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Research Article

Gut microbiota of dung beetles shows taxonomic turnover but relative functional stability

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Abstract

The dung beetle gut microbiota is essential for host health and plays a key role in regulating the biochemical processes underpinning grassland ecosystem functioning. Understanding how its taxonomic and functional composition varies across species may help predict the ecological consequences of changes in dung beetle communities driven by shifts in pastoral practices. Elucidating the mechanisms of microbial acquisition and potential transmission is also crucial. To this end, a multispecies survey of gut-associated prokaryotic and fungal communities was conducted in nine dung beetle species from a valley in the Alps, representing four tribes and three nesting strategies. All individuals were collected from cow dung to minimise dietary variation. Using an amplicon sequence variant (ASV)-based metabarcoding approach targeting the 16S ribosomal RNA (rRNA) gene and the internal transcribed spacer 2 (ITS2) region, taxonomic composition and predicted functional profiles were characterised. Prokaryotic communities exhibited strong host-dependent structuring: both alpha- and beta-diversity analyses revealed significant differentiation among species and tribes, consistent with host phylogeny and suggestive of phylosymbiosis. In contrast, fungal communities were weakly structured by host taxonomy, with extensive overlap among most species and a limited tribal signal, indicating a greater role of environmental acquisition. Despite pronounced taxonomic turnover, predicted functional profiles were relatively consistent across hosts. Fermentation and nitrogen-related pathways dominated prokaryotic communities, whereas fungal communities were largely saprotrophic, with similar trophic distributions among species. This decoupling between taxonomic composition and functional potential suggests substantial functional redundancy in dung beetle gut microbiota, supporting resilient microbial contributions to dung degradation and nutrient cycling in rapidly changing mountain pastoral ecosystems in the Alps.

Key words: Ecosystem functioning, functional redundancy, metabarcoding, microbial community, pasture ecosystems, phylosymbiosis

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Introduction

Pastoral ecosystems, which are largely dominated by grasslands, are structured by trophic interactions between herbaceous vegetation and medium- to large-sized wild and domestic ungulates. Within these systems, dung beetles

(Coleoptera, Scarabaeoidea) play key roles in ecosystem engineering. Through dung relocation and soil incorporation, they enhance nutrient cycling, improve soil structure and aeration, stimulate microbial activity, and reduce parasite transmission (Nichols et al. 2008; Forbes and Scholtz 2024; Hajji et al. 2024). By accelerating dung decomposition, they may also mitigate greenhouse gas emissions from dung pats and promote pasture productivity (Nichols et al. 2008; Slade et al. 2016b; Piccini et al. 2017). These services depend on the composition of local dung beetle assemblages, with larger tunnelling species often having the greatest impact (Nervo et al. 2014, 2017; Piccini et al. 2017). Species identity matters: for instance, spatio-temporal modelling based on species-specific thermal tolerance suggests that some dung beetle species may respond to global warming by increasing dung removal (Nervo et al. 2024).

Despite their ecological importance, pastoral ecosystems worldwide are undergoing profound transformations. Grasslands are increasingly transitioning from herbaceous dominance to woody plant encroachment (Anadón et al. 2014), while in European mountain regions, forest expansion is rapidly progressing because of land abandonment and climate change (Anadón and Sala 2026). The widespread reduction in extensive livestock systems further threatens the persistence of dung-dependent communities. In this context, understanding the biological mechanisms underpinning dung beetle functioning becomes particularly urgent.

Dung beetles contribute directly to ecosystem functioning through their feeding and nesting behaviours. With regard to feeding, both adults and larvae consume dung, but their ecological roles differ: adults actively manipulate and relocate dung for feeding and reproduction, whereas larvae develop within brood balls produced by their parents or directly within the dung mass (Scholtz et al. 2009; Estes et al. 2013). The ways in which dung beetles process and incorporate dung into the soil depend on their nesting strategy, according to which they are characterised as: dwellers, tunnellers, or rollers (Halffter and Edmonds 1982; Jay-Robert et al. 2008; Hanski and Cambefort 2014). Dwellers lay their eggs within the dung mass or at the soil–dung interface, tunnellers dig tunnels directly beneath the dung mass in which they bury brood balls, and rollers shape a quantity of dung into a ball and roll it away from the dung mass to a suitable location for burial (Hanski and Cambefort 2014). The nesting strategies used by dung beetles in the Italian Alps are largely based on phylogenetic differences: Aphodiinae are generally dwellers, the majority of Scarabaeinae are tunnellers, and Sisyphini (Scarabaeinae) are rollers. These behaviours lead to different spatial redistributions of dung-derived nutrients and different effects on the microenvironment in which microbial processes occur (Slade et al. 2016a; Natta et al. 2025b).

The ecosystem services of dung beetles are not solely attributable to their mechanical activity. A substantial component of dung transformation and nutrient recycling is mediated by their gut microbiota (Jones et al. 2025; Natta et al. 2025a). Symbiotic microorganisms facilitate the digestion of complex organic substrates, including lignocellulose and other recalcitrant plant compounds, thereby contributing to nitrogen transformation, and are thought to provide essential metabolites to the host (Jing et al. 2020; Kucuk 2020; Ebert et al. 2021; Jones et al. 2025). In some cases, brood ball construction promotes the vertical transmission of microbial symbionts, reinforcing host–microbe associations

across generations (Estes et al. 2013; Parker et al. 2019; Akamine and Mishima 2023; Chen et al. 2024). For example, female *Onthophagus* beetles emit a faecal secretion called "pedestal" upon which they lay their eggs (Estes et al. 2013). After hatching, the larvae feed on the pedestal, thereby acquiring the maternal microbiota, which is crucial for their survival and proper development (Schwab et al. 2016; Parker et al. 2019). Vertical transmission in the genus *Cephalodesmius* occurs differently: males and females cooperate to collect dung and other decomposing materials to build a brood mass, to which they also add their own faeces, thereby inoculating it with their microbiota (Ebert et al. 2021). Consequently, understanding dung beetle functional ecology requires detailed knowledge of the microbial communities associated with these insects, including their taxonomic composition, transmission routes, and metabolic potential.

The gut microbiota of dung beetles typically comprises two major components: prokaryotes (bacteria and archaea) and fungi. Prokaryotic communities in dung beetles have received the most attention (Kolasa et al. 2019; Ebert et al. 2021; Natta et al. 2024, 2025a), while fungal communities have been studied much less despite playing important roles in lignocellulose degradation and nutrient assimilation (Andlar et al. 2018; Wang et al. 2022). Recent work suggests that microbial transmission in dung beetles may occur both vertically via brood balls and horizontally through the continuous ingestion of dung and soil (Akamine and Mishima 2023; Chen et al. 2024; Natta et al. 2025a; Herrera et al. 2026). The relative importance of these pathways likely influences the stability and host specificity of microbial assemblages.

Host phylogeny has been shown to structure insect-associated bacterial communities, a pattern conceptualised as phylosymbiosis (Brooks et al. 2016). In dung beetles, prokaryotic gut communities may correlate with host taxonomy and phylogenetic relatedness (Kolasa et al. 2019; Natta et al. 2025a), suggesting evolutionary filtering and partially conserved symbiotic associations. In contrast, preliminary evidence indicates that fungal assemblages may follow different structuring rules, showing weaker correspondence with host phylogeny and stronger dependence on diet or environmental acquisition (Franzini et al. 2016; Natta et al. 2025a).

Another key issue concerns the relationship between taxonomic composition and functional potential. Different microbial communities are characterised by enormous compositional diversity, yet the ecosystem processes they mediate may be largely unaffected (Louca et al. 2018). This apparent decoupling between taxonomy and function has been described as "functional redundancy" (Moya and Ferrer 2016). In some insect gut systems, the metabolic roles of different microbial taxa may be similar, resulting in stable functional outputs despite high taxonomic turnover (Natta et al. 2025a; Muñoz-Hernández et al. 2026). Testing whether compositional variability coexists with functional stability across multiple host species within wild dung beetle assemblages is, therefore, crucial for deepening our knowledge of microbiota resilience, namely the capacity of dung beetle microbiota to cope with environmental or dietary disturbances.

From a methodological perspective, advances in high-throughput sequencing have enabled more refined analyses of microbiota. Amplicon sequence variants (ASVs), inferred through error-correcting algorithms such as DADA2 (Callahan et al. 2016), provide higher taxonomic resolution and reproducibility than traditional operational taxonomic units (OTUs). ASVs enable fine-scale

comparisons across studies and reduce clustering artefacts, making them particularly suitable for detecting subtle host-specific patterns in complex microbial assemblages (Fasolo et al. 2024).

