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The endangered northern bettong, *Bettongia tropica*, performs a unique and potentially irreplaceable dispersal function for ectomycorrhizal truffle fungi

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Running Title: Essential role of a fungal specialist

Abstract

Organisms that are highly connected in food webs often perform unique and vital functions within ecosystems. Understanding the unique ecological roles played by highly connected organisms and the consequences of their loss requires a comprehensive understanding of the functional redundancy among organisms. One important, yet poorly understood, food web is that between truffle-forming ectomycorrhizal fungi and their mammalian consumers and dispersers. Mammalian fungal specialists rely on fungi as a food source, and they consume and disperse a higher diversity and abundance of fungi than do mycophagous mammals with generalist diets. Therefore, we hypothesise that mammalian fungal specialists are functionally distinct because they disperse a set of fungal taxa not fully nested within the set consumed by the combined generalist mammalian community (i.e. functional redundancy of fungal dispersal is limited). Using high-throughput sequencing, we compared the fungal composition

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of 93 scats from the endangered fungal specialist northern bettong (*Bettongia tropica*) and 120 scats from nine co-occurring generalist mammal species across three sites and three seasons. Compared with other generalist mammals, *B. tropica* consumed a more diverse fungal diet with more unique taxa. This aligns with our hypothesis that *B. tropica* performs a unique dispersal function for ectomycorrhizal truffle fungi. Additionally, modelling of mammalian extinctions predicted rapid loss of food web connections which could result in loss of gene flow for truffle taxa. Our results suggest that this system is sensitive to the extinction of highly connected specialist species like *B. tropica* and their loss could have consequences for ectomycorrhizal truffle fungal diversity. This suggests that the conservation of fungal specialists is imperative to maintaining ectomycorrhizal fungal diversity and healthy plant-mycorrhizal relationships.

Introduction

Ecological networks can be particularly sensitive to the extinction of highly connected species (Dunne et al. 2002, Memmott et al. 2004). As highly connected organisms can be central within networks and often have disproportionally large number of interactions relative to their abundance, they can provide irreplaceable ecosystem services(Mello et al. 2015). Where functional redundancy is low (i.e. few taxa occupy any given niche and food-web connectance is low) the loss of highly connected species and their ecological roles can induce co-extinction of interconnecting species, because their function is not replaced by other species (Dallas and Cornelius 2015). Alternatively, such central functions may in part be substituted by other less-central organisms (Vernes and McGrath 2009, Wood et al. 2012). Understanding when functions are maintained despite diversity loss requires a comprehensive knowledge of functional redundancy and network structure (Dunne et al. 2002, Memmott et

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al. 2004). Such knowledge is becoming increasingly relevant as many systems continue to lose biodiversity (Thomas et al. 2004, Sax and Gaines 2008, Urban 2015).

One important, yet often overlooked, interaction involves three different groups of organisms; mammals, fungi and plants. Some mammals rely on hypogeous sequestrate fungi (i.e. fungi that form fruit-bodies below ground; truffle fungi) as a major component of their diet (Claridge 2002), while these same fungi rely on mammals for dispersal (Malajczuk et al. 1987, Claridge and May 1994, Johnson 1996). Mycophagy is common in many invertebrates (Fogel and Peck 1975, Reddell and Spain 1991, Nakamori and Suzuki 2005, 2010, Houston and Bougher 2010, Anslan et al. 2018) but, evidence for high spore survival and the long gut-retention times necessary for long-distance dispersal is found mostly in mammalian-truffle interactions (Nuske 2017). In addition, almost all truffle fungi are ectomycorrhizal, making them critical for ecosystem nutrient cycling (Hawkins et al. 2015, van der Heijden et al. 2015), plant health (Scott et al. 2012), and an important component of many forest systems globally (Tedersoo et al. 2010, 2014).

Not all mammals consume and disperse fungi equally (e.g. Meyer et al. 2005, Vernes and Dunn 2009, Schickmann et al. 2012). For example, a recent meta-analysis found that most members of the small marsupial family, Potoroidae, consume higher abundance and diversity of fungi than do other Australian mammals (Nuske et al. 2017). This suggests that Potoroid mammals perform an ecosystem function that no other mammal species do (i.e. Potoridae are not functionally redundant in terms of their fungal dispersal role compared to other mammals with generalist diets). However, the literature upon which this conclusion is based, also contains a significant taxonomic bias, with *Potorous* spp. and *Bettongia* spp. (Potoroidae) having a higher number of studies and samples than other mammal species (Nuske et al. 2017). Additionally, some generalist mammals consume a fungal diet as diverse as that of fungal specialists, indicating that, at least in some systems, functional redundancy may be

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higher. In this study we aimed to remove the influence of this bias and re-examine functional redundancy in the dispersal of fungi by mammals. To do this, we compared the fungal diet of an endangered fungal specialist, *Bettongia tropica* (northern bettong; Potoroidae), to that of other co-occurring generalist mammal species within the same community.

Bettongia tropica is endemic to the Wet Tropics of North Queensland, Australia. The species is endangered and in decline, having already disappeared from a number of localities at the edge of its distributional range (Bateman et al. 2011). *Bettongia tropica* is described as an ecotonal specialist. Its distribution is restricted to wet open sclerophyll forest where the canopy is dominated by host plants that associate with ectomycorrhizal-truffle-bearing fungi that border rainforest (Vernes and Pope 2001, Vernes et al. 2001, Abell et al. 2006).

The main diet of *B. tropica* consists of a diversity of truffle fungi (McIlwee and Johnson 1998, Vernes et al. 2001) and therefore is considered a fungal specialist (Nuske et al. 2017).Fungal generalists, on the other hand, are mammals that only consume fungi seasonally, or as a supplementary food source (Nuske et al. 2017). We hypothesise that the dispersal role of a fungal specialist, *B. tropica*, is unique and not functionally redundant within the combined dispersal roles of fungal generalists. Specifically, that fungal specialists consume (and potentially disperse) a set of fungal taxa not fully nested within the set consumed by the combined generalist mammalian community. We also examine the robustness of this system to mammalian extinction by modelling the loss of connections to truffle taxa (potentially representing loss of dispersal events) after selectively removing mammals from the data set.

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Methods

Field sampling

Mammal diets were examined by collecting scat samples from trapped individuals. Trapping and scat collection were carried out at three locations on the Lamb Range, North Queensland, Australia: Emu Creek (17°6'18.10"S, 145° 31'47.46"E), Danbulla State Forest near Tinaroo Dam (17°9’50.30"S, 145°32’11.56"E; now part of Danbulla National Park) and Davies Creek National Park (17°1’23.28"S, 145°34’55.71"E). These sites are roughly the same locations as used by previous studies on populations of *B. tropica* (Pope et al. 2000, Vernes et al. 2001). Elevation ranged from 600 and 900 m above sea level. The dominant ectomycorrhizal tree species are *Eucalyptus crebra, E. tindaliae, E. mediocris, Corymbia intermedia, Allocasuarina littoralis, A. torulosa* and *Acacia flavescens*.

Either seven or eight cage trap transects were set up in open forest at each location. Cage traps, (30 × 30 × 60 cm treadle traps) designed to capture medium-sized mammals, were spaced 100 m apart, 7-8 cages per transect totalling 53 cage trap locations per site. Three to four Elliot trap transects (10 × 10 × 30 cm aluminium box traps, designed to catch small mammals) were also set with traps 50 m apart in each transect; totalling 50 Elliot trap locations per site. All traps were set at dusk and baited with a mixture of peanut butter, rolled oats, vanilla, honey and canned sardines. Cage traps were checked from midnight until dawn, whereas Elliot traps were checked over a two hour span at dawn. Trapping was carried out across four consecutive nights at each site. Elliot trapping occurred within three weeks of cage trapping. Traps were set in three seasons; November-December 2014 (late dry), February-March 2015 (early wet) and May-June 2015 (late wet). Due to logistical constraints of limited time and personnel, Elliot traps were only set at Tinaroo Dam and Davies Creek, in

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the late dry and early wet seasons. The total number of trap placements was 1908 cage traps (53 traps × 4 nights × 3 sites × 3 seasons) and 800 Elliot traps (50 traps × 4 nights × 2 sites × 2 seasons).

Each animal was handled according to the James Cook University animal ethical guidelines (Approved ethics application A2044). Mammals were identified according to Van Dyck et al. (2013) and marked by either removing a small patch of hair with scissors at the base of the tail or microchipping (*B. tropica* only; Minichips, Micro Products Australia, Canning Vale, WA or ISO FDX-B Microchips, OzMicrochips, Peakhurst, NSW). Scats were collected from the bottom of each Elliot trap or from plastic placed under each cage trap. All traps and plastic were initially cleaned with 70% ethanol and then re-cleaned subsequent to each animal being caught. Scats were stored on ice, or in a portable fridge (4 ºC) in the field and transferred to -20 ºC as soon as possible (≤ 4 days).

