 0.959, P < 0.001*; Figure 3-5).

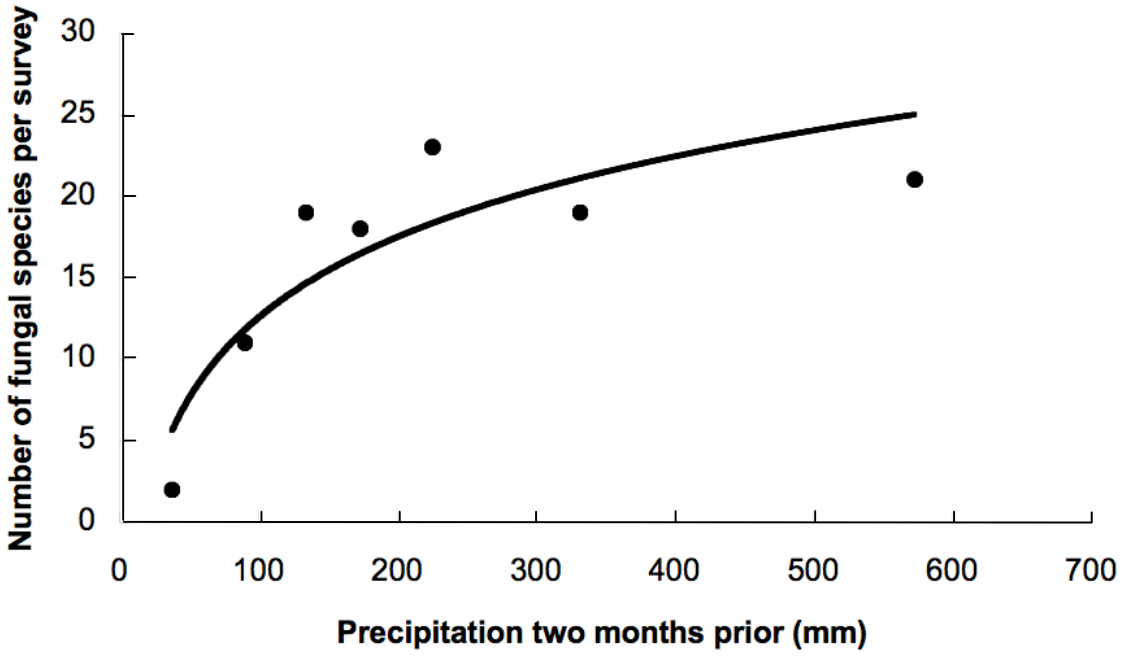


**Figure 3-5:** The positive logarithmic relationship between the number of hypogeous fungal species surveyed and precipitation two months prior.

### *3.3.4 Taxonomic assemblage*

A total of 25 genera and 49 species of hypogeous fungi were recorded from 1161 occurrences across the 18 sites (six per vegetation type), and seven surveys (six for the WSF) (Table 3-1). Observations of species richness (S) indicated that there were 29 species from 339 occurrences in the Dry Season (Early and Late Dry combined) and 40 species from 822 occurrences in the Wet Season (Early and Late Wet combined) (Table 3-1). The Shannon-Wiener diversity index was higher for the Wet season (3.31) compared to the Dry season (3.12) (Table 3-1). The Chao1 and ACE indices were also higher in the wet season (53.5; 48.69) compared to the dry season (33.08; 35.91) (Table 3-1). With both seasons pooled the Shannon-Wiener diversity index was 3.05, Chao1 was 53.9 and ACE was 53.25 (Table 3-1). The Chao1 completeness of the dry season (87.67%) was higher than the wet season (74.77%) survey (Table 3-1). Using the more conservative ACE index, the completeness of the surveys were almost equivalent for the wet (80.76%) and dry (82.15%) seasons (Table 3-1).

**Table 3-1:** Diversity of hypogeous sporocarps in the dry (early and late pooled) and wet (early and late pooled) seasons.

| Season | Abundance | S[a] | Chao1[b] | Chao1 95% CL | Chao1 Completeness (%) | ACE[c] | ACE Completeness (%) | H'[d] |
|---|---|---|---|---|---|---|---|---|
| Dry | 339 | 29 | 33.08 | (29.8, 49.66) | 87.67 | 35.91 | 80.76 | 2.49 |
| Wet | 822 | 40 | 53.5 | 42.89, 102.88 | 74.77 | 48.69 | 82.15 | 2.92 |
| Total | 1161 | 49 | 53.9 | (49.97, 73.69) | 90.91 | 53.25 | 92.02 | 3.05 |

a S refers to observed species richness.

b Species richness estimate according to Chao (1987) based on abundance data. Numbers in brackets refer to 95% confidence intervals.

c Species richness estimate correcting for effect of singletons according to Chao and Lee (1992) based on abundance data.

d H' refers to Shannon-Wiener diversity index.

A total of twenty-one genera were collected in the wet season compared to seventeen in the dry season (Table 3-2). Of the genera that were collected in the wet season, thirteen (61.90%) were also collected in the dry season surveys (Table 3-2). The remaining eight genera (38.10%) were only collected during the wet season surveys (Table 3-2). Of the genera collected in the dry season, thirteen (76.47%) were also collected in the wet season (Table 3-2). In comparison to the wet season there was a lower (4; 23.53%) proportion of genera unique to the dry season surveys (Table 3-2). Overall there was no significant difference between the number of shared or unique genera between the seasons (Pearson: $\chi^2_1 = 1.043;\ P = 0.307$; Table 3-2).

A total of forty species were collected in the wet season surveys compared to twenty-nine species in the dry season (Table 3-2). Twenty (50%) of these species were only collected in the wet season (Table 3-2). The remaining twenty (50%), species were collected in both the wet and dry seasons (Table 3-2). Of the species collected in the dry season, twenty (68.95%) were also collected during the wet season surveys (Table 3-2). There was a lower (9; 31.03%) proportion of species that were unique to the dry season surveys (Table 3-2). However, the number of species that were unique or shared between the wet and dry seasons was not significantly different (Pearson: $\chi^2_1 = 2.482;\ P = 0.115$; Table 3-2).

**Table 3-2:** The total list of species surveyed, indicating the genera and species unique (*u*) or shared (*s*) between the wet and dry seasons.

| Species List | | Dry | Wet |
|---|---|---|---|
| *Aroramyces* | *spA* | *s* | *s* |
| *Castoreum* | *infimiratio* | *u* | |
| *Castoreum* | *sublaeve* | *u* | |
| *Castoreum* | *tasmanicum* | | *u* |
| *Chamonixia* | *vittatispora* | | *u* |
| *Chondrogaster* | *spA* | *s* | *s* |
| *Chondrogaster* | *spB* | *s* | *s* |
| *Chondrogaster* | *spC* | | *u* |
| *Chondrogaster* | *spD* | *u* | |
| *Chondrogaster* | *spF* | | *u* |
| *Chondrogaster pac* | *spE* | | *u* |
| *Dingleya* | *spA* | *u* | |
| *Dingleya* | *spB* | *u* | |
| *Endogone* | *spA* | *s* | *s* |
| *Endogone* | *spB* | | *u* |
| *Gautieria* | *amara* | *s* | *s* |
| *Gelopellis* | *cf thaxteri* | | *u* |
| *Geogyroporous* | *spA* | | *u* |
| *Glomus* | *spB* | | *u* |
| *Glomus* | *spC* | | *u* |
| *Gummiglobus* | *joyceae* | *s* | *s* |
| *Gummiglobus* | *spB* | *s* | *s* |
| *Gymnomyces* | *eildonensis* | | *u* |
| *Hysterangium* | *spA* | | *u* |
| *Hysterangium* | *spB* | | *u* |
| *Hysterangium* | *affine* | | *u* |
| *Hysterangium* | *aggregatum* | *s* | *s* |
| *Hysterangium* | *cf gardneri* | *s* | *s* |
| *Hysterangium* | *inflatum* | *s* | *s* |
| *Hysterangium* | *spC* | *s* | *s* |
| *Hysterangium* | *spD* | | *u* |
| *Macowanites* | *spC* | | *u* |
| *Labyrinthomyces* | *spA cf varius* | *u* | |
| *Malajczukia* | *ingrattissima* | *u* | |
| *Mesophellia* | *clelandii* | *u* | |
| *Mesophellia* | *glauca* | *s* | *s* |
| *Mesophellia* | *oleifera* | *s* | *s* |
| *Mycoamaranthus* | *auriorbis* | *s* | *s* |
| *Octaviania* | *spA* | *u* | |
| *Pogisperma* | *spA* | | *u* |
| *Pogisperma* | *spB* | | *u* |
| *Royoungia* | *boletoides* | | *u* |
| *Scleroderma* | *bougheri* | *s* | *s* |
| *Sclerogaster* | *spA* | *s* | *s* |
| *Stephanospora* | *spA* | *s* | *s* |
| *Zelleromyces* | *spA* | | *u* |
| *Zelleromyces* | *spC* | *s* | *s* |
| *Zelleromyces* | *spD* | *s* | *s* |
| *Zelleromyces* | *spE* | *s* | *s* |

When considering the change in sporocarp biomass and abundance between "normal" and "extreme" rainfall, three distinct groups of species could be distinguished (Discriminant Function Analysis: *Wilks' λ = 0.200*; $F^2_{11} = 22.145$; *P < 0.0001;* Figure 3-6). All (100%) of the cases were correctly classified to each group.

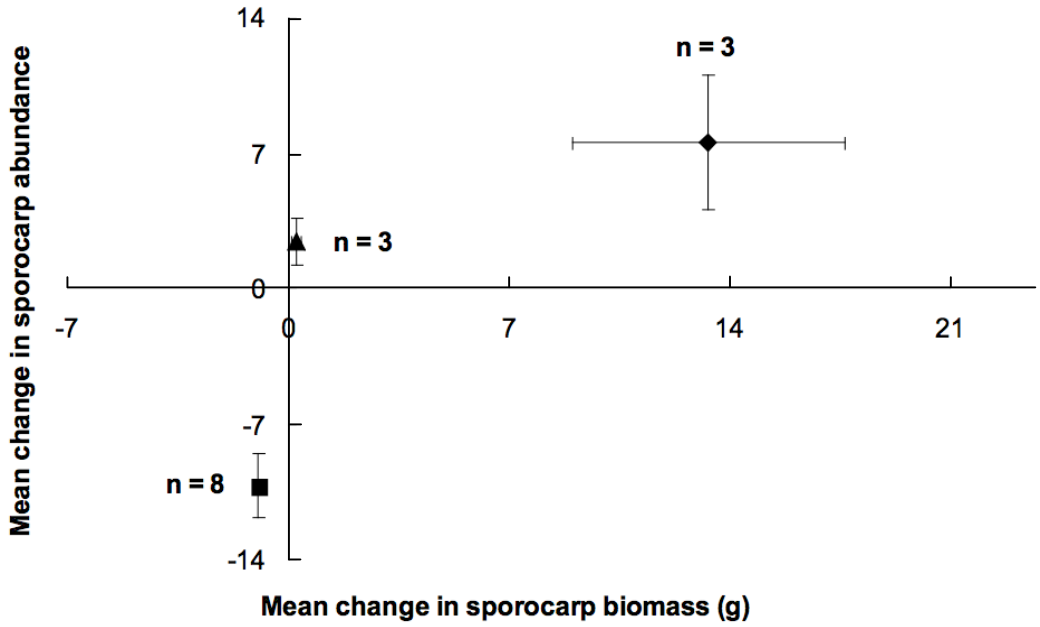


**Figure 3-6:** The change in biomass and abundance (±1SE) between normal and extreme rainfall showing the significant centromeres produced by a K-means analysis; (■) represents eight species that decreased in both abundance and biomass, (◆) three species that increased in abundance and biomass and (▲) three species that were relatively stable and increased only slightly in abundance and biomass.

Three species, *Gautieria amara* Trappe, *Gummiglobus joyceae* Trappe, Castellano & Amar. and *G. spB* increased in both abundance and biomass, and three species *Stephanospora spA*, *Zelleromyces spC* and *Aroramyces spA* maintained a relatively stable abundance and biomass (Figure 3-6). Eight species, *Chondrogaster spB*, *Hysterangium aggregatum* J.W. Cribb, *H. gardneri* E. Fisch., *H. inflatum* Rodway, *H. spC*, *Sclerogaster spA*, *Zelleromyces spD*, *Z. spE* decreased in both abundance and biomass (Figure 3-6). The species that remained relatively stable after extreme rainfall were found to produce few, small sporocarps (Figure 3-7). The species that increased in both abundance and biomass after extreme rainfall produced few, large sporocarps, while the species that decreased in both abundance and biomass after extreme rainfall produced many, small sporocarps (Figure 3-7).

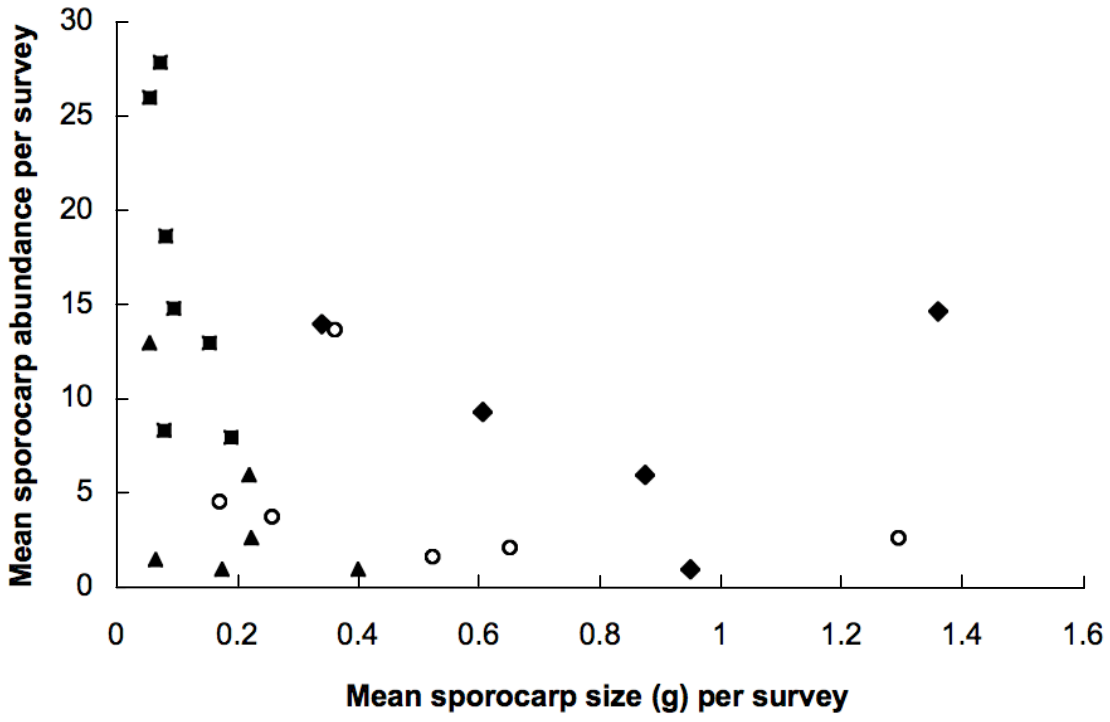


**Figure 3-7:** The mean sporocarp size (biomass/abundance) and mean abundance of species from all surveys. Each data point represents a single survey with species that (■) decreased, (◆) increased, (▲) remained relatively stable or (O) were unable to be assigned a category (were not present) after extreme rainfall.

## 3.4 Discussion

Due to the variability in the level of precipitation during the two years of sampling (Figure 3-1), the hypothesis that precipitation not temperature influenced abundance and biomass of hypogeous fungi in the tropics (Abell *et al.* 2006) was able to be tested. There was a significantly lower abundance and biomass of sporocarps found during the pooled late dry season (spring 2004/05) samples and no difference between the other seasons, including the early wet (summer 2005/2006), late wet (autumn 2005; 2006) and early dry (winter 2004) (Figure 3-1 and 3-2).

This is a very different pattern of sporocarp production compared to the temperate region, where peaks in abundance occur both in spring and autumn. In the tropics, sporocarp production was high and not significantly different in all seasons (autumn, winter and summer), except for spring when it was consistently significantly lower (Figure 3-2). This occurred despite the higher precipitation leading up to the second spring survey (September 2005) (Abell *et al.* 2006) compared to the first spring survey (September 2004) (Figure 3-1). The variability in abundance between the wet and dry seasons indicated a possible correlation with climate variables. However, there were no correlations with temperature, sunlight, cloud cover or soil moisture and initially a correlation between precipitation and sporocarp abundance could not be detected.

Prior to the last survey (May 2006), two extreme climatic events occurred with the passage of two tropical cyclones south (Cyclone Larry; Cat. 5; March 2006), then north (Cyclone Monica; Cat. 3; April 2006) of the study area. Subsequently, precipitation was more than three times above the expected levels (74 year means) for the month of April, immediately prior to the May 2006 survey (Figure 3-1).

These precipitation levels were also the highest of any other survey throughout the study (Figure 3-1). Instead of the highest abundance, sporocarps were recorded at medium levels for that survey (Figure 3-3). This may indicate a non-linear relationship between abundance and precipitation. However, although abundance was lower, biomass was at the highest level after the extreme precipitation (Figure 3-4) and the number of species fruiting also did not decline (Figure 3-5).