Building upon these conceptual and methodological frameworks, the present study extends previous species-level investigations to the scale of dung beetle assemblages. Specifically, the gut microbiota of nine species belonging to four tribes and representing all three nesting strategies typical of mid-altitude pastures in the Alps were analysed. Using an ASV-based metabarcoding approach, the study aimed to (1) test whether the patterns of association between hosts and their prokaryotic and fungal gut communities followed the same rules and (2) evaluate whether high compositional variability was accompanied by relative functional stability across taxonomically diverse dung beetle species. By integrating taxonomic and functional analyses of both prokaryotic and fungal components, this study aimed to elucidate the ecological and evolutionary processes shaping dung beetle gut microbiota to better understand their contribution to ecosystem functioning in rapidly changing pastoral ecosystems.

Methods

Entomological and environmental sampling

In May, live adult dung beetles (*N* = 88) belonging to nine species across four tribes and characterised by three nesting strategies (Table 1) were collected from dung deposited by a herd of Piedmontese cattle. The collection pastures are located in a managed submontane agroecosystem characterised by semi-natural grasslands in the western Italian Alps [Mocchie (TO), Susa Valley, Piedmont (45°8.16'N, 7°17.76'E)], within a radius of approximately 200 m of the specified GPS point and at an elevation of 800–900 m a.s.l. At least eight individuals per species were collected to capture intraspecific variability in the gut microbiota (Natta et al. 2024). The species and sex of the individuals were determined based on morphological features (Baraud 1992; Dellacasa and Dellacasa 2006; Ballerio et al. 2014), and species identification was then confirmed through molecular characterisation using the mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequence as a DNA barcode (https://boldsystems.org/) as described in Natta et al. (2025a).

Dung samples were collected from two randomly selected dung pats and soil samples at a depth of 5 cm from around the same pats. These environmental samples were stored in 1.5 mL Eppendorf tubes and homogenised upon arrival at the laboratory, and absolute ethanol was added to the tubes containing dung to prevent fermentation. All samples were stored at −20 °C until analysis.

Gut removal

To empty the insects' guts as much as possible, the beetles were kept in a terrarium without food for at least 24 h (Zhang et al. 2021; Chen et al. 2024). They were then euthanised by submersion in absolute ethanol and immediately dissected to extract the entire gut. All dissection tools were sterilised using a 30% sodium hypochlorite solution and then washed in distilled water before use. Each removed gut was preserved in absolute ethanol and stored at 4 °C.

Table 1. Number of individuals of each species sampled and their respective tribes, nesting strategies, collection elevations, and collection times. The individuals collected were evenly distributed between the sexes, except for *A. stercorosus*, for which only three males were sampled.

Species	Individuals	Tribe	Nesting strategy	Collection elevation (m a.s.l.)	Collection time
Aphodius pedellus (De Geer, 1774)	8	Aphodiini	dweller	850	May
Colobopterus erraticus (Linnaeus, 1758)	10	Aphodiini	dweller	850	May
Anoplotrupes stercorosus (Scriba, 1791)	10	Geotrupini	tunneler	850	May
Geotrupes mutator Marsham, 1802	10	Geotrupini	tunneler	850	May
Trypocopris pyrenaeus (Charpentier, 1825)	10	Geotrupini	tunneler	850	May
Onthophagus fracticornis (Preyssler, 1790)	10	Onthophagini	tunneler	850	May
Onthophagus medius (Kugelann, 1792)	10	Onthophagini	tunneler	850	May
Onthophagus verticicornis (Laicharting, 1781)	10	Onthophagini	tunneler	850	May
Sisyphus schaefferi (Linnaeus, 1758)	10	Sisyphini	roller	850	May

DNA extraction, amplification, and Illumina NovaSeq sequencing

DNA was extracted from gut and dung samples using the modified cetyltrimethylammonium bromide (CTAB) method described by Natta et al. (2024). DNA from soil samples was extracted using the DNeasy® PowerSoil® Pro Kit (QIAGEN).

A DNA metabarcoding approach was used to investigate the microbiota. For the prokaryotic component, the 16S ribosomal RNA (rRNA) gene was amplified using the primer set 515fB (5'–GTGYCAGCMGCCGCGGTAA–3') (Parada et al. 2016) and 806rB (5'–GGACTACNVGGGTWTCTAAT–3') (Apprill et al. 2015). The polymerase chain reaction (PCR) cycling programme consisted of an initial denaturation step at 94 °C for 3 min, followed by 35 cycles at 94 °C for 45 s, 55 °C for 60 s, and 72 °C for 90 s, with a final extension step at 72 °C for 10 min.

To characterise the total fungal community, the nuclear ribosomal internal transcribed spacer 2 (ITS2) region was amplified using a semi-nested PCR approach. In the first PCR, the entire ITS region (ITS1–5.8S–ITS2) was amplified using the universal fungal primer pair ITS1F (5'–CTTGGTCATTTAGAGGAAGTAA–3') (Gardes and Bruns 1993) and ITS4 (5'–TCCTCCGCTTATTGATATGC–3') (Ihrmark et al. 2012). The amplification protocol consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 54 °C for 45 s, and 72 °C for 60 s, with a final extension at 72 °C for 10 min. PCR products were visualised on an agarose gel, diluted 1:20, and used as templates for the semi-nested PCR targeting the ITS2 region. This second amplification employed the primer pair fITS7 (5'–GTGARTCATCGAATCTTTG–3') and ITS4 (5'–TCCTCCGCTTATTGATATGC–3') (Ihrmark et al. 2012). The semi-nested PCR cycling conditions consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s, followed by a final extension step at 72 °C for 7 min.

Illumina overhang adapter sequences were added to the primers used for library preparation. Specifically, Illumina overhang adapter sequences (forward overhang: 5'–TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG–[locus-specific target primer]; reverse overhang: 5'–GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG–[locus-specific target primer]) were added to primers 515fB and 806rB, which

were used for bacterial 16S rRNA gene amplification, and to primers fITS7 and ITS4, which were used in the semi-nested PCR for fungal ITS2 amplification.

PCR reactions were performed in a final volume of 25 µL using 5 µL of 5× FIREPol® Master Mix (12.5 mM $MgCl_2$; Solis BioDyne), 0.2 µM of each primer, and 20 ng of genomic DNA. Extracted DNA was amplified in triplicate and pooled before purification. Purification and quantification of the amplified DNA were conducted following Natta et al. (2025a). The negative controls for all PCR reactions were pooled, purified, and quantified in the same manner as the samples for both prokaryotic and fungal analyses. Amplicons were subsequently sent to IGA Technologies (Udine, Italy) for Illumina NovaSeq sequencing (2 × 250 bp).

Bioinformatics and statistical analyses

Bioinformatics and statistical analyses were conducted separately for prokaryotes and fungi. The sequences were analysed using QIIME2 (Quantitative Insights Into Microbial Ecology 2, v. 2023.5) microbiome bioinformatics platform (Bolyen et al. 2019) after the removal of adapters and primers. Denoising and quality control, including chimaera removal, were performed using the DADA2 plugin (Callahan et al. 2016) through the "qiime dada2 denoise-paired" command. Chimaera detection was carried out using the "consensus" method. The taxonomic assignment of the prokaryotic community was achieved using the Greengenes2 database (McDonald et al. 2024), whereas the UNITE database v. 19.02.2025 (Abarenkov et al. 2025) was used for fungi. Phylogenetic trees were generated using the QIIME2 plugin "qiime phylogeny align-to-tree-mafft-fasttree".

The outputs of the QIIME2 pipeline ("taxonomy.qza", "otu_table.qza", and "rooted_tree.qza"), together with the corresponding metadata files, were imported into RStudio (Posit team 2025) using the R package *qiime2R* v. 0.99.6 (Bisanz 2022) to generate phyloseq objects. Two phyloseq objects were constructed, corresponding to the prokaryotic and fungal datasets, respectively.

The amplicon sequence variants (ASVs) detected in the negative control were removed from the phyloseq objects before analysis (additional information on the ASVs present in the negative control can be found in Suppl. material 1: fig. S1). ASVs shared by both the beetles and the environmental samples (i.e. dung and soil samples) were subsequently identified by analysing the results of the flower plots generated from all 92 samples using the plot_venn function in the R package *microeco* (Liu et al. 2021). Environmental ASVs were removed from the phyloseq objects, and all subsequent diversity analyses were conducted on the resulting filtered datasets. However, to assess the influence of these environmental ASVs on beta diversity and functional profiles, the results of the analyses carried out by also considering these ASVs in the phyloseq objects are included in the Supplementary materials.

