

Local tree cover predicts mosquito species richness and disease vector presence in a tropical countryside landscape

Johannah E. Farmer (✉)

[data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAZAAAAyAQMAAABI0h/eAAAABIBMVEX///8AAABVwtN+AAAACXBIWXMAAA7EAAAOxAGVKw4bAAAA50IEQVRIiWNgGAWjYBACXuYDYFoOTFYABRoYGAzwa2ILANPGDGxA8gwxWhjYIFoSG4jWwtzG/OxzQcW99Pnzm589OFBxT7aBvXmbBH6HsRnPnnGmOHfDMTZzgwNnio0bel6V4dcyv8GYmbctIXcDG4OZ9Me2hMQGiRwzArawf2bm/ZeQLt/G/k3iIEiL/BtCWniAtjQkJDac4zGDaJHglailmJnnWILhhmM5ZRIHziQYt/GkFVvg02LYxr6ZmacmQV6++fg2iQMVCbL97lc33sC rpQFdha2fchCQJ6RgFlyCUTAKRgEDAEL0RtAfURNyAAAAEIFTkSuQmCC \)](data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAZAAAAyAQMAAABI0h/eAAAABIBMVEX///8AAABVwtN+AAAACXBIWXMAAA7EAAAOxAGVKw4bAAAA50IEQVRIiWNgGAWjYBACXuYDYFoOTFYABRoYGAzwa2ILANPGDGxA8gwxWhjYIFoSG4jWwtzG/OxzQcW99Pnzm589OFBxT7aBvXmbBH6HsRnPnnGmOHfDMTZzgwNnio0bel6V4dcyv8GYmbctIXcDG4OZ9Me2hMQGiRwzArawf2bm/ZeQLt/G/k3iIEiL/BtCWniAtjQkJDac4zGDaJHglailmJnnWILhhmM5ZRIHziQYt/GkFVvg02LYxr6ZmacmQV6++fg2iQMVCbL97lc33sC rpQFdha2fchCQJ6RgFlyCUTAKRgEDAEL0RtAfURNyAAAAEIFTkSuQmCC))

Stanford University

Meghan Howard

Stanford University

Jeffrey R. Smith

Princeton University

Christopher B. Anderson

Planet Labs

Erin A. Mordecai

Stanford University

Research Article

Keywords: mosquitoes, Culicidae, community assembly, tree cover, *Aedes albopictus*, biodiversity

Posted Date: February 19th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3954302/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Context

Land use change drives both biodiversity loss and zoonotic disease transmission in tropical countryside landscapes. Developing solutions for protecting countryside biodiversity, public health, and livelihoods requires understanding the scales at which habitat characteristics such as land cover shape biodiversity, especially for arthropods that transmit pathogens. Evidence increasingly shows that species richness for many taxa correlates with local tree cover.

Objectives

We investigated whether mosquito species richness, community composition, and presence of disease vector species responded to land use and tree cover – and if so, whether at spatial scales similar to other taxa.

Methods

We paired a field survey of mosquito communities in agricultural, residential, and forested lands in rural southern Costa Rica with remotely sensed tree cover data. We compared mosquito community responses to tree cover surrounding survey sites measured across scales, and analyzed community responses to land use and environmental gradients.

Results

Tree cover was positively correlated with mosquito species richness, and negatively correlated with the presence of the common invasive dengue vector *Aedes albopictus*, particularly at small spatial scales of 80 – 200m. Land use predicted community composition and *Ae. albopictus* presence. Environmental gradients of tree cover, temperature, and elevation explained 7% of species turnover among survey sites.

Conclusions

The results suggest that preservation and expansion of tree cover at local scales can protect biodiversity for a wide range of taxa, including arthropods, and also confer protection against disease vector occurrence. The identified spatial range of tree cover benefits can inform land management for conservation and public health protection.

Introduction

Although land use change is a leading cause of loss and endangerment of both biodiversity and ecosystem services (e.g., Sala, 2000; Pereira et al., 2010; Maxwell et al., 2016; Giam, 2017), human-dominated landscapes also have high potential and a pressing need to contribute to conservation (Norris, 2008; Chazdon et al., 2009). Countryside landscapes characterized by natural habitat remnants

patchworked with human residential and agricultural infrastructure are globally dominant, and thus represent both an exceptional threat to and exceptional opportunity for conservation (Norris, 2008; Maxwell et al., 2016). A critical requirement for leveraging the conservation potential of countryside is understanding which landscape features help to retain biodiversity and biodiversity-associated ecosystem services, such as crop pollination and pest control (Frishkoff et al., 2019). Percent tree cover at small spatial scales of < 100m has emerged as a reliable predictor of biodiversity for taxa in Latin American tropical countryside landscapes including birds, non-flying mammals, and bats (Mendenhall et al., 2016). These findings suggest that local tree cover management holds promise as a practicable conservation tool, but major knowledge gaps remain surrounding how local tree cover relates to biodiversity and ecosystem services. A key open question relevant to biodiversity conservation is the extent to which local tree cover correlates with species richness across taxa, particularly for invertebrates. Additionally, because tree cover management may influence human – wildlife contact patterns, developing best practices for this conservation tool requires understanding how tree cover in working landscapes relates to vector-borne and zoonotic disease risk.

The spatial scales at which mosquito communities (family Culicidae) respond to tree cover present a knowledge gap relevant to both biodiversity and human health. In addition to playing roles as prey, predators, and detritivores in aquatic and terrestrial food webs (Addicott, 1974; Heard, 1994; Daugherty, Alto and Juliano, 2000; Poulin, Lefebvre and Paz, 2010), several mosquito species are important vectors of wildlife and human diseases including malaria, dengue, chikungunya, Zika, yellow fever, West Nile fever, and arboviral encephalitis (Garmendia, Van Kruiningen and French, 2001; Lemon, 2008; LaPointe, Atkinson and Samuel, 2012; World Health Organization, 2020). Mosquito community composition and abundance are fundamental to when, where, and how widely such diseases are transmitted (reviewed in Franklinos et al., 2019). Landscape context is likely to have multifaceted effects on mosquito community composition through its influence on biotic and abiotic conditions that affect mosquito life cycles. Differences in temperature, habitat structural complexity, and biotic context associated with tree cover are all likely to affect the presence and abundance of mosquito species that vary in their thermal niches, aquatic breeding habitat requirements, and preferred groups of vertebrates for blood meals (Laird, 1988; Gutman et al., 2004; Mordecai et al., 2019; Prevedello et al., 2019). Indeed, previously documented shifts in mosquito communities across habitats show associations between forest conversion and low mosquito biodiversity, and suggest that high rates of land use change and active invasions by major vector species are together reshaping mosquito communities in ways that increase disease risk (Ferraguti et al., 2016; Meyer Steiger, Ritchie and Laurance, 2016; Burkett-Cadena and Vittor, 2018; Chaves et al., 2021). Determining the spatial scales at which tree cover shapes mosquito communities is a critical next step towards understanding how tree cover in countryside landscapes can be managed to balance biodiversity conservation, public health, and economic needs.

The global range expansions of the dengue vectors *Aedes aegypti* and *Aedes albopictus* exemplify ongoing rapid change to mosquito communities that may be linked to concurrent changes in both land cover and disease transmission (Rezza, 2012; Kraemer et al., 2015). In Central America, the first suspected dengue outbreaks occurred in the 1600s; both the disease and the historically common

introduced vector *Aedes aegypti* and dengue were eradicated in the 1940s; and both vector and virus were reintroduced in the 1970s (Brathwaite Dick et al., 2012). Dengue in this region has increased dramatically in recent decades: from 378,469 cases reported between 1990 and 2000 to 1,338,330 cases reported between 2007 and 2017 (WHO, 2021). Concurrent with this increase, *Ae. albopictus* was introduced to and spread throughout Central America (Benedict et al., 2007). However, how *Ae. albopictus* colonization relates to dengue in this region is unknown, because its distribution in its new range is poorly documented, and its role in disease transmission must be disentangled from that of *Ae. aegypti* (Rezza, 2012; Kraemer et al., 2015). Globally, *Ae. albopictus* and *Ae. aegypti* are predominantly associated with rural and urban human settlements, respectively, suggesting that *Ae. albopictus* and its responses to tree cover may play particularly important roles in shaping disease risk in countryside landscapes where dengue can be prevalent (Braks et al., 2003; Tsuda et al., 2006).

The countryside of Costa Rica is an ideal system in which to study relationships between land cover, mosquito community characteristics, and disease vector occurrence because this system is the subject of intensive long-term research into links between landscape context, biodiversity, and ecosystem services (Burkett-Cadena and Vittor, 2018). This presents a unique opportunity to improve scientific understanding of mosquito community assembly, and resulting disease risk, in comparison with a rich understanding of other taxonomic diversity and ecosystem services. This is particularly relevant to this region because mosquito-borne diseases, including dengue virus, impose a substantial public health burden. *Aedes aegypti* is considered the primary dengue vector in Costa Rica, but the ongoing, patchily described invasion by the globally important vector species *Aedes albopictus* is potentially reshaping disease risk (Troyo et al., 2006; Calderon-Arguedas et al., 2010; Calderón-Arguedas et al., 2015; Rojas-Araya et al., 2017). Finally, a pioneering national Payment for Ecosystem Services program offers a well-established venue for translating research findings into land management policy, and evidence for additional ecological and human health benefits of local tree cover may help motivate enforcement of an existing law that protects riparian buffer zones up to 50m around rivers, which is expected to increase access to clean water for vulnerable populations, and pertains to a spatial scale at which tree cover has previously been shown to protect biodiversity for other taxa (Sánchez-Azofeifa et al., 2007; Langhans et al., 2022).

Here, we combine field observations of mosquito community composition in forested, agricultural, and residential settings in a rural area of southern Costa Rica with remotely-sensed land cover data in order to ask: (Q1) Does mosquito species richness decline with declining tree cover, consistent with patterns previously observed for a wide range of taxa in a tropical countryside landscape? (Q2) Is the presence of *Aedes albopictus*, a key mosquito vector of human disease, negatively correlated with tree cover? (Q3) How does the spatial scale at which tree cover is observed affect the answers to (Q1) and (Q2)? We additionally ask (Q4) How do mosquito communities respond to environmental gradients versus land use types? Based on evidence that species richness for birds, non-flying mammals, bats, reptiles, and amphibians, and plants increases with tree cover (Mendenhall et al., 2014, 2016), we hypothesize that as local tree cover increases, (H1) mosquito species richness increases, (H2) the presence of human-associated *Aedes albopictus* decreases and (H3) these relationships are strongest at spatial scales of < 100 m. We additionally hypothesize that (H4) shifts in mosquito community composition along tree cover

gradients are reflected in distinct forest and residential mosquito species assemblages that are bridged by intermediate agricultural assemblages. By improving scientific understanding of how landscape context influences mosquito community assembly in Costa Rica, the results of this study contribute to a more general understanding of links between land cover, biodiversity, and ecosystem services through the lens of entomological factors that influence disease risk.

