Animal Microbiome

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Metagenomic profiling of bacterial and fungal microbiota and putative pathogens of southern greater gliders (*Petauroides volans*)

Jordyn Clough[1*] and Katarina M. Mikac[1*]

Abstract

Background The microbiome is significant for conservation biology and should be considered in threatened species management programs. Commensal microbes contribute important functions for host health, while pathogenic microbes can negatively impact the host, leading to morbidity, mortality, and population declines. Shotgun metagenomics, involving the agnostic sequencing of all DNA within a sample, has utility for simultaneous microbial community profiling and pathogen detection. Herein, we used shotgun metagenomics to profile the faecal bacteriome and mycobiome of the southern greater glider (*Petauroides volans*), an endangered Australian marsupial, and identify putative pathogens that could represent threats to population health.

Results We analysed faecal samples collected from wild southern greater gliders ($n=48$) across southeastern New South Wales, Australia. Geographic location had significant effects on both bacterial and fungal community composition. The bacteriome was dominated by *Firmicutes*, *Proteobacteria* and *Bacteroidetes*, with 58 core bacterial species shared among all locations. The mycobiome was dominated by the *Ascomycetes*, with 261 core fungal species shared among all locations. Geographic location was associated with significant differences in bacterial and fungal microbiota abundance but not host sex or weight. We identified 18 bacterial pathogens and 41 fungal pathogens of veterinary interest, ranging from low prevalence to ubiquitous. *Bacteroides fragilis* was associated with shifts in the *Firmicutes*:*Bacteroidetes* ratio, and therefore, potential dysbiosis.

Conclusions Geographic location is a key determinant of faecal microbial community structure and abundance in southern greater gliders. Core communities of faecal bacteria and fungi are conserved within and among populations. Southern greater gliders carry genetic material from a variety of putative bacterial and fungal pathogens. These microbes may present threats to the health of greater gliders, their possum relatives, or other animals in Australian forest ecosystems. Scientists and natural resource managers should consider the holobiont, rather than just the individual, when planning for conservation management actions such as translocation.

*Correspondence:
Jordyn Clough
jclough@uow.edu.au
Katarina M. Mikac
kmikac@uow.edu.au

Full list of author information is available at the end of the article

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Backround

Southern greater gliders (*Petauroides volans*) are arboreal, folivorous marsupials of high conservation priority, given they are an endangered species that has experienced ongoing declines for several decades [1]. Greater gliders depend on old growth, hollow-bearing trees for denning and shelter [2], and are heavily restricted in their diet as specialist *Eucalyptus* folivores [3]. Well known threats to greater gliders include bushfires [4, 5], climate change [6, 7], logging [4, 8], and deforestation [9]. However, there is a noted lack of information on greater glider health, especially when it comes to pathogens. Aside from *Chlamydia* [10, 11], there is little knowledge of the pathogens that greater gliders are exposed to, and could potentially be susceptible to infection from, in Australian forest ecosystems. Both bacterial and fungal pathogens alike can have devastating impacts on wildlife, causing morbidity, mortality and significant population declines [12, 13]. While preliminary work has revealed geographic location to be a driver of the gastrointestinal (GI) microbiota of greater gliders [14], species-level resolution microbial profiling has not been achieved to date.

Over recent years, calls have begun for the inclusion of microbiome research to address knowledge gaps and assist in threatened species conservation [15]. Characterisation of the GI microbiota, both locally and across the landscape, is useful for informing conservation strategies. This is especially relevant when considering translocation as a species conservation tool [16]. There is ample evidence that the GI microbiota influence host eating behaviours [17]. In the context of arboreal marsupials, the GI microbiota have been shown to influence diet selection by the koala (*Phascolarctos cinereus*), another specialist *Eucalyptus* folivore [18]. In turn, differences in diet, resulting from the consumption of different *Eucalyptus* spp., are associated with distinct GI microbiomes [19]. Translocated individuals face enforced dietary change when they are moved from one forest to another, but do not necessarily have GI microbiota adapted to the local environment, and importantly the local feed trees, at the release site. Hence, understanding the fine-scale composition of the GI microbiota across different populations within the landscape is a necessary precursor to implementing translocation programs for specialist folivores.

The GI microbiota have important functions for host health [20]. Commensal microbes aid in host digestion, metabolism and immunity [21, 22], without inducing damage to the host [23]. However, not all microbes are beneficial. Pathogenic microbes infect the host and induce damage, which can clinically manifest as disease [23]. Typically harmless members of the normal microbiome can also shift to become pathogenic under particular conditions, such as compromised host immunity or establishment in atypical body sites [24]. Dysbiosis, a state of imbalance in the microbiota, can negatively impact the host. This may feature the loss of commensals and their important microbial functions, proliferation of pathogenic microbes, or reduced microbial diversity [25]. Beyond the individual, microbes from different hosts and environments are interconnected through the cycling of microbial communities [26], and may interact competitively or cooperatively [27], bearing implications for the broader ecosystem. Considering the complex interplay between microbes, their hosts and the environment, the microbiome has significant implications for conservation biology [28].

Shotgun metagenomics is a powerful tool involving next-generation sequencing to identify DNA from all organisms present in a sample, enabling the profiling of microbial communities associated with a host down to species-level resolution [29]. This metagenomic sequencing approach is superior to the commonly used 16S rRNA gene sequencing for microbial profiling, with greater power to detect less abundant taxa and provide a more complete view of the microbial community [30]. Various studies have employed faecal shotgun metagenomics to profile the GI microbiota of wildlife [31–34]. However, applications of shotgun metagenomics to detect pathogenic agents have been largely limited to laboratory [35, 36], livestock [37, 38] and domestic animals [39, 40]. Herein, we explore the use of shotgun metagenomics for simultaneous microbiota profiling and agnostic pathogen detection in a cryptic, endangered Australian marsupial.

The overarching aim of this study was to characterise the faecal bacteriome and mycobiome of southern greater gliders at species-level resolution using shotgun metagenomics. We leveraged a landscape-scale sampling design as a natural experiment to test for geographic variation in these microbial communities and determine the prevalence of pathogenic microbes. The specific objectives of this study were to: (i) assess microbial diversity and community structure, (ii) identify core microbiota conserved within and among geographic locations, and (iii) screen for putative bacterial and fungal pathogens.

Methods

Study area and sample collection

Faecal samples were collected from southern greater gliders ($n = 48$) captured from four geographic locations across southeastern New South Wales, Australia

(Fig. 1). Individuals were sampled from Mount Lindsey (MTL) (34.45158 S, 150.55395 E) ($n = 9$, August–October 2023), Tallaganda National Park and State Conservation Area (TAL) (35.41334 S, 149.54551 E) ($n = 7$, November 2023), a private property bordering Tallaganda National Park and Tallaganda State Forest protected by a conservation agreement with the NSW Biodiversity Conservation Trust (SHA) ($n = 18$, March 2022–November 2023), Seven Mile Beach National Park (SMB) (34.81081 S, 150.75793 E) ($n = 8$, January-April 2024) and Ulladulla (ULL) (35.371020 S, 150.445255 E) ($n = 6$, June 2024). Each sampling location was considered spatially independent based on the small home ranges (1–4 ha) and limited dispersal capabilities of southern greater gliders [41–45]. MTL, SMB, and ULL are geographically isolated from all other sites by many kilometres of unsuitable habitat. While TAL and SHA are located within the same contiguous forest block, the sites are separated by >10 km.