A potential explanation for the lower abundance and higher biomass after the extreme rainfall could be that a different assemblage of species were fruiting in the wet season compared to the dry season. The species richness, Shannon Weiner, Chao1 and ACE indices confirmed that the dry season surveys were lower in richness compared to the wet season (Table 3-1). However, there were no significant differences between the taxonomic assemblage of species sampled in the wet (Early and Late Wet) compared to the dry (Early and Late Dry) seasons (Table 3-2).

In prior studies the taxonomic assemblage did differ between the seasons (autumn and spring) and those differences were attributed to morphological characteristics of the sporocarps (Claridge *et al.* 2000b; Claridge *et al.* 1993; Johnson 1994a). In contrast to the temperate region studies there were no significant differences between the genera or species that fruited in the wet and the dry seasons (Table 3-2). Therefore, the taxonomic assemblage could not explain the difference in abundance and biomass observed after extreme rainfall. The number of species sampled asymptoted after 200mm of precipitation and remained at a relatively constant level thereafter (Figure 3-5). This indicated that the same fungal species responded in a different way after extreme rainfall by producing relatively fewer, but larger sporocarps.

When this was examined closely a more complicated pattern emerged (Figure 3-6). Three different responses were found; three species increased in both abundance and biomass, three species remained relatively stable, and the majority of species (eight) decreased significantly in abundance as well as biomass (Figure 3-6). Extreme rainfall appears to have affected the reproductive ability of the majority of species, explaining the discrepancy between biomass and abundance that was found (Figures 3-3 & 3-4).

The species that increased in abundance and biomass may be tentatively described as being ecologically hydrophilic and the decreasers as hydrophobic. This observation may have a physiological basis as ectomycorrhizal fungi, including hypogeous fruiting species, are known to have hyphae with hydrophobic and hydrophilic physiological properties (Agerer 2001).

The taxa that are known to have hydrophobic hyphae include species from the genus *Hysterangium* as well as *Gautieria* (Agerer 2001). Hydrophobic ectomycorrhizal fungal species may be highly sensitive to water-logging under laboratory conditions, and this is considered to occur only rarely in some temperate forest soils (Stenstrõm 1991). *Hysterangium aggregatum, H. gardneri, H. inflatum, H. spC, Chondrogaster spB, Sclerogaster spA, Zelleromyces spD* and *Z. spE* were found to have a possible hydrophobic response to extreme precipitation. *Stephanospora spA, Zelleromyces spC* and *Aroramyces spA* remained stable while, *Gautieria amara, Gummiglobus joyceae* and *G. spB* were found to have a hydrophilic response to extreme precipitation.

The results of this study indicate a potential negative effect of waterlogging on the reproductive ability of hypogeous hydrophobic taxa in the field (Figure 3-6). These observations require experimental data to confirm a physiological basis to the apparent ecological response.

The ecological responses to extreme rainfall allowed different reproductive strategies for all taxa to be distinguished. The decreasers were found to produce many, small sporocarps, while the increasers produced few, large sporocarps (Figure 3-7). The different reproductive strategies may be related to the water tolerance of those taxa. Hydrophobic species are thought to have a selective advantage in environments that experience periodical drought (Unestam 1991; Unestam and Sun 1995). Producing many, small sporocarps may be a water efficient reproductive strategy. Alternatively, hydrophilic taxa are thought to have a selective advantage in wetter environments (Unestam 1991; Unestam and Sun 1995).

Precipitation was required by all species to initiate reproduction (Figure 3-3, 3-4 and 3-5). The two-month lag effect of precipitation indicates that the fungal symbionts are buffered from current environmental conditions by their plant hosts and explains why there were no correlations for soil moisture that was measured at the time of each survey. Increased precipitation results in an increased photosynthesis rate due to elevated carbon fixation, augmenting the ability of the fungus to undergo sexual reproduction. This relationship between host plant photosynthesis rate and fungal fruiting is confirmed in numerous studies (Godbout and Fortin 1990, 1992; Hacskaylo 1965; Hogberg *et al.* 2001; Lamhamedi *et al.* 1994; Last *et al.* 1979; Nara *et al.* 2003; Norton *et al.* 1990; Simard *et al.* 1997a). In the seasonal tropics there are two extremes of the climate. Water is extremely limiting during the dry season and this dry period is generally followed by high rainfall during the wet season.

Water tolerance may provide a competitive advantage for both hydrophobic and hydrophilic hypogeous fungal species, facilitated by alternative reproductive strategies, at the two extremes of the tropical climate.

# Chapter Four: Nutrient Levels Determine The Spatial Distribution Of Tropical Hypogeous Fungal Sporocarps

## 4.1 Introduction

Environmental gradients correlated with abiotic factors help to determine patterns of animal and plant species diversity (Barbour *et al.* 1987; Begon *et al.* 2006; Lomolino 2001; Rahbek 1995; Rosenzweig 1995; Rosenzweig and Abramsky 1993; Whittaker and Niering 1975; Zobel *et al.* 1976). Fungi provide essential ecosystem services both directly as a resource for mycophagous animals, and indirectly in their role as decomposers and mycorrhizal symbionts. Therefore, understanding the environmental factors that influence the structuring of fungal communities provides important insight into their role in ecosystem function and their influence on both animal and plant spatial distribution. Despite their ecological importance, to date few studies have examined patterns of fungal diversity along environmental gradients in undisturbed natural ecosystems.

In Australia hypogeous (fruiting below-ground) fungal species have a high diversity (est. 2000 spp.) and endemicity (35% genera; 95% species) (Bougher and Lebel 2001). They also contribute significantly to ecosystem function by forming ectomycorrhizal associations with the roots of woody plants. Through these associations they provide water and otherwise unavailable nutrients in exchange for 30 – 60% of the sugars produced by their host plants (Norton *et al.* 1990; Simard *et al.* 1997b).

The fruiting bodies of hypogeous fungi are also an important resource for native mammals (Bougher and Lebel 2001), with many specialist mycophagous mammals, including the northern bettong, *Bettongia tropica*, being listed as endangered (IUCN Species Survival Commission 2007).

The majority of studies on the diversity of hypogeous fungi have been carried out in the Northern Hemisphere and have focused on host specificity (Bougher and Lebel 2001). These studies have shown that in mono-dominant forests consisting of hardwoods and conifers, the distribution of ectomycorrhizal fungi is explained, in part, by host specificity (Bills *et al.* 1986; Nantel and Neumann 1992; Villeneuve *et al.* 1989).

However, these findings are not generally applicable. Many ectomycorrhizal species are host generalists (Bougher and Lebel 2001; Bruns *et al.* 2002; Molina *et al.* 1992) and host specificity is expected to be substantially lower in mixed forest types (Trudell and Edmonds 2004). Hypogeous fungal species in Australian forests appear to have relatively low host plant specificity (Bougher and Lebel 2001; Bougher and Tommerup 1996; Malajczuk *et al.* 1982; Smith and Read 1997).

Laboratory studies have previously correlated abiotic variation with ectomycorrhizal species characteristics. For example, ectomycorrhizal formation (root tip colonisation) is decreased by low temperature (McInnes and Chilvers 1994), reduced soil organic matter (Reddell and Malajczuk 1984) and low light availability (Reid *et al.* 1983), as well as by high moisture availability (Bougher and Malajczuk 1990), high soil pH (Thomson *et al.* 1996), high soil phosphorous (Bougher *et al.* 1990) and high nitrogen (Lilleskov *et al.* 2001).

Excessive nutrients have immediate and more pronounced negative effects on extramatrical mycelium formation as well as sporocarp production (Jonsson *et al.* 2000; Peter *et al.* 2001; Wallenda and Kottke 1998).

Data from the limited number of field studies available on factors influencing ectomycorrhizal fungal sporocarp abundance and distribution are equivocal. A northern hemisphere study carried out along an elevation gradient with uniform host species distribution, found moisture was the most significant factor influencing the spatial distribution of ectomycorrhizal epigeous (fruiting above-ground) sporocarps (O'Dell *et al.* 1999). In another study comparing two sites with uniform host species, decreased soil nitrogen could explain differences in taxonomic assemblage between sites and increased sporocarp abundance in one site (Trudell and Edmonds 2004). This pattern has also been found in disturbed ecosystems, ectomycorrhizal species richness and sporocarp abundance are significantly reduced by high soil nitrogen levels (Lilleskov *et al.* 2001; Wallenda and Kottke 1998).

In Australia, field studies have shown that fruiting of certain hypogeous fungal species may be influenced by broad-scale characteristics of climate, topography, host plant and animal vector, as well as disturbance history (Bougher and Lebel 2001; Claridge *et al.* 2000a; Claridge *et al.* 2000b), but detailed functional relationships were not identified in these studies. Only one study has demonstrated a temporal community level increase in abundance and species richness of hypogeous fungal species with increasing precipitation (Abell *et al.* 2008a; Chapter 3).

The aim of this chapter is to determine the relative importance of different biotic and abiotic factors in determining the spatial distribution and taxonomic assemblage of ectomycorrhizal hypogeous fungal species in three contiguous vegetation types situated along an elevational gradient. If there are no differences in taxonomic assemblage between the vegetation types (minimal host specificity), northern hemisphere studies suggest that in general richness, abundance and biomass of ectomycorrhizal fungal sporocarps will be positively correlated with the altitudinal moisture gradient. However, fine-scale variation may also be associated with changes in microclimate and soil nutrient levels. Therefore, a range of potential driving factors for ectomycorrhizal hypogeous fungal species distribution were examined across this elevational gradient in an hierarchical analysis. These were host association (vegetation type), soil moisture, variation in light availability and temperature, as well as total soil nutrient content (nitrogen and phosphorous).

## 4.2 Methods

### *4.2.1 Sample Design*

The study site was located at Davies Creek on the Lamb Range in the Wet Tropics World Heritage area of far north Queensland, Australia (145°35'E, 17°01'S). Eighteen fungal survey sites were positioned in three vegetation types (six in each) across an altitude gradient approximately 5km wide, in ecotonal wet to moist sclerophyll forest (Figure 4-1).

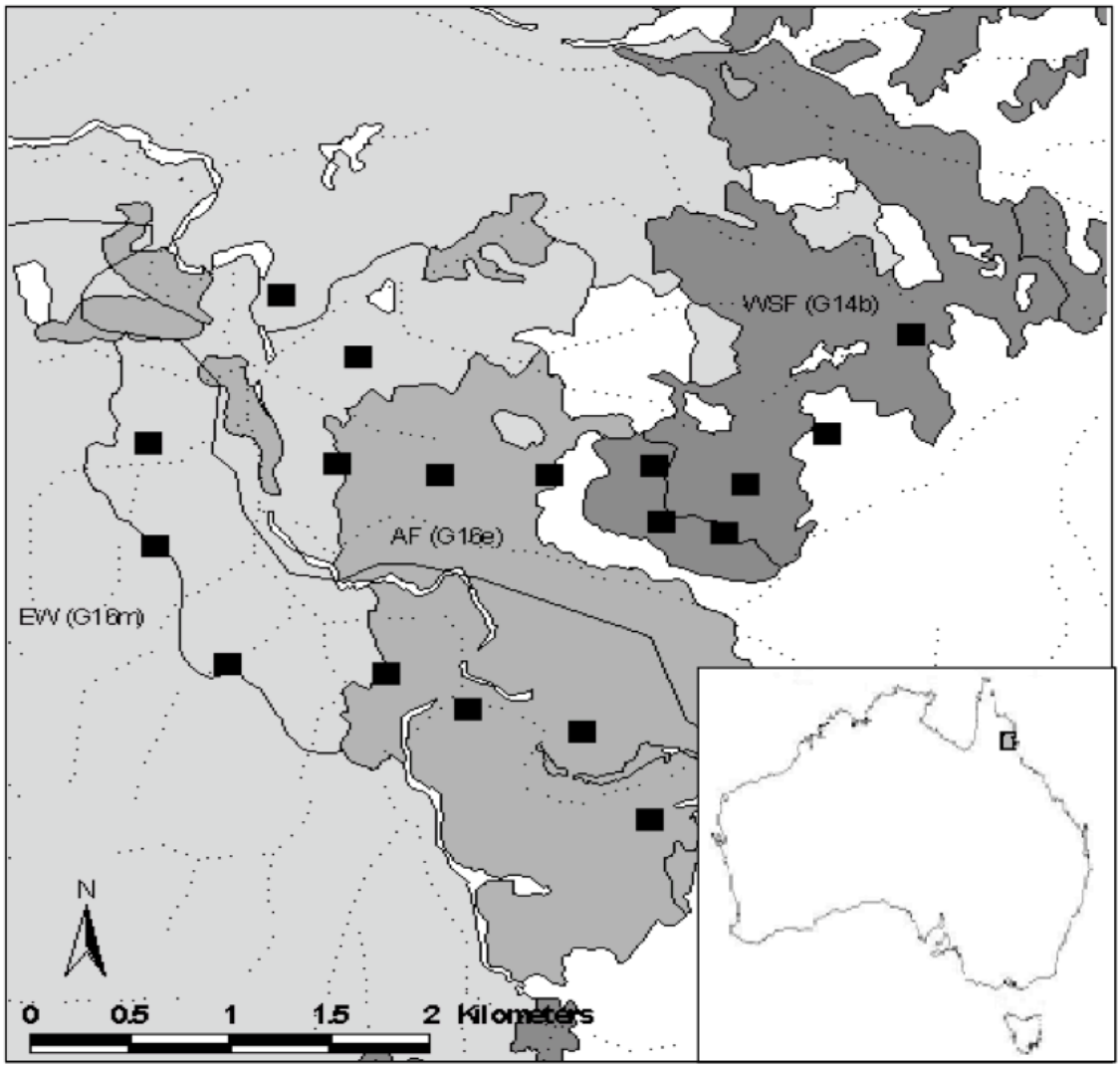


**Figure 4-1:** A map of the study site at Davies Creek on the Lamb Range, showing the location of six sites per vegetation type, including the wet sclerophyll forest (WSF; Type G14b), *Allocasuarina* forest (AF: Type G16e) and *Eucalyptus* woodland (EW: Type G16m). The letter G before the vegetation type number indicates the soil type (granite), the solid line is Davies Creek Road and the broken lines indicate drainage.

Wet sclerophyll forest (WSF) sites ranging in altitude from 1040 to 770 m (a.s.l.) were located in tall open-forest on granite. The dominant tree species were *Eucalyptus resinifera, E. acmenioides, E. intermedia, E. cloeziana, E. grandis* and *Syncarpia glomulifera* (Type 14b: Tracey 1982). *Allocasuarina* forest (AF) sites ranging in altitude between 720 to 670 m (a.s.l.) were located in medium woodland on granite. This forest type had a dense canopy dominated by *Allocasuarina torulosa* and *A. littoralis,* and may also have included *Eucalyptus intermedia, E. tereticornis, Tristania suaveolens, Acacia cincinnata, A. flavescens* and *Banksia compar* (Type 16e: Tracey 1982). *Eucalyptus* woodland (EW) sites ranging in altitude between 700 and 640 m (a.s.l.) were located in medium woodland on granite. This forest type had a relatively open canopy that included *Eucalyptus acmenioides, E. tereticornis, E. intermedia, Syncarpia glomulifera, Tristania suaveolens, Melaleuca viridiflora, Acacia flavescens, Allocasuarina littoralis* and *Xanthorrhoea johnsonii* (Type 16m: Tracey 1982).

Surveys were undertaken at each site in June and September in 2004, January, April (excluding the WSF sites) and September in 2005 as well as January and May 2006. A detailed explanation of the sample design including how the fungal survey sites were selected as well as the hypogeous fungal sampling protocol are given in Abell *et al.* (2006).

### *4.2.2 Assessment of fungal diversity*

Fungal sporocarps were identified (Appendix) using current and unpublished preliminary keys provided by and with the guidance, where possible, of experts in the field; Dr Teresa Lebel of the Royal Botanic Gardens Melbourne Australia and Dr James Trappe of the Department of Forest Science Oregon State University Corvallis, USA.

A detailed description of how the assessment of fungal diversity was carried out is given in Abell (2008a; Chapter 3). The species richness (S) was compared to the Shannon-Weiner Richness Index (H') for the three vegetation types.

### *4.2.3 Light/ Temperature*

Light levels and temperature were not measured directly. Instead a surrogate measurement, canopy cover was used. Canopy cover at each site was determined by estimating the percent coverage when standing at the center of each 50 x 20m quadrat, at the time of each survey. To determine the light level, the opposite of canopy cover, the canopy cover percentages were converted into reciprocal (1/canopy cover) proportions. The mean (SE) light levels for each vegetation type were then calculated. Light availability is generally positively correlated with temperature and the same values of light availability were used to also infer temperature levels for each site.

### *4.2.4 Soil analysis*

Two sealed bags of topsoil were collected from 5m up-slope and 5m down-slope from the centre of each quadrat at the time of each survey (n=7). Upon return to the laboratory the soil was dried, and the mean soil moisture (mL/g) was determined for each site per survey. Samples were then resealed and stored in a cool dry environment awaiting nutrient analysis.

A total of 240 soil samples were collected from 18 sites (12 in one survey) and seven surveys. Pooling of the soil samples (36 WSF; 42 AF, EW) was performed in an attempt to remove the expected effect of temporal (between surveys) and spatial (between quadrats) differences of soil nutrient status within sites.

This was achieved by combining equal portions of soil from each survey into one homogenous soil sample, including two replicates (above and below quadrat centre) for each site. A total of 36 (18 sites x 2 replicates) samples were subsequently sent for analysis to the Tropical Vegetation Dynamics Research Group, School of Marine and Tropical Biology, James Cook University, Townsville campus, Queensland Australia. Nine of the original samples were repeated to ensure accuracy of the chemical analysis.

