Disentangling the drivers of parasite occurrence in a native and a non-native small mammal across a land-use gradient

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Abstract

Land-use change can reorganize host–parasite systems by altering environmental conditions, host communities, and transmission pathways. Yet parasite occurrence remains difficult to predict as it is shaped simultaneously by the environment, host identity, individual host attributes, the within-host microbiome, and parasite biology. We integrated these components within a single framework for two coexisting small mammals along a land-use gradient in northeastern Madagascar: the native, Malagasy-endemic shrew tenrec, *Microgale brevicaudata* (Tenrecidae), and the non-native, invasive black rat, *Rattus rattus* (Muridae). We characterized gut protozoan and nematode parasites in 505 hosts and used Hierarchical Modeling of Species Communities (HMSC) to quantify the relative contributions of environmental, host, microbiome, and parasite-level factors to parasite occurrence. Host species was the dominant driver overall, accounting for approximately half of the weighted explained variance, followed by environmental conditions, but their relative importance varied substantially among parasites. Parasite taxonomic group explained part of this variation, indicating that parasite biology also shaped responses to the same environmental and host-level drivers. Land-use responses differed sharply between hosts: parasite richness in *M. brevicaudata* was highest in the primary forest, whereas richness in *R. rattus* was highest in disturbed habitats. Thus, land-use change did not generate a common increase or decline in parasitism, but redistributed parasite richness between coexisting native and non-native hosts. Together, these results show that responses of host–parasite systems to land-use change emerge from the interaction of host identity, environmental conditions, and parasite biology, and cannot be understood from any single level alone. This multi-level perspective

can improve predictions of infection risk as global change expands the interface between native and non-native hosts.

Introduction

Land-use change is a major driver of biodiversity loss, reshaping ecological communities and altering the processes that regulate disease transmission [1,2]. As natural habitats are converted to agricultural zones and settlements expand, wildlife communities reorganize in composition and diversity, with cascading effects on the gut endoparasites they host (hereafter, parasites) [3–5]. A defining feature of many human-modified landscapes is the expansion of non-native host species, which increasingly co-exist with declining native fauna in novel and artificial assemblages [6,7]. This ecological reassembly can reshape parasite communities by introducing taxa, modifying host availability, and changing infection pathways, including opportunities for cross-species transmission [8,9]. Identifying the drivers of parasite occurrence across native and non-native hosts is therefore essential for understanding how disturbed landscapes alter infection risk, shape disease dynamics in native wildlife, and influence the potential for pathogen transmission to humans.

Quantifying the drivers of infection requires disentangling the processes that shape parasite occurrence across multiple biological scales, from the landscape a host occupies to the symbiont community within it, and assessing how their relative importance varies among parasite taxa [10–13]. Here, we identify five major drivers underlying parasite occurrence. *Environmental conditions* shape infection risk by determining the composition and persistence of the parasite pool, altering transmission pathways and rates, and defining the ecological context in which host–parasite interactions occur [14,15]. Parasite occurrence also depends on host characteristics at two scales. At the *host species* scale, the phylogenetic history of host-parasite coevolution shapes host compatibility, and thereby determines whether parasites can infect, establish, and persist in a given host species [16–18]. Within a single species, *individual host attributes*, such as body condition or sex, can modify infection by affecting both the likelihood of encountering parasites (i.e., exposure) and the host's capacity to resist infection after exposure (i.e., susceptibility) [19–21]. Hosts also rarely harbor a single parasite in isolation. Instead, parasite infection occurs within a broader within-host symbiont community, in which the *microbiome* can interact with co-infecting parasites through competition, immune modulation, and resource use [22,23]. These interactions among parasitic, mutualistic, and commensal symbionts can influence parasite establishment, persistence, and transmission [24–26]. Finally, parasite occurrence is also shaped by *parasite traits*. Parasites differ in life-history and functional traits, including taxonomic group, transmission strategy, and host specificity, all of which can determine how strongly they respond to different environmental and host-related factors [27,28].

Together, environmental factors, interspecific and intraspecific host variation, within-host microbial interactions, and parasite traits are expected to contribute measurably to heterogeneity in parasite occurrence. These dimensions are explicitly integrated within the conceptual *disease pyramid* framework (**Figure 1A**) [29,30]. The pyramid extends the classical disease triangle of host, pathogen, and environment [31] by explicitly adding the within-host microbial community as a fourth vertex and recognizes that infection dynamics emerge from interactions across ecological scales. In human-modified landscapes—where environmental conditions, host assemblages,

and microbial communities are shifting simultaneously—these processes are tightly coupled and are therefore best quantified jointly [32–35]. Framing parasite community assembly within this framework thus provides a powerful conceptual lens for explaining and predicting how parasite communities reorganize in response to environmental change.

Despite growing recognition of these interconnected processes, empirical studies rarely quantify the relative contributions of all drivers within a single framework [29,36]. Instead, most studies focus on one or two drivers in isolation, such as environmental effects on infection [37], host traits associated with susceptibility [38], or microbiome–parasite interactions [39]. Such studies are also typically conducted at a single hierarchical scale: either analyzing host–parasite networks at the species level while overlooking intraspecific variation [40,41], or focusing on individual-level differences within a single host species without considering broader interspecific associations [20]. However, infection patterns often emerge from the interplay between these two scales [36,42,43]. As a result, the independent and joint contributions of multiple drivers to parasite occurrence remain difficult to disentangle. This limitation is especially consequential in rural, economically emerging regions, where land-use change can bring wildlife, livestock, and people into closer contact, potentially altering infection risk for both animals and humans [44,45]. Small mammals are central to these dynamics as they often move between natural habitats, agricultural areas, and human settlements, and can serve as reservoirs for zoonotic pathogens [46]. However, little is known about how land-use variation shapes small-mammal parasite communities [47].

Here, we apply the disease pyramid framework to formally investigate the effects of land-use change on parasitism in two species of small mammals. To do so, we conducted extensive field sampling along a land-use gradient in northeastern Madagascar, focusing our analyses on two coexisting small mammals: the native, Malagasy-endemic shrew tenrec (*Microgale brevicaudata*, Tenrecidae) and the non-native, invasive black rat (*Rattus rattus*, Muridae). This system provides an effective setting for decomposing the drivers of parasite occurrence, as these hosts coexist across a fine-scale mosaic of remnant forest, agricultural land, and villages. Previous work in this region showed that land use influences parasitism in small mammals [5,32,48]. For each host individual, we characterized multiple parasitic protozoan and nematode taxa, gut bacterial communities, environmental conditions, and host attributes. Using Hierarchical Modeling of Species Communities (HMSC), a joint species distribution modeling framework [49,50], we analyzed these parasites jointly within a single model. This comparative approach allowed us to quantify the relative contributions of multiple drivers to parasite occurrence, test whether parasite responses are structured by their taxonomic group, and examine how the parasite communities of the two hosts differ along the land-use gradient.

Using the disease pyramid framework, we investigated three questions. Our first question (**Q1**) asks how the relative importance of the different components of the disease pyramid—environmental conditions, host species, individual host attributes, and the host microbiome—varies across parasites (**Figure 1B**). This provides a broader context for understanding which parts of the system most strongly structure parasite occurrence. The parasite itself, however, is also a component of the disease pyramid, and different parasites are not expected to respond uniformly to the same drivers. Our second question (**Q2**), therefore, asks whether parasite taxonomic group—nematode or protozoan—explains differences in parasite responses to these drivers

(**Figure 1C**). We expected the taxonomic group to capture broad differences in transmission and infection biology, while recognizing that both groups include biologically diverse taxa. Two opposing expectations follow. Protozoan occurrence may track environmental conditions more closely, as transmission often proceeds through fecal contamination and should increase where hosts are dense, whereas chronic nematode infections may decouple occurrence from current conditions [51,52]. Alternatively, nematodes may be filtered more strongly by the environment, since their obligate free-living larval stages are exposed to desiccation and temperature fluctuations, whereas many protozoa persist as resistant cysts or oocysts.

Finally, our third question (**Q3**) incorporates human-driven habitat change into the disease pyramid by considering how the parasite communities of the native *M. brevicaudata* and the non-native *R. rattus* change along the land-use gradient (**Figure 1D**). This comparison links the broader disease-pyramid analysis to a central consequence of land-use change: the redistribution of parasites across coexisting native and non-native hosts [48]. We expected the disturbance-tolerant *R. rattus* to harbor higher parasite occurrence in human-modified habitats [53], where its density and transmission opportunities are highest. In contrast, we expected infection in *M. brevicaudata* to depend less on land use, reflecting stronger host association and a potentially longer co-evolutionary history [54].