Methods

Study area

This study was conducted in the cantons of Coto Brus, Corredores, and Golfito (8°43'14"N, 82°57'20"W), located in the southern Puntarenas region of Costa Rica along the border with Panama (Fig. 1). The region ranges from coastal lowland tropical rainforest (0 m above sea level) to high elevation cloud forest (1500 m above sea level) and has distinct wet and dry seasons. The study area is predominately composed of rural communities surrounded by agriculture interspersed with forest patches, and also includes the protected Las Cruces forest reserve. Dengue reintroduction in Costa Rica was first reported from the Puntarenas region in 1993, where the disease has since been endemic (World Health Organization, 1994; Troyo et al., 2009). *Ae. aegypti* is common and *Ae. albopictus* has a growing presence in this area (Troyo et al., 2008; Rojas-Araya et al., 2017).

Study sites

With landowner permission, we accessed 37 sites located in San Vito, Sabalito, Copa Buena, Ciudad Neilly, and Pavones (Fig. 1). The sites represented three broad land use classes that were determined on-site by the survey team: residential (N = 17), agricultural (N = 12), and forest (N = 8) (Fig. 1). Residential sites included urban and peri-urban areas; agricultural sites included coffee plantations, an oil palm plantation, a pine plantation, one mixed agricultural field, and one pasture; forested sites included primary and secondary forest edges, interiors, and fragments.

Environmental variables

To quantify percent tree cover, we used a 30 m resolution map of tree cover in Costa Rica created by Echeverri et. al (2022) from multi-sensor satellite observations and fine-scale tree cover maps (Fig. 1). From this map, we calculated percent tree cover at different spatial scales surrounding each site using the R package “raster” (Hijmans et al., 2015). Specifically, we began by calculating percent tree cover within a radius of 30 m; we then increased this radius by increments of 10 m up to 200 m, and by increments of 50 m for radii between 200 m and 1000 m (following Mendenhall et. al, 2014). To account for mosquito interspecific variation in sensitivity to thermal conditions (Mordecai et al., 2019), which could affect observed relationships between land cover and mosquito community characteristics along the 1500 m elevational gradient surveyed, we additionally extracted mean annual temperature data from

1970–2000 for each study site from the WorldClim 1 km² resolution mean annual temperature dataset (Fick and Hijmans, 2017).

Sample collection

We trapped mosquitoes at all sites twice between June 19 and August 9, 2017, excepting the sites in Pavones, which were trapped once. At each site, we placed a total of four traps overnight for a 12–16 hour period within an area of 30 m in radius: one unlighted CDC trap baited with carbon dioxide produced by a mixture of Fleischmann’s Dry Active Yeast, household refined sugar, and water; one BG Sentinel baited with a BG-Lure and octanol; and two BG-GAT traps furnished with yellow sticky cards, corn oil, and a mixture of water and local leaf litter. Trap locations within sites were chosen per BioGents recommendations, and square metal frames covered in large black plastic bags were placed over BG Sentinel and BG-GAT traps for protection from rainfall. To supplement the overnight trapping, consistent members of the field team carried out 20 minutes of direct aspiration at each site during each trapping session.

Mosquito identification

Trained personnel identified, sexed, and counted any *Ae. albopictus* and *Ae. aegypti* mosquitoes collected at each site. All other mosquitoes were counted, stored, and transported to Stanford University for molecular identification. We extracted and amplified DNA from the mitochondrial CO1 gene from pooled samples of mosquitoes from each site using MyTaq RedMix (Meridian Bioscience, Cincinnati, OH), following the protocol provided by the manufacturer. Amplified DNA was sequenced via Illumina next-generation sequencing, with samples containing *Aedes albopictus* and *Culex tarsalis* DNA as positive controls. We used the R package “dada2” to filter and trim the DNA sequences to 473 bp, with a minimum overlap of 20 bases and a maximum of 5 expected errors (Callahan et al., 2016). We estimated taxonomic placement for the sequenced mosquitoes by using the R packages “Biostrings” and “DECIPHER” to group DNA sequences into operational taxonomic units (OTUs, henceforth referred to as species) of 97% sequence similarity, and comparing representative sequences for each species to the BOLD and GenBank database records (Altschul et al., 1990; Ratnasingham and Hebert, 2007; Wright, 2016; Pagès et al., 2022). Species were identified based on top matches with sequence similarity $\geq 97\%$ (Hardulak et al., 2020). When sequence similarity to the top match was $< 97\%$, a higher level of taxonomic identification (e.g., genus) was assigned based on placement within a phylogenetic tree of the BOLD database sequences.

Statistical analyses

We described mosquito communities in terms of species richness and species composition by combining presence data from the morphologically identified *Aedes* data and the sequencing data. To quantify relationships between species richness and percent tree cover, we used generalized linear models (GLMs) with negative binomial error corrections for overdispersion and mean-centered independent variables. To assess the spatial scales at which tree cover best predicted species richness, we compared AIC values for GLMs that included percent tree cover surrounding each site calculated at radii ranging from 30 m to

1000 m. We used binomial logistic regression to analyze relationships between *Ae. albopictus* disease vector presence/absence and percent tree cover across spatial scales. For the 1000 m spatial scale where climate data were available, we additionally assessed the relative influence of mean annual temperature on species richness and *Aedes* vector presence with GLMs including mean annual temperature and its interaction with tree cover.

To compare species richness and *Aedes* vector presence between forest, agricultural, and residential land uses, we used Kruskal-Wallis tests with Bonferroni p-value adjustments to account for multiple comparisons.

To compare species composition among land use types and along environmental gradients, we first calculated the Jaccard coefficient of community similarity for each pair of sites for use in statistical tests and ordination. We then tested for differences in community similarity among land uses with permutational analysis of variation (PERMANOVA), first for all land use types, and then with pairwise adonis functions. Because PERMANOVA is sensitive to heterogeneity in dispersion among groups (Anderson and Walsh, 2013), we additionally tested whether dispersion differed among land use types using Tukey's Honest Significant Differences method with betadisper() calculations of group average distances to the median. To visualize community similarity across land uses, we used non-metric multidimensional scaling (NMDS). All the above analyses of compositional similarity among land uses were run using the R package "vegan" (Oksanen et al., 2013); for the pairwise PERMANOVA analyses, we used the R package "ecole" which provides wrapper functions for "vegan" (Smith, 2022). Finally, to quantify compositional turnover along environmental gradients of tree cover, mean temperature, elevation, and geographic distance, we used the R package "gdm" for generalized dissimilarity modeling (GDM), a form of nonlinear matrix regression that is robust to collinearity (Fitzpatrick et al., 2022). As above, we compared GDM models that incorporated tree cover at radii ranging from 30–1000 m to identify the spatial scale at which tree cover best explained compositional turnover.

All analyses were performed in R version 4.2.1. In addition to the R packages cited above, we used the packages "tidyverse" (Wickham et al., 2019), "cowplot" (Wilke et al., 2019), "MASS" (Ripley et al., 2013), "interactions" (Long, 2019), "gridExtra" (Auguie et al., 2017), and "reshape2" (Wickham, 2007) for data analysis and figure generation.

Results

Across sites, tree cover ranged from 0–100% (mean = 27.9%, SD = 34.8%) at the smallest spatial scale we considered, a 30 m radius. Surrounding tree cover within a 1000 m radius, the largest spatial scale considered, ranged from 8.2–74.8% (mean = 33.4%, SD = 20.3%). The lowest and highest site elevations were 12 m and 1451 m above sea level, respectively (mean = 776 m, SD = 473.2 m). Mean annual temperature ranged from 18.8 to 26.4 °C (mean = 22.6 °C, SD = 2.3 °C).

A total of 1,283 mosquitoes representing 48 species in 13 genera were collected from 34 sites (Fig. 2). The number of mosquitoes collected at a site ranged from 0 to 244 (mean = 35, SD = 64). Of these, 108

individuals from 12 residential and four agricultural sites were morphologically identified as *Ae. albopictus*, and 5 individuals from single sites within each land use category were identified as *Ae. aegypti*. *Ae. albopictus* DNA was detected in the pooled samples of molecularly identified mosquitoes from 7 sites where *Ae. albopictus* individuals were also morphologically identified. The five most common species were *Culex quinquefasciatus*, *Ae. albopictus*, *Cx. nigripalpus*, *Wyeomyia adelpha/Guatemala*, and *Limatus durhamii* (Fig. 2, Table S1).

Site-level species richness ranged from one to 19 (mean = 5.2, SD = 4.1). Overall species counts for forest, agricultural, and residential land uses were 33, 29, and 21, respectively. Ten species (21%) were observed in all three land uses. Nineteen species (40%) were shared among forest and agricultural land uses, 13 species (27%) were shared among agricultural and residential land uses, and twelve species (25%) were shared among forest and residential land uses (Fig. 2, Table S1). Eleven species (23%) were found only in forested settings, six species (13%) were found only in agricultural settings, and six species (13%) were found only in residential settings (Fig. 2, Table S1). Two species, *Ae. albopictus* and *Culex quinquefasciatus*, were common (observed at > 50% of sites) in residential settings, no species were common in agricultural settings, and three species—*Culex nigripalpus*, *Wyeomyia complosa*, and *Wyeomyia adelpha/guatemala*—were common in forested settings (Table S1).

At least five of the mosquito species observed are known vectors of human diseases. Three of these— the dengue and chikungunya virus vector *Ae. albopictus* (present at 15 sites) and the St. Louis Encephalitis virus vectors *Cx. quinquefasciatus* (present at 17 sites) and *Cx. nigripalpus* (present at 13 sites)—were the three most frequently observed species (Fig. 2, Table S1) (Reisen, 2003; Simmons et al., 2012). Rarely observed vector species included the dengue, chikungunya, yellow fever, and Zika virus vector *Ae. aegypti* (present at three sites spanning all three land use types) and the malaria vector *Anopheles albimanus* (present at one agricultural site) (Fig. 2, Table S1) (Zimmerman, 1992; Simmons et al., 2012). In contrast to *Ae. aegypti*, *Cx. nigripalpus*, and *Cx. quinquefasciatus*, which were observed in all land use types, *Ae. albopictus* was observed only in residential and agricultural settings associated with intensive human modification (Fig. 2, Table S1).

Mosquito species richness was explained by tree cover, but not by land use type. Comparisons of GLMs using tree cover calculated for radii ranging between 30 m and 1000 m surrounding each site indicated that species richness was positively correlated with tree cover at radii between 80 m and 600 m, and tree cover at a 250 m radius had the largest effect size (estimated effect = 1.29×10^{-2} , SE = 4.75×10^{-3} , z-value = 2.71, p-value = 6.65×10^{-3}) (Fig. 3a, Table S2). At the 1000 m spatial scale where both tree cover and climate data were available, the interaction between tree cover and mean annual temperature had a significant effect on species richness (estimated effect = 7.87×10^{-3} , SE = 3.65×10^{-3} , z-value = -2.16, p-value = 3.11×10^{-2}) (Table S3). Specifically, at high temperatures, species richness was low even when tree cover was high (Figure S1). By contrast, Kruskal-Wallis test results indicated that species richness did not differ significantly among forested, agricultural, and residential sites (chi-squared = 2.60, df = 2, p-value = 0.28) (Fig. 3b). Notably, the highest species richness was observed at a site in the Coto Brus forest reserve (Fig. 3b).

