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RESEARCH

Land use legacies shape wild boar (*Sus scrofa*) diet along a forest-urban gradient in Singapore

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Abstract

Wild boar (*Sus scrofa*) are one of the most widespread mammals on the planet but when overabundant, can cause negative impacts to ecosystems and people. In Singapore, native wild boar were extirpated in the mid-1900s amid rapid habitat loss and hunting, but have since re-established populations naturally and are now highly abundant in local forests where their natural predators remain absent. Understanding the role that their dietary preferences play in their ecological success is thus crucial for managing their populations and human-wildlife conflict in the compact city state. Using DNA metabarcoding of faecal samples, we show that local wild boar exhibit a high degree of dietary flexibility. While there is a high prevalence of soil invertebrates and native plant foods in our samples, a wide variety of cultivated fruit tree species – common remnants of past land use in secondary forests – was also detected. However, the extent of neighbouring vegetated areas was not strongly or consistently correlated with plant and animal dietary diversity or composition. Our study suggests that food resources due to land use legacies and other anthropogenic food sources may play a role in subsidising local wild boar populations, but likely vary spatiotemporally. Management of remnant cultivated tree species in secondary forests can be a potential strategy for regulating wild boar population in the highly urban forests of Singapore, but further quantitative research on the availability and exploitation of anthropogenic foods, and their demographic consequences, will be needed to evaluate the effectiveness of such interventions.

Keywords Faecal samples · Metabarcoding · eDNA · Urbanisation · Tropical forests · Trophic ecology · Urban forests · Urban wildlife

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Introduction

The Eurasian wild boar (*Sus scrofa*) is one of the most widespread of mammal species, with a native range across much of Europe, North Africa and mainland Asia (Keuling and Leus 2019). Occupying a wide range of temperate and tropical habitats, their feeding habits and dietary flexibility have also allowed them to adapt to and thrive in novel environments where they have been introduced, often with detrimental consequences for local biodiversity and ecosystems (Nogueira-Filho et al. 2009; Barrios-Garcia and Ballari 2012; Ballari et al. 2015; McClure et al. 2018; McDonough et al. 2022; Canright et al. 2023). For example, in the forest edges of Southeast Asia, high abundances of wild boar have been implicated in negative outcomes for native forest plants, invasives, and soil microbial communities, and they pose a disease risk for livestock and zoonotic diseases

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for humans (Luskin et al. 2017, 2019; Brearley et al. 2024; Moore et al. 2023). Therefore, understanding the local-scale factors that shape wild boar diets may provide insights for managing their populations, especially where they are overpopulated, unsustainable, and present undesirable impacts on local native ecosystems or to human environments and people.

In the highly urbanised city-state of Singapore, wild boar were extirpated from the main island due to habitat loss and hunting, and remained absent until as recently as the 1990s (Corlett 1992; Lamperty et al. 2023). Wild boar have since successfully recolonized the main island by swimming across the Johor Strait from Peninsular Malaysia and nearby offshore islands such as Ubin Island (Yong et al. 2010; Koh et al. 2019; Lamperty et al. 2023). Having lost about 99% of its original vegetation (Corlett 1992), about half of Singapore is vegetated today, most of which is secondary forest (Fig. 1) (Yee et al. 2011; Gaw et al. 2019). Most of Singapore's forest habitat patches that are large enough for wildlife are concentrated in the central core of the main island around the key water catchment areas, with substantial tracts of forest near coastal margins and offshore islands, and pockets of natural or seminatural vegetation situated within urban areas. Since recolonization, wild boar have expanded their range throughout the main island, seemingly able to cross urban areas to reach isolated forest patches, and populations have considerably increased in size (Yong et al. 2010; Koh et al. 2019; Khoo et al. 2021; Lamperty et al. 2023). Wild boar populations in Singapore are thus often in close proximity to humans and urban environments, even in nature reserves where forest habitats are partly accessible to the public (e.g., trails). Given the extirpation of their main predators throughout Singapore (Corlett 1992) and the loss of hunting pressure, wild boar densities are expected to eventually exceed that in more intact ecosystems due to the ecological release from top-down regulation and bottom-up resources due to supplemental food (Yong et al. 2010; Lamperty et al. 2023; Moore et al. 2023).

Despite the significance of wild boar populations to natural ecosystems and human-wildlife interactions, little is known about the role that diet plays in their recent ecological success in Singapore. This research gap has added importance, as the population density of wild boar is largely dependent on food availability (Massei et al. 1997; Luskin et al. 2017), and so the management of food sources is one option for regulating wild boar populations. Wild boar are generalists and known to have exceptionally broad diets, consuming a wide variety of plant material and invertebrates, and may even predate on vertebrates and scavenge on carrion (Behera and Gupta 2007; Ballari