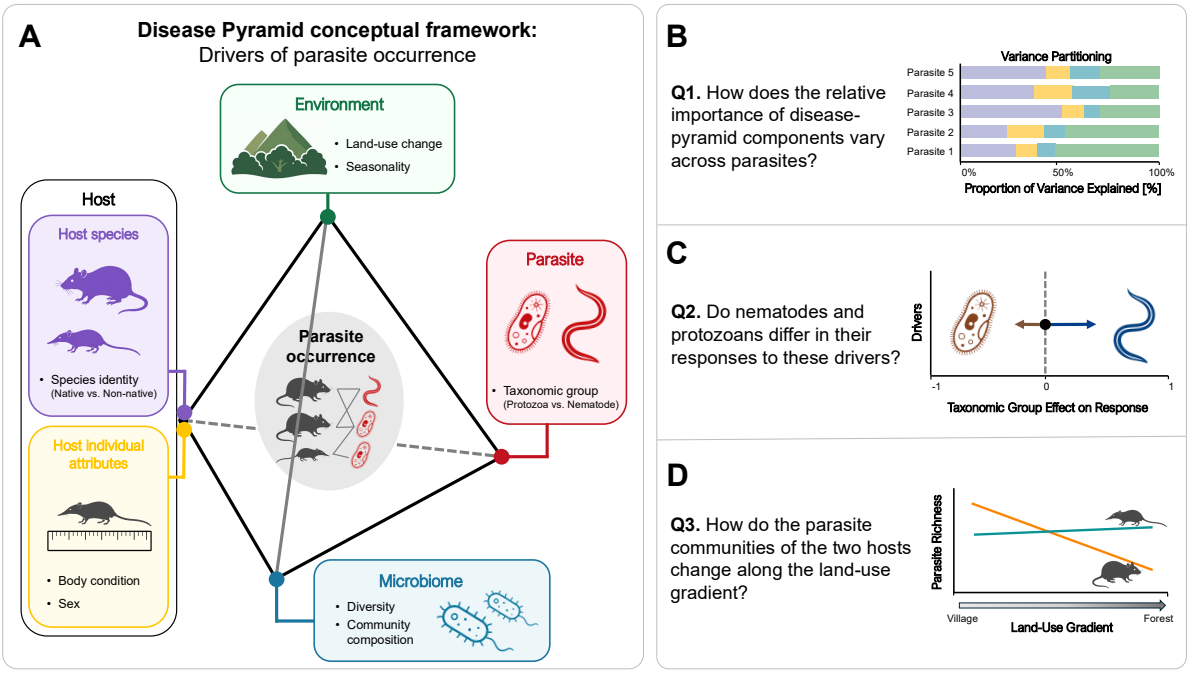

Figure 1: Conceptual framework and analytical approach. (A) The 'disease pyramid' [29] frames parasite occurrence as shaped by multiple, interacting drivers. These drivers comprise environmental conditions (land-use change and seasonality), host-related factors at two scales (the host species and individual attributes such as body condition and sex), the host gut microbiome (diversity and community composition), and the parasite itself, represented by its taxonomic group (protozoan or nematode). We characterized these drivers for each host individual and modeled the occurrence of all parasite taxa jointly using a Hierarchical Modeling of Species Communities (HMSC) framework. Panels **(B)**–**(D)** illustrate the three analyses this framework supports, corresponding to our three questions. **(B)** We partitioned the explained variance in each parasite's occurrence into the disease-pyramid driver categories (colors as in A), yielding a variance-partitioning profile per parasite. Comparing these profiles across taxa shows how the relative importance of the drivers varies among parasites. **(C)** We included parasite taxonomic group (protozoan or nematode) as a proxy for parasite traits in the model to test whether it structures how parasites respond to the drivers. For each driver, the analysis estimates a trait effect: the difference in mean response between protozoa and nematodes, where a value away from zero indicates that the two groups respond differently. **(D)** We used the fitted model to predict each parasite's probability of occurrence along the land-use gradient, from the village to forested habitat, separately in the native *Microgale brevicaudata* and the non-native *Rattus rattus*, and summed these to obtain predicted parasite richness in each host. Panel (D) illustrates the expected change in parasite richness for each host along the gradient.

Methods

Study site and sampling

Small mammals were sampled in the SAVA Region of northeastern Madagascar, in close proximity to the village of Mandena and within the adjacent Marojejy National Park (**SI note 1**). Marojejy comprises moist evergreen forest spanning a broad elevational gradient, from lowland to montane habitats, becoming non-forested in summital zones exceeding 2000 m. We sampled across seven habitat types: (1) semi-intact lowland moist evergreen forest inside the national park, (2) secondary forest, (3) *savoka* (brushy regrowth after forest clearing), (4) agroforest (vanilla plantation), (5) mixed agriculture (sugarcane/coffee plantation), (6) rice fields, and (7)

Mandena village. Sites were spaced at least 500 m apart. At each site, we deployed an 11 × 11 grid of 121 live traps spaced 10 m apart, supplemented by two pitfall lines of 11 buckets each (spaced 10 m apart) located outside the main grid. Each of the seven sites was sampled for a total of 18 nights, consisting of six consecutive nights in each of three seasons: October–November 2019 (before the wet season), March–April 2020 (end of the wet and hot season), and July–September 2020 (dry and cold season).

Our study focused on the two most abundant small mammal species captured (**Figure S1**): the native shrew tenrec (*Microgale brevicaudata*, Tenrecidae) and the non-native black rat (*Rattus rattus*, Muridae). Together, these species accounted for 74% of all sampled small mammals. *R. rattus* is a large (∼90 g) generalist omnivore with a highly adaptable diet [53] that was introduced to Madagascar, likely during the 10th century [55–57]. Ecologically, it acts as a major disruptor of native communities, a primary agricultural pest, and a known vector for several zoonotic diseases on the island [57,58]. In contrast, *M. brevicaudata* is a small (∼10 g) Malagasy-endemic shrew tenrec; it is primarily an insectivore associated with forest habitats, but also occurs in disturbed environments [48,59,60].

Parasite detection and bioinformatic processing

We used DNA metabarcoding of fecal samples to characterize gut protozoan and nematode parasites. Detailed laboratory protocols, filtering steps, and taxonomic procedures are provided in **SI note 2**. DNA was extracted from preserved fecal samples using Zymo MiniPrep Fecal kits following the manufacturer's protocol. Protozoan parasites were screened by amplifying the 18S rRNA region using the G4 and G6 primer sets, whereas nematodes were screened by amplifying the ITS2 region using the NC1/NC2 primer set [61–63]. Amplicons were barcoded with 8-nucleotide forward and reverse tags, pooled with additional samples, purified, and sequenced on an Illumina MiSeq platform (v3, 2 x 300 bp).

Raw reads were demultiplexed with *cutadapt* and processed using the *dada2* pipeline, including quality filtering, error correction, dereplication, paired-read merging, and chimera removal [64,65]. Taxonomy was assigned separately for protozoa and nematodes. Protozoan ASVs were assigned taxonomy using a consensus approach based on *dada2* and *DECIPHER* [66] against the SILVA non-redundant database (v138.1), with conflicting assignments checked against NCBI GenBank. ASVs were then taxonomically filtered to retain only taxa established or suspected to be mammalian parasites, and retained protozoan ASVs were aggregated at the genus level. Reference barcode coverage for the nematodes of Malagasy small mammals is sparse, so most sequences could not be matched to a named species or genus. Nematode units were therefore delimited by sequence clustering rather than by reference assignment. Nematode ASVs were classified against the nemabiome ITS2 database (v1.6), clustered into OTUs at 97% similarity, and then curated with the LULU algorithm [67] to merge over-split variants of the same taxon based on sequence similarity and co-occurrence across samples.

Importantly, each nematode OTU was labeled with the lowest taxonomic rank to which its representative sequence could be confidently assigned, so unit names range from genus to superfamily. A family-level name denotes a single OTU that could not be resolved further, rather than the family as a whole. Names that appear nested, such as *Nippostrongylus* and Heligmonellidae,

therefore refer to separate, non-overlapping OTUs.

Finally, to ensure the robustness and predictive power of our models, we removed relatively rare parasite taxa with fewer than 10 occurrences in the dataset. The final dataset included 333 *R. rattus* and 172 *M. brevicaudata* individuals infected by seven protozoan and four nematode taxa (**Figure S2**).

Environmental and host-level predictors

To investigate the determinants of parasite occurrence, we quantified environmental, host-level, and gut microbiome variables for each captured host individual (**Table S1**; see **SI note 3** for details). These variables were selected because they represent the vertices of the disease pyramid and have the potential to influence both host exposure and susceptibility to infection [29].

Environmental variables We captured environmental variation using land-use and seasonality. We represented the land-use gradient with the distance from each host's capture location to the nearest village center, a proxy for anthropogenic disturbance. This gradient of habitat modification shapes both the survival of free-living parasite stages and host contact rates [15,68]. We verified that this single variable tracks the broader gradient by confirming its association with independently measured vegetation structure and small-mammal community composition (**SI note 3**). We further included season (before the wet season, end of the wet season, and the dry season) to account for temporal variation in climatic conditions that affect parasite survival, transmission, and host physiology. We also included the trapping site as a random effect to absorb unmeasured spatial variation.

Host-level variables We assessed host variables at two scales: species and individual. The host species (*R. rattus* or *M. brevicaudata*) was included at the species level. At the individual level we included body size, body condition, and sex, which are well-established determinants of host behavior (e.g., home-range size and social contacts) and physiology, including immune function [19,20,69]. Body size and condition capture complementary axes: structural size versus energetic state relative to that size.

Microbiome composition Gut microbiome composition was characterized using 16S rRNA gene sequencing of fecal samples (**SI note 3**). To capture biologically meaningful variation among hosts, we derived three predictors spanning complementary dimensions of gut community structure: (1) diversity, as a measure of overall community richness linked to stability and colonization resistance; (2) microbiome uniqueness, quantifying how far an individual's community deviates from that of its conspecifics, a signature of dysbiosis and reduced stability [70]; and (3) the Firmicutes/Bacteroidota ratio (FB ratio), a marker of the balance between the two dominant phyla related to diet and energy harvest [71].

Because the two focal species differ markedly in body size and baseline microbiome, all continuous individual-level variables were standardized within each species (i.e., z-scored) to reflect intraspecific variation rather than interspecific differences.

Individual random effect Given that multiple parasite taxa were measured from each host, we included the host individual as a random effect to account for the hierarchical structure of the data. Beyond absorbing host-specific variation not explained by the measured predictors (e.g.,

unmeasured physiology, immune function, or exposure history), this term allows us to assess residual associations among parasites within hosts after accounting for the measured predictors [49]. Such residual associations provide a way to evaluate whether parasites co-occur or exclude one another beyond what shared predictors would predict, consistent with potential within-host interactions such as facilitation or competition.

Pairwise correlations among continuous predictors ranged from −0.34 to 0.13, with a mean absolute correlation of 0.08 (**Figure S3**), indicating generally weak associations among predictors. To further evaluate potential multicollinearity, we calculated the variance inflation factor (VIF) for each variable. All predictors retained in the final models had VIF values below 2, suggesting low collinearity and supporting the suitability of the predictor set for robust statistical inference [72].

Statistical analysis

Hierarchical Modeling of Species Communities (HMSC) analysis of parasite occurrence

Quantifying the relative contribution of drivers requires analytical approaches that integrate information across multiple parasite taxa and hierarchical levels, while simultaneously assessing multiple drivers of infection. We therefore used a joint species distribution modeling framework that simultaneously models different parasite taxa and estimates species-specific responses to environmental and host-related predictors. To account for the data structure, we included random effects for site and host individual, capturing unmeasured spatial variation among sampling locations and host-level variation shared among parasite taxa within the same individual [49,73,74]. We implemented the joint species distribution modeling using HMSC, a Bayesian framework implemented in the *Hmsc* package in R [49,50]. Parasite infection data were organized as a binary presence–absence matrix ($\mathbf{Y}$) and modeled using a probit link that relates the infection probability to a latent linear predictor that includes fixed effects, parasite traits, hierarchical random effects, and latent factors capturing residual species associations. We used the default shrinkage priors implemented in *Hmsc*, which are recommended for standard applications of the framework, to regularize species-specific responses and reduce overfitting.

The fixed-effect component ($\mathbf{X}$) comprised the host species, individual host attributes (body size, body condition, and sex), microbiome structure (diversity, uniqueness, and the FB ratio), and environmental conditions (distance to the nearest village as a land-use proxy, and season). Because the two focal host species may respond differently to anthropogenic disturbance, we also included an interaction between host species and land-use. Each parasite taxon was assigned a taxon-specific regression coefficient vector ($\boldsymbol{\beta}$) describing its response to these predictors, including the host species × land-use interaction. These taxon-specific responses capture how individual parasite taxa respond to variation in host, microbiome, and environmental conditions [50].

In addition, we incorporated a parasite trait matrix ($\mathbf{Tr}$) that classified each taxon by its taxonomic group (nematode or protozoan). In HMSC, this matrix models the taxon-specific responses ($\boldsymbol{\beta}$) as a function of parasite traits, thereby linking responses to parasite biology [49].

Residual associations among parasite taxa were estimated from the individual-level latent factors

as the taxon-by-taxon correlation matrix, interpreted as co-occurrence beyond that explained by the shared predictors.

Models were fitted using Markov Chain Monte Carlo (MCMC) sampling with four independent chains, each run for 350,000 iterations, with the first 100,000 iterations discarded as burn-in. Posterior samples were thinned every 250 iterations to reduce autocorrelation, resulting in 1,000 retained samples per chain and a total of 4,000 posterior samples across all chains. Convergence was evaluated using the Potential Scale Reduction Factors diagnostic (Gelman–Rubin $\hat{R}$) and by visual inspection of trace plots.

Model performance was assessed using Tjur's coefficient of discrimination (Tjur's R^2), a metric appropriate for binary response models that quantifies how well predicted infection probabilities discriminate between observed presences and absences [75]. Tjur's R^2 ranges from 0 to 1, with higher values indicating the model's greater ability to distinguish infected from uninfected host individuals. We report both fitted and five-fold cross-validated (CV) Tjur's R^2. The fitted value reflects in-sample explanatory power, whereas the cross-validated value evaluates predictive performance on held-out data and provides a more conservative assessment of model generalization, helping to detect potential overfitting.

Quantifying parasite host association

We used each parasite's host-species coefficient in the joint model as a continuous measure of its host association (**Figure S7**). Positive values indicate a higher probability of occurrence in *R. rattus* and negative values in *M. brevicaudata*, with the magnitude reflecting the strength of the association. Because the coefficient is a partial effect, conditional on the environmental, individual-level, and microbiome predictors, as well as the spatial and individual random effects, it isolates host association from the conditions under which each host was sampled—which simpler measures, such as a difference in raw prevalence, cannot do. We use this measure to describe parasites and to consistently order them across the results.

Variance partitioning across parasites

To evaluate the relative importance of model components for each parasite taxon (**Figure 1B**), we performed variance partitioning using the built-in function in the *Hmsc* package, and summarized the contribution of each predictor and random factor to the explained variance. We then aggregated these estimates across parasite taxa. Because predictive performance varied among parasites, we weighted variance fractions by cross-validated (CV) model performance, giving greater influence to taxa whose occurrence was predicted more reliably while limiting the dominance of highly predictable taxa. Specifically, for parasite i, weights were defined as

$$w_i = \frac{\sqrt{R^2_{\mathrm{Tjur},i}}}{\sum_j \sqrt{R^2_{\mathrm{Tjur},j}}} \tag{1}$$

so parasite weights are relative and sum to one. The weighted contribution of predictor k was then computed as $\sum_i w_i \times V_{ik}$, where V_{ik} denotes the variance fraction explained by predictor k for parasite i. This approach provides a balanced summary of the relative importance of the

predictors while accounting for differences in model predictive performance across parasites.

Predictor-level contributions were subsequently aggregated into six broader categories: host species, individual host attributes, microbiome, environment, the host species × land-use interaction, and the individual random effect (**Table S1**).

Taxonomic structuring of parasite responses

The disease pyramid frames parasite occurrence as shaped not only by host-related and environmental factors, but also by the parasites themselves, whose intrinsic biology can determine how they respond to those factors. To capture this parasite component of the pyramid, we tested whether parasite responses to the predictors are structured by broad taxonomic group (nematode or protozoan). Because detailed trait data were not available for all parasites, we used taxonomic group as a proxy for underlying parasite traits. Taxonomic group was included in the HMSC species-trait matrix (**Tr**), allowing us to test whether regression coefficients differed systematically between nematodes and protozoa (**Figure 1C**).

This yields three complementary quantities that move from the general to the specific. We first asked how much the broad taxonomic group contributes to parasite occurrence overall, quantified as the proportion of variance in a parasite's occurrence attributable to its group, averaged across parasite taxa. Because this single value does not identify the drivers through which the group acts, we then examined the responses themselves. For each predictor, we calculated the proportion of the between-parasite variation in the response to that predictor explained by taxonomic group. A high value identifies a driver to which nematodes and protozoa respond in consistently different ways, and a low value identifies a driver to which parasites differ irrespective of group. These proportions indicate which drivers the two groups differ on, but not in which direction. We therefore examined the trait coefficients (γ), which give the difference in mean response between nematodes and protozoa for each predictor (protozoa as the reference). We did not apply a fixed threshold for declaring a difference. Instead, we report each γ with its 90% credible interval and the posterior probability that it differs from zero, so that the evidence for a group difference is treated as graded rather than dichotomous.

Host-specific responses to land-use

To test whether the two focal host species differ in how infection changes along the land-use gradient, we included the host species × land-use interaction in the HMSC model (**Figure 1D**). We examined the interaction coefficients both averaged across all parasite taxa, to test for an overall difference between *R. rattus* and *M. brevicaudata*, and for each taxon individually, to identify which parasites contribute most strongly to this difference. We then used the fitted model to predict how infection changes across the land-use gradient in each host species. For each species, we predicted the probability of occurrence of each parasite taxon along the gradient, indicating whether infection increases or decreases toward the semi-intact forest, and how this differs between the two hosts. We also predicted total parasite richness, calculated as the sum of the predicted occurrence probabilities across taxa. Because the interaction is estimated jointly with all other model terms, it isolates the effect of land-use while accounting for the remaining predictors (e.g., season and body condition) and random effects.

All analyses were conducted in R (v4.4.0) [76]

Results

Parasite occurrence varied substantially among taxa and hosts. Across 333 *Rattus rattus* and 172 *Microgale brevicaudata* individuals, we identified 11 parasite taxa, with mean species richness per host of 2.08 ± 1.18 SD and 2.02 ± 1.08 SD, respectively (**Figure S4**). Parasite prevalence varied substantially across taxa (**Figure S2**). While a subset of parasites was highly prevalent, reaching up to 80% infection rates in their primary host species, the majority were detected at low frequencies ($< 10\%$).

