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Abstract

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Canine vaccination campaigns have substantially reduced rabies cases worldwide. However, in Latin America, dog vaccination has failed to eliminate endemic rabies as the zoonotic virus has persisted in a vampire bat-cattle cycle that appears enhanced by forest conversion for livestock. To improve the understanding of this dynamic, I analyzed spatial autocorrelation of documented bovine rabies outbreaks from 1985 to 2020 (n=109) in Costa Rica. Logistic modeling of known outbreaks with land-use indicators extracted from national spatial data throughout the study period was used to evaluate the impact of anthropogenic activity on rabies incidence. Such analysis showed positive spatial autocorrelation among outbreaks (Moran's I=0.038, $P < 0.05$), but no temporal relationship was discovered. Modeling showed an inverse association between elevation and outbreak probability (p=0.01) and deforestation represented a potential predictor for bovine rabies outbreak (p=0.05). The effects of land-use on vampire bat (*Desmodus rotundus*) foraging patterns and diet were also evaluated using stable isotope analyses of tissue samples (n=227) from 12 caves throughout Costa Rica. Principal component analysis (PCA) was used to determine the variance and effect of correlated land-use predictors. Multivariate modeling of PCs revealed significant differences in $\delta^{15}C$ between caves and highlighted land-use areas (forest, agriculture, urban), proximities (distance to town, distance to road), and population densities (cattle, human, farm) in the 10km surrounding each cave as significant predictors of vampire bat $\delta^{13}C$ values and, consequently, prey preference. PC1 and 2 variables described the intensity and/or proximity of/to agricultural and human development at each cave and were associated with feeding choices of *D. rotundus* and predicted higher $\delta^{13}C$ values, indicating feeding on livestock (GLMM, PC1: Estimate = 0.52, $P < 0.001$; PC2: Estimate = 0.21, $P = 0.38$). Overall, these findings demonstrate a connection between human land-use and changing vampire bat ecology, with implications for rabies transmission.

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Introduction

Rabies virus (family Rhabdoviridae, genus *Lyssavirus*) is a preventable, yet fatal zoonosis, transmitted to humans via the bite of an infected animal. The virus is primarily transmitted by domestic dogs, responsible for about 99% of human cases (World Health Organization, 2018a). In 1983, the Pan American Health Organization (PAHO) and the World Health Organization (WHO) named rabies a neglected tropical disease and committed to canine rabies surveillance and vaccination campaigns in Latin America and the Caribbean (Pan American Health Organization, 1983; Vigilato et al., 2013). The result was a 95% case reduction in canine and human rabies (PAHO & WHO, 2018). Costa Rica benefited from this effort, enjoying over three decades without a human case (Badilla et al., 2003); however, the management of canine rabies has failed to eliminate endemic rabies.

While human and canine cases have decreased in past decades, rabies has persisted in a vampire bat-cattle cycle and resulted in rare, but repeated spillover to humans, demonstrating the potential for rabies re-emergence as a human disease (Badilla et al., 2003). With fewer canine rabies vaccination campaigns, the latent risk to humans in Costa Rica and elsewhere in Latin America may increase, presenting a public health risk. If canine vaccination campaigns are not sustained, rabies transmission beyond the scope of *D. rotundus* should be expected.

The common vampire bat (*Desmodus rotundus*), native to the Americas, is named for its diet of mammalian blood and represents a wildlife reservoir of rabies. In Costa Rica, rabies has persisted in cattle populations, whose blood has become the preferred meal of *D. rotundus* as forests have been transformed to pasture (Voigt & Kelm, 2006). As dead-end hosts, unable to transmit the virus once infected, cattle serve as sentinels of rabies prevalence within a geographical area and within a vampire bat population (Baum, 2008; Johnson et al., 2014). The bite of a vampire bat infects prey with sylvatic rabies, causing encephalitis, a swelling of the brain. In humans, infection manifests in symptoms such as painful sensations at the wound site, fever, headache, aggressive behavior, seizures, and paralysis (World Health Organization, 2018a, 2018b). Infected cattle exhibit some of the same human symptoms, like aggressive behavior, but generally display unique symptoms like anorexia, hypersalivation, and impaired movement and coordination (Centers for Disease Control and Prevention, 2019). Rabies can be prevented by providing pre-exposure immunization to at-risk populations and post-exposure prophylaxis. After observable symptoms, and without immunization or intensive care, the disease has a near 100% fatality rate within days of exposure (Pieracci et al., 2019; WHO, 2020).

In efforts to reduce the spread of rabies, many Latin American countries, including Costa Rica, have implemented culling campaigns targeting vampire bats to limit disease transmission. However, culling programs may actually amplify rabies transmission by targeting adult *D. rotundus*, leaving juveniles that are more susceptible to the virus as a larger proportion of the population (Olival, 2016; Streicker & Allgeier, 2016). As vampire bats are social creatures, culling forces *D. rotundus* migration to new caves and communities, presenting a potential for further distribution of the rabies virus (Blackwood et al., 2013; Johnson et al., 2014).

Livestock intensification is a primary source of land-use conversion and deforestation in Central America (Chacón Navarro, 2020; FAO, 2021; Holmann et al., 2008; Nicholson et al., 1995;

World Bank, CIAT, et al., 2014). After rapid forest conversion to pasture in the late 20th century, agricultural land still represents 47 percent of Costa Rica's landscape (OECD, 2017). Such anthropogenic landscapes increase overlap of wildlife with humans and domesticated animals, and alter the distribution and abundance of wildlife food resources. In the case of vampire bats, the conversion of forest to agricultural pasture and livestock farming has the capacity to shift foraging patterns away from forest-dwelling animals toward the abundant and stationary blood-meal sources that cattle represent (Becker et al., 2018; Streicker & Allgeier, 2016; Voigt & Kelm, 2006). Previous studies have shown that vampire bat populations are significantly larger in cattle-rearing areas compared to undisturbed ecosystems (Delpietro et al., 1992; Hampson et al., 2015; Jones et al., 2013; León et al., 2021). In essence, changes to the natural habitat have resulted in a shift in vampire bat food web structure and the geographical expansion of *D. rotundus* (Jones et al., 2013; Streicker et al., 2019). Such changes may promote sylvatic rabies prevalence and increase the probability of spillover to humans (Benavides et al., 2016). Persistent investment in livestock farming indicates that these development trends will continue, underscoring the risk of rabies surrounding fragmented habitats.

Rabies prevention and elimination once served as a model for a concerted "One Health" approach that combined animal and human health; however, the current trend of increasing bovine rabies cases coupled with declining canine rabies vaccination signal a shift from proactive to reactive mitigation strategies. In 2001, human rabies cases surfaced in Costa Rica, ending the nation's 31-year period without human infection (Badilla et al., 2003). Endemic rabies proximal to agricultural communities presents a novel opportunity for disease spillover and disproportionately affects rural areas, many without the resources or rabies education to properly report and mitigate an outbreak (Grace et al., 2017; Pieracci et al., 2019). Human and cattle death from rabies is completely preventable with vaccination, yet the death toll rises each year, further skewed by underreporting (Hampson et al., 2015). Surveillance efforts are often hindered by diminished local knowledge of disease awareness, prevention, and mitigation strategies. In addition, the economic burden of rabies exceeds 30 million US dollars annually; the loss of human and livestock life to infection accounts for the majority of this cost, and further expenses include vaccinations, laboratory testing, and vampire bat management (Jones et al., 2013).

The objective of this research is to improve the understanding of vampire bat ecology and landscape-related risk factors to improve the efficacy of rabies awareness campaigns and interventions. With the resurgence of human rabies, further research on this topic is imperative to improve mitigation efforts in Costa Rica and beyond. Specifically, I examined the effect of anthropogenic land-use change on rabies virus transmission between *D. rotundus* and cattle in Costa Rica, using cattle as sentinels of rabies prevalence. Through spatial analysis, I investigated the relationship between land-use change and bovine rabies outbreak rate in the seven administrative provinces of Costa Rica from 1985 to 2020. I hypothesized that bovine rabies outbreak frequency would be positively associated with human and cattle density. In addition, since anthropogenic land-use change was expected to facilitate rabies transmission, I hypothesized that geographical clustering of outbreaks would align with anthropogenic environments and not be spatially random. Additionally, I investigated the role of human land conversion on vampire bat foraging patterns and diet by stable isotope analysis. Using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios, I identified feeding trends in vampire bats (n=227) across twelve sampled caves in Costa Rica. I hypothesized that anthropogenic indicators (i.e., human, cattle, and farm density) would be positively associated with vampire bat $\delta^{13}C$ isotopic ratios since this ratio is elevated for bovine compared to wildlife blood-meals. The overarching goal of my work is to determine anthropogenic landscape factors that impact rabies disease transmission in order to inform preventative public health interventions.

Materials and Methods

Historical Bovine Rabies Analyses

Data Collection: Reported livestock outbreaks of rabies from 1985 to 2020 were obtained from National Animal Health Service (SENASA), Costa Rica's veterinary authority. SENASA has maintained national rabies virus surveillance on domestic animals since the implementation of canine rabies elimination strategies in 1985. For this study, only bovine rabies outbreaks (n=119) were considered. Recorded outbreak incidents were confirmed by direct fluorescent antibody testing (Hutter et al., 2016).

Variables of interest were date and latitude-longitude coordinates of outbreaks. An outbreak point or incident referred to one event of rabies infection with at least one infected host. As each documented outbreak had laboratory confirmation of rabies infection, all points were considered verified bovine rabies outbreak incidents. Ten outbreaks were excluded from analysis due to inconsistent or missing coordinate data. Outbreaks were mapped using QGIS, an open-source geographical information system, and analyzed with neighboring land activity. Land-use was quantified using spatial data from the 2014 Atlas of Costa Rica and the National Territorial Information System updated in 2018.

Vampire bats fly a maximum distance of 10-kilometers to feed (Medina et al., 2007), so land-use was characterized within the 10 km radius around each outbreak point. Within this radius, area of forest cover and distance to nearest forest patch were calculated, as well as outbreak point elevation. Data was extracted from open-source landscape orthophotos provided by the 2014 Atlas of Costa Rica and the National Territorial Information System in 2018. Outbreaks were sorted into four groups of ranging years (forest coverage data were only available for 1989, 1997, 2005, and 2017) and analyzed using the landscape data most relevant to those years. Distance to forest calculations excluded forest patches < 100 m^2 due to scaling accuracy of QGIS. This variable was an indicator of neighboring land-use change and revealed aspects of the relationship between forest proximity and bovine rabies outbreaks. Human and cattle population densities were extracted for each outbreak at the district level, obtained through the National Institute of Statistics and Census (INEC) for 2011 and 2014. For human populations, this data provided estimations of future population densities from 2011 to 2025 based on growth trends. For outbreaks after 2011, estimated human population density was used.

Spatial Autocorrelation: I evaluated spatial autocorrelation among bovine rabies outbreaks over the 35-year study period in Costa Rica. Using GeoDa software and R analysis, a global and local univariate Moran's I was conducted to detect areas of high and low autocorrelation at different spatial scales among outbreaks by measuring autocovariance. Spatial autocorrelation measures the degree to which similar observations occur near each other, detecting groups of spatially related points with similarly high or low values. A local Moran’s I, or Local Indicators of Spatial Association (LISA) analysis, measures spatial autocorrelation within sub-regions of the study area. The global Moran's I detected the trend of outbreak autocorrelation throughout the whole country. Moran’s I values are expressed between -1 and 1, with zero indicating complete spatial randomness.

Kernel Density Estimations: To evaluate outbreak distribution and density probability of a bovine rabies outbreak I used Kernel Density Estimations (KDE). KDE created a spatial probability estimation based on known outbreaks using a kernel function that both smoothed and interpolated probabilities for an outbreak across the study area. The kernel weighted its interpolation based on the distance to an outbreak point, applying Tobler’s Law that near things are more related than distant things. A kernel radius of ten kilometers was selected for this test to represent the foraging range of the vampire bats (Medina et al., 2007; Rocha et al., 2020); this limited interpolation to the determined kernel radius. The KDE bandwidth parameter selected, which determines the level of data smoothing within the function, was the default value to QGIS.

Regression Analyses: In addition to documented bovine rabies outbreaks (n=109), additional points were randomly sampled within the boundaries of Costa Rica to provide non-event data for comparisons with outbreaks (n=120). The same variables—forest proximity, forest cover, elevation, human and cattle population—were analyzed for randomly sampled points without a rabies outbreak. All points were compiled, and a binary generalized linear mixed model (GLMM) regression was applied to evaluate the statistical effect of land-use factors on bovine rabies outbreaks. A mixed model approach was selected to model fixed and random effects for longitudinal data and create a regression equation that predicts the effects of each variable on a potential bovine rabies outbreak. Variable coefficients included distance to forest, elevation, human, and cattle population densities. District was set as a random effect to account for site randomness within the arbitrary administrative boundaries. Distance to the forest was used as a proxy for deforestation as it indicates the spatial relationship between outbreak and forest cover despite the limitations of inaccurate or incomplete forest coverage data for the study timeframe (1985 to 2020).

Stable Isotope Analyses

Data Collection: Vampire bat wing tissue samples (n=205) were collected in 2019 from 12 caves in Costa Rica with varying anthropogenic perturbation and theoretical availability to both wildlife and cattle blood-meal sources (Figure 1). All cave sites were at least 20 km apart from one another and included both natural and artificial bat roosts. Vampire bat wing tissue samples were stored in 96% ethanol solution and analyzed for carbon and nitrogen stable isotope ratios at the University of Georgia Stable Isotope Laboratory using a Thermo Delta V isotope ratio mass spectrometer. Isotopic quantities were expressed in δ notation; both $\delta^{13}C$ and $\delta^{15}N$ were calculated using [(Rsample/Rstandard) - 1] x 1000 where R is the ratio of ^{13}C to ^{12}C or ^{15}N to ^{14}N, respectively. Sampled isotope analysis utilized three standards per 12 samples: spinach ($\delta^{13}C = -27.53 \pm 0.03‰$ and $\delta^{15}N = -0.26 \pm 0.06‰$), bovine liver ($\delta^{13}C = -17.50 \pm 0.01‰$ and $\delta^{15}N = 8.15 \pm 0.02‰$), and blue harbor ($\delta^{13}C = -16.78 \pm 0.01‰$ and $\delta^{15}N = 14.62 \pm 0.05‰$).

Stable isotopic ratios of carbon and nitrogen reveal the blood diet of vampire bats, illuminating feeding trends and host preferences. Grazing livestock (i.e., cattle) generally consume C4 grasses that are high in ^{13}C. C3 plants, consumed by wildlife, utilize a different photosynthetic pathway that results in lower levels of ^{13}C. Differences in carbon isotopic ratios depict vampire bat reliance on wildlife and livestock, respectively, providing insight into their foraging patterns. Nitrogen isotopic ratios were also measured and depicted trophic positioning; vampire bat nitrogen isotopic ratios are enriched by 3‰ to 4‰ relative to its prey blood meal, though these values are similar for domestic and wildlife vampire bat prey (Post, 2002). Becker et al. 2017 used stable isotope analysis to evaluate vampire bat ecology in Belize, demonstrating the efficacy of such analyses in evaluating vampire bat feeding patterns. The overall mean of $\delta^{13}C$ values for the sample size (n=205) was compared to the expected carbon isotopic ratio of *D. rotundus* in a pristine forest ($\delta^{13}C = -27.4‰$) established by Herrera M. et al., 1998 using a 2-tailed paired t-test (Voigt & Kelm, 2006).

ANOVA: $\delta^{13}C$ and $\delta^{15}N$ values for individual *D. rotundus* were grouped by cave and analyzed using a one-way Welch's ANOVA to evaluate differences in isotopic ratios at each cave site. Welch's ANOVA analysis was selected over the traditional ANOVA because the data did not meet the assumption of equal variance between groups, as evaluated using Bartlett and Levene's tests of variance homogeneity. Post-hoc analysis was performed using Games-Howell which analyzed point-wise comparisons between the 12 sampled caves. $\delta^{15}N$ stratified by cave was analyzed to detect appropriate sample variability but was excluded from larger analyses as $\delta^{15}N$ does not differ significantly between livestock and wildlife hosts due to their similar trophic positioning.

Stable Isotope Regression Analysis: The same methodology used to extract spatial data surrounding historical bovine rabies outbreaks was applied to each sampled cave (n=12). In the 10 km radius surrounding each cave, land-use spatial data were extracted in square meters and grouped into general categories: area of agricultural coverage (including pasture and farmland), area of forest coverage, forest fragmentation index (fragment area divided by fragment perimeter), area of urban coverage (including human-developed infrastructure), cave elevation, cave distance to the nearest town, cave distance to the nearest road, road density, and river density. Additionally, just as with bovine rabies outbreaks, human and cattle population densities were extracted for each cave at the district level using census data.

Analysis of stable isotope ratios was focused on $\delta^{13}C$ to distill differences in vampire bat feeding trends between wildlife (C3 pathway; lower ^{13}C; smaller $\delta^{13}C$) and bovine hosts (C4 pathway; higher ^{13}C; larger $\delta^{13}C$). Unlike previous studies utilizing stable isotope methodology, isotopic values for prey sources were unavailable for direct comparison. Instead, *D. rotundus* prey dependence and foraging patterns were approximated based on the current understanding of $\delta^{13}C$ values associated with bovine livestock and wildlife (Becker et al., 2018; Streicker & Allgeier, 2016; Voigt & Kelm, 2006).

As land-use variables were correlated—for example, urban area was linked to human density, principal component analysis (PCA) was used to determine the variance of land-use predictors and identify relationships between variables. PCA employs linear transformation to reduce the complexity of independent variables in the study dataset (Jolliffe & Cadima, 2016). This method produced new orthogonal variables, known as principal components (PCs), that depict the variance and effect of correlated land-use factors at each cave. Each PC accounts for a proportion of the total variance in the original dataset and the most significant PCs were tested with $\delta^{13}C$ to determine the effect of complex land-use variables.

To test whether land-use and land-use indicators (i.e., human, cattle, and farm density) within close foraging range of caves predicted vampire bat $\delta^{13}C$, and thus host selection, multivariate modeling was used. Sensitivity analyses demonstrated generalized linear mixed modeling (GLMM), including both random and fixed effects, as the best fit. I used the GLMM approach to measure the associations of PC1 and PC2 variables with individual *D. rotundus* $\delta^{13}C$ values at each cave, using cave membership as a random effect.

Results

Historical Bovine Rabies

Analysis produced a local and global Moran's I value of $I = 0.138$ ($P < 0.05$), indicating a positive autocorrelation between outbreaks. A univariate Global Moran's I analysis of the data showed significant spatial autocorrelation among 20 outbreak points (16.8 percent of total points (n=109)). Of these points, only five neighboring points shared similarly high values. Neighboring high-high outbreaks illuminated the southern region of Costa Rica as having high outbreak spatial autocorrelation (Figure 2).

KDE also highlighted higher probable outbreak density in the Southern region (Figure 3). The highest estimated densities, seen in red, were found there. These analyses provided insight into the spatial relationship among outbreaks. Additional analyses were run to evaluate spatial-temporal relatedness (i.e., Kulldorff Space-Time Permutation and K Means Clustering), but no significant cluster events were detected; however, retrospective clustering analyses can still inform disease surveillance and mitigation strategies moving forward.

Elevation decreased the probability of an outbreak event (GLMM, $R^2 = 0.13$: Estimate = -1.65×10^{-3}, SE = 3.72×10^{-1}, Z-value = -3.27, $P < 0.01$). A one-meter increase in elevation decreased the probability of a bovine rabies outbreak by 0.16%, while a one-kilometer increase in elevation decreased the probability of a bovine rabies outbreak by more than 100%. Distance from a forest patch increased the probability of an outbreak (GLMM: Estimate = 4.33×10^{-4}, SE = 3.32×10^{-1}, Z-value = 1.95, $P = 0.05$). A one meter increase in distance from a forest patch increased the probability of a bovine rabies outbreak by 0.04%, while a one-kilometer increase in distance from a forest patch increased the probability of a bovine rabies outbreak by 4.00%. Human and cattle densities were not associated with bovine rabies outbreaks (GLMM, Human Density: Estimate = -3.93×10^{-5}, SE = 1.93×10^{-5}, Z-value = -1.53, $P = 0.13$; GLMM, Cattle Density: Estimate = -3.76×10^{-5}, SE = 3.23×10^{-5}, Z-value = -0.93, $P = 0.35$).

Stable Isotope Results

The 205 tissue samples revealed an average of 8.40 ± 0.13‰ for $\delta^{15}N$ and -11.06 ± 0.17‰ for $\delta^{13}C$. Carbon isotope values differed from the expected value of $\delta^{13}C = -27.4$‰ for a pristine tropical forest (Herrera M. et al., 1998; Voigt & Kelm, 2006), supporting my hypothesis that vampire bats preferentially feed on bovine cattle of the C4 food chain resulting in lower levels of $\delta^{13}C$ (2-tailed paired t-test: $t = 95.94$, $df = 204$, $P < 0.001$). However, $\delta^{13}C$ values varied greatly among caves (Welch's one-way ANOVA: $F = 31.61$, $P < 0.001$), ranging from -14.765 to -9.368‰ (Figure 4). Notably, the cave site with the largest proportion of agricultural land within 10 km, Túnel Arenal, had the highest mean $\delta^{13}C$. Post-hoc analysis indicated differences among 34 out of the 66 cave-to-cave $\delta^{13}C$ comparisons, supporting my alternate hypothesis that the proportion of natural versus developed land around each cave was effector of vampire bat feeding trends and that cave-specific land-use may drive *D. rotundus* prey selection and feeding. $\delta^{15}N$ values differed significantly among all caves, but all values matched the known range for the trophic level of *D. rotundus* prey (Welch's one-way ANOVA: $F = 11.255$, $P < 0.001$). Collectively, these $\delta^{13}C$ and $\delta^{15}N$ results indicate foraging heterogeneity by *D. rotundus* within the same trophic level (Figure 5).

PC1 and PC2 explained 40.61% and 22.43% of the overall variance, respectively, and, therefore, estimated the complexity and significance of predictor variables (Figure 6). Eigenvalues depicted PC1 and PC2 as the primary effector variables for multivariate analysis. PC1 was loaded positively by road density (0.79), farm density (0.79), agricultural area (0.75), human density (0.73), and cattle density (0.67), and loaded negatively by forest area (-0.79) and distance to the road (-0.67) (Figure 7). Additional variables contributed to PC1 but did not make significant inputs. PC1, therefore, described the level of, and proximity to, agricultural and human development at each cave. PC2 was loaded positively by cattle density (0.63), river density (0.48), and distance to town (0.48), and loaded negatively by urban area (-0.75), human density

(-0.55), and cave elevation (-0.51). Additional variables contributed to PC2 but did not constitute significant inputs. The PC2 variable, thus, described farming development, indicated by cattle density, and depicted the distance from urban areas with high human density (Figure 7).

PC1 was associated with feeding choice by *D. rotundus* and predicted higher $\delta^{13}C$ values, indicating livestock feeding (GLMM, $R^2 = 0.33$: Estimate = 0.52, SE = 0.18, T = 2.95, $P < 0.001$). In the context of the regression results, PC1 revealed that higher road density; proportion of agricultural area; and farm, human, and cattle density; were associated with higher $\delta^{13}C$ levels and greater reliance on livestock prey. Likewise, decreased forest area and distance to the nearest road contributed to higher $\delta^{13}C$ levels. PC2 showed no association with livestock feeding (GLMM: Estimate = 0.21, SE = 0.24, T = 0.88, P = 0.38).

Discussion

Moran's I and KDE analysis revealed positive spatial autocorrelation of historical bovine rabies outbreaks throughout Costa Rica, with the highest spatial relatedness and interpolated probability of outbreak in the Southern region. This highlights the South of Costa Rica as an area of focus for future rabies research and preventative intervention. Proximity to forest patch was positively associated with bovine rabies outbreaks, indicating land-use areas as predictive of future outbreaks. Elevation was negatively associated with bovine rabies outbreaks, as well as a partial driver of *D. rotundus* feeding on livestock and resultant $\delta^{13}C$ values. PCA and multivariate analyses revealed that the degree of and proximity to human and agricultural development significantly predicted *D. rotundus* reliance on livestock, with increased development associated with higher δ^{13}C values among sampled individuals (n=205).

Spatial modeling of historical bovine rabies cases from 1985 to 2020 indicated elevation as a preventative factor in rabies outbreaks. *D. rotundus* primarily feed on prey at lower elevations to reduce energy costs (Rocha et al., 2020). Related to this, elevation was negatively associated with δ^{13}C values in *D. rotundus* and bovine rabies outbreaks, and positively associated with consumption of wildlife prey by *D. rotundus*. Human and cattle density did not affect bovine rabies outbreak probability as expected. Since human and cattle population data was only available for 2011 and 2014, respectively, this may be an underestimate of the predictive effect of these population densities, since agricultural intensification was rapidly increasing until the 1996 Forest Law, which slowly drove down investments and developments in agricultural land (Chacón Navarro, 2020; Nicholson et al., 1995; World Bank, CIAT, et al., 2014). By comparing outbreaks from all years to data from 2011 and 2014, I could not accurately account for realistic shifts in population densities throughout Costa Rica, especially those that occurred prior to 2011. However, human and cattle density were positively associated with δ^{13}C values in *D. rotundus*, demonstrating that these land-use indicators influence vampire bat feeding and thus, likely have an effect on bovine rabies transmission. Additionally, elevation findings from bovine outbreak modeling may be correlated with population density variables. Logistical modeling also depicted proximity to forest as positively associated with bovine rabies outbreaks, implying that closeness to forest patches reduces the likelihood of bovine rabies outbreaks. This finding aligns with the current understanding of *D. rotundus* feeding and rabies transmission, as increased proximity to and area of forest indicates greater *D. rotundus* access to wildlife hosts and, generally, decreased proximity to agricultural areas (Bobrowiec et al., 2015). However, further investigation is necessary to better evaluate the relationship between forest cover and agricultural land and thus the effect of anthropogenic forest conversion and fragmentation on rabies transmission.

Variation in land-use correlates around focal caves was associated with vampire bat $\delta^{13}C$ values, suggesting that *D. rotundus* feeding patterns are affected by anthropogenic development. In general, analyses showed increased reliance on cattle in Costa Rica which proved significant compared to previous isotopic estimates from purely wildlife hosts; these findings support the hypothesis that *D. rotundus* has become reliant on blood meals from available livestock. Still, feeding is likely mixed between wildlife and bovine livestock. Other studies have investigated isotopic ratios at disturbed and undisturbed cave sites, however, this study used sampled data from multiple different caves that provided a gradient of perturbation to determine the underlying effects of specific land-use trends. These findings support previous research demonstrating that vampire bat feeding is greatly impacted by localized land-use change (Becker et al., 2018; Becker & Hall, 2014; Ingala et al., 2019; Streicker & Allgeier, 2016), and specifically highlight increases in agricultural area, road density, and human, farm and cattle density as the primary drivers of feeding pattern shifts.

Effects of correlated land-use variables were examined by PCA, reducing complexity and confounding in order to distill the joint effects of land-use change on the isotopic carbon composition of *D. rotundus*. Vampire bat tissue samples used for stable isotope analysis were collected in 2019 and compared to spatial data from the 2014 Atlas of Costa Rica and the 2018 National Territorial Information System, which provided land-use insights from 1987 to 2017.

Lags in updated spatial data present limitations to this study design, as analyses may have missed newer associations with $\delta^{13}C$. Historical bovine rabies cases were compared to the most relevant year of spatial data, which limited predictor analysis as only forest coverage and fragmentation data spanned the surveillance period of 1985 to 2020. Additionally, ten outbreaks were not considered for analysis due to reporting errors; this limited sample size, reducing statistical power and potentially contributing to missed spatial-temporal associations among outbreaks.

This study provides novel insight into land-use factors and indicators that may drive bovine rabies outbreaks and *D. rotundus* reliance on livestock, respectively. Since vampire bat feeding is the primary mechanism of rabies transmission to cattle (World Health Organization, 2018a), increased feeding success on bovine prey could promote rabies transmission and present avenues for novel rabies virus mutation and spillover to humans (Badilla et al., 2003; Becker & Hall, 2014; Streicker & Allgeier, 2016). These results contribute to the body of research demonstrating that vampire bat foraging patterns respond to environmental changes. Priority must be placed on implementing sustainable development solutions that reduce the effects of anthropogenic land-use change on *D. rotundus* feeding and rabies transmission. As rabies remains endemic in Latin America, it is important to continue monitoring anthropogenic land-use changes, as well as vampire bat dietary composition and bovine rabies outbreaks to detect potential shifts driven by human and agricultural development. Such data and investigation will allow health agencies to implement preventative policies to reduce the rabies burden as interactions between humans and wildlife become more frequent with continued land-use change and human population growth. Future studies integrating spatial, dietary, and surveillance data for *D. rotundus* are needed to provide additional insights into land-use effects on rabies transmission. Moving forward, scientific inquiry must focus on vampire bat ecology to improve rabies virus interventions in Latin America.

References

Badilla, X., Pérez-Herra, V., Quirós, L., Morice, A., Jiménez, E., Sáenz, E., Salazar, F., Fernández, R., Orciari, L., Yager, P., Whitfield, S., & Rupprecht, C. E. (2003). Human Rabies: A Reemerging Disease in Costa Rica? *Emerging Infectious Diseases*, *9*(6), 721–723. https://doi.org/10.3201/eid0906.020632

Baum, S. G. (2008). Zoonoses-With Friends Like This, Who Needs Enemies? *Transactions of the American Clinical and Climatological Association*, *119*, 39–52.

Becker, D. J., Czirják, G. Á., Volokhov, D. V., Bentz, A. B., Carrera, J. E., Camus, M. S., Navara, K. J., Chizhikov, V. E., Fenton, M. B., Simmons, N. B., Recuenco, S. E., Gilbert, A. T., Altizer, S., & Streicker, D. G. (2018). Livestock abundance predicts vampire bat demography, immune profiles and bacterial infection risk. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1745), 20170089. https://doi.org/10.1098/rstb.2017.0089

Becker, D. J., & Hall, R. J. (2014). Too much of a good thing: Resource provisioning alters infectious disease dynamics in wildlife. *Biology Letters*, *10*(7), 20140309. https://doi.org/10.1098/rsbl.2014.0309

Benavides, J. A., Valderrama, W., & Streicker, D. G. (2016). Spatial expansions and travelling waves of rabies in vampire bats. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1832). https://doi.org/10.1098/rspb.2016.0328

Blackwood, J. C., Streicker, D. G., Altizer, S., & Rohani, P. (2013). Resolving the roles of immunity, pathogenesis, and immigration for rabies persistence in vampire bats. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(51), 20837–20842. https://doi.org/10.1073/pnas.1308817110

Bobrowiec, P. E. D., Lemes, M. R., & Gribel, R. (2015). Prey preference of the common vampire bat (Desmodus rotundus, Chiroptera) using molecular analysis. Journal of Mammalogy, 96(1), 54–63. https://doi.org/10.1093/jmammal/gyu002

Centers for Disease Control and Prevention. (2019, February 22). *Veterinarians: Clinical Signs of Rabies in Animals—Rabies*. Centers for Disease Control and Prevention. https://www.cdc.gov/rabies/specific_groups/veterinarians/clinical_signs.html

Chacón Navarro, M. (2020, March 11). *Costa Rica: Balancing environmental impacts and socio-economic performance of the livestock sector |Livestock Environmental Assessment and Performance (LEAP) Partnership|FAO*. FAO. http://www.fao.org/partnerships/leap/news-and-events/news/detail/en/c/1322072/

Delpietro, H. A., Marchevsky, N., & Simonetti, E. (1992). Relative population densities and predation of the common vampire bat (Desmodus rotundus) in natural and cattle-raising areas in north-east Argentina. *Preventive Veterinary Medicine*, *14*(1), 13–20. https://doi.org/10.1016/0167-5877(92)90080-Y

FAO. (2021). *World Food and Agriculture – Statistical Yearbook 2021*. FAO. https://doi.org/10.4060/cb4477en

Grace, D., Lindahl, J., Wanyoike, F., Bett, B., Randolph, T., & Rich, K. M. (2017). Poor livestock keepers: Ecosystem–poverty–health interactions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1725). https://doi.org/10.1098/rstb.2016.0166

Hampson, K., Coudeville, L., Lembo, T., Sambo, M., Kieffer, A., Attlan, M., Barrat, J., Blanton, J. D., Briggs, D. J., Cleaveland, S., Costa, P., Freuling, C. M., Hiby, E., Knopf, L., Leanes, F., Meslin, F.-X., Metlin, A., Miranda, M. E., Müller, T., … Prevention, on behalf of the G. A. for R. C. P. for R. (2015). Estimating the Global Burden of Endemic Canine Rabies. *PLOS Neglected Tropical Diseases*, *9*(4), e0003709. https://doi.org/10.1371/journal.pntd.0003709

Holmann, R. L., Perez, E., Castro, C., Schuetz, P., & Rodríguez, J. (2008). *The beef chain in Costa Rica: Identifying critical issues for promoting its modernization, efficiency, and competitiveness*. Livestock Research for Rural Development. https://www.lrrd.cipav.org.co/lrrd20/4/holmb20051.htm

Ingala, M. R., Becker, D. J., Holm, J. B., Kristiansen, K., & Simmons, N. B. (2019). Habitat fragmentation is associated with dietary shifts and microbiota variability in common vampire bats. *Ecology and Evolution*, *9*(11), 6508–6523. https://doi.org/10.1002/ece3.5228

Johnson, N., Aréchiga-Ceballos, N., & Aguilar-Setien, A. (2014). Vampire Bat Rabies: Ecology, Epidemiology and Control. *Viruses*, *6*(5), 1911–1928. https://doi.org/10.3390/v6051911

Jolliffe, I. T., & Cadima, J. (2016). Principal component analysis: A review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *374*(2065), 20150202. https://doi.org/10.1098/rsta.2015.0202

Jones, B. A., Grace, D., Kock, R., Alonso, S., Rushton, J., Said, M. Y., McKeever, D., Mutua, F., Young, J., McDermott, J., & Pfeiffer, D. U. (2013). Zoonosis emergence linked to agricultural intensification and environmental change. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(21), 8399–8404. https://doi.org/10.1073/pnas.1208059110

León, B., González, S. F., Solís, L. M., Ramírez-Cardoce, M., Moreira-Soto, A., Cordero-Solórzano, J. M., Hutter, S. E., González-Barrientos, R., & Rupprecht, C. E. (2021). Rabies in Costa Rica – Next Steps Towards Controlling Bat-Borne Rabies After its Elimination in Dogs. *The Yale Journal of Biology and Medicine*, *94*(2), 311–329.

Medina, A., Harvey, C. A., Merlo, D. S., Vílchez, S., & Hernández, B. (2007). Bat Diversity and Movement in an Agricultural Landscape in Matiguás, Nicaragua: Bats in Agricultural Landscapes. *Biotropica*, *39*(1), 120–128. https://doi.org/10.1111/j.1744-7429.2006.00240.x

Nicholson, C. F., Blake, R. W., & Lee, D. R. (1995). Livestock, Deforestation, and Policy Making: Intensification of Cattle Production Systems in Central America Revisited. *Journal of Dairy Science*, *78*(3), 719–734. https://doi.org/10.3168/jds.S0022-0302(95)76684-X

OECD. (2017). *Agricultural Policies in Costa Rica*. OECD. https://doi.org/10.1787/9789264269125-en

Olival, K. J. (2016). To Cull, Or Not To Cull, Bat is the Question. *EcoHealth*, *13*(1), 6–8. https://doi.org/10.1007/s10393-015-1075-7

PAHO, & WHO. (2018, September 21). *Rabies persists in only four countries of Latin America* *and the Caribbean*. Pan American Health Organization / World Health Organization. https://www.paho.org/hq/index.php?option=com_content&view=article&id=14665:rabies-persists-in-only-four-countries-of-latin-america-and-the-caribbean&Itemid=135&lang=en

Pan American Health Organization. (1983). *Estrategia y plan de acción para la eliminación de la rabia urbana en América Latina para el final de la década 1980* [Technical reports]. PAHO. https://iris.paho.org/handle/10665.2/51202

Pieracci, E. G., Pearson, C. M., Wallace, R. M., Blanton, J. D., Whitehouse, E. R., