Does deforestation beget disease?: Leishmania host and vector communities across a gradient of forest loss on the Amazonian deforestation frontier

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Abstract

There is ongoing debate concerning whether there exists a generalizable effect of land-use change on biodiversity and consequently zoonotic disease risk. Strong data informing this debate is sparse because ecological and sampling complexities make it challenging to establish direct links between vertebrate hosts (and non-hosts), vectors, and pathogens across landscapes. However, emerging molecular methods using invertebrate-derived DNA (iDNA) can now measure species diversity and interactions from vector bloodmeals, which has the potential to improve mechanistic understanding of the effects of land-use change on zoonotic disease risk. Here, we used iDNA metabarcoding of vectors and their bloodmeals to disentangle the complex relationships between Leishmania parasites, known sandfly vectors, and potential wildlife hosts. We collected 56,775 sandflies during 3,159 trap nights at 39 forested sites across the southern Amazon 'Arc of Deforestation', which exemplifies global patterns of deforestation and fragmentation at the borders of tropical forest ecosystems due to agricultural expansion. We found that vector community composition was influenced by forest cover and pasture cover, and the most common vector, Nyssomia spp., was encountered less frequently in forests surrounded by pasture. Sandflies fed on a diversity of vertebrates, but the edge-loving nine-banded armadillo, Dasypus novemcinctus, was overwhelmingly the most prevalent host, followed by the greater long-nosed armadillo, Dasypus kappleri. The probability of a host being detected in sandfly bloodmeals was lower at sites with higher forest cover, which was overwhelmingly due to reduced bloodmeals arising from D. novemcinctus. Armadillos were also the most prevalent sylvatic vertebrate taxon in sandfly pools that were positive for Leishmania, further suggesting that these xenarthrans are a key host pathway for zoonotic disease transmission.

Introduction

Land-use change is hypothesized to be a key driver of the emergence of infectious disease (Foley et al., 2005; Lambin et al., 2010; Norris, 2004; Patz et al., 2000). Deforestation and the expansion of human activity into forests can alter ecological communities and interactions (Aguirrea & Taborb, 2008; Roque & Jansen, 2014) and thus potentially increase the risk of infectious disease emergence from wildlife reservoirs and vectors (Lambin et al., 2010; T. Lima et al., 2017; McCauley et al., 2015; Vanwambeke et al., 2007). However, there is debate on whether there is a generalizable effect of land-use patterns and consequently changes in biodiversity on increased disease risk (Levi et al., 2016; Ostfeld, 2013; Ostfeld & Keesing, 2013; Randolph & Dobson, 2012; Wood et al., 2014; Wood & Lafferty, 2013). The question of whether dilution, amplification, or neutralizing effects predominate for particular infectious diseases (or entire suites of pathogens) across gradients of disturbance and biodiversity is a challenging empirical problem as the mechanisms are rarely

clear and often non-linear. This is particularly the case for vector-borne pathogens, which land-use change can influence by modifying the composition, density, and transmission traits of the hosts, vectors, and pathogens (Burkett-Cadena & Vittor, 2018; Kocher et al., 2022).

Improved mechanistic understanding of how land-use change influences host, vector, and pathogen networks is particularly critical in tropical forests where anthropogenic impacts are rapidly altering landscapes and the burden of disease is disproportionately high. Large-scale deforestation and/or forest fragmentation can influence biodiversity by several mechanisms that likely influence pathogen dynamics and ultimately disease risk. For example, vertebrates at lower trophic levels can become hyperabundant in fragmented forest due to energy-rich subsidies from forest edges and the agricultural matrix (Luskin et al., 2017; Marczak et al., 2007; Wilcox & Gubler, 2005), and due to ecological release when habitat loss and/or fragmentation leads to the decline of larger-bodied competitors and predators (Debinski & Holt, 2000; Nupp & Swihart, 1998; Ripple et al., 2014; Wilmers & Levi, 2013). These mechanisms are supported by field surveys in the southern Amazon showing a strong negative association between the abundance of common reservoir species and forest patch size, and the extirpation of apex predators and other large-bodied taxa in the smallest forest fragments (Michalski & Peres, 2007). However, as the context of tropical deforestation changes from agricultural expansion by many smallholders to large-scale agribusiness monocultures, the impacts of this change on vertebrate community composition may disrupt the patterns of forest loss and/or fragmentation on biodiversity witnessed thus far.

While disturbance-tolerant vertebrate reservoir hosts are likely to play a key role in influencing disease prevalence and emergence as tropical forest systems are fragmented (Johnson et al., 2013), this mechanism alone is not sufficient to predict how disease risk changes as tropical forests are degraded given how the intricate ecological network between hosts, vectors, and pathogens can respond to forest loss in complex ways. For vector-borne pathogens, even if forest edge increases the abundance of reservoir hosts in tropical forests, pathogen reproduction will be stymied if vector populations decline in edge habitats, if there is spatial mismatch between hosts and vectors, or if vectors feed disproportionately on species that are not competent reservoirs. While studies have demonstrated some of the effects of land-use change on host species (Guo et al., 2019; LoGiudice et al., 2003), little work has been done to simultaneously examine the effects of deforestation on vectors, hosts, and pathogens.

To tease apart the effects of large-scale land-use change on host and vector communities, and consequently disease risk, we implemented a multifaceted, landscape epidemiology approach using field surveys and DNA metabarcoding of sandfly vectors and their bloodmeals to disentangle the complex relationships between *Leishmania* parasites, the known sandfly vectors, and the potential wildlife hosts (see Fig. 1) in response to rapid deforestation across the Amazonian 'Arc of Deforestation'. Numerous *Leishmania* species cause cutaneous and visceral leishmaniasis (Akhoundi et al., 2016), neglected tropical diseases that are associated with both intact tropical forest (Jones et al., 1987; Lainson, 1983; Travi et al., 1998) and forest fragments (De Luca et al., 2003; Jones et al., 1987). The natural mammalian hosts are diverse, but small mammals and armadillos (*Dasypus* spp.) are thought to be strongly associated with *Leishmania* transmission (Lainson et al., 1979; Lainson & Shaw, 1989; B. S. Lima et al., 2013; Travi et al., 1994, 1998, 2002). Domesticated species, particularly dogs (*Canis lupus familiaris*), are also important hosts (Courtenay et al., 2002; Gramiccia & Gradoni, 2005; Quinnell & Courtenay, 2009), and may play a key role as conduits of disease transmission if forest fragmentation leads to increased interactions between sylvatic and domesticated species. Given that many *Leishmania* species that cause leishmaniasis in humans are multi-host parasites (Roque & Jansen, 2014), their prevalent transmission pathways in deforested landscapes remain an open question. Kocher et al. (2022) recently found that mammal diversity, which declined with greater human footprint, was correlated with lower reservoir host abundance, lower prevalence of*Leishmania* spp. in sandflies, but high sandfly abundance. The context for this work was the intrusion of small landholders into otherwise vast, continuous tropical forest across 19 forest sites in French Guiana. Here, we use a similar landscape epidemiology approach but, in contrast to Kocher et al. (2022), we sampled a gradient of forest loss and fragmentation that represents the most typical deforestation pattern witnessed across much of the Amazon and other tropical forest ecosystems worldwide, where >70% of all remaining forests is now within 1 km of a forest

edge (Haddad et al., 2015) primarily due to agricultural expansion (Geist & Lambin, 2002). Within this context, we ask how large-scale deforestation influences *Leishmania* hosts, vectors, their interactions, and pathogen prevalence. Specifically, we hypothesized that deforestation would be associated with increased bloodmeals derived from competent hosts that proliferate due to matrix subsidies and relaxed top-down control, and that sandfly vector abundance may increase as a result of higher host density in degraded forests.

Methods

Study area

We conducted this study near Sinop, Mato Grosso, Brazil (11.8608°S, 55.5095°W; Fig. 2) at the southern edge of the Amazon forest biome. This area is largely defined by seasonally dry evergreen tropical forest (NT0140) at the transition zone between the Cerrado scrubland savannah and Amazon biomes (Fig. 2). The climate is classified as neotropical with a fairly consistent mean temperature all year-round (24-25°C), but with great variation in mean precipitation between the dry (mean July rainfall = 2 mm) and the wet season (mean February rainfall = 309 mm) . The study area was nearly completely forested until the 1970s when cattle ranching, logging, and more recently soybean agriculture began fragmenting the once contiguous forest and promoted rapid development of urban areas. This is consistent with patterns of deforestation across the Brazilian Amazon where soybean monoculture is one of the primary drivers of deforestation (Fearnside, 2017; Nepstad et al., 2006). Thus, a mosaic of primarily soybean agriculture (that is typically intercropped with maize), exotic pastures, and forest fragments have replaced primary forest.

Fieldwork and sandfly collection

Fieldwork took place across 39 forest sites across a deforestation gradient (Fig. 2) and was conducted primarily during the wet-to-dry transition and dry seasons (April to August) of 2015 and 2016. At each site, we established three parallel transects 50 m apart, each with nine UV LED CDC light traps (BioQuip; Catalog Number: 2770) set 30 m apart amounting to 27 traps per site to capture sandflies. Trapping grids were selected to be at least 3 km apart. We ran all traps for four days and three nights. We collected insects after each 24-hour period and replaced each collection pot with a sterile collection pot. The collection pots from the previous 24 hours were immediately placed into a portable refrigerator in which cold temperatures immobilized the insects. At the end of each day, insect collections were transferred to a -20°C freezer at UFMT lab facilities. At the completion of work at each individual site, sandflies were separated from other insects and stored into 2 ml Eppendorf tubes and labeled by site and date. These collections were placed in a -80°C freezer until they were shipped using dry ice to our home laboratory at Oregon State University where they were once again frozen at -80°C until molecular processing.

Molecular analysis

We sorted sand flies from each site into pools of 50 individuals (with the final pool for each site containing more or fewer than 50 individuals ranging from 31 to 68 individuals) using sterilized petri dishes and forceps, and then transferred each pool into sterile 1.7 ml tubes where sandflies were macerated in lysis buffer using bead beaters. We then extracted DNA from the pooled sandfly samples with the Qiagen Blood and Tissue Kit with slight modifications. Briefly, 200 ul of Buffer ATL and 20 ul of Proteinase K were added to the sample in a 1.7 ml Eppendorf tube and the sample incubated for 3-5 hours at 56°C. Post-incubation, samples were vortexed for 10 min and then purified through washing. The DNA was eluted in a final volume of 100 ul.

Vertebrate amplification

Following extractions, we amplified each pooled sample of sandflies in two separate reactions using a modification of the primer pair 12SV5F/12SV5R (Riaz et al., 2011). We used the reverse primer (TTAGATACCCCACTATGC) as Riaz et al. (2011) and a modified version of the forward primer to allow for broader binding of vertebrate targets (YAGAACAGGCTCCTCTAG). These primers target approximately 100 base pairs in the 12S rRNA gene region of the vertebrate mitochondrial genome. Additionally, we used twin-tags to better

detect tag jumping. Briefly, we carried out PCR reactions in a volume of 20 ul using 10 ul AmpliTaq Gold 360 Master Mix (final concentration of 1x), 5 ul of forward and reverse primers (final concentration of 0.25 uM), 3 ul of water, and 2 ul of DNA template. PCR cycling was as follows: initial denaturing at 95°C for 10 min; 40 cycles: 95°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec; and a final extension at 72°C for 7 min.

Sandfly amplification

We amplified each sandfly DNA extraction in two separate reactions using the ANML primer pair from Jusino et al. (2019) with the forward primer (GGTCAACAAATCATAAAGATATTGG) and the reverse primer (GGWACTAATCAATTTCCAAATCC). These primers target the COI gene and amplify a broad range of arthropods. Although the primers do not bind without mismatches to all sandflies, after pilot testing we found that this primer pair readily amplified in sandflies and was appropriate for distinguishing sandfly species. Again, we used twin-tags unique to each sample within a library. PCR reactions were carried out in a volume of 15 ul using 3 ul GoTaq Green Master Mix (final concentration of 1x), 0.1 ul of GoTaq DNA Polymerase (final concentration of 0.033 u/ul), 3 ul of forward and reverse primers (final concentration of 0.2 uM), 5.48 ul of water, 0.12 ul of BSA, 0.3 ul of dNTPs, and 3 ul of DNA template. PCR cycling was as follows (Hebert et al., 2003; Jusino et al., 2019): initial denaturing at 94°C for 60 sec; 5 cycles: 94°C for 60 sec, 45°C for 90 sec, 72°C at 90 sec; 35 cycles: 94°C for 60 sec, 50°C for 90 sec, 72°C for 60 sec; and a final extension of 72°C for 7 min.

Library preparation

After the initial PCR, we cleaned all amplicons using PCRClean DX solid-phase reversible immobilization magnetic beads (Aline Biosciences, Woburn, MA). Each PCR reaction was quantified using Accublue High Sensitivity dsDNA Quantitation kit (Biotium, Fremont, CA) and normalized to 6 ng/ul. For 12S preparation, each group of 384 PCR products was then pooled into a single library for a total of four libraries. For COI preparation, each group of 192 PCR products was pooled into a single library for a total of eight libraries. Individual libraries were then tagged with an additional 6 base pair index using the NEBnext Ultra II DNA Library Prep kit (New England Biolabs, Ipswich, MA). Pooled samples were analyzed on a Bioanalyzer to confirm fragment size. The libraries were then sequenced using the Illumina HiSeq 3000 2 x 150 bp PE at the Center for Genome Research and Biocomputing at Oregon State University.

Leishmania screening

We tested each extracted pool of sandflies for the presence of*Leishmania* species with real-time quantitative PCR (qPCR) using primer pairs kDNA1 and *L. braziliensis* kDNA3 as described by Weirather et al. (2011). The kDNA1 primers amplify DNA for the species*L. amazonensis* , *L. chagasi* , *L. donovani* , *L. infantum* , *L. major* , *L. mexicana* , and *L. tropica* , while the kDNA3 primers primarily amplify DNA for *L. braziliensis*(Weirather et al., 2011). PCR reactions were carried out in a volume of 10 ul using 5 ul PowerTrack SYBR Green Master Mix (final concentration of 1x), 0.05 ul of the forward primer (final concentration of 500 nM), 0.05 ul of the reverse primer (final concentration of 500 nM), 2.9 ul of water, and 2 ul of the DNA template. The PCR cycling was as follows (Weirather et al., 2011): 95°C for 10 min; 40 cycles of 95°C for 15 sec; and 60°C for 1 min. We carried out a total of 2,260 reactions (1,130 reactions using the kDNA1 primers and 1,130 reactions using the *L. braziliensis* kDNA3 primers) to screen for the presence of*Leishmania* species.

Sequence analysis

Raw sequence reads were analyzed using a bioinformatics pipeline designed to trim and sort the sequence reads according to sample identification. An outline of the bioinformatic process is as follows: (1) raw reads were paired using PEAR; (2) followed by demultiplexing using 8 basepair index sequences unique to each sample (mismatches discarded); (3) lastly, OTUs from each sample were taxonomically assigned using BLAST against 12S vertebrate sequences available in GenBank and using BLAST against COI arthropod sequences available in MIDORI (Leray et al., 2018; Machida et al., 2017).

A series of filtering and quality control measures were carried out on taxonomically assigned sequences. For 12S vertebrate data, we initially removed OTUs that were identified as human DNA or contaminants

(if total number of reads per sample was less than 100 or averaged less than the number of reads in the negative controls). We then removed non-amplifying samples determined by a 500 read sample replicate threshold. Within the remaining sample replicates, we removed OTUs with a percentage identity score less than 95%. With this filtered data, we additionally removed OTUs that totaled less than 1% of the total number of remaining sequences in a sample. Finally, we eliminated species that were not found in both sample replicates. We then compared taxonomic assignment with the known regional fauna to reassign non-regional species with closely related, regional matches. If no suitable species-level matches were discovered, these taxa were then assigned at the genus or family level or removed from the dataset.

