A comparison of the fungal diets of sympatric small mammals and the methods used to study them

A Thesis submitted by

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ABSTRACT

Fungi play a significant role in ecosystems as decomposers, nutrient recyclers, plant pathogens and as symbionts of plants. Knowledge of how fungi function and interact within their given habitat is essential to understanding ecosystem functioning and will inform management approaches. The dispersal of fungi can be mediated by wind, water or by animals through consumption, an interaction known as mycophagy. Mammalian mycophagists are important in many ecosystems, particularly in the Australian context. However, the methods used to study the fungal diets of mammals remain inconsistent. The project aimed to compare the fungal diets of sympatric small mammal species in south-east Queensland. Additionally, this project aimed to compare spore morphology and DNA metabarcoding, the two predominant methods used in studies of this kind, in their efficiency and effectiveness. Mammals were live trapped to collect scat samples. Spore morphology analysis was conducted using light microscopy with spore traits examined using an existing key and comparison with known taxa observed at field sites. DNA was extracted from scat samples and sent for sequencing at the Australian Genome Research Facility (AGRF) with returned sequences phylogenetically analysed. The two methods were compared by analysing the fungal species richness able to be identified in each sample and a cost efficiency analysis was conducted encompassing cost and time taken to achieve fungal identifications. All mammals tested were shown to consume fungi and two novel mycophages were identified with 19 taxa identified through spore morphology analysis and 20 taxa identified through DNA metabarcoding. Results differed slightly in the species and functional groups of fungal taxa detected between the two methods with DNA metabarcoding revealing a higher median richness of fungi detected in samples but cost-efficiency showed no significant differences between the methods. The research presented here shows that mammals are important vectors for fungal dispersal in southeast Queensland, with the results highlighting that all mammal species considered are comparably mycophagous. It is hoped that this will further highlight the need for dynamic environmental management approaches and that further research will be conducted into small mammal mycophagy.

CERTIFICATION OF THESIS

I Georgia Fox certify that the Honours Thesis entitled *A comparison of the fungal diets of sympatric small mammals and the methods used to study them* is my own work except where otherwise acknowledged, with the majority of the contribution to the chapters presented as manuscripts undertaken by me. The work is original and contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma.

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Student and supervisors' signatures of endorsement are held at the University.

STATEMENT OF CONTRIBUTION

Paper 1: Review article

Fox, G., Dearnaley, J. & Edwards, M. (2025). Mammalian Mycophagy in the Australian Context: Importance, Prevalence and Methodology, A Review, *Austral Ecology* (to be submitted)

Student contributed 90% to this paper. Collectively Dr Meg Edwards and Associate Professor John Dearnaley contributed the remainder.

Paper 2: Original research article

Fox, G., Dearnaley, J. & Edwards, M. (2025). Fungal consumption by sympatric small mammals in southeast Queensland, *Australian Mammalogy* (to be submitted)

Student contributed 80% to this paper. Dr Meg Edwards contributed 15% and Associate Professor John Dearnaley contributed 5%

Paper 3: Original research article

Fox, G., Dearnaley, J., & Edwards, M. (2025). Looking at the scat, man: comparing scat analysis methods for studies of small mammal mycophagy, *Ecology and Evolution* (to be submitted)

Student contributed 90% to this paper. Collectively Dr Meg Edwards and Associate Professor John Dearnaley contributed the remainder.

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Additionally, I would like to acknowledge the Queensland Mycological Society and all my fellow citizen scientists within this group for their suggestions, questions and technical knowledge when discussing the project.

Lastly, I would like to thank my family and friends for their unwavering support. I thank them for indulging me on late nights talking about fungi and animals and why I think they should be more interested. I acknowledge that I would never have been able to complete a project like this without an amazing group of people surrounding me.

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CHAPTER 1: INTRODUCTION TO PROJECT

1.1. Background Information

The role of fungi in ecosystem functioning cannot be understated. Fungi play a number of important roles in decomposition, nutrient cycling (Pouliot & May 2010), as plant pathogens (Carnegie 2007) and as mutualistic organisms associated with plants (Brundrett 2004). In particular, mycorrhizal fungi contribute to the ongoing health of ecosystems by forming symbioses with plants, providing water and nutrients to their host in exchange for sugars (Stuart & Plett 2019). As a result, the overall health of plant communities is strengthened by these associations.

The colonisation of new hosts and environments by mycorrhizal fungi is predicated on reproduction and spore dispersal, particularly over long distances (Golan & Pringle 2017). Animal-mediated dispersal is a key driver of this, with the consumption of fungal fruiting bodies by animals, hereafter ‘mycophagy’, contributing significantly to spore dispersal (Vasutova et al. 2019).

A large proportion of existing research on mammalian mycophagy regards obligately mycophagous species (Elliott et al. 2022), with comparatively little consideration given to opportunistic fungi consumers. In the Australian context, there has been a continuing interest in this area of research, with the literature beginning to consider the role opportunistic mycophages play (Nest et al. 2025). Whilst the contributions of obligate mycophages to fungal dispersal cannot be understated, opportunistic mycophagous species also have a role to play in this process, particularly in areas where obligate species are absent (Tory et al. 1997)

However, studies of this kind can be inconsistent in their methodology making it difficult to compare results across regions and internationally (Elliott et al. 2022). The majority of studies employ some form of spore morphology analysis, whether using faecal sample material or stomach contents (Decker et al. 2023; Elliott et al. 2025). Samples are prepared and mounted as microscope slides which are subsequently examined for the presence of spores which are identified to the lowest taxonomic level possible (Reddell, Spain & Hopkins 1997; Nest et al. 2023). In recent years, an increasing number of papers employ DNA metabarcoding to determine the fungal taxa consumed by mammals (Nuske et al. 2018; Bradshaw et al. 2022). This method has been shown experimentally to produce clear and consistent results for fungal identifications from faecal samples (Cloutier et al. 2019). However, papers published

as recently as July 2025 still employ spore morphology techniques (Nest et al. 2025), potentially due to the high costs involved with DNA metabarcoding. A global review on studies of mammalian mycophagy has called for more consistency in methodology, particularly with the increased accessibility to DNA sequencing (Elliott et al. 2022).

1.2. Contribution to Broader Field

The number of fungal taxa identified in scat samples will be compared across mammalian species. This analysis will contribute to a broader understanding of the role mammalian mycophages play in ecosystems. It is hoped that the comparison will quantify the contributions made by various species to the dispersal of fungal spores in a given ecosystem. In future, this data may also contribute to modelling that predicts spore dispersal distance spread by mammal species (Danks et al. 2020). A greater understanding of the presence and contribution of mycophagous species to ecosystem functioning will hopefully also contribute to more comprehensive habitat analyses and inform management practices.

This project will also use DNA metabarcoding in addition to spore morphology analysis and will aim to compare the two methods in effectiveness and intensity of resource use. This will help to highlight gaps in previous research (Cloutier, et al. 2019) and to confirm the suitability of DNA metabarcoding for the study of mammalian mycophagy. It is hoped that insights from this comparison will inform future studies of mycophagy by highlighting the advantages and disadvantages of existing methods. If the methodology used in studies of this kind can be made consistent across regions, larger data sets could be collated, and meta-analysis of these results could be conducted. This could have significant implications for our understanding of how mycophagous mammals contribute to ecosystem functioning as well as the dispersal and distributions of mycorrhizal fungal taxa (Bradshaw et al. 2022).

1.3. Objectives and Hypotheses

This project aimed to describe the fungal diets of four small mammal species from sympatric habitat types at two sites in southeast Queensland. In addition, the project aimed to compare two methods used in studies of mammalian mycophagy, namely spore morphology analysis and DNA metabarcoding of scat samples. The methods

will be compared to determine which is most efficient and accurate in studies of this kind.

It is hypothesised that the small mammal species examined in this study will be highlighted as opportunistic mycophages and that species not previously known to consume fungi will have some fungi present in their diets. It is expected that the DNA metabarcoding analysis will be more accurate for fungal identification and provide greater time efficiencies than spore morphology analysis. It is also hypothesised that DNA metabarcoding will reveal higher fungal species richness in the diet than spore morphology analysis.

A review manuscript was prepared and it highlighted several existing gaps in mammalian mycophagy research, particularly in the Australian context. Several Australian mammal species and families which are known to eat fungi but have not had their fungal diets investigated thoroughly. The review also highlighted the need for consistent methodology both in spore morphology and DNA metabarcoding analysis techniques as current protocols differ between research groups.

1.4. Scope and Limitations

The work described in this thesis is based on methodological considerations for studies of mammalian mycophagy, but the fungal diets of the species involved will also be described and compared. Novel data may emerge regarding the status of some species as mycophages, as well as regarding fungal taxa consumed by mammals in southeast Queensland. The methods which were employed and are considered in this thesis are based on existing methodology in the literature and were refined in consultation with other researchers.

Due to the time considerations associated with the completion of this thesis, there are some limitations to the scope of the work described herein. Data collection occurred over a relatively short period of time and therefore the effect of seasonality on both consumption rates of fungi and the fungal taxa consumed cannot be compared. Cost and time limitations also influence the number of samples able to be analysed for inclusion in this thesis.

The scope of the review manuscript covers mammalian mycophagy research in Australia within the broader context of mycology research. The review is limited by the relatively small number of Australian studies published on mammalian mycophagy and the different methods used within these studies. As such a narrative

review was selected rather than a meta-analysis to avoid misinterpretation of data collected using different protocols.

1.5. Overview

This thesis is comprised of five chapters, including the introduction, a review manuscript, two manuscripts based on the original research carried out to be submitted for peer-review and publication, and a brief conclusion. The introduction highlights the context in which this research was conducted. Chapter Two, comprising a narrative review manuscript, focuses on the existing research regarding mammalian mycophagy in an Australian context. The review also aims to heighten the profile of this body of research whilst also exploring existing gaps as a direction for further study. It is necessary to highlight the importance of mammalian mycophagy research to provide incentive for the calls to action for future research outlined in the review, and to emphasise the impacts this research could have on multiple disciplines such as mycology and wildlife ecology. Chapters Three and Four discuss the primary research project around which this thesis is based and are presented as two research article manuscripts for submission.

CHAPTER 2: PAPER 1 - Fungi and Mammals in Australia: The Importance of Mycophagy for Conservation and Directions for Future Research

2.1 Introduction

The review manuscript is to be submitted to *Austral Ecology* for consideration for publication. This is a narrative review with a focus on mammalian mycophagy in the Australian context. A narrative review style was chosen for this paper rather than a systematic review because a comprehensive systematic global review of mammalian mycophagy was published recently (Elliott and Truong et al. 2022). Some consideration was given to conducting a meta-analysis, however due to inconsistencies in methodology within the published research, meta-analysis was ultimately not suitable for the purposes of this review. There has been consistent publication of studies on mammalian mycophagy from Australian researchers (Vernes & Dunn 2009; Nuske et al. 2018), many of which were discussed in the global review referred to above (Elliott and Truong et al. 2022). However, our review places Australian research as the primary focus rather than as a subset of global research. The review aims to highlight the significance of mycophagy as an ecological interaction in the context of broader mycological research in Australia. It is hoped that through highlighting this, further consideration will be given to animal vectors in future mycological research and heightened attention will be paid to these interactions in studies of community ecology from both wildlife ecology and mycology perspectives. The review also includes articles which were published after the 2022 review (Elliott and Truong et al. 2022) and covers research published as recently as October 2025 (Quah et al. 2025).

The author guidelines for *Austral Ecology* can be found at the following link: https://onlinelibrary.wiley.com/page/journal/14429993/homepage/forauthors.html

Fungi and Mammals in Australia: The Importance of Mycophagy for Conservation and Directions for Future Research

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The authors declare no conflicts of interest.

Fungi and Mammals in Australia: The Importance of Mycophagy for Conservation and Directions for Future Research

A review manuscript

12/11/2025

Dear Dr Andrew,

We wish to submit an original research article entitled “Fungi and Mammals in Australia: The Importance of Mycophagy for Conservation and Directions for Future Research” for consideration by *Austral Ecology.*

We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

In this paper, we review the existing body of literature on mycophagous mammals in Australia within the broader context of Australian mycology research. It is hoped that this review will prompt interest from both wildlife and mycology researchers. The review highlights several gaps in the current literature and calls for further research into these areas.

We believe that this manuscript is appropriate for publication by Austral Ecology as it addresses ecosystem interactions which are vital to continued ecological health as well as the conservation of mammals, fungi and the communities they inhabit. The review focuses specifically on the Australian context for this area of research and highlights the importance of understanding intra-species relationships in terrestrial ecosystems.

By dispersing fungi, particularly mycorrhizal taxa which form symbiotic associations with economically and environmentally important plant species, mammals ensure the continued health of the ecosystems in which they live. A better understanding of the existing state of play in studies of mammalian mycophagy will help ecologists to act upon these insights in broader assessments of ecosystem health and to continue to advocate for conservation.

We have no conflicts of interest to disclose.

Please address all correspondence concerning this manuscript to Dr Meg Edwards at the University of Southern Queensland through the following email address meg.edwards@unisq.edu.au

Thank you for your consideration of this manuscript.

Sincerely,

Georgia Fox

Fungi and Mammals in Australia: The Importance of Mycophagy for Conservation and Directions for Future Research

Abstract

Mammalian mycophagy is an important ecosystem interaction which facilitates dispersal of fungal spores. This interaction has been studied well both globally and in an Australian context. Animal-mediated spore dispersal is particularly important for hypogeous ectomycorrhizal (ECM) taxa due to their subterranean habit. This review examines the context of mycological research in Australia, the existing research on mammalian mycophagy, the mammal groups which have been studied and the methodology used to do so. Additionally, the review aims to summarise the body of research on native Australian mycophagous mammals and to highlight its importance in the broader context of mycological research. Gaps in the existing research, such as species which lack any published data on their fungal diets and inconsistent research methodology, are discussed, Additionally, the disproportionality of studies published on obligate mycophages compared to opportunistic mycophages is examined. In highlighting these gaps, the authors hope to provide information which will inform further studies, as well as to cement mycophages as keystone species and argue for their conservation and consideration in broader ecosystem assessments.

Key Words

Fungi, Ecological Methods, Community Ecology, Spore Dispersal

Introduction

Mammalian mycophagy plays an important role in maintaining ecosystem function by contributing to fungal spore dispersal, particularly for (truffle-like) hypogeous taxa. Not only are hypogeous fungi important food sources for a variety of mammal species, many form symbiotic myccorhizal partnerships with plants promoting resilience through the increased provision of water and nutrients. Additionally mycorrhizal fungi confer other beneficial traits to their plant hosts such as disease resistance and salinity tolerance (Brundrett & Tedersoo 2018). Without the perpetuation of these fungal-plant relationships by mammalian vectors it is

hypothesised that several types of terrestrial ecosystems would be threatened due to impaired regeneration, reduced productivity and overall species diversity decline (Maser et al. 2008).