Laboratory

Scats were only used from the first capture of an individual per trapping session (i.e. only one sample was taken per individual per sampling season to maintain independent samples and avoid possible influence of bait). Obvious soil contamination was removed with forceps from each scat before processing. Each boluse of scat was broken in half and a small sample of faecal material removed from the inside with sterile forceps. This material was homogenised manually with a sterilised blunt probe in a weight boat and 0.25 g of homogenate taken for DNA extraction.

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DNA was extracted by PowerLyser PowerSoil DNA Isolation kit following the manufacturer's instructions (Mo Bio, Carlsbad, CA USA), except that the samples were lysed using a Qiagen Tissue Lyser for 2 x 30 secs at 30 Hz, swapping the position of the samples between runs. DNA was amplified with ITS3-Mix1-5 (5'CTAGACTCGTCANCGATGAAGAACGYRG-3') and ITS4ngs (5'-TCCTSCGCTTATTGATATGC-3') primers (Tedersoo et al. 2014). The latter primers were tagged with 10-11 base unique molecular identifiers (MIDs) to later distinguish samples with sequencing runs (Table S1). We included negative controls for all DNA extractions and negative and positive controls for all PCR reactions. We used FavorPrep™ GEL/PCR Purification Kit (Favorgen Biotech Corp., Taiwan, China) to purify the amplicons, following manufactures instructions, except two FAfDF Columns were used per plate, doubling the elution with milliQ water to 80 µl which we let stand for 5 mins. Normalized amplicons were subjected to ligation of Illumina adaptors using the TruSeq DNA PCR-free HT Sample Prep kit (Illumina Inc., San Diego, CA, USA) to allow DNA strands to be read by the sequencing machine. All 242 samples were sequenced in Illumina MiSeq 2 × 300 paired-end runs.

Because Australian truffle sequences are underrepresented in existing large-scale public databases, we generated a reference sequence data set by sequencing representative specimens of multiple fungal species obtained from an extensive truffle survey at Davies Creek (Abell-Davis 2008). Two to three specimens were selected per truffle morpho-group for DNA extraction. A small section from the gleba of dried sporocarps was taken and DNA was extracted using DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions. As some specimens were harder to lyse because of their tough texture, we performed additional lysis steps where samples were frozen in liquid N in between bead-beating. DNA was amplified using ITS1 or ITS5 forward primers and ITS4 reverse primer

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(White et al. 1990). The PCR cocktail consisted of 1.0 µl DNA, 0.4 µl each of the primers (0.2 µmol each), 2.5 µl (1.25X) Kappa BufferB (Kapa Biosystems, Massachusetts, USA), 1.25 mM MgCl2, 0.2 mM dNTPs, 2% BSA, 0.12 µg/µl DMSO, 1.25 U Taq (Kapa Biosystems) and made up to 20 µl with MilliQ water. PCR was carried out using the following thermocycling conditions: an initial 1 min at 95 °C, followed by 36 cycles at 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, and a final cycle of 8 min at 72 °C. The relative quantity of PCR products was estimated by 1% agarose gel electrophoresis. The amplicons were cleaned with Exo-AP Mix (Enzyonmics, Daejeon, Korea). Amplicons were Sanger sequenced by AGRF or Macrogen.

The truffle sequences were processed using Geneious (v8-9.2) and the quality of the ITS sequences were checked using the guidelines outlined in Nilsson et al. (2012). ITS2 sequences were extracted to construct the local database and compared against Illumina sequences generated (see below). Raw Illumina data is deposited in Sequence Read Archive (SRA; bioproject SRP150847) and Sanger data is available in GenBank (Accessions KY686200-KY686202, KY697566-KY697576, KY697578-KY697619; Table S2).

Bioinformatics

Bioinformatics analysis for the paired-end Illumina data were performed using PipeCraft (v1.0; Anslan, Bahram, Hiiesalu, & Tedersoo, 2017) as follows. Paired-end reads were merged and quality filtered using vsearch (v1.11.1; https://github.com/torognes/vsearch) with minimum overlap = 10, allowing no ambiguous base pairs, and expected errors = 1 (allowmergestagger = T, minlen = 50). These high-quality sequences were allocated to samples (demultiplexed) based on MIDs using mothur (v1.36.1; Schloss et al. 2009), no

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primer or MID differences were allowed. Putative chimeric reads were detected and removed using *de novo* and based on reference (UNITE uchime reference dataset v7.0; Abarenkov et al. 2010) as implemented in vsearch (v1.9.10). Fungal ITS2 sequences were verified using ITS Extractor (v1.0.11; Bengtsson-Palme et al. 2013). Full ITS2 reads without flanking gene fragments were then clustered to Operational Taxonomic Units (OTUs) with CD-HIT (v4.6; Li & Godzik 2006) at 97% similarity threshold. Global singletons were removed from further analyses. Representative sequences were chosen using the 'abundance' method of mother and compared against UNITE database (v7.0), GenBank ITS database and our local truffle database to obtain taxonomic affiliation using BLASTn (Camacho et al. 2009).

We considered OTUs accurate at kingdom level if BLASTn matched to known species at <e-50, identity >75% and coverage >70% (Tedersoo et al. 2014). Other putatively non-fungal OTUs were removed from analyses. We removed OTUs from samples if they were present in negative and positive controls. OTUs were further filtered manually based on BLASTn values. Taxonomic groups were assigned to functional categories using FUNGuild (v1.0; Nguyen et al. 2015). All Glomeromycota taxa were assigned as arbuscular mycorrhizal.

Statistics

Statistics were conducted with the phyloseq package (McMurdie and Holmes 2013) in R (R Core Team 2012). We did not rarefy our sequencing data as this can lead to a high rate of false positives for species abundances across samples (McMurdie and Holmes 2014). Altogether 29 samples (12%) were removed from further analyses, because these comprised <500 filtered sequences (6 *B. tropica* samples and 18 samples from 6 mammal species with fungal generalist diets). The fungal data were examined at three broad levels; at the whole

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OTU community level, only examining the ectomycorrhizal (ECM) fungal OTUs (4.1% of all taxa) and only examining truffle taxa (2.0% of all taxa). The ECM subset of the data included only taxa that were assigned as 'highly probable' and 'probable' based on FUNGuild output. Truffle taxa are listed in Table S4. We also defined an 'ambiguous fruiting' category for secotioid taxa (fungi with enclosed fruiting bodies, which may have active spore dispersal and therefore are not solely mammal dispersed; Bougher and Lebel 2001) and taxa listed with ambiguous fruiting habit because of uncertain taxonomic assignment (e.g. Russulaceae which includes both truffle and wind-dispersed mushroom taxa). Only three taxa were matched to OTUs at species level and had secotioid fruiting habit (*Cortinarius globuliformis*, *C. porphyroideus* and *Scleroderma spB/spC*; Table S4). All three of these taxa are 'semi-hypogeous' and may also deposit spores on the surface of the soil or into the air (after erosion of the peridium for *Scleroderma spB/spC*). All other taxa we lumped together with secotioid species matched at genus or family level and we could not conclusively define fruiting habit (although these genera or families contain truffle or secotioid species). We excluded these taxa from the truffle analysis because mammal consumption is not the sole form of dispersal. Sporocarpic arbuscular mycorrhizal fungi were not included as truffle taxa. Only 4 OTUs of arbuscular mycorrhizal fungi could be distinguished to a genus or species (three matching sporocarpic *Glomus macrocarpus* and one matching *Scutellospora* sp.).

We compared *B. tropica* (fungal specialist) samples with other mammal species sampled (listed in Table S4 and Figure 1, thereafter referred to as fungal generalist mammals) within the same site and season. We also compared all *B. tropica* samples (n = 93) to all fungal generalist mammal species combined (9 species, n = 120) by merging OTU matrices for either fungal specialist or generalist samples by average read count per OTU. Not all mammal species were captured in all sites and seasons.

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To assess the potential role of mammal species and specialisation groups (fungal specialist versus generalists) as dispersers of truffle ECM species, the fungal community data from their scats were assessed in the following ways: 1) community composition of all fungal taxa via multi-dimensional statistics, 2) raw OTU richness of all taxa, ECM taxa and truffle taxa per sample, 3) rarefaction curves of truffle OTU richness across read count (depth) per sample or specialisation group and 4) unique and shared OTUs.