For COI sandfly species data, there was a similar set of quality control measures. We again removed non-amplifying samples with a 500 read threshold for a sample replicate. We then removed non-sandfly sequences based on family and genus taxonomic designations so that only sandfly species from family Psychodidae were retained. OTUs with a percentage identity score less than 95% and query sequences that totaled less than 1% of the total number of sequences in that sample were removed. Finally, species that were not present in both sample replicates were removed. With this curated dataset, we manually blasted each individual OTU to examine if there were other local taxa with equal or nearly equal query percentage matches. If so, we reassigned these species to the genus level, which was the case for all *Nyssomyia* species.

Map generation and landscape analysis

We used a geotiff layer depicting land-use and land cover (LULC) of Mato Grosso in 2015 made available by the MapBiomas platform (*Project MapBiomas - Collection 5.0 of Brazilian Land Cover & Use Map Series* , 2020) to create the land cover map of our study landscape (Fig. 2). We modified the original LULC map by reclassifying land cover classes to reduce the number of classes to forest, savannah, cropland (primarily soybean), pasture, open-water, and urban (the savannah land cover class was excluded in subsequent analyses as the total area of this habitat type was minimal). We primarily used the *raster* (Hijmans, 2019),*rgeos* (Bivand & Rundel, 2020), and *sp* (Bivand et al., 2013; Pebesma & Bivand, 2005) packages in R for reading and manipulating the spatial data.

From this LULC map, we quantified the deforestation surrounding each site by measuring the percentage cover of forest, pasture, and cropland (using the *landscapemetrics* (Hesselbarth et al., 2019) package in R) within a 2500 m circular buffer (as shown on the map in Fig. 2) of the trapping grid at each site as the centroid. We selected a 2500 m buffer to capture the effects of deforestation and encroachment of non-forest cover surrounding each forest patch while also reducing the overlap between neighboring buffer windows. Proportions of forest and cropland cover within a 2500 m circular buffer around each trapping grid were highly correlated (r = -0.97) so we included only forest cover in evaluating the effects of deforestation in the study region.

Data analyses

We profiled species diversity and relative abundance across the landscape using a relative abundance index (RAI) calculated from the metabarcoding results. The RAI of a species is equal to the sum of occurrences at a site (or over the entire landscape) for species$i$ divided by the total number of pooled samples at that site (or number of pooled samples across the landscape). Occurrence of a species in a sample was determined by the presence of sequence reads for that taxon post quality control of sequence reads.

We used NMDS ordinations to examine the potential separation of the vector and the non-vector communities and the host and the non-host communities. The function *envfit* was used to determine if any of the environmental metrics were significantly associated with the community composition of sandfly or vertebrate species. Amount of forest and pasture were logit transformed and all environmental variables were rescaled prior to analysis. We also included Julian day as a predictor for the sandfly species ordination due to the association of leishmaniasis (and other zoonotic diseases) incidence with the wet season. To better tease apart patterns between landscape structure and the host and the vectors, we used generalized linear models to test the hypothesis that the measures of forest cover, pasture cover, and distance to the major urban center (and Julian day for the sandfly models) predict the likelihood and density of disease-competent taxonomic

groups. We used a Poisson regression model to ask if the counts of sandfly pools were influenced by phenology (Julian day), percentage forest, percentage pasture, and distance to the urban center. We then built binomial models with a random effect for site to assess whether Julian day, percentage forest, percentage pasture, and distance to urban affected the probability that a sandfly pool contained a medically important vector. Lastly, we built binomial models with a random effect for site to assess whether the environmental variables affected the probability that any sandfly pool contained a host or non-host species.

We constructed bipartite networks to examine how vector-host interactions restructure between the most forest intact sites (>60% forest cover) and the most deforested sites (<30% forest cover). We first subsetted samples to only include those in which vector species accounted for the majority of DNA sequences in that sample (samples where more than 50% of the reads were vector species) because our aim was to better understand changing vector-host relationships due to deforestation. This subsetted dataset contained six sites, with five sites containing 14 samples categorized as "intact" and three sites containing 24 samples categorized as "deforested". The bipartite networks were constructed using the*bipartite* package in R (Dormann et al., 2011) and display a weight that is equal to the number of interactions at the pooled sample level between a sandfly and any vertebrate species identified in the same sample.

Results

Sandfly iDNA metabarcoding

We captured 56,775 sand flies and sorted them into 1,137 pools of 50 flies. The number of sandflies captured at each site ranged substantially from 131 individuals at site E19 to 8,469 individuals at site B5 (site level data summary available in Appendix S1: Table S1). Metabarcoding results filtered for sandfly species (Family: Psychodidae) revealed 34,598,149 total paired sequence reads from 927 pooled sandfly samples (the number of molecularly processed samples is less than the total number of samples due to the exclusion of some pooled samples from sites with disproportionately high number of sand flies (see Appendix SI: Table S1 for details). After quality control measures designed to remove non-amplified samples and clean the raw sequencing data, the final dataset used for analysis had 19,334,956 total paired sequence reads from 851 pooled samples from across all 39 sites. Metabarcoding results for vertebrate species revealed 25,093,673 total paired sequence reads from 976 pooled sandfly samples. After quality control measures designed to remove non-amplified samples and clean the raw sequencing data, the final dataset used for analysis had 4,753,773 total paired sequence reads from 481pooled samples which represented 38 of the 39 sites.

Sandfly diversity and identified vector species

DNA metabarcoding identified 11 sandfly taxa (Table 1). We categorized the identified sandfly species based on their known medical importance. Of the 11 sandfly taxa, seven are considered medically important vectors (Table 1; Fig. 3). The most abundant sandfly species was*Psathyromyia aragaoi* (RAI = 0.86). High abundance of this sandfly species is correlated with proximity to armadillo holes and domestic animal dwellings (Margonari et al., 2010). This species is not currently confirmed as a medically important species for the transmission of*Leishmania* to humans but multiple studies have confirmed the presence of *Leishmania* spp. in *Psathyromyia aragaoi*samples and one recent study found a human blood meal from *Pa. aragaoi* (Araujo-Pereira et al., 2020) so we classified this species as a possible vector. Of all known vectors of *Leishmania* spp.,*Nyssomyia* spp. (RAI = 0.64) were the most prevalent followed by*Psychodopygus davisi* (RAI = 0.12), both of which are vectors of cutaneous leishmaniasis. Two vectors of visceral leishmaniasis,*Lutzomyia longipalpis* and *Migonemyia migonei* , were also present but at low relative abundance (RAI < 0.01) (Table 1).

Vertebrate diversity and identified host species

The sandfly metabarcoding data revealed 50 vertebrate taxa of which 48 were identified to the genus or species level (Table 2; Fig. 3). Of the 50 identified vertebrate taxa, 43 were considered sylvatic species (results for taxa considered to be domesticated species are shown in Table 2 and Appendix S1: Fig S1). We categorized the vertebrate taxa based on their known host and/or reservoir status for *Leishmania*parasites

after an extensive review of the literature (Table 2). The most abundant sylvatic vertebrate species in sandfly pools were armadillos (*Dasypus novemcinctus* (RAI = 0.18), *Dasypus kappleri* (RAI = 0.04)), tapir (*Tapirus terrestris* (RAI = 0.04)), and lesser anteater (*Tamandua tetradactyla* (RAI = 0.02)). Host and/or reservoir species dominated the sylvatic species diversity with 27 species categorized as host or probable host and only three species categorized as non-host or unlikely host (Table 2). Too little is currently known about the host status of the remaining 13 vertebrate taxa, most of which were birds (n=10); tapir, giant otter, and red brocket deer are the only non-bird species designated as 'unknown'.

Species assemblages across the deforestation gradient: winners and losers

Ordinations based on Bray-Curtis dissimilarity of sandfly species relative abundance (k = 2; stress = 0.11) and vertebrate species relative abundance (k = 2; stress=0.18) show that there is no clear separation of the vector and the non-vector communities nor the host and the non-host communities in ordination space (Fig. 4 left side panels). However, these ordinations reveal that the relative abundance of individual species differed across the environmental gradients with most of the sandfly species ordinated closer to increased forest intactness and away from the most heavily deforested sites with minimum forest cover (Fig. 4 top left panel) and that many important host species align along the percentage pasture vector (Fig. 4 bottom left panel). Regression models more clearly show the responses of individual species to deforestation (Fig. 4 right side panels; Appendix S1: Table S2). Higher pasture land cover was a significant negative predictor of the probability of encountering a vector (p = 0.04) and the vector genus*Nyssomyia* spp. (p = 0.03), while higher forest cover was an important predictor of the probability of encountering the vector species *Psychodopygus davisi* (p = 0.10). The most important finding from sandflies feeding on vertebrate species showed that decreasing forest cover was a significant predictor of the probability of finding a sylvatic host in a sandfly pool ($p < 0.05$), as well as the probability of finding the most competent host species (nine-banded armadillo) in a sandfly pool ($p < 0.05$) (Fig. 4 bottom right panel; Appendix S1: Table S2).

Species interaction networks

Sites categorized as 'intact' had four sandfly species (three of which were known vectors) and five host species, and sites categorized as 'deforested' had three sandfly species (two of which were known vectors) and four host species. Bipartite interaction networks showed evidence of restructuring of interactions across sites categorized by their amount of forest cover (Fig. 5). Namely, the large armadillo (*Dasypus kappleri*), which is a known interior forest species, was less prevalent in sandfly bloodmeals in deforested sites compared to intact sites. Sandflies in those highly deforested sites fed primarily upon the lesser anteater (*Tamandua tetradactyla*). The nine-banded armadillo (*Dasypus novemcinctus*) was an important host for sandflies in both intact and deforested sites even though its presence was significantly associated with higher levels of deforestation (Figure 4).

Leishmania incidence

Of the 1,130 total sandfly pools, 42 samples tested positive for*Leishmania* (3.7% positivity rate). The proportion of infected pools at a site varied substantially (Appendix S1: Table S3) across all sites but there was no significant effect of any of the measured environmental metrics on the likelihood of a pool testing positive for*Leishmania* (Appendix S1: Fig. S2). Eighteen of the 39 sites had at least one infected pool with one site (E19) having 100% of pools (n=3) infected. Aside from this outlier site, sites with infected pools had RAI values that range from 0.01 – 0.20. Of the 42 positive samples, 11 samples contained sylvatic vertebrates, each of which were known host species for *Leishmania* . In those samples, armadillos (n=6) were the most found taxa and five samples had co-occurrences of sylvatic host species (two host species found in the same sample) (Fig. 6).

Discussion

There has been a robust debate about whether there is a generalizable effect of changes in biodiversity (as a consequence of habitat loss and/or fragmentation) on the emergence and prevalence of infectious diseases (Levi et al., 2016; Ostfeld, 2013; Ostfeld & Keesing, 2013; Randolph & Dobson, 2012; Wood et al., 2014;

Wood & Lafferty, 2013). This debate has been stymied by a lack of landscape-scale empirical data across land-use gradients and observational approaches that are unable to deduce the mechanistic underpinning to changing disease risk. This can be especially complex for vector-borne pathogens where land-use change can differentially influence hosts, vectors, and pathogens (Burkett-Cadena & Vittor, 2018). We addressed this debate with a landscape scale epidemiology approach across a forest habitat loss gradient within the world's largest tropical deforestation frontier induced by large-scale agricultural commodity production.