The HMSC model demonstrated robust convergence, with potential scale reduction factors (PSRFs) for all parameters consistently below 1.1 (mean = 1.001 ± 0.003 SD). Evaluation of model performance revealed substantial variation in explanatory and predictive power across the parasite community (**Figure S5**). Fitted Tjur's R^2 values ranged from 0.06 to 0.67 (mean $= 0.34 \pm 0.21$ SD), while cross-validated R^2 values ranged from 0.001 to 0.53 (mean $= 0.23 \pm 0.19$ SD). This variation in predictive power was primarily associated with parasite prevalence and host association (**Figure S6**).

Host species and the environment are the dominant drivers of parasite occurrence

Variance partitioning revealed substantial differences among parasites in the relative importance of the disease-pyramid components (**Figure 2A**). For example, *Hypotrichomonas* and *Eimeria* were predominantly determined by host species, whereas *Entamoeba* was much more strongly structured by land use. Other parasites, such as *Tetratrichomonas* and *Blastocytis*, showed substantial contributions from several levels of the disease pyramid, including environmental, host-level, and microbiome predictors. Across these contrasting parasite-specific patterns, host species was the largest single contributor to variation in occurrence. Its contribution, however, was far from uniform, ranging from 4.4% to 84.9% of the explained variance across parasites.

To summarize the hierarchy of drivers across the community, we averaged each variance component across parasites, weighting each parasite by its cross-validated predictive performance (see *Methods*; **Figure 2B**). Grouping predictors into the broad disease-pyramid categories reinforced this hierarchy (**Figure 2C**): host species alone explained 44.6% of the weighted variance, followed by the environment (land-use and season combined, 26.3%) and the host $\times$ land-use interaction (10.9%), while host attributes (6.2%), the microbiome (3.5%), and the individual random effect (8.5%) played secondary roles. Across the community, parasite occurrence was thus shaped predominantly by the host species and environmental conditions, with only a small contribution from individual attributes and the microbiome.

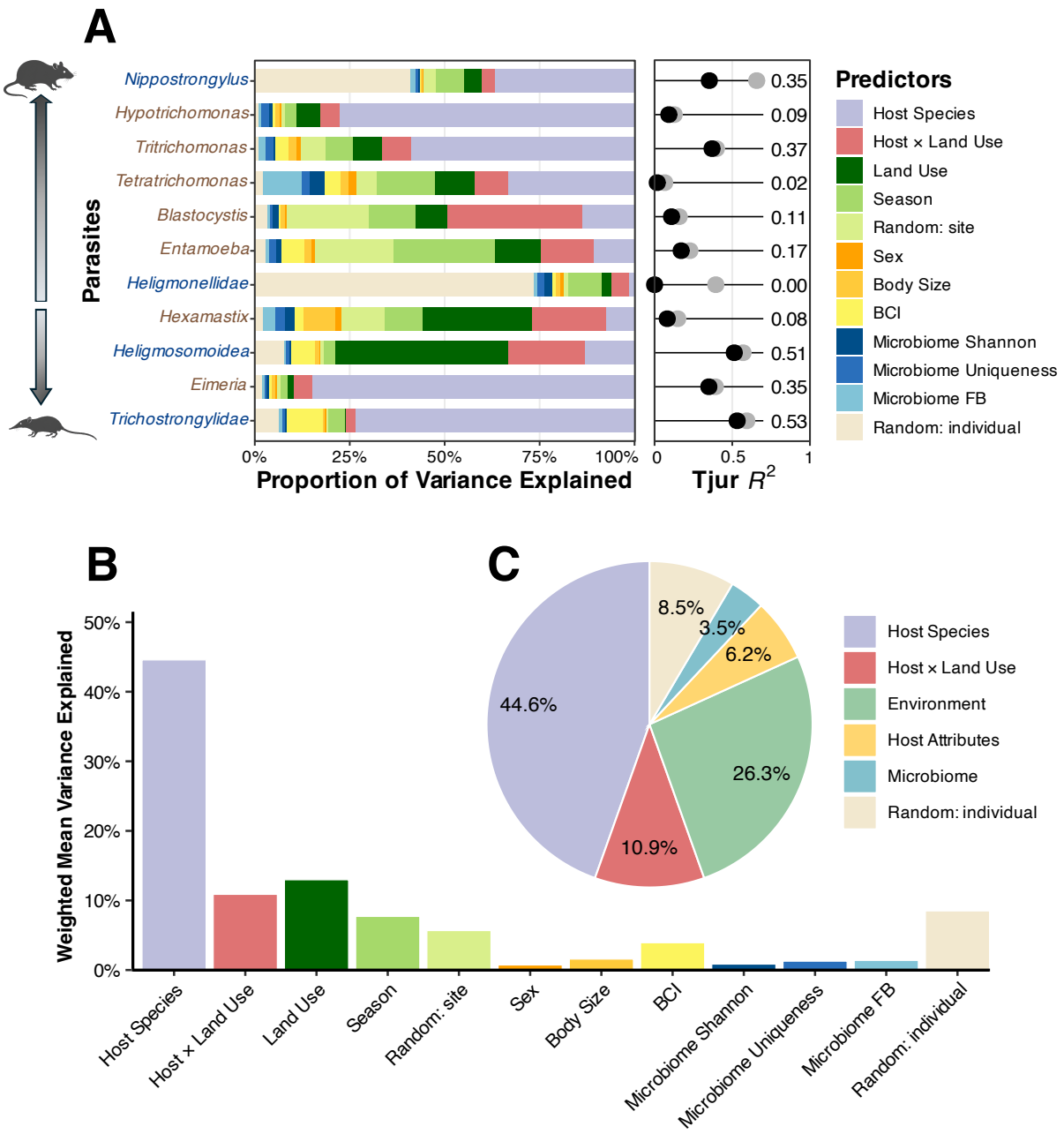

Figure 2: Variance partitioning of drivers of parasite occurrence. (A) Each stacked bar represents the relative contribution of host species, host attributes, environmental predictors, microbial predictors, and their random effects to the total explained variance for a single parasite. To the right of each bar, model performance is shown as the cross-validated Tjur's R^2 (black points) and the fitted Tjur's R^2 (grey points), with the value next to each pair is the cross-validated Tjur's R^2. The distance between the fitted and cross-validated values indicates how well model performance generalizes to unseen data, with larger differences suggesting greater overfitting and lower out-of-sample predictive performance. Parasites are ordered by host association, from those most strongly associated with the non-native *Rattus rattus* at the top to those most strongly associated with the native *Microgale brevicaudata* at the bottom. The intersection of the two arrows indicates a host association of zero (no strong association with either host species). Parasite names are color-coded by taxonomic group: protozoa in brown and nematodes in blue. **(B)** Community-level importance of each predictor, calculated as the mean variance explained across the parasite community, weighted by each parasite's cross-validated predictive performance (CV Tjur's R^2). **(C)** Aggregated variance partitioning across the broad predictor categories. The host species was the most important category, accounting for 44.6% of the total weighted explained variance, followed by the environment (26.3%) and the host × land-use interaction (10.9%).

Taxonomic group structures how parasites respond to environmental and host-related predictors

We next tested whether parasite responses to the environmental, host, and microbial drivers depend on their broad taxonomic group—nematode or protozoan—by including group as a proxy for traits in the joint HMSC model (**Figure 1C**).

Across the community, taxonomic group accounted for 12.1% of the variance in parasite occurrence, a modest but non-negligible contribution averaged over taxa. This value reflects the trait's overall effect on parasite distributions, but does not distinguish among individual drivers. A separate quantity does so by measuring, for each predictor, how much of the variation among parasites in their response to it is attributable to the group. These values varied markedly between predictors (**Figure 3A**). Taxonomic group accounted for the largest share of the between-parasite variation in responses to BCI (27.6%), land-use (24.8%), and the dry season relative to the before-the-wet season (24.1%; season 3), and for less of the variation for the other predictors. Thus, a substantial part of the differences among parasites in how they respond to several drivers is structured by taxonomic group.

These proportions identify the predictors for which the two groups diverge, but not which group responds more strongly, or in which direction. This is given by the trait coefficients (γ), which express the difference in mean response between nematodes and protozoa, with positive values indicating a more positive response in nematodes (**Figure 3B**). The evidence for a group difference was strongest for two predictors. Nematodes responded more positively than protozoa to land-use ($\gamma = 0.49$, 90% CrI [0.05, 0.93]), with 92% posterior probability of being positive, and to the dry season ($\gamma = 0.62$, 90% CrI [0.07, 1.18]; 93% above zero). For the remaining predictors, the posterior distributions were centered near zero, providing weaker evidence of a systematic difference between the groups. Therefore, the positive land-use contrast indicates that, relative to protozoa, nematode occurrence increased toward more forested habitats, away from the village.