Samples were first passed through a 2mm sieve to separate the fine earth fraction from gravel, then the single digestion method of Anderson and Ingram (1989) for the determination of N, P and cations was performed. Total Nitrogen (μg/g) was determined colorimetrically by the salicylate-hypochlorite method of Baethgen & Alley (1967), and Total Phosphorus (μg/g) by an adaptation of Murphy and Riley's (1967) single solution method (Anderson and Ingram 1989).

### *4.2.5 Data analysis*

The data obtained for sporocarp abundance, biomass, soil moisture and light availability did not conform to expectations of normality (Levene's equality of variances: $F_{2, 117} = 1.779$; $P = 0.173$; $F_{2, 117} = 4.233$; $P < 0.05$; $F_{2, 117} = 6.389$; $P < 0.01$; $F_{2, 117} = 14.292$; $P < 0.01$ respectively). Therefore, Kruskal–Wallis one-way non-parametric analysis of variance (ANOVA) were used to compare mean sporocarp abundance, biomass and light availability across vegetation types. When the data were normal (soil nutrients, genera and species), one-way parametric ANOVA and Bonferroni post-hoc tests were used. When comparing frequency of genera and species occurrence in the different vegetation types, Pearson one-way non-parametric chi square ($\chi^2$) analyses were used.

## 4.3 Results

### *4.3.1 Fungal species richness*

A total of 49 species of hypogeous fungi were recorded from 1161 occurrences across the 18 sites (six per vegetation type), and seven surveys (six for the WSF) (Table 4-1). Observations of species richness (S) indicated that there were 26 species from 197 occurrences in the WSF, 29 from 491 in the AF and 27 from 473 in the EW (Table 4-1). Shannon-Wiener diversity indices ranged from 2.38 in the WSF, 2.60 in the AF and 2.54 in the EW. With all vegetation types pooled the Shannon-Wiener diversity index was 3.05.

The total number of genera collected in the AF was higher compared to the other vegetation types (Table 4-1). Eighteen genera were collected in the AF compared to fifteen in both the WSF and EW (Table 4-1).

Of the genera that were collected thirteen (86.67%) from the WSF, fourteen (77.78%) from the AF and twelve (80%) from the EW were shared between vegetation types (Table 4-1). The remaining genera were collected solely from each vegetation type, two (13.33%) from the WSF, four (22.22%) from the AF and three (20%) from the EW (Table 4-1). However, there was no significant effect of vegetation type on the mean number of genera (One-way ANOVA: $F_2 = 2.309$; $P = 0.134$).

A similar pattern to the number of genera was found for the number of species (Table 4-1). Of the species that were collected nineteen (70.37%) from the WSF, eighteen (69.23%) from the AF and twenty (68.97%) from the EW were shared between vegetation types (Table 4-1).

**Table 4-1:** The total list of species that were surveyed in three vegetation types: wet sclerophyll forest (WSF), *Allocasuarina* forest (AF) and *Eucalyptus* Woodland (EW)

| Genus | Species | Vegetation Type |
|---|---|---|
| *Aroramyces* | *spA* | all |
| *Castoreum* | *tasmanicum* | AF |
| *Castoreum* | *infimiratio* | WSF |
| *Castoreum* | *sublaeve* | WSF |
| *Chamonixia* | *vittatispora* | WSF |
| *Chondrogaster* | *spF* | AF |
| *Chondrogaster* | *spB* | all |
| *Chondrogaster* | *spC* | EW |
| *Chondrogaster* | *spD* | EW |
| *Chondrogaster* | *spE* | WSF |
| *Chondrogaster* | *spA* | WSF/EW |
| *Dingleya* | *spA* | AF/EW |
| *Dingleya* | *spB* | EW |
| *Endogone* | *spB* | AF |
| *Endogone* | *spA* | AF/EW |
| *Gautieria* | *amara* | WSF/AF |
| *Gelopellis* | *cf thaxteri* | EW |
| *Geogyroporous* | *spA* | EW |
| *Glomus* | *spB* | EW |
| *Glomus* | *spC* | WSF/AF |
| *Gummiglobus* | *joyceae* | AF |
| *Gummiglobus* | *spB* | AF |
| *Gymnomyces* | *eildonensis* | AF |
| *Hysterangium* | *spC* | AF |
| *Hysterangium* | *aggregatum* | AF/EW |
| *Hysterangium* | *spB* | AF/EW |
| *Hysterangium* | *spD* | AF/EW |
| *Hysterangium* | *cf gardneri* | all |
| *Hysterangium* | *inflatum* | all |
| *Hysterangium* | *spA* | WSF/AF |
| *Hysterangium* | *affine* | WSF/EW |
| *Labyrinthomyces* | *spA cf varius* | AF |
| *Macowanites* | *spC* | EW |
| *Malajczukia* | *ingrattissima* | WSF |
| *Mesophellia* | *clelandii* | WSF |
| *Mesophellia* | *glauca* | WSF |
| *Mesophellia* | *oleifera* | WSF/EW |
| *Mycoamaranthus* | *auriorbis* | AF/EW |
| *Octaviania* | *spA* | AF |
| *Pogisperma* | *spB* | WSF |
| *Pogisperma* | *spA* | WSF/AF |
| *Royoungia* | *boletoides* | all |
| *Scleroderma* | *bougheri* | WSF/EW |
| *Sclerogaster* | *spA* | all |
| *Stephanospora* | *spA* | WSF/AF |
| *Zelleromyces* | *spC* | all |
| *Zelleromyces* | *spD* | all |
| *Zelleromyces* | *spE* | all |
| *Zelleromyces* | *spA* | EW |

The remaining species were collected solely from each vegetation type, eight (29.63%) from the WSF, eight (30.77%) from the AF and nine (31.03%) from the EW (Table 4-1). There was no significant difference for the proportion of unique species between the vegetation types (Pearson: $\chi^2_2 = 0.014;\ P = 0.993$). There was no significant effect of vegetation type on the mean number of species (One-way ANOVA: $F_2 = 1.952;\ P = 0.176$).

### *4.3.2 Fungal abundance and biomass*

There was a significant effect of vegetation type on sporocarp abundance (Kruskal-Wallis: $\chi^2_2 = 8.246;\ P < 0.05$). The lowest ranking vegetation type overall was the WSF (Figure 4-2). When this category was removed there was no significant effect of vegetation type found (Kruskal-Wallis: $\chi^2_1 = 0.588;\ P = 0.443$). Post-hoc pairwise comparisons confirmed that the WSF had significantly less sporocarps compared to the other vegetation types (Figure 4-2).

There was a significant effect of vegetation type on sporocarp biomass (Kruskal-Wallis: $\chi^2_2 = 9.982;\ P < 0.01$). The highest ranking vegetation type overall was the AF (Figure 4-2). When this category was removed there was no significant effect of vegetation type found (Kruskal-Wallis: $\chi^2_1 = 1.392;\ P = 0.238$). Post-hoc pairwise comparisons confirmed that the AF had significantly higher sporocarp biomass than both the EW and WSF and there was no difference between the latter vegetation types (Figure 4-2).

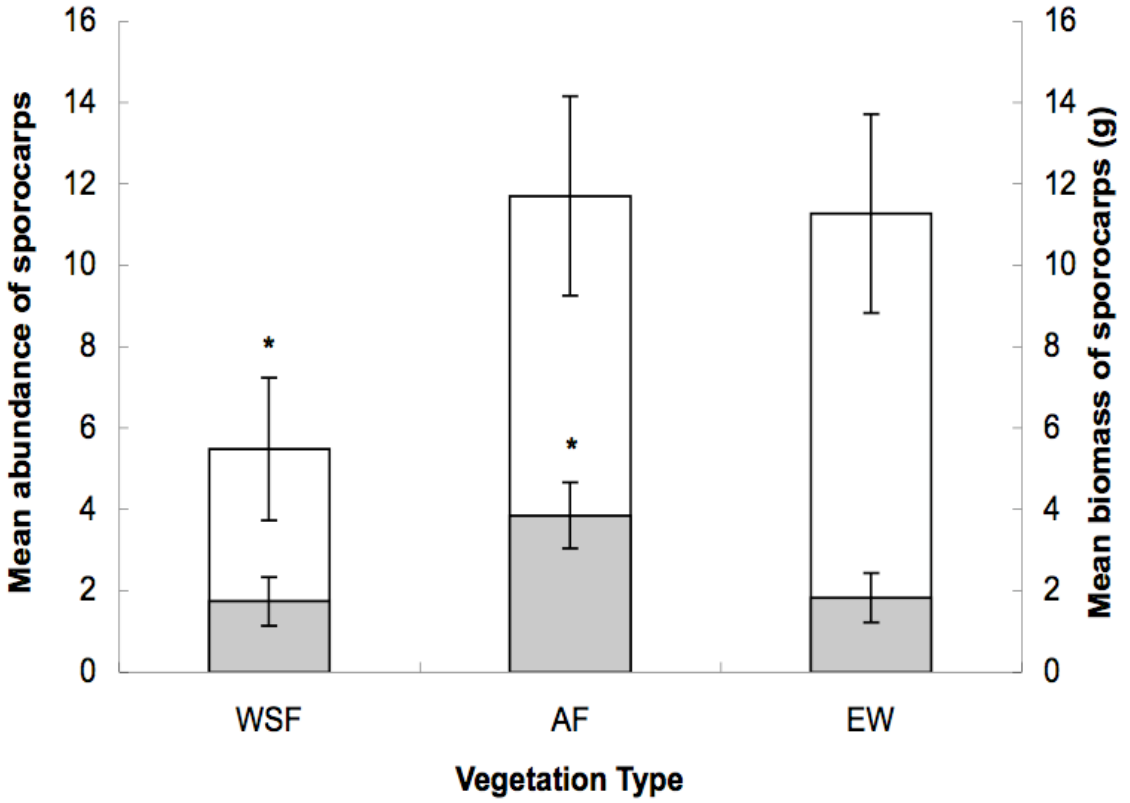


**Figure 4-2:** The mean (±1SE) sporocarp abundance (white columns) and biomass (grey columns) surveyed in the wet sclerophyll forest (WSF; n=36), *Allocasuarina* forest (AF; n=42) and *Eucalyptus* woodland (EW; n=42). * Significant difference

### *4.3.3 Moisture*

As expected the observed mean soil moisture differed significantly among the vegetation types (Figure 4-3a, Kruskal-Wallis: $\chi^2_2 = 37.428;\ P < 0.01$). The highest ranking vegetation type was the WSF followed by the AF (Figure 4-3a). When the lowest ranking vegetation type (EW) was removed there was no significant difference between the AF and WSF vegetation types (Kruskal-Wallis: $\chi^2_1 = 2.859;\ P = 0.091$).

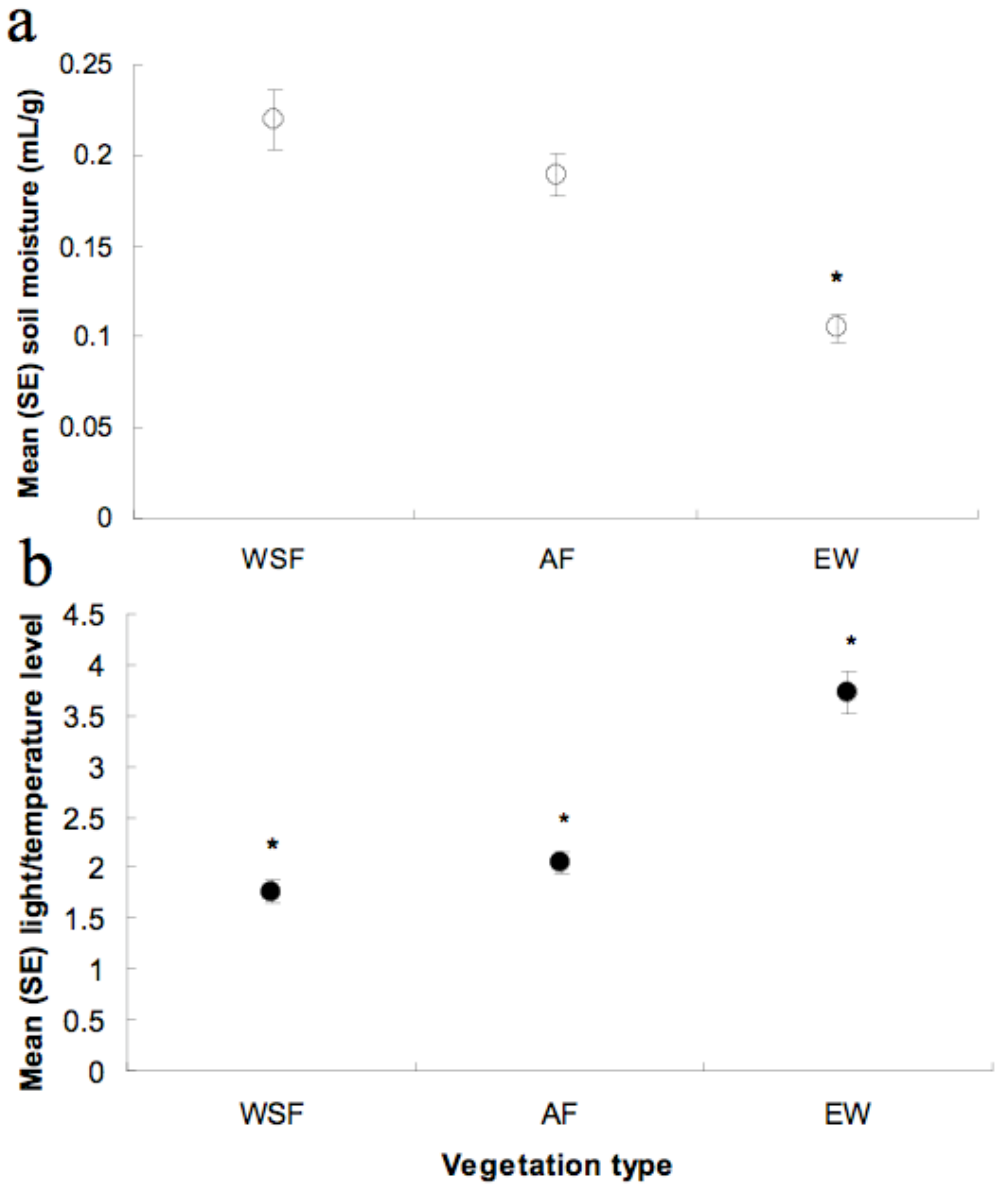


**Figure 4-3:** The mean (±1SE) **a)** soil moisture and **b)** light availability (inverse proportion of canopy cover) from the wet sclerophyll forest (WSF), *Allocasuarina* forest (AF) and *Eucalyptus* woodland (EW). * Significant difference

### *4.3.4 Light/ Temperature*

The observed mean light/ temperature availability (inverse proportion of canopy cover) increased significantly across the altitude gradient (Figure 4-3b). Post-hoc pair-wise comparisons confirmed that there was a significant difference in light availability between all three vegetation types (Kruskal-Wallis: EW/AF/WSF $\chi^2_2 = 61.340$; $P < 0.01$; EW/AF $\chi^2_1 = 39.716$; $P < 0.01$: EW/WSF $\chi^2_1 = 45.213$; $P < 0.01$; AF/WSF $\chi^2_1 = 7.810$; $P < 0.01$; Figure 4-3b).

### *4.3.5 Nutrients*

There was a significant effect of vegetation type on total soil nitrogen (µg/g) (One-way ANOVA: F2 = 6.119; P < 0.01). The EW vegetation type had significantly less nitrogen compared to the other vegetation types (Figure 4-4a). There was also a significant effect of vegetation type on total soil phosphorous (P) (µg/g) (One-way ANOVA: F2 = 8.247; P < 0.01), with the WSF vegetation type having significantly more phosphorous compared to the other vegetation types (Figure 4-4b).

## 4.4 Discussion

The number (S) and species richness (H') of hypogeous ectomycorrhizal sporocarps did not vary between the vegetation types. The number and species richness were similar in the WSF (26; 2.38), AF (29; 2.60) and the EW (27; 2.54). The fungal community structure observed suggests little if any host association (potential host specificity) at this location. The spatial distribution of ectomycorrhizal fungi did not vary significantly across the ecotonal gradient from WSF, through AF to EW, with the majority of genera and species shared between the vegetation types (Table 4-1).