This review aims to synthesise the existing research on mammalian mycophagy in an Australian context and identify where this body of research sits within the broader global field of fungal ecology. In particular, the literature reviewed focuses on the importance of mycophagy as an ecosystem interaction and for spore dispersal, the mammal groups for which there is existing research, and the methodology used to gather data and determine fungal taxon identifications. Fungi play a variety of roles in ecosystems as saprotrophs, pathogens, mutualistic partners of plants and as food sources for animals (Buchanan & May 2003; Frew et al. 2025). The intrinsic ecological value of fungi and, by extension, mycophagy in analyses of ecosystems has historically been overlooked, particularly in Australian research (Pouliot & May 2010). This review will highlight the urgent need for further research into mammalian mycophagy in Australia, particularly in light of local extinctions for critical weight range mammals, including obligately mycophagous species (Short 1998). The gaps in the literature highlighted throughout the review may provide a strategic starting point for future research.

Fungal Research in Australia

The State of Play

The kingdom Fungi has long been overlooked in the field of ecology and is only tacitly referred to in scientific literature and governmental publications when compared with fauna and flora. This is particularly true in Australia where the estimated diversity of fungal species is between 50,000 to 250,000 and the number of formally described species is estimated to be 11,846 (Pouliot & May 2010). The true diversity of fungi in Australia is difficult to ascertain, with many of the type specimens collected in the 19th and 20th centuries only known to occur at the collection location (Buchanan and May 2003). A singular theme emerges among the wide variety of studies published on fungi in Australia, which is that there is always a need for more research. It should be noted that studies of Australia's macrofungi, which produce large fruiting structures such as mushrooms for the purposes of spore dispersal, are being published at an increasing rate. These include taxonomic

reviews (Guard et al. 2024) and studies of fungal diversity in some ecosystems (Packham et al. 2008; Gates et al. 2011). Researchers are also developing methodology for consistent data collection and databases to improve recognition of Australian fungi in the global context (Frew et al. 2025).

Most fungi are protected under federal and state laws in Australia, however very few are referred to in the legislation which often omits the term fungi, where flora and fauna are explicitly referred to (Buchanan & May 2003). It has also been historically difficult to include fungal species in formal threat lists such as the International Union for Conservation of Nature (IUCN) Red List due to the existing requirements for threat level classifications. The assessment criteria for the IUCN red list are designed for flora and fauna species. Whilst there are difficulties with the assessment criteria which hinder efficient and appropriate listing for the plant and animal species, these difficulties are heightened for fungal species given their ephemeral and sometimes cryptic nature. For example, there is no current benchmark for survey efforts to establish rarity (Buchanan & May 2003). However, this is set to change with the IUCN setting up a Microbial Conservation Specialist Group which will aim to appropriately assess conservation priorities for microbial organisms including fungi (Gilbert et al. 2025). More data on fungal species presence can also now be collected using eDNA, and databases have been created to collate this data in accessible forms for future research (Bissett et al. 2016). As such, it is important that research into the fungal kingdom is continued and broadened in scope, particularly in the face of a changing climate (Xue et al. 2025).

Much of the research on fungi conducted in Australia regards plant pathogenic fungi (Hyde et al. 2010; Kiss et al. 2020). Long-term field studies of fungal diversity in Australia are rare but where available may also focus on plant pathogenic fungi (Carnegie 2007), with limited focus on species that are symbiotic or pose no specific threat to agriculture (Gates et al. 2011). Research into fungal ecology and the collection of presence/absence records is primarily undertaken by citizen science groups in place of institutions who struggle to obtain funding for studies in these fields (Pouliot & May 2010). Groups such as the Australian Mycological Society, FungiMap and state-based organizations conduct organised forays at a variety of locations and organize national citizen science projects such as the annual ‘Great

Aussie Fungi Hunt' with an aim to increase the number of occurrence records and overall knowledge of Australian fungal species (FungiMap 2025). Australian mycology, therefore, relies heavily on the continued interest and dedication of citizen scientists. This model has limitations as many groups are restricted by resources and access to emerging technologies such as DNA metabarcoding. As a result, the knowledge and records collected by these groups relate primarily to the macrofungi that are easily observed in field situations. Despite their apparent diversity (Bougher & Lebel 2001), knowledge of hypogeous, mycorrhizal and other cryptic species remains sparse in the Australian context (Pouliot & May 2010; Egidi et al. 2019).

The Role of Mycorrhizal Fungi

Mycorrhizas are complex symbiotic associations between fungi and plants and may be defined as a symbiotic relationship between a fungus and a living plant wherein the fungus supplies the plant with increased uptake of nutrients and water in addition to other benefits in exchange for sugars supplied by the plant (Brundrett 2004). Mycorrhizal fungi which colonise the roots of host trees confer many benefits to the host including increased drought and salinity tolerance, as well as increased nutrient acquisition and uptake (Stuart & Plett 2019). In return, the colonising fungus receives carbon in the form of sugars from the host (Stuart & Plett 2019). This relationship contributes significantly to the health of ecosystems, and it is estimated that over 250,000 plant species rely on mycorrhizal fungi (van der Heijden et al. 2015)

There are several types of mycorrhizal fungi. The most prominent worldwide, are the arbuscular mycorrhizal (AM) fungi (Mathieu et al. 2018). AM fungi belong to the Glomeromycota and form complex hyphal structures called arbuscules inside the roots of their host plants. The complexity of the arbuscules results in an increased surface area for the fungus to facilitate nutrient exchange (Farhaoui et al. 2025). AM fungi are broad in distribution and are prevalent in most ecosystems in the tropical to temperate zones and are particularly important for agricultural production (Zhang et al. 2019).

The second type of mycorrhiza is formed by ectomycorrhizal (ECM) fungi. ECM fungi do not form intracellular associations as with AM fungi but instead form a mantle of hyphal connections with the exterior of the plant host's roots known as a Hartig net

(Stuart & Plett 2019). In Australia, ectomycorrhizal fungal associations are more common than other mycorrhizal associations, and Australia is a hotspot for ECM associations with approximately 33% of species globally (Brundrett & Tedersoo 2018).

The remaining two prominent types of mycorrhizal fungi are the ericoid and orchid mycorrhizas. Ericoid mycorrhizas are extremely specialized and are only formed between plants of the order Ericaceae and fungi of the order Leotiales (Cairney 2000). Orchids are initially myco-heterotrophic, meaning they must form associations with fungi at the early stage of their life in order to survive (Brundrett 2004). Orchid mycorrhizal fungi also penetrate the roots of their hosts to facilitate nutrient exchange (Cameron, Leake & Read 2006).

The majority of predominant Australian forest trees form ectomycorrhizal associations, including *Eucalyptus* spp. and *Acacia* spp. (Bougher & Lebel 2001). Many widespread genera of fungi are ECM colonisers of Australia's dominant tree species, including *Amanita, Cortinarius,* and *Russula.* Species of these genera may form either (i) above-ground, epigeous, or (ii) below-ground, hypogeous, fruiting bodies. ECM hypogeous fungi are more diverse in some southern hemisphere locations than in the entirety of the northern hemisphere (Bougher & Lebel 2001). Whilst ECM associations are more prominent in Australia, AM associations still play a critical role in a variety of native ecosystems as well as in agricultural cropping land (Zhang et al. 2019; Frew et al. 2025).

Dispersal of Fungi

There are three primary methods of fungal spore dispersal which are primarily discussed in the literature – wind, water, and animal mediated dispersal. Wind-mediated dispersal or anemochory is perhaps the most discussed method of spore dispersal and is considered to be a widespread dispersal strategy in the fungal kingdom (Peay et al. 2012). Some of the mould-forming fungi such as *Penicillium* spp. produce lightweight conidia which can be picked up and carried by air currents (Segers et al. 2023). Other species of fungi, particularly those belonging to the Basidiomycota, may employ forcible spore discharge methods when conditions are better suited for anemochory (Pringle et al. 2005). This is particularly important for

many ectomycorrhizal species who produce fruiting bodies at ground level. In these species, changes in surface tension caused by wind, trigger forcible discharge of spores from the basidia, resulting in spores being carried further than could be achieved with passive spore dispersal (Pringle et al. 2005).

Water-mediated dispersal or hydrochory is more commonly employed in aquatic and damp environments and is an essential perpetuation strategy for some fungal species (Yafetto et al. 2008). Members of the Chytridiomycota, for example, have motile spores which require sufficient water/moisture to facilitate movement through the environment (Gleason et al. 2008). Other fungi which colonise marine environments are often only known from spores with dispersal relying almost exclusively on hydrochory (Golan & Pringle 2017). Arbuscular mycorrhizal fungi spores have also been recorded to be dispersed by water, with spores found in freshly deposited river sediments in areas where vegetation is not yet established (Yafetto et al. 2008). Hydrochory is less prominent in literature surrounding the dispersal of ectomycorrhizal fungi, however it is likely that some ECM spores are dispersed by water where it is an available vector (Pickles et al. 2012).

Animal-mediated dispersal or zoochory can be achieved by endozoochory, that is the consumption of fungi and subsequent dispersal of spores through faeces, or ectozoochory, the dispersal of fungal spores on the exterior of an animal (Vasutova et al. 2019). Both arbuscular mycorrhizal fungi and ectomycorrhizal fungi have been shown to be dispersed by animals, although there is a stronger focus in the literature on ECM dispersal by animals with only a few examples noted of AM zoochory (Bueno & Moora 2019; Paz et al. 2021).

Mammalian Mycophagy in Australia

Importance of Mycophagy

The consumption of fungi by animals, hereafter ‘mycophagy’, is an important interaction contributing to efficient ecosystem functioning (Decker et al. 2023; Elliott et al. 2023). Through consumption, animals can spread fungal spores further than the fruiting body itself (Johnson 1996). This contributes to colonisation of fungi in new areas which can have significant impacts, particularly in early successional ecosystems (Johnson 1996; Komur et al. 2021). Mycophagy can also significantly

alter fungal composition of ecosystems as animals spread both native and invasive fungal species (Soteras et al. 2017). As such, a thorough understanding of this interaction is essential for both fungal and wildlife ecologists. Because of the ecological roles fungi play in ecosystems, the dispersal of fungi by mycophages can have a significant influence on ecosystem health. This may be particularly true in fragmented or highly disturbed landscapes, where mycophagous mammals may be one of the few remaining vectors contributing to fungal dispersal and colonisation. It should be noted that several classes of animal are known to participate in mycophagy, including birds (Costa et al. 2024), reptiles (Cooper & Vernes 2011), members of the phylum Mollusca (Ori et al. 2021) and the mammals (Elliott and Truong et al. 2022). Whilst the importance of these interactions with multiple classes of organisms cannot be understated, the focus for the remainder of this review will be on mycophagy with reference to Australian mammals. Several species of Australian mammal have been shown to consume fungi, often significant amounts (Elliott et al. 2025). These mammals should be considered keystone species due to their mycophagous habits, however apart from studies focusing specifically on these interactions, fungal-mammal relationships are rarely given consideration in broader studies of community ecology.

Mycophagy is particularly important for fungi with sequestrate and secotioid forms. As aforementioned, many of these fungi cannot forcibly or passively discharge spores as with their above-ground counterparts. However, there are several advantages for producing hypogeous fruiting bodies, including superior protection from the elements and therefore a longer ‘lifespan’, as well as a decreased likelihood of being interfered with or consumed by animals prior to sporulation (Claridge 2002). Historically, the suggestion was that fungi initially existed as truffle-like fruiting bodies and evolved epigeous forms – however recent work has shown that many of the hypogeous species may have evolved from epigeous forms (Nilsen et al. 2024). In tandem with this, it has been argued that mammalian mycophagy has been a substantial driving factor in the evolution of a diverse range of hypogeous fruiting species (Elliott and Truong et al. 2022).

Mycophagy by mammals is an important means of spore dispersal for many fungi species. Quantifying and comparing successful dispersal between the various

strategies is difficult as there are a variety of factors contributing to successful transport and eventual germination of spores. Spores relying on abiotic dispersal can travel long distances (Golan & Pringle 2017), however the majority of spores produced by a sporocarp do not, with only 5% of spores achieving a dispersal distance of greater than one metre (Golan & Pringle 2017). As such mammalian mycophagy may contribute to successful dispersal over longer distances when compared with other strategies due to the likelihood of high numbers of spores being consumed and transported by animals throughout their home ranges (Reddell, Spain & Hopkins 1997). It is difficult to know if being deposited in faeces assists with colonisation of new substrates, however, there is some evidence to suggest improved spore germination rates following their passing through mammal digestive tracts (Colgan & Claridge 2002).

Mammalian mycophagy confers other benefits to ecosystems aside from the dispersal of fungi. Where sequestrate fruiting bodies are consumed, digging behaviours to access the fungi are observed. Digging mammals contribute significantly to the bioturbation of soils, resulting in increased aeration and organic matter decomposition (Newell 2008). For mammals that consume truffle-like taxa, the depth of fruiting bodies may impact an animals' ability to detect and/or reach the fruiting body (Vernes & Lebel 2011). Notably, a previous study has shown that the depth of the fruiting body in the soil may contribute to the types and amounts of volatile organic compounds (VOC) produced (Allen & Bennett 2021). Rodents have also been shown to select for deeper fruiting truffle species with distinct VOC profiles (Stephens 2020).

Digging behaviours exhibited by mycophagous mammals can prevent soils from becoming hydrophobic and contributes to nutrient cycling in ecosystems (Decker et al. 2023), the increased decomposition rate of leaf litters can also lessen fuel load during bushfires (Palmer et al. 2020). The scale at which mammals dig differs between species, but many Australian mammals have been shown to displace and turn-over significant quantities of soil (Robley 2001, Hopkins et al. 2021), for example burrowing bettongs have been shown to turn-over up to 30 tonnes per individual per year (Robley 2001; Newell 2008; Hopkins et al. 2021). Digging to access fruiting bodies can also disperse other soil organisms both at the dig site and

through ectozoochory (Dundas et al. 2018). This likely assists in maintaining habitat suitability for a variety of species, including the fungi being consumed.

Mammal Groups Studied

Mammalian mycophagy is not a purely Australian phenomenon. Many mammal species around the world are known to consume fungi. North America has been a centre of research into mammalian mycophagy and has many species which have been noted as consumers of fungi, this includes a high diversity of mycophagous rodents (Elliott and Truong et al. 2022). American red squirrels (*Tamiasciurus hudsonicus*) have been recorded eating up to 89 species of fungi (Fogel & Trappe 1978) and are known to carefully dry fungal fruiting bodies, including truffles, to cache them for later use (Vernes & Poirier 2007). Despite an overwhelming majority of research into mycophagy occurring in North America (Elliott and Truong et al. 2022), there has been a solid body of mycophagy research published regarding Australian mammals, examples of which can be seen in Table 1.

Table 1: Notable Australian mammal species previously examined in studies of mycophagy or with fungi recorded in their diet.

Family	Species	Reference
Potoroidae	*Aepyprymnus rufescens*	Reddell, Spain & Hopkins 1997
	Bettongia gaimardii	Johnson 1996
	Bettongia lesseur	Newell 2008
	Bettongia penicillata	Garkaklis, Bradley & Wooller 2004
	Bettongia tropica	Nuske et al. 2018
	Potorous gilbertii	Bougher, Friend & Bell 2008; Quah et al. 2025
	Potorous longipes	Nuske et al. 2017
	Potorous tridactylus	Tory et al. 1997; Vernes & Jarman 2011
Peramelidae	*Isoodon fusciventer*	Hopkins et al. 2021; Elliott et al. 2023
	Isoodon macrourus	Reddell, Spain & Hopkins 1997
	Isoodon obesulus	Maclagan et al. 2020
	Perameles gunnii	Elliott et al. 2023

	Perameles nasuta	Reddell, Spain & Hopkins 1997
Muridae	*Melomys burtoni*	Elliott and Elliott et al. 2022
	Melomys capensis	Elliott and Elliott et al. 2022
	Melomys cervinipes	Elliott and Elliott et al. 2022
	Pseudomys fumeus	Nuske et al. 2017
	Pseudomys novaehollandiae	Vernes & Dunn 2009
	Pseudomys oralis	Elliott et al. 2020; Elliott and Elliott et al. 2022
	Rattus fuscipes	Elliott and Elliott et al. 2022; Quah et al. 2025
	Rattus lutreolus	Elliott and Elliott et al. 2022
	Rattus tunneyi	Reddell, Spain & Hopkins 1997
	Uromys caudimaculatus	Elliott and Elliott et al. 2022
	Zyzomys argurus	Elliott and Elliott et al. 2022
Dasyuridae	*Antechinus flavipes*	Nest et al. 2023; Nest et al. 2025; Vernes 2007
	Antechinus godmani	Reddell, Spain & Hopkins 1997
	Antechinus mimetes	Nest et al. 2025
	Antechinus minimus	Nest et al. 2025
	Antechinus stuartii	Nest et al. 2023; Nest et al. 2025
	Sminthopsis murina	Nest et al. 2023
Macropodidae	*Notamacropus parma*	Elliott & Vernes 2020a
	Notamacropus rufogriseus	Vernes & Jarman 2011
	Petrogale penicillata	Vernes 2007
	Setonix brachyurus	Quah et al. 2025
	Thylogale stigmatica	Elliott & Vernes 2020a
	Thylogale thetis	Elliott & Vernes 2020a
	Wallabia bicolor	Danks et al. 2020
Phalangeridae	*Trichosurus caninus*	Claridge & Lindenmayer 1998
	Trichosurus vulpecula	Elliott et al. 2025
Burramyidae	*Cercartetus nanus*	Vernes & Dunn 2009

Much of the historical focus in this area of research in Australia has been on members of the Potoroidae. Many members of this family are obligately mycophagous with large amounts of fungi forming part of their natural diet (Reddell,

Spain & Hopkins 1997; Tory et al. 1997; Vernes & Jarman 2011). For example, the Tasmanian Bettong has been shown to have a diet of up to 90% fungi during times of peak availability of fruiting bodies (Johnson 1996). Consequently, these species are seen as ecosystem engineers and a significant body of literature has examined their fungal diets in detail. Members of the Peramelidae have been examined at length regarding their role as mycophages and are also considered to play a significant role in fungal dispersal. For example, a 1997 study found comparable fungal diversity in scats collected from *I. macrourus* and *P. nasuta* when compared with *A. rufescens* (Reddell, Spain & Hopkins 1997). More recently, increased attention has been paid to the role of smaller mammals such as the murid rodents in studies of mycophagy. The most comprehensive study of fungal diets in Australian rodents was published in 2022 and found that 38 unique taxa were consumed across 10 species of native rodent (Elliott and Elliott et al. 2022). However, with over 60 native rodent species in Australia, further research is needed to fully understand their importance as vectors for fungal dispersal. Similarly, the Dasyuridae have also been examined more closely as mycophages in recent years. Species such as *Antechinus flavipes* and *A. stuartii* have historically been known to consume fungi (Reddell, Spain & Hopkins 1997; Vernes 2007). However other members of this genus such as *A. mimetes* and *A. minimus* were only confirmed as mycophagous in 2025 (Nest et al. 2025). This demonstrates that gaps in the research remain and that we do not yet have a full understanding of the roles Australian native species play in dispersing fungi within and between ecosystems.

Some invasive mammal species in Australia are known to be mycophagous and likely contribute to fungal spore dispersal. Invasive pigs, *Sus scrofa,* have been shown to disperse fungal spores in Patagonia, Argentina (Soteras et al. 2017) and are speculated to do the same in Australia (Laurance & Harrington 1997) although no studies outline the type and diversity of taxa consumed in Australian ecosystems. Introduced fallow deer, (*Dama dama*) have been shown to consume and disperse *Cyttaria gunii,* an ascomycete fungus associated with Nothofagaceae plants in New Zealand (Nugent 1990). Similarly to pigs, they are hypothesised to consume and disperse fungi in Australia also (Forsyth & Davis 2011) but have not had their fungal diets examined in Australian ecosystems. Black rats (*Rattus rattus*) have been shown to consume a high diversity of fungi at a New South Wales site where they

are considered an introduced pest (Vernes & McGrath 2009). As such, the role invasive mammal species play may need to be considered in assessments of fungal diversity and dispersal, particularly in habitats where native species are declining or absent, such as in highly urbanised environments.

Methodology for Studying Mycophagy

Prominent Methodology

Several methods have been used to identify fungi in mammalian diets. Of the research papers considered in this review which used faecal material, 23 employed spore morphology, five DNA metabarcoding and one study used both techniques identify fungi. Typically, studies using spore morphology analysis apply a variety of methods to wash spores out of the scat sample and filter out other larger particulate matter (Tory et al. 1997; Elliott and Elliott et al. 2022; Nest et al. 2023). Identification of fungi from spores alone can be difficult, particularly in regions where detailed knowledge of fungal species distributions is absent (Elliott and Truong et al. 2022). Similar methodology for spore morphology analysis has been employed for stomach samples collected from wet specimens in museum collections (Vernes & Lebel 2011; Elliott & Vernes 2020b; Elliott et al. 2023). Identification keys can be helpful to some extent and multiple studies refer to a spore key developed with special reference to mammalian mycophagy (Castellano et al. 1989). However, many studies still record ‘unknown’ spore types in their data (Claridge & Lindenmayer 1993; Maclagan et al. 2020; Nest et al. 2023). Whilst this is not necessarily problematic for studies with a primary focus of identifying fungal diversity in diets as unknown species are often given monikers (e.g. Unknown 1) (Nest et al. 2023), the opportunity to increase knowledge of fungal species occurrence, particularly for hypogeous taxa, may be lost in these cases.

Observational data collected in field situations has also been referred to in the literature, although often anecdotally in broader studies. Previous studies have employed camera traps specifically to detect mycophagous behaviour (Vernes & Jarman 2011; Vernes, Smith & Jarman 2014), whilst others have reported mycophagous behaviour observed incidentally from camera traps employed for other purposes (Elliott & Vernes 2020a). Camera traps have been used most often when the purpose is to examine detectability of mycophagous mammals, particularly

through bait preference tests (Vernes & Jarman 2011; Claridge, Paull & Cunningham 2016). However, the use of camera traps for studies of mammalian fungal consumption is limited and cannot provide consistent data on the type or diversity of fungi consumed due to both the ephemeral nature of fungal fruiting bodies as well as methodological considerations of camera trap usage.

Emergence of DNA Metabarcoding

Recently, studies of mammalian mycophagy which employ DNA metabarcoding for fungal identification have emerged in the literature. This technique has been employed for scats collected passively and identified fungal species through DNA sequencing in Canada (Cloutier et al. 2019). Similar techniques have also been employed successfully in the Australian context (Nuske et al. 2018; Kanishka et al. 2025; Quah et al. 2025), however DNA sequencing remains expensive, and studies published as recently as 2025 continue to employ spore morphology analysis (Elliott et al. 2025). DNA metabarcoding can be cost-prohibitive whereas microscopy of spores is relatively inexpensive, particularly for institutions who already have the necessary equipment. The identification of fungal DNA in scats is also not necessarily evidence of direct consumption as there are many species of coprophilous fungi that may colonise faecal pellets and many mammal species may also consume mycophagous insects resulting in secondary consumption (Elliott and Truong et al. 2022, Quah et al. 2025). DNA results are also likely to contain pathogens, yeasts and other gut microbiotic taxa (Quah et al. 2025). However, the use of DNA techniques may be more definitive than spore morphology in cases where morphotypes are unable to be identified. It is also assumed that DNA analysis is more efficient in terms of the person-hours required to identify species, however there is little in the literature currently to quantify this.

Analysing Dispersal and Distribution

Most Australian mammals have recorded estimates for home range, being the area an animal lives in and moves through periodically, but there may not be detailed data available regarding individual movements and regular distance travelled. In response to this gap in the research, Danks et al. (2020) published a method to model the dispersal of fungal spores by mammalian mycophages, using the swamp wallaby as a model organism. This involved capturing and subsequently tracking individuals and

the use of previously collected spore gut passage data to statistically model dispersal distance (Danks et al. 2020). This methodology provides a good baseline to determine potential spore dispersal distance and could be applied to other mycophagous species. Previous work has also shown that fungi respond strongly to environmental cues such as aridity and bioturbation (Decker et al. 2023) which may influence spore viability after dispersal.

Whilst there have been several studies in the Australian context comparing diversity of vascular plants and macrofungi (Packham et al. 2008; Gates et al. 2011), vectors of fungal dispersal, including mycophagous mammals, are rarely considered. This misses a crucial element of ecosystem function, with previous work demonstrating that up to 88% of fungal species in soil samples are also found in scat samples of co-occurring mycophagous mammals (Nuske et al. 2019). Comparative studies conducted in fenced reserves are useful for monitoring the impact of mycophagous mammals on fungal species diversity. Previous work at Karakamia Sanctuary in Western Australia showed that ectomycorrhizal species were dominant within the fenced reserve where woylies are present, whereas outside of the fence the fungal species were functionally absent and AM taxa were dominant (Dundas et al. 2018). It has also been suggested that mycophagous mammals may contribute significantly to the dispersal of mycorrhizal fungi that associate with Australian native orchids as well as *Eucalyptus* spp. (Dearnaley & Le Brocque 2006). However, there are no large-scale studies of the effectiveness of mammal-mediated spore dispersal and further work is needed to determine the role mycophagous mammals play in influencing fungal diversity on a broader scale.

Conclusion

Mammalian mycophagy plays an important role in dispersing and perpetuating fungal species. Whilst some Australian mycophagous mammal species such as the northern brown bandicoot (*I. macrourus*) and long-nosed bandicoot (*P. nasuta*) are species of ‘least concern’ for conservation under current legislation (Woinarski & Burbidge 2016), others such as the northern bettong (*B. tropica*) are endangered and declining (Woinarski & Burbidge 2016). Given their important contributions to fungal dispersal and ecosystem services, endangered mycophagous species are important to consider in ecosystem regeneration and protection projects. It becomes

increasingly clear that conservation of these mammal species is unlikely to be achieved without considering the fungi and vice versa. This is also true for those species of least concern and mycophagous relationships should warrant consideration during ecosystem surveys and assessments, particularly in areas where obligately mycophagous species are now absent.

Many native Australian mammals are known to eat fungi but have not had their fungal diets examined to determine the species and diversity consumed. The most well studied mycophages in Australia tend to be obligately mycophagous, such as the bettongs and potoroos. However, it is difficult to know how much opportunistic mycophages contribute to fungal spore distribution. Notably, many of the small mammals including several species of rodent and dasyurid have not been included in studies of mycophagy. The methods used to study mammalian mycophagy must be examined and standardised across institutions and/or regions so that data is comparable, this should be done for both spore morphology analysis and DNA metabarcoding. Researchers have an opportunity with the advent and increasing accessibility of DNA metabarcoding to develop consistent protocols for studies of mammalian mycophages. However, it is noted that spore morphology analysis will remain important until such a time as DNA metabarcoding is less cost-prohibitive, and it is recommended that standard protocols for this type of study be developed and published. Standardisation of methods will then allow for broad-scale meta-studies of available data regarding fungal species richness in mammal diets as well as quantification of the level of mycophagy displayed between mammal species. Building on this, further research into using these methods for modelling fungal dispersal and distributions should be encouraged to gain a better understanding of fungal ecology and biodiversity in Australia, particularly for more cryptic species which are difficult to survey using traditional methods. Furthering knowledge in this field, particularly on a broad-scale and using consistent methodology, will help to provide evidence for mycophagous species as keystone species and will substantiate the need for increased conservation action to protect not only the mammal species themselves but the ecological communities they help to perpetuate.

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CHAPTER 3: PAPER 2 – Fungal consumption by sympatric small mammals in southeast Queensland

3.1 Introduction

This manuscript is a research paper comparing the fungal diet of four small mammal species across two properties in southeast Queensland. Fungal species richness data is statistically compared across both mammal species and group (i.e. rodent, marsupial). Previous studies of mammalian mycophagy in Australia have been primarily conducted in the southern states (Vernes & Jarman 2011) or in far north Queensland (Nuske et al. 2018). This study contributes to the literature by examining and reporting the fungal taxa consumed by two rodents, including one known mycophage, and two antechinus species within a southeast Queensland context. Understanding not only which taxa of fungi are consumed and spread by mammals, but also the diversity of fungal species consumed is essential to a holistic understanding of the ecosystems inhabited by these mammals. If preferences are shown for specific fungal taxa this is likely to influence the make-up of complex plant communities due to their mycorrhizal symbiont preference, thus significantly influencing their own habitat as ecosystem engineers. It is hypothesised that all mammal species in this study will be shown to consume fungi to varying degrees and that the three opportunistic mycophages may consume fungi on a comparable level with the obligately mycophagous species. This manuscript is intended for submission to the journal *Australian Mammalogy*.

The author guidelines for *Australian Mammalogy* can be found at the following link: https://connectsci.au/am/pages/author-instructions

3.2 Original research manuscript 1

Fungal consumption by sympatric small mammals in southeast Queensland

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The authors declare no conflicts of interest.

Fungal consumption by sympatric small mammals in southeast Queensland

A research article manuscript

20/11/2025

Dear Dr Goldingay,

We wish to submit an original research article entitled “Fungal consumption by sympatric small mammals in southeast Queensland” for consideration by *Australian Mammalogy*.

We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere. We confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

In this paper, we examine the fungal diets of four sympatric small mammal species across two properties (four sites) in southeast Queensland. We identify two species of Antechinus (*Antechinus mysticus* and *A. subtropicus*) as novel mycophages. Notably, Bush Rats (*Rattus fuscipes*) have historically been considered the ‘most’ mycophagous of the native rodents however, we identify that fungi consumption of all mammals considered here as comparable.

We believe that this manuscript is appropriate for publication by Australian Mammalogy as it contributes to the broader knowledge base of mammalian mycophagy in Australia. The dispersal of fungi, particularly mycorrhizal taxa, by mammals is important in maintaining ecosystem health. The research presented here demonstrates that there is still more to learn about these ecologically significant interactions between fungi and mammals. It is hoped that this study will inspire further research into mammalian mycophagy particularly for mammal species which are not necessarily considered to be mycophagous.

We have no conflicts of interest to disclose.

Please address all correspondence concerning this manuscript to Dr Meg Edwards at the University of Southern Queensland through the following email address meg.edwards@unisq.edu.au

Thank you for your consideration of this manuscript.

Sincerely,

Georgia Fox

Fungal consumption by sympatric small mammals in southeast Queensland

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Abstract

Mammalian mycophagy, or the act of a mammal consuming fungi, is an important ecological interaction which leads to the dispersal of fungal spores, including both arbuscular mycorrhizal and ectomycorrhizal species. The dispersal and subsequent germination of these spores by mammals contributes to not only the continued existence of the fungus and the mammal itself but to the ongoing maintenance and diversity of the ecosystems they inhabit. At present, research into Australian mammal mycophages has been primarily based in southern states and in far-north Queensland, with very little research occurring in the subtropics. This study presents data on the fungal diets of four sympatric small mammal species in a mixture of subtropical notophyll vine forest and wet sclerophyll across two properties in southeast Queensland during autumn of 2025. Individuals were trapped using Elliott traps and scats collected on first capture for subsequent extraction and analysis of fungal spores. The study includes one well-known mycophagous species, the bush rat, (*Rattus fuscipes*), and three species with mixed data availability regarding fungal diet; fawn-footed melomys (*Melomys cervinipes*), subtropical antechinus (*Antechinus subtropicus*), and the buff-footed antechinus (*A. mysticus*). Generalised Linear Mixed Models were used to compare fungal species richness across species and between rodent and marsupial groups. No statistically significant difference in fungal species richness was found between mammal species, but a slight difference was noted between groups using a significance level of p=0.05. Our results indicate that the small mammals in this study consume a comparable diversity of fungi and that fawn-footed melomys and *Antechinus* spp. warrant further consideration as mycophages.

Additional Keywords

Antechinus, Community Ecology, Dasyuridae, Diet, Fungivory, Mycophagy, Rodentia, Spore Morphology

Introduction

The act of mammals consuming fungi, hereafter referred to as mycophagy, is an important ecosystem interaction which contributes to the spread and colonisation of fungal species (van der Heijden et al. 2015). This is particularly important for ectomycorrhizal species with hypogeous fruiting habits, as animal vectors feeding on these fruiting bodies spread fungal spores much further than the fungus itself could achieve (Vasutova et al. 2019). Mycorrhizal partnerships between fungi and their plant hosts are essential to ecosystem functioning as they confer many benefits to both partners (Claridge 2002) and perpetuate the ongoing existence of complex plant communities (Brundrett 2004). The digging behaviours employed by mammals to access fungal fruiting bodies, also confer other benefits to ecosystems such as increased soil health (Decker et al. 2023). Many Australian mammals are known to be mycophagous with several, such as the Rat-Kangaroos (Family: Potoroidae), regarded as obligately mycophagous with fungi comprising up to 90% of diet during certain times of the year (Tory et al. 1997). Other taxa of native Australian mammals known to be mycophagous include several other species of macropod, possums, dasyurids and rodents (Elliott et al. 2022; Nest et al. 2025). Implementing understanding of these interactions into environmental management practices could guide appropriate conservation actions.

Whilst many mammal species have been recorded consuming fungi, there is insufficient data available on the type and diversity of fungi in their diets. Fine scale understanding of these relationships is also lacking in some geographic locations and ecosystems. This study aimed to compare the diversity of fungal taxa consumed by four sympatric small mammal species in southeast Queensland. The ecosystems in this geographical area are important to study due to their conservation status, world heritage value and proximity to urban development, particularly in the face of a changing climate. Much of the Australian research into mammalian mycophagy focuses on areas in New South Wales, Victoria and Far North Queensland (Vernes & Jarman 2011; Elliott et al. 2022). The mammal species considered in this study

included two species of native rodent, bush rats (*Rattus fuscipes*) and fawn-footed melomys, (*Melomys cervinipes*) as well as two species of antechinus, subtropical antechinus (*Antechinus subtropicus*) and buff-footed antechinus (*A. mysticus*). The data collected for this study will contribute to the broader understanding of mycophagy in Australian mammals by highlighting the contributions of animals in the critical weight range to spreading a diversity of both ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungal species. It is hypothesised that all species will consume fungi and that, when compared statistically, they will consume a comparable diversity of fungal taxa.

Materials and Methods

Trapping and Sample Collection Methods

Small mammal surveys were conducted across two private properties at Bellthorpe (26°49'44"S 152°42'28"E) and Mount Byron (27°12'22"S 152°40'33"E) in southeast Queensland in Autumn of 2025 following significant rainfall; approximately 1030.8 mm total for January to April, which was the sixth wettest Autumn on record (Bureau of Meteorology 2025). Both properties contain gradients of subtropical notophyll vine forest and wet sclerophyll forest. Two sites were selected at each property and were at least 500 m apart and were chosen as they contained a mixture of typical arbuscular mycorrhizal (AM) fungi associated plants (e.g. *Neolitsea* spp.) and typical ectomycorrhizal (ECM) fungi associated plants (e.g. *Eucalyptus* spp.) (Neldner et al. 2023).

Each site had 50 Elliott traps (30 x 10 x 9 cm, Elliott Scientific, Upwey VIC) set in transects for 4–5 nights. Traps were set late in the afternoon and were baited with standard bait (peanut butter and oats). Traps were checked early in the morning, and mammals were identified and temporarily marked prior to release to identify recaptures. As much scat as was available was collected from each individual upon their first capture and was stored frozen in 2 mL microfuge tubes using a portable cooler prior to laboratory analysis. Once in the laboratory, scat was stored at -20˚C. Scat was collected using forceps whilst wearing gloves. The amount of scat used for further analysis from each sample was later standardised. Traps had all scat removed and were cleaned between captures to prevent cross-contamination of scat samples.

A total of 50 samples, 25 from each property, were selected for further analysis. Samples chosen for each property consisted of ten bush rat, ten fawn-footed melomys and five antechinus scats (with antechinus species grouped for analysis).

Slide Preparation and Spore Morphology Analysis

Approximately two whole faecal pellets from each sample were prepared following methodology outlined in Nest et al. (2023). Pellets were macerated in 5% KOH and were then rinsed through a 125 µm aperture sieve using sterile water. Filtrate (approximately 20 µL) from the scat solution was placed onto a slide which was placed on a slide warmer until dry. Slides were mounted with 80% glycerol and a coverslip. Slides were then systematically scanned at 400× magnification using a Nikon E600 photomicroscope to check for spores. Images were taken of three random fields of view for subsequent analysis. Images were labelled with their sample ID but not with species name to minimise observer bias when examining the presence of spore types in images.

Spores were identified to the lowest taxonomic level possible (e.g. Russulales 1) using the key developed by Castellano et al. (1989) as well as through morphological comparison with known spore characteristics for macrofungal species observed during the trapping period using existing images. Spore types that could not be identified were given consistent labels (e.g. Unknown 1). Spore types which only appeared once were not counted and to avoid bias, spore types were only considered in the analysis if they appeared in at least 2 fields of view for each sample. The exception was AM fungi spore types which were considered as part of the analysis if found in one field of view. This is due to the substantially larger size of these spores which were in some cases only partially visible in the field of view at the 400× magnification level.

Statistical Analysis

To compare overall fungal species richness data between species and group (i.e. rodent, marsupial), Generalised Linear Mixed Models (GLMM) were fitted using the *lme4* package (Bates et al. 2015) in R (version 4.5.1) with a Poisson distribution. Models were checked for overdispersion (dispersion ratio ~ 0.82), and site was included in the models as a random effect to account for site-to-site variation. Estimated marginal means (EMMs) for species and groups were obtained using the

emmeans package (Lenth et al. 2025) and pairwise comparisons were performed. The EMMs were then back-transformed to the response scale (rather than log scale) for interpretation and visualisation. The significance value for all statistical tests was set at p = 0.05.

Fungal composition differences between species and groups were also conducted to determine if different species or groups consumed different types of fungi. Samples that contained no fungi were removed for this analysis. This analysis used non-metric multidimensional scaling (NMDS) based on a Jaccard dissimilarity matrixes for both species and group calculated using presence-absence data. This was done using the *vegan* package (Oksanen et al. 2025) to visualise patterns in fungal community similarity. During initial NMDS ordination of fungal composition, one *Melomys cervinipes* sample was identified as an extreme outlier (Fig. 4a-b). This sample contained only two unique taxa (*Glomeromycota and Unknown 1*), resulting in near-complete dissimilarity from all other samples. To address this, the outlier was removed and NMDS were rerun. The revised ordination (Fig. 5a-b) showed tighter clustering and reduced distortion, and thus the outlier was not included in further compositional analysis.

To test for differences among species and group, permutational multivariate analysis of variance (PERMANOVA) were conducted using the ‘adonis2’ function with 999 permutations.

Results

Fungal taxa consumed

All species considered in this study were shown to consume fungi, with 19 unique fungal taxa observed across all samples (Table 1). Of these 19 taxa, two were identified to genus level, six were identified to family level, four were identified to order, two were identified to phylum and five spore types were unable to be identified past Kingdom level. Of the 50 samples, only ten individuals were not considered to have consumed fungi according to the protocol outlined in the methods above, although the authors note that spores were also visible in these samples but not in multiple fields of view. Example images of fungal spores can be seen below in Fig. 1a-1b

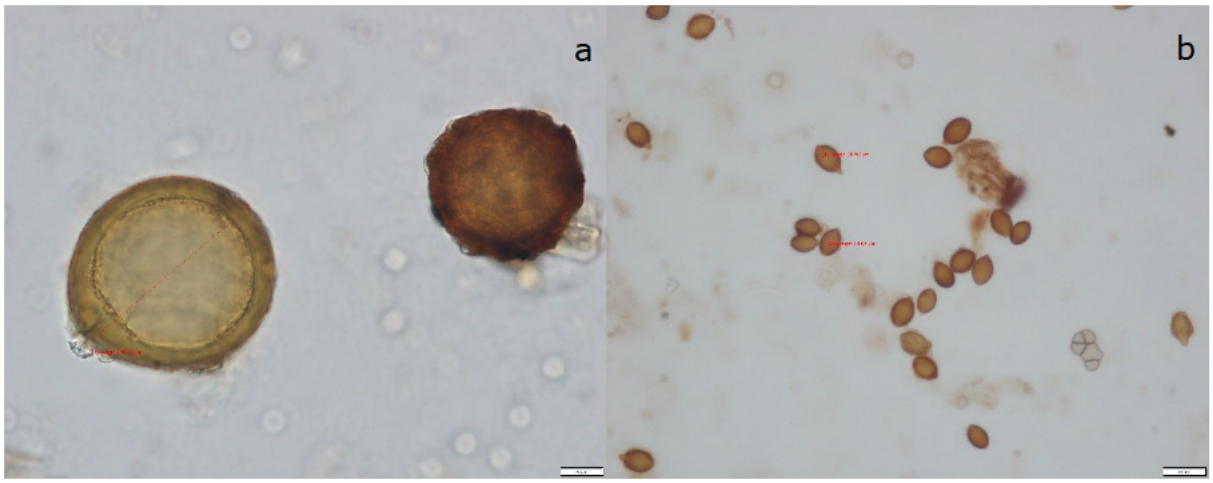

Figure 1: (a) Glomeromycotaspores suspended from *R. fuscipes* scat sample and (b) Cortinariaceae spores suspended from *R. fuscipes* scat sample. 20 µm white scale bars shown in bottom right corner of each image.

Table 1: Fungal taxa consumed by mammal species across all sites at the Bellthorpe and Mount Byron Properties, presence of taxa in mammal species diet indicated by ×

Fungal Taxa	Melomys cervinipes	Rattus fuscipes	Antechinus subtropicus	Antechinus mysticus
Russulales 1	×	×	×	
Russulales 2	×	×		×
Glomerales 1	×	×	×	
Glomerales 2		×		
Glomeromycota	×			
Scleroderma 1		×		
Scleroderma 2	×	×		
Cortinariaceae 1	×	×	×	
Cortinariaceae 2	×	×		
Cortinariaceae 3	×			

Psathyrellaceae 1	×	×		×
Psathyrellaceae 2			×	×
Physalacriaceae	×	×		
Basidiomycota		×		
Unknown 1	×			
Unknown 2		×		
Unknown 3		×		×
Unknown 4				×
Unknown 5		×	×	
Total Number of Taxa Consumed	11	14	5	5

Fungal species richness

Fungal species richness in the diet did not show statistically significant differences across species. Mean predicted fungal species richness was slightly higher for *R. fuscipes* (2.15 types of fungi per scat) compared to *M. cervinipes* (1.2) and *Antechinus spp.* (1.4) (Fig. 2). For the group comparison, marsupials had a slightly lower mean predicted richness (1.4) than rodents (1.68) (Fig. 3). However, results of the pairwise comparisons following post-hoc tests between both species and group yielded no significant differences on the response scale (Table 2).