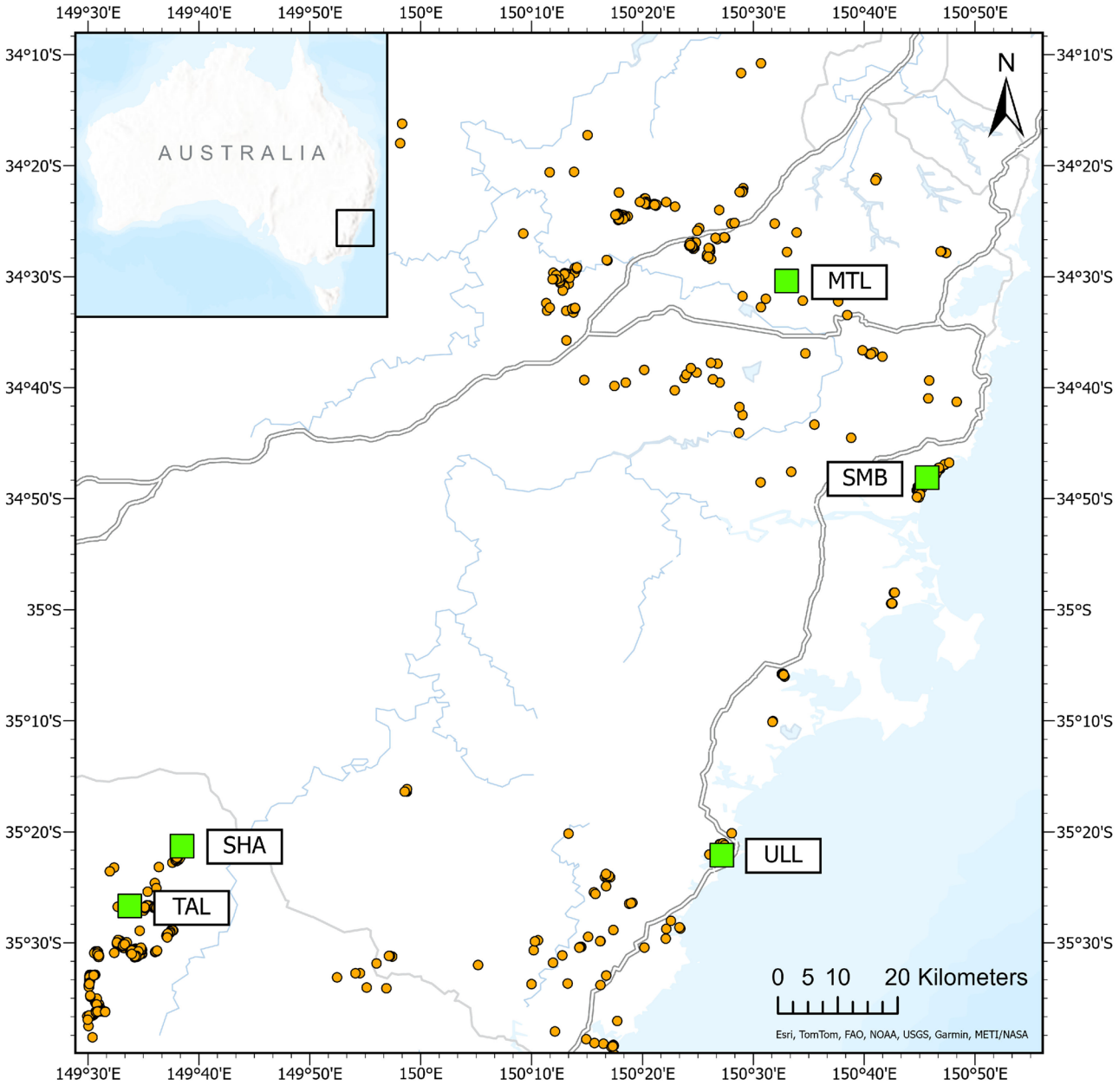

Fig. 1 Study area map showing southern greater glider (*Petauroides volans*) faecal sampling locations across southeastern NSW. Sampling locations are indicated by green squares. Species occurrence records from May 2020 onwards (orange circles) were sourced from the BioNet atlas database (http://www.bionet.nsw.gov.au, accessed on 2/8/25) and overlaid on the map for distributional context. Location abbreviations: MTL = Mount Lindsey; SMB = Seven Mile Beach National Park; TAL = Tallaganda National Park and State Conservation Area, SHA = private property connected to Tallaganda National Park and Tallaganda State Forest; ULL = Ulladulla

Live capture of southern greater gliders was conducted by direct capture from hollows or using the branch-shake method, as previously described [46]. Upon capture, faecal pellets were collected directly from the cloaca in a sterile 1.5 mL Eppendorf tube and stored dry at −20 °C.

DNA extraction and shotgun metagenomic sequencing

Extraction of microbial DNA and whole metagenome shotgun next-generation sequencing (mNGS) was performed at the Australian Genomics Research Facility (AGRF, Australia). Briefly, library preparation was completed using the Illumina DNA Preparation (M) kit, producing a high-quality library of ~ 350 bp inserts with uniform coverage. Sequencing was performed on the Illumina NovaSeqX Plus platform with 150 bp paired-end chemistry, generating 150 bp paired-end reads. The raw reads were processed by trimgalore (v. 0.4.5; a wrapper tool around Cutadapt [47] and FastQC [48]) to remove sequencing adapters and low-quality bases. The filtered reads were then processed using Kraken2 (v. 2.0.8) [49] in paired-end mode (–paired flag) and Bracken (v. 2.5) [50] to taxonomically classify and profile the composition of microbial communities based on the NCBI genome and nucleotide (nt) database (downloaded 15/04/2021). Both tools were run using default parameters, as recommended by the developers. A summary of sequencing read counts and classification rates for each sample is provided in Supplementary Table S1.

Data filtering

All data analysis was performed in R (v. 4.4.1). The absolute abundance output from Bracken was filtered to generate bacterial and fungal operational taxonomic unit tables, which were combined with taxonomy and sample metadata information into 'phyloseq' objects using the *phyloseq* function from the phyloseq R package (v. 1.48.0) [51]. Contaminant reads were identified using the *isContaminant* function from the decontam R package (v. 1.24.0) [52], specifying the water control as the negative control. Contaminants were removed from each 'phyloseq' object using the *prune_taxa* function from phyloseq [51]. The decontaminated bacterial and fungal 'phyloseq' objects were then analysed independently using the methods described below. Hereafter, we use the term 'microbiome' to refer to the complete community of microbes. When discussing specific components of the community, we refer to the 'bacteriome' (bacterial community) and the 'mycobiome' (fungal community).

Alpha diversity analysis

Alpha diversity metrics (Shannon diversity, Simpson diversity, observed species) were calculated at the species level using the *diversity* function from the vegan R package (v. 2.6.10) [53]. Kruskal Wallis tests (*kruskal.*test) were performed to test for significant differences among geographic locations and between the sexes. Spearman's rank correlation (*cor.test*) was used to test for correlations with body weight. Violin plots showing Shannon diversity across locations were generated using the *ggplot* function from the ggplot2 R package (v. 4.0.0) [54].

Beta diversity analysis

Beta diversity was assessed using both presence-absence (Jaccard dissimilarity) and abundance-weighted (Bray-Curtis dissimilarity) metrics, calculated at the species level using the *vegdist* function from vegan [53]. Principal coordinates analysis (PCoA) was performed for visualisation of beta diversity using the *pco* function from the ecodist R package (v. 2.1.3) [55]. Permutational multivariate analysis of variance (PERMANOVA) was performed on Jaccard dissimilarities using the *adonis2* function from vegan [53], with 999 permutations, to test for significant effects of location, sex, and body weight on microbial community composition. Homogeneity of multivariate dispersion was assessed using the *betadisper* function from vegan [53], with 999 permutations, to test for differences in within-group variance.

Taxonomic composition

Taxa were agglomerated to the phylum and genus level using the *tax_glom* function from phyloseq [51]. Data were then transformed from absolute abundance to relative abundance using the *transform* function from the microbiome R package (v. 1.26.0) [56]. Bar plots visualising phylum-level and genus-level microbial community profiles were generated using the *ggplot* function from ggplot2 [54].

Differential abundance analysis

Differential abundance analysis was performed using the Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC2) method [57]. The *ancombc2* function from the ANCOMBC R package (v. 2.6.0) [58] was run using the default parameters. Analysis was performed at the phylum and genus levels based on: (i) geographic location, (ii) sex, and (iii) host weight. All three covariates were included as fixed effects in the 'fix_formula' parameter, to control for variability in microbial abundances that could be attributed to differences in geographic location, sex and weight. The 'struc_zero' parameter was set to 'TRUE' for analysis based on location and sex (categorical); and set to 'FALSE' for analysis based on weight (continuous). Only taxa that were flagged as significantly differentially abundant in the global test ($p_{adj} < 0.05$) and passed the sensitivity analysis to pseudo-count addition were considered to be differentially abundant.

Core microbiome analysis

To identify core microbes, relative abundance data were first inspected to identify which species were the most prevalent using the *prevalence* function from microbiome [56]. A heatmap showing prevalence at different relative abundance detection thresholds was generated using the *plot_core* function from microbiome [56] and used to inform the selection of an appropriate detection threshold (0.1%). Relative abundance data were then screened using the *core_members* function from phyloseq [51], setting a detection threshold of 0.1% and prevalence threshold of 50%. The output was visualised as a Venn diagram using the *venn* function from the gplots R package (v. 3.2.0) [59]. Core species detected among all locations were visualised on a heatmap using the *Heatmap* function from the ComplexHeatmap R package (v. 2.20.0) [60]. Relative abundances were $\log_{10}$-transformed prior to visualisation.

Screening for putative pathogens

A reference list of bacterial and fungal pathogens of veterinary interest was compiled from the VetBact database [61] and established veterinary textbooks [62–65]. The metagenomic data were then screened against this list to identify putative pathogens. For each putative pathogen identified, a literature search was conducted using the PubMed, Scopus and Google Scholar search engines to determine its known Australian wildlife hosts. The search for bacterial pathogens focused on Australian possum and glider hosts, while the search for fungal pathogens was extended to include all Australian marsupial hosts, due to the lack of sufficient records in possums and gliders. Search terms included the scientific name of the pathogen (e.g. "*Escherichia coli*") and host (e.g. "*Trichosurus vulpecula*"). Putative pathogens were classified as zoonotic based on documented evidence of animal-to-human transmission, primarily referencing Bauerfeind et al. [66]. This was supplemented by a literature search using the scientific name of the pathogen combined with the terms "zoonotic" or "zoonosis".

The prevalence of each putative pathogen was calculated as the percentage of samples in which it was detected out of the total sample size ($n = 48$). For visualisation, a presence-absence heatmap of pathogens detected in more than one sample was generated using the *Heatmap* function from ComplexHeatmap [60]. Linear regression models were used to assess associations between the presence of each detected pathogen and shifts in the *Firmicutes*: *Bacteroidetes* (F:B) ratio, a marker of GI dysbiosis [67]. Prior to statistical analysis, the F:B ratio was log-transformed to address its skewed distribution and meet the assumptions of linear regression models. Models were ran using the *lm* function, specifying binary presence/absence as the independent variable and the log-transformed F:B ratio as the dependent variable, with geographic location included as a fixed effect to control for potential confounding. To correct for multiple comparisons, *p*-values were adjusted using the Benjamini-Hochberg False Discovery Rate (FDR) correction [68]. An FDR-adjusted $p < 0.05$ was considered significant. For significant associations, effect size was quantified by calculating Cohen's *d* with a 95% confidence interval (CI) using the *cohens_d* function from the effectsize R package (v. 1.0.1) [69]. Estimated fold-changes in the F:B ratio were exponentiated from the model coefficients.