From the *Aedes* disease vector survey, we present results only for *Ae. albopictus* because observations of *Ae. aegypti* were insufficient for statistical analysis. Both tree cover and land use type predicted *Ae. albopictus* presence. Comparisons of GLMs using tree cover calculated for radii ranging between 30 m and 1000 m surrounding each site indicated that *Ae. albopictus* presence was negatively correlated with tree cover at radii between 50 m and 200 m, and was best explained by tree cover at a 110 m radius (estimated effect = -4.23×10^{-2} , SE = 1.96×10^{-2} , z-value = -2.16, p-value = 3.08×10^{-2}) (Fig. 4a, Table S3). At the 1000 m spatial scale where we additionally assessed the influence of climate, *Ae. albopictus* presence was negatively correlated with tree cover and positively correlated with temperature (tree cover estimated effect = -7.76×10^{-2} , SE = 3.74×10^{-2} , z-value = -2.07, p-value = 3.81×10^{-2} ; mean annual temperature estimated effect = 6.62×10^{-1} , SE = 3.35×10^{-1} , z-value = 1.97, p-value = 4.814×10^{-2}) (Table S5). Land use type also predicted *Ae. albopictus* presence (Kruskal-Wallis chi-squared = 9.58, p-value = 8.311×10^{-3}). Specifically, *Ae. albopictus* was significantly more likely to be observed in residential settings (present at 13/17 sites) than in forested settings (present at 0/8 sites) (Kruskal-Wallis chi-squared = 9.03, Bonferroni-adjusted p-value = 7.98×10^{-3}), and its presence in agricultural settings (present at 4/12 sites) did not differ significantly compared to either residential (Kruskal-Wallis chi-squared = 2.41, adjusted p-value = 3.6×10^{-1}) or forested (Kruskal-Wallis chi-squared = 3.04, adjusted p-value = 2.43×10^{-1}) settings. Fourteen of the 15 sites where *Ae. albopictus* was present were surrounded by less than 25% tree cover within a 50 m radius. The only site within a pine plantation was a clear outlier, where *Ae. albopictus* was present under 85% tree cover (Fig. 4B).

In contrast to species richness, community composition and dispersion were predicted by land use type. PERMANOVA results comparing all three land uses showed that land use significantly affected community composition (Sum of squares = 2.113, $R^2 = 0.16$, F-value = 3.03, p-value = 0.001), and pairwise PERMANOVAs showed that agricultural and forest mosquito communities differed significantly from residential communities (Table 1). Wider dispersion among agricultural compared to residential mosquito communities (average distance to the median: agriculture = 0.612, forest = 0.572, residential = 0.497; Tukey test adjusted p-values: residential – agriculture = 0.0422, residential – forested = 0.303, forested – agriculture = 0.736) likely contributed to the community dissimilarity detected between these land uses (Anderson and Walsh, 2013) (Fig. 5). NMDS visualization of communities grouped by land use type suggests that more variable agricultural communities bridge relatively distinct forest and residential communities (NMDS stress = 0.12), in agreement with the statistical test results (Fig. 5, Table 1). The wider variation among agricultural sites is also evident from the species observation table: no single species was observed at more than 1/3 of all agricultural sites, whereas *Ae. albopictus* and Unidentified Culicidae 1 were both observed at > 70% of residential sites, and *Culex nigripalpus*, *Wyeomyia adelpha/guatemala*, and *Wyeomyia complosa* were each observed at > 60% of forested sites (Table S1).

Table 1
PERMANOVA results for community composition compared among land use types. Asterisks indicate $p < 0.05$.

Land use pair	Sum of squares	F-Value	R^2	Bonferroni-adjusted p-value
agriculture vs. forest	0.591	1.45	0.0785	0.291 *
agriculture vs. residential	0.788	2.22	0.0846	0.021 *
forest vs. residential	1.56	4.63	0.181	0.003 *

Finally, generalized dissimilarity modeling (GDM) indicated that environmental gradients explained little of the species turnover among sites. Among the spatial scales for which tree cover was calculated, the model using the 50m radius explained the highest amount of species turnover among sites (Table S6). The model that included mean annual temperature, geographic distance, and tree cover at the 50m radius explained 7% of species turnover among sites. Elevation showed no relationship with species turnover. Whereas increasing tree cover was associated with a consistent increase in community turnover, increasing temperature was associated with a steep increase in community turnover up to a plateau around 22 °C (Fig. 6).

Discussion

We found that local tree cover, but not land use (residential, agricultural, or forest), predicted mosquito species richness: more diverse communities occurred at higher tree cover (supporting H1) (Fig. 3, Table S2). By contrast, community composition was more predictable for forested and residential land uses, and more variable among agricultural sites (supporting H4) (Fig. 5, Table 1). Environmental gradients of tree cover, climate, and geographic distance explained 7.2% of species turnover among sites (Fig. 6, Table S6). *Ae. albopictus* presence varied significantly with both tree cover and land use, but in the opposite direction from mosquito diversity: *Ae. albopictus* occurrence probability increased with lower tree cover and in residential compared to forested sites (supporting H2) (with intermediate probability in agricultural sites; Fig. 4, Table S4). Overall, our results add to support from other taxa for the value of both natural and semi-natural habitat in sustaining biodiversity and ecosystem services—here, in the form of protection against an invasive mosquito that is a major vector of human disease.

Mosquito diversity

The spatial scales at which tree cover predicted mosquito species richness were small, and comparable to previous findings for other taxa in the same study area, highlighting the disproportionately positive impact of small patches of trees on biodiversity. The radius at which tree cover best predicted species richness was 250 m; by comparison, biodiversity was correlated with tree cover at small spatial scales for non-flying mammals (70 m), bats (50–60 m), birds (30 m), reptiles (50 m), and amphibians (80 m) in the same region of Costa Rica (Mendenhall et al., 2014, 2016; Frank et al., 2017). Although the radius at which mosquito species richness responded most strongly to tree cover was larger compared to

previously studied taxa, the spatial scale remained local, and significant effects of tree cover were found at radii as small as 80 m (Fig. 3). Our observation of a positive relationship between species richness and tree cover aligns with those of many other studies of mosquito diversity along land cover gradients in locations including Latin America, Asia, and Europe (e.g., Johnson, Gómez and Pinedo-Vasquez, 2008; Thongsripong et al., 2013; Ferraguti et al., 2016; Chaverri et al., 2018), and clarifies the spatial scales at which tree cover shapes mosquito communities.

Our observation that mosquito community composition was distinct among different land uses is consistent with patterns observed both for other taxa in this system, and for mosquitoes in other regions (Mendenhall et al., 2014, 2016; Meyer Steiger, Ritchie and Laurance, 2016; da Silva Pessoa Vieira et al., 2022). In agricultural settings, relatively high species richness and community similarity with forested settings support the argument that farmlands can contribute substantially to biodiversity conservation (Fig. 5, Table 1) (e.g., Norris, 2008). However, the high proportion of species unique to forest habitats and the high species richness observed inside the large Las Cruces forest reserve also reaffirm the singular importance of forests and protected areas as refugia for biodiversity (Coetzee, Gaston and Chown, 2014; Mendenhall et al., 2016) (Fig. 3, Table S1). Additionally, the compositional variability among agricultural settings and the close community resemblance between some agricultural and residential sites indicate a need for additional research on how mosquito communities respond to specific land uses, crop assemblages, or management practices that can result in similar levels of tree cover (Fig. 5, Table 1). For example, organic farming methods are associated with higher arthropod diversity globally compared to conventional methods, and Kenyan ricelands that rely on natural rather than artificial irrigation have higher mosquito species richness Kenya (Muturi et al., 2006; Lichtenberg et al., 2017). Costa Rican croplands that are less intensively farmed may similarly support greater mosquito biodiversity, a pattern already observed for bird species richness (Hendershot et al., 2020).

Environmental gradients of tree cover and temperature shaped species turnover, but explained only 7% of variance in community composition, suggesting that additional habitat characteristics may play important roles in determining species composition. Such factors might include local microclimates, differences among types of tree cover (e.g., agricultural types, primary versus secondary forest), and/or the presence of vertebrate hosts preferred by different mosquito species. Differences in species abundances and community evenness, which were not quantified here, might also respond more strongly to gradual environmental change than the identities of the species present. However, our result that land use predicts species composition, while land cover predicts diversity, aligns with patterns of abundance-based Dipteran and Culicid diversity observed in the tropical Australian countryside (Smith and Mayfield, 2015; Meyer Steiger, Ritchie and Laurance, 2016).

Disease vectors

The most frequently observed disease vector, *Ae. albopictus*, was more likely to be observed in sites with lower surrounding tree cover and agricultural or residential land uses, suggesting that rural landscapes with more forest and tree cover may be more resistant to invasion by this species. These observations align with this species' well-established preferences for taking blood meals from humans and livestock

(Niebylski et al., 1994; Richards et al., 2006), and its association with rural, agricultural, suburban, and/or deforested settings in the Americas, Asia, and Africa (Gilotra, Rozeboom and Bhattacharya, 1967; Braks et al., 2003; Young et al., 2017; Câmara et al., 2020; Canelas et al., 2023). The 40–200 m distances where tree cover negatively affected *Ae. albopictus* presence fell within the 80–600 m range where tree cover positively affected species richness, suggesting promise for local tree cover management as a means of supporting both public health and biodiversity conservation. Protection against mosquito disease vectors conferred by tree cover may extend beyond *Ae. albopictus* to include at least 16 other significant vectors of human diseases that are favored by deforestation, including *Ae. aegypti*, multiple Anopheline malaria vectors, and the Amazonian malaria vector *Nyssorhynchus darlingi* (Burkett-Cadena and Vittor, 2018; Chaves et al., 2021).

Our finding that *Ae. albopictus* was associated with, but inconsistently observed in, agricultural settings (present in 33% of agriculture sites) (Fig. 4, Table S1) reinforces that agricultural lands have the potential to either harbor or resist invasive species, and suggests vector associations with agricultural subtypes as a key future research direction. In our survey, factors that differentiated the high tree-cover pine plantation and two of the six surveyed coffee plantations as suitable habitat for *Ae. albopictus* are of particular interest (Fig. 4). Understanding vector responses to agriculture is particularly important because this land use is the most likely candidate for local tree cover management in Costa Rica due to its spatial extent, the established Payment for Ecosystem Services program infrastructure for incentivizing landowner forest retention and tree planting, and a previous finding that urban tree cover is correlated with dengue incidence in this region (Sánchez-Azofeifa et al., 2007; Troyo et al., 2009).