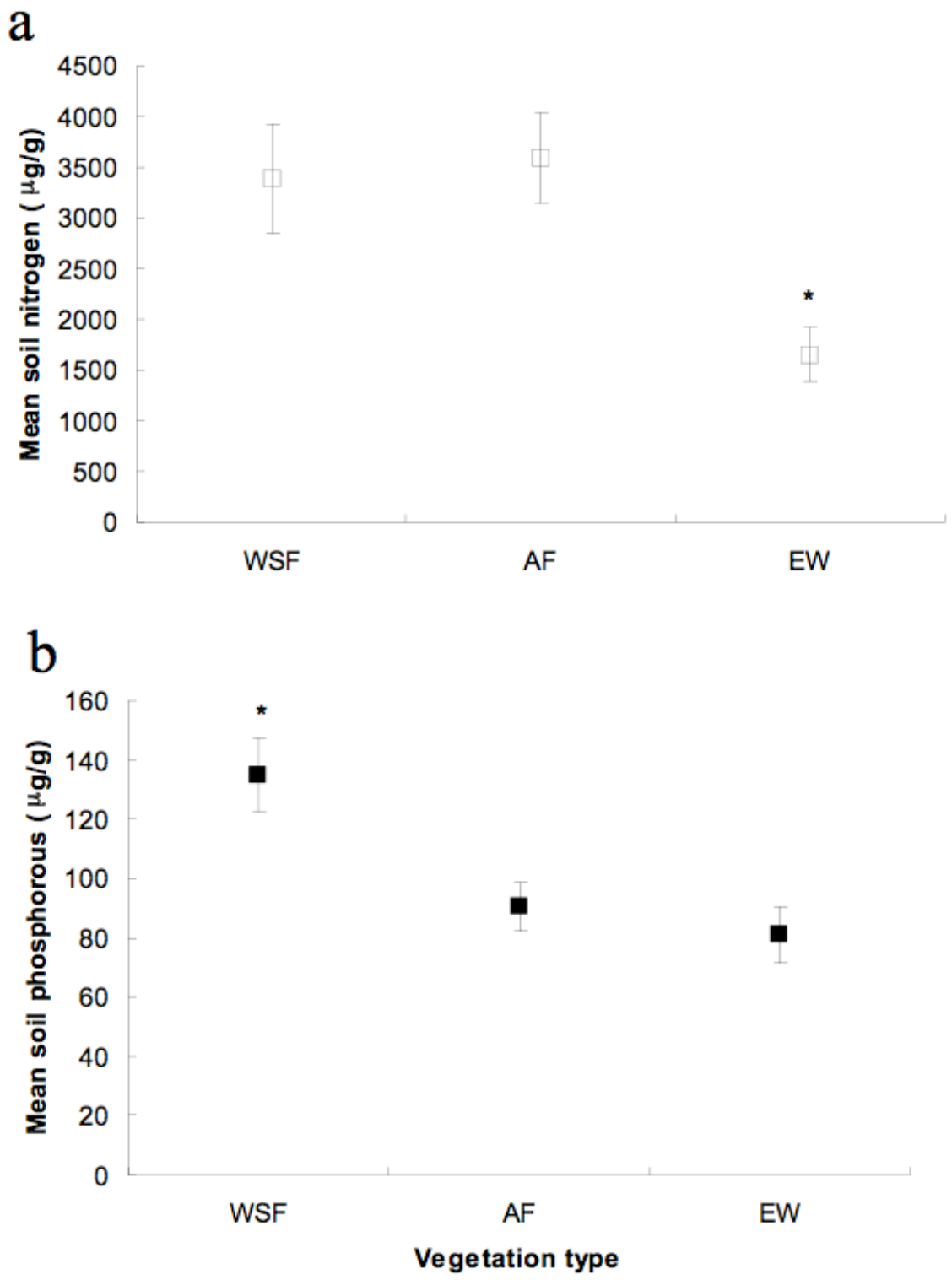


**Figure 4-4:** The mean (±1SE) **a)** total soil nitrogen and **b)** soil phosphorous (µg/g) sampled from the wet sclerophyll forest (WSF), *Allocasuarina* forest (AF) and *Eucalyptus* woodland (EW). * Significant difference

Genera that were ubiquitous in all vegetation types included *Aroramyces*, *Austrohysterangium*, *Chondrogaster*, *Royoungia*, *Sclerogaster* and *Zelleromyces* (Table 4-1).

Genera shared between the WSF and the AF included *Castoreum*, *Gautieria*, *Pogisperma* and *Stephanospora* (Table 4-1). Genera shared between the AF and EW included *Dingleya*, *Endogone* and *Mycoamaranthus* (Table 4-1). *Mesophellia* and *Scleroderma* were shared only by the WSF and the EW (Table 4-1). This latter association was the only indication of potential host specificity found. *Mesophellia* and *Scleroderma* appear to prefer vegetation dominated by *Eucalypts* rather than *Allocasuarina*. This is consistent with current distribution records. *Mesophellia* are found to be associated with natural and planted *Eucalyptus* forests in Australia and like *Scleroderma* are associated with *Eucalyptus* plantations outside of Australia (Bougher and Lebel 2001; Giachini *et al.* 2004; Trappe *et al.* 1996). Low host specificity is also consistent with the findings of previous studies of hypogeous fungi in Australia (Bougher and Lebel 2001; Bougher and Tommerup 1996; Malajczuk *et al.* 1982; Smith and Read 1997).

While richness and taxonomic assemblage did not vary between vegetation types, abundance of sporocarps was significantly lower in the WSF compared to the other forest types, and biomass was significantly higher in the AF (Figure 4-2). This finding appears to contradict previous studies showing a clear link between average monthly precipitation and fungal fruiting at a seasonal scale (Abell *et al.* 2008a; Chapter 3).

Previous studies predict both species richness and productivity should have been equivalent and higher in the WSF and AF forest types (high soil moisture), than in the EW (low soil moisture) (Figure 4-3a). The EW did have a lower biomass than the AF, which could be attributed to the significant difference in soil moisture between these two vegetation types (Figure 4-3a). However, biomass was equivalent between the WSF and EW (Figure 4-2). Overall, the observed pattern of productivity could not be due to a positive correlation with soil moisture (Figure 4-3a). Other abiotic factors that may influence productivity include light/temperature and nutrient levels.

Higher light availability has been found to increase the formation of ectomycorrhizas (Reid *et al.* 1983) and is expected to increase the availability of sporocarps due to the positive association between increased current photosynthate supply and fruiting found in numerous studies (Godbout and Fortin 1990, 1992; Hacskaylo 1965; Hogberg *et al.* 2001; Lamhamedi *et al.* 1994; Last *et al.* 1979; Nara *et al.* 2003; Norton *et al.* 1990; Simard *et al.* 1997a).

Light availability can also be used as a surrogate measurement of temperature. Increased temperature has also been found to improve ectomycorrhizal formation (McInnes and Chilvers 1994). Light availability/ temperature were significantly lower in the WSF than in the other two vegetation types (Figure 4-3b), a pattern consistent with the observed differences in sporocarp production (Figure 4-2). However, the AF also had significantly lower light/temperature levels as compared to the EW (Figure 4-3b) and the highest sporocarp production (both abundance and biomass) occurred at intermediate light/temperature levels (Figure 4-2).

There was a higher production of sporocarps found within the intermediate light/temperature forest type, AF (Figure 4-3b). Therefore, light/ temperature variation could not explain the pattern of productivity observed.

High nutrient levels, especially of nitrogen (Lilleskov *et al.* 2001) and phosphorous (Bougher *et al.* 1990) are known to influence ectomycorrhizal formation, production of extramatrical mycelium and sporocarps (Wallander 1995; Wallander and Nylund 1992). According to the carbohydrate hypothesis (Bjorkman 1940, in Wallander 1995), a host will allocate less carbohydrates to the fungus at higher levels of nutrient supply (phosphorous and nitrogen), because of greater carbon demand by the host for shoot production. In contrast, Wallander (1995) suggests that it is the fungus, not the host that partitions carbon supply. Even relatively small increases in nitrogen are predicted to reduce the ability of the fungus to allocate carbon away from nitrogen assimilation, to its own reproduction and growth (Trudell and Edmonds 2004; Wallander 1995). Regardless of the mechanism involved, both models predict that fungal growth is reduced as soil nitrogen levels increase.

On the other hand, low phosphorous is thought to counteract the effect of high nitrogen levels (Wallander 1995; Wallander and Nylund 1992). It does this by reducing plant shoot growth and thus the nitrogen demand of the host (Wallander 1995; Wallander and Nylund 1992). This means that under low phosphorous conditions fungi will be better able to partition carbon into reproduction and growth, as well as into nitrogen assimilation (Wallander 1995; Wallander and Nylund 1992).

The negative effect of nitrogen on ectomycorrhizal communities have been well documented along anthropogenic nitrogen deposition gradients and in nitrogen fertilisation experiments (see Trudell and Edmonds 2004). Aspects of these hypotheses have only once been tested in natural ecosystems (Trudell and Edmonds 2004) and never across an undisturbed ecotonal gradient.

In the current study, significantly higher levels of both nitrogen and phosphorous were found within the WSF soils (Figure 4-4). Soils around the nitrogen fixing *Allocasuarina* spp. within the AF also had higher nitrogen levels, compared to the levels found for the EW (Figure 4-4a). Unlike the WSF, the AF had a low level of phosphorous (Figure 4-4b). As discussed previously, low phosphorous counteracts the effect of high nitrogen by reducing plant shoot growth, thus the nitrogen demand of the host, allowing the fungus to partition carbon to reproduction and growth as well as nitrogen assimilation (Wallander 1995; Wallander and Nylund 1992). The higher abundance and biomass of fruiting bodies in the AF can be explained by the significantly lower availability of phosphorous in the soil (Figure 4-4b). This is the first time that the spatial distribution of hypogeous ectomycorrhizal fungal organisms could be explained in terms of their physiological functioning along a natural moisture and nutrient gradient.

The relative levels of soil nutrients observed are also consistent with what is known of the ecological process associated with the different over-story vegetation types. Mineralisation of nitrogen and phosphorous can occur at a faster rate with higher moisture levels (Trudell and Edmonds 2004). Therefore, higher estimated soil moisture (Figure 4-3a) in the WSF forest, may explain the increased level of nitrogen and phosphorous found in the soil there (Figure 4-4).

The WSF also contains a high proportion of rainforest tree species (Harrington and Sanderson 1994) and rainforest leaves are known to be more easily degraded than sclerophyll leaves (Read and Perez-Moreno 2003). Higher moisture (mineralisation), reduced fire frequency and subsequent invasion by rainforest species, may have contributed to the increased nutrient load in the soil of the WSF.

# Chapter Five: Micro-spatial Distribution Of Tropical Ectomycorrhizal Hypogeous Fungi

## 5.1 Introduction

Despite the ecological importance of hypogeous fungi in many ecosystems, few studies have attempted to identify factors responsible for either the availability or richness of fungi within specific communities (Abell *et al.* 2008b; Chapter 4; Claridge *et al.* 2000a). The scale at which species distributions are analysed is an important ecological consideration (Levin 1992 ; Schneider 2001; Wiens 1989) as hypogeous fungal communities may be shaped by multiple abiotic and biotic variables with different factors being more or less important at different temporal and spatial scales (Meyer and North 2005).

At the largest spatial scale of landscape, studies in temperate ecosystems have shown precipitation (North 2002), elevation and moisture gradients (Claridge *et al.* 2000a) to be the factors most likely to affect hypogeous fungal species occurrence. In the tropics little is known about the processes influencing spatial variation at this scale but at one location season-to-season variation in sporocarp availability, richness and taxonomic richness were correlated with annual patterns of precipitation (Abell *et al.* 2008a; Chapter 3; Abell *et al.* 2006).

At the more localised spatial scale of between forest-stands, temperate studies suggest that differences in forest structure (North *et al.* 1997; Smith *et al.* 2002), host specificity (Bills *et al.* 1986; Loeb *et al.* 2000; Nantel and Neumann 1992; Villeneuve *et al.* 1989) and forest age (Luoma *et al.* 1991; Vogt *et al.* 1981 ) are the most important determinants of hypogeous fungal distribution and abundance.

In contrast, in tropical ecotonal sclerophyll forest phosphorous levels have been shown to be more important determinants of hypogeous sporocarp availability (abundance and biomass) but not of taxonomic richness (Abell *et al.* 2008b; Chapter 4). It is clear that the factors driving hypogeous fungal community structure in temperate regions are not necessarily the same as those in the tropics.

At the finest micro-spatial scale of individual sites, the factors responsible for hypogeous fungal sporocarp availability and taxonomic richness are less clear-cut. Instead the relationships appear to be taxon or species-specific. Many different microhabitat variables have been documented influencing the distribution and diversity of individual hypogeous fungal taxa. For example, fungal species can be influenced by the presence/absence of decaying logs (Amaranthus *et al.* 1994; Claridge *et al.* 2000a; North and Greenberg 1998), orientation and size of fallen logs within a site (Maser and Trappe 1984), depth and cover of leaf litter (Beaton *et al.* 1985; Claridge *et al.* 2000a; Claridge *et al.* 1993; Johnson 1994a), slope and aspect (Claridge *et al.* 2000a; Claridge *et al.* 1993), soil moisture and nutrient status (Perry *et al.* 1987) (Claridge *et al.* 1993; Johnson 1994a), altitude (North 2002) as well as topography (Claridge *et al.* 2000a; Claridge *et al.* 1993). Site-specific interactions among these variables have been tested in very few of those studies. As with results for other spatial scales these findings have been generated primarily from temperate ecosystems.

Data at other spatial scales clearly suggests tropical fungal communities function differently and that data from temperate studies cannot easily be extrapolated to tropical systems. Currently there are no data available to determine which, if any, of the above factors are important at the micro-spatial scale in the tropics.

Previous data at the larger forest-stand scale show that phosphorous is the most likely determinant of overall hypogeous sporocarp availability in the tropics (Abell *et al.* 2008b; Chapter 4). This may also be true at the smaller micro-spatial scale but is, as yet, untested. No clear picture of the factors determining taxonomic richness in the tropics has previously been obtained from studies at larger spatial scales (Abell *et al.* 2008b; Chapter 4). Therefore, variables operating at the finest micro-spatial scale may also be more important in explaining patterns at these larger scales.

The aim of this chapter is to identify the relative importance of different environmental variables in influencing the micro-spatial scale distribution of both availability (sporocarp abundance and biomass) and richness (genus and species) of ectomycorrhizal hypogeous fungi across an ecotonal altitude gradient. Since previous evidence points to low phosphorous levels being an important determinant of hypogeous fungal availability at larger scales (Abell *et al.* 2008b; Chapter 4), the primary analysis in this chapter will focus on establishing if this is also true at smaller spatial scales.

In an hierarchical analysis this chapter will first examine how much variation in fungal availability and richness can be explained by **a)** soil phosphorous using simple regression analysis and then **b)** multiple microhabitat variables that have been known to influence the distribution of individual hypogeous fungal species in temperate systems. This second analysis will be undertaken using an Information Theoretic Model Comparison (ITMC) approach (Burnham and Anderson 1998). This will allow interactions between variables to be examined as well as the most plausible and parsimonious model(s) to be identified to help explain the factors driving hypogeous fungal species distribution at the micro-spatial scale within tropical ecotonal sclerophyll forest.

## 5.2 Methods

The study site was located at Davies Creek on the Lamb Range in far North Queensland, Australia (145°35'E, 17°01'S). Six fungal survey sites were positioned in each of three forest types (n =18); wet sclerophyll Forest (WSF) (Type 14b: Tracey 1982), *Allocasuarina* forest (AF) (Type 16e: Tracey 1982) and *Eucalyptus* woodland (EW) (Type 16m: Tracey 1982) that comprised ecotonal wet to moist (respectively) vegetation along an altitude gradient.

A detailed explanation of the sample design, including how the fungal survey sites were selected as well as the hypogeous fungal sampling protocol are given in Abell *et al.* (2006). A description of how the assessment of fungal diversity was carried out is detailed in Abell *et al.* (2008a; Chapter 3) and a site map is available in Abell *et al.* (2008b; Chapter 4). Surveys were undertaken at each site in June and September 2004, January, April (excluding the WSF sites) and September 2005 and January, May 2006.

At the time of each survey abiotic and biotic microhabitat measurements were recorded following the protocol of Claridge *et al.* (2000a). Abiotic factors included soil nitrogen, phosphorous and moisture (as per Abell *et al.* (2008b; Chapter 4)), topographic category (ridge, upslope, midslope, downslope or gully), site aspect (North, East, South or West), slope measured using an inclinometer and rock cover (%) estimated at site center.

The biotic variables were measured as either an estimated vegetation percent cover from site centre, or by counting within a 2 x 50m transect that ran through the centre (one metre either side) on the long axis of each quadrat.

Percent cover measurements included canopy cover, understorey cover (above head-height), ground cover (below head-height), litter cover and coarse woody debris. Transect variables included number of potential host species including *Eucalyptus* and *Allocasuarina* stems the number of non-ectomycorrhizal host species including *Acacia* and rainforest stems, as well as number of stags (standing dead trees), fallen trees, fallen trees lying across the slope and large (>50cm diameter) fallen trees. The number of small animal (bettong/ bandicoot) diggings within the transect were also recorded within the transect. Litter depth (mm) was measured by averaging thirteen random measurements taken within each quadrat.

### *5.2.1 Data Analysis*

Previous evidence suggests soil phosphorous levels may significantly influence the spatial availability of hypogeous fungi (Abell *et al.* 2008b; Chapter 4; Bougher *et al.* 1990; Johnson 1994a). Therefore, prior to any other analyses multiple curve-fits were analysed relating soil phosphorous with the dependent variables being fungal abundance, biomass, number of genera and species. The equation (i.e. linear, logarithmic, exponential etc.) that fitted best to the data (highest $r^2$ value and lowest P value) for each dependent variable was subsequently presented.

As previous studies provide multiple potential hypotheses to explain the distribution of hypogeous fungi, traditional null hypothesis testing with regression analyses were not appropriate to use in this instance (Burnham and Anderson 1998). The Information Theoretic Model Comparison (ITMC) approach is suitable to use when there are multiple hypotheses to test (Burnham and Anderson 1998). Using ITMC means that it is possible to simultaneously compare different plausible models leading to the selection of one (or a smaller set) best approximating model (Burnham and Anderson 1998).

This was performed by fitting general linear regression models (GLM) to the data that combined multiple hypotheses to explain the dependent variables abundance and biomass (g) (sporocarp availability) as well as species and genera number (richness).

The independent microhabitat variables used in the analyses, were chosen based on a previous study by Claridge *et al.* (2000a). A reduced version of the data set was derived by averaging the data from seven surveys for each site (18). This removed the expected temporal variability associated with rainfall levels (Abell *et al.* 2008a; Chapter 3) as well as reduced the number of zeros contributed by particular surveys. This also normalised the data but there were more independent than dependent variables.