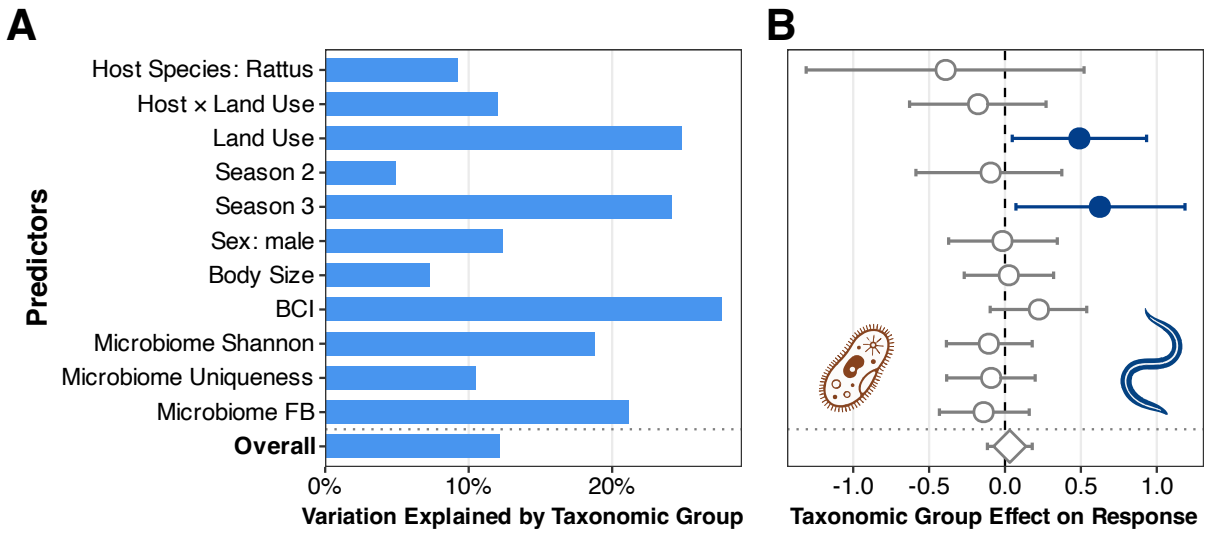

Figure 3: Variation in parasite responses explained by taxonomic group. The trait component of the HMSC model estimates how parasites' broad taxonomic group (nematode or protozoan) structures their responses to each predictor. **(A)** Proportion of the variation among parasites in their response to each predictor that is attributable to taxonomic group. For example, 24.8% of the among-parasite variation in land-use response is attributable to group membership. "Overall" (bottom) is a distinct quantity, the trait's total contribution to explaining parasite occurrence, and is not the average of the per-predictor values above it. **(B)** Trait coefficients (γ), the difference in mean response between nematodes and protozoa. Positive values indicate a more positive response in nematodes, negative values the reverse, and the vertical dashed line marks no difference. Points are posterior means, and horizontal bars are 90% credible intervals. Filled points denote predictors for which the posterior provides clearer evidence of a group difference, with at least 90% of the posterior probability on one side of zero; open points denote predictors for which the evidence was weaker. The diamond (bottom) shows the community-wide mean contrast between the two parasite groups. For example, $\gamma = 0.49$ for land use indicates that nematode occurrence increases more steeply toward forested habitat than protozoan occurrence.

A second signature of the taxonomic group appeared in the way parasites co-occurred within hosts, after accounting for the measured predictors (**Figure S10**). Residual associations were markedly stronger among nematode pairs than among protozoan pairs (difference in mean association = 0.93, 90% CrI [0.60, 1.14]; $P > 0.99$) or than between the two groups (0.71, 90% CrI [0.32, 1.11]; $P > 0.99$). Nematodes therefore co-occur within hosts more often than expected from the measured drivers alone, which may reflect shared transmission routes, common within-host conditions, or direct facilitation among taxa.

Native and non-native parasite communities respond oppositely to the land-use gradient

A central aim of our study was to compare how parasite communities in native and non-native hosts respond to the land-use gradient. Our variance partitioning analysis reinforced the relevance of this question, as host species and land-use were the two dominant drivers shaping host-parasite associations, and their interaction accounted for a distinct component of the explained variation. We therefore examined this interaction directly.

At the community level, the host × land-use interaction was negative ($\beta = -0.48$, 90% CrI [-0.67, -0.30]), with a posterior probability of being negative of $P > 0.99$, indicating that

the two host communities responded to the gradient in opposite directions (**Figure 4A**). In *R. rattus*, community-level occurrence decreased with distance from the village (slope = −0.29, 90% CrI [−0.41, −0.18], $P > 0.99$). In *M. brevicaudata*, occurrence tended in the opposite direction, increasing toward more forested habitat (slope = 0.19, 90% CrI [0.03, 0.36], 94% posterior probability of being positive), though the evidence for this response was weaker than in *R. rattus*. Per-parasite interaction terms were consistently negative but varied in magnitude (**Figure S8**), suggesting that the community-level pattern was largely driven by a subset of taxa.

To determine which parasites drove this contrast, we examined the predicted occurrence of each taxon along the land-use gradient in each host (**Figure 4A**). In *R. rattus*, the most strongly rat-associated parasite (*Nippostrongylus*) remained nearly constant across the gradient, while two others (*Tritrichomonas* and *Blastocystis*) declined sharply from the village toward forested habitat, and several more declined modestly, together producing the overall decline. In *M. brevicaudata*, the two most strongly *Microgale*-associated parasites (*Trichostrongylidae* and *Eimeria*) were nearly constant, while the next (*Heligmosomoidea*) increased sharply toward forested habitat; one rat-associated parasite (*Nippostrongylus*) also increased sharply, and several others increased modestly, generating the overall increase. Latent-scale responses confirmed these patterns and were not distorted by ceiling effects in high-prevalence taxa (**Figure S9**).

These taxa-specific parasite responses scaled up to whole-community parasite richness (**Figure 4B**). Predicted richness in *R. rattus* was highest near the village and declined toward forested habitat, whereas richness in *M. brevicaudata* showed the reverse, increasing away from the village—so that the two hosts harbored their most diverse parasite communities at opposite ends of the land-use gradient.