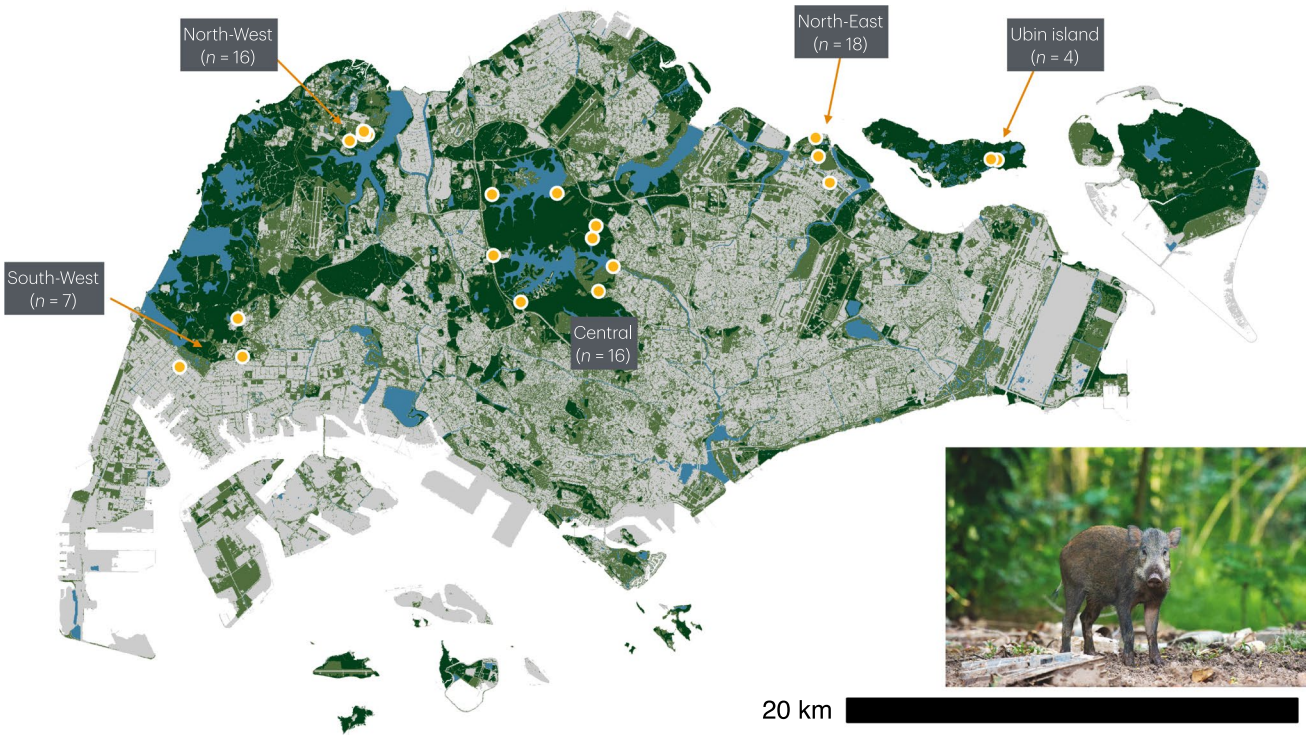


Fig. 1 Distribution of wild boar faecal sample localities (yellow points) across Singapore (n = 61). Sample size among localities for each region indicated in parentheses. Base map represents land cover (30 x 30 cm resolution) from Gaw et al. 2019, which was used to calculate forest cover (dark green pixels only) and vegetation cover (light and dark green pixels) associated with each sampling locality (proportion of terrestrial pixels within 1 km). We hypothesised that the amount of non-forest or non-vegetated cover in the proximity of samples may be a proxy for the degree of urban influence on wild boar diets

and Barrios-García 2014; Robeson et al. 2018; McDonough et al. 2022). However, how they adapt to the mixed tropical forest-urban landscapes in Singapore is unclear. Furthermore, diets of wild boar populations in Singapore may also be supplemented by anthropogenic foods (e.g., food waste in rubbish, community gardens, food provisioning) (Ang 2021; Abdullah 2022), but the relative contribution of such sources to their diets is unknown.

Here, we assess wild boar diets in Singapore using faecal samples collected across the country in areas with varying degrees of urbanisation. We employed DNA metabarcoding to capture a wide spectrum of potential plant and animal dietary items and the gut microbiome. We hypothesised that faecal samples collected in areas in closer proximity to natural or semi-natural vegetation such as forests, fields, or parks are more likely to exhibit a greater diversity of putative diet items and more diverse gut microbiomes than faecal samples collected in areas with urban environments, due to the relatively limited nature and homogeneity of anthropogenic food sources. We additionally examine the degree to which prior land use continues to influence contemporary wild boar diets through the persistence of cultivated tree species within exotic-dominated secondary forests (Yee et al. 2016, 2019). Because such forests are often situated closer to urban environments than core native-dominated forest habitats in the Central parts of Singapore, we expected these formerly cultivated species to represent important dietary resources for wild boar. Specifically, we predicted that wild boar would frequently consume cultivated species characteristic of former plantations, orchards, and village landscapes and that detections of these species in wild boar diets would spatially correspond with the distribution of exotic-dominated secondary forests in Singapore.

Materials & methods

Sample collection

We collected 61 wild boar faecal samples between July 2019 and December 2021. We located scats non-invasively through opportunistic sampling (n = 35) and supplemented them with samples collected by hand rectally from individuals trapped as part of an official population monitoring programme (n = 26) (Supplementary Table S1). Only one rectal sample was collected per individual. Scat samples were opportunistically collected when they appeared moist and undisturbed, indicating recent deposition (likely <1 day), and although the exact time cannot be determined, the crepuscular activity of wild boar (Khoo et al. 2021) suggests the samples would have been likely deposited on the same or the previous day. Samples were stored separately in sterile resealable bags and kept at -20°C (Kolodziej et al. 2013), usually within a few hours of collection, until DNA extraction.

Faecal samples were broadly grouped into five geographic regions (Fig. 1), each varying in landscape connectivity and quality, and potential exposure of populations to urban environments (Supplementary Information, Supplementary Fig. S2): Central (forests in the core central area of Singapore which are surrounded by urban residential areas), North-east (forests in the Punggol area and surrounding urban matrix), North-west (forested areas in Kranji, far from urban areas), South-west (forest fringe areas in the West of the island), and Ubin island (forested, offshore island with low urbanisation).

Library preparation and DNA sequencing

Material from the interior of the faecal samples was extracted for DNA extraction to reduce the amount of environmental contamination which would be the highest on the exterior of the stools. DNA was extracted from samples using the QIAamp PowerFecal Pro DNA Kit (Qiagen) according to the manufacturer's protocol.

Amplicon libraries were prepared using a two-stage PCR, dual-indexing strategy (Lange et al. 2014; Lim et al. 2022), where target regions are first amplified via PCR using custom primers, and Illumina compatible adapters and indexes (both forward and reverse) were then incorporated to amplicons through a second round of PCR.

To obtain a comprehensive profile of wild boar diets, we amplified three different genetic loci, targeting both plants (the chloroplast trnL-P6 loop region, trnL, and internal transcribed spacer 2, ITS2) and animals (cytochrome oxidase I, COI) (Supplementary Table S2). For plants, we amplified ITS2 and trnL barcode regions as their short length improves their recovery from faecal samples where DNA may be highly fragmented (Kartzinel et al. 2015; Ando et al. 2020; Kolter and Gemeinholzer 2021). For animals, we used widely used COI primers (Supplementary Table S2) that are broadly indiscriminate to maximise taxonomic representation of diet items (Leray et al. 2013). However, to reduce the amount of wild boar DNA being co-amplified in the process, we also used a taxon-specific blocking primer (Robeson et al. 2018) and bead-based size selection (Krehenwinkel et al. 2017a) to improve the amplification of non-wild boar reads. The former technique reduces the likelihood of primer binding to wild boar DNA, whereas the latter treatment tries to reduce the amount of host DNA in the extract by selectively filtering for DNA of low molecular weight (which is more likely to be of diet items) (Krehenwinkel et al. 2017a). Blocking primers were used in a 2:1 ratio in conjunction with the COI amplification primers. Bead-based