Accordingly, a principal components analysis (PCA) using SPSS software (Version 16.0, http://www.spss.com) was used to summarise the environmental variables into a smaller subset (23 reduced to 5) of independent variables and also remove the confounding effects of colinearity (Graham 2003). The strength of the PCA was checked by repeating the analysis using standardised variables and this did not change the factor scores or loadings.

Multiparameter candidate models were developed using different combinations of the principal coordinate variables. Akaike Information Criterion (AIC) were used to determine the most parsimonious model(s) for each of two dependent variables (the number of genera and the number of species) (Burnham and Anderson 1998). As the sample size (n=18) was small (n/K>40) compared to the number of variables (K=5), the second order Akaike bias correction parameter ($AIC_c$ = AIC + 2K(K+1)/n-K-1) was used, as recommended by Burnham and Anderson (1998). The model with the lowest $AIC_c$ value was designated as the $AIC_{min}$.

The relative support for each model was determined by comparing the $AIC_{min}$ with each models own AIC value ($\Delta_i = AIC_i - AIC_{min}$) (Burnham and Anderson 1998). Those with the lowest $AIC_c$ values that were within 2 AIC units ($\Delta_i$ ) of the lowest value were considered as candidate models (Burnham and Anderson 1998). Akaike weights ($w_i = \exp(-\Delta_i/2)/ \Sigma^R_{r=1} \exp(-\Delta_r/2)$) were used as a measure of the relative likelihood (probability) of each model (Burnham and Anderson 1998).

The smallest subset of candidate models that summed to 0.95 ($w_i$), were used to generate the 95% confidence limits for each model (Burnham and Anderson 1998). The probability that an independent variable was contained in the best approximating model was obtained by calculating the sum of the $w_i$ (Selection Probability) and the associated confidence limits (95% CL Selection probability) by summing the 95% CL $w_i$, for each model containing that variable (Burnham and Anderson 1998). Lastly, the maximum log likelihood $\chi^2$ values for each model were examined for significance.

Linear regressions were then fitted, testing each dependent variable including abundance, biomass, number of genera and number of species against the significant and most plausible AIC determined principal component(s).

## 5.3 Results

### *5.3.1 Soil phosphorous regressions*

There were significant correlations between mean soil phosphorous and mean sporocarp abundance ($y = 38.042e^{0.016x}$; $r^2 = 0.4713$; $F_{1,17} = 14.263$; $P < 0.01$: *exponential*) (Figure 5-1a). Mean soil phosphorous and mean sporocarp biomass were also negatively correlated ($y=19.28e^{-0.0247x}$; $r^2 = 0.388$; $F_{1,17} = 10.146$; $P < 0.01$: *exponential*) (Figure 5-1b).

There was a weak correlation between mean soil phosphorous (x) and the mean number of genera ($y = 2.7094e^{-0.0079x}$; $r^2 = 0.238$; $F_{1,17} = 4.986$; $P < 0.05$: e*xponential*) (Figure 5-1c). The mean number of species (y) was also weakly correlated with the mean soil phosphorous ($y = 4.073e^{-0.0102x}$; $r^2 = 0.375$; $F_{1,17} = 9.588$; $P < 0.01$: e*xponential*) (Figure 5-1d).

When the outlier was removed the relationships for phosphorous and the mean number of genera ($r^2 = 0.001$; $F_{1,16} = 0.017$; $P = 0.898$) and also the mean number of species ($r^2 = 0.052$; $F_{1,16} = 1.216$; $P = 0.381$) were no longer significant.

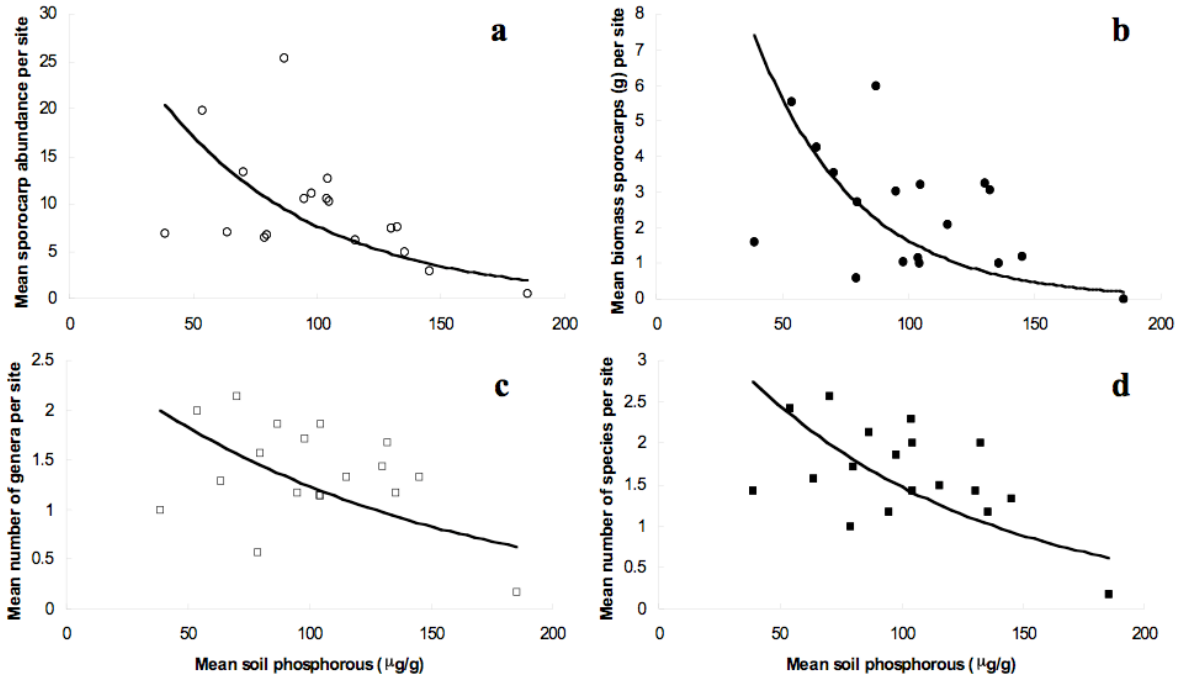


**Figure 5-1:** Inverse exponential relationships between **a)** mean sporocarp abundance, **b)** mean sporocarp biomass, **c)** mean number of genera and **d)** mean number of species with mean soil phosphorous per site.

### *5.3.2 Principal components interpretation*

The PCA with varimax raw rotation summarised 23 independent variables to five (as determined by a scree plot) principal components (Table 5-1). The first five principal components of the PCA explained 81.32% of the total environmental variance among sites (Table 5-1). The first principal component (PC1) was mainly defined by a negative association with the number of *Eucalyptus* stems and a positive correlation with the number of *Allocasuarina* stems (Table 5-1). This axis was interpreted as explaining the change in vegetation type across the gradient from the WSF (High *Eucalyptus,* Low *Allocasuarina*), through the AF (Low *Eucalyptus*, High *Allocasuarina*) to the EW (High *Eucalyptus,* Low *Allocasuarina*). The second principal component (PC2) was related to the abiotic site characteristics and was defined by a negative association with slope and rock cover (Table 5-1). Principal component three (PC3) was defined simply by aspect (Table 5-1).

Principal component four (PC4) was defined by soil phosphorous, altitude, the number of rainforest stems, understorey vegetation percent cover and soil moisture (Table 5-1). This axis was interpreted as explaining the increase in altitude across the environmental gradient as it was considered to be the most likely causative variable within the principal component (Table 5-1). Principal component five was interpreted as the axis explaining the biotic structure of the site as it was defined by a negative correlation with the percent of ground cover present as well as a positive relationship with the amount of coarse woody debris within the sites.

**Table 5-1:** Principal component scores of the environmental variables measured at seven surveys from 18 sites (12 in one survey). The main structuring variables (≥0.70) determined after varimax raw rotation for each principal component are indicated in bold type.

| Variables | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| Eigen Values (%) | 8.79 (38.24) | 3.52 (15.29) | 2.43 (10.55) | 2.35 (10.22) | 1.61 (7.02) |
| Soil nitrogen (μg/g) | 0.42 | -0.23 | 0.43 | 0.55 | -0.21 |
| Soil phosphorous (μg/g) | 0.03 | -0.60 | -0.04 | **0.70** | 0.04 |
| altitude | -0.29 | -0.36 | -0.06 | **0.70** | 0.44 |
| topographic category | 0.66 | -0.06 | 0.22 | -0.34 | 0.26 |
| aspect | -0.21 | 0.09 | **0.91** | 0.10 | -0.02 |
| slope | -0.11 | **-0.79** | -0.06 | 0.07 | 0.44 |
| litter depth (mm) | 0.58 | -0.23 | 0.04 | 0.56 | 0.45 |
| number of *Eucalyptus* stems | **-0.87** | 0.24 | 0.09 | -0.21 | -0.07 |
| number of *Allocasuarina* stems | **0.85** | 0.22 | -0.15 | 0.17 | -0.04 |
| number of *Acacia* stems | -0.31 | 0.26 | 0.49 | -0.25 | -0.20 |
| number of rainforest stems | 0.04 | 0.22 | 0.11 | **0.88** | 0.21 |
| canopy cover (%) | 0.36 | -0.22 | 0.03 | 0.68 | 0.46 |
| understorey cover (%) | 0.31 | 0.08 | 0.07 | **0.83** | 0.28 |
| ground cover (%) | 0.03 | 0.23 | -0.14 | -0.34 | **-0.77** |
| number of stags | -0.03 | -0.13 | -0.29 | 0.34 | 0.68 |
| number of fallen trees | 0.49 | 0.06 | 0.68 | 0.19 | 0.39 |
| number of fallen trees crosslope | 0.49 | 0.44 | 0.46 | 0.28 | 0.25 |
| number of large fallen trees | 0.14 | -0.51 | 0.28 | 0.00 | 0.57 |
| litter cover (%) | 0.68 | 0.03 | 0.21 | 0.63 | -0.10 |
| rock cover (%) | 0.12 | **-0.90** | -0.08 | 0.04 | -0.02 |
| coarse woody debris (%) | 0.13 | 0.05 | 0.11 | 0.32 | **0.88** |
| number of animal diggings | -0.30 | 0.30 | -0.53 | -0.07 | -0.45 |
| soil moisture (mL/g) | 0.20 | -0.10 | 0.06 | **0.92** | 0.22 |

### *5.3.3 Statistical Modelling*

The number of significant plausible models explaining the distribution of hypogeous fungi at the micro-spatial scale, as determined by a $\Delta i$ of less than two and the significance of the maximum log-likelihood ratio was relatively small compared to the potential models available (Table 5-2). Out of a set of 31 potential models for each dependent variable, three (9.7%) were considered plausible for the mean number of genera and three (9.7%) for the mean number of species (Table 5-2). Plausible models consistently included the index of vegetation type PC1 (83.3%), followed by the index of increasing altitude PC4 (66.7%) (Table 5-2). Represented less frequently (16.7%) were PC2 and PC5, which were the abiotic and biotic site structure indices respectively (Table 5-2). The index of site aspect PC3 was not included in any plausible model (Table 5-2).

Consistent second-order interactions were found between vegetation type PC1 and altitude PC4, with this interaction occurring three times within plausible models (Table 5-2). Less consistent second-order interactions included vegetation type PC1 with abiotic site structure PC2 (16.7%) as well as PC1 with abiotic site structure PC2 (16.7%). There were no significant interactions for PC1 with either site aspect PC3, or biotic site structure PC5 (Table 5-2). Only a single significant third-order interaction was observed between vegetation type PC1, altitude PC4 and biotic site structure PC5 (Table 5-2).

Overall, the most significant model explaining mean number of genera (y) per site was the positive relationship with vegetation type PC1 (x) ($AIC_{min}$: $\chi^2 = 8.0332; P < 0.01$) ($y = 0.3036x + 1.3639; r^2 = 0.379; P < 0.01$: *Linear*) (Table 5-2a; Figure 5-2). This single variable model had the highest selection probability at 95.41% (± 0.0536). Other less likely models included a first-order interaction between vegetation type PC1 and altitude PC4 (*AIC*: $\Delta i = 1.2493$; $\chi^2 = 9.3339; P < 0.01$), as well as PC1 and abiotic site structure PC2 (*AIC*: $\Delta i = 1.9661$; $\chi^2 = 8.6171; P < 0.05$) (Table 5-2a). The selection probabilities for these models were well below 50% (Table 5-2a) and could therefore be excluded.

**Table 5-2:** Plausible models generated to explain the distribution of hypogeous fungi using the ITMC approach for **a)** number of genera and **b)** number of species, based on 23 microhabitat variables that were summarised into five principal component independent variables (PC1–5). The table includes $AIC_c$ values, $AIC_c$ differences ($\Delta_i$), AIC weights ($w_i$), 95% confidence limits (95% CL $w_i$), log likelihood ratio (L.Ratio $\chi^2$) and associated P values. Significant models are in bold type.

| a) number of genera | Independent variable | | | | | | | | 95%CL | L.Ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Model | PC1 | PC2 | PC3 | PC4 | PC5 | AICc | $\Delta_i$ | $w_i$ | $w_i$ | $\chi^2$ | p |
| $AIC_{min}$ | * | | | | | 20.8045 | **0.0000** | 0.2848 | 0.0160 | 8.0332 | **0.0046** |
| 2 | * | | | * | | 22.0538 | **1.2493** | 0.1525 | 0.0086 | 9.3339 | **0.0094** |
| 3 | * | * | | | | 22.7706 | **1.9661** | 0.1066 | 0.0060 | 8.6171 | **0.0135** |
| 4 | * | | | | * | 23.1607 | 2.3562 | 0.0877 | 0.0049 | 8.2270 | **0.0164** |
| 5 | * | | * | | | 23.2604 | 2.4559 | 0.0834 | 0.0047 | 8.1273 | **0.0172** |
| 6 | * | * | | * | | 24.1762 | 3.3717 | 0.0528 | 0.0030 | 10.1258 | **0.0175** |
| 7 | * | | * | * | | 24.7894 | 3.9849 | 0.0388 | 0.0022 | 9.5126 | **0.0232** |
| 8 | * | | | * | * | 24.8784 | 4.0739 | 0.0371 | 0.0021 | 9.4236 | **0.0242** |
| 9 | * | * | | | * | 25.4825 | 4.6780 | 0.0275 | 0.0015 | 8.8195 | **0.0318** |
| 10 | * | * | * | | | 25.6089 | 4.8044 | 0.0258 | 0.0014 | 8.6931 | **0.0337** |
| 11 | * | | * | | * | 25.9673 | 5.1628 | 0.0216 | 0.0012 | 8.3347 | **0.0396** |
| 12 | * | * | * | * | | 27.3723 | 6.5678 | 0.0107 | 0.0006 | 10.2924 | **0.0358** |
| 13 | * | * | | * | * | 27.4370 | 6.6325 | 0.0103 | 0.0006 | 10.2276 | **0.0368** |
| 14 | * | | * | * | * | 28.0427 | 7.2382 | 0.0076 | 0.0004 | 9.6219 | **0.0473** |
| 15 | * | * | * | | * | 28.7604 | 7.9559 | 0.0053 | 0.0003 | 8.9042 | 0.0635 |
| (global model) 16 | * | * | * | * | * | 31.1772 | 10.3727 | 0.0016 | 0.0001 | 10.4105 | 0.0644 |
| 17 | | | | * | | 27.7044 | 6.8999 | 0.0090 | 0.0005 | 1.1333 | 0.2871 |
| 18 | | * | | | | 28.0668 | 7.2623 | 0.0075 | 0.0004 | 0.7709 | 0.3800 |
| 19 | | | | | * | 28.6490 | 7.8445 | 0.0056 | 0.0003 | 0.1887 | 0.6640 |
| 20 | | | * | | | 28.6504 | 7.8459 | 0.0056 | 0.0003 | 0.1873 | 0.6652 |
| 21 | | * | | * | | 29.6913 | 8.8868 | 0.0033 | 0.0002 | 1.6964 | 0.4282 |
| 22 | | | | * | * | 30.1102 | 9.3057 | 0.0027 | 0.0002 | 1.2775 | 0.5279 |
| 23 | | | * | * | | 30.1396 | 9.3351 | 0.0027 | 0.0002 | 1.2481 | 0.5358 |
| 24 | | * | | | * | 30.4592 | 9.6547 | 0.0023 | 0.0001 | 0.9285 | 0.6286 |
| 25 | | * | * | | | 30.4647 | 9.6602 | 0.0023 | 0.0001 | 0.9230 | 0.6303 |
| 26 | | | * | | * | 31.0162 | 10.2117 | 0.0017 | 0.0001 | 0.3715 | 0.8305 |
| 27 | | * | | * | * | 32.4735 | 11.6690 | 0.0008 | 0.0000 | 1.8285 | 0.6088 |
| 28 | | * | * | * | | 32.4950 | 11.6905 | 0.0008 | 0.0000 | 1.8070 | 0.6134 |
| 29 | | | * | * | * | 32.9049 | 12.1004 | 0.0007 | 0.0000 | 1.3971 | 0.7062 |
| 30 | | * | * | | * | 33.2302 | 12.4257 | 0.0006 | 0.0000 | 1.0718 | 0.7839 |
| 31 | | * | * | * | * | 35.7263 | 14.9218 | 0.0002 | 0.0000 | 1.9383 | 0.7471 |
| Selection Probability ($\Sigma$ $w_i$) | 0.9541 | 0.2583 | 0.2094 | 0.3318 | 0.2133 | | | | | | |
| 95% CL Selection probability ($\Sigma$ 95% CL $w_i$) | 0.0536 | 0.0145 | 0.0118 | 0.0186 | 0.0120 | | | | | | |

| b) number of species | Independent variable | | | | | | | | 95%CL | L.Ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Model | PC1 | PC2 | PC3 | PC4 | PC5 | AICc | $\Delta_i$ | $w_i$ | $w_i$ | $\chi^2$ | p |
| $AIC_{min}$ | * | | | * | | 30.3124 | **0.0000** | 0.1831 | 0.0114 | 6.9766 | **0.0306** |
| 2 | * | | | * | * | 32.2945 | **1.9821** | 0.0680 | 0.0042 | 7.9088 | **0.0479** |
| 3 | | | | * | | 30.8337 | **0.5213** | 0.1411 | 0.0088 | 3.9053 | **0.0481** |
| 4 | * | * | | * | | 32.7766 | 2.4642 | 0.0534 | 0.0033 | 7.4267 | 0.0595 |
| 5 | * | | * | * | | 32.9389 | 2.6264 | 0.0492 | 0.0031 | 7.2644 | 0.0639 |
| 6 | * | | | | | 31.8659 | 1.5535 | 0.0842 | 0.0052 | 2.8731 | 0.0901 |
| 7 | | | | * | * | 32.8575 | 2.5451 | 0.0513 | 0.0032 | 4.4315 | 0.1091 |
| 8 | * | * | | * | * | 35.2169 | 4.9045 | 0.0158 | 0.0010 | 8.3490 | 0.0796 |
| 9 | * | | * | * | * | 35.4407 | 5.1283 | 0.0141 | 0.0009 | 8.1252 | 0.0871 |
| 10 | | * | | * | | 33.1677 | 2.8552 | 0.0439 | 0.0027 | 4.1213 | 0.1274 |
| 11 | | | * | * | | 33.2639 | 2.9514 | 0.0419 | 0.0026 | 4.0251 | 0.1336 |
| 12 | * | * | * | * | | 35.8533 | 5.5409 | 0.0115 | 0.0007 | 7.7126 | 0.1027 |
| 13 | * | * | | | | 34.0282 | 3.7157 | 0.0286 | 0.0018 | 3.2608 | 0.1958 |
| 14 | * | | | | * | 34.0850 | 3.7725 | 0.0278 | 0.0017 | 3.2040 | 0.2015 |
| 15 | * | | * | | | 34.2573 | 3.9449 | 0.0255 | 0.0016 | 3.0317 | 0.2196 |
| 16 | | * | | * | * | 35.5456 | 5.2331 | 0.0134 | 0.0008 | 4.6577 | 0.1986 |
| (global model) 17 | * | * | * | * | * | 38.9129 | 8.6005 | 0.0025 | 0.0002 | 8.5761 | 0.1272 |
| 18 | | | * | * | * | 35.6707 | 5.3583 | 0.0126 | 0.0008 | 4.5326 | 0.2094 |
| 19 | | * | | | | 34.2441 | 3.9317 | 0.0256 | 0.0016 | 0.4949 | 0.4818 |
| 20 | | * | * | * | | 35.9603 | 5.6479 | 0.0109 | 0.0007 | 4.2430 | 0.2364 |
| 21 | | | | | * | 34.5172 | 4.2047 | 0.0224 | 0.0014 | 0.2218 | 0.6376 |
| 22 | | | * | | | 34.5346 | 4.2222 | 0.0222 | 0.0014 | 0.2044 | 0.6512 |
| 23 | * | * | | | * | 36.5969 | 6.2845 | 0.0079 | 0.0005 | 3.6064 | 0.3072 |
| 24 | * | * | * | | | 36.8050 | 6.4926 | 0.0071 | 0.0004 | 3.3983 | 0.3342 |
| 25 | * | | * | | * | 36.8514 | 6.5390 | 0.0070 | 0.0004 | 3.3519 | 0.3405 |
| 26 | | * | * | * | * | 38.7989 | 8.4865 | 0.0026 | 0.0002 | 4.7670 | 0.3120 |
| 27 | | * | | | * | 36.5322 | 6.2198 | 0.0082 | 0.0005 | 0.7568 | 0.6850 |
| 28 | | * | * | | | 36.6206 | 6.3081 | 0.0078 | 0.0005 | 0.6684 | 0.7159 |
| 29 | | | * | | * | 36.8548 | 6.5424 | 0.0070 | 0.0004 | 0.4342 | 0.8049 |
| 30 | * | * | * | | * | 39.8261 | 9.5137 | 0.0016 | 0.0001 | 3.7398 | 0.4424 |
| 31 | | * | * | | * | 39.2578 | 8.9454 | 0.0021 | 0.0001 | 0.9455 | 0.8144 |
| Selection Probability ($\Sigma$ $w_i$) | 0.5872 | 0.2428 | 0.2254 | 0.7152 | 0.2640 | | | | | | |
| 95% CL Selection probability ($\Sigma$ 95% CL $w_i$) | 0.0364 | 0.0151 | 0.0140 | 0.0444 | 0.0164 | | | | | | |

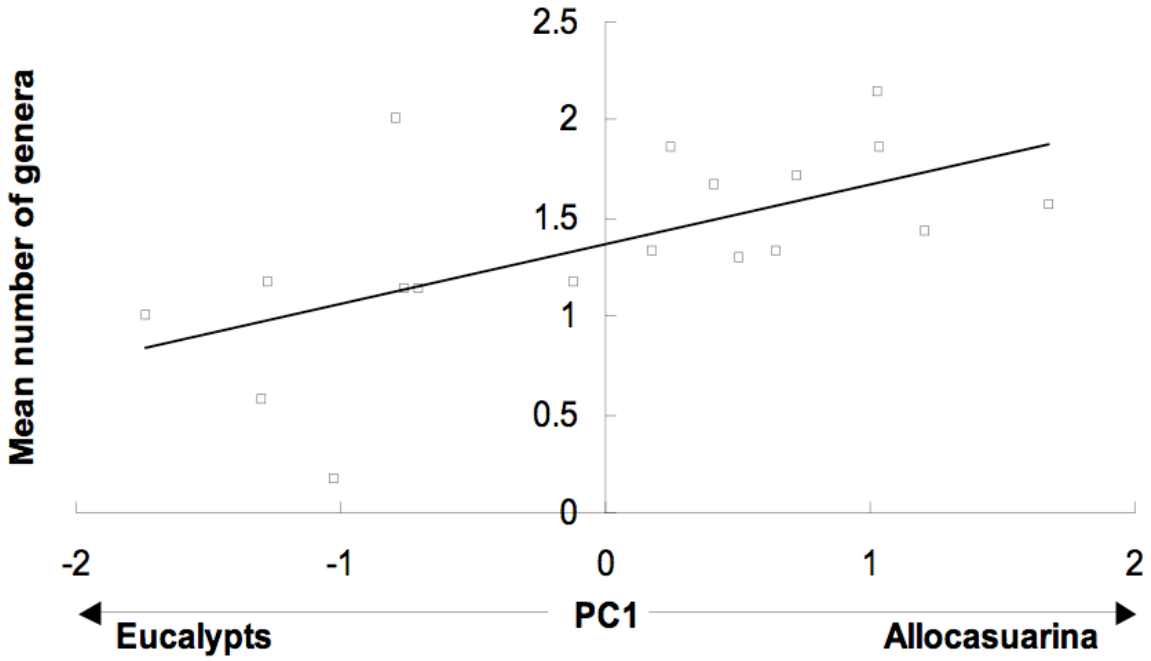


**Figure 5-2:** The positive correlation between vegetation type PC1 with the mean number of genera. The number of genera increase with the number of *Allocasuarina* and decrease with the number of *Eucalyptus* stems.

The most plausible model relating site characteristics to the mean number of fungal species was an interaction between vegetation type PC1 and altitude PC4 (*AIC*: $\Delta i = 0.000$; $\chi^2 = 6.9766$; $P < 0.05$) (*Linear:* $y = 1.62 + -0.27PC4 + 0.23PC1$; $r^2 = 0.374$; $P < 0.05$) (Table 5-2b; Figure 5-3). Other less plausible models included a third-order interaction between vegetation type PC1, altitude PC4 and biotic site structure PC5 (*AIC*: $\Delta i = 1.9821$; $\chi^2 = 7.9088$; $P < 0.05$) (Table 5-2b). A first order interaction with altitude was also found (*AIC*: $\Delta i = 0.5213$; $\chi^2 = 3.9053$; $P < 0.05$) (Table 5-2b). The highest selection probabilities for the number of species were altitude PC4 at 71.52% (± 0.0444), followed by vegetation type PC1 at 58.72% (± 0.0364) (Table 5-2b).

There were no significant models that related microhabitat site variables to sporocarp abundance ($AIC_{min}$: $\chi^2 = 2.5273;\ P = 0.119$) or biomass ($AIC_{min}$: $\chi^2 = 2.8328;\ P = 0.0924$).

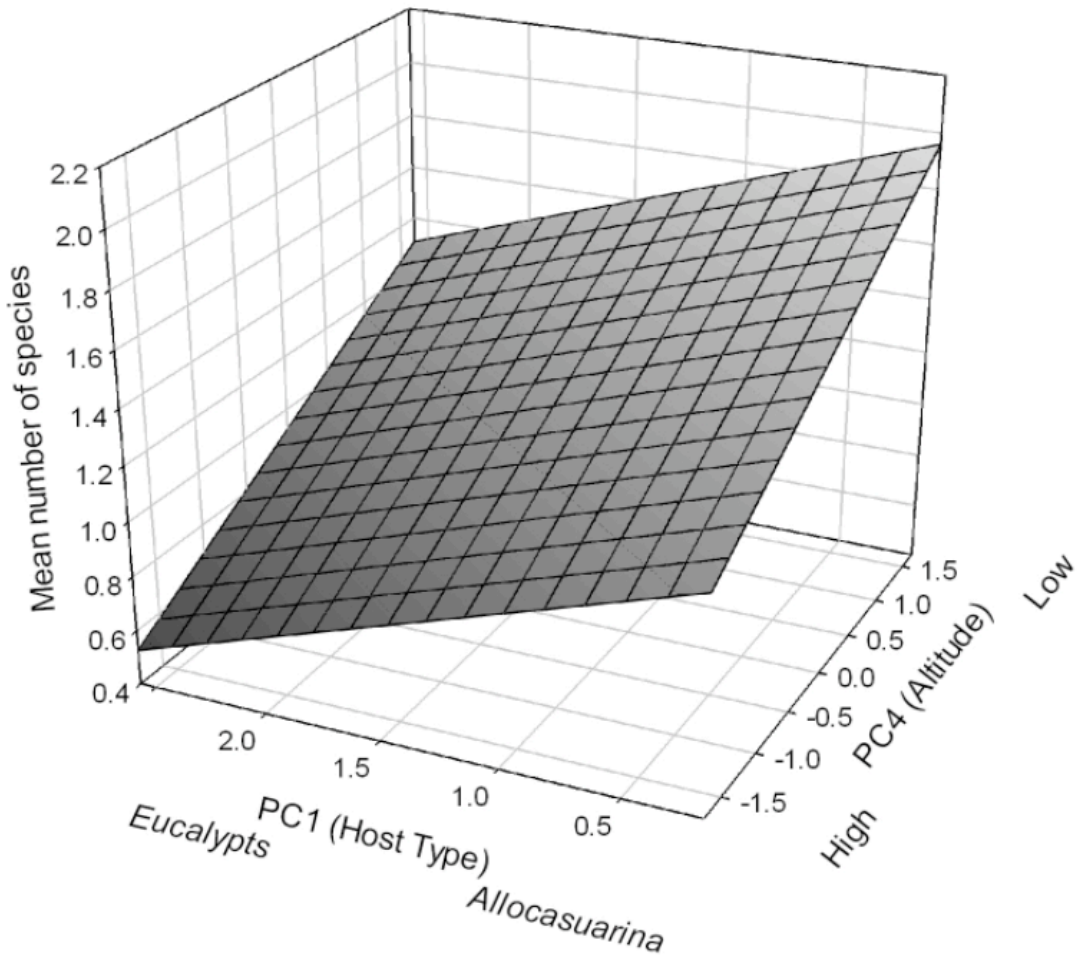


**Figure 5-3:** The interactive effect of proximity to rainforest PC4 and vegetation type PC1 on the mean number of species. Mean number of species increases with the number of *Allocasuarina* stems and an increase in altitude.

## 5.4 Discussion

At the micro-spatial scale, significant negative relationships were found between phosphorous concentration and both the availability (abundance and biomass) and richness (genera and species) of hypogeous fungi (Figure 5-1). There were no plausible models found for abundance or biomass using the ITMC approach. While this relationship still requires further *in-situ* experimental confirmation, it provides further evidence that in general both among forest stands and sites high phosphorous levels significantly decrease sporocarp production of hypogeous fungi in tropical ecotonal sclerophyll forest (Figure 5-1; (Abell *et al.* 2008b; Chapter 4). The functional mechanisms behind this observed association have been discussed previously in Abell *et al.* (2008b; Chapter 4).

In contrast the correlations detected between phosphorous levels and fungal species and genera richness were weak and obviously a function of only a single data point in each case (Figures 5.1c; 5.1d). This suggests that at best phosphorous concentration is only one of a number of factors affecting fungal taxonomic diversity at the micro-spatial scale. Similarly, when the distribution of species and genera were examined using the ITMC analysis the selection probabilities for the abiotic (PC1), biotic (PC5) and aspect (PC3) axes were all well below fifty percent. Therefore, factors associated with these axes (Table 5-1; 5-2) were not considered to significantly influence hypogeous fungal distribution.

The strongest correlate of fungal richness at the site scale was the index of host-type availability (Table 5-2). Both the mean number of fungal genera and species increased with the number of *Allocasuarina* stems and decreased with the number of *Eucalyptus* stems among sites (Table 5-1 and 5-2; Figure 5-2 and 5-3).

*Allocasuarina* species are known to form ectomycorrhizal associations (Reddell *et al.* 1986; Warcup 1980), but a lack of long-term fungal sporocarp surveys in forests dominated by *Allocasuarina* has meant that the relative importance of these associations has been unknown. The current data confirm that *Allocasuarina* are important ectomycorrhizal hosts in this system. They also suggest that *Allocasuarina* are able to form ectomycorrhizal relationships with more fungal genera and species and may have broader host receptivity than the *Eucalyptus* species present.

This finding is significant, as it suggests that previously the relative importance of *Allocasuarina* as host species may have been substantially underestimated.

*Allocasuarina* is considered to be an invasive climax species that utilise allelopathy to increase its dominance within sclerophyll ecosystems (Lunt 1998; Withers 1978). This effectively reduces the ability of other plant species to recruit in the absence of disturbance, especially fire (Lunt 1998; Withers 1978) and defines *Allocasuarina* forests as monodominant. Our findings further suggest that the ability of *Allocasuarina* species to invade and then dominate patches within sclerophyll forest may also be a function of their higher ectomycorrhizal fungal receptivity.

Ectomycorrhizal communities are generally characterised as having a low host diversity and high fungal diversity compared to endomycorrhizal communities (Allen *et al.* 1995; Connell and Lowman 1989; Janos 1985). There are many examples of ectomycorrhizal communities with poor plant host diversity. These include northern hemisphere conifer and oak forests, southern hemisphere *Nothofagus* forests and also monodominant forest stands that even occur alongside, or within, diverse tropical rainforests (Allen *et al.* 1995). Monodominant stands also tend to occur on nutrient poor soils, deficient in nitrogen and/or phosphorous (Allen *et al.* 1995).

Unlike endomycorrhizal fungi, ectomycorrhiza are able to scavenge as well as liberate (hydrolyse) otherwise unavailable organic forms of nitrogen and phosphorous (Lambers *et al.* 2008). The improved access to nutrients that ectomycorrhizal plants have due to fungal scavenging may explain their ability to colonise, then maintain monodominance within nutrient poor soils (Janos 1985) (Allen *et al.* 1995; Connell and Lowman 1989). The most plausible model relating the test variables to the number of hypogeous fungal species was an interaction between the index of host availability and altitude (Table 5-2; Figure 5-3).

As well as increasing with the number of *Allocasuarina* stems, the number of hypogeous fungal species increased at higher altitudes (Figure 5-3). At higher altitudes moisture levels were also higher and there was an associated increase in soil phosphorous, number of rainforest stems and understorey cover (Table 5-1). Therefore, fungal species diversity was related to soil phosphorous levels in a pattern opposite to that observed for sporocarp abundance and biomass (Figure 5-1a; 5-1b). Instead of a decrease, fungal species number increased with increasing phosphorous levels (Figure 5-3).

At higher altitudes an increase in *Allocasuarina* stems is correlated with an increase in phosphorous, soil moisture (Abell *et al.* 2008b; Chapter 4), number of rainforest stems and understorey cover (pers. obs.). Whereas, with an increase in *Eucalyptus* stems at a lower altitude (*Eucalyptus* woodland and not wet sclerophyll) there is a decrease in phosphorous, soil moisture (Abell *et al.* 2008b; Chapter 4), number of rainforest stems and understorey cover (pers. obs.). Therefore, due to the correlation between PC1 and PC4 it is difficult to determine which is the causative factor.

The altitude axis (Figure 5-3) may simply reinforces the positive correlation between the number of hypogeous fungal species and the number of *Allocasuarina* stems.