To enable comparisons between samples with non-uniform coverage, the ASV tables were normalised using the rarefy_even_depth function in the R package *phyloseq* v. 1.36.0 (McMurdie and Holmes 2013). Rarefaction curves of the rarefied ASV tables were obtained using the rarecurve function in the R package *vegan* v. 2.6-4 (Oksanen et al. 2022). Absolute counts (i.e. the number of reads) and relative abundances (i.e. the ratio of the number of reads

belonging to an ASV in a sample to the total number of reads in that sample) were then calculated for each ASV.

Alpha diversity was assessed using the "Observed ASVs", "Shannon", and "Faith's Phylogenetic Diversity (Faith's PD)" indices, calculated with the estimate_richness function in the R package *phyloseq* (McMurdie and Holmes 2013). Pairwise relationships among diversity indices were evaluated using Spearman's rank correlation coefficients, computed with the cor function in the R package *corrplot* (Wei et al. 2024). As all three indices were highly correlated, only one, namely Faith's PD, was used. The effects of species, tribe, nesting strategy, and sex on alpha diversity were then tested using analysis of variance (ANOVA), and variance homogeneity was checked using Levene's test. In cases of unequal variances, a linear model was fitted using generalised least squares with the gls function in the R package *nlme* (Pinheiro et al. 2025).

Dissimilarity among individuals in terms of both prokaryotes and fungi was visualised by means of distance-based redundancy analysis (dbRDA) based on a Bray–Curtis distance matrix of ASV composition, using the R package *vegan*. Before running the dbRDA, the Hellinger transformation was applied to the ASV table. The dbRDA results were plotted using the R package collection *tidyverse* (Wickham et al. 2019). An *envfit* analysis was performed on the dbRDA ordination to determine which factors had the greatest ability to differentiate among individuals based on the R^2 values and *p*-values. An ANOVA was then performed on the full model plotted in the dbRDA.

Flower plots (generated using a custom script available upon request) were obtained to visualise the shared (i.e. core) and exclusive ASVs associated with each species, tribe, and nesting strategy. To visualise the taxonomic diversity of the different samples, percentage-stacked bar charts were created using the R package collection *tidyverse* (Wickham et al. 2019). To facilitate their representation in the bar charts, only the 15 most abundant genera (i.e. those with the highest number of reads out of the total) were considered, and the other genera were grouped under "Others".

The predicted functions of the identified prokaryotic and fungal communities were assessed using FAPROTAX (Functional Annotation of Prokaryotic Taxa) (Louca et al. 2016) and FUNGuild (Nguyen et al. 2016), respectively, both of which were implemented in the R package *microeco* (Liu et al. 2021). As with taxonomic diversity, percentage-stacked bar charts were used to visualise differences in trophic behaviour among the samples, with only the 15 most abundant functions represented. Differences among species or tribes in taxonomic and functional composition were tested using *t*-tests or chi-squared tests.

Results

Alpha diversity

Bioinformatic analysis identified 12,501 prokaryotic ASVs at a rarefaction depth of 6,167 reads per sample and 3,301 fungal ASVs at a rarefaction depth of 3,326 reads per sample. Rarefaction curves based on observed ASVs indicated that all 88 beetle samples reached an asymptote for both prokaryotes and fungi, indicating that the sequencing depth was sufficient to capture gut microbiota diversity (Suppl. material 1: fig. S2A, B).

Concerning prokaryotes, significant differences in the Faith's PD index were detected among species (F = 8.85, P < 0.001), tribes (F = 14.17, P < 0.001), and nesting strategies (F = 11.27, P < 0.001), whereas no significant differences were observed between sexes (F = 0.948, P = 0.341). Regarding fungi, significant differences in the Faith's PD index were detected among tribes (F = 3.39, P < 0.05) and nesting strategies (F = 4.92, P < 0.01), but no significant differences were found among species (F = 1.87, P = 0.077) or between sexes (F = 1.06, P = 0.307).

Beta diversity

The dbRDA ordination of prokaryotes showed that the gut communities of the sampled individuals differed consistently according to host species and tribe. The nine dung beetle species were well separated across the two-dimensional plot, forming four clusters of species that corresponded to the four tribes (Fig. 1). The four clusters did not overlap, whereas the convex hulls of the species within each cluster showed partial overlap but still maintained a clear degree of interspecific differentiation. Specifically, *A. pedellus* and *C. erraticus* (tribe Aphodiini) were grouped together and clearly separated from the other three clusters formed by *A. stercorosus*, *G. mutator*, and *T. pyrenaeus* (tribe Geotrupini); *O. fracticornis*, *O. medius*, and *O. verticicornis* (tribe Onthophagini); and *S. shaefferi* (tribe Sisyphini). The envfit analysis confirmed this result, as species produced a significantly higher R^2 value (R^2 = 88.1%; P = 0.001), followed by tribe (R^2 = 79.7%; P = 0.001). Nesting strategy accounted for 44.7% (P = 0.001), and sex, the only non-significant factor, accounted for 2.74% (P = 0.087). Intraspecific dispersion varied among species, with some species showing compact clusters of individuals and others showing dispersed clusters of individuals. The ANOVA performed on the full model plotted in the dbRDA yielded a statistically significant result (F = 3.07, P < 0.001). The comparative analysis conducted while preserving the environmental ASVs largely mirrored these results, both in terms of spatial ordination and the envfit R^2 values (Suppl. material 1: fig. S3).

Conversely, the dbRDA ordination of fungal communities revealed no consistent differences in gut community composition among host species or tribes. The nine dung beetle species were not well separated across the two-dimensional plot: seven were plotted in the upper-right corner and showed extensive overlap, one was plotted in the right portion of the graph, and one was plotted in the left portion (Fig. 2). The plot revealed no evident differentiation among the four tribes. Specifically, *A. pedellus* and *A. stercorosus* formed well-defined and largely separate clusters, indicating marked differences in fungal community composition compared with the other species. The other seven species occupied overlapping regions of the ordination space, reflecting similar gut fungal assemblages. Nevertheless, when only these seven species were considered, reduced overlap among the polygons was observed (Suppl. material 1: fig. S4). Indeed, on the first constrained axis (dbRDA1), *C. erraticus* and *T. pyrenaeus* were clearly distinguished from other species, such as *G. mutator*, *S. shaefferi*, *O. fracticornis*, and *O. medius*, whereas the second axis (dbRDA2) further distinguished among the species, particularly within the genus *Onthophagus*, with *O. fracticornis* and *O. medius* grouped together

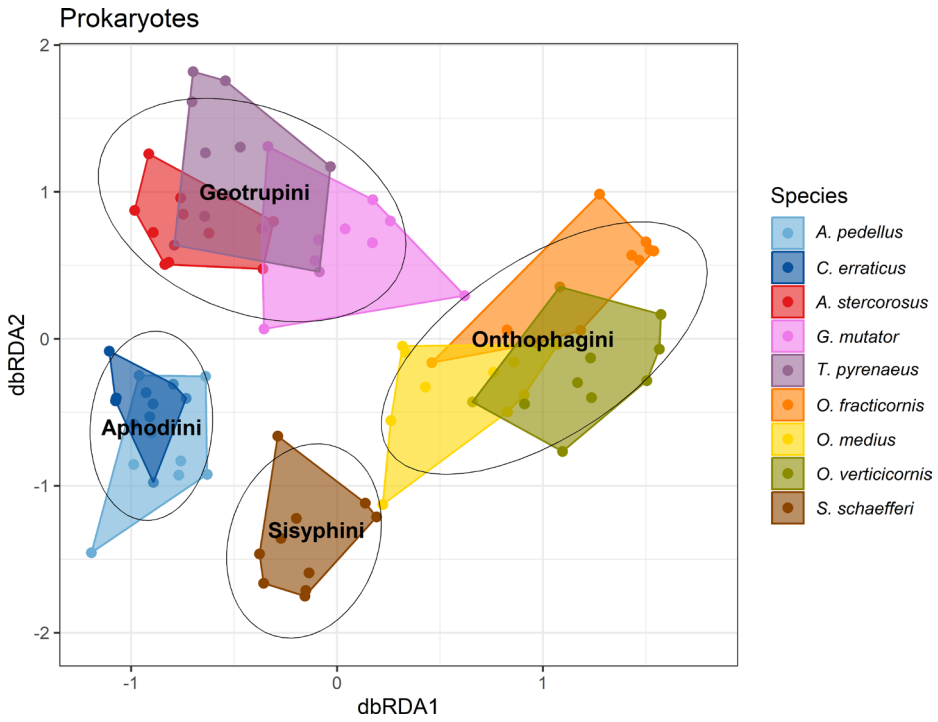

Figure 1. dbRDA ordination plot of prokaryotic gut communities from 88 dung beetles, grouped according to species and tribe.