Table 2: Results of post-hoc pairwise comparisons for species and group.

Comparison	Estimate	SE	z-ratio	P-value
Antechinus sp. – Melomys. cervinipes	0.154	0.336	0.4583774	0.891
Antechinus sp. – Rattus. fuscipes	-0.429	0.308	-1.3941630	0.344

Melomys cervinipes – Rattus. fuscipes	-0.583	0.255	-2.2886529	0.057
Marsupial – Rodent	-0.179	0.294	-0.610	0.5417

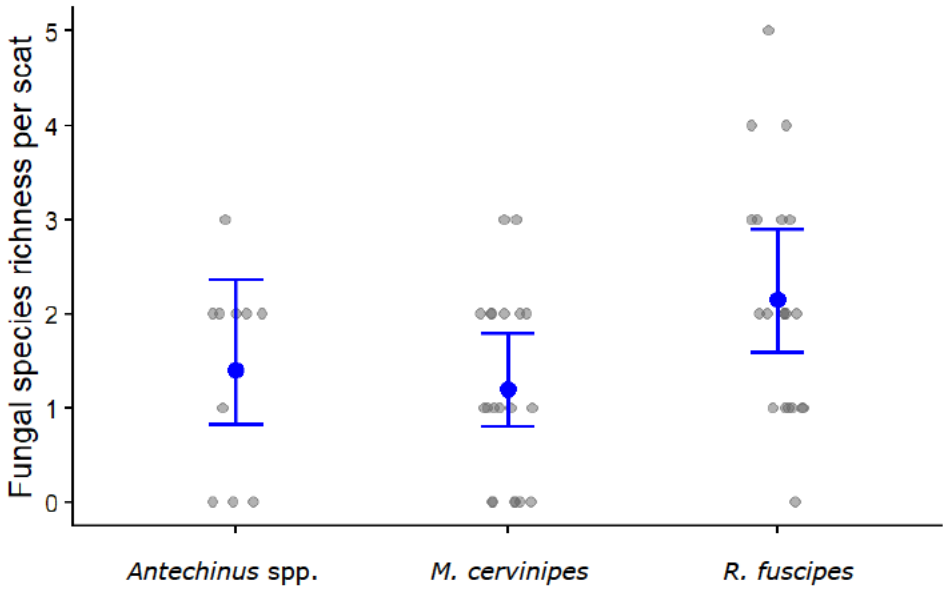

Figure 2: Fungal species richness across mammalian species, mean and 95% confidence intervals shown in blue, with raw data of fungal taxa represented by grey dots

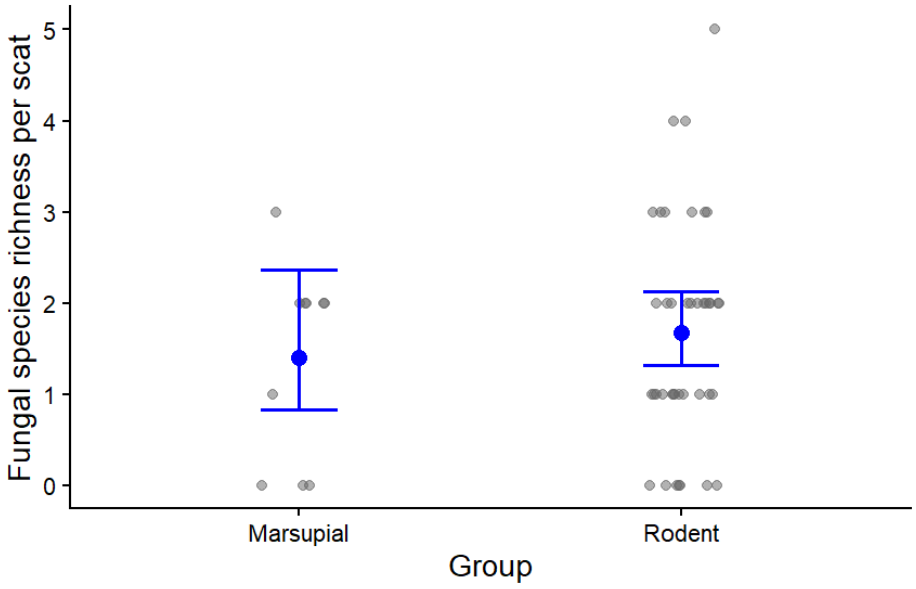

Figure 3: Fungal species richness across mammalian group, mean and 95% confidence intervals shown in blue, with raw data of fungal taxa represented by grey dots

Fungal Composition Analysis

NMDS plots initially included an outlier with minimal clustering shown, however following the removal of the outlier, revised NMDS ordinations showed clustering for both group and species (Fig. 4a-d).

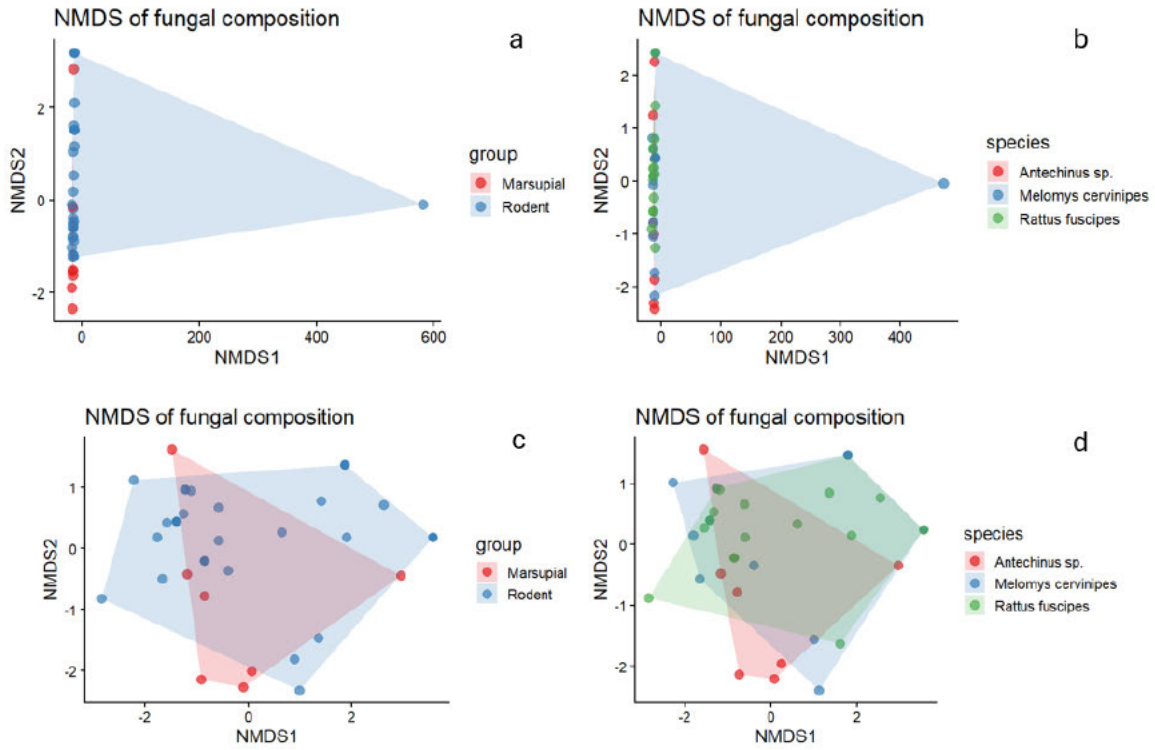

Figure 4: NMDS plots representing Bray-Curtis dissimilarity matrices for fungal taxa consumed by (a) group with outlier present; (b) species with outlier present; (c) group with outlier removed showing broader ordination of the rodent group; and (d) species with outlier removed showing tight clustering of all species fungal diets.

A statistically significant difference in dietary fungal composition was observed between mammalian group (p=0.019), but not between species (p=0.083) (Table 3).

Table 3: Summary of PERMANOVA results for fungal species richness across species and group using 999 permutations

Variable	Df	Sum of Squares	R^2	F	P-value
Species	2	1.702	0.07625	1.5271	0.083
Group	1	0.8699	0.05668	2.2834	0.019

Discussion

This study highlights that several species of sympatric small mammals in southeast Queensland consume a comparable diversity of fungal species, including 2 novel mycophages. It should be highlighted that the bush rat is a historically well noted mycophage (Vernes & Dunn 2009) but that when compared with another rodent and small marsupial species, no statistically significant difference was noted in the diversity of fungi consumed. A 2022 study of rodent mycophagy on Australia's east coast considered both *R. fuscipes* and *M. cervinipes* and also found comparable fungus consumption across habitats (Elliott et al. 2022). Our findings for the two rodent species in a southeast Queensland context are consistent with the results outlined in this study (Elliott et al. 2022).

One *M. cervinipes* individual consumed two completely unique taxa, not consumed by any other individuals in the study, and was considered an outlier for the purposes of NMDS ordination. Reasons for this drastic difference in taxa consumed are unclear as the individual was captured in areas adjacent to other melomys individuals observed in this study. As such, the fungal taxa observed in this sample were considered genuine observations and the data point was still considered as part of the PERMANOVA. For transparency NMDS of the Bray-Curtis dissimilarity matrices with this data point included were still reported. In line with these results, the fawn-footed melomys warrants further consideration in discussions of mammalian mycophagy and should be studied further in this context.

The overall diversity of fungal taxa in *Antechinus spp.* diets was lower than that of the rodent species and a slight but statistically significant difference was found between the rodent and marsupial groups ($p=0.019$). The NMDS plot demonstrated a broader range of ordination space for the rodent group, with the taxa consumed by the Antechinus exhibiting clustering over a smaller area. This suggests a more limited range of fungal taxa in the antechinus species diet. A recent study compared the fungal diets of four different antechinus species (Nest et al. 2025) and the number of taxa consumed by those species was comparable with the number of taxa consumed by the species considered in our study. Elliott et al. (2025) recorded the following number of fungal taxa in antechinus diets; *A. flavipes* (4), *A. mimetes* (3), *A. minimus* (4), and *A. stuartii* (14). It should be noted that Elliott's study was

conducted using preserved museum specimens, so collection methodology differs slightly to our study. However, even with smaller sample sizes of the two antechinus species considered here, comparable fungal taxa diversity was observed with *A. subtropicus* and *A. mysticus* consuming 5 fungal species each. As far as the authors are aware, this is the first recorded evidence of mycophagy for both *Antechinus subtropicus* and *A. mysticus*. Despite being considered 'carnivorous' marsupials, dasyurid species, particularly those in the critical weight range, warrant further consideration and study as mycophagous mammals. Although not consuming as high a diversity of fungi as rodents, the contributions these species make to fungal dispersal are still important to maintaining ecosystem functioning with two of the taxa identified in this study being found only in antechinus samples and not in rodent samples.

The fungal species which have been identified here include both ectomycorrhizal and arbuscular mycorrhizal taxa which are important symbionts for many Australian plant species, including those noted at the survey sites. Species from the Cortinariaceae family and Russulales order for example, are both important ECM symbionts of *Eucalyptus* spp. (Bougher 1994). Both the Cortinariaceae and the Russulales have hypogeous and epigeous fruiting species, but as species identifications for the taxa observed in this study were unable to be achieved, the nature of their fruiting bodies remains unknown. However, the presence of AM taxa in the diets of both rodent and marsupial species indicates that the small mammals considered here are likely to consume both hypogeous and epigeous taxa. The spread of spores by mammal vectors is important for both epigeous and hypogeous fruiting fungi, however the importance is heightened for hypogeous taxa, which are unable to be dispersed significant distances by other means. Previous studies of mammalian mycophagists have indicated that the digging behaviours associated with consumption of hypogeous taxa also confer numerous benefits to soil health including increased aeration, drainage and nutrient cycling (Fleming et al. 2013; Dundas et al. 2018).

Not all taxa consumed were mycorrhizal fungi, with all mammal species consuming saprotrophic fungi from the Psathyrellaceae family also. Whilst the majority of previous mammalian mycophagy research has focused on the dispersal of mycorrhizal spores, the dispersal of saprotrophic fungi is also an important

ecosystem service. Saprotrophic fungi are important nutrient recyclers, breaking down plant materials such as litter and wood and returning the nutrients to the soil (Lebreton et al. 2021). Previous instances of mammalian mycophagy of fungal saprotrophs have been noted (Vernes, Cooper & Green 2015), however this interaction is more prevalent in studies of invertebrates (Santamaria, Verbeken & Haelewaters 2023).

The effect of seasonality on fungal consumption by mammals has been shown experimentally in several studies (Tory et al. 1997; Pyare & Longland 2001; Vernes, Cooper & Green 2015). Our study offers a snapshot in time, providing a picture of fungus consumption across four small mammal species during the sixth-wettest autumn on record for southeast Queensland (Bureau of Meteorology 2025). The effect of seasonality on small mammal mycophagy should be investigated further, particularly with reference to hypogeous taxa which are difficult to detect using traditional survey techniques but contribute significantly to ecosystem functioning (Bougher & Lebel 2001). Not only this but gaining further understanding of fine scale fungal dietary preferences will be influential in understanding how fungi move through ecosystems and establish in new locations. So far modelling of fungal dispersal by mammals in Australia has only been conducted for one species, the swamp wallaby (*Wallabia bicolor*) (Danks et al. 2020), but consideration should be given to smaller mammals for future modelling studies so that these fine scale relationships can be better understood.

Conclusion

Our study highlights the fungal diet of four small mammals in southeast Queensland and provides further evidence for the role of mammals in fungal spore dispersal. In particular, it highlights that fawn-footed melomys and *Antechinus* spp. consume a comparable diversity of fungi when compared with bush rats, historically considered to be the most mycophagous of the Australian native rodent species (Vernes & Dunn 2009). It also provides the first evidence for both *Antechinus subtropicus* and *A. mysticus* as mycophages. Both ECM and AM fungi spores were prevalent in scat samples, as were saprotrophic taxa, highlighting that mammals contribute to the dispersal of both mycorrhizal symbionts and fungal decomposers. The movement of fungal spores by mammals on a fine scale is not only important for the perpetuation

of fungal species but also for painting an interconnected picture of ecosystem health. Studies of this kind can provide valuable insights about both animal dietary preferences and fungal ecology. We hope that the research presented here will prompt further investigation into small mammal mycophagy with particular reference to rodent and dasyurid species as well as modelling of fungal dispersal by these mammal vectors. Understanding these relationships could contribute to explaining where degraded, riparian and regenerating ecosystems require more targeted management, particularly where obligately mycophagous mammals are absent, and provide an ecological argument for species and ecosystem conservation.