However, further research is needed to understand how tree cover management could be applied for such public health protections. For example, future studies should aim to capture the seasonality of vector presence and abundance along tree cover gradients (Calderón-Arguedas et al., 2008; Troyo et al., 2008; Romero-Vega et al., 2023); assess relationships between vector presence and other factors in habitats that have similar tree cover but differ in aspects such as crop type or tree cover geometry; and use reforestation efforts or experimental tree cover additions at relevant spatial scales to test mosquito community responses. In the study region, tree cover responses of *Ae. aegypti* and *An. albimanus* require clarification, as we observed these regionally important vectors too rarely for statistical analysis, and saw unexpected rarity and apparent habitat generalism in the canonically widespread and urban vector *Ae. aegypti* (Fig. 2, Table S1) (Troyo et al., 2006; Cáceres et al., 2012). Additionally, the potential disease control benefits of local tree cover should be tested with human pathogen surveillance in field-captured mosquitoes from different environments, and by comparison of disease case data to mosquito community data. In addition to dengue, Zika, and malaria, St. Louis Encephalitis Virus should be considered for surveillance in rural areas where humans and animals live in close proximity, because two potential vectors—*Cx. quinquefasciatus* and *Cx. nigripalpus*—were common, and this unmonitored disease is already widespread among both domesticated and wild animal hosts in Costa Rica (Medlin et al., 2016; A. Chaves et al., 2021; Piche-Ovares et al., 2023).

Conclusions

Overall, our results join a large body of evidence for the importance of local tree cover to maintaining both biodiversity and ecosystem services, adding prevention of disease vector proliferation to a list of benefits including water quality, crop pollination, carbon sequestration, and soil health, among others (Millennium Ecosystem Assessment, 2005; Jose, 2009; Barrios et al., 2018; Frishkoff et al., 2019). Our findings follow patterns observed repeatedly across the globe associating tree cover with higher mosquito species richness and lower disease vector presence, suggesting broad applicability of local tree cover management as a combined vector control and biodiversity conservation method. Further, we shed light on the contexts in which tree cover both increases mosquito species richness and decreases *Ae. albopictus* presence, namely at spatial scales of 80–200m (Figs. 2,3; Tables S2, S4). We also find that at larger spatial scales of 1000m, warm mean annual temperatures increase habitat suitability for *Ae. albopictus* and limit tree cover contributions to species richness, but note that other factors that covary with climate across the study region may contribute to this result (Figure S1, Tables S3, S5). Although the specific mechanisms and characteristics by which tree cover inhibits disease vectors remain unclear, the regularity with which simple measures of local tree cover correlate with both biodiversity and ecosystem services represents a practicable solution for fostering both human and ecosystem health in countryside landscapes.

Declarations

Funding

This work was funded by National Institutes of Health 331 (R35GM133439, R01AI168097, R01AI102918), the National Science Foundation (DEB3322011147, with Fogarty International Center), the Stanford Center for Innovation in Global Health, King Center on Global Development, Woods Institute for the Environment, the Ward Wilson Woods Jr Environmental Study Fund, the Lewis and Clark Fund from the American Philosophical Society, and the Bing-Mooney Fellowship.

Permits

Field collections and sample exports were carried out under general insect collecting permit R-036-2017-OT-CONAGEBIO, Costa Rica export permit 434-DGVS-2016, USDA veterinary import permit 134109, and Health and Human Services permit 2017-08-079.

Author contributions

All authors contributed to the study conception and design. MH, JS, and CA collected the data. MH and JF prepared the data, and JF analyzed the data. The first draft of the manuscript was written by JF, and all authors contributed to subsequent versions of the manuscript. All authors read and approved the final manuscript.

The authors have no competing interests to declare.

Data availability

The data presented in this manuscript are available in the DRYAD repository at <https://doi.org/10.5061/dryad.p8cz8w9xg>.

Acknowledgements

We thank the many landowners who generously provided access to research sites; Henry Sandi Amador, Esteban Calderon, and Erick Barrantes of the Organization for Tropical Studies for field assistance; Jamieson O'Marr for field and laboratory assistance; Gretchen Daily for advice on the project design; and Mordecai lab members for laboratory assistance and helpful feedback throughout all stages of the project. This work was funded by National Institutes of Health 331 (R35GM133439, R01AI168097, R01AI102918), the National Science Foundation (DEB3322011147, with Fogarty International Center), the Stanford Center for Innovation in Global Health, King Center on Global Development, Woods Institute for the Environment, the Ward Wilson Woods Jr Environmental Study Fund, the Lewis and Clark Fund from the American Philosophical Society, and the Bing-Mooney Fellowship.

References

1. Addicott, J.F. (1974) 'Predation and prey community structure: An experimental study of the effect of mosquito larvae on the protozoan communities of pitcher plants', *Ecology*, 55(3), pp. 475–492. Available at: <https://doi.org/10.2307/1935141>.
2. Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) 'Basic local alignment search tool', *Journal of Molecular Biology*, 215(3), pp. 403–410. Available at: [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
3. Anderson, M.J. and Walsh, D.C.I. (2013) 'PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing?', *Ecological Monographs*, 83(4), pp. 557–574. Available at: <https://doi.org/10.1890/12-2010.1>.
4. Auguie, B., Antonov, A. and Auguie, M.B., 2017. 'Package 'gridExtra'', *Miscellaneous Functions for "Grid" Graphics*.
5. Barrios, E., Valencia, V., Jonsson, M., Brauman, A., Hairiah, K., Mortimer, P.E., and Okubo, S. (2018) 'Contribution of trees to the conservation of biodiversity and ecosystem services in agricultural landscapes', *International Journal of Biodiversity Science, Ecosystem Services & Management*, 14(1), pp. 1–16. Available at: <https://doi.org/10.1080/21513732.2017.1399167>.
6. Benedict, M.Q., Levine, R.S., Hawley, and W.A., Lounibos, L. P. (2007) 'Spread of the tiger: Global risk of invasion by the mosquito *Aedes albopictus*', *Vector-Borne and Zoonotic Diseases*, 7(1), pp. 76–85. Available at: <https://doi.org/10.1089/vbz.2006.0562>.

7. Braks, M.A.H., Honório, N.A., Lourenço-De-Oliveira, R., Juliano, S.A., and Lounibos, L.P. (2003) 'Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Southeastern Brazil and Florida', *Journal of Medical Entomology*, 40(6), pp. 785–794. Available at: <https://doi.org/10.1603/0022-2585-40.6.785>.
8. Brathwaite Dick, O., San Martín, J.L., Montoya, R.H., del Diego, J., Zambrano, B., and Dayan, G.H. (2012) 'The history of dengue outbreaks in the Americas', *The American Journal of Tropical Medicine and Hygiene*, 87(4), pp. 584–593. Available at: <https://doi.org/10.4269/ajtmh.2012.11-0770>.
9. Brosi, B.J., Daily, G.C. and Ehrlich, P.R. (2007) 'Bee community shifts with landscape context in a tropical countryside', *Ecological Applications*, 17(2), pp. 418–430. Available at: <https://doi.org/10.1890/06-0029>.
10. Burkett-Cadena, N.D. and Vittor, A.Y. (2018) 'Deforestation and vector-borne disease: Forest conversion favors important mosquito vectors of human pathogens', *Basic and Applied Ecology*, 26, pp. 101–110. Available at: <https://doi.org/10.1016/j.baae.2017.09.012>.
11. Cáceres, L., Rovira, J., Torres, R., García, A., Calzada, J., and De La Cruz, M. (2012) 'Characterization of *Plasmodium vivax* malaria transmission at the border of Panamá and Costa Rica', *Biomedica*, 32(4), pp. 557–569. Available at: <https://doi.org/10.1590/S0120-41572012000400011>
12. Calderón-Arguedas, O., Troyo, A., Solano, M.E., Avendaño, A., and Beier, J.C. (2008) 'Urban mosquito species (Diptera: Culicidae) of dengue endemic communities in the Greater Puntarenas area, Costa Rica', *Revista de Biología Tropical*, 57(4). Available at: <https://doi.org/10.15517/rbt.v57i4.5459>.
13. Calderón-Arguedas, O., Avendaño, A., López-Sánchez, W., and Troyo, A. (2010) 'Expansion of *Aedes albopictus* skull in Costa Rica', *Rev. Ibero-Latinoam. Parasitol.*, 69(2), pp. 220–222.
14. Calderón-Arguedas, O., Troyo, A., Moreira-Soto, R.D., Marín, R., and Taylor, L. (2015) 'Dengue viruses in *Aedes albopictus* Skuse from a pineapple plantation in Costa Rica', *Journal of Vector Ecology*, 40(1), pp. 184–186. Available at: <https://doi.org/10.1111/jvec.12149>.
15. Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P.(2016) 'DADA2: High-resolution sample inference from Illumina amplicon data', *Nature Methods*, 13(7), pp. 581–583. Available at: <https://doi.org/10.1038/nmeth.3869>.
16. Câmara, D.C.P., Pinel, C.S., Rocha, G.P., Codeço, C.T., and Honório, N.A. (2020) 'Diversity of mosquito (Diptera: Culicidae) vectors in a heterogeneous landscape endemic for arboviruses', *Acta Tropica*, 212, p. 105715. Available at: <https://doi.org/10.1016/j.actatropica.2020.105715>.
17. Canelas, T., Thomsen, E., KAmgang, B., and Kelly-Hope, L.A. (2023) 'Demographic and environmental factors associated with the distribution of *Aedes albopictus* in Cameroon', *Medical and Veterinary Entomology*, 37(1), pp. 143–151. Available at: <https://doi.org/10.1111/mve.12619>.
18. Chaverri, L.G., Dillenbeck, C., Lewis, D., Rivera, C., Romero, L.M., and Chaves, L.F. (2018) 'Mosquito species (Diptera: Culicidae) diversity from ovitraps in a Mesoamerican tropical rainforest', *Journal of Medical Entomology*, 55(3), pp. 646–653. Available at: <https://doi.org/10.1093/jme/tjx254>.
19. Chaves, A., Piche-Ovares, M., Ibarra-Cerdeña, C.N., Corrales-Aguilar, E., Suzán, G., Moreira-Soto, A., and Gutiérrez-Espeleta, G. (2021) 'Serosurvey of nonhuman primates in Costa Rica at the human–wildlife