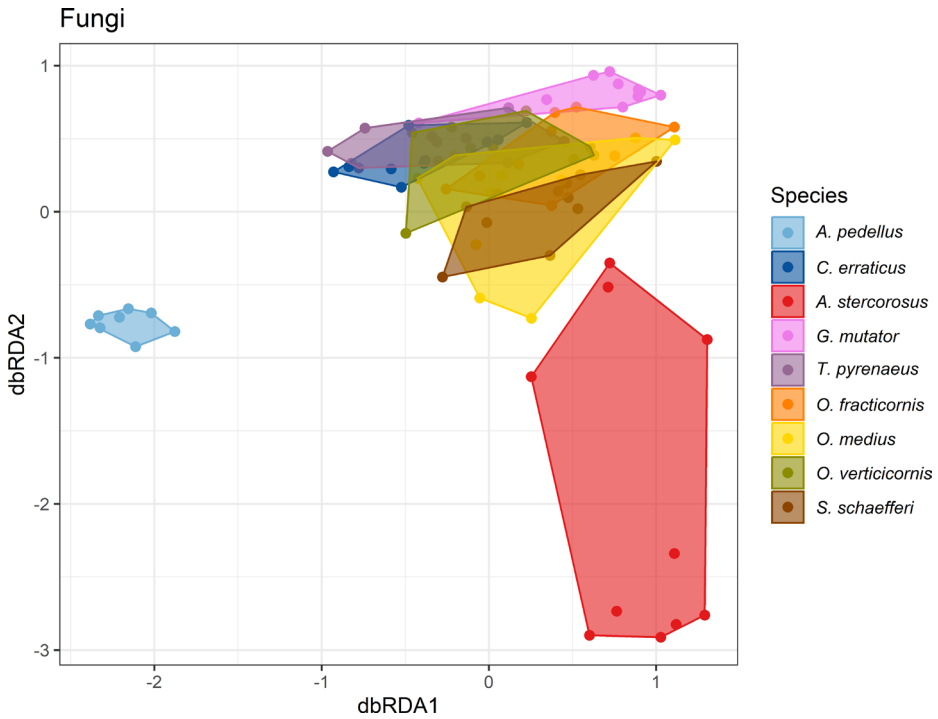

Figure 2. dbRDA ordination plot of fungal gut communities from 88 dung beetles, grouped according to species.

and separated from *O. verticicornis* at higher dbRDA2 values. The envfit analysis, performed on the ordination of all nine species, identified species as the factor with the highest explanatory power (R^2 = 80.6%; P = 0.001). Tribe and nesting strategy were both significant factors, accounting for 26.4% (P = 0.001) and 23.2% (P = 0.001), respectively. Sex was the factor with the lowest

R^2 value and was also non-significant (R^2 = 0.40%; P = 0.725). Intraspecific dispersion varied among species, with one species showing a very compact cluster of individuals (*A. pedellus*), whereas the individuals of another species were highly dispersed (*A. stercorosus*). The ANOVA performed on the full model returned a statistically significant result (F = 1.58, P < 0.001). The comparative analysis conducted while preserving the environmental ASVs showed similar results, except for the position of *A. stercorosus* in the main dbRDA plot (Suppl. material 1: fig. S5). Indeed, when the environmental ASVs were retained, *A. pedellus* showed a marked distance from the other species in the plot, which otherwise remained highly overlapping. This considerable similarity in the results between the two approaches (i.e. with and without the environmental ASVs) was also evidenced by the analogous envfit R^2 values (Suppl. material 1: fig. S5). Furthermore, when *A. stercorosus* and *A. pedellus* were excluded from the analyses, the similarity in the results between the two approaches was even more pronounced (Suppl. material 1: fig. S6).

Exclusive and core ASVs

The extent of exclusive and core ASVs was assessed by considering both (i) the number of ASVs and (ii) the number of reads per ASV, expressed as relative percentages (i.e. the relative abundance of exclusive and core ASVs). Exclusive ASVs were those unique to each of the nine species, whereas core ASVs were those shared by all nine species.

Overall, exclusive prokaryotic ASVs (Fig. 3, petals) accounted for 10,677 of the 12,501 detected ASVs (85.4%) but represented only 21.3% of the total reads. Similarly, exclusive fungal ASVs represented 2,761 of the 3,301 detected ASVs (83.6%) but represented only 34.1% of the total reads. The mean number of ASVs exclusive to each species was significantly higher in prokaryotes than in fungi (paired t-test: $t_{(8)}$ = 3.55, P < 0.01). However, the mean relative abundance of exclusive ASVs was significantly lower in prokaryotes than in fungi (paired t-test: $t_{(8)}$ = −3.33, P < 0.05). In addition, most species-exclusive ASVs were detected in a single individual of the species considered (on average, 96.6 ± 1.7% and 96.8 ± 1.8% for prokaryotes and fungi, respectively). The remaining fraction of these exclusive ASVs detected in two or more individuals of the same species was, therefore, very small. In particular, for prokaryotes, only one ASV was found in nine individuals of *A. stercorosus*, whereas for fungi, only one ASV was found in four individuals of *G. mutator*, and two ASVs were found in four individuals of *S. shaefferi* (Suppl. material 1: fig. S7). Core ASVs (i.e. those shared by all nine species) were rare, with only 47 detected in prokaryotes and nine in fungi (Fig. 3, centre), corresponding to 0.4% and 0.3% of all ASVs, respectively. Despite their low numbers, core ASVs accounted for a disproportionately higher fraction of the total reads, representing 19.5% of prokaryotic and 5.7% of fungal ASV abundances. The three most abundant prokaryotic genera among the core ASVs were *Vagococcus_B*, *Apibacter*, and *Acinetobacter* (Suppl. material 1: fig. S8A). With regard to fungi, over 50% of the core ASVs were assigned to the genus *Candida*, followed by an unclassified Ascomycota and *Lithohypha* (Suppl. material 1: fig. S8B).

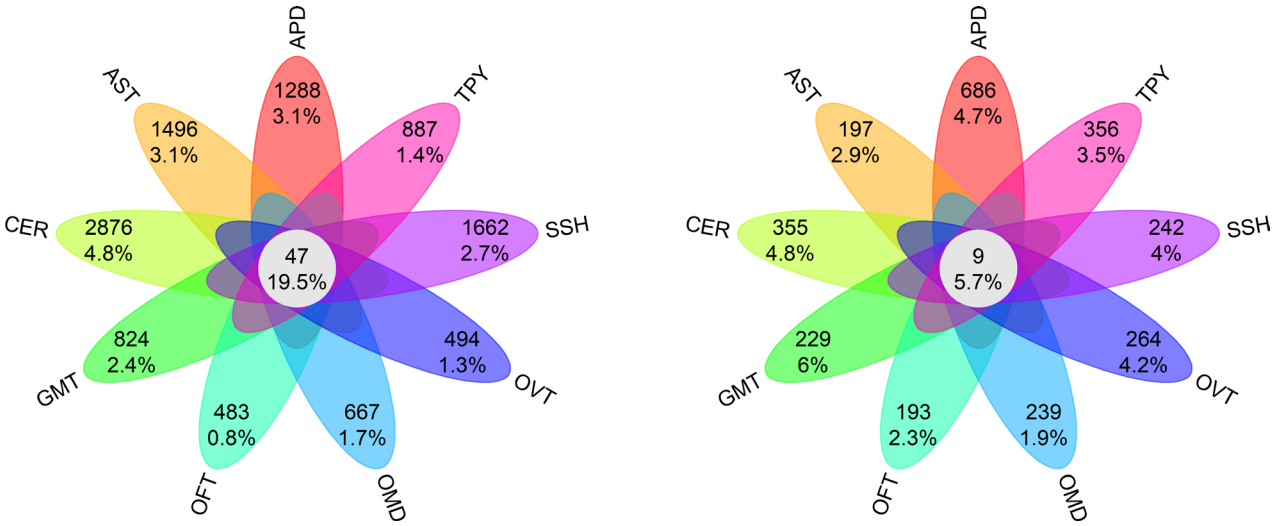

Figure 3. Flower plots presenting the relative abundances (percentages) of core and exclusive ASVs for prokaryotes (left) and fungi (right) in each dung beetle species (APD: *A. pedellus*; AST: *A. stercorosus*; CER: *C. erraticus*; GMT: *G. mutator*; OFT: *O. fracticornis*; OMD: *O. medius*; OVT: *O. verticicornis*; SSH: *S. shaefferi*; TPY: *T. pyrenaeus*). The numbers indicate the number of ASVs, whereas the percentages refer to the number of ASV reads (i.e. the relative abundances expressed as percentages). The circles in the centre of each flower plot represent the ASVs shared by all nine species, i.e. the core ASVs, whereas the petals represent the ASVs exclusive to each species.