size selection was conducted on faecal DNA extracts before amplification PCR using solid phase reversible immobilization beads, with modifications to the protocol outlined by Krehenwinkel et al. (2017a). We first used AMPure XP Beads (Agencourt Biosciences) at a ratio of 0.75 to clean up 40 μL of genomic DNA (diluted to approximately 30 ng/μL of DNA) for each sample. The supernatant, containing low molecular weight DNA, was then precipitated using 300 μL of isopropanol mixed with GlycoBlue (15 mg/mL; Thermo Fisher Scientific) in a ratio of 300:0.75. After spinning down the tubes at maximum speed for 3 minutes to form a tight DNA pellet, the isopropanol-GlycoBlue mixture was discarded. Thereafter, the pellet was washed with 300 μL of 70% ethanol. Once the ethanol had fully evaporated and the pellet was dry, 20 μL of TE buffer was used to dissolve the DNA pellet overnight. Lastly, we also amplified the 16S rRNA gene region (Supplementary Table S2) to investigate whether dietary differences between individuals may be reflected in gut microbiome composition (Clayton et al. 2016; Dillard et al. 2022; Littleford-Colquhoun et al. 2019). Negative PCR controls were performed for all loci, and most did not show any visible band when verified using gel electrophoresis, except for 16S. We sequenced the negative control for 16S and removed any ASVs recovered in the negative control from subsequent analysis.

After both PCR steps were completed, amplicon libraries were cleaned separately using AMPure XP beads (Agencourt Biosciences). A 1:1 ratio by volume was used, but a higher ratio was used for the trnL locus (1.8:1 ratio) to reduce loss of yield due to its small indexed amplicon size (approximately 200 bp). Libraries were then pooled at equimolar concentrations and sequenced on an Illumina MiSeq platform (Illumina) with 250 paired-end reads by Genewiz (Azenta Life Sciences).

Sequence analysis and taxonomic assignment

We pre-processed demultiplexed sequences by removing all primer sequences using "cutadapt" (v4.3) (Martin 2011), except for trnL amplicons. The amplicon length of the trnL locus is small, varying from 10–143 bp (Taberlet et al. 2007), so removing the primer sequences prevents sufficient overlap between forward and reverse reads, which hinders their merging and results in low sequence recovery. We then dereplicated the reads and performed quality filtering of the reads using the DADA2 R package v.1.32.0 (Callahan et al. 2016), which uses estimated error rates for the forward and reverse reads respectively to generate amplicon sequence variants (ASVs) from the sequence data, unique DNA sequence variants at single-nucleotide resolution. Following the denoising process, forward and reverse reads were merged, requiring 10 bp of overlap for trnL and 50 for the other loci. Chimeric sequences were then removed using the "removeBimeraDenovo" function with the consensus method to generate an ASV table, reporting the number of times each ASV was present in each sample.

Putative taxonomic identities were assigned using the NCBI's BLASTn tool (Mcginnis and Madden 2004) for recovered trnL, ITS2 and COI ASVs, with the GenBank nucleotide database as the reference. We first filtered out non-target taxa, retaining only Streptophyta for trnL and ITS2, and only animal phyla for COI. Finer taxonomic assignments were then made using a consensus-based approach. For each ASV, the top three taxonomic hits with an E-value < 0.001 were selected based on sequence similarity. Species-level assignments were made only if all three top hits had a similarity of 99% or higher and matched the same species. Similar criteria were applied for higher taxonomic ranks, with similarity thresholds set at 95% for genus and family, and 90% for order, provided the hits were consistent across the top three matches. Only ASVs with at least an order-level assignment were retained for further analysis. Plant taxonomic assignments for trnL ASVs were further checked against whole chloroplast data from the Singapore Flora Genome Sequencing project (Niissalo et al. 2025), and species identities from this reference database were prioritized over GenBank matches.

Data analysis

We characterised the diversity of food items and microbiome within faecal samples using the number ("richness") and proportion ("composition") of unique ASVs from the loci examined as a proxy. We interpret greater ASV richness within a faecal sample as reflecting consumption of a wide variety of diet items, while samples that share many ASVs are interpreted to reflect a greater number of shared diet items. While ASVs may be clustered into operational taxonomic units (OTUs) using arbitrary thresholds, ASVs can offer greater resolution in diversity patterns over OTU-based analyses (Glassman and Martiny 2018; Fasolo et al. 2024). We do not analyse read abundance as it can be greatly affected by various factors, such as PCR amplification biases (Krehenwinkel et al. 2017b), as well as the opportunistic nature of the faecal sampling. For our analysis of COI, reads identified as either *Sus scrofa* or *Homo sapiens* were omitted. Bead-based size selection did not increase the recovery of non-wild boar DNA in faecal samples, but the use of a blocking primer increased the relative proportion of non-wild boar reads in recovered sequences greatly (Supplementary Information, Fig. S1). We thus used the recovered non-wild boar reads from the combined treatment (bead-based size selection and blocking primer applied simultaneously) for all analyses (mean of 60.2% of

reads originating from wild boar vs. 74.4% in the negative control). To normalise for differences in sequencing depth between samples for each locus, we further subsampled each ASV dataset 100 times to the minimum sequencing depth (Lim et al. 2022).

To examine whether the extent of natural or semi-natural environments influences wild boar diets, we calculated the proportion of terrestrial area within 1 km of each sampling locality that is vegetated or forested, roughly corresponding to previous estimates of wild boar home range size in Singapore (Koh et al. 2018). While we acknowledge that the spatial scale considered is going to be context-specific (Moll et al. 2020), we believe that our choice of home range size is an appropriate benchmark as faecal samples only provide a short-term snapshot of diet (Castle and Castle 1956). Land cover information was obtained using a high-resolution ground-truthed land cover raster of Singapore (Gaw et al. 2019). We defined vegetated areas as terrestrial pixels (marine areas and water bodies excluded) that are covered by any sort of vegetation, regardless of whether they are managed (e.g., parks, road verges) or natural, ranging from open fields to forests. Non-vegetated terrestrial areas thus consist of terrestrial pixels classified as either "buildings", "impervious surfaces" or "non-vegetated pervious surfaces". We defined forested areas as vegetated pixels with "limited human management" that were covered by tree canopy (Gaw et al. 2019) (Fig. 1). We used two different cover variables because while both non-forest and forest vegetated areas likely both offer natural food sources for wild boar, non-forest vegetated areas (e.g., parks, roadside trees and verges, gardens) have a greater degree of anthropogenic influence (e.g., likelihood of human contact, plant composition). Thus, while non-vegetated areas can be considered highly urban (buildings, roads, bare ground), the presence of non-forested vegetated areas may indicate a lesser degree of urban exposure. We then used the inverse of either metric as a proxy for the degree of urban influence on wild boar diets.