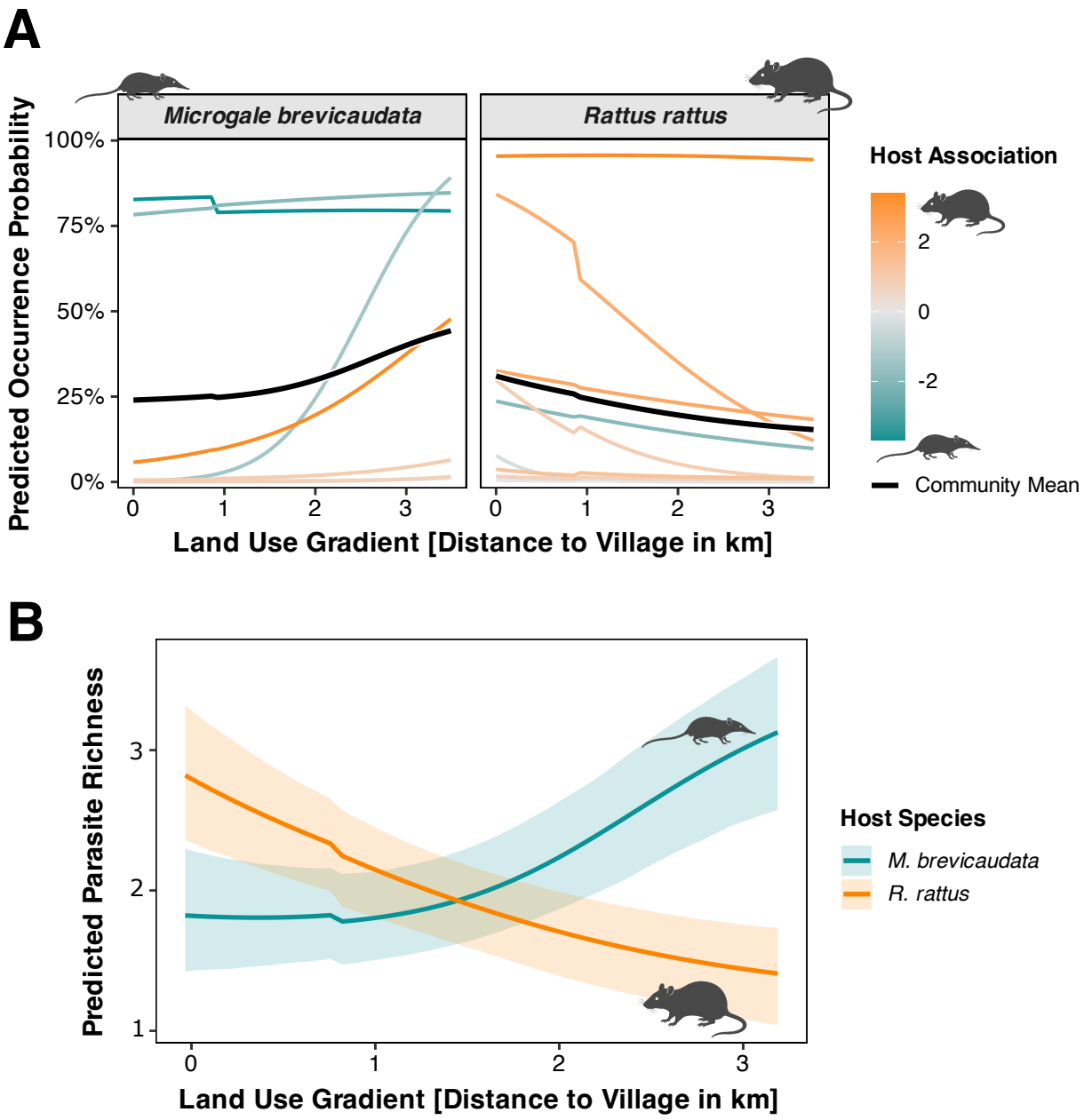

Figure 4: Predicted parasite occurrence and richness along the land-use gradient in each host. Predictions are derived from the HMSC model along the land-use gradient (distance to village in km; higher values on the x-axis indicate more forested habitat, farther from the village). **(A)** Predicted occurrence probability of each parasite taxon along the gradient, shown separately for the native *Microgale brevicaudata* (left) and non-native *Rattus rattus* (right). Each line is one parasite taxa, colored by its host association—the parasite's tendency to occur in one host over the other, taken from the host-species coefficients of the model (teal, associated with *M. brevicaudata*; orange, associated with *R. rattus*). The black line represents the community mean across parasites. To reduce clutter, in each panel, only parasites present in at least 5 individuals of the corresponding host are shown. **(B)** Predicted parasite richness (summed occurrence across taxa) along the land-use gradient for each host species, with shaded bands showing 95% credible intervals. Richness in *R. rattus* was predicted to be highest near the village and declined toward forested habitat, whereas richness in *M. brevicaudata* showed the opposite pattern.

Discussion

Anthropogenic land-use change can reorganize host–parasite systems by altering host communities, creating new interfaces between native and non-native species, and reshaping pathways of

parasite transmission and infection [2,3,9]. However, predicting these changes remains difficult, as parasite occurrence is shaped by multiple drivers that operate across biological levels and, in most studies, are examined separately [29]. We addressed this gap through the lens of the disease pyramid framework to decompose the drivers of protozoan and nematode parasite occurrence in a native, Malagasy-endemic shrew tenrec, *Microgale brevicaudata*, and a globally important non-native and invasive rodent, *Rattus rattus*, along a land-use gradient in northeastern Madagascar. By modeling multiple drivers within a single framework, we quantified their relative importance and found that host species was the dominant axis of parasite occurrence, followed by environmental conditions, though this varied among parasites and taxonomic groups. Notably, the two host species showed contrasting responses to land-use, with their parasite communities shifting in opposite directions along the land-use gradient.

The explanatory power of host species was not uniform across the community. The share of variance it accounted for varied several-fold among parasites, with environmental and individual-level drivers explaining more of the remaining variation. This pattern is consistent with the view that host specificity reflects compatibility filters that vary in strength among parasites [27,28]. Accordingly, host association is better viewed as a continuous property of each parasite rather than as a binary attribute. This distinction is particularly relevant in invaded systems, where parasites are often classified into discrete categories according to their origin or invasion history, such as native, acquired, or co-introduced parasites [77]. Although useful for describing invasion history, these categories do not capture the strength of current host association. A parasite found almost exclusively in one host differs ecologically from one commonly shared between hosts, particularly in its potential for cross-host transmission [78,79].

Quantifying host association as a continuous measure allowed us to identify which parasites generated the contrasting responses of the two hosts to land-use. We had expected *R. rattus*-associated parasites to increase toward the village and *M. brevicaudata*-associated parasites to be structured primarily by host species, but these expectations were only partly supported. Responses were instead strongly parasite-specific: parasites with comparable host associations differed substantially in how their occurrence changed along the gradient, from taxa that tracked it closely to taxa that changed little. How strongly a parasite is tied to a host, therefore, says little about how it will respond to land-use.

Despite this variation among parasites, a consistent pattern emerged at the level of the hosts: occurrence tended to shift toward the village when a parasite infected *R. rattus* and toward the forest when it infected *M. brevicaudata*, so that each host harbored its richest community where the other harbored its poorest. Some divergence between the two communities is unsurprising, given the phylogenetic distance between Tenrecidae and Muridae. However, the hosts' evolutionary differences do not, by themselves, explain the opposing direction of change along the land-use gradient. This contrast is better explained by their ecology. *R. rattus* is a generalist tolerant of human disturbance [53] that reaches its highest densities across human-modified habitats, where anthropogenic food and shelter support large, often dense populations that share contaminated substrates, elevating both contact rates and environmental transmission. *M. brevicaudata*, in contrast, is a predominantly forest-associated insectivore [80] that occurs at lower densities throughout the gradient and encounters parasites largely through a soil- and

litter-associated diet rather than through crowding. Its richer parasite community in forested habitats may therefore reflect a long-standing local association with parasites of native habitats rather than density-driven transmission. Each host thus reached its highest predicted parasite richness where it is ecologically most successful.

These infection patterns may also have implications for human exposure to zoonotic parasites. Because *R. rattus* is a recognized reservoir of zoonotic pathogens in this region [57,58], its greater parasite richness in disturbed habitats, where rats, people, and domestic animals are in closest contact, may increase opportunities for transmission to humans. In contrast, parasites associated with the forest-associated *M. brevicaudata* are concentrated away from villages, although regular human activity in forested habitats may still create opportunities for exposure.

The components of the disease pyramid considered so far describe the host (host species, individual traits, and microbiome) and its environment, but the pyramid also includes the parasite itself, and parasites that differ in biology need not respond in the same way to the same drivers. We examined this using a broad taxonomic group, nematode or protozoan, as a proxy for a set of correlated traits such as transmission mode and infection duration. Taxonomic group accounted for 12.1% of the variance in parasite occurrence and an appreciable share of the between-parasite variation in drivers' responses. The clearest group differences, in the responses to land-use and season, were consistent in direction, but group membership explained only part of the variation among parasites: both groups included taxa that responded steeply to land-use and others that changed little along the gradient. This is consistent with the biological diversity within each group and the absence of a single transmission strategy in either group. Among protozoa, some taxa produce environmentally resistant cysts or oocysts (e.g., *Entamoeba*, *Blastocystis*, *Eimeria*) while others, such as the trichomonad flagellates (e.g., *Tritrichomonas*, *Tetratrichomonas*), survive poorly outside the host [51]. Nematodes are similarly varied. They are transmitted as free-living larvae that are sensitive to moisture and temperature, which could closely link their occurrence to environmental conditions. Many also cause chronic infections that persist long after exposure, so that presence at capture may instead reflect accumulated susceptibility and exposure history rather than conditions at the time of sampling [52]. The two groups are also delimited differently, as protozoan units are genera whereas nematode units are single sequence clusters (OTUs), so part of the difference between them may reflect how the units were defined rather than parasite biology.

A second, complementary pattern appeared in the way parasites co-occurred within hosts. After accounting for the measured predictors, well-supported positive associations were concentrated almost entirely among nematode pairs, and nematodes also showed a relatively high contribution of the individual random effect [49]. These patterns suggest that variation in nematode occurrence arises partly at the level of the individual host, beyond what the measured predictors capture. Such variation could arise from the parasites themselves, if chronic infections accumulate over a host's lifetime or if establishment by one nematode facilitates the establishment of others [81]. Alternatively, differences could arise from unmeasured variation in exposure and susceptibility among individual hosts. Longitudinal or experimental data would be needed to distinguish among these possibilities.

By integrating multiple drivers across host and parasite scales, this study advances our under-

standing of how parasite occurrence is structured among native and non-native hosts. Several limitations should nonetheless be considered. First, fecal metabarcoding detects parasite DNA rather than evidence of established infection and does not quantify infection intensity, so our results reflect occurrence rather than burden [82,83]. Whether burden shifts along the land-use gradient in the same way remains to be tested. Second, the endoparasites of Malagasy small mammals remain poorly documented [84], and our data inherit that gap. Most taxa could not be identified to species, their life cycles and transmission modes are inferred rather than observed, and their origins are unknown, so we cannot establish whether a given taxon is long-resident in Madagascar or arrived with *R. rattus*. With that knowledge, parasite traits could be included directly rather than approximated by taxonomic group. Third, several potentially relevant variables were not measured, including host immune function, diet, movement, social contacts, microbiome functionality, host genetics, and fine-scale microhabitat conditions [85–87]. Model performance was also moderate for several parasite taxa, so some variation in occurrence remains unexplained. That variation is itself informative: it points to the parasites for which important drivers remain to be measured. Finally, although *R. rattus* and *M. brevicaudata* are the most abundant host species in this system and provide a strong comparison between coexisting non-native and native hosts, our inference is based on two host species. Testing whether these patterns generalize will require studies that include more host species across additional communities, regions, and disturbance contexts.

In summary, we brought the full disease pyramid into a single analytical framework to test how it shapes host-parasite interactions in a native and a non-native host along a land-use gradient. This integrated approach revealed that host species was the dominant predictor of host-parasite interactions, followed by the environment. However, the relative importance of these drivers varied markedly among parasites. Most strikingly, the two host species responded to land-use in opposite directions, so that disturbance redistributed parasites between the native and non-native hosts rather than uniformly increasing infection across the community. This perspective is particularly important as land-use change brings native and non-native species into new contact and reshapes the opportunities for parasite transmission between them. More generally, anticipating disease dynamics in changing landscapes will require moving beyond calculating mean community-level responses to identify the conditions under which different parasites persist, spread, or shift between native and non-native hosts, including the pathways that may increase the risk of transmission to humans.