Distance-based Redundancy Analysis (db-RDA) was with Hellinger distance was used to examine the relationships between site, season and mammal species or specialisation groups on the fungal community sequenced from the scats. Constrained ordination was preferred in our application to unconstrained (such as NMDS) as it provides a good compromise between power limitations of unconstrained ordination and computational limitations of multivariate GLM extensions (ter Braak and Šmilauer 2015). Of the four distance metrics examined – Bray-Curtis, Chi-square (Canonical Correspondence Analysis), Chord and Hellinger – Hellinger distance yielded the highest fraction of explained variation by constraints (Legendre and Gallagher 2001). Partial db-RDA was used on the community data to partition out the variation within site and season and to only examine the relationship between mammal species or specialisation groups. Permutation tests were used to establish the significance of the terms in constrained ordinations using 500 permutations, equivalent in its implementation by our software to a PERMANOVA using Hellinger distance.

Fungal OTU richness per sample between *B. tropica* (fungal specialist) and fungal generalist mammal species were compared using Tukey HSD tests at all levels of the data (all taxa, ECM only and truffle only OTUs). To estimate the accumulation of OTUs across samples,

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we created rarefaction OTU accumulation curves and their 95% confidence intervals for each mammal species and all samples using EstimateS 9.1.0 (Colwell 2013). We also created rarefaction curves to estimate the accumulation of OTUs across sequence read counts (depth) for each sample within each site and season using the 'ggrare' function (richness.R, phyloseq extensions; https://github.com/mahendra-mariadassou/phyloseq-extended with added ggplot2 graphics). Unique fungal OTUs within *B. tropica* and fungal generalist mammal scats were examined by creating Venn diagrams using limma and VennDiagram packages in R.

To examine the functional redundancy in this truffle-mammal dispersal network, we calculated the 'secondary extinction slope' (Memmott et al. 2004) and robustness index (R; Burgos et al. 2007). The 'secondary extinction slope', which could represent loss of dispersal events for truffle taxa, was calculated using the 'second.extinct' function in the bipartite R package (Dormann et al. 2008) by deleting the most-to-least connected mammal species in the network and plotting the corresponding proportional decrease in truffle connections. Connectedness is defined as the sum of interactions divided by the number of possible interactions. The robustness index is the area under the extinction slope; values close to 0 correspond to fragile networks where small decreases in diversity have large consequences for secondary loss of network connections, whereas values close to 1 indicate more robust systems (Dunne et al. 2002, Burgos et al. 2007, Jonsson et al. 2015). Since species connectance and network structure is highly related to species abundance (Dormann et al. 2017), we compared results with those found after randomly subsampling for an equal number of samples for each mammal species. We repeated this 60 times (only for mammal species with $n \geq 12$ total samples) so that within each iteration the truffle diets of each mammal species were represented from 10 samples.

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Results

Fungal OTUs across all 213 scat samples totalled 7505. Twenty-one percent of OTUs could be assigned to functional guilds by FUNGuild; of these, the most diverse were undefined saprotrophs (728 OTUs, 9.7 %; Table S3) followed by ectomycorrhizal taxa (323 OTUs, 4.3%). Even though just under 50% of the ECM fungal OTUs could be assigned as truffle fungi (150/323 OTUs), truffle ECM OTUs had a higher relative abundance (>50% of sequence reads) compared to other ECM OTUs for most mammal species (Fig. S1; Table S4), except *Antechinus flavipes* and *Isoodon obesulus*. These OTUs with high relative abundance matched truffle taxa *Mesophellia* and *Malajcukia* in *B. tropica, Isoodon macrourus, Uromys caudimaculatus, Trichosurus vulpecula and Zyzomys argurus* scats; *Chondrogaster sp.* in *U. caudimaculatus* and *Rattus sp.* scats and *Rhizopogon pseudoroseolus* in *Perameles nasuta* scats (Fig. 1; Table S4). The most OTU-rich truffle genera were *Mesophellia* and *Malajcukia* (35% of all truffle OTUs).

Scats of *B. tropica* and fungal generalist mammal species displayed distinct fungal communities (Fig. 2). With site and season conditioned out using partial-RDA, specialist *B. tropica* fungal diets were significantly different from all generalist mammal species combined (pseudo-$F_{1, 209}$ = 5.6108, P = 0.001; Fig. 2). A permutation significance test on db-RDA (PERMANOVA) revealed significant interactions between site and season (Table S5). Variation tended to be greater in fungal communities from the specialist than generalists (Fig. 2). The fungal diets of mammals grouped within families in partial RDA constraining for mammal species and conditioning out site and season (Fig. S2).

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The number of OTUs obtained from the total number of sequenced samples did not reach an asymptote (Fig. S3), suggesting that more samples would be needed to fully characterise the fungal community within the scats. This is common for high throughput sequencing studies for fungi (e.g. Taylor et al. 2010, Unterseher et al. 2011, Anslan et al. 2016). Nevertheless, for a given number of samples (e.g. 50), samples of fungal specialist mammal species contained more ECM OTUs (mean ± 95% lower and upper confidence interval: 183 ± 167, 199) than all scat samples from the fungal generalist species combined (101 ± 90, 113; Fig. S3). This is also reflected in truffle data; *B. tropica* scats contained more truffle OTUs per read count than fungal generalist mammals consistently across sites and seasons (Fig. 3). When OTU matrices were merged (for average read count) for fungal specialist and generalist samples, specialist samples contained a higher species richness and read count than generalists in all sites and seasons except for one (Tinaroo Dam, late dry). This latter exception was the only example where we had a much greater number of fungal generalist samples (n = 22 for 6 mammal species) than specialist samples (n = 7; Fig. S4).

Compared to the fungal generalist mammals, specialists (*B. tropica*) consumed truffles of relatively greater diversity (Fig. 1). The average fungal OTU richness per sample from *B. tropica* scats was significantly greater than in all generalist mammals combined (Table 1). This trend was most notable when only examining ECM OTUs or OTUs from truffle taxa (Table 1; Fig. S5). The percentage of samples that contained ECM and truffle OTUs was higher in *B. tropica* scats (99% and 96%, respectively) compared to fungal generalist mammal species (90% and 71%, respectively). Further, *B. tropica* samples contained more unique ECM and truffle OTUs compared with samples from the fungal generalist mammal species (Fig. 4).

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The 'secondary extinction slope' showed a sharp decline in connections to unique truffle taxa for the progressive 'extinction' of mammal species in the data set (Fig. 5). This resulted in a robustness index value below 0.5 (0.24) indicating that this network is sensitive to mammalian extinctions. *Bettongia tropica* was the most connected within the food web; indeed over 50% of truffle connections were lost when *B. tropica* was removed from the data (Fig. 5). The robustness index was similar when all data with uneven sampling was used compared to repeated subsampling for the same number of samples per mammal species (0.24 and 0.253 ± 0.003, respectively; Fig. S6), indicating that network structure was not overly sensitive to sampling bias.

Discussion

Our data suggest that the fungal specialist, *B. tropica*, potentially performs a unique ecosystem function through dispersing truffle fungi. We found that the diet of *B. tropica* contained a significantly different fungal community structure, higher diversity per sample and more unique ECM and truffle fungal taxa than the combined diets of nine generalist mycophagous mammal species. *B. tropica* diets also contained truffle fungal taxa more often and at higher relative abundances than fungal generalist mammals. The truffle diversity in fungal specialist diets exceeded the cumulative diversity consumed by generalists at all sites and seasons but one (where threefold more generalist individuals were sampled than *B. tropica* individuals), indicating that the dispersal role of specialists can only be matched by a greater abundance of generalists. These results are consistent with our hypothesis and previous findings that potoroid mammals (fungal specialists) consume a higher diversity of fungi than generalist mycophagists across Australia (Nuske et al. 2017).

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Modelling the extinction of mammal species from this food web resulted in a rapid loss of connections for truffle fungi, representing a loss of potential dispersal events. A low (below 0.5) ecosystem robustness index indicates that networks are sensitive to loss of diversity (Dunne et al. 2002, Burgos et al. 2007, Jonsson et al. 2015). More than 50% of truffle connections were lost when only *B. tropica* was removed from the dataset, which resulted in a robustness index of 0.24. Taken together, these results suggest that this food web is sensitive to the extinction of mammals and the fungal dispersal role of specialists is not functionally redundant within generalists. Whether the extinction of mammalian dispersers (particularly highly connected fungal specialists) results in the co-extinction of truffle taxa in the long term requires further verification.