To evaluate the influence of forest and vegetation cover on plant and animal diet and gut microbiome richness, we fitted spatial autoregressive linear regression models (Dormann et al. 2007) with either trnL, 16S, COI or 16S ASV richness as the response variable and either forest or vegetation cover as the explanatory variable, using the "errorsarlm" function from the "spatialreg" R package v1.4-2 (Bivand et al. 2019). A spatial model with inverse distance weighting was fitted to account for the potential spatial autocorrelation in diversity patterns as many of our samples were geographically clustered and samples in close proximity may come from the same sampling event (Fig. 1). ASV richness for each locus was calculated as the average across randomly subsampled datasets.

In addition, we tested to see if compositional variation in diets and gut microbiome could be explained by differences in vegetation and forest cover associated with faecal samples using Mantel tests. Partial Mantel tests were also fitted separately to account for variation in ASV composition due to geographic proximity. For each sequenced locus, ASV compositional dissimilarity between samples was quantified using Jaccard's dissimilarity index. Mantel and partial Mantel tests were performed for each of the 100 randomly subsampled ASV datasets separately to control for the effect of sequencing depth on ASV diversity and composition. Mantel and partial Mantel tests were implemented using the "mantel" function from the "vegan" R package v2.6-6.1 (Oksanen et al. 2024).

As there may be compositional differences between rectal and opportunistically ("ground") collected faecal samples, we conducted several sensitivity analyses. Compositional differences between rectal and ground faecal samples across sequenced loci were tested using permutational multivariate analyses of variance (PERMANOVA). As species-level taxonomic identities may not be reliably assigned to all ASVs, we also used the proportion of sequencing reads in each sample that could be attributed to flies (Diptera) as a coarse proxy for post-depositional contamination of faecal samples, as we expected flies to be a relatively small dietary component based on other studies (Robeson et al. 2018). This was tested using the afore-mentioned spatial autoregressive model approach with logit-transformed fly DNA proportion as a response variable and collection type as a explanatory variable. Next, as rectal faecal samples were largely limited to a few sites in the Central and North-east regions (Supplementary Table S1), we repeated all other analyses on just data limited to ground samples to test the influence of collection type on our results.

Lastly, to further examine the degree to which prior land use may be shaping wild boar diets, we examined the degree to which formerly cultivated tree species in secondary forests are represented in wild boar diets. We focused primarily on species of exotic-dominated secondary forests (*sensu* Yee et al. 2019) – secondary forests largely composed of non-native tree species that develop on former rubber (*Hevea brasiliensis*) plantations, fruit orchards or villages – as they often retain mature trees of various cultivated species that also produce fruit or seeds that may represent anthropogenic food for wild boar populations and therefore of management concern. Exotic-dominated secondary forests in Singapore largely occur as isolated fragments or are largely peripheral to core old-growth and secondary forest areas dominated by native tree species, and are thus in closer proximity to urban environments in Singapore than afore-mentioned forest habitats. We examined the incidence of three formerly cultivated tree species previously identified to be characteristic

of exotic-dominated forests (Yee et al. 2016): durian (*Durio zibethinus*), rambutan (*Nephelium lappaceum*) and rubber. We then compared the geographic distribution of species detections with the occurrence of these tree species in post-cultivation secondary forests across Singapore (Neo et al. 2017). Forest plot data was accessed using the "novelforestSG" R package (https://github.com/hrlai/novelforestSG).

Results

Plant and animal diversity in wild boar diets

We assigned putative taxonomic identities (at least to order-level) to 411 trnL ASVs, 1,995 ITS2 ASVs, and 654 COI ASVs. Using our consensus approach, we assigned taxonomic families to a high proportion of ASVs (96%, 71% and 35% of trnL, ITS2 and COI ASVs, respectively). There were fewer genus- and species-level assignments (Supplementary Table S3).

For plants, a total of 96 families, 205 genera and 60 species were identified using trnL and ITS2 sequencing. The most prevalent genera and families were similar between loci (Fig. 2; Supplementary Tables S4–5, S7–S8), although taxonomic overlap was generally low (family = 44; genera = 37, species = 4) (Fig. 2). Highly prevalent families (i.e., at least half of samples) that were detected by both plant loci include: Arecaceae, Combretaceae, Convolvulaceae, Moraceae, Myrtaceae, Poaceae, and Zingiberaceae. Only 4 genera were both highly prevalent and detected using both loci: *Artocarpus* (Moraceae), *Ficus* (Moraceae), *Syzygium* (Myrtaceae) and *Terminalia* (Combretaceae). A variety of cultivated herbaceous and tree species were also detected in the faecal samples, notably *Allium sativum* (e.g., onion), *Ananas comosus* (pineapple), *Artocarpus heterophyllus* (jackfruit), *Artocarpus camansi* (breadnut), *Brassica rapa* (mustard), *Durio zibethinus* (durian), *Mangifera indica* (mango), *Nephelium lappaceum* (rambutan), and *Theobroma subincanum* (cocoa relative) (Supplementary Tables S6, S9). *Oryza sativa* (rice) was detected in 3 samples using the trnL loci (Supplementary Table S6). Some non-native invasive species such as *Miconia* sp. were also detected (11% of samples using trnL locus; Supplementary Table S5).

For animals, 44 families, 34 genera and 18 species could be identified using our consensus approach. Highly prevalent (i.e., at least half of samples) taxonomic families detected using the COI locus include insects such as flies (Anthomyiidae, Muscidae, Psychodidae & Sphaeroceridae: 100% of samples; Drosophilidae: 98%; Stratiomyidae: 90%; Cecidomyiidae: 77%), assassin bugs (Reduviidae: 74%); termites (Termitidae: 95%), earthworms (Glossoscolecidae: 100%, Megascolecidae: 84%) (Fig. 2; see Supplementary Table S10–S11). The earthworms, *Pontoscolex corethurus* and *Amynthas morrisi* are among the only species that were found in more than half of samples (100% and 82% of samples, respectively) and make up an average of 17% reads across samples (Fig. 2; Supplementary Table S12). Mammals were also notably detected in wild boar scat, including mouse-deer (*Tragulus*: 100%) and leopard cat (*Prionailurus bengalensis*, 32%).