Acknowledgments

We are grateful to the members of the Madagascar Health team for their support and insightful feedback throughout this work. We also thank the Mention Zoologie et Biodiversité Animale, Université d'Antananarivo, Madagascar National Parks, and the Direction des Aires Protégées, des Ressources Naturelles Renouvelables et des Ecosystèmes for administrative assistance and research permits. We thank Jacquis A. Tahinarivony for botanical expertise during the vegetation surveys. We especially thank the Duke Lemur Center–SAVA Conservation office for logistical support. Finally, we warmly acknowledge the local communities, authorities, and the many field assistants from the study villages for their welcome and help in the field.

Funding

This work was supported by the Israel Science Foundation (grants 1281/20 and 491/25 to SP), the U.S. National Science Foundation (grant DEB 2308460 to GT and CLN), the U.S.-Israel Binational Science Foundation (grant 2022721 to SP), the NIH-NSF-NIFA Ecology and Evolution of Infectious Diseases program (award R01-TW011493 to CLN), and the Human Frontier Science Program (grant RGY0064/2022 to SP).

Author contributions

Conceptualization: MM, SP; **Formal analysis:** MM; **Project administration:** TMR, VS, SMG, CLN, GT, SP; **Resources:** TMR, VS, SMG, GT; **Visualization:** MM; **Writing – original draft:** MM, CLN, GT, SP; **Writing – review & editing:** MM, TMR, VS, SMG, CLN, GT, SP.

Competing interests

The authors declare that they have no competing interests.

Data and code availability

Data and code are also available on the GitHub repository https://github.com/MadagascarEEID/parasite_disease_pyramid.

References

1. Newbold, T. *et al.* Global effects of land use on local terrestrial biodiversity. *Nature* **520,** 45–50 (2015).
2. Gibb, R. *et al.* Zoonotic host diversity increases in human-dominated ecosystems. *Nature* **584,** 398–402 (2020).
3. Budria, A. & Candolin, U. How does human-induced environmental change influence host-parasite interactions? *Parasitology* **141,** 462–474 (2014).
4. Kołodziej-Sobocińska, M. Factors affecting the spread of parasites in populations of wild European terrestrial mammals. *Mamm. Res.* **64,** 301–318 (2019).
5. Herrera, J. P. *et al.* Effects of land use, habitat characteristics, and small mammal community composition on Leptospira prevalence in northeast Madagascar. *PLoS Negl. Trop. Dis.* **14,** e0008946 (2020).
6. Sánchez-Ortiz, K. *et al.* Effects of land-use change and related pressures on alien and native subsets of island communities. *PLoS One* **15,** e0227169 (2020).
7. Liu, D. *et al.* The impact of land use on non-native species incidence and number in local assemblages worldwide. *Nat. Commun.* **14,** 2090 (2023).
8. Young, H. S., Parker, I. M., Gilbert, G. S., Sofia Guerra, A. & Nunn, C. L. Introduced Species, Disease Ecology, and Biodiversity–Disease Relationships. *Trends Ecol. Evol.* **32,** 41–54 (2017).
9. Telfer, S. & Bown, K. The effects of invasion on parasite dynamics and communities. *Funct. Ecol.* **26,** 1288–1299 (2012).

10. Sweeny, A. R., Albery, G. F., Venkatesan, S., Fenton, A. & Pedersen, A. B. Spatiotemporal variation in drivers of parasitism in a wild wood mouse population. *Funct. Ecol.* **35,** 1277–1287 (2021).

11. Tompkins, D. M., Dunn, A. M., Smith, M. J. & Telfer, S. Wildlife diseases: from individuals to ecosystems. *J. Anim. Ecol.* **80,** 19–38 (2011).

12. Pedersen, A. B. & Fenton, A. Emphasizing the ecology in parasite community ecology. *Trends Ecol. Evol.* **22,** 133–139 (2007).

13. Poulin, R. Are there general laws in parasite ecology? *Parasitology* **134,** 763–776 (2007).

14. Rohr, J. R. *et al.* Frontiers in climate change-disease research. *Trends Ecol. Evol.* **26,** 270–277 (2011).

15. Gottdenker, N. L., Streicker, D. G., Faust, C. L. & Carroll, C. R. Anthropogenic land use change and infectious diseases: a review of the evidence. *Ecohealth* **11,** 619–632 (2014).

16. Cardoso, T. D. S., Andreazzi, C. S. d., Maldonado Junior, A. & Gentile, R. Functional traits shape small mammal-helminth network: patterns and processes in species interactions. *Parasitology* **148,** 947–955 (2021).

17. Dallas, T. A. *et al.* Host traits associated with species roles in parasite sharing networks. *Oikos* **128,** 23–32 (2019).

18. Dáttilo, W. *et al.* Species-level drivers of mammalian ectoparasite faunas. *J. Anim. Ecol.* **89,** 1754–1765 (2020).

19. Grear, D. A., Perkins, S. E. & Hudson, P. J. Does elevated testosterone result in increased exposure and transmission of parasites? *Ecol. Lett.* **12,** 528–537 (2009).

20. Sánchez, C. A. *et al.* On the relationship between body condition and parasite infection in wildlife: a review and meta-analysis. *Ecol. Lett.* **21,** 1869–1884 (2018).

21. Sallinen, S., Norberg, A., Susi, H. & Laine, A.-L. Intraspecific host variation plays a key role in virus community assembly. *Nat. Commun.* **11,** 5610 (2020).

22. Ezenwa, V. O. & Jolles, A. E. From host immunity to pathogen invasion: the effects of helminth coinfection on the dynamics of microparasites. *Integr. Comp. Biol.* **51,** 540–551 (2011).

23. Keegan, S. P., Pedersen, A. B. & Fenton, A. The impact of within-host coinfection interactions on between-host parasite transmission dynamics varies with spatial scale. *Proc. Biol. Sci.* **291,** 20240103 (2024).

24. Leung, J. M., Graham, A. L. & Knowles, S. C. L. Parasite-Microbiota Interactions With the Vertebrate Gut: Synthesis Through an Ecological Lens. *Front. Microbiol.* **9,** 843 (2018).

25. Rosshart, S. P. *et al.* Wild mouse gut Microbiota promotes host fitness and improves disease resistance. *Cell* **171,** 1015–1028.e13 (2017).

26. Erazo, D., Sweeny, A. R., Pedersen, A. B. & Fenton, A. Parasite responses to resource provisioning can be altered by within-host co-infection interactions. *Proc. Biol. Sci.* **292,** 20251840 (2025).

27. Poulin, R., Krasnov, B. R. & Mouillot, D. Host specificity in phylogenetic and geographic space. *Trends Parasitol.* **27,** 355–361 (2011).

28. Park, A. W. *et al.* Characterizing the phylogenetic specialism-generalism spectrum of mammal parasites. *Proc. Biol. Sci.* **285** (2018).

29. Bernardo-Cravo, A. P., Schmeller, D. S., Chatzinotas, A., Vredenburg, V. T. & Loyau, A. Environmental factors and host microbiomes shape host-pathogen dynamics. *Trends Parasitol.* **36,** 616–633 (2020).

30. Vega Thurber, R. *et al.* Deciphering Coral Disease Dynamics: Integrating Host, Microbiome, and the Changing Environment. *Frontiers in Ecology and Evolution* **8** (2020).

31. Scholthof, K.-B. G. The disease triangle: pathogens, the environment and society. *Nat. Rev. Microbiol.* **5,** 152–156 (2007).

32. Markfeld, M. *et al.* Host traits and environmental factors shape infection heterogeneity in wild rat–protozoa networks. *ISME Commun.* **6,** ycag026 (2026).

33. Becker, C. G., Longo, A. V., Haddad, C. F. B. & Zamudio, K. R. Land cover and forest connectivity alter the interactions among host, pathogen and skin microbiome. *Proc. Biol. Sci.* **284** (2017).

34. Teles, W. S. *et al.* Host diversity mediates the influence of landscape structure on parasite communities in Cerrado agricultural landscapes. *Ann. N. Y. Acad. Sci.* **1545,** 105–115 (2025).

35. Markfeld, M. *et al.* Differential assembly of core and non-core host-microbe network structures along a land-use change gradient. *Ecol. Lett.* **28,** e70255 (2025).

36. Johnson, P. T. J., de Roode, J. C. & Fenton, A. Why infectious disease research needs community ecology. *Science* **349,** 1259504 (2015).

37. Bordes, F. *et al.* Habitat fragmentation alters the properties of a host–parasite network: rodents and their helminths in South-East Asia. *J. Anim. Ecol.* **84,** 1253–1263 (2015).

38. Beldomenico, P. M. & Begon, M. Disease spread, susceptibility and infection intensity: vicious circles? *Trends Ecol. Evol.* **25,** 21–27 (2010).

39. Schmid, D. W. *et al.* A framework for testing the impact of co-infections on host gut microbiomes. *Anim Microbiome* **4,** 48 (2022).