Results

Bacteriome

Bacterial alpha diversity did not vary significantly among geographic locations ($p > 0.05$) (Supplementary Table S2). However, the variance was not uniform among locations, with samples from SMB exhibiting the widest range of Shannon diversity (Fig. 2A). Neither host sex nor body weight were significant factors affecting bacterial alpha diversity ($p > 0.05$) (Supplementary Table S2). Principal coordinates analysis on Jaccard dissimilarities revealed clustering of bacterial communities by location (Fig. 2B). Samples from TAL and SHA clustered together and away from MTL, SMB and ULL samples (Fig. 2B). PERMANOVA revealed that location was a significant factor explaining bacterial beta diversity using both presence-absence (Jaccard dissimilarity) ($F(4, 48) = 2.91$, $R^2 = 0.21$, $p = 0.001$) (Fig. 2B) and abundance-weighted (Bray-Curtis dissimilarity) ($F(4, 48) = 2.39$, $R^2 = 0.19$, $p = 0.004$) metrics (Supplementary Table S2). The effect of location was confirmed to not be due to differences in within-group variance, based on tests for homogeneity of multivariate dispersion ($p > 0.05$). Host sex and body weight were not significant factors explaining bacterial beta diversity using either dissimilarity metric ($p > 0.05$) (Supplementary Table S2).

A total of nine phyla and 12 genera were detected at >1% mean relative abundance across the samples (Fig. 3A, B). The most abundant phyla were *Firmicutes* (35.06% ± 6.30%), *Proteobacteria* (28.62% ± 7.75%) and *Bacteroidetes* (11.83% ± 3.29%) (Fig. 3A). The most abundant genera were *Escherichia* (3.01% ± 3.09%), *Clostridium* (2.13% ± 0.38%), and *Lachnoclostridium* (1.94% ± 0.92%) (Fig. 3B). The majority of bacterial microbes comprised low-abundance (<1%) genera and this was consistent across all samples (Fig. 3B).

Using ANCOM-BC2, we identified 43 bacterial phyla and 737 bacterial genera that showed significant differential abundance among locations in the global test ($p_{adj} < 0.05$) (Supplementary Table S3). These findings indicate that geographic location was associated with differences in bacterial microbiota abundance. Neither sex

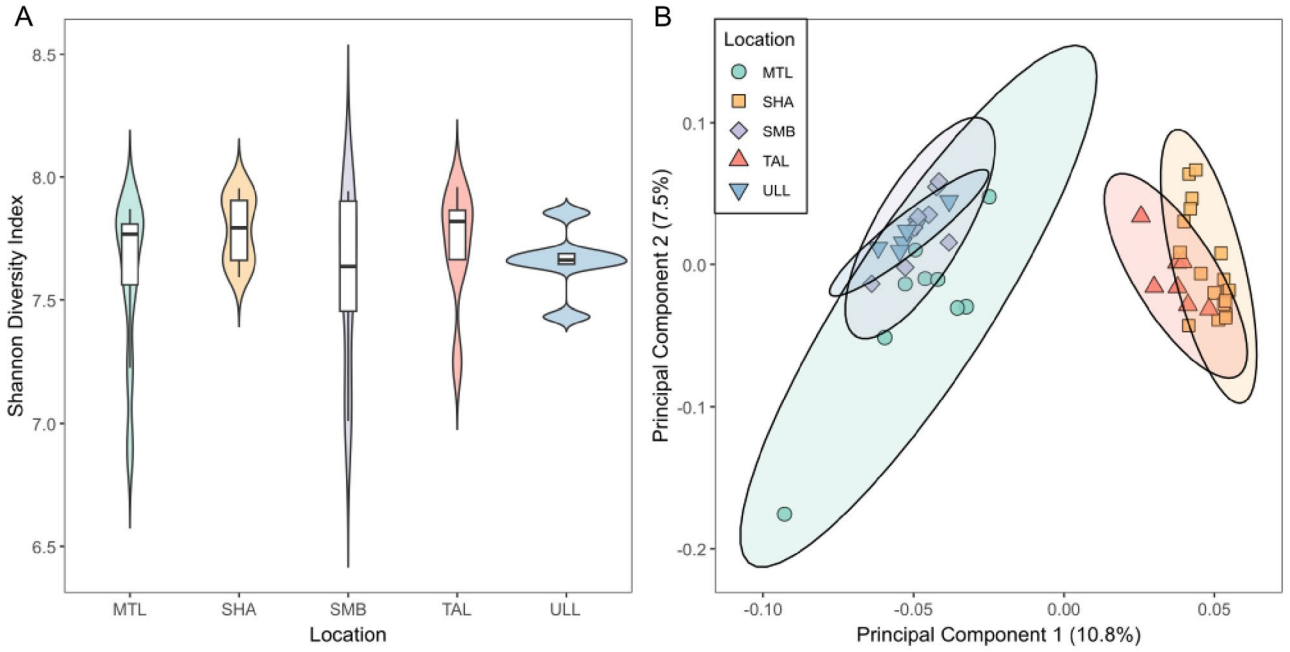

Fig. 2 Diversity of southern greater glider (*Petauroides volans*) faecal bacteriomes from different locations across southeastern NSW. (**A**) Shannon diversity (alpha diversity). Boxplots show the median and interquartile range of the Shannon diversity index at each location. Violin plots visualise the variation in Shannon diversity within and among sites. (**B**) PCoA based on Jaccard dissimilarity (beta diversity). Ellipses represent 95% CIs around the group centroids based on the beta diversity index. See Fig. 1 for location details

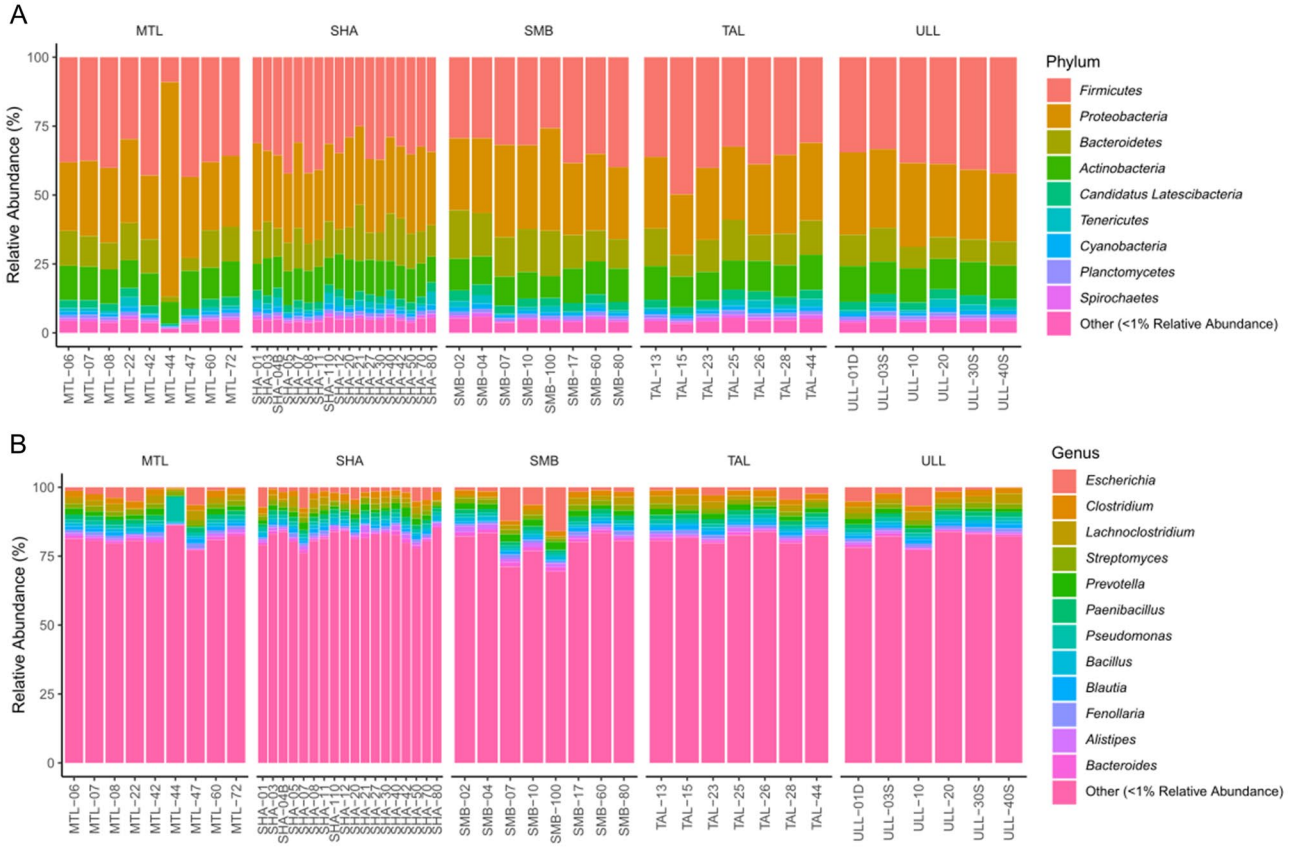

Fig. 3 Taxonomic composition of the southern greater glider (*Petauroides volans*) faecal bacteriome. (**A**) Phylum-level plot. (**B**) Genus-level plot. Taxa with <1% mean relative abundance have been agglomerated under the designation 'other'. See Fig. 1 for location details

nor host weight had significant effects on the abundance of bacterial phyla or genera.

Core bacterial species were found both conserved within and shared among different locations. Nine species were detected with 100% prevalence (Supplementary Table S4). Uncultured Bacteria, given as a designation for bacteria that could not be taxonomically classified, were also detected in every sample. A total of 58 core bacteria were shared among all locations (Fig. 4A, B, Supplementary Table S4). MTL recorded the highest count of core species (97) and SMB recorded the highest count of unique core species (those found as core only at this location) (9) (Fig. 4A, Supplementary Table S4). The relative abundances of the core members of the bacteriome varied considerably, with *Escherichia coli* identified as the most dominant species overall (Fig. 4B).

Mycobiome

Fungal alpha diversity significantly varied among geographic locations ($p < 0.05$) (Supplementary Table S5). The variance also differed between sites, although in contrast to the bacterial result, samples from SMB had the narrowest range of Shannon diversity (Fig. 5A). Host sex and body weight were not significant factors affecting fungal alpha diversity ($p > 0.05$) (Supplementary Table S5). Principal coordinates analysis on Jaccard dissimilarities revealed clustering of fungal communities by location (Fig. 5B). Samples from SMB and ULL formed a cluster within the broader MTL distribution, while samples from SHA and TAL clustered together (Fig. 5B). PERMANOVA revealed that location was a significant factor explaining fungal beta diversity using both presence-absence (Jaccard dissimilarity) ($F(4, 48) = 2.52$, $R^2 = 0.19$, $p = 0.001$) (Fig. 5B) and abundance-weighted (Bray-Curtis dissimilarity) ($F(4, 48) = 3.37$, $R^2 = 0.24$, $p = 0.001$) metrics (Supplementary Table S5). The effect of location was confirmed to not be due to differences in within-group variance, based on tests for homogeneity of multivariate dispersion ($p > 0.05$). Neither host sex nor body weight were significant factors explaining fungal beta diversity ($p > 0.05$) (Supplementary Table S5).

Four phyla and 13 genera were detected at >1% mean relative abundance across the samples (Fig. 6A, B). *Ascomycetes* was the predominant phylum by a wide margin (81.00% ± 5.81%), followed by *Basidiomycota* (15.50% ± 4.84%), *Zoopagomycetes* (1.42% ± 0.42%) and *Mucoromycota* (1.37% ± 0.53%) (Fig. 6A). The most abundant genera were *Aspergillus* (9.23% ± 3.68%), *Debaryomyces* (4.34% ± 10.80%) and *Fusarium* (3.93% ± 4.64%) (Fig. 6B). Low-abundance (<1%) genera comprised the bulk of fungal microbes, although some samples from MTL, SHA, and to a lesser extent TAL, showed reduced proportions of low-abundance genera and elevated proportions of higher abundance genera (Fig. 6B).

Using ANCOM-BC2, we identified six phyla and 356 genera that showed significant differential abundance among locations in the global test ($p_{adj} < 0.05$) (Supplementary Table S6). These findings indicate that geographic location was associated with differences in fungal microbiota abundance. Meanwhile, no significant effect of sex or weight was found on the abundance of fungal phyla or genera.

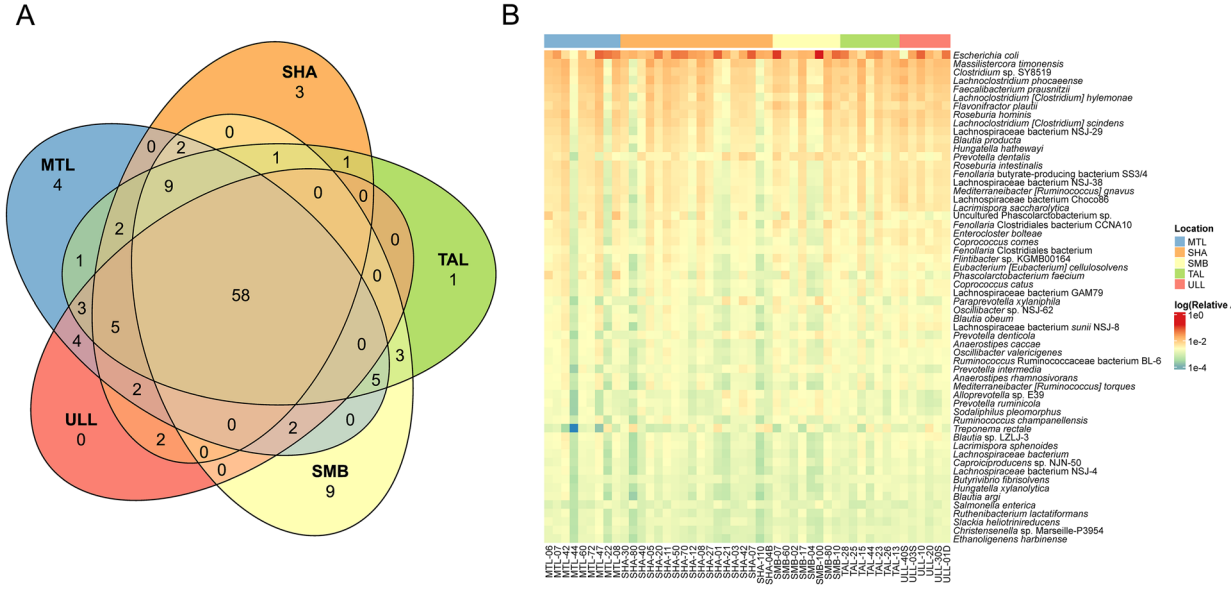

Fig. 4 Core species identified in southern greater glider (*Petauroides volans*) faecal bacteriomes. (**A**) Venn diagram showing core species (>50% prevalence, >0.1% relative abundance) across different locations. The intersection of circles represents species shared by locations. (**B**) Heatmap showing relative abundance of the 58 core species shared among all locations. See Fig. 1 for location details

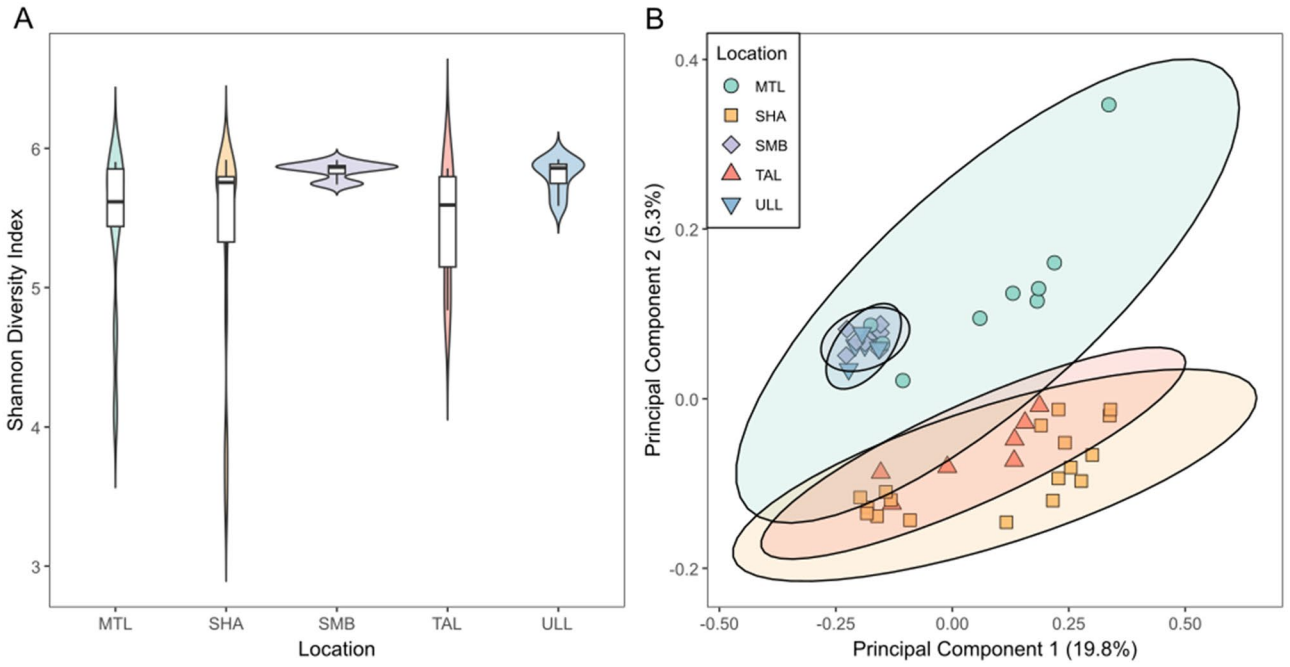

Fig. 5 Diversity of southern greater glider (*Petauroides volans*) faecal mycobiomes from southeastern NSW. (**A**) Shannon diversity (alpha diversity) box plot. Boxes represent the interquartile range, with the median indicated by the horizontal line and whiskers extending to 1.5× the interquartile range. Violin plots visualise the variation in Shannon diversity within and among sites. (**B**) PCoA visualisation based on Jaccard dissimilarity (beta diversity). Ellipses represent 95% CIs around the group centroids based on the beta diversity index. See Fig. 1 for location details

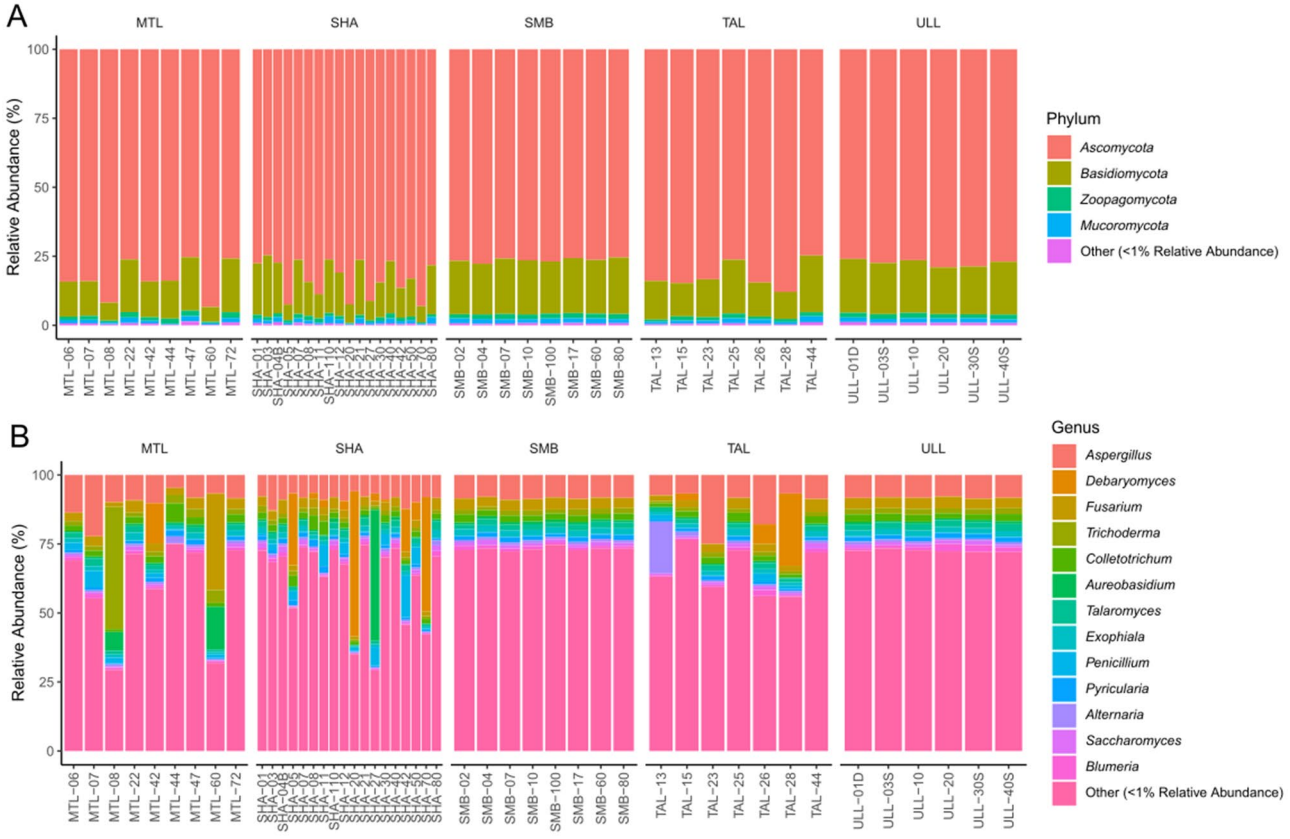

Fig. 6 Taxonomic composition of the southern greater glider (*Petauroides volans*) faecal mycobiome. (**A**) Phylum-level plot. (**B**) Genus-level plot. Taxa with <1% mean relative abundance have been agglomerated under the designation 'other'. See Fig. 1 for location details

Core fungal species were found both conserved within and shared among different locations. Forty-four fungal species were detected at 100% prevalence (Supplementary Table S7) and a total of 261 core fungi were shared between all locations (Fig. 7A, Supplementary Table S7). SMB recorded the highest count of core species (326) and unique core species (9) (Fig. 7A, Supplementary Table S7). The relative abundances of the core members of the mycobiome were generally low, with no fungus consistently detected at high abundance across the samples (Fig. 7B).

Putative pathogens of veterinary interest

DNA from a variety of putative pathogenic bacteria and fungi was detected. To remain conservative in our reporting, we focused on the putative pathogens detected in more than one sample at a > 0.1% relative abundance threshold, which included 18 bacterial and 41 fungal species (Tables 1 and 2, Fig. 8). Among the bacteria, the most well-represented families were *Enterobacteriaceae* (with *Escherichia* and *Salmonella*) and *Listeriaceae* (with *Bronchothrix* and *Listeria*), with *Clostridium* the most well-represented genus (3 spp.) (Table 1). Among the fungi, *Aspergillus* was the most well-represented genus (12 spp.), followed by *Candida* (5 spp.) and *Malassezia* (5 spp.) (Table 2).

Several putative pathogens were highly prevalent (Fig. 8, Tables 1 and 2). For bacteria, *E. coli* was ubiquitous (100% prevalence), while *Salmonella enterica* (79.17%) and *Clostridium perfringens* (70.83%) showed high prevalence. Moderate prevalence bacterial species included *Bacteroides fragilis* (56.25%), *Prevotella melaninogenica* (37.50%), *Pseudomonas fluorescens* (22.92%), *Helicobacter pylori* (16.67%), and *Enterococcus faecalis* (16.67%) (Fig. 8A). For fungi, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Aspergillus nidulans* were ubiquitous. Furthermore, 13 fungal taxa showed very high prevalence (>90%), nine were detected at high prevalence (>50–90%), and eight were found at moderate prevalence (>10–50%), including *Batrachochytrium dendrobatidis* (45.83%) (Fig. 8B). Other bacterial and fungal taxa were detected at low prevalences (<10%) (Fig. 8, Tables 1 and 2). The relative abundances of these microbes of veterinary interest are provided in Supplementary Table S8 and Supplementary Table S9.

Pathogen-associated signatures of GI dysbiosis

Finally, we assessed whether the presence of any of the detected putative pathogens was associated with shifts in the F:B ratio, a marker for GI dysbiosis. After controlling for geographic location, *B. fragilis* was the only bacterial pathogen significantly associated with shifts in the log-transformed F:B ratio (FDR-adjusted $p = 1.5 \times 10^{-6}$). Specifically, the presence of *B. fragilis* was linked to a 39.5% reduction in the F:B ratio (estimated fold-change = 0.605), with a large effect size (Cohen's $d = 1.97$, 95% CI: 1.26, 2.66). This pattern was consistent across all sampled locations (Fig. 9). No fungal pathogens were significantly associated with shifts in the F:B ratio.

Discussion

This study reports the application of shotgun metagenomics to characterise the faecal bacteriome and mycobiome of southern greater gliders, achieving species-level resolution microbial profiling. We discuss bacterial and fungal diversity, core members and putative pathogens

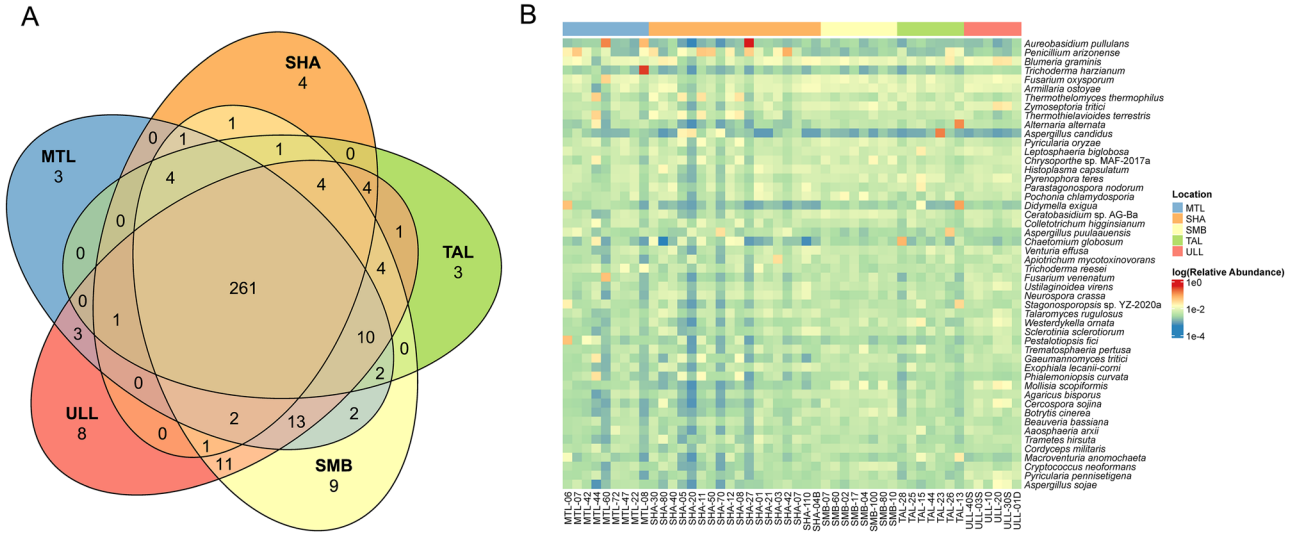

Fig. 7 Core species identified in southern greater glider (*Petauroides volans*) faecal mycobiomes. (**A**) Venn diagram showing core species (>50% prevalence, >0.1% relative abundance) across different locations. The intersection of circles represents species shared by locations. (**B**) Heatmap showing the relative abundance of core fungal species shared among all locations (only the top 50 most abundant species are shown). See Fig. 1 for location details

Table 1 Putative bacterial pathogens detected in southern greater glider (*Petauroides volans*) faecal samples by mNGS

Pathogen		Prevalence	Australian possum or glider hosts	Zoonotic transmission
Bacillus	Bacillus cereus	8.33%	Common brushtail possum [70]	—
Bacteroides	*Bacteroides fragilis*	56.25%	Yellow-bellied glider [71]	—
Bronchothrix	*Brochothrix thermosphacta*	6.25%	NR	—
Clostridioides	*Clostridioides difficile*	8.33%	NR	Yes [72, 73]
Clostridium	*Clostridium chauvoei*	4.17%	NR	—
	Clostridium perfringens	70.83%	NR	Possible [74]
	Clostridium tetani	4.17%	NR	—
	†*Clostridium* spp.	NA	†Common brushtail possum [75]	—
Enterococcus	*Enterococcus faecalis*	16.67%	NR	Possible [76]
	†*Enterococcus* spp.	NA	†Sugar glider [77]	—
Erysipelothrix	*Erysipelothrix rhusiopathiae*	4.17%	NR	Yes [66, 78]
Escherichia	*Escherichia coli*	100%	Common ringtail possum [71] Common brushtail possum [70, 71, 79] Mountain brushtail possum [80, 81] Sugar glider [82] Squirrel glider [71] Leadbeater's possum [71]	Yes [66]
Helicobacter	*Helicobacter pylori*	16.67%	NR	Possible [66]
	†*Helicobacter* spp.	NA	†Common ringtail possum [83] †Common brushtail possum [84]	—
Listeria	*Listeria monocytogenes*	4.17%	Common brushtail possum [85] Common ringtail possum [65, 85] Sugar glider [86, 87]	Yes [66]
Porphyromonas	*Porphyromonas gingivalis*	4.17%	NR	—
	†*Porphyromonas* spp.	NA	a†Common brushtail possum [88, 89]	—
Prevotella	*Prevotella melaninogenica*	37.50%	NR	—
	†*Prevotella* spp.	NA	†Common brushtail possum [75]	—
Pseudomonas	*Pseudomonas fluorescens*	22.92%	NR	—
	†*Pseudomonas* spp.	NA	†Common brushtail possum [75]	—
Salmonella	*Salmonella enterica*	79.17%	bCommon brushtail possum [71, 90] bCommon ringtail possum [71] cWestern ringtail possum [91]	Yes [66, 78]
Staphylococcus	*Staphylococcus haemolyticus*	8.33%	NR	Possible [66]
	Staphylococcus spp.	NA	†Common brushtail possum [75]	—
Streptococcus	*Streptococcus suis*	6.25%	NR	Yes [66, 92]
	Streptococcus spp.	NA	†Common brushtail possum [75] †Western ringtail possum [93]	—

For each bacterial pathogen detected in > 1 sample, the overall prevalence (%), reported Australian marsupial hosts and evidence of zoonotic transmission are indicated. Evidence of a genus-level identification in the host is indicated by †. NR = possum or glider host not reported

[a]Reported "*Porphyromonas gingivalis*-like species"

[b]Reported "*Salmonella typhimurium*" (now *Salmonella enterica* subsp. *enterica* serovar Typhimurium)

[c]Reported *Salmonella enterica* subsp. *diarizonae*

associated with the southern greater glider, an endangered arboreal marsupial found in eastern Australia [1].

Microbial diversity and composition

Our analysis revealed the bacteriome was dominated by *Firmicutes*, *Proteobacteria* and *Bacteroidetes*, with the *Proteobacteria* exhibiting a higher relative abundance than reported previously [14]. This can be explained by the increased sensitivity of shotgun mNGS compared to 16S rRNA sequencing, as it has greater power to detect low abundance taxa and captures a more complete profile of the microbiota [30]. The most abundant bacterial genera were *Escherichia*, *Clostridium* and *Lachnoclostridium*. *Clostridium* has previously been flagged as the most common bacterial genus in the Tasmanian devil gut [129]. In addition to protein degradation activities [130], *Clostridium* spp. have documented ability to degrade tannins [131], complex compounds found in high concentrations in *Eucalyptus* leaves [132]. Meanwhile, *Lachnoclostridium* spp. are recognised for their ability to degrade plant polysaccharides like cellulose and xylans [133, 134]. Considering greater gliders are specialist folivores with a diet consisting almost exclusively of *Eucalyptus* leaves [3], high abundances of these genera in the gut

Table 2 Putative fungal pathogens detected in southern greater glider (*Petauroides volans*) faecal samples by mNGS

Pathogen		Prevalence	Australian marsupial hosts	Zoonotic transmission
Alternaria	Alternaria alternata	95.83%	NR	—
Aspergillus	*Aspergillus fumigatus*	95.83%	Julia Creek dunnart [65]	—
	Aspergillus clavatus	93.75%	NR	—
	Aspergillus felis	97.92%	NR	—
	Aspergillus fischeri	10.42%	NR	—
	Aspergillus flavus	58.33%	NR	—
	Aspergillus glaucus	95.83%	NR	—
	Aspergillus lentulus	75.00%	NR	—
	Aspergillus nidulans	100%	NR	—
	Aspergillus niger	95.83%	NR	—
	Aspergillus terreus	97.92%	NR	—
	Aspergillus thermomutatus	93.75%	NR	—
	†*Aspergillus* spp.	NA	†Koala [65, 94] †Red kangaroo [65, 95]	—
Batrachochytrium	*Batrachochytrium dendrobatidis*	45.83%	NR	—
Blastomyces	*Blastomyces gilchristii*	97.92%	NR	—
Candida	*Candida albicans*	93.75%	Common ringtail possum [71] Short-beaked echidna [65, 96] Eastern grey kangaroo [65, 97] Koala [65, 98] Common brushtail possum [99] Macropods (unspecified) [100] Possums (unspecified) [101]	Possible [102]
	Candida tropicalis	93.75%	Eastern grey kangaroo [65]	—
	Candida dubliniensis	89.58%	NR	—
	Candida orthopsilosis	68.75%	NR	—
	Candida parapsilosis	68.75%	NR	Possible [103]
	†*Candida* spp.	NA	†Red kangaroo [65] †Red-necked wallaby [65] †Agile wallaby [65] †Wallaroo [65] †Musky rat-kangaroo [65]	—
Cladosporium	*Cladosporium cladosporioides*	4.17%	NR	—
Cryptococcus	*Cryptococcus gattii*	64.58%	Long-nosed potoroo [104] Gilbert's Potoroo [105] Quokka [105] Common brushtail possum [93] Feathertail glider [105] Koala [106–108] Eastern-barred bandicoot [109]	—
	Cryptococcus neoformans	100%	Leadbeater's possum [90] Gilbert's potoroo [104] Quokka [110, 111] Sugar glider [105] Koala [108, 112] Short-beaked echidna [113] Red kangaroo [114] Numbat [111] Parma wallaby [111]	Yes [115, 116]
	†*Cryptococcus* spp.	NA	†Bilby [109] †Dusky antechinus [117] †Common brushtail possum [71] †Common ringtail possum [71] †Leadbeater's possum [71] †Striped possum [71, 111] †Mountain pygmy possum [71] †Feathertail glider [71]	—
Exophiala	*Exophiala oligosperma*	97.92%	NR	—

Table 2 (continued)

Pathogen		Prevalence	Australian marsupial hosts	Zoonotic transmission
Fusarium	*Fusarium graminearum*	77.08%	NR	—
	Fusarium verticillioides	95.83%		—
Histoplasma	*Histoplasma capsulatum*	100%	NR	—
Malassezia	*Malassezia furfur*	72.92%	NR	—
	Malassezia globosa	58.33%		—
	Malassezia pachydermatis	25.00%		Yes [118, 119]
	Malassezia restricta	27.08%		—
	Malassezia sympodialis	27.08%		—
Microsporum	*Microsporum canis*	77.08%	Red kangaroo [120] Western grey kangaroo [120] Yellow-footed rock wallaby [120]	Yes [66, 121]
	†*Microsporum* spp.	NA	†Platypus [65] †Koala [122] †Common brushtail possum [123] †Short-eared possum [123] †Possum (unidentified) [65] †Eastern grey kangaroo [123]	—
Paracoccidioides	*Paracoccidioides brasiliensis*	14.58%	NR	—
	Paracoccidioides lutzii	4.17%		—
Pithomyces	*Pithomyces chartarum*	8.33%	NR	—
Pneumocystis	*Pneumocystis carinii*	31.25%	Red kangaroo [124] Common wombat [125]	Possible [66]
Pseudogymnoascus	*Pseudogymnoascus destructans*	87.5%	NR	—
Scopulariopsis	*Scopulariopsis brevicaulis*	8.33%	NR	Possible [126]
Sporothrix	*Sporothrix schenckii*	95.83%	Bilby [127]	Yes [121]
Trichophyton	*Trichophyton benhamiae*	4.17%	NR	Yes [121]
	Trichophyton rubrum	31.25%	NR	Yes [66]
	†*Trichophyton* spp.	NA	†[a] Common brushtail possum [123] †[a] Northern brown bandicoot [123] †[a] Eastern grey kangaroo [123] †Red kangaroo [128]	—

For each fungal pathogen detected in > 1 sample, the overall prevalence (%), reported Australian marsupial hosts and evidence of zoonotic transmission are indicated. Evidence of a genus-level identification in the host is indicated by †. NR = host not reported.

[a] *Triochophyton* spp. and *Chrysosporium* spp. reported as a collective group

might assist in the breakdown of plant compounds found in *Eucalyptus*. A core bacteriome conserved across locations suggests that the GI microbiota contribute essential microbial functions for greater glider physiology. The strong representation of the *Lachnospiraceae*, accounting for over half of the core species detected, was expected considering the *Lachnospiraceae* are major contributors to the metabolism of dietary compounds present in the *Eucalyptus* diet of koalas [34]. Some of the core bacteria have also been directly isolated from koala faeces, such as *Lonepinella koalarum*, a tannin-degrading bacterium [135], and *Phascolarctobacterium faecium*, a bacterium that converts succinate to propionate (an energy source for the host) [136]. Of the three ubiquitously detected bacteria, *E. coli* is common in the GI tract of many herbivorous marsupials and has been detected at up to 100% prevalence in short-eared possums (*Trichosurus caninus*) [137], while *Ruminococcus gnavus* has been isolated from northern brown bandicoot (*Isoodon macrourus*) faeces [138] and *Blautia producta* from red kangaroo (*Macropus rufus*) faeces [139].

While the bacterial microbiota of Australian marsupials is being increasingly characterised [140–142], the fungal microbiota remain understudied [143]. We found that the mycobiome was dominated by *Ascomycota*, with *Aspergillus*, *Debaryomyces* and *Fusarium* the most abundant fungal genera. Various *Aspergillus* spp. and *Fusarium* spp. are associated with *Eucalyptus* trees and the soil beneath them [144, 145]. Mycophagy, the consumption of fungi in the diet, is widespread among Australian mammals [146] and recorded in various possums, including the common brushtail possum [147], mountain brushtail possum [148] and short-eared possum [149]. But given the exclusive folivorous diet of greater gliders [3], the diversity of fungi in southern greater glider faeces likely reflects environmental exposure, rather than purposeful consumption. As greater gliders forage on *Eucalyptus*, it is conceivable that they ingest fungal spores present on the leaf surfaces [150, 151] or acquire them from tree

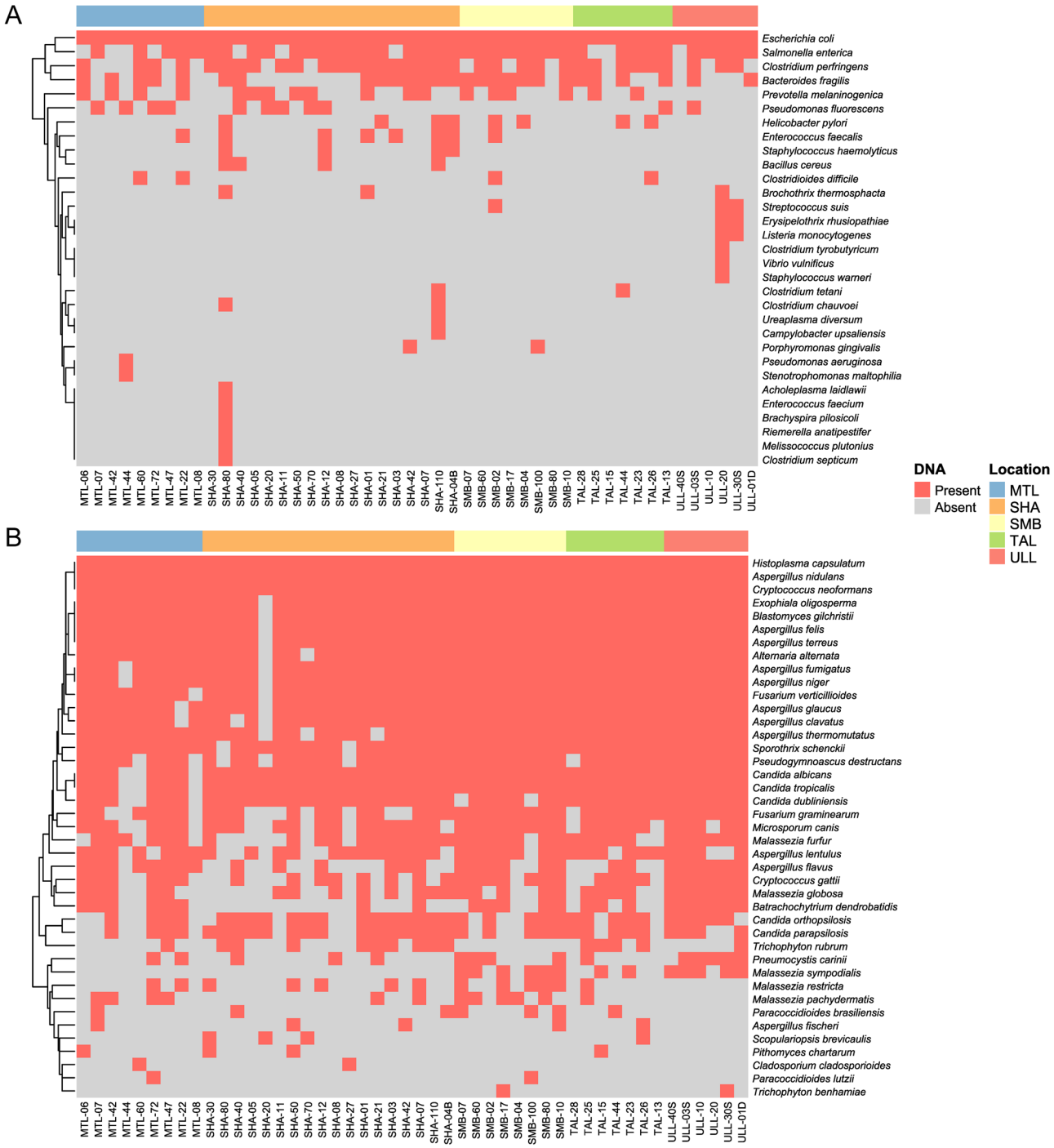

Fig. 8 Prevalence of putative bacterial (**A**) and fungal (**B**) pathogens detected in southern greater glider (*Petauroides volans*) faecal samples collected from southeastern NSW. Heatmaps show presence (red) or absence (grey) of pathogen DNA at a 0.1% relative abundance detection threshold. See Fig. 1 for location details

branches or bark [144, 152] when traversing the arboreal environment. Greater gliders, as hollow-dependent species [2], are also regularly in contact with confined tree hollow environments undergoing fungal decay [153, 154], creating a likely route for fungal transmission from hollow to glider. Other transmission routes, such as intra- or interspecies transmission, may also contribute to the dissemination of fungi through greater glider populations. The only fungus previously recorded from greater gliders, *Arthroderma cuniculi* [123], was not detected in our study. The broader core mycobiome, relative to the bacteriome, likely comprises many transient fungi that are

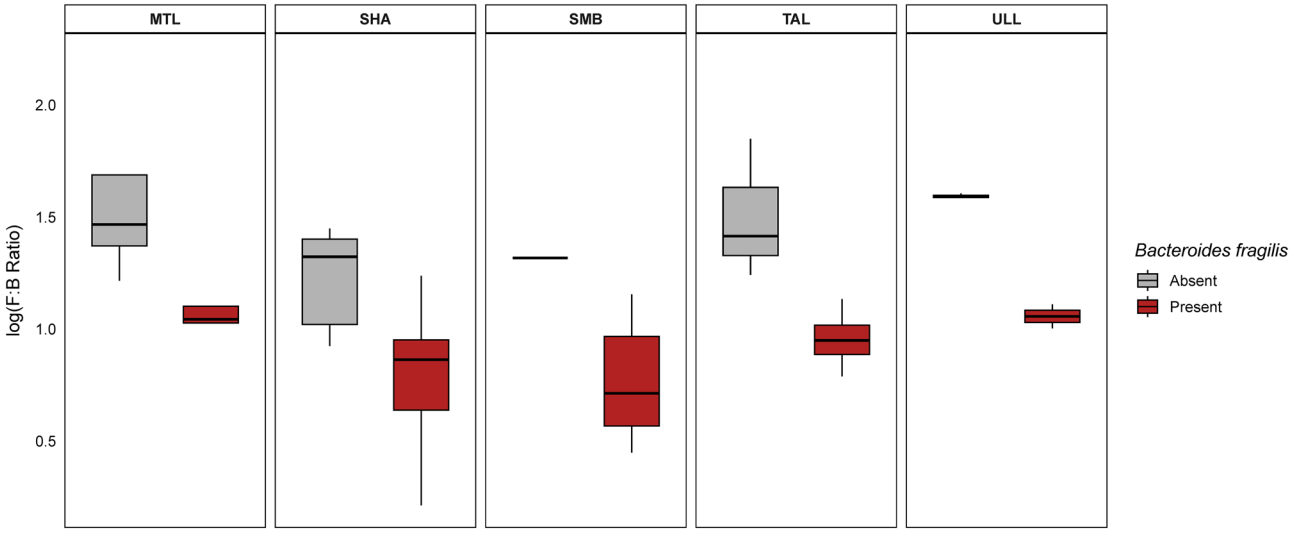

Fig. 9 Shifts in the log-transformed *Firmicutes:Bacteroidetes* (F:B) ratio associated with the presence of *Bacteroides fragilis* in southern greater glider (*Petauroides volans*) faecal samples. Boxplots indicate the presence (red) or absence (grey) of *B. fragilis* in samples collected from different geographic locations. Each box represents the interquartile range, with the median indicated by the horizontal line and whiskers extending to 1.5× the interquartile range. See Fig. 1 for location details. FDR-adjusted $p = 1.5 \times 10^{-6}$

commonly acquired from Australian forest environments and pass through, rather than colonise, the GI tract.

In agreement with preliminary work [14], geographic location was found to be a strong driver of GI microbial communities in our study. Specifically, bacterial and fungal community structure and composition showed significant variation among locations, which aligns with findings in other wild mammals [155–157]. This is further supported by the results of the ANCOM-BC2 differential abundance analysis, which identified geographic location, but not host sex or weight, as having a significant effect on microbial abundance. Collectively, these findings demonstrate that greater glider GI microbiomes are geographically structured.

Bacterial pathogens

Understanding the pathogen landscape, and therefore potential disease risks, is important to ensuring the health of native wildlife and promoting successful conservation efforts. We identified a range of putative bacterial pathogens in southern greater glider faeces by mNGS, of which many genera have known Australian possum hosts. Colibacillosis, caused by pathogenic *E. coli*, and salmonellosis, caused by *Salmonella enterica*, are known GI diseases of possums [71]. The ubiquity of *E.coli* in southern greater glider faeces is unsurprising, considering most strains act as gut commensals of mammals and *E.coli* has been reported from a number of possum species [70, 79–82, 113]. However, certain strains can act as opportunistic pathogens and cause enteric disease [158]. Meanwhile, the high prevalence of *S. enterica* in southern greater glider faeces appears unusual, considering it is not a typical gut commensal, and a previous study reported a low prevalence of *S. enterica* in Australian wildlife [159]. *Clostridium perfringens*, a common environmental bacterium and member of the normal flora [160, 161], was also prevalent. Although it has not been described in possums, *C. perfringens* strains are associated with enterotoxic and other enteric disease in various animals [162]. These high carriage rates suggest that greater gliders may act as reservoirs of important bacterial enteropathogens.

Other, less prevalent bacteria in southern greater glider faeces are also of known importance for wildlife health. For instance, listeriosis, caused by *Listeria monocytogenes* [163], has been described in the common brushtail possum [85], common ringtail possum [85] and sugar glider [86]. Other bacteria, such as *Streptococcus suis* and *Erysipelothrix rhusiopathiae*, are opportunistic pathogens known to cause disease in other wild animals [92, 164], but their clinical relevance in possums remains unknown. To date, chlamydial infection is the only infectious disease described in greater gliders [11]. *Chlamydia* spp. are widespread pathogens of Australian wildlife [11, 165] that are notorious for causing debilitating ocular, urinary and reproductive tract disease in koalas [13, 166]. Consistent with the findings of our recent screening study using conjunctival and cloacal swabs [10], *Chlamydia* was not detected in any southern greater glider faeces and is unlikely to be a prevalent threat to greater gliders in the study area. Further research is needed to ascertain the pathogenicity of many of the detected bacteria in greater gliders and their possum relatives.