- interface reveals high exposure to Flaviviruses', *Insects*, 12(6), p. 554. Available at: <https://doi.org/10.3390/insects12060554>.
20. Chaves, L.S.M., Bergo, E.S., Conn, J.E., Laporta, G.Z., Prist, P.R., and Sallum, M.A.M. (2021) 'Anthropogenic landscape decreases mosquito biodiversity and drives malaria vector proliferation in the Amazon rainforest', *PLOS ONE*, 16(1), p. e0245087. Available at: <https://doi.org/10.1371/journal.pone.0245087>.
 21. Chazdon, R.L., Harvey, C.A., Komar, O., Griffith, D.M., Ferguson, B.G., Martínez-Ramos, M., *et. al.* (2009) 'Beyond reserves: A research agenda for conserving biodiversity in human-modified tropical landscapes', *Biotropica*, 41(2), pp. 142–153. Available at: <https://doi.org/10.1111/j.1744-7429.2008.00471.x>.
 22. Coetzee, B.W.T., Gaston, K.J. and Chown, S.L. (2014) 'Local scale comparisons of biodiversity as a test for global protected area ecological performance: A meta-analysis', *PLOS ONE*, 9(8), p. e105824. Available at: <https://doi.org/10.1371/journal.pone.0105824>.
 23. Daugherty, M.P., Alto, B.W. and Juliano, S.A. (2000) 'Invertebrate carcasses as a resource for competing *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae)', *Journal of Medical Entomology*, 37(3), pp. 364–372. Available at: <https://doi.org/10.1093/jmedent/37.3.364>.
 24. Echeverri, A., Smith, J.R., MacArthur-Waltz, D., Lauck, K.S., Anderson, C.B., Monge Vargas, R., *et. al.* (2022) 'Biodiversity and infrastructure interact to drive tourism to and within Costa Rica', *Proceedings of the National Academy of Sciences*, 119(11), p. e2107662119. Available at: <https://doi.org/10.1073/pnas.2107662119>.
 25. Ferraguti, M., Martínez-de la Puente, J., Roiz, D., Ruiz, S., Soriguer, R., and Figuerola, J. (2016) 'Effects of landscape anthropization on mosquito community composition and abundance', *Scientific Reports*, 6(1), p. 29002. Available at: <https://doi.org/10.1038/srep29002>.
 26. Fick, S.E. and Hijmans, R.J. (2017) 'WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas', *International journal of climatology*, 37(12), pp. 4302–4315.
 27. Fitzpatrick, M., Mokany, K., Manion, G., Nieto-Lugilde, D., and Ferrier, S. (2022) 'gdm: Generalized Dissimilarity Modeling', *R package*. Available at: <https://CRAN.R-project.org/package=gdm>.
 28. Frank, H.K., Frishkoff, L.O., Mendenhall, C.D., Daily, G.C., and Hadly, E.A. (2017) 'Phylogeny, traits, and biodiversity of a Neotropical bat assemblage: Close relatives show similar responses to local deforestation', *The American Naturalist*, 190(2), pp. 200–212. Available at: <https://doi.org/10.1086/692534>.
 29. Franklino, L.H.V., Jones, K.E., Redding, D.W., and Abubakar, I. (2019) 'The effect of global change on mosquito-borne disease', *The Lancet Infectious Diseases*, 19(9), pp. e302–e312. Available at: [https://doi.org/10.1016/S1473-3099\(19\)30161-6](https://doi.org/10.1016/S1473-3099(19)30161-6).
 30. Frishkoff, L.O., Ke, A., Martins, I.S., Olimpi, E.M., and Karp, D.S. (2019) 'Countryside biogeography: The controls of species distributions in human-dominated landscapes', *Current Landscape Ecology Reports*, 4(2), pp. 15–30. Available at: <https://doi.org/10.1007/s40823-019-00037-5>.

31. Garmendia, A.E., Van Kruiningen, H.J. and French, R.A. (2001) 'The West Nile virus: Its recent emergence in North America', *Microbes and Infection*, 3(3), pp. 223–229. Available at: [https://doi.org/10.1016/S1286-4579\(01\)01374-0](https://doi.org/10.1016/S1286-4579(01)01374-0).
32. Giam, X. (2017) 'Global biodiversity loss from tropical deforestation', *Proceedings of the National Academy of Sciences*, 114(23), pp. 5775–5777. Available at: <https://doi.org/10.1073/pnas.1706264114>.
33. Gilotra, S.K., Rozeboom, L.E. and Bhattacharya, N.C. (1967) 'Observations on possible competitive displacement between populations of *Aedes aegypti* Linnaeus and *Aedes albopictus* Skuse in Calcutta', *Bulletin of the World Health Organization*, 37(3), pp. 437–446.
34. Gutman, G., Janetos A. C., Justice, C.O., Moran, E.F., Mustard, J.F., Rindfuss, R.R. *et. al.* (eds) (2004) *Land Change Science: Observing, Monitoring and Understanding Trajectories of Change on the Earth's Surface*. Dordrecht: Springer Netherlands (Remote Sensing and Digital Image Processing). Available at: <https://doi.org/10.1007/978-1-4020-2562-4>.
35. Hardulak, L.A., Morinière, J., Hausmann, A., Hendrich, L., Schmidt, S., Doczkal, D., *et. al.* (2020) 'DNA metabarcoding for biodiversity monitoring in a national park: Screening for invasive and pest species', *Molecular Ecology Resources*, 20(6), pp. 1542–1557. Available at: <https://doi.org/10.1111/1755-0998.13212>.
36. Heard, S.B. (1994) 'Pitcher-plant midges and mosquitoes: A processing chain commensalism', *Ecology*, 75(6), pp. 1647–1660. Available at: <https://doi.org/10.2307/1939625>.
37. Hendershot, J.N., Smith, J.R., Anderson, C.B., Letten, A.D., Frishkoff, L.O., Zook, J.R., *et al.* (2020) 'Intensive farming drives long-term shifts in avian community composition', *Nature*, 579(7799), pp. 393–396. Available at: <https://doi.org/10.1038/s41586-020-2090-6>.
38. Hijmans, R.J., Van Etten, J., Cheng, J., Mattiuzzi, M., Sumner, M., Greenberg, J.A., Lamigueiro, O.P., Bevan, A., Racine, E.B., Shortridge, A. and Hijmans, M.R.J., 2015. Package 'raster'. *R package*, 734, p.473.
39. Hughes, J.B., Daily, G.C. and Ehrlich, P.R. (2002) 'Conservation of tropical forest birds in countryside habitats', *Ecology Letters*, 5(1), pp. 121–129. Available at: <https://doi.org/10.1046/j.1461-0248.2002.00294.x>.
40. Johnson, M.F., Gómez, A. and Pinedo-Vasquez, M. (2008) 'Land use and mosquito diversity in the Peruvian Amazon', *Journal of Medical Entomology*, 45(6), pp. 1023–1030. Available at: [https://doi.org/10.1603/0022-2585\(2008\)45\[1023:LUAMD\]2.0.CO;2](https://doi.org/10.1603/0022-2585(2008)45[1023:LUAMD]2.0.CO;2).
41. Jose, S. (2009) 'Agroforestry for ecosystem services and environmental benefits: An overview', *Agroforestry Systems*, 76(1), pp. 1–10. Available at: <https://doi.org/10.1007/s10457-009-9229-7>.
42. Karp, D.S., Mendenhall, C.D., Sandí, R.F., Chaumont, N., Ehrlich, P.R., Hadly, E.A., and Daily, G.C. (2013) 'Forest bolsters bird abundance, pest control and coffee yield', *Ecology Letters*, 16(11), pp. 1339–1347. Available at: <https://doi.org/10.1111/ele.12173>.
43. Kraemer, M.U., Sinka, M.E., Duda, K.A., Mylne, A.Q.N., Shearer, F.M., Barker, C.M., *et. al.* (2015) 'The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*', *eLife*, 4, p. e08347.

Available at: <https://doi.org/10.7554/eLife.08347>.

44. Laird, M. (1988) *The natural history of larval mosquito habitats*. Academic Press Ltd, London, UK. ISBN: 9780124340053. Available at: <http://www.cabdirect.org/cabdirect/abstract/19892056420> (Accessed: 5 July 2021).
45. Langhans, K.E., Schmitt, R.J.P., Chaplin-Kramer, R., Anderson, C.B., Vargas Bolaños, C., Vargas Cabezas, F., *et al.* (2022) 'Modeling multiple ecosystem services and beneficiaries of riparian reforestation in Costa Rica', *Ecosystem Services*, 57, p. 101470. Available at: <https://doi.org/10.1016/j.ecoser.2022.101470>.
46. LaPointe, D.A., Atkinson, C.T. and Samuel, M.D. (2012) 'Ecology and conservation biology of avian malaria: Ecology of avian malaria', *Annals of the New York Academy of Sciences*, 1249(1), pp. 211–226. Available at: <https://doi.org/10.1111/j.1749-6632.2011.06431.x>.
47. Lemon, S.M., Sparling, P.F., Hamburg, M.A., Relman, D.A., Choffnes, E.R., and Mack, A. (eds) (2008) *Vector-borne diseases - understanding the environmental, human health, and ecological connections: workshop summary*. Washington, D.C.: National Academies Press.
48. Lichtenberg, E.M., Kennedy, C.M., Kremen, C., Batáry, P., Berendse, F., Bommarco, R., *et al.* (2017) 'A global synthesis of the effects of diversified farming systems on arthropod diversity within fields and across agricultural landscapes', *Global Change Biology*, 23(11), pp. 4946–4957. Available at: <https://doi.org/10.1111/gcb.13714>.
49. Long, J.A. and Long, M.J.A., 2019. Package 'interactions'. See <https://interactions.jacob-long.com>.
50. Maxwell, S.L., Fuller, R.A., Brooks, T.M., and Watson, J.E.M. (2016) 'Biodiversity: The ravages of guns, nets and bulldozers', *Nature*, 536(7615), pp. 143–145. Available at: <https://doi.org/10.1038/536143a>.
51. Medlin, S., Deardorff, E.R., Hanley, C.S., Vergneau-Grosset, C., Siudak-Campfield, A., Dallwig, R., *et al.* (2016) 'Serosurvey of selected arboviral pathogens in free-ranging, two-toed sloths (*Choloepus hoffmanni*) and three-toed sloths (*Bradypus variegatus*) in Costa Rica, 2005–07', *Journal of wildlife diseases*, 52(4), pp. 883–892.
52. Mendenhall, C.D., Fishkoff, L.O., Santos-Barrera, G., Pacheco, J., Mesfun, E., Quijano, F. M., *et al.* (2014) 'Countryside biogeography of Neotropical reptiles and amphibians', *Ecology*, 95(4), pp. 856–870. Available at: <https://doi.org/10.1890/12-2017.1>.
53. Mendenhall, C.D., Shields-Estrada, A., Krishnaswami, A.J., and Daily, G.C. (2016) 'Quantifying and sustaining biodiversity in tropical agricultural landscapes', *Proceedings of the National Academy of Sciences*, 113(51), pp. 14544–14551. Available at: <https://doi.org/10.1073/pnas.1604981113>.
54. Meyer Steiger, D.B., Ritchie, S.A. and Laurance, S.G.W. (2016) 'Mosquito communities and disease risk influenced by land use change and seasonality in the Australian tropics', *Parasites & Vectors*, 9(1), p. 387. Available at: <https://doi.org/10.1186/s13071-016-1675-2>.
55. Millennium ecosystem assessment, M. (2005) *Ecosystems and human well-being*. Island press Washington, DC.