By using DNA metabarcoding of sandflies and their vertebrate bloodmeals, we were able to link medically important hosts and vectors to deforestation at large scales (56,775 sandflies collected from 27 light traps set for 3 days at each of the 39 sites). Metabarcoding allowed us to identify thousands of sandflies at the species or genus level so that the ecology of vectors and non-vectors could be investigated. Species level sandfly data is usually only possible with painstaking morphological identification of sandfly species, which requires rare taxonomic expertise. In contrast, previous research has shown that DNA derived from sandflies are a good measure of the vertebrate diversity in this landscape and requires significantly less resources and identification effort (Massey et al., 2022). Ideally, sample pools would have included fewer individuals, or we would have a larger sample size, so that direct vector to host comparisons and interaction networks could be quantified without data contamination from multiple sandflies sequenced as part of the same pool. However, metabarcoding of sandfly pools allowed for sufficient cost reduction to allow sequencing of over 50,000 individual sandflies to taxonomically identify both sandfly species and any vertebrate bloodmeal remnants.

Fundamentally, sandfly responses to deforestation reported here were nuanced. Although total sandfly abundance did not vary with deforestation, the relative abundance of vector species ordinated in the direction of greater forest cover with the most significant positive response to forest cover found with *Psychodopygus davisi* , a known vector of *Leishmania braziliensis* (Fig. 4). Further, the probability of a sandfly pool containing any vector or the dominant sandfly vector genus, *Nyssomyia* spp*.,* was higher at sites surrounded by less cattle pasture (Fig. 4). *Psychodopygus davisi*and *Nyssomyia* spp. are vectors known for transmitting species of*Leishmania* responsible for cutaneous leishmaniasis in Brazil, which while treatable, causes disfiguring and painful skin lesions. We found no significant positive responses of sandfly density or the probability of finding a vector species to increasing deforestation which runs counter to the hypothesis that vector amplification (as a consequence of increased host density) occurs in response to deforestation.

However, disease risk is a product of both vector and host ecology. Despite the heavily deforested nature of this region, we found that this landscape supports a large diversity of terrestrial and arboreal vertebrate species. The majority of sylvatic vertebrate taxa we detected using sandflies as a source of iDNA are known host species for*Leishmania* parasites. The responses of sylvatic vertebrate taxa in sandfly bloodmeals were driven by the high prevalence of armadillos in the genus *Dasypus,* particularly the disturbance-tolerant nine-banded armadillo, *D. novemcinctus* , which was by far the most common source of bloodmeals (Fig. 3). These data suggest that sandflies strongly select for armadillos, which are among the most important hosts for *Leishmania* spp., which cause leishmaniasis in humans (Christensen et al., 1982; Kocher et al., 2017; Lainson & Shaw, 1989). Armadillos were the only prevalent vertebrate taxa to show a significant relationship to the deforestation gradient with increased probability of finding *D. novemcinctus* in sandfly bloodmeals as forest cover decreased (Fig. 4). Bipartite networks, which directly measured sandfly × vertebrate interactions, and the *Leishmania* -positive samples suggest that armadillos drive the feeding ecology of sandflies and*Leishmania* transmission dynamics across a degraded forest landscape. However, we excluded domesticated species from our analyses because (i) we were primarily concerned with how land use change influences wild vertebrate communities, and (ii) possible routine lab contamination. Even with conservative read thresholds, we found that domestic dog (*Canis lupus familiaris*) occurred in just over 20% of the sandfly samples and was ubiquitous across the landscape (Appendix SI: Fig. S1), including in some samples that also contained*Dasypus* (Appendix SI: Fig. S3). Given that pathogen spillover to humans increases when domesticated species are in close proximity to sylvatic hosts, domestic dogs may play a key role in peridomestic transmission of *Leishmania* to humans. Our findings support this potential mechanism of zoonotic disease transmission primarily through the observed pattern of armadillo and dog co-occurrences from sample pools across the

study area.

While our sampling scheme allowed us to sample across a landscape-scale deforestation gradient, it is important to note that we did not sample the extremes of vast tracts of continuous, undisturbed forests (compared with Kocher et al. 2022) nor entirely deforested landscapes lacking forest remnants. Consequently, while there was a gradient of deforestation across our study region, it is likely that the current forest landscape structure has not resulted in the same level of extirpation of vertebrate species as other studies have documented. Instead, our study system represents a landscape-scale deforestation gradient resulting from rapid and recent forest conversion into seed crop agriculture. As discussed previously, this expansion of human activity into tropical forests can alter ecological communities and species interactions particularly at transition zones between forests and peri-urban areas (Aguirrea & Taborb, 2008; Roque & Jansen, 2014), thereby potentially increasing the risk of infectious disease emergence from wildlife reservoirs and vectors into domestic vertebrate hosts and/or humans (Lambin et al., 2010; T. Lima et al., 2017; McCauley et al., 2015; Vanwambeke et al., 2007). Our findings support this given the high diversity of vertebrate hosts and sandfly vectors found across the landscape and the lack of localized extinctions at even the most forest degraded sites.