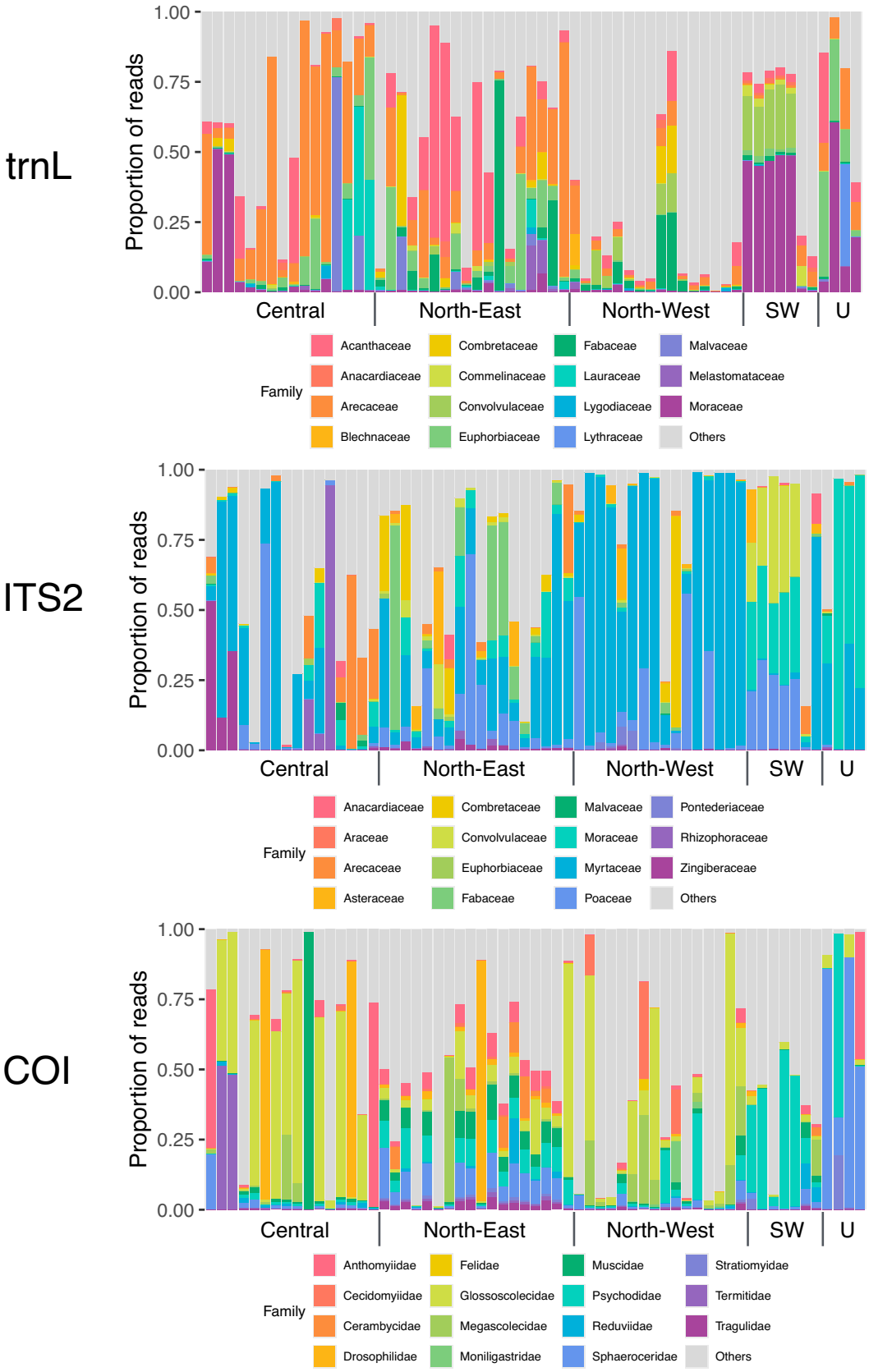
Fig. 2 Relative read abundance of the most prevalent plant (trnL and ITS2) and animal (COI) taxonomic families across wild boar faecal samples. Only the fifteen most prevalent families for each locus shown in colour. Grey bars represent the relative read abundance of ASVs that were among the most prevalent families or were not assigned to a family using our criteria (see Methods). SW = South-West, U = Ubin Island

Faecal collection type, however, has a strong impact on the composition (identity and relative abundance) of ASVs recovered across all loci (Supplementary Fig. S2) (PERMANOVA with 999 permutations: trnL, F=6.61, p=0.001; ITS2, F=5.33, p=0.001; COI, F=3.14, p=0.001; 16S, F=10.03, p=0.001). However, while the proportion of fly DNA recovered was higher in opportunistically collected samples (median = 0.89; mean = 0.62) than in rectal samples (median = 0.46; mean = 0.43) (Supplementary Fig. S3), the effect was not statistically significant when the spatial proximity of samples has been taken into account (spatial autoregressive model, z=-1.82, p=0.07).

Dietary diversity and composition along a forest-urban gradient

Plant, animal and gut microbiome sequence richness in faecal samples showed mixed responses with vegetation cover (Fig. 3). Vegetation and forest cover were not significantly correlated with the plant ASV richness recovered in faecal samples, with the exception of ASV richness in ITS2, which was positively correlated with forest cover. Animal ASV richness, as estimated using COI, was strongly negatively correlated with both vegetation and forest cover. Microbial diversity showed no significant relationship with either vegetation or forest cover. These relationships were largely similar when only non-rectal samples were analysed (Supplementary Fig. S4).

Plant and animal ASV composition in faecal samples generally varied with vegetation cover (all vegetation or forest only), even after geographic distance between samples was accounted for using partial Mantel tests (Table 1). Differences in vegetation or forest cover were not correlated with dissimilarity in 16S ASV composition when geographic distance between samples was accounted for. When only non-rectal / opportunistic samples were analysed, the effect of vegetation cover (all vegetation or forest only)


trnL
Proportion of reads
1.00
0.75
0.50
0.25
0.00
Central
North-East
North-West
SW
U
Family
Acanthaceae
Anacardiaceae
Arecaceae
Blechnaceae
Combretaceae
Commelinaceae
Convolvulaceae
Euphorbiaceae
Fabaceae
Lauraceae
Lygodiaceae
Lythraceae
Malvaceae
Melastomataceae
Moraceae
Others
ITS2
Proportion of reads
1.00
0.75
0.50
0.25
0.00
Central
North-East
North-West
SW
U
Family
Anacardiaceae
Araceae
Arecaceae
Asteraceae
Combretaceae
Convolvulaceae
Euphorbiaceae
Fabaceae
Malvaceae
Moraceae
Myrtaceae
Poaceae
Pontederiaceae
Rhizophoraceae
Zingiberaceae
Others
COI
Proportion of reads
1.00
0.75
0.50
0.25
0.00
Central
North-East
North-West
SW
U
Family
Anthomyiidae
Cecidomyiidae
Cerambycidae
Drosophilidae
Felidae
Glossoscolecidae
Megascolecidae
Moniligastridae
Muscidae
Psychodidae
Reduviidae
Sphaeroceridae
Stratiomyidae
Termitidae
Tragulidae
Others