Microbial taxonomic composition

Among the prokaryotes, Proteobacteria, Firmicutes_D, and Bacteroidota accounted for approximately 80% of all ASVs, whereas archaea were detected at very low abundance. At the genus level (Fig. 4), the three most abundant genera across all samples were *Wolbachia*, *Apibacter*, and *Vagococcus_B*. However, the relative abundances of the 15 most prevalent prokaryotic genera varied considerably among the nine host species, indicating pronounced species-specific structuring of the gut microbiota. In *A. pedellus*, *C. erraticus*, *G. mutator*, and *S. shaefferi*, low-abundance taxa grouped as "Others" comprised a substantial fraction of the community, representing approximately half of the total relative abundance. In contrast, *O. fracticornis*, *O. medius*, *O. verticicornis*, and *T. pyrenaeus* exhibited microbial profiles with strong dominance by one or two genera, which reached relative abundances of approximately 30%. *Apibacter* was the dominant component of the microbiota in two *Onthophagus* species, namely *O. fracticornis* and *O. medius*, where it accounted for approximately one third of the total (on average, 30 ± 8%). *Wolbachia* was a prominent component of the gut microbiota in *O. verticicornis* (24%) and was also present in *O. fracticornis*, *O. medius*, *A. stercorosus*, and *S. shaefferi*, accounting for 14 ± 4% of the community, while remaining negligible in the other species. *Enterococcus_G* was particularly abundant in *T. pyrenaeus*, contributing a quarter of the total microbiota (25%), whereas its relative abundance remained below 5% in all other species except *A. stercorosus* (9%). Additional genera, such as *Vagococcus_B* and *Faecalibacter*, displayed pronounced interspecific variability, reaching 15–20% in some hosts but remaining at low relative abundances in others. *G. mutator* and *T. pyrenaeus* exhibited a high abundance of *Vagococcus_B* (16% each), whereas *Faecalibacter* occurred at a high percentage only in *C. erraticus* (18%). Overall,

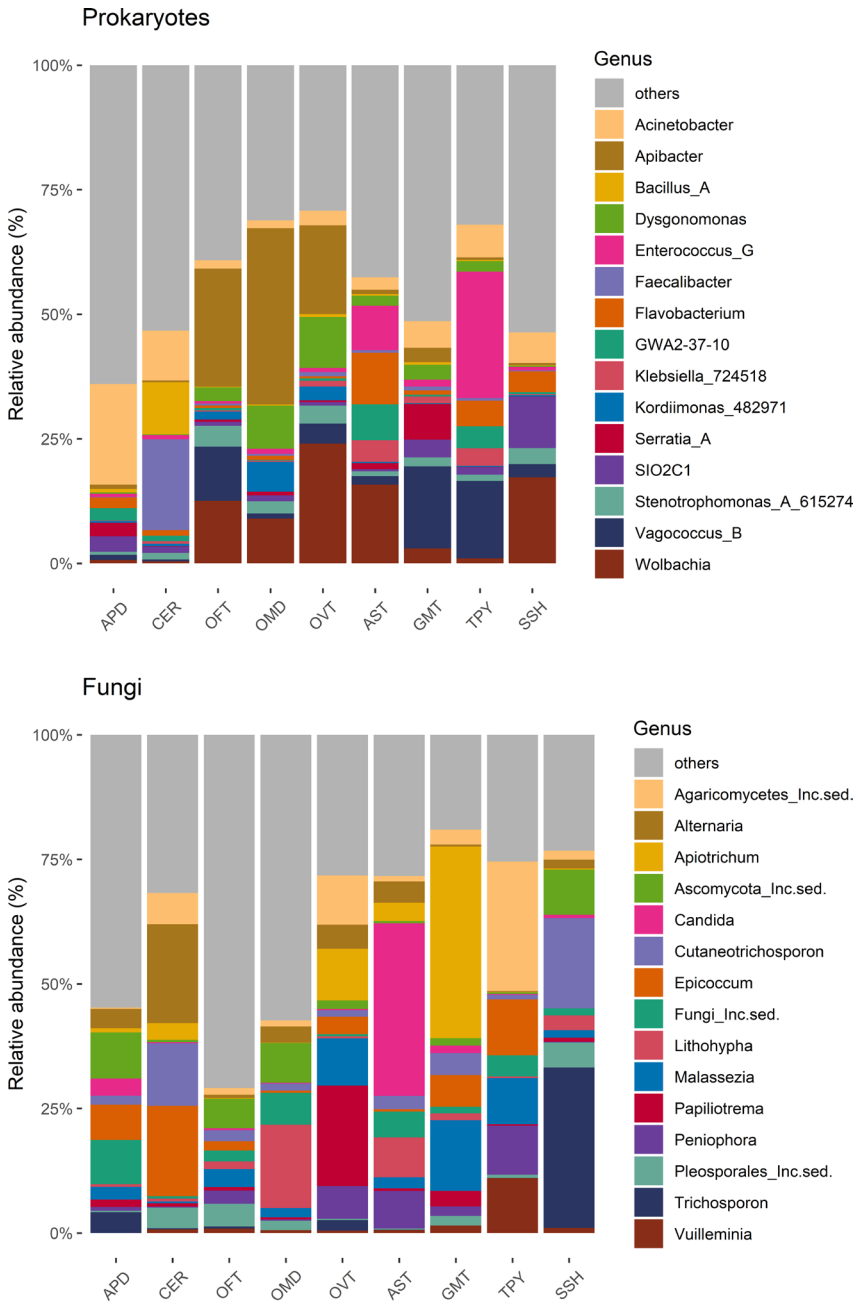

Figure 4. Stacked bar chart of the 15 most abundant microbial genera of prokaryotes and fungi in the nine dung beetle species (APD: *A. pedellus*; AST: *A. stercorosus*; CER: *C. erraticus*; GMT: *G. mutator*; OFT: *O. fracticornis*; OMD: *O. medius*; OVT: *O. verticicornis*; SSH: *S. shaefferi*; TPY: *T. pyrenaeus*). Genera that were not among the 15 most abundant were pooled and labelled as "Others".

despite the presence of shared dominant genera across species, their relative contributions differed markedly, resulting in distinct gut microbiota profiles for each species. Nevertheless, a pattern emerged when the host species were clustered into tribes according to their phylogenetic relationships (Suppl. material 1: fig. S9A). Specifically, the tribe Aphodiini displayed the highest abundance of *Acinetobacter* ($\chi^2 = 11.67$, $P < 0.01$), the tribe Onthophagini had the highest abundance of *Apibacter* ($\chi^2 = 68.86$, $P < 0.001$), the tribe Geotrupini had the highest levels of *Enterococcus_G* ($\chi^2 = 24.20$, $P < 0.01$), and Sisyphini, represented only

by *S. shaefferi*, presented a combination of genera that distinguished it from the remaining tribes. These results were confirmed by the analysis of the core ASVs among species of the same tribe, in which *Acinetobacter* accounted for 42% of the core ASVs of Aphodiini, *Apibacter* accounted for 38% of the core ASVs of Onthophagini, and *Enterococcus_G* and *Vagococcus_B* accounted for 24% and 22% of the core ASVs of Geotrupini, respectively (Suppl. material 1: fig. S10).

With regard to fungi, Ascomycota and Basidiomycota were the most abundant phyla, accounting for 95% of the ASVs. At the genus level (Fig. 4), the three most abundant genera across all samples were *Apiotrichum*, *Epicoccum*, and an unclassified genus of Agaricomycetes. The composition of the fungal gut microbiota varied considerably among the nine species analysed. Clear interspecific differences were observed in both the relative abundance and dominance of the 15 most prevalent fungal genera. In some species, the fungal community was strongly dominated by a limited number of taxa, with *Apiotrichum* representing 38% of the total relative abundance in *G. mutator* and *Trichosporon* accounting for 32% in *S. shaefferi*. In contrast, *O. fracticornis* exhibited a more diverse community structure, with no individual genus exceeding 10% relative abundance and low-abundance taxa, grouped collectively as "Others", accounting for approximately 70% of the total community. Genera such as *Papiliotrema*, *Malassezia*, and *Cutaneotrichosporon* were detected across multiple species, generally contributing between 5% and 20%, depending on the host. Other genera, including *Epicoccum*, *Alternaria*, and *Lithohypha*, reached moderate relative abundances in specific hosts, in some cases accounting for approximately 15–20% of the fungal community. For instance, *Epicoccum* and *Alternaria* were mostly found in *C. erraticus* (18% and 20%, respectively), whereas *Lithohypha* was most dominant in *O. medius* (17%). The dbRDA ordination plot suggested that *A. stercorosus* and *A. pedellus* hosted distinct fungal communities. The former was the only species to show a substantial presence of *Candida* (35%), whereas the latter hosted genera such as *Hannaella* and *Nigrospora*, included in the "Others" group, which were abundant only in this species while representing less than 1% in a few other species. Overall, the relative contributions of fungal genera varied among species, indicating pronounced species-specific differences in gut microbiota composition in a few cases. When the host species were grouped into tribes, the tribe Aphodiini displayed the highest abundance of *Alternaria* ($\chi^2 = 17.20$, $P < 0.001$), the tribe Geotrupini had the highest levels of *Candida* ($\chi^2 = 27.50$, $P < 0.001$), and the tribe Sisyphini, represented only by *S. shaefferi*, showed a significantly higher abundance of *Trichosporon* than the other tribes ($\chi^2 = 82.60$, $P < 0.001$) (Suppl. material 1: fig. S9B). Moreover, analysis of only the core ASVs among species of the same tribe revealed notable details. For example, Aphodiini were dominated by *Hannaella* (34%), Sisyphini were dominated by *Trichosporon* (32%), and Geotrupini were dominated by *Candida* and an unclassified genus of Agaricomycetes (26% and 21%, respectively). By contrast, the tribe Onthophagini showed a mixture of genera, with no clearly dominant taxon (Suppl. material 1: fig. S11).

Microbial functional composition

Despite interspecific differences in taxonomic composition, the functional composition of prokaryotes and fungi remained relatively consistent across all samples, regardless of species (Fig. 5).

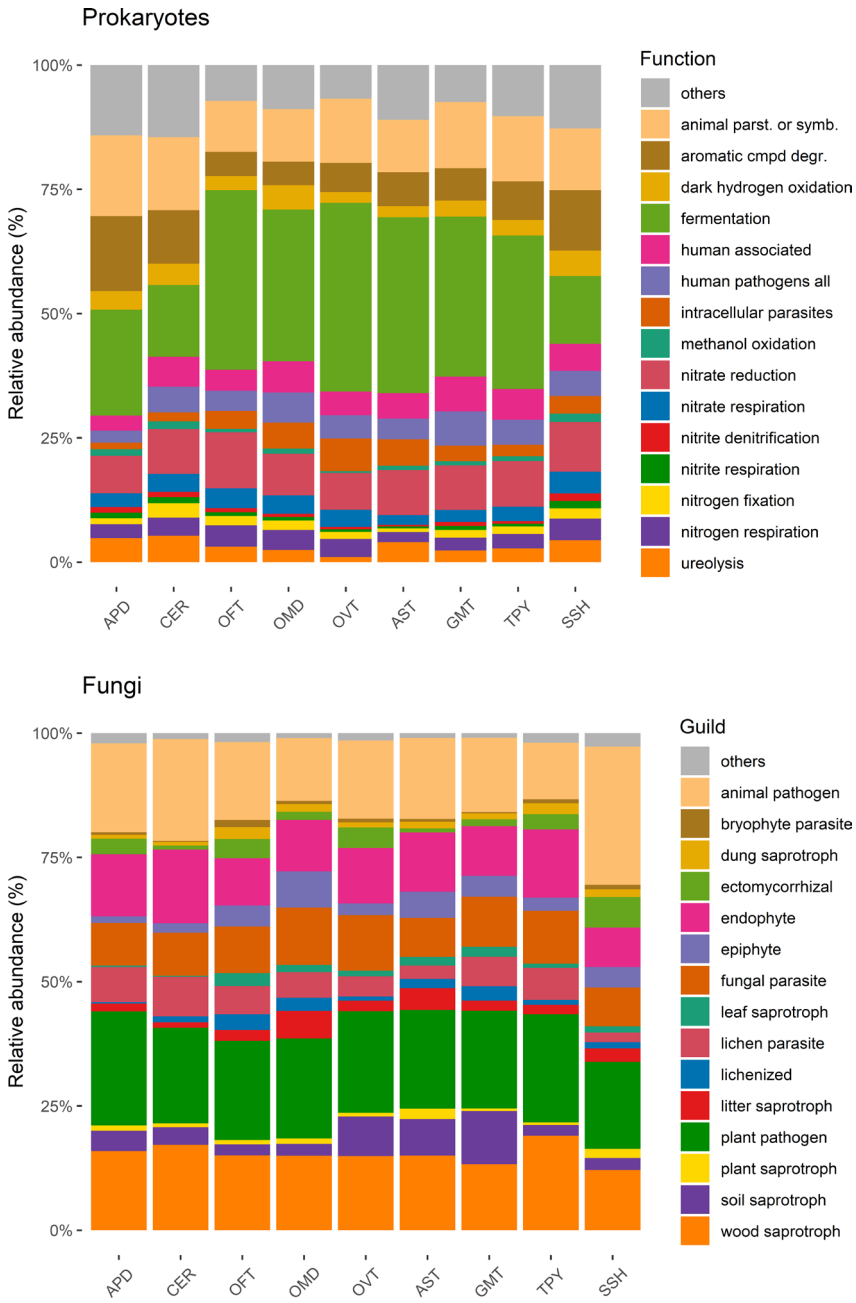

Figure 5. Stacked bar chart of the 15 most abundant prokaryotic functions and fungal guilds in the nine dung beetle species (APD: *A. pedellus*; AST: *A. stercorosus*; CER: *C. erraticus*; GMT: *G. mutator*; OFT: *O. fracticornis*; OMD: *O. medius*; OVT: *O. verticicornis*; SSH: *S. shaefferi*; TPY: *T. pyrenaeus*). Functions and guilds that were not among the 15 most abundant were pooled and labelled as "Others". (animal parst. or symb.: animal parasites or symbionts; aromatic cmpd. degr.: aromatic compound degradation).

Fermentation was identified as the predominant metabolic process among the prokaryotes hosted in the guts of the nine species, with an average relative abundance of 28 ± 9%. Nitrogen-related metabolic pathways (i.e. nitrate reduction, nitrate respiration, nitrite denitrification, nitrite respiration, nitrogen fixation, nitrogen respiration, and ureolysis) represented nearly one quarter of the total functional profile in each species and were relatively constant among species, with an average contribution of 23 ± 4%. The comparative analysis conducted

while preserving the environmental ASVs did not reveal any substantial differences from the results obtained through dataset cleaning (Suppl. material 1: fig. S12). The three species belonging to the tribe Onthophagini (*O. fracticornis*, *O. medius*, and *O. verticicornis*) and the three species belonging to the tribe Geotrupini (*A. stercorosus*, *G. mutator*, and *T. pyrenaeus*) exhibited very similar functional profiles, in which fermentation was predominant, accounting for at least 30% of the total abundance. Despite this relatively constant pattern, slight differences were also detected among species. For instance, *A. pedellus*, *C. erraticus*, and *S. shaefferi* displayed lower levels of fermentation than the other species (Welch's *t*-test: $t_{(3.10)} = -6.68$, $P < 0.01$; mean = 16.33 vs. 33.83) but higher levels of aromatic compound degradation (Welch's *t*-test: $t_{(2.85)} = 4.93$, $P < 0.05$; mean = 12.67 vs. 6.17).

Regarding fungi, from a broader functional perspective, the most common trophic mode (i.e. the way by which fungi obtain nutrients) was saprotrophy, accounting for approximately half of the total relative abundance (on average, 46 ± 4%), followed by pathotrophy (35 ± 4%) and symbiotrophy (19 ± 1%). Among the saprotrophs, the dominant fungal guild was wood saprotrophs (15 ± 2%), followed by soil saprotrophs (5 ± 3%), litter saprotrophs (3 ± 2%), and plant-, leaf-, and dung-associated saprotrophs, which were present at low relative abundances (1 ± 1%). These proportions were largely consistent across species, except for *O. verticicornis*, *A. stercorosus*, and *G. mutator*, which exhibited higher relative abundances of soil saprotrophs (Welch's *t*-test: $t_{(2.51)} = 4.71$, $P < 0.05$; mean = 8.67 vs. 2.67). Plant and animal pathogens were abundant in all species, with comparable relative contributions (20 ± 1% and 17 ± 5%, respectively). The comparative analysis conducted while preserving the environmental ASVs did not reveal any substantial differences from the results obtained through dataset cleaning (Suppl. material 1: fig. S13).

Discussion

This study conducted a comprehensive comparative analysis of gut-associated prokaryotic and fungal communities from 88 dung beetles belonging to nine different species differing in phylogenetic affiliation and nesting strategy. All beetles were collected from the same dung to minimise the effect of diet.

Host specificity and structuring of gut communities

Approximately 12,000 prokaryotic ASVs and 3,000 fungal ASVs were detected, indicating that prokaryotic alpha diversity was four times greater than fungal diversity. This finding aligns with previous studies indicating higher prokaryotic diversity in the dung beetle gut microbiota (Ziganshina et al. 2018; Natta et al. 2025a). Alpha-diversity analyses also revealed contrasting patterns between prokaryotic and fungal communities. Prokaryotic phylogenetic diversity varied significantly among species, tribes, and nesting strategies, whereas fungal diversity was significantly associated only with tribe and nesting strategy. The lack of sex-related effects on alpha diversity observed here further corroborates earlier findings in dung beetles, suggesting that sex plays a marginal role in shaping gut microbial diversity compared with host phylogeny and diet (Suárez-Moo et al. 2020; Chen et al. 2024; Natta et al. 2025a).

Beta-diversity analyses further emphasised the contrasting patterns between prokaryotes and fungi. Regarding prokaryotes, the dbRDA results showed that species from the four tribes considered clearly hosted different bacterial communities and that, even within the same tribe, the species tended to maintain distinct microbiota, demonstrating that host-species taxonomy was reflected in their microbiota. The presence of different bacterial microbiota among dung beetle tribes suggests a stable host–microbe association, potentially mediated through long-term cooperative association. Comparable patterns have been described in dung beetles and other insects, in which prokaryotic communities show evidence of host-specific filtering and partial evolutionary conservation (Yun et al. 2014; Brooks et al. 2016; Parker et al. 2019; Ebert et al. 2021). For example, Ebert et al. found that differences in the bacterial microbiota of dung beetles from the genera *Onthophagus* and *Cephalodesmius* were associated not only with phylogeny but also with gut morphophysiology (Ebert et al. 2021). Similarly, two species of *Onthophagus*, namely *O. gazella* and *O. sagittarius*, were found to harbour species-specific bacterial communities despite sharing the same diet (Parker et al. 2019). Similar results were also found for three other phylogenetically close species of *Onthophagus* that nonetheless exhibited distinct bacterial microbiota (Natta et al. 2025a).

In contrast, fungal communities appeared to be weakly structured. The dbRDA results showed that only *A. pedellus* and *A. stercorosus* formed well-defined and largely non-overlapping clusters, whereas the other seven species occupied overlapping regions. Although species identity explained a considerable proportion of the variation observed in the composition of the gut fungal community, it should be noted that *A. pedellus* and *A. stercorosus* determined the ordering of points in the dbRDA space and occupied a significant portion of the space, pushing the other species into the remaining space and thus influencing the R^2 values. In fact, when these two species were excluded from the analysis, the remaining species showed reduced overlap among polygons. The fungal microbiota appeared to be less strongly associated with host phylogeny and nesting strategy than the prokaryotic microbiota, consistent with the view that fungi are more frequently acquired from the environment (Franzini et al. 2016; Natta et al. 2025a). Given the intimate association of dung beetles with microbially rich substrates such as dung and soil, these environments likely act as major sources of fungal propagules, which the beetles ingest during feeding, resulting in more variable and less host-constrained fungal assemblages. Moreover, the results obtained when environmental ASVs were retained also confirmed these drivers of variability in prokaryotes and fungi: for prokaryotes, no substantial differences were observed between analyses with and without environmental ASVs – suggesting that these communities were more closely linked to phylogeny than to the environment and diet – whereas for fungi, some differences emerged, particularly in *A. stercorosus*. This species is known to have a highly diversified diet (Weithmann et al. 2020; Arias-Álvarez and Piquero-Rodriguez 2025), which probably explains why its fungal microbiota may be more strongly influenced by the presence of environmental ASVs commonly found in soil and dung.

For both prokaryotic and fungal gut communities, dung beetle nesting strategies showed moderate and significant R^2 values. Nevertheless, it is hypothesised that these results merely reflect the fact that individuals with different

nesting strategies also belong to different species and tribes, which, as discussed above, exert a much greater influence on gut communities.

Similar to the results for alpha diversity, sex did not play a significant role in differentiating individuals in the dbRDA ordination for either prokaryotes or fungi, in agreement with previous results for dung beetles (Natta et al. 2024).

The observed variation in microbiota composition among individuals of the same species further suggests differences in the degree of host control over microbial communities. Species that form compact clusters in the ordination space may possess more specialised or tightly regulated gut microenvironments, whereas broader clusters may reflect greater dietary breadth, as previously hypothesised for beetles with diverse ecological niches (Franzini et al. 2016; Kolasa et al. 2019; de Beer et al. 2026).

Exclusive vs. core microbiota

The analysis of exclusive and core ASVs revealed a highly diverse and largely species-specific microbial assemblage. The majority of the detected ASVs were, in fact, exclusive to individual species, particularly among prokaryotes, yet these taxa accounted for only a minor fraction of the total reads. Similar patterns of high taxonomic turnover have previously been documented, suggesting that rare, host-specific microbial taxa contribute disproportionately to interspecific differentiation without dominating the overall community structure (Natta et al. 2025a). Moreover, most species-exclusive ASVs were detected in only a single individual of the species in question, casting doubt on the true biological exclusivity of these ASVs. In the case of ASVs represented by one or very few reads, their presence in only a single individual is inevitable, representing a form of "statistical exclusivity". This high level of intraspecific variability, which is also supported by previous findings (Natta et al. 2024), serves as a reminder that it is advisable to consider at least 8–10 individuals per species when conducting analyses of this kind.

In contrast, the core microbiota shared across all nine species was numerically scarce but disproportionately abundant, especially among prokaryotes. This pattern closely resembles findings from previous work on dung beetles and other detritivorous insects, in which a limited set of widespread taxa dominated gut communities, in accordance with the minimal core model (Franzini et al. 2016; Natta et al. 2025a). The higher relative abundance of core ASVs in prokaryotes compared with fungi supports the idea that bacterial communities are more conserved across dung beetles, whereas fungal assemblages are more variable and potentially transient. These differences are consistent with the interpretation that bacteria form relatively stable mutualistic associations involved in digestion and nutrient cycling, whereas fungi are more often ingested from dung and soil and may act as opportunistic or short-lived gut associates (Estes et al. 2013; Schwab et al. 2016; Kucuk 2020; Šigut et al. 2022).

Taxonomic variability and functional redundancy

The prokaryotic gut microbiota was dominated by Proteobacteria, Firmicutes_D, and Bacteroidota, a pattern widely reported in dung beetles and other detritivorous beetles (Franzini et al. 2016; Kolasa et al. 2019; Suárez-Moo et al. 2020;

Ebert et al. 2021; Chen et al. 2024). Previous studies have interpreted their dominance as evidence of adaptation to protein-rich and high-fibre diets such as dung (Chen et al. 2024; Jones et al. 2025). At the genus level, pronounced interspecific differences were observed despite the recurrence of a limited set of dominant taxa across hosts, as emphasised by the analysis of the core microbiota. *Apibacter* has been reported as a frequent and sometimes dominant component of the gut microbiota in *Onthophagus* beetles and other insects, and it has been hypothesised to play roles in carbohydrate metabolism and host nutrition (Kwong et al. 2018; Natta et al. 2025a). *Acinetobacter*, known to be a highly flexible decomposer of organic compounds, including dung, is also common in the microbiota of dung beetles (Kolasa et al. 2019; Jácome-Hernández et al. 2023; Chen et al. 2024; Natta et al. 2024, 2025a). Similarly, lactic acid bacteria such as *Enterococcus* and *Vagococcus*, which are also abundant in *Thorectes lusitanicus* (Geotrupini), are predominantly saccharolytic (i.e. they improve energy recovery by breaking down otherwise indigestible sugars), generate an acidic environment, and protect against putrefactive and sulphate-reducing bacteria that produce gas (Hernández et al. 2015).

The frequent detection of *Wolbachia* in several species also warrants consideration. Although *Wolbachia* is typically regarded as an intracellular endosymbiont rather than a gut-associated bacterium, its presence has been repeatedly reported in amplicon-based surveys of dung beetles and other insects (Hilgenboecker et al. 2008; Kolasa et al. 2019; Parker et al. 2020; Ebert et al. 2021). The effects of *Wolbachia* infections on host insects can be both beneficial, such as nutrient supplementation and protection against viruses, and deleterious, including feminisation and the killing of males (Parker et al. 2020).

In prokaryotes, a high degree of correspondence was observed between the dbRDA and taxonomic composition analyses, with both indicating that different dung beetle species harboured different microbial communities. By contrast, the results for fungi showed less concordance between the two approaches because, as mentioned above, the presence of two dung beetle species with very different microbiota reduced the dbRDA differences among the other species. This highlights the usefulness of the taxonomic composition results. In fact, despite the limitation that many groups of microorganisms were grouped into a generic "Others" category, the taxonomic composition analysis indicated that even species that completely overlapped in spatial ordination could still harbour partially distinct fungal communities.

Fungal communities were dominated by Ascomycota and Basidiomycota, in agreement with earlier work on dung beetles (Franzini et al. 2016; Chen et al. 2024; Natta et al. 2025a). Fungal assemblages showed weaker correspondence with host phylogeny than bacterial communities, as previously reported, but noteworthy differences were nevertheless highlighted. For example, the pronounced presence of *Candida* in *A. stercorosus*, coupled with its absence in the other species, likely explains the isolated position of this species in the two-dimensional dbRDA space. *Candida* yeasts are common inhabitants of beetle digestive tracts and are frequently reported in plant- or fungus-feeding beetles and dung beetles (Suh et al. 2008; Nwaefuna et al. 2023). They play important roles in nutrient breakdown and microbial community dynamics and, in some cases, form host-specific associations (Suh et al. 2008). Similarly, the isolated position of *A. pedellus* in the dbRDA space can be explained by the

fact that this species harbours a heterogeneous assemblage of fungal taxa, including genera such as *Hannaella* and *Nigrospora*, which are almost exclusive to *A. pedellus*. *Hannaella* yeasts are widely distributed on the leaf surfaces of various plants (Li et al. 2021), and some species are believed to play an important role in promoting plant growth and biological control (Ji et al. 2024). *Nigrospora*, like many other fungi found in the guts of dung beetles, is a known plant pathogen that primarily infects leaves (Liu et al. 2024). Previous studies have reported *Nigrospora* to be a frequent component of the diets of various insects and to play a role in cellulose degradation (Zhang et al. 2018; Olivier et al. 2026). Hypothetically, *Nigrospora* may be acquired from plants consumed by cattle, subsequently excreted in dung, and finally ingested by individuals of *A. pedellus*. Following this hypothesis, it can be speculated that the dung beetle food chain may contribute to controlling these pathogenic fungi, promoting their removal from dung and potentially limiting their spread to other plants. The case of *A. pedellus* highlights the fact that two dung beetle species may have similar microbiota in terms of the 15 most abundant genera while differing greatly in the composition of the genera grouped into the "Others" category. In fact, the presence of high percentages of "Others" could limit the usefulness of these analyses unless a species-by-species study of the most abundant taxa in the microbiota is conducted, as in this case.

The absence of a clear tribal signal suggests that fungi are less strongly influenced by host evolutionary history and are instead shaped mostly by environmental acquisition, i.e. from dung, soil, or plant material. Nevertheless, the recurrent presence of genera such as *Apiotrichum*, *Trichosporon*, and *Epicoccum* across multiple species indicates that some fungi may be frequently ingested and are able to persist, at least transiently, in the gut environment. If gut fungi are acquired through the diet, differences in trophic preferences among dung beetle species (Urrutia et al. 2022; Rolando et al. in preparation) would explain how a wide range of beneficial fungal taxa is introduced into their gut microbiota. For example, fungal genera such as *Apiotrichum* and *Trichosporon* are known to be capable of assimilating or degrading lignocellulose (Akamine and Mishima 2023).

Despite the taxonomic differences among host species, the predicted functional profiles of both prokaryotic and fungal communities remained remarkably stable. This finding, although based on predicted functional profiles rather than measurements obtained through metatranscriptomics or metabolomics, corroborates previous studies reporting a decoupling between taxonomic composition and functional potential (i.e. functional redundancy) in dung beetles and other insects. These findings led to the hypothesis that microbial consortia could be assembled from different taxonomic pools yet still maintain functional outcomes (Moya and Ferrer 2016; Natta et al. 2025a). In prokaryotes, fermentation emerged as the dominant metabolic process across all species, underscoring the central role of gut bacteria in mediating the breakdown of complex organic substrates present in dung. The relative stability of nitrogen-related pathways among species further supports a conserved contribution of gut microbiota to nitrogen transformation and recycling. Similarly, fungal functional guilds were largely conserved among species, with saprotrophy being the most dominant trophic mode in all hosts. This pattern aligns with previous observations that dung beetle-associated fungi largely reflect saprotrophic lifestyles linked to dung and soil environments (Akamine and Mishima 2023; Natta et al. 2024, 2025a).

Conclusion

This study aimed to test whether the patterns of association between hosts and their microbial communities were the same or differed between prokaryotes and fungi. At the same time, it aimed to determine whether the high interspecific variability in microbial composition was associated with high variability in predicted functions. The results demonstrated that dung beetle gut microbiota varied among species, with contrasting patterns for prokaryotes and fungi. Species from the four tribes considered hosted very different prokaryotic communities, and even within the same tribe, different species tended to maintain distinct microbiota. This indicates that prokaryotic communities are strongly structured by phylogeny, including tribe affiliation and host species identity. By contrast, the fungal communities of the sampled individuals did not differ consistently according to host species or tribe. Fungal communities were much more variable and appeared to be environmentally driven. Further studies on the environmental factors that may have influenced the fungal community will need to be conducted, for example, by comparing individuals collected from different sites, in different seasons, or at different elevations.

At the same time, the functional similarity observed across hosts in both prokaryotes and fungi, despite their high taxonomic turnover, suggests that dung beetle gut microbiota are resilient systems capable of maintaining key metabolic roles under changing taxonomic compositions. This means that even if major changes occur in the composition of the gut microbiota, microbial functions are likely to remain stable, implying a high degree of functional redundancy. These findings reinforce the emerging view that host-associated microbiota can exhibit high taxonomic flexibility while maintaining functional stability (Moya and Ferrer 2016; Louca et al. 2018).

This functional redundancy may reflect two evolutionary pressures: the need to maintain host health and the need to maintain the efficiency of dung degradation and nutrient cycling. However, considering that microbial functions were predicted and not measured experimentally in this study, future work integrating metagenomics or metatranscriptomics, together with experimental manipulation of the diet and microbiota, will be essential to clarify the functional significance of specific microbial taxa and disentangle the relative contributions of host filtering and environmental acquisition in shaping dung beetle gut ecosystems.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
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Author contributions

Conceptualization: AR, CP, GN. Data curation: EL, AL, SV. Formal analysis: SV, EL, AR, AL, GN. Funding acquisition: CP. Investigation: GN. Methodology: SV, AR, EL. Project administration: CP. Resources: GN, EL. Supervision: AR. Validation: AL, AR. Visualization: SV. Writing - original draft: EL, CP, AL, AR, GN, SV. Writing - review and editing: AR.

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Data availability

The raw data used to generate the dataset for this study can be found in the NCBI Sequence Read Archive (SRA) under accession nos. PRJNA1436194 and PRJNA1436940 for the prokaryotic and fungal communities, respectively.

Supplementary material 1

Additional figures

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