40. Runghen, R., Poulin, R., Monlleó-Borrull, C. & Llopis-Belenguer, C. Network analysis: Ten years shining light of host-parasite interactions. *Trends Parasitol.* **37,** 445–455 (2021).

41. Dallas, T. A. & Jordano, P. Spatial variation in species' roles in host-helminth networks. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **376,** 20200361 (2021).

42. Wu, Q. *et al.* Scaling up to understand disease risk: distinct roles of host functional traits in shaping infection risk of avian malaria across different scales. *Proc. Biol. Sci.* **292,** 20242175 (2025).

43. Pilosof, S., Morand, S., Krasnov, B. R. & Nunn, C. L. Potential parasite transmission in multi-host networks based on parasite sharing. *PLoS One* **10,** e0117909 (2015).

44. Spencer, L. A. & Irwin, M. T. Cryptosporidium and Giardia prevalence amongst lemurs, humans, domestic animals and black rats in Tsinjoarivo, Madagascar. *Heliyon* **6,** e05604 (2020).

45. Dubrulle, J. *et al.* Effect of land-use on Hantavirus infection among introduced and endemic small mammals of Madagascar. *Ecol. Evol.* **15,** e70914 (2025).

46. Keesing, F. & Ostfeld, R. S. Emerging patterns in rodent-borne zoonotic diseases. *Science* **385,** 1305–1310 (2024).

47. Hunter-Barnett, S. & Viney, M. Gut protozoa of wild rodents - a meta-analysis. *Parasitology* **151,** 594–605 (2024).

48. Rajaonarivelo, J. A. *et al.* Leptospira prevalence and lineages vary across land-use types due to shifts in small mammal communities. *Appl. Environ. Microbiol.* **92,** e0206125 (2026).

49. Ovaskainen, O. *et al.* How to make more out of community data? A conceptual framework and its implementation as models and software. *Ecol. Lett.* **20,** 561–576 (2017).

50. Tikhonov, G. *et al.* Joint species distribution modelling with the r-package Hmsc. *Methods Ecol. Evol.* **11,** 442–447 (2020).

51. Wang, X., Wang, X. & Cao, J. Environmental factors associated with Cryptosporidium and Giardia. *Pathogens* **12,** 420 (2023).

52. Reynolds, L. A., Filbey, K. J. & Maizels, R. M. Immunity to the model intestinal helminth parasite Heligmosomoides polygyrus. *Semin. Immunopathol.* **34,** 829–846 (2012).

53. Dammhahn, M., Randriamoria, T. M. & Goodman, S. M. Broad and flexible stable isotope niches in invasive non-native Rattus spp. in anthropogenic and natural habitats of central eastern Madagascar. *BMC Ecol.* **17,** 16 (2017).

54. Penczykowski, R. M., Laine, A.-L. & Koskella, B. Understanding the ecology and evolution of host-parasite interactions across scales. *Evol. Appl.* **9,** 37–52 (2016).

55. Brouat, C *et al.* Invasion genetics of a human commensal rodent: the black rat Rattus rattus in Madagascar. *Mol. Ecol.* **23,** 4153–4167 (2014).

56. Scobie, K. *et al.* Reproductive ecology of the black rat (Rattus rattus) in Madagascar: the influence of density-dependent and -independent effects. *Integr. Zool.* **19,** 66–86 (2024).

57. Randriamoria, T. M., Ramasindrazana, B, Soarimalala, V & Goodman, S. M. in *The new natural history of Madagascar* (ed Goodman, S. M.) 2006–2008 (Princeton University Press, Princeton, NJ, 2014).

58. Rahelinirina, S. *et al.* First isolation and direct evidence for the existence of large small-mammal reservoirs of Leptospira sp. in Madagascar. *PLoS One* **5,** e14111 (2010).

59. Soarimalala, V, Rasolobera, F & Goodman, S. M. Diversité et distribution des petits mammifères terrestres dans le Parc National de Marojejy, Nord-est de Madagascar: Gradient altitudinal et variation temporelle. *A floral and faunal inventory of the Parc National de Marojejy: altitudinal gradient and temporal variation. Malag. Nat* **17,** 211–228 (2023).

60. Rasolobera, F, Randriamoria, T. M., Soarimalala, V & Goodman, S. Ecologie de la reproduction de Microgale brevicaudata (Tenrecidae) dans le Nord-Est de Madagascar. *Bull Soc Zool Fr* **147,** 143–151 (2022).

61. Gogarten, J. F. *et al.* Metabarcoding of eukaryotic parasite communities describes diverse parasite assemblages spanning the primate phylogeny. *Mol. Ecol. Resour.* **20,** 204–215 (2020).

62. Krogsgaard, L. R. *et al.* Characteristics of the bacterial microbiome in association with common intestinal parasites in irritable bowel syndrome. *Clin. Transl. Gastroenterol.* **9,** 161 (2018).

63. Gasser, R. B., Chilton, N. B., Hoste, H & Beveridge, I. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Res.* **21,** 2525–2526 (1993).

64. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* **17,** 10–12 (2011).

65. Callahan, B. J. *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13,** 581–583 (2016).

66. Murali, A., Bhargava, A. & Wright, E. S. IDTAXA: a novel approach for accurate taxonomic classification of microbiome sequences. *Microbiome* **6,** 140 (2018).

67. Frøslev, T. G. *et al.* Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nat. Commun.* **8,** 1188 (2017).

68. Patz, J. A., Graczyk, T. K., Geller, N & Vittor, A. Y. Effects of environmental change on emerging parasitic diseases. *Int. J. Parasitol.* **30,** 1395–1405 (2000).

69. Cornell, S. J., Bjornstad, O. N., Cattadori, I. M., Boag, B. & Hudson, P. J. Seasonality, cohort-dependence and the development of immunity in a natural host-nematode system. *Proc. Biol. Sci.* **275,** 511–518 (2008).

70. Wilmanski, T. *et al.* Gut microbiome pattern reflects healthy ageing and predicts survival in humans. *Nat. Metab.* **3,** 274–286 (2021).

71. Bensch, H. M., Tolf, C., Waldenström, J., Lundin, D. & Zöttl, M. Bacteroidetes to Firmicutes: captivity changes the gut microbiota composition and diversity in a social subterranean rodent. *Anim. Microbiome* **5,** 9 (2023).

72. Hair, J. F. & Hair, J. F. *Multivariate Data Analysis* (Prentice Hall, 2010).

73. Warton, D. I. *et al.* So many variables: Joint modeling in community ecology. *Trends Ecol. Evol.* **30,** 766–779 (2015).

74. Pollock, L. J. *et al.* Understanding co-occurrence by modelling species simultaneously with a Joint Species Distribution Model (JSDM). *Methods Ecol. Evol.* **5,** 397–406 (2014).

75. Tjur, T. Coefficients of determination in logistic regression models—A new proposal: The coefficient of discrimination. *Am. Stat.* **63,** 366–372 (2009).

76. R Core Team. *R: A Language and Environment for Statistical Computing* Vienna, Austria, 2024.

77. Thakur, M. P., van der Putten, W. H., Cobben, M. M. P., van Kleunen, M. & Geisen, S. Microbial invasions in terrestrial ecosystems. *Nat. Rev. Microbiol.* **17,** 621–631 (2019).

78. Chalkowski, K., Lepczyk, C. A. & Zohdy, S. Parasite Ecology of Invasive Species: Conceptual Framework and New Hypotheses. *Trends Parasitol.* **34,** 655–663 (2018).

79. Dunn, A. M. & Hatcher, M. J. Parasites and biological invasions: parallels, interactions, and control. *Trends Parasitol.* **31,** 189–199 (2015).

80. Rasolobera, F., Rajemison, B., Randriamoria, T. M., Soarimalala, V. & Goodman, E. S. M. Diet of Microgale brevicaudata (Tenrecidae) in northeast Madagascar. *Bull. Soc. Zool. Fr.* **146,** 167–173 (2021).

81. Kasal, D. N., Warner, L. M., Bryant, A. S., Tait Wojno, E. & von Moltke, J. Systemic immune modulation by gastrointestinal nematodes. *Annu. Rev. Immunol.* **42,** 259–288 (2024).

82. Miller, M. L., Rota, C. & Welsh, A. Transforming gastrointestinal helminth parasite identification in vertebrate hosts with metabarcoding: a systematic review. *Parasit. Vectors* **17,** 311 (2024).

83. Patch, M. E., Goodman, G. B. & Weinstein, S. B. Scoop that poop: Optimising faecal sample pre-processing for parasite metabarcoding. *Mol. Ecol. Resour.* **26,** e70112 (2026).

84. Li, C.-H. & Poulin, R. Biased availability of genetic data for parasites: it's all about the host. *Int. J. Parasitol.* **55,** 657–663 (2025).

85. Berland, F. *et al.* Effects of host sex, age and behaviour on co-infection patterns in a wild ungulate. *Parasitology* **152,** 1282–1296 (2025).

86. Briard, L. & Ezenwa, V. O. Parasitism and host social behaviour: a meta-analysis of insights derived from social network analysis. *Anim. Behav.* **172,** 171–182 (2021).

87. Oyesola, O. O., Souza, C. O. S. & Loke, P. The influence of genetic and environmental factors and their interactions on immune response to helminth infections. *Front. Immunol.* **13,** 869163 (2022).