In summary, the results at the micro-spatial scale are the same as the patterns previously found among forest stands (Abell *et al.* 2008b; Chapter 4). Soil phosphorous levels are also essential environmental determinants of patterns of sporocarp abundance and biomass at this smaller scale. The optimal conditions for promoting hypogeous fungal diversity in this tropical ecosystem appear to be within *Allocasuarina* forest, between the rainforest invaded wet sclerophyll forest at higher altitudes, and the *Eucalyptus* dominated woodland at lower altitudes. The results highlight the apparent broader host receptivity of *Allocasuarina* species and that ecotonal sclerophyll monodominant forests, in comparison to mixed forest types, support a higher hypogeous sporocarp fungal diversity.

# Chapter Six: Thesis Synthesis

The aim of this thesis was to identify the relative importance of different biotic and abiotic factors in determining both the **a)** temporal and **b)** spatial distribution of hypogeous fungal taxa in a tropical ecotonal landscape. As predicted, factors that contributed to changes in the distribution and abundance of tropical hypogeous fungi differed in both time and space and were consistently different to phenomena previously identified as being important in temperate studies. This chapter presents summaries of the main findings of this thesis and the significance of those findings for managing the endangered mammal species *Bettongia tropica*. Potential future research directions are also outlined.

## 6.1 Diversity of hypogeous fungi

In total 49 morphological groups (species) of fungi were identified from 1161 occurrences recorded in this the first long-term study of hypogeous fungi in a tropical ecosystem. While identification to genus was possible (with only one exception) using current published and unpublished preliminary keys, a large proportion (84%) of morphological groups were not able to be assigned species names. Preliminary comparison to the literature suggests that at least one third (31%) of the morphological groups are likely to be new species to science. This level of diversity is equivalent to that found in Australian temperate regions, although this may need to be re-evaluated after more rigorous examination of the samples. Taxonomic descriptions of these species are ongoing and will be published elsewhere.

## 6.2 Temporal distribution of sporocarps

In temperate northern and southern hemisphere ecosystems fluctuations in fruiting intensity and differences in the availability of ectomycorrhizal hypogeous fungal species have been linked to both temperature variation and between season changes in moisture availability. In contrast, the current study found that in the tropics, season *per se*, or temperature alone were not directly associated with fungal fruiting, but that precipitation was the sole factor driving the temporal distribution of tropical hypogeous sporocarps. Both sporocarp abundance, and biomass (availability) were significantly correlated with precipitation; the magnitude of each parameter being significantly reduced during the late 'dry-season' in most years. This is the first study to document an overall temporal shortage of sporocarps in the tropics and an annual pattern of production that appears to be unique to tropical systems.

Precipitation patterns also influenced fungal taxonomic assemblage. Extreme rainfall contributed to a decrease in abundance and biomass of species from the genera *Chondrogaster*, *Hysterangium*, *Sclerogaster* and *Zelleromyces*. Species of *Gautieria* and *Gummiglobus* increased in biomass and abundance. The species/genera that decreased in abundance all had similar life-history characteristics. They were always smaller in size and at relatively greater densities than other taxa, characteristics suggesting that they require less water to reproduce and may have a possible competitive advantage during the dry season. In comparison, fungi that increased in abundance during high rainfall periods were always relatively larger species that consistently occurred at lower densities. Taxa that are likely to require more water to reproduce and so would have a reproductive advantage during the wet season.

## 6.3 Spatial distribution of sporocarps

At the more localised spatial scale of between forest-stands, fungal taxonomic assemblage did not differ among the three contiguous vegetation types; wet sclerophyll, *Allocasuarina* forest and *Eucalyptus* woodland. The fungal community structure observed suggested little if any host association (potential host specificity or preference) at this scale and location. Previous studies identifying soil moisture as the principal determinant of fungal abundance predicted that both species richness and productivity should have been equivalent and higher in the wet sclerophyll and *Allocasuarina* forest types (high soil moisture), than in the *Eucalyptus* woodland (low soil moisture). Reduced sporocarp abundance and biomass were observed in the *Eucalyptus* woodland as expected. However, counter-intuitively, less fungal productivity occurred in the wettest forest type, wet sclerophyll forest than in the *Allocasuarina* forest.

Fine scale spatial analysis of relative differences in soil nutrient levels identified the reason for this apparent anomaly. Both fungal abundance and biomass were reduced in the presence of high soil nitrogen and phosphorous in the wet sclerophyll forest, while the effect of high nitrogen availability was counteracted by low phosphorous levels in the *Allocasuarina* forest. Importantly, these finding were consistent with laboratory based studies on the relative importance of, and synergistic interactions between, these two soil nutrients. This is the first time that field observations on the spatial distribution of hypogeous ectomycorrhizal fungal organisms could be explained in terms of their physiological functioning along a natural moisture and nutrient gradient.

These patterns were then examined at the finest micro-spatial scale of individual sites. As expected the same relationships were found at this spatial scale as had been observed at the forest-stand scale; there being negative correlations between soil phosphorous levels and sporocarp abundance/biomass. However, at the finer scale, changes in soil phosphorous did not adequately explain differences in patterns of species richness observed among sites. Therefore, multivariate models incorporating a range of other potentially important environmental variables at each site were compared using an Information-Theoretic approach. There were no significant multivariate models found linking any of these new factors to changes in abundance and/or biomass, thus confirming that relative differences in phosphorous and nitrogen levels were the most plausible explanation for variation in fungal availability at both the between forest-stand and the micro-spatial scales.

In contrast, variations in the mean number of genera were best explained by an index of vegetation type; a positive correlation with the number of *Allocasuarina* stems and a negative correlation with the number of *Eucalyptus* stems. The most plausible model describing changes in the mean number of fungal species was one that involved an interaction between the indices of vegetation type and altitude. Species number increased with an increase in altitude (along with soil moisture, phosphorous, number of rainforest stems and understorey) and also by the number of *Allocasuarina* stems. That the number of fungal taxa (both species and genera) was consistently correlated with the number of *Allocasuarina* stems, suggests that *Allocasuarina* species may have a broader ectomycorrhizal host receptivity than *Eucalyptus* species.

Ectomycorrhizal plant communities although low in host diversity (monodominant) are generally more diverse in fungal taxa compared to mixed forest types. Ecotonal sclerophyll forests become increasingly dominated by *Allocasuarina* in the absence of fire or other disturbances. In this ecosystem an increase in host monodominance appears to also increase fungal richness. This pattern of plant-fungus taxonomic assemblage (i.e. the Ectomycorrhizal Hypothesis) has been found previously for monodominant ectomycorrhizal rainforest stands (Connell and Lowman 1989; Janos 1985) but never for ectomycorrhizal sclerophyll forest.

## 6.4 Implications and recommendations for *B. tropica*

The endangered northern bettong, *B. tropica* is thought to be restricted to habitats where the availability of hypogeous fungi, their principal food resource, remains consistently high. In this study a relationship was found between annual precipitation patterns and fungal availability. Fungal availability correlated strongly with the seasonal rainfall pattern determined from 74-year monthly means and was significantly lower in the late dry-season. This contrasted with a previously published study where mycophagy, measured by bettong faecal analysis, remained high across all seasons, presumably because of aseasonal rainfall during that study period. Based on my findings, hypogeous fungi could not remain consistently available to bettongs throughout an average rainfall year. This means that an alternative resource must be available to *B. tropica* during the typical annual fungal shortage.

*Alloteropsis semialata* (cockatoo grass) use by bettongs increased significantly during the period of low fungal availability. This suggests that the importance of cockatoo grass as an alternative food resource during annual and extended dry periods was previously underestimated. An important and possibly equivalent dependence of *B. tropica* on both hypogeous fungi and *A. semialata* helps to explain the bettongs restricted distribution, previously identified habitat preferences (Laurance 1997; Winter 1997) and identifies this species as a true ecotonal specialist.

The principal findings of this thesis have important implications for the management of *B. tropica.* The results have already been used to inform a new northern bettong recovery plan. Understanding the factors influencing hypogeous sporocarp production at both the temporal and spatial scales provides novel information including **a)** potential reasons for historic and current population declines, **b)** the importance of alternative resources during periods of low fungal availability as well as **c)** explanations for bettong habitat restrictions.

Since European settlement, drought has interacted with a multitude of other anthropogenic factors to cause the extinction of three out of five rat–kangaroo species in New South Wales, Australia (Short 1998). Increasing drought frequency (or intensity) may already have affected the range of *B. tropica* in Queensland, with population contraction occurring to higher rainfall areas. Contraction appears to have occurred from south to north (‘‘extinction’’ of the subtropical Rockhampton population and more recently the decline in the tropical Coane Range population), and west to east (‘‘extinction’’ of the low rainfall Kaban–Ravenshoe population).

The close link between hypogeous fungal availability and precipitation found in this study suggests that this population contraction can be expected to continue as the intensity and/or frequency of drought in Australia increases with global climate-change (Hughes 2003; Kothavala 1999). My findings also suggest that small, relatively isolated *B. tropica* populations will be especially vulnerable during times of drought. This specifically includes the Coane Range, Carbine and Mt Windsor populations. Monitoring rainfall patterns, may allow managers to implement drought mitigation protocols for these populations as required.

The results presented here also highlight the importance of *A. semialata* as a critical supplementary resource for *B. tropica*. This grass species does not respond well to long-term over-grazing due to a low seed-bank abundance and early wet season flowering (Crowley and Garnett 2001). After only moderate defoliation (as a surrogate for grazing) in the early wet season, inflorescence production can be reduced for at least two years (Crowley and Garnett 2001). It is believed that the recent change in tenure from State Forest to National Park throughout most of *B. tropica's* range may have reduced the problems associated with over-grazing of this resource by cattle. However, this assumption may require re-evaluation after modelling (using the precipitation/fruiting relationship), establishes core habitat of *B. tropica*. An additional threat to the ground-cover resources of *B. tropica* may be the feral pig *Sus scrofus*.

The reason that wet sclerophyll appears to be marginal habitat for *B. tropica* in the Lamb Range area, may be due to associated changes in vegetation structure caused by a lack of an adequate fire regime. Almost all wet sclerophyll sites in this study were found to have a rainforest understorey.

This is thought to result from variable fire intensity failing to remove the rainforest saplings, a process that results in sclerophyll trees dominating the canopy and rainforest saplings dominating the understorey (Harrington and Sanderson 1994). Although this does not appear to have a short-term effect on fungal abundance, it does affect the distribution of other important food resources including *A. semialata* and *Hypoxis* lilies in the understorey. This marginalisation of habitat also appears to be occurring within the higher altitude *Allocasuarina* forests at Davies Creek. A lack of fire increases the canopy cover of *Allocasuarina* trees making them the dominant species, again shading out the groundcover resources of *B. tropica*.

While the increase in *Allocasuarina* monodominance may be detrimental to the availability of bettong ground-cover resources, the importance of *Allocasuarina* hosts should not be underestimated. The evidence suggests *Allocasuarina* trees are important hosts in this ecosystem, significantly contributing to hypogeous sporocarp diversity.

*Alloteropsis semialata* is sensitive to fires during the growing season (early wet season) (Everson *et al.* 1988). The managers should develop an adequate fire regime ensuring that the fire intensity and regularity is suited, in particular, to the requirements of *A. semialata* and other important groundcover species. Specifically fire should be avoided during the early wet season. If there has been a particularly dry wet season, fire should be avoided during the subsequent dry season, as stores in the shoot base of *A. semialata* would most likely be depleted during growth, following fire. This will ensure that adequate resources are available for *B. tropica* when fungal resources are limiting.

Alternatively, fire should be encouraged during wetter dry seasons when fungal resources are available to make up for the depletion in the *A. semialata* resource during the time of fire.

Relationships driving the spatial availability of hypogeous sporocarps determined in this thesis also helps to clarify the habitat preference or restriction of *B. tropica* within a narrow band (>10km) of wet to moist sclerophyll forest on the western margin of Wet Tropics rainforest in Far North Queensland, Australia. *Bettongia tropica* relies on hypogeous sporocarps for as much as 67% of their diet (Johnson and McIlwee 1997). Previous studies have shown that *B. tropica* has a preference for ecotonal *Eucalyptus* woodland and *Allocasuarina* Forest and tends to avoid wet sclerophyll forest (Laurance 1997; Winter 1997). Until now the reasons why *B. tropica* does not utilise wet sclerophyll forest were unknown. Despite the availability of ectomycorrhizal plant hosts as well as high moisture levels, expected to be beneficial for sporocarp production, high nutrient levels appear to reduce the reproductive potential of hypogeous fungi in the wet sclerophyll forest. Hypogeous fungal resource availability, in synchrony with other crucial resources, clearly defines the habitat restriction of *Bettongia tropica* at both the wet and dry end of the ecotonal gradient.

## 6.5 Future Research

### *6.5.1 Hypogeous fungi and their plant hosts*

A number of new hypotheses have been generated from this thesis. Precipitation appears to drive temporal availability as well as diversity of tropical hypogeous ectomycorrhizal fungal sporocarps. With the available data it is not known whether the relationship between precipitation and mean sporocarp abundance plateau's or decreases at high rainfall. It is predicted that there is an upper limit to the production of sporocarps. A trade-off seems to be evident in the data with a subsequent increase in biomass rather than in abundance at the highest rainfall level. This trade-off between size and abundance has been examined extensively for plants (i.e. seed size/number trade-off theory) but not for hypogeous fungi or fungi in general.

Nutrient levels appear to have a strong influence on the spatial availability (abundance and biomass) of tropical hypogeous sporocarps. This is not a new hypothesis for fungi as there have been many studies on, for instance the effects of anthropogenic nitrogen deposition on fungal sporocarp production. However, it is a new hypothesis for hypogeous fungi and it appears that even small differences in phosphorous and nitrogen levels in a natural ecosystem significantly affect the number of hypogeous sporocarps that are produced. This hypothesis now requires experimental confirmation.

At the micro-spatial scale of site, taxonomic diversity of sporocarps appears to be associated with the number of *Allocasuarina* stems and this may be related to host monodominance.

The "Ectomycorrhizal Hypothesis" has been used to explain the presence and maintenance of monodominant forest stands of ectomycorrhizal rainforest plants amidst or adjacent to hyper-diverse stands of tropical endomycorrhizal rainforests (Connell and Lowman ; Janos 1985). This hypothesis appears to be equally applicable to ecotonal, monodominant *Allocasuarina* sclerophyll forest and also warrants further study.

### *6.5.2 The bettongs*

The relationship between fungal abundance and precipitation can now be used to develop population viability and habitat models under different climate change and drought scenarios. Such models will indicate to managers the areas of vulnerable (presumably lower altitude/rainfall) and core *B. tropica* habitat. Currently, little is known of the relationship between feral pig, *A. semialata* or hypogeous sporocarp abundance and these relationships require careful investigation.

More data are required to determine the impact of fire on fungal and ground-cover resources in both the short and long term within tropical ecotonal sclerophyll forest. One of the biggest questions is whether bettongs re-colonise or increase population numbers in wet sclerophyll forest after the rainforest is replaced with a grassy understorey that includes *A. semialata* and *Hypoxis spp*. If this does occur appropriate fire management may significantly increase the area of suitable habitat that is currently available, improving the now dire prospects of this endangered mycophagous marsupial, *Bettongia tropica*.