56. Mordecai, E.A., Caldwell, J.M., Grossman, M.K., Lippi, C.A., Johnson, L.R., Neira, M., *et al.* (2019) 'Thermal biology of mosquito-borne disease', *Ecology Letters*. Edited by J. (Jeb) Byers, p. ele.13335. Available at: <https://doi.org/10.1111/ele.13335>.
57. Muturi, E.J., Shililu, J., Jacob, B., Gu, W., Githure, J., and Novak, R. (2006) 'Mosquito species diversity and abundance in relation to land use in a riceland agroecosystem in Mwea, Kenya', *Journal of Vector Ecology*, 31(1), pp. 129–137. Available at: [https://doi.org/10.3376/1081-1710\(2006\)31\[129:MSDAAI\]2.0.CO;2](https://doi.org/10.3376/1081-1710(2006)31[129:MSDAAI]2.0.CO;2).
58. Niebylski, M.L., Savage, H.M., Nasci, R.S., and Craig, G.B. (1994) 'Blood hosts of *Aedes albopictus* in the United States', *Journal of the American Mosquito Control Association*, 10(3), pp. 447–450.
59. Norris, K. (2008) 'Agriculture and biodiversity conservation: opportunity knocks', *Conservation Letters*, 1(1), pp. 2–11. Available at: <https://doi.org/10.1111/j.1755-263X.2008.00007.x>.
60. Oksanen, J., Blanchet, F.G., Kindt, R., Legendr, P., Minchin, P.R., O'Hara, R.B., *et al.* (2013) 'Package "vegan"', *Community ecology package, version*, 2(9), pp. 1–295.
61. Pagès, H., Aboyoun, P., Gentleman, R., and DebRoy, S. (2022) 'Biostrings: Efficient manipulation of biological strings'. (R package). Available at: <https://bioconductor.org/packages/Biostrings>.
62. Pereira, H.M., Leadley, P.W., Proença, V., Alkemade, R., Scharlemann, J.P.W., Fernandez-Manjarrés, J.F., *et al.* (2010) 'Scenarios for global biodiversity in the 21st Century', *Science*, 330(6010), pp. 1496–1501. Available at: <https://doi.org/10.1126/science.1196624>.
63. Piche-Ovares, M., Romero-Vega, M., Vargas-González, D., Murillo, D.F.P, Soto-Garita, C., Francisco-Llamas, J., *et al.* (2023) 'Serosurvey in two dengue hyperendemic areas of Costa Rica evidence active circulation of WNV and SLEV in peri-domestic and domestic animals and in humans', *Pathogens*, 12(1), p. 7. Available at: <https://doi.org/10.3390/pathogens12010007>.
64. Poulin, B., Lefebvre, G. and Paz, L. (2010) 'Red flag for green spray: adverse trophic effects of Bti on breeding birds', *Journal of Applied Ecology*, 47(4), pp. 884–889. Available at: <https://doi.org/10.1111/j.1365-2664.2010.01821.x>.
65. Prevedello, J.A., Winck, G.R., Weber, M.M., Nichols, E., Sinervo, B., and Xu, Y. (2019) 'Impacts of forestation and deforestation on local temperature across the globe', *PLOS ONE*. Edited by Y. Xu, 14(3), p. e0213368. Available at: <https://doi.org/10.1371/journal.pone.0213368>.
66. Ratnasingham, S. and Hebert, P.D.N. (2007) 'bold: The Barcode of Life data system (<http://www.barcodinglife.org>)', *Molecular Ecology Notes*, 7(3), pp. 355–364. Available at: <https://doi.org/10.1111/j.1471-8286.2007.01678.x>.
67. Reisen, W.K. (2003) 'Epidemiology of St. Louis encephalitis virus', *Advances in virus research*, 61, pp. 139–184.
68. Rezza, G. (2012) '*Aedes albopictus* and the reemergence of dengue', *BMC Public Health*, 12(1), p. 72. Available at: <https://doi.org/10.1186/1471-2458-12-72>.
69. Richards, S.L., Ponnusamy, L., Unnasch, T.R., Hassan, H.K., and Apperson, C.S. (2006) 'Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) in relation to availability of human and domestic

- animals in suburban landscapes of central North Carolina', *Journal of Medical Entomology*, 43(3), pp. 543–551. Available at: <https://doi.org/10.1093/jmedent/43.3.543>.
70. Ricketts, T.H., Daily, G.C., Ehrlich, P.R., and Michener, C.D. (2004) 'Economic value of tropical forest to coffee production', *Proceedings of the National Academy of Sciences*, 101(34), pp. 12579–12582. Available at: <https://doi.org/10.1073/pnas.0405147101>.
71. Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, A., Firth, D. and Ripley, M.B., 2013. Package 'mass'. *Cran r*, 538, pp.113-120.
72. Rojas-Araya, D., Marín-Rodríguez, R., Gutiérrez-Alvarado, M., Romero-Vega, L.M., Calderón-Arguedas, O., and Troyo, A. (2017) 'New records of *Aedes albopictus* (Skuse) in four locations of Costa Rica', *Revista Biomédica*, 28(2), pp. 79–88. Available at: <https://doi.org/10.32776/revbiomed.v28i2.575>.
73. Romero-Vega, L.M., Piche-Ovares, M., Soto-Garita, C., Barantes Murillo, D.F., Chaverri, L.G., Alfaro-Alarcón, A., *et al.* (2023) 'Seasonal changes in the diversity, host preferences and infectivity of mosquitoes in two arbovirus-endemic regions of Costa Rica', *Parasites & Vectors*, 16(1), p. 34. Available at: <https://doi.org/10.1186/s13071-022-05579-y>.
74. Sala, O.E. (2000) 'Global biodiversity scenarios for the year 2100', *Science*, 287(5459), pp. 1770–1774. Available at: <https://doi.org/10.1126/science.287.5459.1770>.
75. Sánchez-Azofeifa, G.A., Pfaff, A., Robalino, J.A., and Boomhower, J.P. (2007) 'Costa Rica's Payment for Environmental Services program: intention, implementation, and impact', *Conservation Biology*, 21(5), pp. 1165–1173. Available at: <https://doi.org/10.1111/j.1523-1739.2007.00751.x>.
76. da Silva Pessoa Vieira, C.J., Steiner São Bernardo, C., Ferriera da Silva, D.J., Rigotti Kubiszkeski, J., Serpa Barreto, E., de Oliveira Monteiro, H.A., *et al.* (2022) 'Land-use effects on mosquito biodiversity and potential arbovirus emergence in the Southern Amazon, Brazil', *Transboundary and Emerging Diseases*, 69(4), pp. 1770–1781. Available at: <https://doi.org/10.1111/tbed.14154>.
77. Simmons, C.P., Farrar, J.J., van Vinh Chau, N., and Wills, B. (2012) 'Dengue', *New England Journal of Medicine*, 366(15), pp. 1423–1432. Available at: <https://doi.org/10.1056/NEJMra1110265>.
78. Smith, R.J. (2022) *R package 'ecole', phytomosaic/ecole: ecole: School of Ecology Package*. Available at: <https://rdr.io/github/phytomosaic/ecole/>.
79. Smith, T.J. and Mayfield, M.M. (2015) 'Diptera species and functional diversity across tropical Australian countryside landscapes', *Biological Conservation*, 191, pp. 436–443. Available at: <https://doi.org/10.1016/j.biocon.2015.07.035>.
80. Thongsripong, P., Green, A., Kittayapong, P., Kapan, D., Wilcox, B., and Bennett, S. (2013) 'Mosquito vector diversity across habitats in central Thailand endemic for dengue and other arthropod-borne diseases', *PLoS Neglected Tropical Diseases*, 7(10), p. e2507. Available at: <https://doi.org/10.1371/journal.pntd.0002507>.
81. Troyo, A., Porcelain, S.L., Calderón-Arguedas, O., Chadee, D.D., and Beier, J.C. (2006) 'Dengue in Costa Rica: the gap in local scientific research', *Revista Panamericana de Salud Pública*, 20, pp. 350–360. Available at: <https://doi.org/10.1590/S1020-49892006001000012>.

82. Troyo, A., Calderón-Arguedas, O., Fuller, D.O., Solano, M.E., Avendaño, A., *et al.* (2008) 'Seasonal profiles of *Aedes aegypti* (Diptera: Culicidae) larval habitats in an urban area of Costa Rica with a history of mosquito control', *Journal of Vector Ecology*, 33(1), pp. 76–88. Available at: [https://doi.org/10.3376/1081-1710\(2008\)33\[76:SPOAAD\]2.0.CO;2](https://doi.org/10.3376/1081-1710(2008)33[76:SPOAAD]2.0.CO;2).
83. Troyo, A., Fuller, D.O., Calderón-Arguedas, O., Solano, M.E., and Beier, J.C. (2009) 'Urban structure and dengue incidence in Puntarenas, Costa Rica', *Singapore Journal of Tropical Geography*, 30(2), pp. 265–282. Available at: <https://doi.org/10.1111/j.1467-9493.2009.00367.x>.
84. Tsuda, Y., Suwonkerd, W., Chawprom, S., Prajakwong, S., and Takagi, M. (2006) 'Different spatial distribution of *Aedes aegypti* and *Aedes albopictus* along an urban-rural gradient and the relating environmental factors examined in three villages in northern Thailand', *Journal of the American Mosquito Control Association*, 22(2), pp. 222–228. Available at: [https://doi.org/10.2987/8756-971X\(2006\)22\[222:DSDOAA\]2.0.CO;2](https://doi.org/10.2987/8756-971X(2006)22[222:DSDOAA]2.0.CO;2).
85. Wickham, H (2007). "Reshaping data with the reshape package." *Journal of Statistical Software*, **21**(12), 1–20. <http://www.jstatsoft.org/v21/i12/>.
86. Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., *et al.* (2019) 'Welcome to the Tidyverse', *Journal of Open Source Software*, 4(43), p. 1686. Available at: <https://doi.org/10.21105/joss.01686>.
87. Wilke, C.O., Wickham, H. and Wilke, M.C.O., 2019. Package 'cowplot'. *Streamlined plot theme and plot annotations for 'ggplot2*, 1.
88. World Health Organization (1994) 'Outbreak of classic dengue, Costa Rica', *Weekly Epidemiological Record*, 69, pp. 85–86.
89. World Health Organization (2021) *Dengue data application*. Available at: <https://ntdhq.shinyapps.io/dengue5>.
90. Wright, E. (2016) 'Using DECIPHER v2.0 to analyze big biological sequence data in R', *The R Journal*, 8(1), pp. 352–359.
91. Young, K.I., Mundis, S., Widen, S.G., Wood, T.G., Tesh, R.B., Cardoso, J., *et al.* (2017) 'Abundance and distribution of sylvatic dengue virus vectors in three different land cover types in Sarawak, Malaysian Borneo', *Parasites & Vectors*, 10(1), p. 406. Available at: <https://doi.org/10.1186/s13071-017-2341-z>.
92. Zimmerman, R.H. (1992) 'Ecology of malaria vectors in the Americas and future direction', *Memórias do Instituto Oswaldo Cruz*, 87, pp. 371–383. Available at: <https://doi.org/10.1590/S0074-02761992000700064>