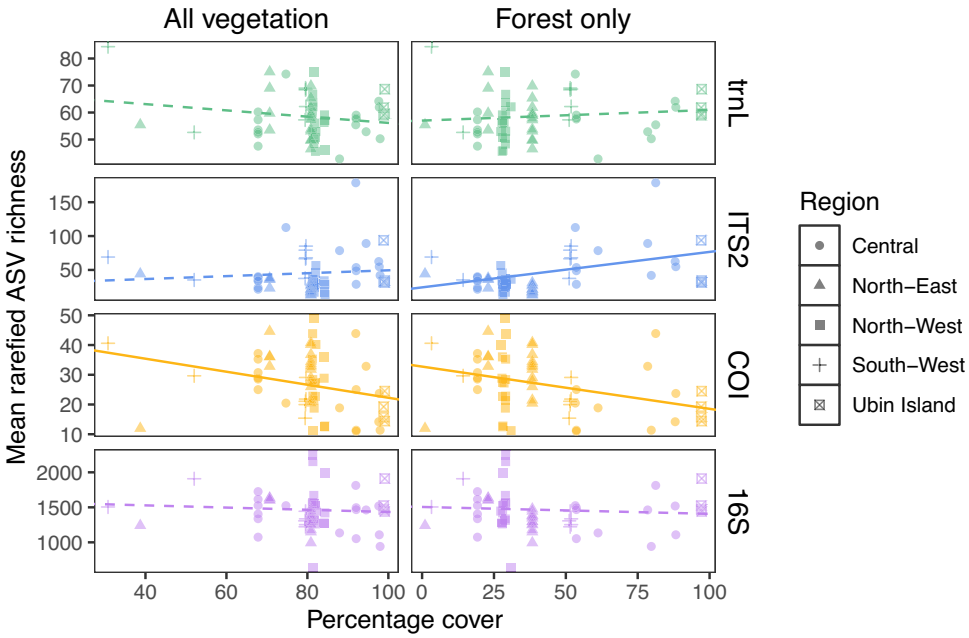


Fig. 3 Relationship between plant, animal and microbiome ASV richness, and the extent of vegetation (all vegetation or forest only) in the vicinity (within 1 km) of each faecal sample. Lines represent the best-fit relationship estimated using spatial autoregressive linear regressions with an inverse distance weighting (solid = statistically significant, dashed = not statistically significant)

Table 1 Effect of vegetational differences and geographic distance on plant, animal and microbiome ASV composition in wild boar faecal samples. Strength of correlation (r) of each variable was determined using separate Mantel correlation tests. The effect of forest cover and vegetation cover controlling for geographic distance was determined using partial Mantel tests. G = effect of geographic distance only, F = effect of difference in forest cover, F|G = effect of difference in forest cover after controlling for geographic distance. V = effect of difference in vegetation cover, V|G = effect of difference in vegetation cover after controlling for geographic distance. To account for differences in sequencing depth (see Methods), each test was performed on 100 rarefied matrices (random sequencing read sub-sampling). Number of matrices with significant correlations (α = 0.05) presented in parentheses

	G	F	F|G	V	V|G
Plant (trnL)	0.22 (100)	0.19 (99)	0.15 (67)	0.20 (96)	0.17 (66)
Plant (ITS2)	0.25 (100)	0.37 (100)	0.33 (100)	0.31 (100)	0.28 (100)
Animal (COI)	0.21 (100)	0.41 (100)	0.38 (100)	0.18 (68)	0.15 (32)
Microbiome (16S)	0.41 (100)	0.16 (100)	0.07 (0)	0.15 (39)	0.09(0)

after geographic distance was accounted for, was generally weaker across most loci (Supplementary Table S13).

Key formerly cultivated tree species characteristic of secondary forests were detected across faecal samples, albeit to varying degrees. *Durio zibethinus* (durian) was detected in all faecal samples. *Nephelium lappaceum* (rambutan) and *Hevea brasiliensis* (rubber) were only detected in a few samples, but detections were geographically distant from each other (Fig. 4a). While *Hevea brasiliensis* detections were consistent with fine-scale information of the distribution of the species within secondary forest plots; *Nephelium lappaceum* and *Durio zibethinus* detections extended beyond the Central region (Fig. 4b).

Discussion

Singapore's wild boar exhibited high dietary breadth, as evidenced by the diversity and variability observed in the plant and animal items detected via faecal metabarcoding. This is in line with work from other parts of their native and introduced range (Ballari and Barrios-García 2014). While dietary metabarcoding studies have been used to investigate wild boar diets across natural, rural and urban settings (Stillfried et al. 2017b; Robeson et al. 2018; Mysterud et al. 2024; Wilson et al. 2026), our study represents the first study within a mixed urban tropical landscape in its native range. Dietary metabarcoding provides a dietary snapshot, and will depend on the temporal variability of various food items relative to sampling, and the time since consumption of diet items. Furthermore, the taxonomic composition and relative sequencing read abundance of different diet items can be influenced by many factors, including differences in the discriminatory ability of each locus (CBOL Plant Working Group et al. 2009), differences in the coverage of reference sequences available for each loci (Kolter and Gemeinholzer 2021; Kartzinel et al. 2015), differences in PCR amplification efficiency (Pompanon et al. 2012; Krehenwinkel et al. 2017b), and potential differences in the recovery success of amplicons of different length (Taberlet et al. 2007) when dealing with potentially fragmented DNA samples like those derived from faecal material (Pompanon et al. 2012; Hollingsworth et al. 2016). As such, we have