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# Appendix One: Key to the Genera of Hypogeous Fungi Surveyed in Far North Queensland, Australia

1. Spores large >60 μm..... 2 **Zygomycete/Glomeromycete (A)***
1. Spores small <60 μm ..... 3

2. Peridium well developed (~60 μm), thick spore walls (>2 μm) and >1 spore hyphal attachment often difficult to detect at maturity ..... ***Endogone***
2. Peridium thin, thin spore walls <2 μm and one obvious terminal hyphal attachment to each spore ..... ***Glomus***

3. Spores formed within an ascus..... 4 **Ascomycete (B)***
3. Spores formed on a basidium..... 5 **Basidiomycete (C)***

4. Peridium dark brown, lightly tomentose (woolly), reticulate with raised ridges on mature specimens, (mature specimens: paraphyses half as long as asci)..... ..... ***Labyrinthomyces***
4. Peridium light brown, scabrous and tessellated, (mature specimens: paraphyses the same length as asci)..... ***Dingleya***

5. Spores globose and ornamented..... 6
5. Spores with all other shapes, with or without ornaments .....13

6. Spore ornaments turning blue/black (amyloid reaction) in Meltzers reagent or iodine solution ..... 7
6. Spore ornaments with no amyloid reaction to Meltzers reagent or iodine solution..... 9

7. Stipe columella present..... ***Macowanites***
7. Columella absent, vestigial stipe sometimes present..... 8

*** See relevant sections within the species key Appendix Two**

8. Latex milky to watery in fresh specimens, lactiferous tissue abundant in the peridium .....***Zelleromyces***
8. No latex and no lactiferous tissue present in the peridium ..... ***Gymnomyces***

9. Spores dark brown in water .....10
9. Spores hyaline or light brown in water.....11

10. Peridium light yellow to brown, smooth and lacking a crust, solid black tar-like gleba, small spores (<14 μm) with short ornaments (<2 μm) . ***Scleroderma***
10. Peridium brown to black with yellow, orange or brown hyphae forming a carbonaceous crust, black powdery spore mass at maturity, relatively large spores (20 μm) with long spines (>2 μm) **only shells found**..... ***Elaphomyces***

11. Latex milky to watery in fresh specimens, lactiferous tissue present in the peridium, gel-filled chambers, ornamented spores with spines forming conspicuous cones ..... ***Octaviania***
11. Latex absent, globose spores spinose .....12

12. Spores with coalescing spines around the spore base forming a corona (crown) locules visible through the thin peridium.....***Stephanospora***
12. Small (5 μm) spores, thick white or light brown peridium.....***Sclerogaster***

13. Utricle present .....14
13. Utricle absent.....15

14. Spores lacking ornaments, utricle either inflated or appressed to spore wall and not covering the spore apex .....***Hysterangium***
14. Spores ornamented, utricle inflated and surrounding the spore apex..... ..... ***Aroramyces***

15. Spores lacking ornamentation .....16
15. Spores with ornamentation.....21

16. Spores yellow .....17

16. Spores hyaline .....18

17. Spores fusiform, yellow to light brown in 5% KOH and water, peridium bright chrome-yellow, gleba orange-yellow and gelatinous ..... ***Royoungia***

17. Spores broadly ovoid, obvious sterigmal attachment, yellow spores in 5% KOH and water, peridium yellow-brown, gleba bright yellow and dry with small open locules..... ***Geogyroporous***

18. Spores smooth oblong-elliptical (pill-shaped), rounded at both the spore base and apex, white peridium when fresh bruising brown, gleba cream or brown ... ..... ***Pogiesperma***

18. Spores fusiform with obvious sterigmal attachment .....19

19. Spores ellipsoid, 4-6 angles in polar view, peridium staining blue, gleba pale yellow..... ***Chamonixia***

19. Spores ellipsoid or ovate, not angular in polar view .....20

20. Spores ellipsoid or ovate, gleba shades of olive-greens or browns, dendritic cartilaginous columella present but no solid glebal core ..... ***Hysterangium***

20. Spores ellipsoid, rounded in polar view, peridium crusty and carbonaceous, spore mass olive green, surrounding a solid glebal core ..... ***Mesophellia***

21. Spores not ellipsoid .....22

21. Spores ellipsoid .....23

22. Spores lemon-shaped, ornamented with warts or ridges except for the smooth apical hump ..... ***Hymenogaster***

22. Spores broadly ovoid with minute (<1 μm) spines, dark brown-yellow spores, peridium bright chrome-yellow, gleba dark brown and gelatinous..... ..... ***Mycoamaranthus***

23. Ornaments are prominent longitudinal ridges, in polar view rounded, peridium bright canary yellow, gleba cinnamon brown ..... ***Gautieria***

23. Ornaments spines, rods or warts.....24

24. Spores warty, fusiform and ellipsoid without a utricle, peridium tightly adherent to the surrounding soil and rootlets ..... ***Chondrogaster***

24. Spores minutely warty and ellipsoid without a utricle, peridium consisting of gummy mycelium surrounding the spore mass .....25

25. Columella present..... ***Gummiglobus***

25. Columella absent ..... ***Castoreum***

# Appendix Two: Key to the Species of Hypogeous Fungi Surveyed

**(A)** *Spores large >60 µm, well developed peridium (~60 µm), thick spore walls (>2 µm) and >1 spore hyphal attachment often difficult to detect at maturity* ***Endogone***... 1

1 Peridium white, yellow gleba densely packed with globose spores 80 x 85 µm .....***Endogone* spA**

1 Peridium white, apricot-orange gleba, spores arranged in packages with white subtending tissue, orange globose spores 93 x 97 µm..... .....***Endogone* spB**

*Spores large >60 µm, thin peridium, thin spore walls <2 µm and one obvious terminal hyphal attachment to each spore* ***Glomus*** ..... 1

1 Peridium and gleba rusty-brown ..... 2

1 Gleba tan to white..... 4

2 Peridium/gleba rusty-brown, gleba densely packed with globose spores 77 µm x 82 µm .....***Glomus* spC**

2 Peridium/gleba rusty-brown, gleba densely packed with obovate spores ..... 3

3 Spores small and obovate 65 x 89 µm.....***Glomus* spA**

3 Spores large and obovate 81 x 106 µm.....***Glomus* spB**

4 Peridium rusty-brown, tan gleba, large obovate spores 102 x 122 µm..... .....***Glomus* spD**

4 Peridium white-cream, tan gleba with open locules, small hyaline spores 35 x 65 µm .....***Glomus* spE**

**(B)** *Spores formed within an ascus, peridium dark brown, lightly tomentose (woolly), reticulate with raised ridges on mature specimens, (mature specimens: paraphyses half as long as asci)* ..... ***Labyrinthomyces*** **spA (*cf varius*)**

*Spores formed within an ascus, peridium light brown, scabrous and tessellated,(mature specimens: paraphyses the same length as asci)* ***Dingleya***..... 1

1. Peridium light brown – orange, intersected with white-cream tessellations ..... .....***Dingleya*** **spA (*cf tesselata*)**
1. Peridium light brown-cream, carbonaceous – sandy in mature specimen, white-cream tessellations in immature specimen.....***Dingleya*** **spB**

**(C)** *Spores globose and ornamented formed on a basidium, spore ornaments turning blue (amyloid reaction) in Meltzers reagent or iodine solution, stipe columella present* ***Macowanites***..... 1

1. Spores 7 µm diameter, basidiocarp brown..... 2
1. Spores 9 µm diameter, basidiocarp white-cream, gleba cream-yellow...***M.*** **spC**

2. Ornaments spinose, <1 µm, peridium brown, gleba light brown.....***M.*** **spA**
2. Ornaments reticulate, <0.5 µm, peridium absent .....***M.*** **spB**

*Spores globose and ornamented formed on a basidium, spore ornaments turning blue (amyloid reaction) in Meltzers reagent or iodine solution, columella absent, vestigial stipe sometimes present, fresh specimens exuding a milky to watery latex, lactiferous tissue abundant in the peridium* ***Zelleromyces***..... 1

1. Spores diameter >9 µm, ornament height >1 µm..... 2
1. Spores 6 x 7 µm, ornaments 0.1 µm high, minute reticulated dots and lines, peridium brown and up to 300 µm thick consisting of appressed hyphae, cream gleba.....***Z.* spA**

2. Spore ornaments consisting of spines and rods often coalescing into long ridges ..... 3
2. Spore ornaments consisting only of individual rods and spines, spores 9 x 9 µm, ornaments <1 µm, cream peridium with orange patches, cystidia present on the peridial surface, cream gleba.....***Z.* spB**

3. Spores 9–13 (15) µm in diameter and most ornaments 2 µm or more high..... 4
3. Spores 7–10 µm in diameter, ornaments 1–2 µm high, locules visible through the thin brown (100–200 µm) peridium, gleba tan/cream .....***Z.* spC**

4. Gleba dark, rusty-red to dark brown, mean spore size 11 x 11 µm, peridium dark red/maroon to brown.....***Z.* spD**
4. Gleba pale, tan-cream to light brown, mean spore size 10 x 11 µm, peridium dark orange to brown.....***Z.* spE**.

*Spores globose and ornamented 7 x 7 µm, formed on a basidium, low (<0.8 µm) spore ornaments turning blue (amyloid reaction) in Meltzers reagent or iodine solution, columella absent, vestigial stipe sometimes present, fresh specimens not exuding latex and no lactiferous tissue present in the peridium, peridiopellis a turf of hyphal tips, large hymenial cystidia present 32–54 µm long, light brown peridium, pale brown gleba .....**Gymnomyces eildonensis***

*Spores globose and ornamented formed on a basidium, spore ornaments with no amyloid reaction to Meltzers reagent or iodine solution, spores dark brown in water and small (<14 µm) with short ornaments (<2 µm), peridium light yellow to brown, smooth and lacking a crust, solid black tar-like gleba* ***Scleroderma*** ..... 1

1. Spore wall rigid (not collapsing in 5% KOH), globose, spore mass pulverant at maturity ..... 2

1. Spores subglobose to globose with irregular shapes, spore walls often collapsing inwards in 5% KOH..... 3

2. Spores with a continuous reticulum, black in 5% KOH, 8–9µm diameter including ornaments of 1 µm high .....***S.* spD**
2. Spores without a reticulum, dark brown in 5% KOH, 9–11 µm diameter including rods/spines up to 1 µm high .....***S. bougheri***

3. Spores with short spines (<1 µm), 10–12 µm diameter.....***S.* spB**
3. Spores with conspicuous spines/cones (>2 µm), 11–14 µm diameter.....***S.* spC**

*Spores globose and ornamented formed on a basidium, spore ornaments with no amyloid reaction to Meltzers reagent or iodine solution spores dark brown in water and relatively large (20 µm) with long spines (>2 µm), peridium brown to black with yellow, orange or brown hyphae forming a carbonaceous crust, black powdery gleba (spore mass) at maturity* .....***Elaphomyces***

*Spores globose and ornamented formed on a basidium, spore ornaments with no amyloid reaction to Meltzers reagent or iodine solution, spores hyaline or light brown in water and ornamented with spines forming conspicuous cones, fresh specimens exuding a milky to watery latex, lactiferous tissue present in the peridium, gel-filled chambers*..... ***Octaviania***

*Spores globose and ornamented 7–11 µm x 7–11 µm, formed on a basidium, spore ornaments with no amyloid reaction to Meltzers reagent or iodine solution, hyaline or light brown in water and spinose, coalescing spines around the spore base forming a corona (crown), locules visible through the thin peridium, latex absent*.....
.....***Stephanospora flava***

*Spores globose and ornamented formed on a basidium, spore ornaments with no amyloid reaction to Meltzers reagent or iodine solution, spores hyaline or light brown in water, spinose and small (5 x 5 µm), thick white (in youth) to brown peridium, gleba orange, latex absent*..... ***Sclerogaster*** **spA**

*Spores formed on a basidium, utricle present or absent, without ornaments, utricle either inflated or appressed to spore wall and not covering the spore apex, gleba olive green, brown or dark brown/black and gelatinous* ***Hysterangium***..... 1

1. Spores oval to ovate, tapering only at one end ..... 2
1. Spores fusiform, tapering at both ends ..... 4

2. Utricle present, gleba brown ..... 3
2. Utricle absent, gleba green to dark brown/black, peridium staining pink, spores 10–14 x 5–6 µm..... ***H.*** **spD**

3. Utricle thin and hyaline, tightly appressed to the spores, 10–14 x 6–7 µm, peridium brown, gleba tan brown ..... ***H.*** **spB**
3. Utricle thick, up to 2 µm thick, spores 12–14 x 5–7 µm, peridium brown and wrinkled upon drying, gleba dark tan to brown..... ***H.*** **spC**

4. Utricle present, small sporocarps 2–15 mm in diameter ..... 5
4. Utricle absent, large sporocarps 10–30 mm in diameter, spores 9–14 x 3–5 µm, peridium brown, gleba dark chocolate brown (some specimens olive-green).... ..... ***H.*** **spA**

5. Spore size average 10 x 4 µm (8–13 x 3–5 µm)..... 6

5. Spore size average 12 x 5 µm (10–14 x 4–5 µm), very small sporocarps 2–4 mm in diameter, peridium embedded in soil and rootlets, distinctive grey-green gleba ..... ***H. aggregatum***

6. Peridium consisting of thickened (<20 µm) hyphae ..... 7

6. Peridium consisting of inflated cells >20 µm in diameter, dark olive-green to black gleba ..... ***H. affine***

7. Utricle often inconspicuous and always partially appressed to the spore wall (especially evident in spore polar view), gleba pale olive-green to dark olive-green/black..... ***H. cf gardneri***

7. Utricle always conspicuous and often inflated up to 2 µm around the spore wall, (especially evident in spore polar view), gleba dark olive-green to dark brown/black..... ***H. inflatum***

   Spores formed on a basidium, utricle present, spores ornamented, 11 x 6 µm, utricle inflated and surrounding the spore apex, gleba tan to dark brown ..... ***Aroramyces cf gelatinosporus***

*Spores formed on a basidium 9–12 x 4–5 µm fusiform, utricle absent and lacking ornamentation, spores yellow to light brown in 5% KOH and water, peridium bright chrome-yellow, gleba orange-yellow to light brown and gelatinous*..... ..... ***Royoungia boletoides***

*Spores formed on a basidium, utricle absent, spores lacking ornamentation, broadly ovoid, obvious sterigmal attachment, yellow in 5% KOH and water, peridium yellow-brown, gleba bright yellow and dry with small open locules*..... ***Geogyroporous***

*Spores formed on a basidium, utricle absent, spores lacking ornamentation, hyaline, smooth oblong-elliptical (pill-shaped), rounded at both the spore base and apex, white peridium, when fresh bruising brown, gleba cream or brown* ***Pogisperma*****.....** 1

1. Spores large, 8–10 x 3–4 µm, brown gleba, thick white peridium consisting of a turf of hyphal ends.....***P.*** **spA**

1. Spores small, 6–7 x 2–3 µm, cream gleba, thin brown peridium.....***P.*** **spB**

*Spores formed on a basidium, utricle absent, spores 10–13 x 4–6 µm, lacking ornamentation, hyaline, fusiform ellipsoid, with obvious sterigmal attachment, 4-6 angles in polar view, peridium staining blue, gleba pale yellow.....*

..... ***Chamonixia vitatispora***

*Spores formed on a basidium, utricle absent, spores lacking ornamentation, hyaline and fusiform ellipsoid with obvious sterigmal attachment and rounded in polar view, spore mass olive green surrounding a solid glebal core, peridium crusty and carbonaceous* ***Mesophellia/Malajczukia*** ..... 1

1. Spore mass pulverant..... 2

1. Spores borne on basidia lining locules radiating from the solid glebal core, large spores 12 x 5 µm ..... ***Malajczukia ingrattissima***

2. Spores large 9–12 x 4–5 µm ..... 3

2. Spores short 8–9 x 3–5µm ..... ***Mesophellia clelandii***

3. Trabeculae few and large 0.5–2 mm+ wide, firmly attaching glebal core to the peridium ..... ***Mesophellia glauca***

3. Trabeculae small, many (hundreds) <0.7 mm wide, glebal core movable and able to be easily detached from the peridium..... ***Mesophellia oleifera***

*Spores 17 x 9 µm, formed on a basidium, utricle absent, spores lemon-shaped with ornamentation of warts or ridges except for the smooth apical hump, brown peridium and gleba.....* ***Hymenogaster* spA**

*Spores formed on a basidium, utricle absent, spores 12–16 x 7–9 µm broadly ovoid, hyaline to dark brown-yellow with minute (<1 µm) spines, peridium bright chrome-yellow, gleba light to dark brown and gelatinous .....* ***Mycoamaranthus auriorbis***

*Spores formed on a basidium, utricle absent, spores 16–22 x 7–10 µm (average 18 x 8 µm), ellipsoid with ornaments that are prominent longitudinal ridges rounded in polar view, peridium bright canary yellow, gleba cinnamon brown .....*
..... ***Gautieria amara***

*Spores formed on a basidium, utricle absent, spores fusiform ellipsoid with ornaments of minute spines or warts, peridium tightly adherrent to the surrounding soil and rootlets* ***Chondrogaster*** ..... 1

1. Spore length/width ratio Q = 2.0–3.0, isolated warts or smooth ..... 2

1. Spore length/width ratio Q < 2, prominent ornaments/spore coating ..... 4

2. Spores fusiform and warty 12 x 4 µm, Q = 2.7–3.0, peridium white and consisting of dirt and rootlets, gleba light olive-green..... ***C.* spB**

2. Spores ovate, tapering only at the base..... 3

3. Spores 10 x 5 µm, most spores with warts, Q = 2.0–2.4, peridium bruising dark pink and consisting of dirt and rootlets, dark olive-green gleba..... ***C.* spA**

3. Spores 12 x 6 µm and smooth, Q = 2.1, peridium consisting of gummy mycelium, gleba pale green..... ***C.* spC**

4. Spores surrounded by long (5 µm) crowded ornaments and enclosed by a utricle, oval spores 13 x 8 µm, Q = 1.5, peridium consisting of gummy mycelium, orange-brown gleba..... ***C.* spD**

4. Spores enclosed with a thick amorphous covering making it difficult to detect the spore outline, oval spores 15 x 9 µm, Q = 1.8, peridium white and encrusted with soil, gleba dark brown/black..... ***C.* spE**

*Spores formed on a basidium, utricle absent, spores ellipsoid with ornaments of minute spines or warts, peridium consisting of gummy mycelium surrounding the spore mass, columella present* ***Gummiglobus***..... 1

1. Spores ellipsoid and ornamented 9–13 x 4–6 µm, Q = 2–2.1, cinnamon brown spore mass, pulverant at maturity..... ***G. joyceae***

1. Spores sub-fusoid to ellipsoid and predominantly smooth 10–13 x 4–5 µm, Q = 2.5–2.7, olive green spore mass, adherrent at maturity..... ***G.* spB**

*Spores formed on a basidium, utricle absent, spores ellipsoid with ornaments of minute spines or warts, peridium consisting of gummy mycelium surrounding the spore mass,columella absent, gleba a powdery spore mass lacking veins of gummy tissue* ***Castoreum*** ..... 1

1. Q < 2, majority of spores with warts, ellipsoid 9–15 x 5–8 µm, (mean: 11 x 7 µm) Q = 1.6–1.8, cinnamon brown spore mass..... ..... ***C. infimiratio sp. nov.*** **(Trappe provisional key 2003)**

1. Q>2, majority of spores smooth..... 2

2. Spores large, 13–17 x 5–8 µm (mean: 16 x 7 µm), Q = 2.4, some spores with low warts, spore mass cinnamon brown..... ***C. tasmanicum***

2. Spores small and smooth 8–12 x 3–5 µm (mean: 10 x 4 µm), Q = 2.3, spore mass olive green.....***C. sublaeve sp. nov.*** **(Trappe provisional key 2003)**