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CHAPTER 4: PAPER 3 – Looking at the scat, man: comparing scat analysis methods for studies of small mammal mycophagy

4.1 Introduction

The research manuscript presented below discusses methodological considerations for studies of small mammal mycophagy. Two methods which have previously been employed in studies of this kind (Cloutier et al. 2019; Nest et al. 2023) are used to obtain fungal species richness data for the four mammal species considered. The species richness data is then statistically compared between methods. Cost-efficiency analysis is also included in the manuscript to provide useful information regarding the advantages and disadvantages of each method. It is hoped that the results from this analysis could be used to inform methodology selection for future research. This manuscript is intended for submission to the journal *Ecology and Evolution.*

The author guidelines for *Ecology and Evolution* can be found at the following link: https://onlinelibrary.wiley.com/page/journal/20457758/homepage/forauthors.html

4.1 Original research manuscript 2

Looking at the scat, man: comparing scat analysis methods for studies of small mammal mycophagy

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The authors declare no conflicts of interest.

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Looking at the scat, man: comparing scat analysis methods for studies of small mammal mycophagy

A research article manuscript

20/11/2025

Dear Dr Moore,

We wish to submit an original research article entitled “Looking at the scat, man: comparing scat analysis methods for studies of small mammal mycophagy” for consideration by *Ecology and Evolution*.

We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere. We confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

In this paper, we employ two methods to analyse the fungal diets of four sympatric small mammal species and compare these methods in effectiveness for detection and identification of fungal taxa from scat samples. Additionally, we also conduct a cost-efficiency analysis to determine whether either method is more resource intensive.

We believe that this manuscript is appropriate for publication by Ecology and Evolution as it addresses key methodological considerations for studies of mammalian mycophagy. These ecosystem interactions between fungi and mammals are vital to continued ecological health. By dispersing fungi, particularly mycorrhizal taxa which form symbiotic associations with economically and environmentally important plant species, mammals ensure the continued health of the ecosystems in which they live. This research article will provide key insights into how these interactions may be studied and will hopefully assist researchers in determining which methodology suits their purpose best.

We have no conflicts of interest to disclose.

Please address all correspondence concerning this manuscript to Dr Meg Edwards at the University of Southern Queensland through the following email address meg.edwards@unisq.edu.au

Thank you for your consideration of this manuscript.

Sincerely,

Georgia Fox

Looking at the scat, man: comparing scat analysis methods for studies of small mammal mycophagy

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Abstract

Understanding the fungal diets of small mycophagous mammals is important to ascertaining a broader understanding of ecosystem functioning. Traditionally, studies of mammalian fungal diet have employed spore morphology techniques involving the identification of fungal taxa using microscopy to analyse spores retrieved from scat. More recently, the use of DNA metabarcoding has been employed to determine the fungal taxa present in scat samples. This study compared these two methods by assessing the fungal diets of four sympatric small mammal species. Samples were collected from live-trapped animals and 28 out of 50 scat samples were selected for further analysis as only 28 of the 50 samples had fungal DNA detected. The samples were subdivided with half used for spore morphology analysis and the remainder used for DNA metabarcoding. A comparison of time and resource costs of the methods using a cost-efficiency analysis was performed. In total, 19 unique fungal taxa were identified using spore morphology analysis and 20 unique taxa were identified using DNA metabarcoding. DNA metabarcoding revealed a higher median richness of fungal taxa in samples than spore morphology analysis. There were no significant differences in cost efficiency between methods. The results presented here may be used to inform future research approaches for studies of mammalian mycophagy.

Additional keywords

Dasyuridae, DNA Metabarcoding, Rodentia, Spore Morphology

Introduction

Small mammal mycophagy has emerged as an important interaction which has significant implications for plant and fungal communities as well as ecosystem

functioning (Dundas et al. 2018; Bradshaw et al. 2022). The methods used to research these interactions fall broadly into two categories. Historically, papers published in this area of research have employed some form of spore morphology analysis from faecal samples to determine fungal diet composition (Tory et al. 1997; Nest et al. 2023). It should also be noted that whilst this methodology is used broadly, laboratory techniques and analysis parameters are inconsistent in the published literature (Maclagan et al. 2020; Elliott et al. 2025), for example where some studies may use potassium hydroxide to macerate and mount spores onto microscope slides (Nest et al. 2023), where others use water alone (Vernes & McGrath 2009).

More recently, DNA metabarcoding has been employed to determine the presence and identity of fungal species from mammal scats (Cloutier et al. 2019; Quah et al. 2025). DNA metabarcoding has broad applications for studies of community ecology in both aquatic and terrestrial ecosystems (Yuan et al. 2025) including species/community monitoring programs (Liu et al. 2025), pollutant and ecosystem health monitoring (Li et al. 2018), and broader dietary studies of specific organisms (Guillerault et al. 2017; Buglione et al. 2018). Whilst this method has also proved successful for studies of mammalian mycophagy (Nuske et al. 2018; Bradshaw et al. 2022), it remains potentially cost-prohibitive due to the high costs of sequencing, and recently published papers continue to employ morphological identifications of spores as the primary method for determining fungal diets (Nest et al. 2025).

However, the difficulty of identifying fungi from spores alone is a consistent theme in existing research of this kind (Reddell, Spain & Hopkins 1997). It has been suggested that some combination of the two methods may be preferable for future research to mitigate the potential failings of either method when solely used (Elliott and Truong et al. 2022). Therefore, this study aimed to compare the two methods by comparing the fungal diet data collected from two rodent and two antechinus species in south-east Queensland, Australia. The time and resources used for both methods are also considered, and a cost-benefit analysis conducted. It is hypothesised that DNA metabarcoding will identify a greater number of fungal taxa from scat samples and that spore morphology analysis will be the less cost-efficient of the two methods. The results of this study will provide guidance surrounding methodology for future

research into mammalian mycophagy with a view to standardising methods for ease of comparison and data sharing on a broader scale.

Materials and methods

Sample Collection and Processing Methods

Two properties in southeast Queensland had small mammal surveys conducted in May of 2025. To ensure effective comparison of the two methods being considered 50 scat samples, 25 from each property, were selected. Samples chosen for each site consisted of scats collected from ten bush rat (*Rattus fuscipes*), ten fawn-footed melomys (*Melomys cervinipes*) and five antechinus scats (*Antechinus mysticus* and *A. subtropicus*). As much scat as was available for each individual was collected and each sample was then halved, with sub-samples prepared for analysis using one of the two methods.

For spore morphology analysis, samples were prepared and analysed as per the methodology outlined in Fox et al. (2025) (Chapter 3). For the DNA metabarcoding, the Qiagen DNeasy PowerLyser PowerSoil Kit was used according to manufacturer's instructions to extract DNA from up to 0.25 g of scat per sample. Where insufficient or extremely thick supernatant was present, steps 2–7 as outlined in the protocol instructions were repeated. Concentrations were checked using a DeNovix DS11+ spectrophotometer to ensure that they met minimum concentration requirements. Extracted DNA (approximately 20 µL for each sample) was stored frozen (-20˚C) prior to being sent to the Australian Genome Research Facility for sequencing using their PacBio Revio Microbial Profiling service, targeting the ITS1 and ITS2 regions. Sequences were then classified using the UNITE database.

Statistical analyses

Fungal ASV counts were compiled for each sample from a total of 20129 reads and were analysed using Shannon's diversity index and subsequent PERMANOVAs for pairwise comparisons of species and group using the *phyloseq* (McMurdie & Holmes 2013) and *vegan* (Oksanen et al. 2025) packages in R (version 4.4.2). Tukey post-hoc tests were performed to determine if statistically significant differences occurred with the significance value set at p=0.05. As this paper is examining fungal

consumption, identifications of fungal taxa determined to be plant pathogens and gut microbiota, including yeasts and members of the Cryptomycota, were excluded from the analysis. Instances of suspected secondary consumption, such as for entomopathogenic taxa, were still included as mammals may still disperse these taxa as a result of consumption.

For the two methods of laboratory analysis, the fungal species richness in a given sample was calculated for both methods. Following sequencing, only 28 of the 50 samples had fungal DNA detected. As a result, only 28 samples were used for comparison of the two methods, with each sample being analysed using both spore morphology analysis and DNA metabarcoding results. It should be noted that the same 28 samples were previously considered for analysis as part of the larger data set (50 samples) in Fox et al. 2025 (Chapter 3) but were reanalysed here as their own subset to compare with the metabarcoding results.

DNA metabarcoding results were converted to presence/absence data for ease of comparison with the spore morphology analysis results. Generalised Linear Mixed Models (GLMM) were used for both the datasets. Models were checked for overdispersion in both cases. Spore morphology data was slightly underdispersed (dispersion ratio ~ 0.70), so GLMMs were fitted with a Poisson distribution using the *lme4* package (Bates 2015) in R (version 4.5.1). The DNA metabarcoding data was overdispersed (dispersion ratio ~1.4) so GLMMs were fitted with a negative binomial distribution using the *glmmtmb* package (McGillycuddy et al. 2025). Estimated marginal means (EMMs) were then calculated for both species and group (i.e. rodent/marsupial) for both data sets using the *emmeans* package (Lenth et al. 2025) to perform pairwise comparisons. To visualise fungal composition across species and group non-metric multidimensional scaling (NMDS) was performed and plots were created using the *vegan* (Oksanen et al. 2025) and *ggplot2* (Wickham 2016) packages respectively. Subsequently PERMANOVAs were performed on the presence/absence data using the adonis2 function. After comparing fungal richness and community composition for mammal species and group for each method individually, summary statistics were calculated for both methods, and it was determined that the data were not normally distributed. As such, a Wilcoxon signed-

rank test was performed to compare species richness data collated from both methods for each sample.

Cost-Efficiency Analysis

Following this, a cost-efficiency analysis was conducted for each of the two methods and encompassed both the material and labour costs associated. As both methods were performed on each sample, costs and time involved in collecting samples in the field was not considered as part of the analysis. The cost of laboratory equipment (e.g. microscope, centrifuge) was also not included in this analysis as it was assumed that much of this equipment will already be available in a research laboratory. Material costs for both microscopy and DNA extractions were calculated by taking the total cost of all materials and dividing by the number of samples. Labour was also calculated per sample with an estimated hourly rate of AU$40 per hour. Mean and median costs per sample were calculated for both methods and paired samples were analysed using the Wilcoxon signed-rank test in R.

Results

Spore Morphology

All mammal species considered in this study were shown to consume fungi, with some individuals showing particularly high spore loads throughout the fields of view examined. Of the original 50 samples, 40 individuals were considered to have consumed fungi according to the protocols outlined in Fox et al. (2025) (Chapter 3). Across all species 19 unique fungal taxa were identified in samples including 14 taxa for *R. fuscipes,* 11 taxa for *M. cervinipes* and five taxa for each of the antechinus species with several taxa present in the diet of multiple mammal species (Fox et al. 2025) (Chapter 3).

DNA Metabarcoding Results

Following sequencing, 20 unique fungal taxa (Figure 1) were identified across the 28 samples which returned fungal amplicon sequence variances (ASVs). Of these, seven were samples from the two *Antechinus* spp., 11 were from *M. cervinipes* and 10 were from *R. fuscipes*. ASV abundance in each of the samples (Figure 1) did not vary significantly between species/group. Relative ASV abundance was also calculated for fungal functional groups across the mammal species (Figure 2).

Shannon's diversity showed low to moderate diversity of fungal taxa consumed by mammal species (Table 1), but no significant difference was found between the species or groups when compared (Table 2).

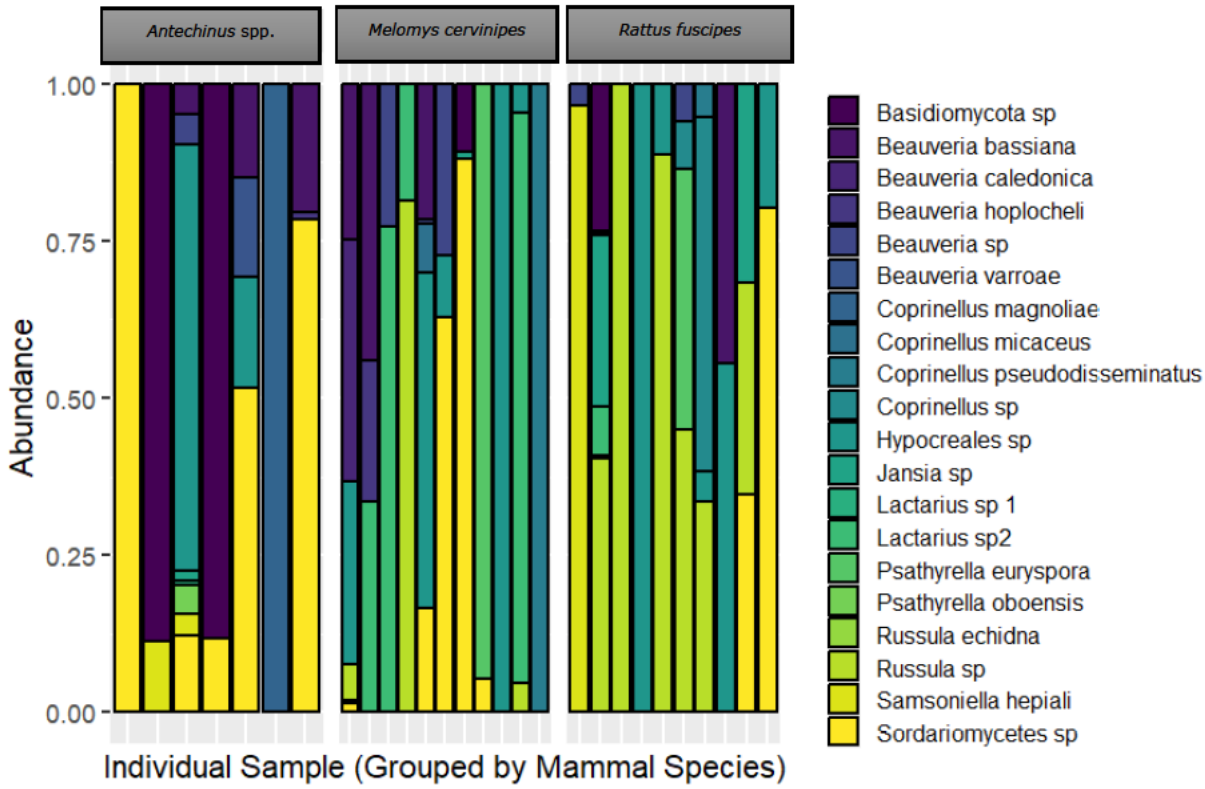

Figure 1: Abundance of fungal taxa in the diet of mammal species: *Antechinus mysticus, A. subtropicus, Melomys Cervinipes* and *Rattus fuscipes* (with the two antechinus species grouped for analysis) across all study sites at both Mount Byron and Bellthorpe.