As truffle fungi rely on zoochory, it is likely that *B. tropica* and other mycophagous mammals heavily influence the gene flow and possibly the population structure of these fungal taxa (e.g. *Mesophellia/Malajczukia, Chondrogaster, Hysterangium, Soliocaccasus polychromus,* truffle taxa of *Russula* and *Cortinarius*). It is not yet known how important the frequency of mammalian spore dispersal is for genetic structuring of truffle species amongst other local factors like compatibility and distribution mating types and mycorrhizal hosts, propensity to spread and inoculate new plant roots via extra-radial hyphae or spores and variation in abiotic soil characteristics (Grubisha et al. 2007, Douhan et al. 2011, Molinier et al. 2016, Taschen et al. 2016). On larger scales, physical barriers like large water bodies, mountain ranges or habitat fragmentation influence mammal movement (Pope et al. 2000, Dietz et al. 2018) and hence are likely to also influence truffle dispersal and genetic structure. Future studies on population genetics of truffle fungi need to consider these factors as well as differing mammal communities' to verify the strength of dispersal processes by mammals on the genetic population structure of truffle taxa.

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An assumption underpinning our conclusions is fungal generalist mammals will not or cannot change their truffle diets in the absence of a fungal specialist. The extent to which generalists can increase their intake of fungi is unknown and is liable to vary between mammal groups (Claridge et al. 1999). However, mammal species tend to consume fungi in proportion to their abundance (Johnson 1994, North et al. 1997), suggesting that if a fungal specialist becomes locally extinct, generalist mammal species may increase the intake of truffle fungi as they become more available. This hypothesis is yet to be tested.

If our results are representative of the situation in other truffle-mammal networks, reduced spore dispersal from mycophagous mammals may, over time, reduce the relative abundance and species richness of truffle fungi, making Australia of particular concern for truffle fungal conservation. Other members of the Potoroidae family in Australia (*Bettongia* spp. and *Potorous* spp.) and mammal species worldwide (e.g. flying squirrels, *Glaucomys sabrinus* in North America and bank voles, *Myodes glareolus* in Europe) are also considered fungal specialists (Pyare and Longland 2001, Schickmann et al. 2012, Nuske et al. 2017). Australia's history of mammal extinction and decline, including members of Potoroidae (Claridge et al. 2007), far exceeds other continents (Woinarski et al. 2015). Australia also has a high diversity of truffle species, most of which are endemic (Bougher and Lebel 2001). In terms of biomass and carbon stocks, ECM trees (especially *Eucalyptus* spp.) are dominant in much of Australia's forests (Reddell et al. 1999, Wood et al. 2015) and reduced ECM abundance and diversity has been linked to declines in *Eucalyptus* tree health (Scott et al. 2012, Ishaq et al. 2013, Horton et al. 2013). The already substantial loss of *Bettongia* spp. and *Potorous* spp. throughout much of the Australian continent (Claridge et al. 2007) could have long-lasting detrimental, yet undocumented, consequences for the diversity of truffle fungi. This raises concern about the vulnerability of these ecosystems, because consumer networks are most

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disrupted by the extinction of highly connected species (Dunne et al. 2002, Burgos et al. 2007, Bellingeri et al. 2013).

Whether reduction of mammal diversity results in compromised plant productivity via loss of truffle diversity has not yet been empirically shown, but it has been suggested by many authors (Maser et al. 1978, Malajczuk et al. 1987, Johnson 1996, Vernes 2007). This link between mycophagous mammals and plant productivity assumes that 1) lowered mammal diversity inhibits gene flow for truffle species, resulting in decline of truffle diversity and that 2) truffles form important and irreplaceable components of functioning ectomycorrhizal fungal communities. Neither of these assumptions have been tested; further studies are needed.

Studying how ecosystem functioning responds to diversity loss is becoming increasingly relevant in light of ever-increasing extinction rates (Thomas et al. 2004, Morris 2010, Urban 2015). Here we show that an endangered mammalian fungal specialist performs a unique and potentially irreplaceable function for dispersing truffle ECM fungi. We propose that this truffle dispersal system is fragile to the loss of mammalian dispersers, and conservation of fungal specialists may be necessary for adequate gene flow of many truffle species. Further research is urgently needed to verify links between mammalian truffle dispersers and truffle ECM diversity, ECM-plant dynamics and healthy ecosystem functioning.

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References

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

Abell-Davis, S. E. 2008. Tropical hypogeous fungal sporocarp distribution in time and space. Implications for an endangered specialist mycophagous marsupial, *Bettongia tropica*. PhD thesis. School of Marine and Tropical Biology. James Cook University.

Abell, S. E., P. Gadek, C. Pearce, and B. C. Congdon. 2006. Seasonal resource availability and use by an endangered tropical mycophagous marsupial. Biological Conservation 132:533–540.

Anslan, S., M. Bahram, I. Hiiesalu, and T. Tedersoo. 2017. PipeCraft: flexible open-source toolkit for bioinformatics analysis of custom high- throughput amplicon sequencing data. Molecular Ecology Resources May:online first.

Anslan, S., M. Bahram, and L. Tedersoo. 2016. Temporal changes in fungal communities associated with guts and appendages of Collembola as based on culturing and high-throughput sequencing. Soil Biology and Biochemistry 96:152–159.

Anslan, S., M. Bahram, and L. Tedersoo. 2018. Seasonal and annual variation in fungal communities associated with epigeic springtails (Collembola spp.) in boreal forests. Soil Biology and Biochemistry 116:245–252.

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Accepted Article

Accepted Article

Bateman, B. L., S. E. Abell-Davis, and C. N. Johnson. 2011. Climate-driven variation in food availability between the core and range edge of the endangered northern bettong (*Bettongia tropica*). Australian Journal of Zoology 59:177–185.

Bellingeri, M., D. Cassi, and S. Vincenzi. 2013. Increasing the extinction risk of highly connected species causes a sharp robust-to-fragile transition in empirical food webs. Ecological Modelling 251:1–8.

Bengtsson-Palme, J., M. Ryberg, M. Hartmann, S. Branco, Z. Wang, A. Godhe, P. De Wit, M. Sánchez-García, I. Ebersberger, F. de Sousa, A. Amend, A. Jumpponen, M. Unterseher, E. Kristiansson, K. Abarenkov, Y. J. K. Bertrand, K. Sanli, K. M. Eriksson, U. Vik, V. Veldre, and R. H. Nilsson. 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods in Ecology and Evolution 4:914–919.

Bougher, N. L., and T. Lebel. 2001. Sequestrate (truffle-like) fungi of Australia and New Zealand. Australian Systematic Botany 14:439–484.

ter Braak, C. J. F., and P. Šmilauer. 2015. Topics in constrained and unconstrained ordination. Plant Ecology 216:683–696.

Burgos, E., H. Ceva, R. P. J. Perazzo, M. Devoto, D. Medan, M. Zimmermab, and A. M. Delbue. 2007. Why nestedness in mutualistic networks? Journal of Theoretical Biology 249:307–313.

Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, and T. L. Madden. 2009. BLAST plus: architecture and applications. BMC Bioinformatics 10:421.

Claridge, A. W. 2002. Ecological role of hypogeous ectomycorrhizal fungi in Australian forests and woodlands. Plant and Soil 244:291–305.

Claridge, A. W., and T. W. May. 1994. Mycophagy among Australian mammals. Australian Journal of Ecology 19:251–275.

Claridge, A. W., J. H. Seebeck, and R. Rose, editors. 2007. Bettongs, Potoroos and the Musky Rat-Kangaroo. First Edit. CSIRO Publishing, Collingwood, VIC.

Claridge, A. W., J. M. Trappe, S. J. Cork, and D. L. Claridge. 1999. Mycophagy by small mammals in the coniferous forests of North America: nutritional value of sporocarps of Rhizopogon vinicolor, a common hypogeous fungus. Journal of Comparative Physiology B 169:172–8.

Colwell, R. K. 2013. EstimateS: Statistical estimation of richness and shared species from samples. Version 9.

Dallas, T., and E. Cornelius. 2015. Co-extinction in a host-parasite network: identifying key hosts for network stability. Scientific Reports 5:13185.

Dietz, M., S. Büchner, J. Hillen, and B. Schulz. 2018. A small mammal's map: identifying and improving the large-scale and cross-border habitat connectivity for the hazel dormouse Muscardinus avellanarius in a fragmented agricultural landscape. Biodiversity and Conservation 27:1891–1904.

Dormann, C. F., J. Fründ, and H. M. Schaefer. 2017. Identifying Causes of Patterns in

This article is protected by copyright. All rights reserved.

Accepted Article

Ecological Networks: Opportunities and Limitations. Annual Review of Ecology, Evolution, and Systematics 48:annurev-ecolsys-110316-022928.