In summary, we found that it was most important to examine the responses of individual species even when investigating the generality of biodiversity and disease risk to land-use change. While overall sandfly abundance (including non-vectors) was unrelated to deforestation, sandfly vectors were more strongly associated with more intact forest landscapes (either more forest or less pasture), which was largely driven by the response of the dominant vector taxa (*Nyssomyia*spp.). Likewise, changes in the relative abundance of sylvatic hosts (namely armadillos) were apparent despite no significant response of the aggregate vertebrate host community across the deforestation gradient. Additionally, samples that tested positive for the presence of*Leishmania* species also failed to show any response to forest cover, pasture cover, or distance to the urban center, suggesting that*Leishmania* transmission can occur across both intact and degraded forests in this system. In conclusion, the complex combined responses of vectors and hosts within the context of partly deforested landscapes did not support the generality of the 'dilution effect' hypothesis. However, patterns of individual species responses to deforestation and vector × host interactions across the deforestation gradient show disease risk consequences of forest conversion and increased human encroachment into Amazonian primary forest.

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Data Accessibility and Benefit-Sharing Statement

Sequencing results that were used for all analyses available as separate Excel files in the Supporting Information.

Figure 1: Conceptual diagram outlining important questions to address with the combination of insect-derived DNA data and landscape-scale land use and land cover change data.

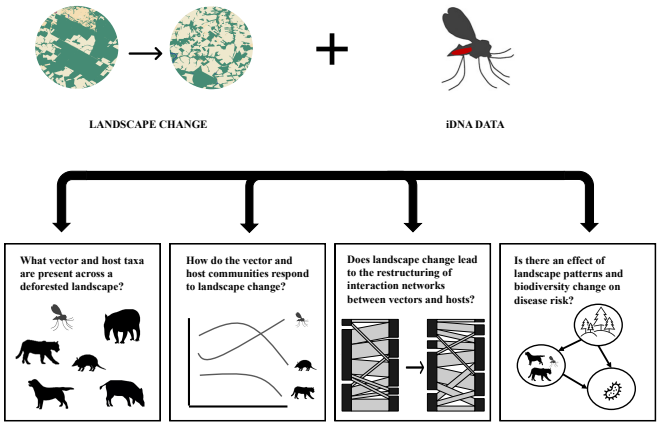


Figure 2: Map of the study area located near Sinop, Mato Grosso, Brazil. Sinop is an urban area (shown in maroon on the map) with more than 160,000 people in 2015. The remainder of the landscape is described by a matrix of closed-canopy forest, cerrado scrublands, croplands, and cattle pastures. Our study sites and a 2500-m circular buffer are shown in black. This study area map was created using 2015 LULC map of Mato Grosso from MapBiomas (*Project MapBiomas - Collection 5.0 of Brazilian Land Cover & Use Map Series*, 2020). The inset map shows the Amazonian biome (shaded green) as it extends across northern Brazil with the location of our study area indicated by the black square. This map was sourced from IBGE (Instituto Brasileiro de Geografia e Estatistica) from the *geobr* (Pereira & Goncalves, 2020) package in R.

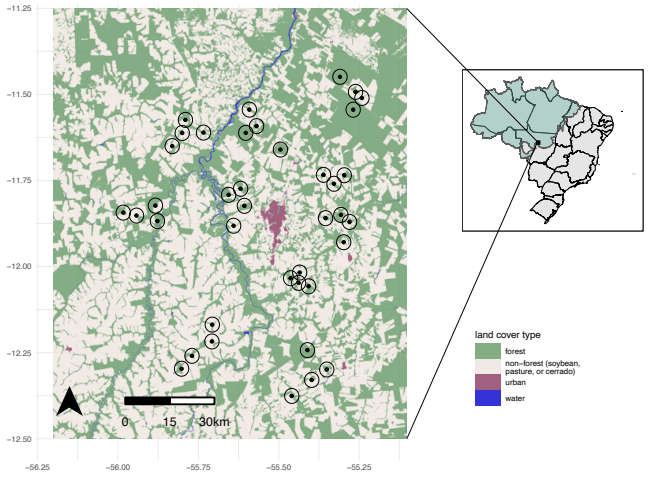


Figure 3: Sandfly (top) and vertebrate (bottom) species diversity as revealed by metabarcoding of sandfly DNA extractions. The relative abundance index (RAI) was calculated as the total number of occurrences for species *i* divided by the total number of pooled samples from across the entire study landscape. Domesticated species found with DNA metabarcoding have been excluded.