Bacteroides fragilis emerged as the only putative pathogen significantly associated with GI dysbiosis. Exposure

to the microbe was linked to a decreased F:B ratio, irrespective of where the glider was sampled from. The F:B ratio is a general indicator of GI microbial balance, representing the relative abundances of two dominant phyla found in the gut [67]. We acknowledge that, as a member of the phylum *Bacteroidetes*, an increase in *B. fragilis* abundance will inherently contribute to a decrease in the F:B ratio. However, the presence of the bacterium itself appears to be a driver of this phylum-level shift. In humans, decreased F:B ratios are observed in patients with inflammatory bowel diseases [167, 168] and heart failure [169]. While commensal strains of *B. fragilis* are found in the normal GI tract, enterotoxigenic strains are associated with diarrhoeal and inflammatory diseases in various animals [170–172]. The only other possum *B. fragilis* has been reported from is the yellow-bellied glider (*Petaurus australis*), isolated from an individual presenting with sinusitis [71]. However, the implications of its presence remain unclear, as *B. fragilis* has also been found to be enriched in the faecal microbiomes of koala joeys immediately after pap feeding [173]. Nevertheless, the strength and direction of the association we observed support the role of *B. fragilis* as a potential driver or indicator of GI dysbiosis.

Fungal pathogens

We also detected a diverse array of putative fungal pathogens, which while not as well studied as their bacterial counterparts in Australian possums, are nonetheless important to consider in the context of wildlife health. Moulds belonging to *Aspergillus* and yeasts belonging to *Candida* and *Malazessia* were particularly well represented in southern greater glider faeces. *Aspergillus* spp. occur primarily in the environment (e.g. soil, vegetation) [174], while *Candida* spp. and *Malazessia* spp. are members of the normal flora [175], but all three genera contain opportunistic fungi that can become invasive and cause disease in mammals [176–178]. Considering greater gliders are obligate hollow-dwellers [2], the ubiquity of *Cryptococcus neoformans* in their faeces is of little surprise, with the yeast known to have a strong environmental association with eucalypt tree hollows [153, 179]. Nevertheless, cryptococcosis caused by *C. neoformans* is a potentially lethal mycosis that affects mammals [175], which has been described previously in the koala [106] and several Australian possums [71, 90, 105]. *Histoplasma capsulatum*, a dimorphic fungus found primarily in soil [180], was also ubiquitous in southern greater glider faeces. Bats are noted carriers of *H. capsulatum* [181], but to our knowledge this is the first report of *H. capsulatum* from an Australian marsupial.

Warranting careful attention is the detection of the chytrid fungus *Batrachochytrium dendrobatidis* at moderate prevalence in southern greater glider faeces, given it causes chytridiomycosis, a lethal skin disease of amphibians [182]. While there is growing evidence that *B. dendrobatidis* can persist in diverse, non-amphibian hosts [183–185] and environmental reservoirs like water [186], plant leaf surfaces [187] and fog [188], there have been no published reports of *B. dendrobatidis* from mammals to date. This might be attributed to the lack of *B. dendrobatidis* screening in mammals or lack of agnostic sequencing studies that focus on fungi. As *B. dendrobatidis* cannot tolerate mammalian body temperatures, showing mortality above 30 °C [189], it does not pose a threat to the health of greater gliders. Nor is it likely that greater gliders contribute to the transmission of *B. dendrobatidis* zoospores as reservoirs of infection. However, from a surveillance standpoint, the detection of *B. dendrobatidis* in various eucalypt forests of southeastern NSW suggests a risk of chytridiomycosis for the resident amphibian populations. This is consistent with previous studies that have established *B. dendrobatidis* as a widespread pathogen in eastern Australia [190, 191]. We demonstrate that faecal metagenomic screening of arboreal marsupials, such as greater gliders, can detect pathogens of high relevance to other animal groups and the broader ecosystem, irrespective of whether the host species is directly affected.

Implications for greater glider health and conservation

The health and disease ecology of southern greater gliders, like that of many cryptic arboreal marsupials, remains poorly understood due to the inherent challenges associated with their capture and study [46]. Shotgun mNGS of faecal samples can provide a comprehensive snapshot of the microbiota [34], including pathogenic microbes [192]. This offers insights into host health without the need for extensive physical restraint or anaesthesia required by other diagnostic methods (e.g. biopsy, swabs), enabling an assessment of pathogen exposure in wild animals under minimal stress. The detection of bacterial and fungal pathogens in southern greater glider faeces holds significant implications for veterinary medicine, wildlife care, and conservation. This information provides a baseline for understanding the pathogens that greater gliders may carry or be susceptible to upon entering veterinary or wildlife care. The detection of zoonotic microbes also underscores the risk of pathogen transmission to wildlife handlers. Consequently, we recommend adherence to the veterinary standard and transmission-based precautions outlined by the Australian Veterinary Association [193] when handling greater gliders to mitigate the risk of contracting zoonoses. Our findings suggest that greater gliders may act as reservoirs for certain pathogens within Australian forest ecosystems, a critical consideration for conservation managers when evaluating the risks of intra- and interspecies pathogen

transmission, as well as indirect pathogen acquisition from the environment. As such, our study contributes to an understanding of wildlife health within the broader One Health framework, which considers the interrelatedness of animal, human and environmental health [194].

Our findings underscore the necessity of considering the microbiome when planning conservation actions for greater gliders. Understanding the resident and transient microbial communities carried by an individual is especially important when considering translocation as a conservation strategy [16]. As specialist *Eucalyptus* folivores [3] with small home ranges [41], the GI microbiota of greater gliders are likely adapted to digesting forage from locally available *Eucalyptus* spp., contributing to the microbial community variation observed among gliders from different locations. Even fine-scale differences in diet, between koalas feeding on different congeners of *Eucalyptus*, are associated with compositionally distinct gut microbiomes [19]. Thus, a key consideration for greater glider welfare and survival post-translocation is the potential for a mismatch between the GI microbiome and the forage available at the release site. Faecal inoculations have been successfully attempted on koalas to reshape their GI microbiomes and alter their feeding preferences, enabling dietary expansion to less-preferred *Eucalyptus* spp. [18]. This procedure could be useful for overcoming the challenge of the microbiome being locally adapted to the source site, rather than release site, and help translocated individuals quickly adapt to new forage. Beyond this, translocation carries significant epidemiological risks, including the transmission of pathogenic microbes from sourced individuals to the release site and vice versa [195]. This raises concern for the welfare of the translocated animals, resident conspecifics, and broader ecosystem at the release site. Therefore, from a conservation management perspective, we advocate for consideration of the holobiont, that is the host and its associated microbial communities [196]. In line with recent recommendations [197], conservation actions should be evaluated on how they influence the transmission of both beneficial and pathogenic microbes. This approach is critical for maximising the success of future conservation interventions, such as translocation, for greater gliders.

Limitations

An important consideration of this study is that shotgun mNGS detects DNA [29], which confirms host exposure but does not differentiate viable microbes from remnant genetic material, nor is it necessarily indicative of active infection. While our results confirm the presence of potentially pathogenic bacteria and fungi, we caution against interpretation of their clinical relevance without additional validation. Future research aiming to establish clinical relevance in southern greater gliders would benefit from confirmatory testing of mNGS-detected pathogens using validated, gold-standard diagnostics (e.g. qPCR, antigen assays, microbiological culture). Screening the mNGS data for virulence factors or functional gene pathways implicated in pathogenicity could also help differentiate pathogenic from commensal strains of species where such a spectrum exists, thereby enabling a more accurate assessment of their health risks for the host. In turn, comparing the efficacy of faecal mNGS against such diagnostic tests would be a valuable next step in working towards the wider implementation of mNGS as a tool for pathogen detection in wildlife. Finally, the scope of this study was limited to the bacteriome and mycobiome, but there are other key components of the microbiome, such as the virome [198] and archaeome [199], which could be explored in future research to attain a more holistic understanding of the greater glider microbiota.

Conclusions

This study reports the first species-resolution data on bacterial and fungal microbiota of southern greater gliders. Faecal bacterial and fungal profiles are strongly influenced by geographic location. Core sets of GI microbial communities are both conserved within and shared among southern greater glider populations. The faecal carriage of genetic material from putative bacterial and fungal pathogens by southern greater gliders represents potential risks to wildlife health. Collectively, these results highlight the utility of faecal mNGS for simultaneous microbiota profiling and agnostic pathogen detection in wildlife. This microbiome data should be considered when making conservation management decisions for the endangered southern greater glider.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42523-026-00564-7.

Supplementary Material 1

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Author contributions

JC and KM conceptualised the study and obtained the funding. JC conducted the fieldwork to collect samples from southern greater gliders, analysed the data and wrote the first draft of the manuscript. KM supervised the study. All authors read and approved the final manuscript.

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Data availability
The dataset generated during the current study is available in the NCBI Sequence Read Archive (SRA) repository under bioproject #1308267 (https://www.ncbi.nlm.nih.gov/bioproject/1308267). Additional data supporting the conclusions of this article are available in the Supplementary Information files.

Declarations

Ethics approval and consent to participate
All procedures were approved by the University of Wollongong animal ethics committee (approval number AE19/02) and conducted under a NSW Scientific Licence (SL101968) granted by the Department of Climate Change, Energy, the Environment and Water.

Consentfor publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Environmental Futures, School of Science, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong 2500, Australia

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