Figures

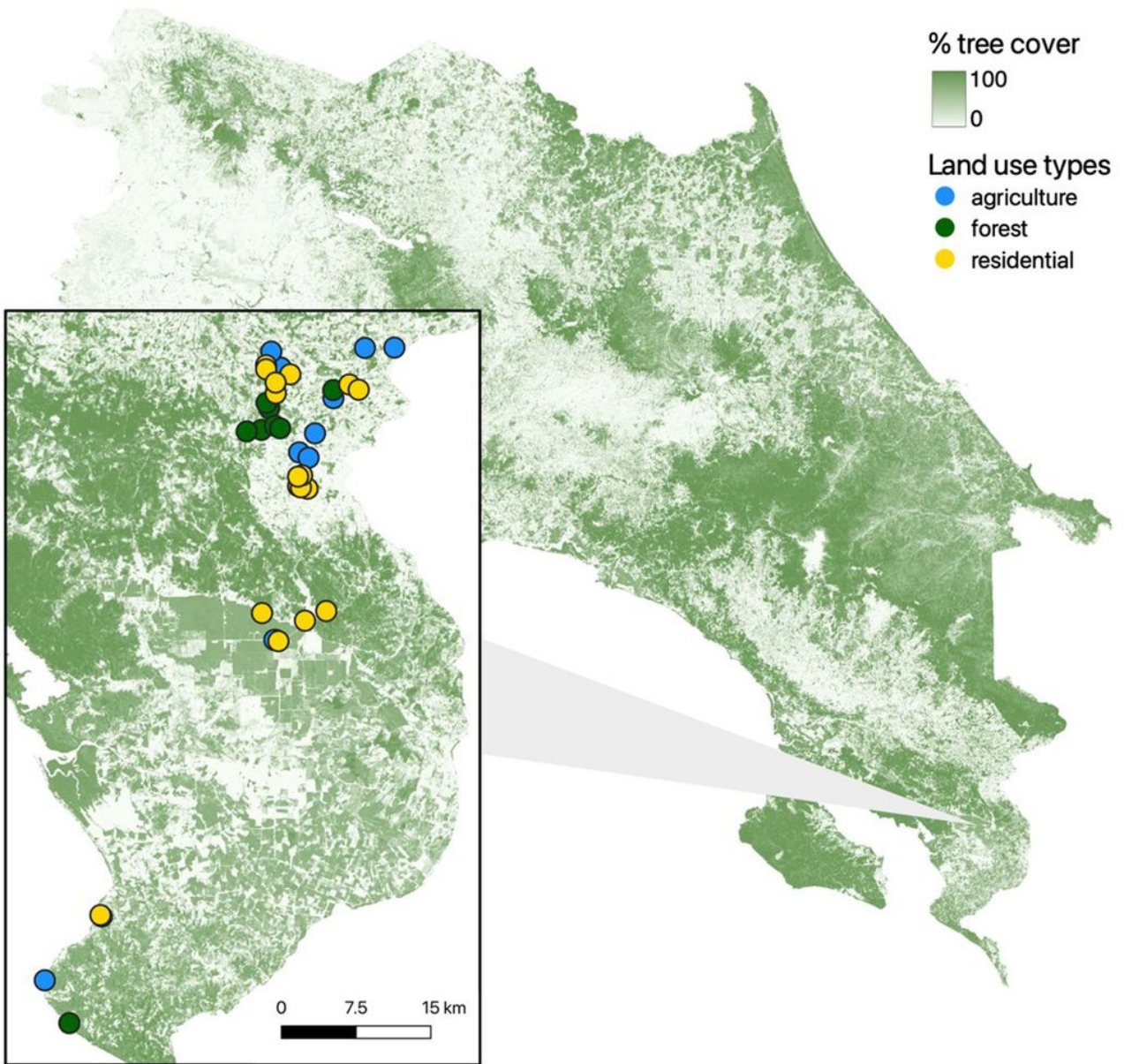


Figure 1

Tree cover and land use varied across study sites in Costa Rica. *Darker green indicates higher percent tree cover, remotely observed at 30 m resolution. Inset shows study sites, categorized by land use type. Blue, green, and yellow points denote agricultural, forest, and residential land uses, respectively.*

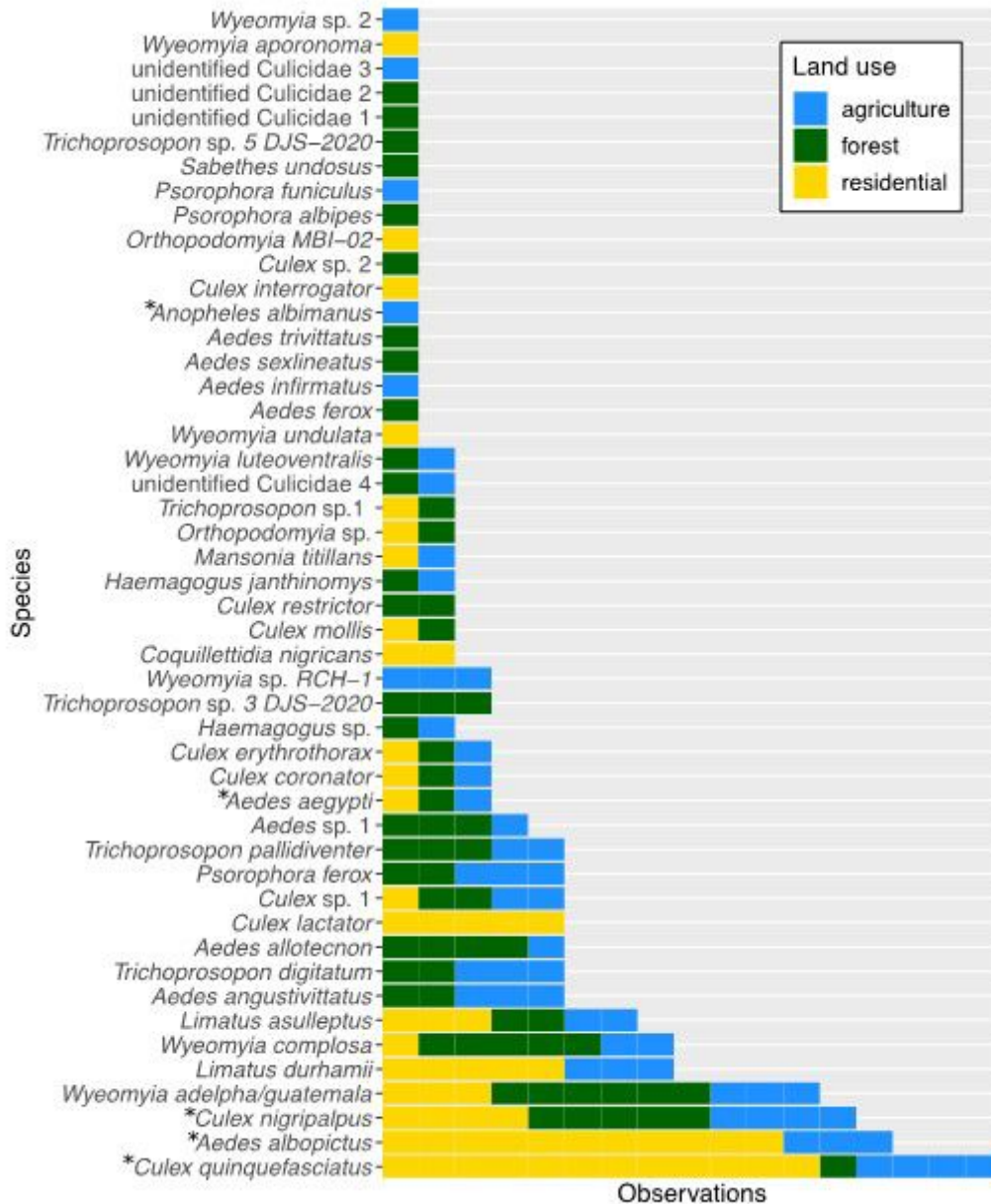


Figure 2

The commonness of 48 observed mosquito species varied both overall and among land use types. Blue bars show the number of presences observed for each species in agricultural sites, green bars show forest sites, and yellow bars show residential sites. Asterisks indicate species known to vector human diseases.

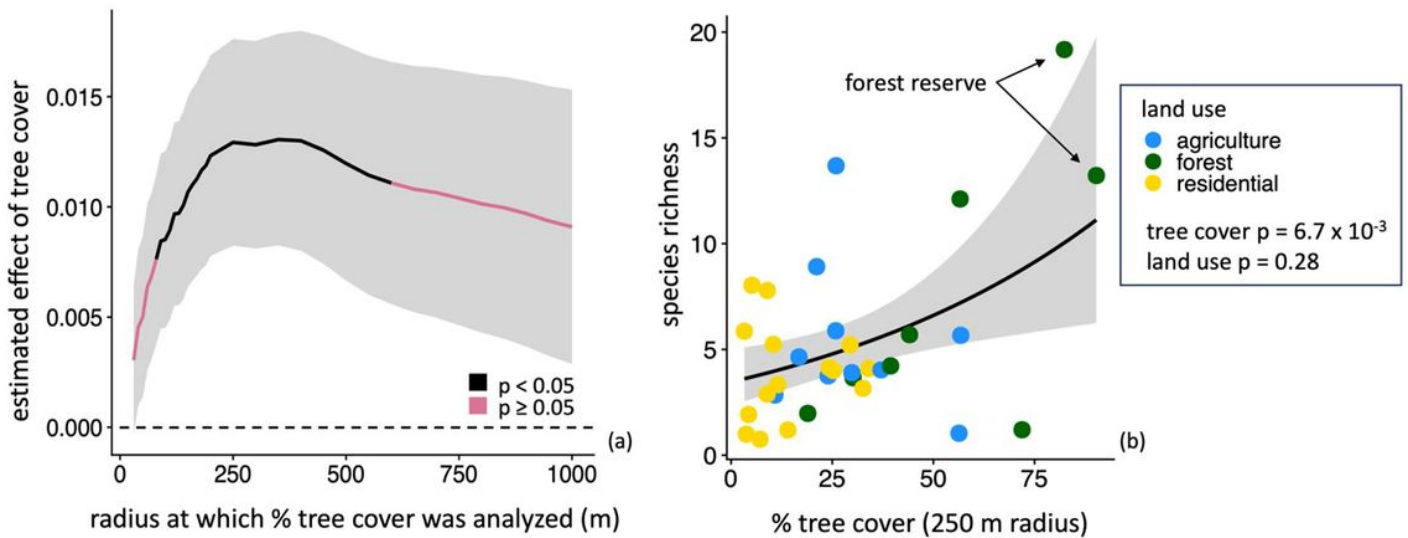


Figure 3

Species richness is correlated with tree cover surrounding survey sites for radii between 80 m and 600 m. (a) The estimated effect of surrounding tree cover calculated across spatial scales on species richness. Radii where the relationship between tree cover and species richness is significant ($p < 0.05$) are shown in black. Values above and below the dashed line are positive and negative, respectively. (b) Species richness increases with percent tree cover at the 250 m radius: the scale identified as having the strongest effect. Land use (colored points) and species richness are not significantly correlated. The site with the highest species richness and the site with the highest surrounding tree cover were both located in the Las Cruces forest reserve (arrows). In both panels, gray shading shows ± 1 SE.