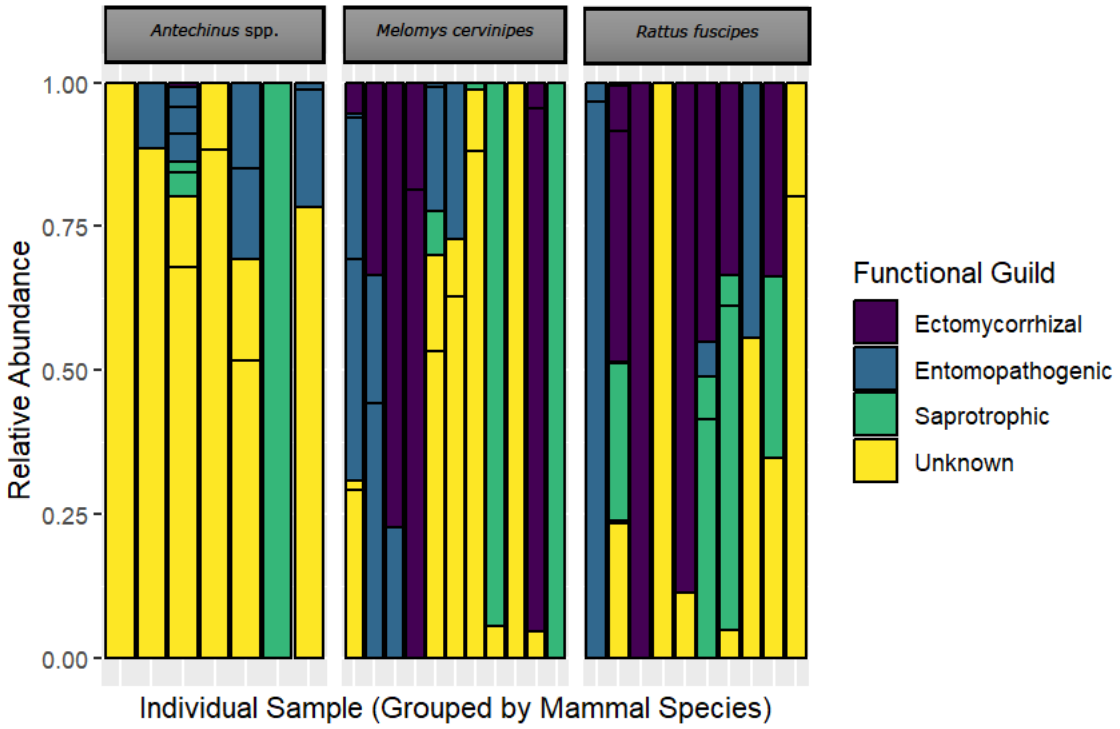

Figure 2: Abundance of fungal functional groups in the diet of mammal species (with the two antechinus species grouped for analysis) across all study sites.

Table 1: Shannon's Diversity Index results (mean and standard deviation) for fungal taxa in the diet of mammal species/group (with the two antechinus species grouped for analysis)

Species/Group	**Mean**	**Std Deviation**
Antechinus spp.	0.526	0.502
Melomys cervinipes	0.587	0.464
Rattus fuscipes	0.620	0.491
Marsupial	0.526	0.502
Rodent	0.603	0.466

Table 2: Results of Tukey post-hoc tests for comparison of Shannon's diversity index results.

Comparison Parameter	**Estimate**	**Std. Error**	**z-value**	**p-value**
Melomys.cervinpes – Antechinus spp.	0.062	0.234	0.263	0.962

Rattus. fuscipes – Antechinus spp.	0.094	0.239	0.396	0.917
Rattus. fuscipes – Melomys. cervinipes	0.033	0.211	0.155	0.987
Rodent-Marsupial	0.077	0.207	0.373	0.709

Comparison of fungal richness and composition across the two methods

There were some differences in the type of fungal taxa detected between the two methods (Table 3). However, fungal species richness was similar across samples

forboth spore morphology and DNA metabarcoding despite the differences in taxa detected (Figure 3a-d).

Table 3: Consumption of fungi from relevant fungal guilds by mammal species as identified by spore morphology analysis and DNA metabarcoding

		Fungal Functional Guild				
Method	**Mammal Species**	**ECM**	**AMF**	**Ento**	**Saprotrophic**	**Unknown**
Spore Morphology	*Melomys cervinipes*	×	×		×	×
	Rattus fuscipes	×	×		×	×
	Antechinus spp.	×	×		×	×
DNA Metabarcoding	*Melomys cervinipes*	×		×	×	×
	Rattus fuscipes	×		×	×	×
	Antechinus spp.			×	×	×

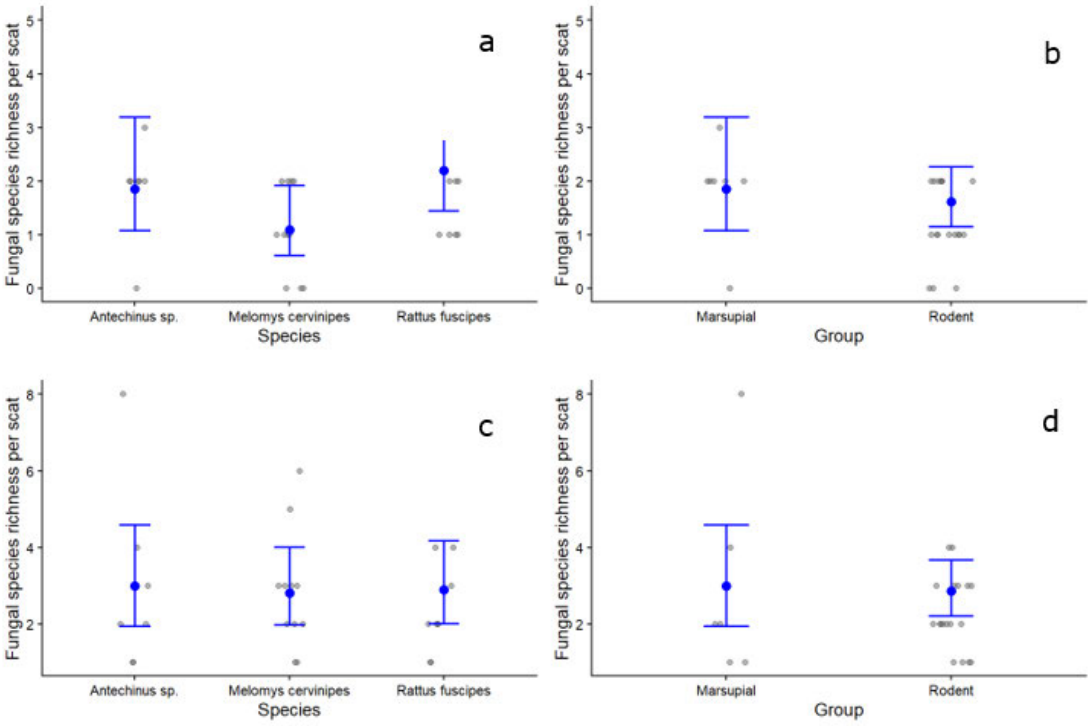

Figure 3: Fungal species richness compared between **a**: mammal species using spore morphology, **b**: mammal group using spore morphology, **c**: mammal species using DNA metabarcoding, **d**: mammal group using DNA metabarcoding. Grey dots representing individual scat samples, with the mean and confidence intervals shown in blue.

No significant differences for species richness were detected between species and groups following post-hoc tests (Table 4) for either method individually. However, when the two methods were compared DNA metabarcoding revealed a higher median richness (-1) of fungal taxa in samples than spore morphology analysis. This difference was shown to be statistically significant using the Wilcoxon signed rank test ($V = 55$, $p = 0.01$).

Table 4: p-values from post-hoc pairwise comparisons between species and group for both spore morphology analysis and DNA metabarcoding

Comparison Parameter	**p-value Morphology**	**p-value DNA**
Melomys cervinipes – Antechinus spp.	0.379	0.973
Rattus fuscipes – Antechinus spp.	0.879	0.992
Rattus fuscipes – Melomys cervinipes	0.124	0.993
Rodent-Marsupial	0.674	0.847

NMDS ordinations were performed to visualise clustering of fungal composition in the diets of species and group for both methods (Figure 4a-d).

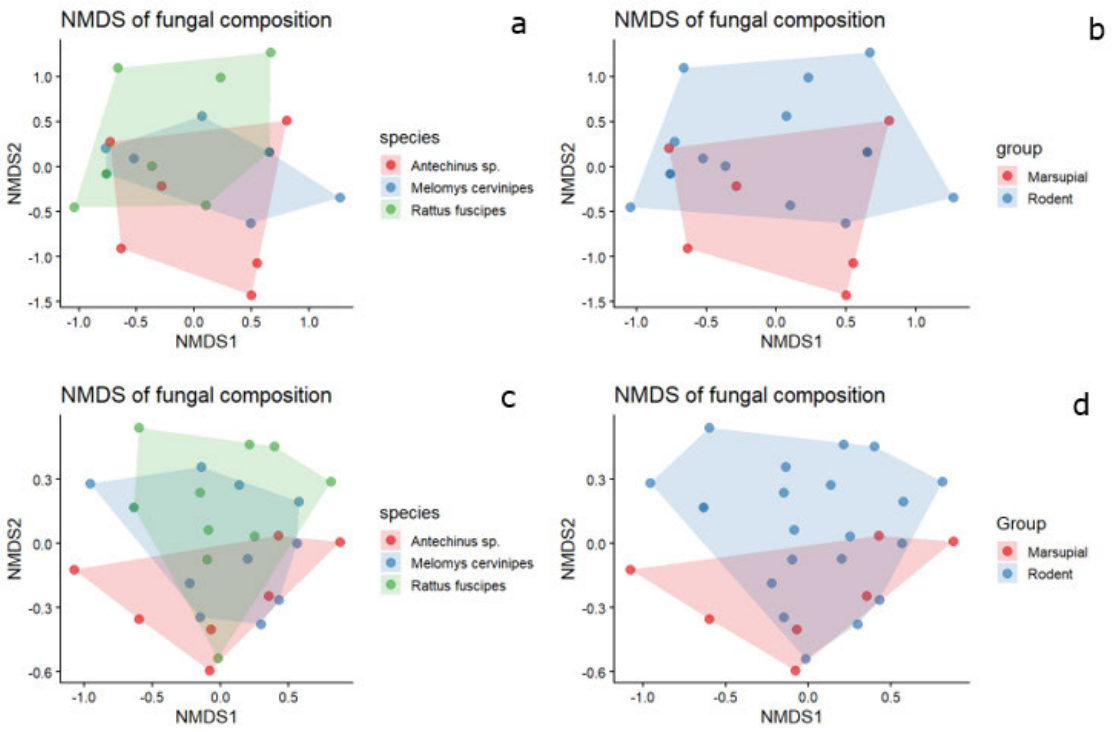

Figure 4: NMDS ordinations of Bray-Cutris dissimilarity matrices for **a**: mammal species using spore morphology analysis data **b**: mammal group using spore morphology analysis data **c**: mammal species using DNA metabarcoding data **d**: mammal group using DNA metabarcoding data

Cost-efficiency analysis

Differences in cost efficiency between the two methods were not statistically significant when compared using a Wilcoxon signed rank test (V=250, p=0.29). However, the median cost efficiency was slightly higher for spore morphology analysis (0.0405 fungal taxa identified per dollar) than for DNA metabarcoding (0.0299 fungal taxa identified per dollar). Cost efficiency comparisons for each individual sample can be seen in Figure 5 with comparative boxplots for the two methods seen in Figure 6.

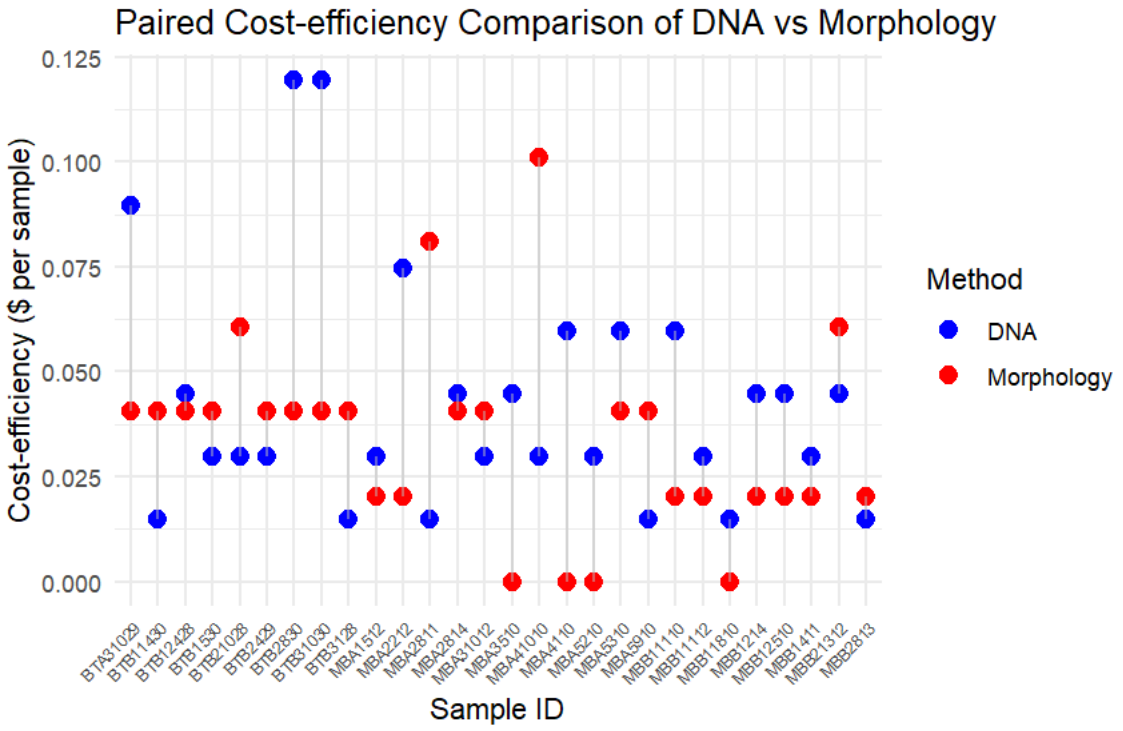

Figure 5: Paired cost-efficiency scores for individual samples, blue dots representing cost efficiency of DNA metabarcoding, red dots representing cost efficiency for spore morphology analysis.