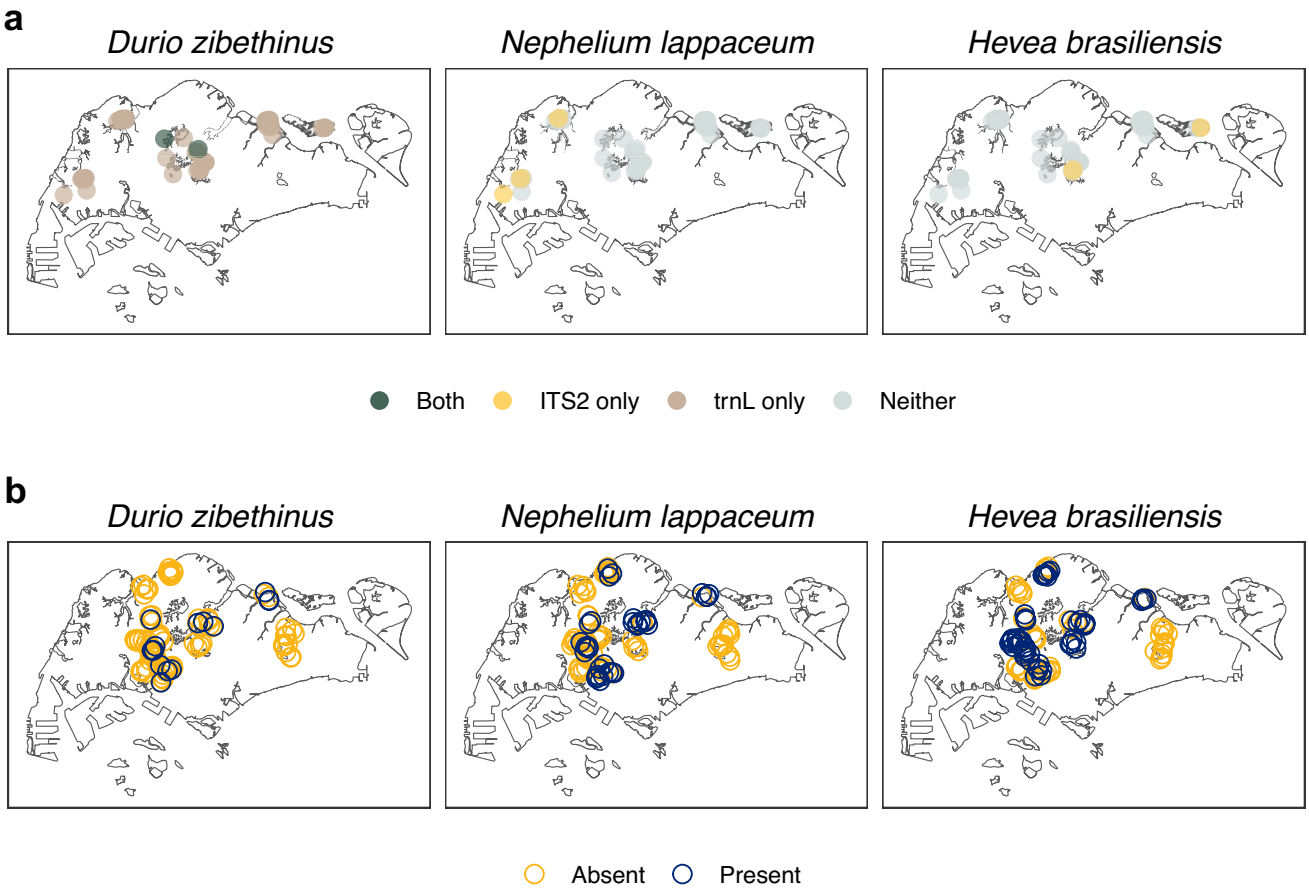


Fig. 4 Prevalence of three common formerly cultivated tree species that persist in Singapore's secondary forests (*Durio zibethinus*, durian; *Nephelium lappaceum*, rambutan; *Hevea brasiliensis*, rubber) in wild boar faecal samples and post-cultivation secondary forest plots in Singapore. (**a**) Geographic distribution of samples where tree species was detected across the two sequenced plant loci. (**b**) Occurrence of each tree species in secondary forest plots across a range of secondary forest plots (Neo et al. 2017)

primarily interpreted the relative importance of various diet items from their prevalence across our samples, rather than the proportion of reads recovered.

At least some of the plant and animal species detected can be explained by environmental exposure experienced by faecal samples after deposition and before collection (e.g., flies and other coprophagous insects), as we demonstrated in the differences in the relative abundance of fly sequenced reads recovered between rectally extracted and opportunistically collected samples. However, even directly extracted samples will be in contact with the skin of the wild boar during the sampling process, and so the introduction of DNA from non-food sources would be unavoidable except possibly the case of gut dissections. While the coprophily of some invertebrates may at least explain their high prevalence in opportunistically collected samples, many animal and plant taxa were also consistently present even in rectally extracted faecal samples, suggesting their importance as food resources. Grasses (Poaceae) and gingers (Zingiberaceae) were detected across all samples, suggesting the importance of herbivory, and are consistent with studies of wild boar faecal matter (Khan and Ilyas 2018) and other metabarcoding studies (Anderson et al. 2018). By contrast, native species of *Syzygium* and *Ficus*, as well as cultivated fruit tree species, are more likely to be exploited by wild boar for their fruit.

Among animals detected through COI metabarcoding, the high prevalence of earthworms (the invasive *Pontoscolex corethurus* and native *Amynthas morrisi*; Shen and Yeo 2005) is not unusual, as earthworms are well-documented diet items (Khan and Ilyas 2018; Ballari and Barrios-García 2014. Wild boar are known to rely on invertebrates for diet (Ballari and Barrios-García 2014; Anderson et al. 2018; Robeson et al. 2018) and some families were detected in high frequency in our dung samples (e.g., Cerambycidae beetles, Reduviidae bugs). However, we also detected mouse-deer and leopard cat DNA, suggesting that wild boar may either be predating (for mouse-deer) or scavenging on carrion (Chua et al. 2026).

The relationship between forest or vegetation cover on plant sequence richness was mixed and generally weak. There was a slight positive correlation between ITS2 plant

sequence diversity and forest cover, but there was no significant correlation between trnL plant sequence diversity with either vegetation or forest cover. This difference may be explained by differences in the coverage of sequence reference databases between loci (1,995 ITS2 ASVs could be assigned at least to taxonomic family vs. 411 ASVs for trnL). However, we found a negative relationship between neighbouring forest and vegetation cover and recovered animal COI sequence diversity, even when we limited our analysis to opportunistic faecal samples. This may suggest that wild boar may rely less on animal dietary sources when in forest areas. Differences in the extent of forest or vegetated habitats did not appear to have a consistent effect on wild boar diet composition and the effect was partly confounded by faecal collection method. Plant and animal diets were more dissimilar when they differed in extent of neighbouring forest or vegetation cover, even after geographic distance between samples was accounted for, but the effect was much weaker when only opportunistic samples were analysed. While the restricted geographic coverage of our rectal samples precludes a direct comparison of patterns between collection types, a possible explanation for the weak patterns may be explained by the high dietary flexibility of wild boar leading to an uncoupling of putative diet diversity patterns with their immediate landscape context. In addition, wild boar individuals may have complex spatial foraging patterns and so the immediate landscape context of faecal samples may not be fully representative of the time spent foraging in different habitats. Future studies should consider coupling individual-level information on wild boar movements to faecal samples to gain a higher-resolution picture of how habitat use may be shaping wild boar diets.