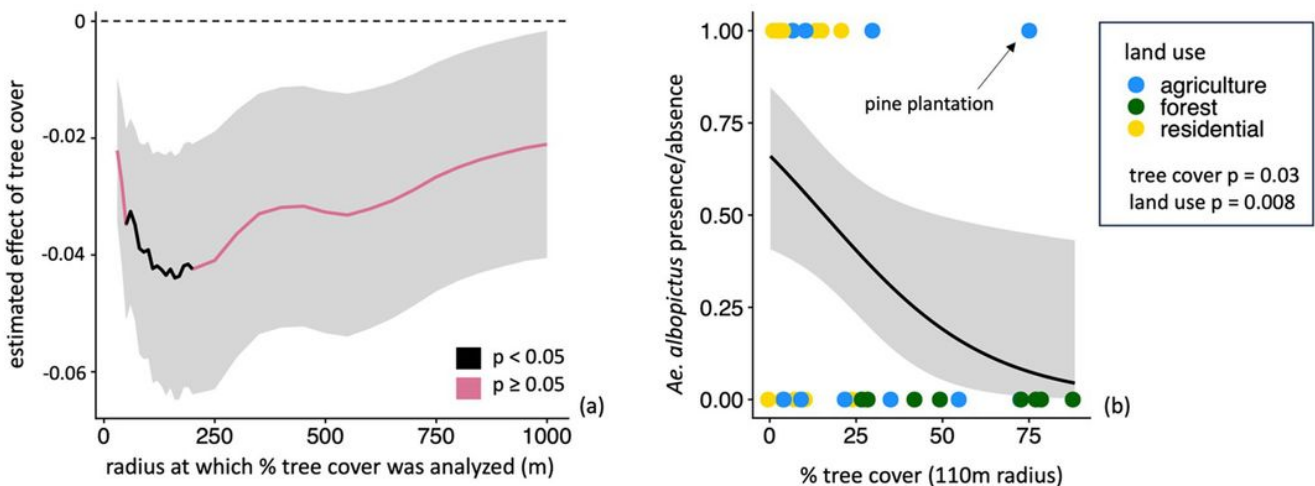


Figure 4

Aedes albopictus is most likely to be found at low tree cover levels and in residential settings. (a) The estimated effects of tree cover across spatial scales on *Ae. albopictus* presence, where black lines

indicate statistically significant ($p < 0.05$) effects and pink lines indicate non-significant ($p > 0.05$) relationships. Values above and below the dashed line are positive and negative, respectively. (b) Land use type (colored points), percent tree cover, and *Ae. albopictus* presence/absence at the highest-significance 110 m radius. In both panels, gray shading shows ± 1 SE.

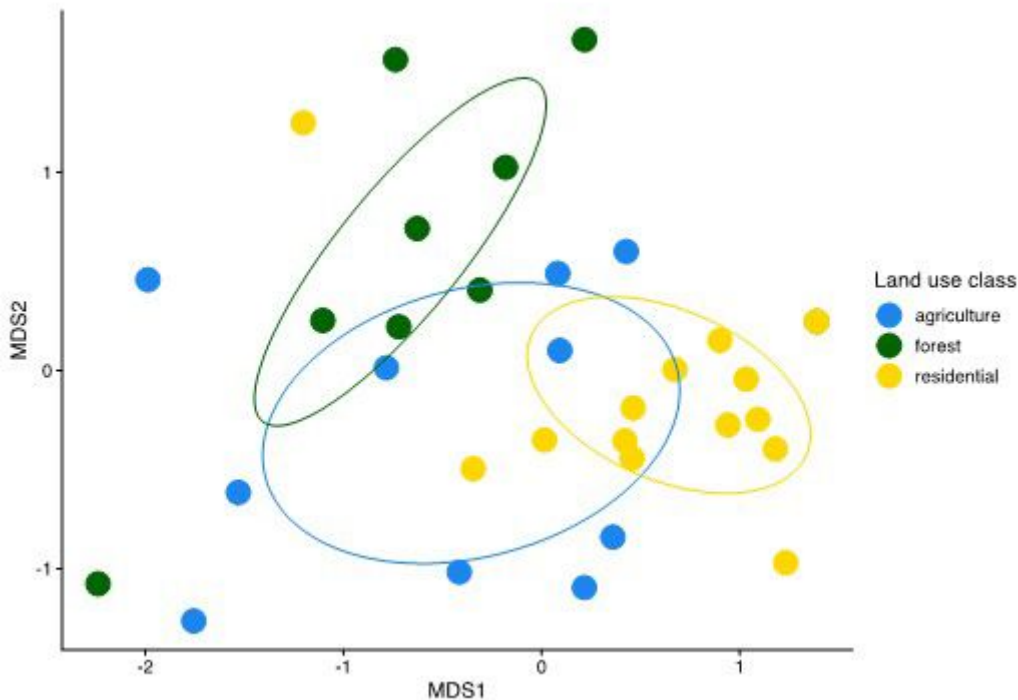


Figure 5

Distinct mosquito communities were observed in forest and residential land uses, while communities in agricultural settings were more variable. NMDS ordination visualization groups sites by community similarity, with colors indicating land use types. Each point represents the community at one study site, and the distance between points is smaller for more similar communities. Ellipses show 95% confidence intervals for ordination of agriculture, forest, and residential mosquito communities.

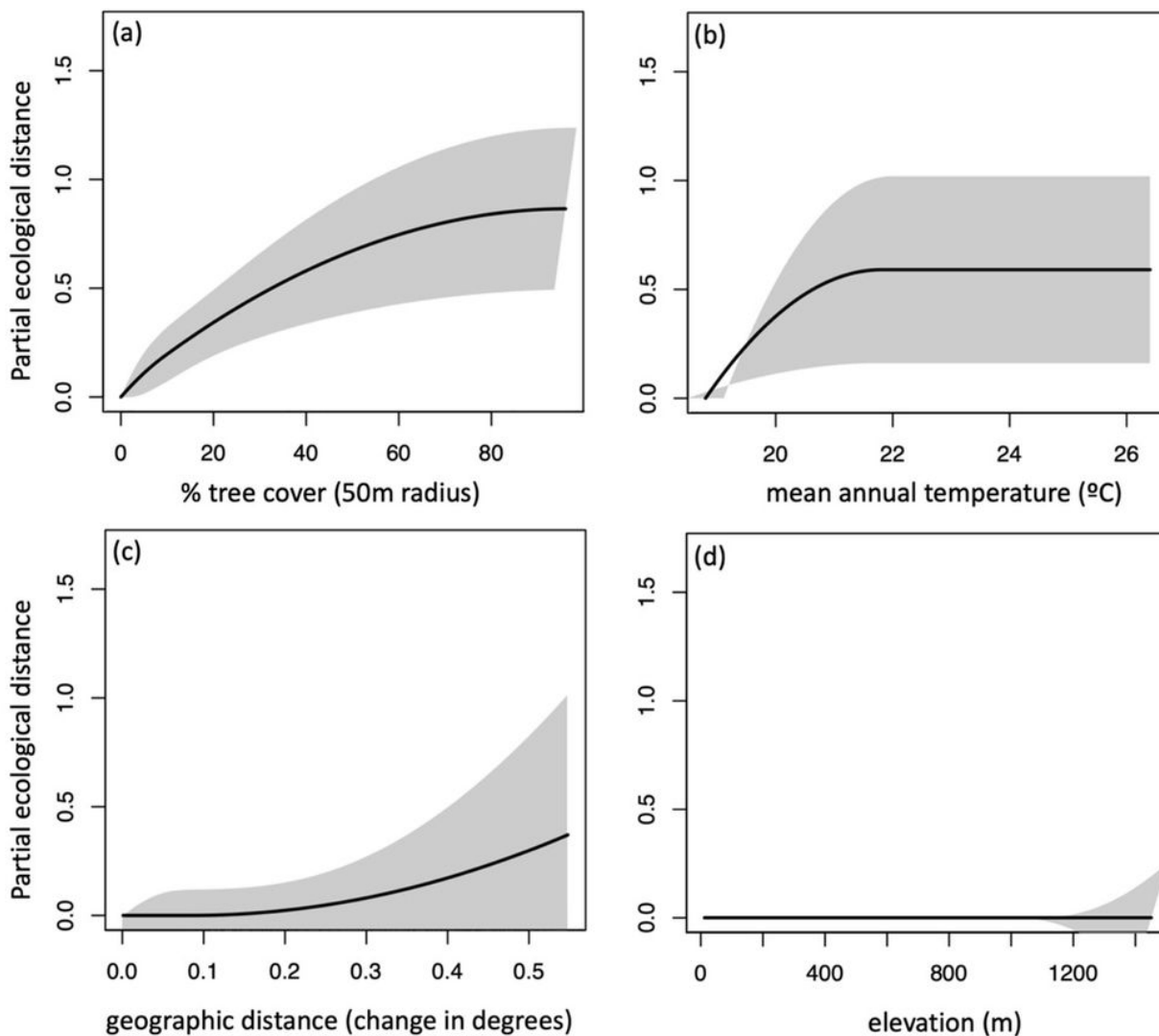


Figure 6

Generalized dissimilarity modelling (GDM) of community dissimilarity indicated that (a) percent tree cover and (b) mean annual temperature explained 7% of deviation from the null. (c) geographic distance and (d) elevation did not significantly contribute to community turnover. In each panel, the x-axis shows the environmental gradient and the y-axis shows the amount of compositional turnover, measured as partial ecological distance. The maximum height the spline reaches on the y-axis indicates the total amount of compositional turnover the gradient is associated with, and the slope shows how the rate of compositional turnover varies along the environmental gradient. The difference in height between any two points along the l-spline corresponds to the modeled contribution of that predictor variable to the difference between those points. Grey shading shows +/- 1 standard deviation when 70% of sites are sampled 10 times.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementCRMosquitoes29Nov2023.docx](#)