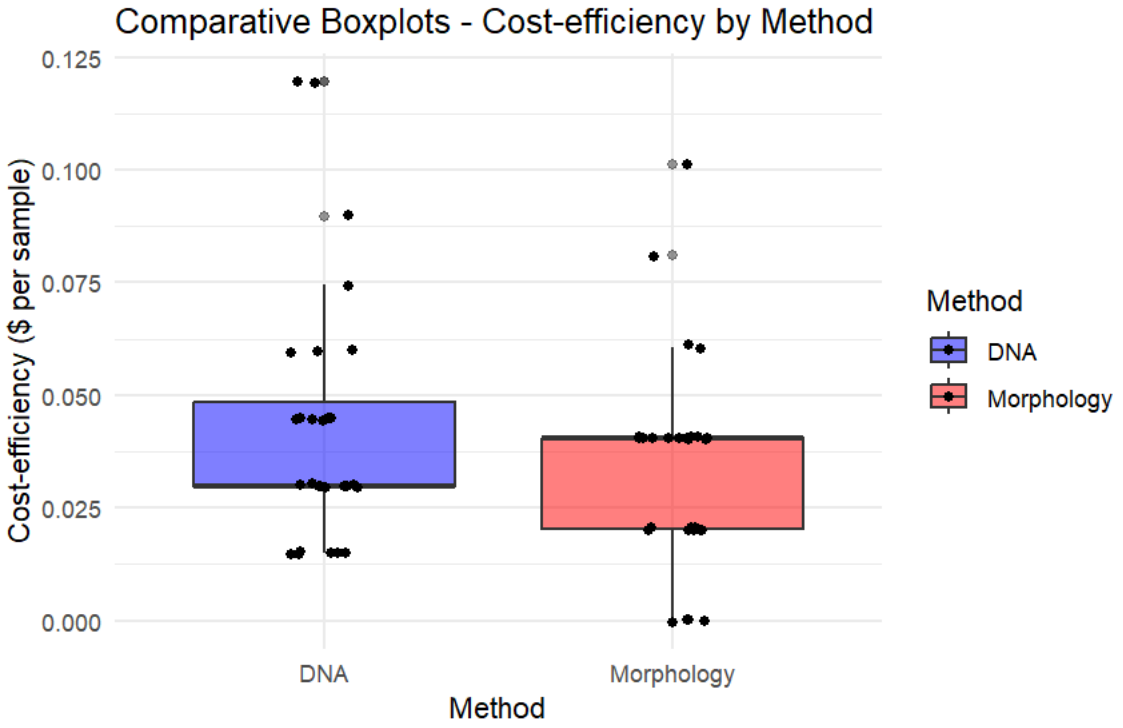

Figure 6: Cost efficiency (in dollars per sample) across samples compared between DNA metabarcoding and spore morphology analysis

Discussion

This study aimed to compare two analysis methods for studies of mammalian mycophagy, namely spore morphology analysis and DNA metabarcoding. Analysis of samples showed that the two methods revealed varying results for fungal community compositions in mammal scats. Some similar fungal taxa were detected between the two methods, there were two Russulales species identified in the spore morphology results and four Russulales species identified using DNA metabarcoding. This suggests there may be some deficiencies in differentiation of spore types using morphology. Members of the Psathyrellaceae were also detected in samples across both methods.

Interestingly, spore morphology analysis revealed several taxa that were not detected from the DNA metabarcoding results such as members of the Cortinariaceae and the Glomeromycota. As the ITS1 and ITS2 primers were used, this likely explains the absence of AMF taxa in sequencing results, as detection of these fungi would usually use different primers such as AML1-AML2. However, reasons for the absence of other taxa such as the Cortinariaceae are difficult to determine but may include degradation of DNA in transport, this could also potentially explain why only 28 samples returned results out of the original 50 submitted for sequencing, with the remaining 22 not yielding any fungal DNA reads. Conversely, DNA metabarcoding detected several taxa of entomopathogenic fungi (e.g. *Beauveria* spp., *Samsoniella hepiali*), none of which were identified morphologically during spore morphology analysis. Five taxa identified in the spore morphology analysis were unable to be identified past kingdom, whilst all taxa identified using DNA metabarcoding were identified to at least division level and usually further with 11 taxa able to be identified to species. Several taxa identified from both analysis methods were unable to be placed into a functional group (e.g. ectomycorrhizal, saprotrophic) as their identifications remained at higher taxonomic levels which may contain many taxa with varying functions (e.g. *Basidiomycota* sp.). Whilst they were not included in the analysis presented above as they were not suspected to have been deliberately consumed, DNA metabarcoding also detected several taxa of plant pathogenic fungi, members of the Cryptomycota and other fungi likely to be associated with the gut microbiome of the mammal species examined (Quah et al. 2025).

Abundance of fungal taxa from each of the samples with DNA metabarcoding results showed compositional differences across the species. When fungal abundance was examined as part of functional groups, the largest abundance for all species was from the unknown category. Without further identifications of the taxa contributing to this category it is difficult to ascertain the true abundance of the various functional groups within mammal diets. Ectomycorrhizal (ECM) taxa were the next most abundant for the two rodent species, providing further evidence to support that rodents are vectors for dispersal of these taxa (Elliott and Elliott et al. 2022). The DNA metabarcoding results showed found no ECM fungi in *Antechinus* spp. scats which contrasts with the spore morphology results. This may be due to misidentification of spores, however multiple ECM spore types were noted in antechinus scats, so this seems unlikely. Other potential causes of this include low abundance of these fungal taxa in samples or degradation of DNA samples during transport. Saprotrophic taxa were prominent across all mammal species, with one antechinus sample and one *M. cervinipes* sample containing only saprotrophic fungi. Studies of mycophagy often focus on mycorrhizal species (Elliott and Truong et al. 2022) but the role of mammals in dispersing saprotrophs should be examined more closely as these fungi are instrumental for nutrient cycling in ecosystems (Lebreton et al. 2021). Entomopathogenic fungi were also present in all samples. As far as the authors are aware, this is the first report noting the presence of entomopathogenic fungi in mammalian scat samples. All mammal species considered in this research are known to consume insects, so it is likely that the presence of the entomopathogenic taxa is a result of secondary consumption. Additionally, it is unknown if contamination of baits occurred but likely contaminants such as the mould-like fungi were excluded from the analysis. The role of mammals in dispersing these fungi warrants further investigation to determine the prevalence of these taxa in mammal diets as well as the viability of spores following dispersal. Previously carnivorous apex-level predators have been shown to disperse fungi through secondary consumption (Elliott et al. 2023).

Whilst the 28 samples considered in this study had previously been analysed for fungal species richness and community composition in Fox et al. in preparation (Chapter 3), abundance was not able to be calculated without doing full spore counts of each sample. This is not standard practice in studies of mycophagy employing

spore morphology analysis and previously spore morphology studies have estimated abundance qualitatively (Reddell, Spain & Hopkins 1997). DNA metabarcoding provides an advantage in estimating the abundance of individual fungal taxa (and other dietary items) in mammal scats in that ASVs can be calculated following sequencing. Abundance can then be calculated by computing the ratio of the total number of reads belonging to an ASV to the total number of reads in the sample (Quah et al. 2025).

We hypothesised that DNA metabarcoding would reveal higher fungal species richness in samples than spore morphology analysis. No significant differences in fungal species richness were detected between mammal species and group for either method following post-hoc tests. However, when species richness was compared between the two methods DNA metabarcoding was shown to consistently produce higher species richness scores than spore morphology analysis. The difference between the two methods was statistically significant, $p=0.01$, which suggests that DNA metabarcoding may be the preferable method for studies which seek to examine fungal species richness in mammal diets.

Additionally, we hypothesised that spore morphology analysis would provide reduced cost-efficiency for sample analysis. However, no statistically significant difference was found for cost efficiency between the two methods. Despite this, it was identified that the median cost efficiency was slightly higher for the spore morphology method. This is likely due to the very low cost of materials required for spore morphology analysis (e.g. microscope slides) and the higher costs associated with sequencing services for DNA metabarcoding.

Analyses were performed on relatively small data sets, meaning that the results are more susceptible to being skewed by outliers and random variability. Further research should be conducted across broader areas and over longer periods of time to contribute to larger data sets. Furthermore, whilst methodology for studies of this kind falls broadly into the two categories of spore morphology analysis and DNA metabarcoding, the exact protocols used can vary (Tory et al. 1997; Vernes & Dunn 2009). As such this is not an exhaustive comparison of all methods used for this type of research. However, it is hoped that the results presented here will provide some

guidance for researchers interested in investigating mammalian mycophagy as to which method may best suit their purpose.

Conclusion

Overall, both spore morphology analysis and DNA metabarcoding provide valuable insights into fungal consumption by mammalian mycophages. DNA metabarcoding reveals higher species richness for individual samples that spore morphology analysis. However, with several differences in the fungal taxa identified between these methods neither appears to provide a complete picture of a mammal's fungal diet. As such, further research should be conducted to see if a combination of both techniques would be most effective in identifying fungal traces found in mammal scats. Future research should also evaluate which combination of ITS regions to target in order to identify the broadest range of relevant fungal taxa in studies employing DNA metabarcoding. The results provided here should contribute to broader discussions amongst the research community regarding standardising methodology for ease of data comparison across geographical regions and between institutions. Whilst neither method provides statistically greater cost-efficiencies to researchers, this may change as DNA metabarcoding becomes more accessible and less cost-prohibitive. Understanding the fungal diets of mammal species also confers understanding of how fungi are dispersed through ecosystems. Data collected from studies of mycophagy may contribute to modelling distributions of fungal species, particularly for cryptic taxa which are difficult to detect by traditional means. We hope that this study will provide some guidance around the benefits of each analysis method and inspire further investigation into the ecological relationships between fungi and mammalian mycophages. Additionally, it is hoped that the DNA metabarcoding results may be used to inform studies in other fields which employ the use of environmental DNA analysis.

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Authors' Contributions

GF wrote the manuscript and conducted all statistical analyses, MCE and JD assisted with trapping, data collection, slide preparation and DNA extractions. All authors reviewed the final manuscript.

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CHAPTER 5: CONCLUSION

This honours project had two primary research aims. The first was to examine and describe the fungal diets of four sympatric small mammal species in southeast Queensland. The second was to compare two methods which are commonly used in studies of mammalian mycophagy, namely spore morphology analysis and DNA metabarcoding in both their fungal identification capabilities and cost-efficiency.

Notably, the research conducted here identified two species of small dasyurid (*Antechinus mysticus* and *A. subtropicus*) as novel mycophages with neither species previously noted as consuming fungi. Additionally, when the consumption of fungi by the small mammal species was compared no significant difference was found in species richness highlighting that both antechinus species as well as fawn-footed melomys (*Melomys* cervinipes) consume a comparable diversity of fungi with bush rats (*Rattus fuscipes*), previously considered to be the most mycophagous of the Australian native rodents. The comparison between spore morphology analysis and DNA metabarcoding highlighted several key considerations that researchers can take into account when conducting studies on mammalian mycophagy. DNA metabarcoding yielded higher fungal species richness results than spore morphology analysis and no significant difference was noted in cost-efficiency between the two methods. Therefore further research is needed to determine which method is best suited to given applications, an a combination of the two methods should be investigated as a standard methodology to encompass the broadest range of fungal taxa detected in samples.

Studies of mammalian mycophagy contribute to broader ecosystem knowledge by highlighting dietary preferences of mammals, providing information regarding fungal presence and distributions, particularly for cryptic species, and by illustrating the influence mammals have on their habitat through the dispersal of fungal spores. Historically, very little research into this interaction has occurred in southeast Queensland, with the majority of Australian research being conducted in the southern east coast states (New South Wales, Victoria and Tasmania) as well as far north Queensland. The research presented in Chapter 3 has provided further

knowledge surrounding fungal diets of the mammal species considered in a southeast Queensland context. Southeast Queensland is important to study in this context due to its high mammal diversity and the biodiverse and unique habitats found throughout the region, particularly those which are susceptible to urbanisation.

The methods used to study mammalian mycophagy, whilst falling broadly into the categories of spore morphology analysis and DNA metabarcoding, remain somewhat inconsistent across regions and institutions. The research presented in Chapter 4 provided helpful information to researchers about the potential pros and cons of employing either method, highlighting not only the challenges of cost but the different fungal taxa detected using each method. It is hoped that not only will this study inspire further, more informed research into mammalian mycophagy, but that the information provided may also eventually help to develop standardised methods so that data and results can be easily compared on a broader scale.

The primary limitations of the research presented in this thesis are related to small samples sizes. Due to the time constraints and resource availability for conducting an honours project, larger sample sizes were not able to be considered. Additionally, the results reported here are based on a small snapshot of time during which trapping and sample collection was able to be conducted, meaning that effects such as seasonality were also not able to be considered in the analysis. For the method comparison, examination of the results showed that several taxa which were identified during spore morphology analysis were not subsequently identified in the DNA metabarcoding results. As such the paper presented in Chapter 4 is unable to make clear recommendations regarding which method may be best suited for any given research question.

Both aims of the presented research were achieved. It was hypothesised that, when compared, all mammals considered would be shown to consume a comparable diversity of fungi. The research presented in Chapter 3 has validated this hypothesis. Additionally, it was hypothesised that DNA metabarcoding would identify a greater number of fungal taxa from scat samples and that spore morphology analysis will be the less cost-efficient of the two methods. DNA metabarcoding did reveal a higher number of taxa in samples as well as a higher median fungal species richness.

However, with discrepancies in the taxa identified between the two methods and no significant differences in cost-efficiency, further research will be needed to determine the most effective methodological approaches, particularly as DNA metabarcoding becomes less cost-prohibitive.

Future research should focus on furthering knowledge into the fungal diets of native mammals, particularly with a view to modelling how fungi are dispersed within ecosystems by these vectors. This knowledge could lead to a more fine scale understanding of intra-species relationships and could eventually be used to inform environmental management approaches. Fungi are often overlooked in environmental management practices, with the majority of the focus being placed on flora and fauna. By understanding how fungi connect all of these elements within a given ecosystem, we can implement this understanding and ensure that a more rounded approach is taken to habitat management and conservation. It is hoped that the research presented as part of this honours project will lead to further developments in how we study mycophagy so that researchers from across regions and institutions can work together to understand and protect our native species.

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