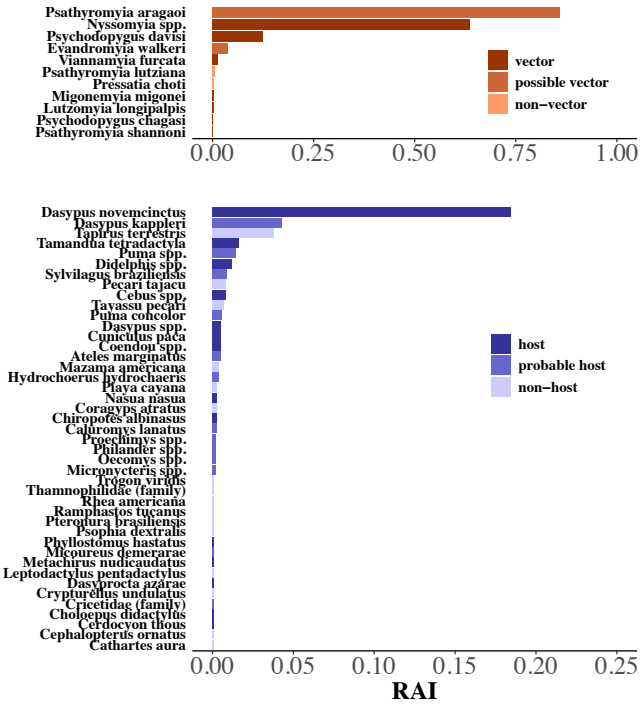


Figure 4: (Top left and bottom left panels) NMDS ordinations of the sandfly (vector and non-vector) community and the vertebrate (host and non-host) community. Percentage forest, percentage pasture, and Julian Day are significant environmental metrics (p-value ≤ 0.10) for the sandfly species ordination and shown by vectors. Amount of pasture cover was the only significant environmental metric for the vertebrate community as shown by the corresponding vector. (Top-right and bottom-right panels) Regression coefficients and their 95% confidence intervals. The top-right panel displays the model results for the effects of percentage forest, percentage pasture, distance to urban center, and Julian day on sandfly density (quasipoisson model) and the probability of encountering (binomial models each with a random effect for site) a vector species (*Nyssomyia* spp. and *Psychodopygus davisi*) or a possible vector species (*Psathyromyia aragaoi*) in a pooled sample of sandflies. The bottom right panel displays the effect of percentage forest, percentage pasture, and distance to urban on the probability of encountering (binomial models each with a random effect for site) a host, a sylvatic host, *Dasypus novemcinctus*, *Dasypus kappleri*, *Tamandua tetradactyla, Puma concolor,* a non-host, and *Tapirus terrestris* from a pooled sample of sandflies. Significant results are bolded and colored. Domesticated species found with DNA metabarcoding have been excluded.

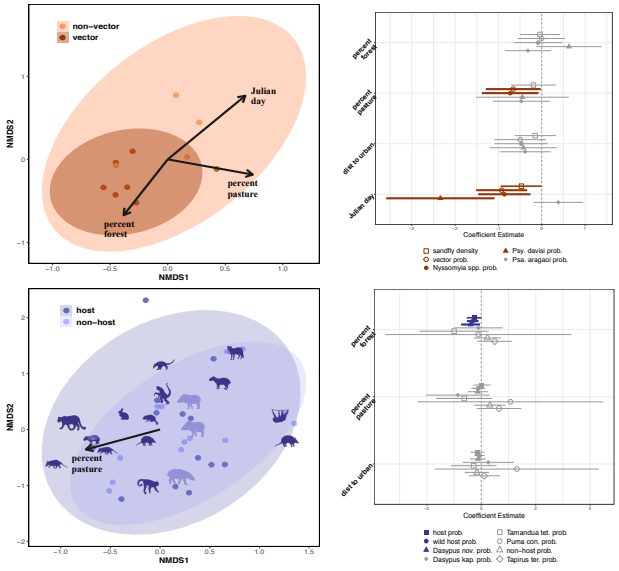


Figure 5: Bipartite plots showing changes in sandfly × vertebrate interactions based on sites binned by the amount of forest cover and categorized as intact (>60% forest) and deforested (<30% forest). The width of links is based on the strength (or number) of interactions between a sandfly species and the corresponding vertebrate species. The width of the nodes is based on the total number of interactions attributed to the taxon within the subsetted data. Domesticated species found with DNA metabarcoding have been excluded.

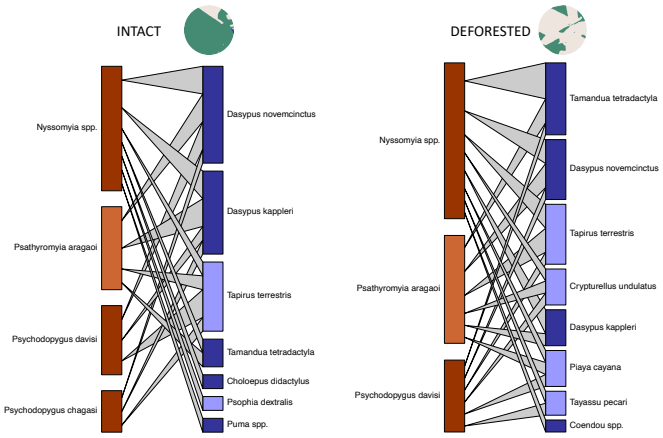
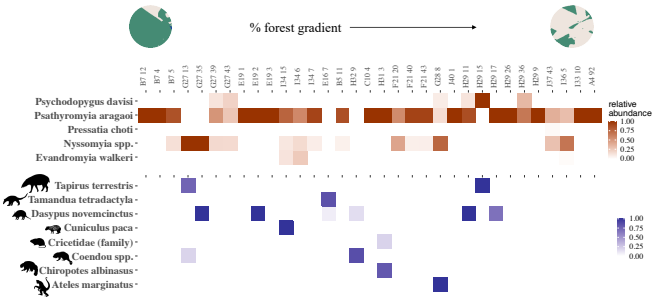


Figure 6: Of 1130 samples, 42 were positive for the presence of at least one *Leishmania* species, 33 of which containing either sandfly and/or vertebrate data from DNA metabarcoding. The samples that tested positive for *Leishmania* are organized on the horizontal axis by amount of forest cover at their corresponding site. The sandfly and vertebrate taxa that were found in these samples are shown with either orange (sandfly) or purple (vertebrate) color showing the relative abundance of sequence reads for each taxon. Domesticated species found with DNA metabarcoding have been excluded.



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LeishTable2.docx available at https://authorea.com/users/410648/articles/649341-does-deforestation-beget-disease-leishmania-host-and-vector-communities-across-a-gradient-of-forest-loss-on-the-amazonian-deforestation-frontier