Dormann, C. F., B. Gruber, and J. Fründ. 2008. Introducing the bipartite Package: Analysing Ecological Networks. R News 8:8–11.

Douhan, G. W., L. Vincenot, H. Gryta, and M. A. Selosse. 2011. Population genetics of ectomycorrhizal fungi: From current knowledge to emerging directions. Fungal Biology 115:569–597.

Dunne, J. A., R. J. Williams, and N. D. Martinez. 2002. Network structure and biodiversity loss in food webs: robustness increase with connectance. Ecology Letters 5:558–567.

Van Dyck, S., I. Gynther, and A. Baker, editors. 2013. Field companion to the mammals of Australia. New Holland Publishers, Sydney.

Fogel, R., and S. B. Peck. 1975. Ecological studies of hypogeous fungi. I. Coleoptera associated with sporocarps. Mycologia 67:741–747.

Grubisha, L. C., S. E. Bergemann, and T. D. Bruns. 2007. Host islands within the California Northern Channel Islands create fine-scale genetic structure in two sympatric species of the symbiotic ectomycorrhizal fungus *Rhizopogon*. Molecular Ecology 16:1811–22.

Hawkins, B. J., M. D. Jones, and J. M. Kranabetter. 2015. Ectomycorrhizae and tree seedling nitrogen nutrition in forest restoration. New Forests 46:747–771.

van der Heijden, M. G. A., F. M. Martin, M.-A. Selosse, and I. R. Sanders. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist 205:1406–1423.

Horton, B. M., M. Glen, N. J. Davidson, D. Ratkowsky, D. C. Close, T. J. Wardlaw, and C. Mohammed. 2013. Temperate eucalypt forest decline is linked to altered ectomycorrhizal communities mediated by soil chemistry. Forest Ecology and Management 302:329–337.

Houston, T. F., and N. L. Bougher. 2010. Records of hypogeous mycorrhizal fungi in the diet of some Western Australian bolboceratine beetles (Coleoptera: Geotrupidae, Bolboceratinae). Australian Journal of Entomology 49:49–55.

Ishaq, L., P. A. Barber, G. E. S. J. Hardy, M. Calver, and B. Dell. 2013. Seedling mycorrhizal type and soil chemistry are related to canopy condition of *Eucalyptus gomphocephala*. Mycorrhiza 23:359–71.

Johnson, C. N. 1994. Mycophagy and spore dispersal by a rat-kangaroo: consumption of ectomycorrhizal taxa in relation to their abundance. Functional Ecology 8:464–468.

Johnson, C. N. 1996. Interactions between mammals and ectomycorrhizal fungi. Trends in Ecology and Evolution 11:503–507.

Jonsson, T., S. Berg, A. Pimenov, C. Palmer, and M. Emmerson. 2015. The reliability of R<inf>50</inf> as a measure of vulnerability of food webs to sequential species deletions. Oikos 124:446–457.

Legendre, P., and E. D. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280.

Li, W., and A. Godzik. 2006. Cd-hit: A fast program for clustering and comparing large sets

This article is protected by copyright. All rights reserved.

of protein or nucleotide sequences. Bioinformatics 22:1658–1659.

Malajczuk, N., J. M. Trappe, and R. Molina. 1987. Interrelationships among some ectomycorrhizal trees, hypogeous fungi and small mammals: Western Australian and northwestern American parallels. Australian Journal of Ecology 12:53–55.

Maser, C., R. A. Nussbaum, and J. M. Trappe. 1978. Fungal-small mammal interrelationships with emphasis on Oregon coniferous forests. Ecology 59:799–809.

McIlwee, A. P., and C. N. Johnson. 1998. The contribution of fungus to the diets of three mycophagous marsupials in *Eucalyptus* forests, revealed by stable isotope analysis. Functional Ecology 12:223–231.

McMurdie, P. J., and S. Holmes. 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8:e61217.

McMurdie, P. J., and S. Holmes. 2014. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Computational Biology 10:e1003531.

Mello, M. A. R., F. A. Rodrigues, L. da F. Costa, W. D. Kissling, Ç. H. Şekercioğlu, F. M. D. Marquitti, and E. K. V. Kalko. 2015. Keystone species in seed dispersal networks are mainly determined by dietary specialization. Oikos 124:1031–1039.

Memmott, J., N. M. Waser, and M. V. Price. 2004. Tolerance of pollination networks to species extinctions. Proceedings of the Royal Society B: Biological Sciences 271:2605–2611.

Meyer, M. D., M. P. North, and D. A. Kelt. 2005. Fungi in the diets of northern flying squirrels and lodgepole chipmunks in the Sierra Nevada. Canadian Journal of Zoology 83:1581–1589.

Molinier, V., C. Murat, A. Baltensweiler, U. Büntgen, F. Martin, B. Meier, B. Moser, L. Sproll, U. Stobbe, W. Tegel, S. Egli, and M. Peter. 2016. Fine-scale genetic structure of natural Tuber aestivum sites in southern Germany. Mycorrhiza 26:895–907.

Morris, R. J. 2010. Anthropogenic impacts on tropical forest biodiversity: a network structure and ecosystem functioning perspective. Philosophical Transactions of the Royal Society of London. Series B 365:3709–18.

Nakamori, T., and A. Suzuki. 2005. Spore-breaking capabilities of collembolans and their feeding habitat within sporocarps. Pedobiologia 49:261–267.

Nakamori, T., and A. Suzuki. 2010. Spore resistance and gut-passage time of macrofungi consumed by *Ceratophysella denisana* (Collembola: Hypogastruridae). Fungal Ecology 3:38–42.

Nguyen, N. H., Z. Song, S. T. Bates, S. Branco, L. Tedersoo, J. Menke, J. S. Schilling, and P. G. Kennedy. 2015. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology:1–8.

Nilsson, R. H., L. Tedersoo, K. Abarenkov, M. Ryberg, E. Kristiansson, M. Hartmann, C. Schoch, J. Nylander, J. Bergsten, T. Porter, A. Jumpponen, P. Vaishampayan, O. Ovaskainen, N. Hallenberg, J. Bengtsson, M. Eriksson, K.-H. Larsson, E. Larsson, and U. Koeljalg. 2012. Five simple guidelines for establishing basic authenticity and reliability of newly generated fungal ITS sequences. MycoKeys 4:37–63.

This article is protected by copyright. All rights reserved.

Accepted Article

North, M., J. Trappe, and J. Franklin. 1997. Standing crop and animal consumption of fungal sporocarps in Pacific Northwest forests. Ecology 78:1543–1554.

Nuske, S. J. 2017. The importance of declining mammalian fungal specialists for ectomycorrhizal fungal dispersal. PhD thesis. College of Science and Engineering, James Cook University, Cairns, Australia.

Nuske, S. J., K. Vernes, T. W. May, A. W. Claridge, B. C. Congdon, A. Krockenberger, and S. E. Abell. 2017. Redundancy among mammalian fungal dispersers and the importance of declining specialists. Fungal Ecology 27:1–13.

Pope, L. C., A. Estoup, and C. Moritz. 2000. Phylogeography and population structure of an ecotonal marsupial, *Bettongia tropica*, determined using mtDNA and microsatellites. Molecular Ecology 9:2041–53.

Pyare, S., and W. S. Longland. 2001. Patterns of ectomycorrhizal-fungi consumption by small mammals in remnant old-growth forests of the Sierra Nevada. Journal of Mammalogy 82:681–689.

R Core Team. 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reddell, P., V. Gordon, and M. S. Hopkins. 1999. Ectomycorrhizas in *Eucalyptus tetrodonta* and *E. miniata* forest communities in tropical northern Australia and their role in the rehabilitation of these forests following mining. Australian Journal of Botany 47:881–907.

Reddell, P., and A. V Spain. 1991. Earthworms as vectors of viable propagules of mycorrhizal fungi. Soil Biology and Biochemistry 23:767–774.

Sax, D. F., and S. D. Gaines. 2008. Species invasions and extinction: The future of native biodiversity on islands. Proceedings of the National Academy of Sciences 105:11490–11497.

Schickmann, S., A. Urban, K. Kräutler, U. Nopp-Mayr, and K. Hackländer. 2012. The interrelationship of mycophagous small mammals and ectomycorrhizal fungi in primeval, disturbed and managed Central European mountainous forests. Oecologia 170:395–409.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn, and C. F. Weber. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75:7537–7541.

Scott, P. M., B. L. Shearer, P. A. Barber, and G. E. S. J. Hardy. 2012. Relationships between the crown health, fine root and ectomycorrhizae density of declining *Eucalyptus gomphocephala*. Australasian Plant Pathology 42:121–131.

Taschen, E., F. Rousset, M. Sauve, L. Benoit, M. P. Dubois, F. Richard, and M. A. Selosse. 2016. How the truffle got its mate: insights from genetic structure in spontaneous and planted Mediterranean populations of Tuber melanosporum. Molecular Ecology 25:5611–5627.

Taylor, D. L., I. C. Herriott, K. E. Stone, J. W. McFarland, M. G. Booth, and M. B. Leigh. 2010. Structure and resilience of fungal communities in Alaskan boreal forest soils.

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

Canadian Journal of Forest Research 40:1288–1301.

Tedersoo, L., M. Bahram, S. Põlme, U. Kõljalg, N. S. Yorou, R. Wijesundera, L. V Ruiz, A. M. Vasco-Palacios, P. Q. Thu, A. Suija, M. E. Smith, C. Sharp, E. Saluveer, A. Saitta, M. Rosas, T. Riit, D. Ratkowsky, K. Pritsch, K. Põldmaa, M. Piepenbring, C. Phosri, M. Peterson, K. Parts, K. Pärtel, E. Otsing, E. Nouhra, A. L. Njouonkou, R. H. Nilsson, L. N. Morgado, J. Mayor, T. W. May, L. Majuakim, D. J. Lodge, S. S. Lee, K.-H. Larsson, P. Kohout, K. Hosaka, I. Hiiesalu, T. W. Henkel, H. Harend, L. Guo, A. Greslebin, G. Grelet, J. Geml, G. Gates, W. Dunstan, C. Dunk, R. Drenkhan, J. Dearnaley, A. D. Kesel, T. Dang, X. Chen, F. Buegger, F. Q. Brearley, G. Bonito, S. Anslan, S. Abell, and K. Abarenkov. 2014. Global diversity and geography of soil fungi. Science 346:125668-1-1256688–10.

Tedersoo, L., T. W. May, and M. E. Smith. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263.

Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. F. de Siqueira, A. Grainger, L. Hannah, L. Hughes, L. Huntley, A. S. van Jaarsveld, G. F. Midgley, L. Miles, M. A. Ortega-Huerta, A. T. Peterson, O. L. Phillips, and S. E. Williams. 2004. Extinction risk from climate change. Nature 427:145–148.

Unterseher, M., A. Jumpponen, M. Öpik, L. Tedersoo, M. Moora, C. F. Dormann, and M. Schnittler. 2011. Species abundance distributions and richness estimations in fungal metagenomics--lessons learned from community ecology. Molecular Ecology 20:275–285.

Urban, M. C. 2015. Accelerating extinction risk from climate change. Science 348:571–573.

Vernes, K. 2007. Are diverse mammal communities important for maintaining plant-fungal associations and ecosystem health? Australasian Plant Conservation 15:16–18.

Vernes, K., M. Castellano, and C. N. Johnson. 2001. Effects of season and fire on the diversity of hypogeous fungi consumed by a tropical mycophagous marsupial. Journal of Animal Ecology 70:945–954.

Vernes, K., and L. Dunn. 2009. Mammal mycophagy and fungal spore dispersal across a steep environmental gradient in eastern Australia. Austral Ecology 34:69–76.

Vernes, K., and K. McGrath. 2009. Are introduced black rats (*Rattus rattus*) a functional replacement for mycophagous native rodents in fragmented forests? Fungal Ecology 2:145–148.

Vernes, K., and L. C. Pope. 2001. Stability of nest range, home range and movement of the northern bettong (*Bettongia tropica*) following moderate-intensity fire in a tropical woodland, north-eastern Queensland. Wildlife Research 28:141–150.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. Pages 315–322 *in* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, editors. PCR Protocols A Guide to Methods and Applications. Elsevier Inc.

Woinarski, J. C. Z., A. A. Burbidge, and P. L. Harrison. 2015. Ongoing unraveling of a continental fauna: Decline and extinction of Australian mammals since European settlement. Proceedings of the National Academy of Sciences of the United States of

This article is protected by copyright. All rights reserved.

America 112:4531–4540.

Wood, J. R., J. M. Wilmshurst, T. H. Worthy, A. S. Holzapfel, and A. Cooper. 2012. A Lost Link between a Flightless Parrot and a Parasitic Plant and the Potential Role of Coprolites in Conservation Paleobiology. Conservation Biology 26:1091–1099.

Wood, S. W., L. D. Prior, H. C. Stephens, and D. M. J. S. Bowman. 2015. Macroecology of Australian tall eucalypt forests: Baseline data from a continental-scale permanent plot network. PLoS ONE 10:1–24.

Data Accessibility

- Raw Illumina data is deposited in Sequence Read Archive (SRA; bioproject SRP150847)
- Sanger data is available in GenBank (Accessions KY686200-KY686202, KY697566-KY697576, KY697578-KY697619; Table S2).

Tables

Table 1: Sample numbers (N) and mean ± SE OTU richness per sample (total OTU richness per mammal species) for the Fungal Specialist (*Bettongia tropica*) within Potoroidae and all other mammal species with generalist diets samples combined (Fungal Generalists) across different subsets of the data (all OTUs, ectomycorrhizal OTUs and truffle OTUs).

	All OTUs		ECM OTUs		Truffle OTUs	
Mammal species	N	Mean ± se (total)	N	Mean ± se (total)	N	Mean ± se (total)
Bettongia tropica (Fungal Specialist)	93	188.1 ± 9.34[b] (4176)	92	10.0 ± 0.75[b] (254)	89	8.6 ± 0.80[b] (135)
Mammals with generalist diets	120	101.2 ± 8.25[a] (5266)	108	4.1 ± 0.32[a] (159)	85	3.8 ± 0.44[a] (73)

[a,b:] Different superscript letters represent significant differences in *Tukey* HSD comparisons between *B. tropica* and generalist mammal species ($P < 0.05$).

Figure Legends

Figure 1: Bipartite network of mammal species (upper level) and the truffle OTUs within each mammal species' diet (lower level). Truffle OTUs are labelled according to truffle family (see Table S4 for which species these represent). Each interaction between mammal and truffle taxa is represented by a green line or triangle filled with blue. Width of boxes and lines is proportional to the relative sequence read count (depth) for each truffle-mammal interaction. Therefore, interactions that have higher relative abundance are wider and appear blue and green lines represent a lower rate of interaction. *Bettongia tropica* are the central hub of this network in that they are the most connected species. The sample numbers for each mammal species are n = 89 for *Bettongia tropica* (fungal specialist), n = 1 for *Antechinus flavipes*, n = 25 for *Isoodon macrourus*, n = 4 for *I. obesulus*, n = 12 for *Melomys sp.*, n = 4

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for *Perameles nasuta,* n = 1 for *Rattus sp.,* n = 12 for *Trichosurus vulpecula,* n = 20 for *Uromys caudimaculatus,* n = 6 for *Zyzomys argurus.*

Figure 2: Ordination plot for the whole OTU dataset on Hellinger-transformed data; Partial-RDA where variation of site and season are partitioned out (2.4% variation) and only specialist groups examined (2.6% variation) (fungal community ~ specialist groups + Condition (site, season)). Permutational significance tests on this ordination (comparable to a conditioned PERMANOVA using Hellinger distance) indicate that fungal communities are significantly different between fungal specialist and fungal generalist scats ($F_{1, 209} = 5.611$, $P = 0.001$). Black circles: fungal specialist samples (*Bettongia tropica*), red triangles: samples from all fungal generalists combined. Grey squares are the mean values for each specialist group and error bars are standard deviations. Note that one constrained axis (RDA1) and one unconstrained axis (PC1) result from constraining the RDA on a factor with two levels (one degree of freedom gives one constrained axis that maximises separation between the two factor levels).

Figure 3: Rarefaction curves for individual mammalian scat samples showing average species (OTU) richness from repeated subsampling across sequence read counts (depth) within each site (Davies Creek, Emu Creek and Tinaroo Dam) and season (early wet; February-January, late dry; November-December, late wet; May-June). *Bettongia tropica* diets (red lines, fungal specialists) more often contained more truffle OTUs per read count than fungal generalist mammals across sites and seasons.

Figure 4: Venn diagrams comparing numbers of unique and shared OTUs from a fungal specialist (*Bettongia tropica*) and all fungal generalist mammal species' samples for a) all OTUs, b) ectomycorrhizal OTUs, and c) truffle OTUs.

Figure 5: Proportional loss of truffle food web connections when mammals are selectively removed from the dataset from most (*Bettongia tropica,* fungal specialist)-to-least connected species. The line represents the mean and standard error (using loess method). The robustness index value is below 0.5 (calculated as the area under the curve) indicating that this network is sensitive to mammalian extinctions.

Author contributions

Nuske, SJ: designed study; collected data; sequenced DNA; analysed data; wrote manuscript

Anslan, S: preformed bioinformatics; edited manuscript

Tedersoo, L: contributed laboratory space, primers and reagents; provided advice on high-throughput sequencing; edited manuscript

Bonner, MTL: provided advice on data analysis and wrote some of the R scripts; edited manuscript

Congdon, BC: provided advice on design of study and data analysis; edited manuscript

Abell, SE: provided advice on design of study and data analysis; edited manuscript

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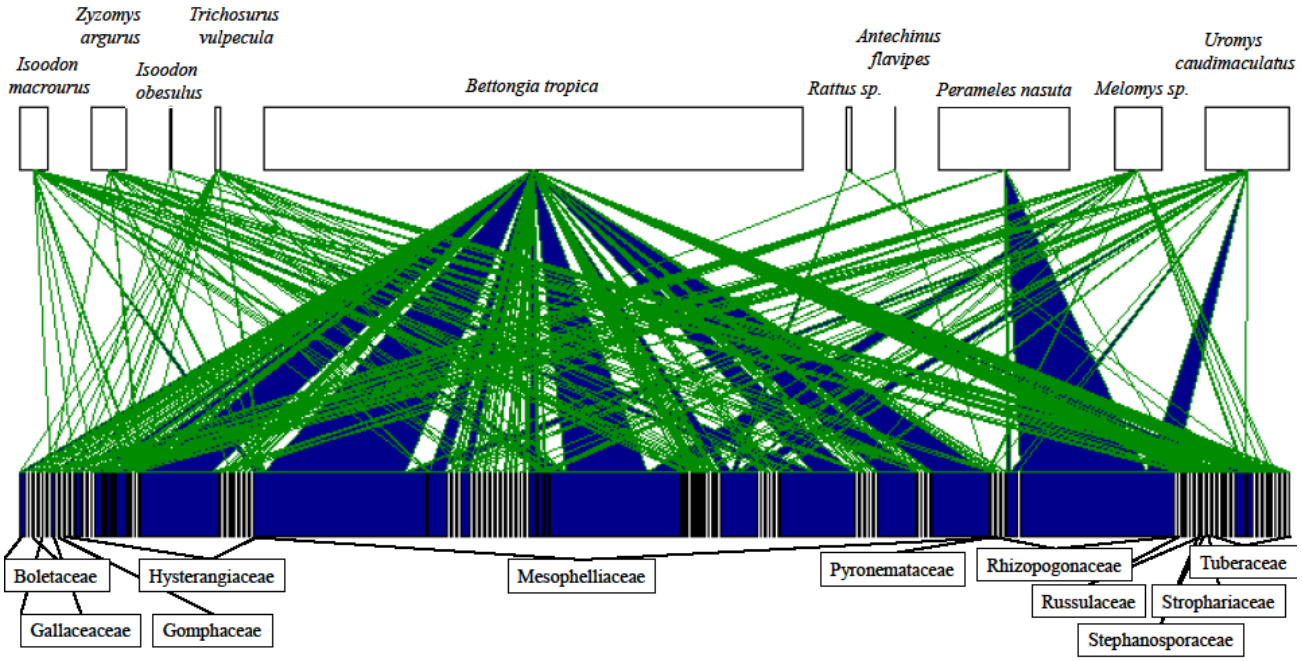

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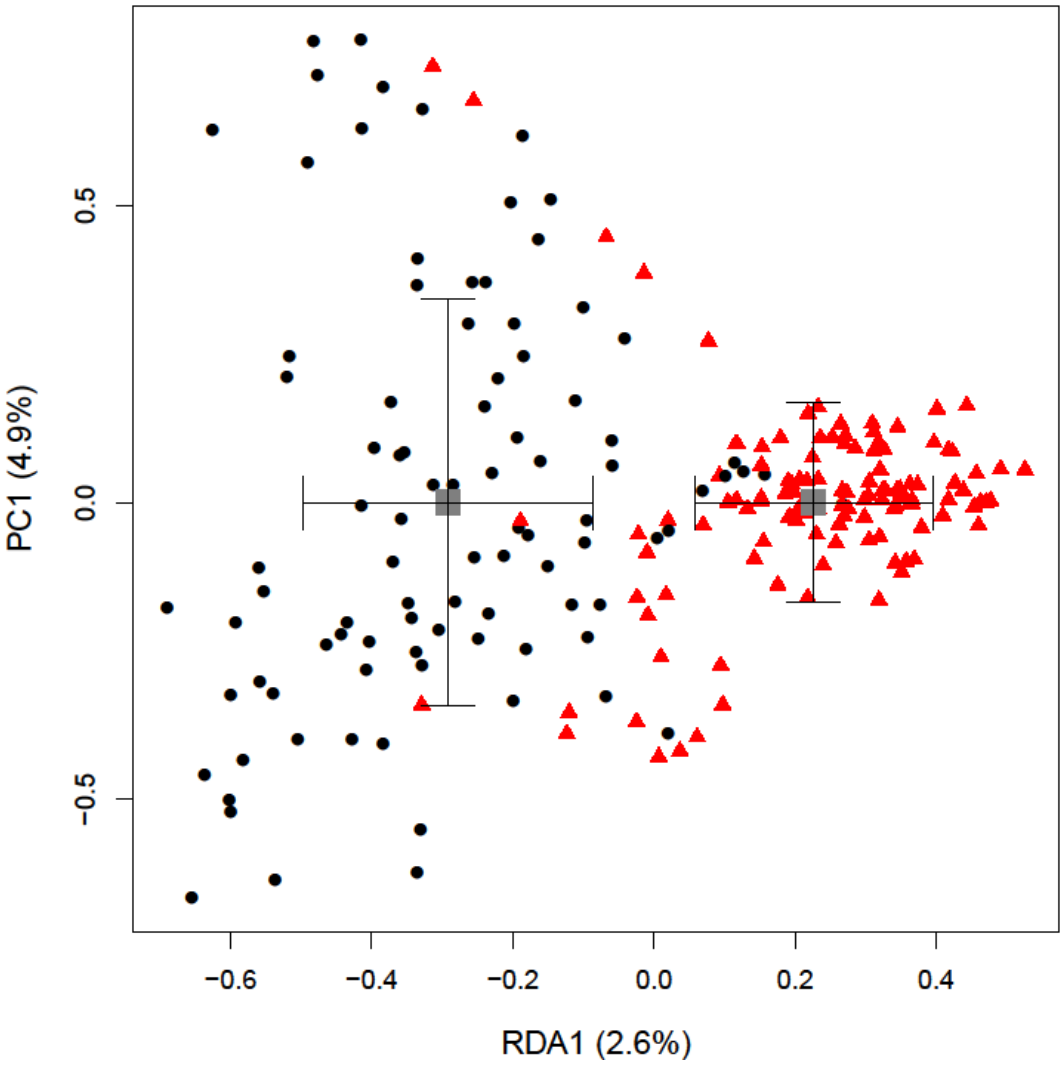

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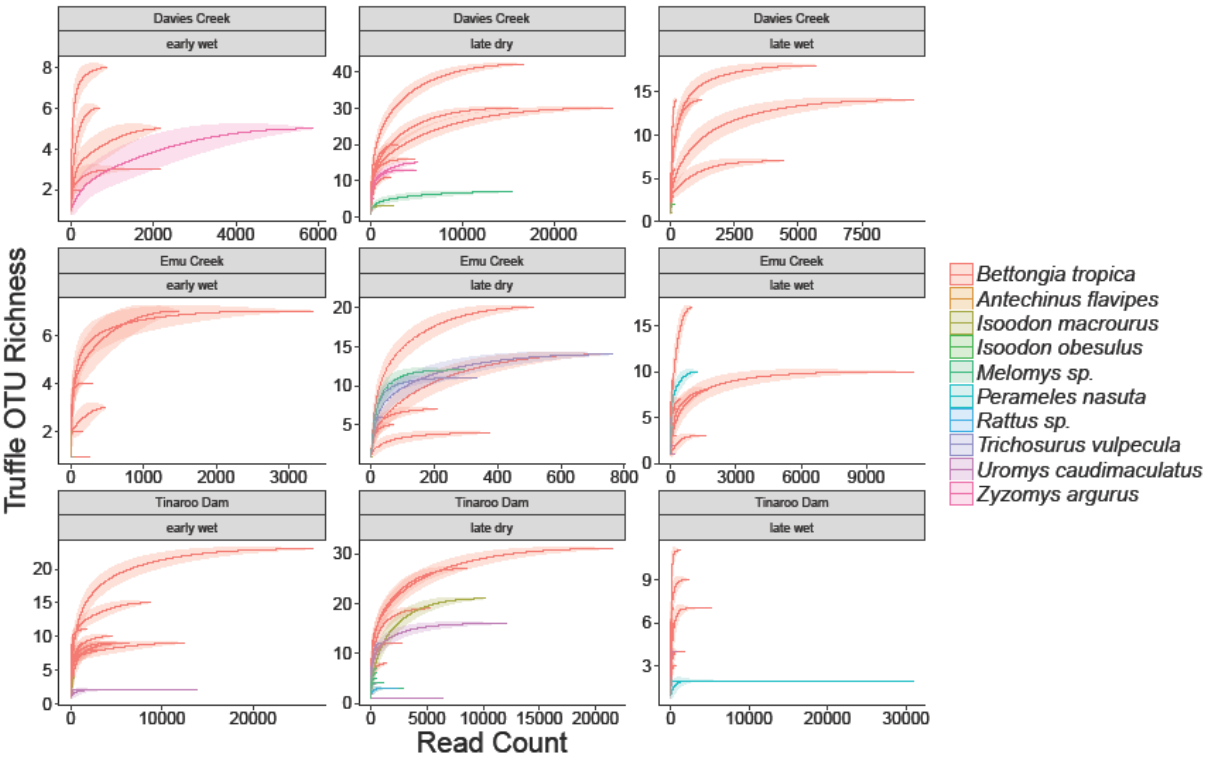

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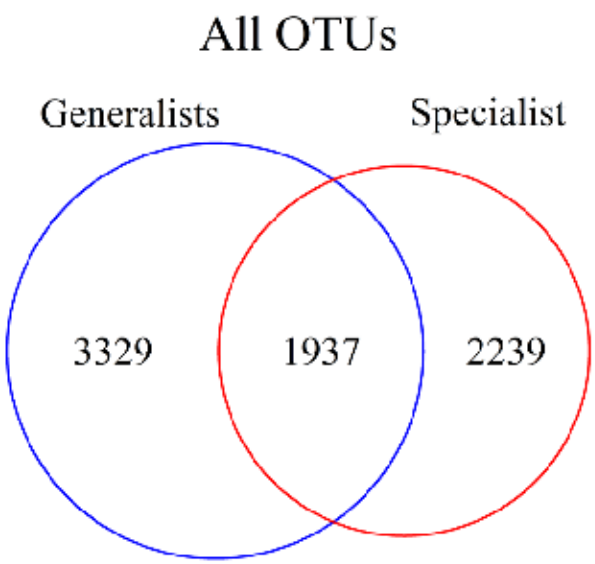
a)



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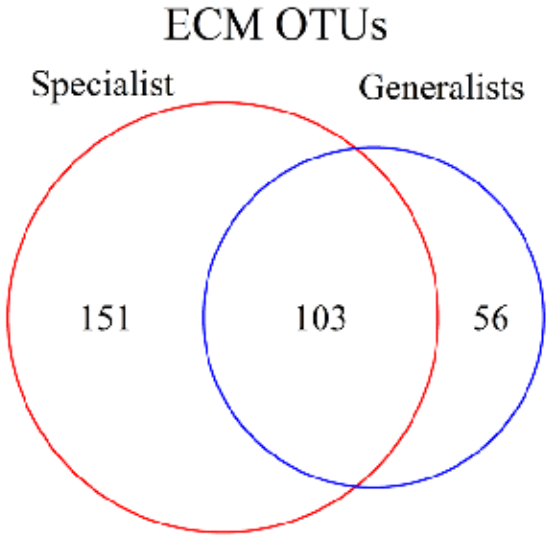

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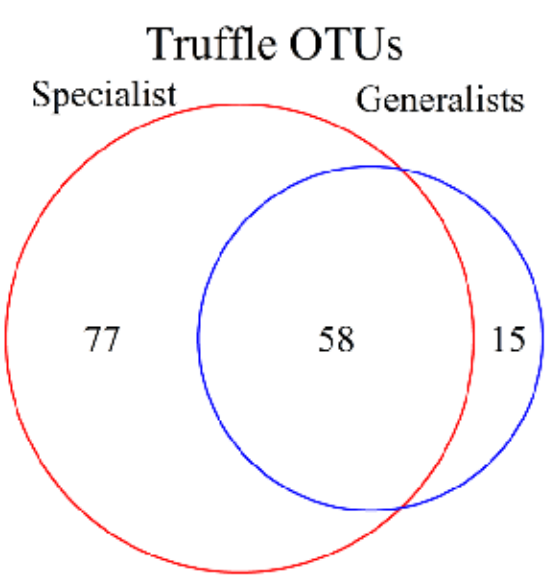

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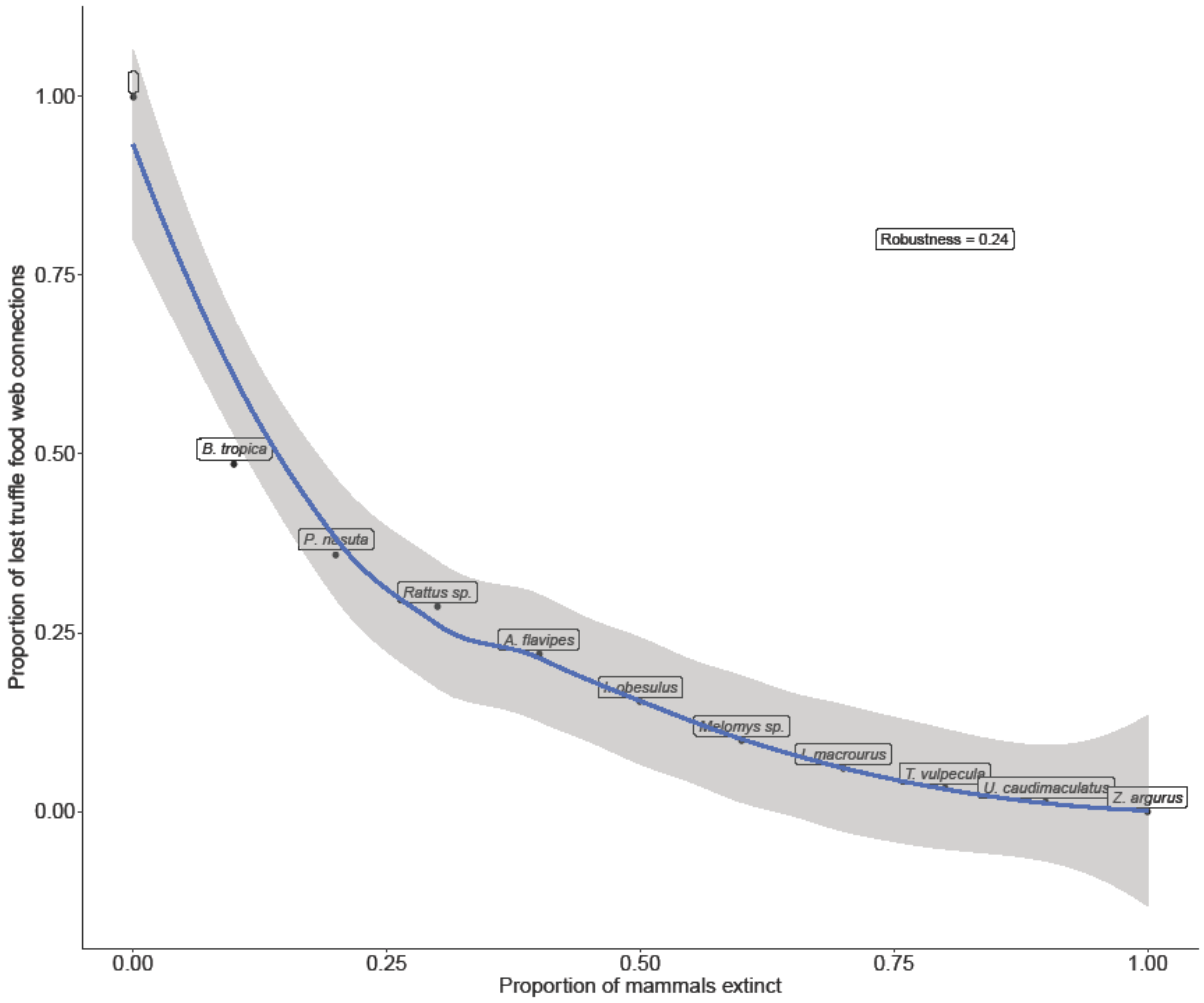

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