Consistent with the afore-mentioned factors, we also did not find a strong relationship between gut microbiome richness and composition and forest or vegetation cover, contrary to some studies which have found more diverse gut microbiomes in urban adapted animals compared to their less urban counterparts (Phillips et al. 2018; Littleford-Colquhoun et al. 2019; Nguyen et al. 2024). This may be because the contribution of human food sources to wild boar diets is relatively low, unlike other studies of urban wild boar populations (Castillo-Contreras et al. 2021). Rice (*Oryza*) was only detected in 8% of samples and probably originates from human food sources, as the small amount of rice cultivation in Singapore is limited to urban high-tech farms that are inaccessible to wild animals. The presence of *Brassica rapa* (3.3% of samples), *Allium sativum* (4.9% of samples) and *Coriandrum sativum* (1.6% of samples), may also originate from the consumption of human foods, but could also indicate crop raiding (e.g., community allotments and gardens). In these cases, the prevalence across samples was low suggesting that the frequency of human food consumption among wild boar was generally low, but DNA degradation of food items from cooking processes may cause human food items to be underrepresented in the recovered sequences.

The high dietary flexibility of wild boar and the local ecological context present unique management challenges for Singapore's forests. As local forests no longer host their main predators (Corlett 1992), the abundance of cultivated tree species in secondary forest areas, and the supplementation of wild boar diets from anthropogenic sources, wild boar will likely achieve densities much higher than in intact forest landscapes (Moore et al. 2023). In other Southeast Asian forests, inflated wild boar populations due to crop raiding in agricultural landscapes has had negative spillover effects on tree regeneration in adjacent forest ecosystems due to the higher wild boar densities (Luskin et al. 2017, 2019; Milson et al. 2025).

Our analysis suggests that cultivated tree species such as durian, rambutan and rubber may play a minor role in supporting local wild boar populations likely through the fruits and seeds they produce, though they may represent temporally variable resources. Detections were generally low for rambutan and rubber, contrary to our expectations, but in geographically distant areas suggesting they are general food items and that their prevalence in faecal samples may have been influenced by the timing of sample collection relative to the timing of fruiting events. As these species are prevalent in Singapore's secondary forests as remnants of former plantations or fruit orchards (Yee et al. 2016), they provide a potential case study for the role of former land use in influencing urban wildlife populations. We acknowledge that the forest survey data we present are not geographically comprehensive (largely forests in the central region) and the occurrence of such fruit trees can vary greatly even at small spatial scales, but they represent the best dataset of post-cultivated secondary forest composition in Singapore (Neo et al. 2017). However, we note that durian and rambutan are also present outside of forest habitats (e.g., cultivated in private gardens) (personal observations) or may be sourced from human environments (e.g., discarded fruits from shops). Further studies such as camera traps on fruiting trees in non-forested residential areas will be needed to determine the relative contribution of trees in different settings (e.g., urban vs. forest) to wild boar diets.

When wild boar become overabundant, their ecological impacts can extend across multiple components of both forest and urban ecosystems. Their foraging behaviour can directly alter vegetation structure through rooting disturbance and herbivory (Luskin et al. 2019), while also facilitating the spread of invasive plants such as *Miconia* (Ho et al. 2023). In systems like Singapore, where many native large-bodied seed dispersers and predators of wild boar have

already been lost (Corlett 1992), these effects may be amplified, making forests more vulnerable to regeneration failure and shifts in plant community composition. Overabundance of wild boar may also be disrupting populations of other wildlife species. When wild boar populations experienced declines due to African Swine Fever in 2022 (Lieb et al. 2025), greater mouse-deer numbers quintupled, consistent with release from competition and predation pressure (Chua et al. 2026). Furthermore, the tendency of wild boar to forage in forest edges and urban areas (Castillo-Contreras et al. 2018) has also led to human-wildlife conflict in Singapore (Woon 2022) and may make it harder to achieve human-wildlife co-existence (Conejero et al. 2024; Wong 2024). Locally, a variety of physical measures (e.g., fencing, cattle guards) have been implemented to contain wild boar within natural areas, including strategic culling (Begum and Lim 2025), as commonly employed on other urban wild boar populations (Stillfried et al. 2017a).

Ultimately, our study suggests that the diet of wild boar mostly originate from natural sources and highlights the indirect role that prior land use history can play in supporting wildlife populations. However, the relatively low prevalence of some anthropogenic foods may suggest the low predictability and availability of such resources and thus they may only have a minor influence on population dynamics, even though the degree of consumption of some human food items may be underestimated given the degradation of DNA from cooking processes. Nevertheless, their diet could shift towards higher anthropogenic food consumption if such resources become more abundant and easy to exploit (Stillfried et al. 2017b; Ang 2021; Abdullah 2022) or when wild boar populations further increase and encroach more into urban areas. Our study suggests that the management of formerly cultivated trees in secondary forest and the accessibility of anthropogenic food sources in urban areas could be a complementary long-term, and cost-effective strategy to reduce wild boar carrying capacities to desirable levels, and it may also help regulate the populations of other urban-tolerant wildlife species such as the long-tailed macaque (*Macaca fascicularis*) (Sha et al. 2009). While the power and scalability of dietary metabarcoding has offered us some insight into wild boar diets, its limitations mean that the integration of other information such as camera traps (e.g., specific food items) and animal movement will be crucial to further our understanding of wild boar foraging behaviour in complex landscapes such as Singapore.

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Author Contributions J.Y.L., J.O., M.S.L. and M.D.Y.K. conceived and designed the study. Sample collections were overseen by M.D.Y.K., W.X.C., G.K.P. and M.S.L.. Molecular work was performed by J.O. and M.A.S.T. Bioinformatic processing and statistical analysis were performed by J.O. and J.Y.L., with some additional support from C.J.M.. The first draft of the manuscript was written by J.O. and J.Y.L., and all authors commented and contributed to subsequent drafts. All authors read and approved the final manuscript.

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Declarations

Competing Interests The authors declare no competing interests.

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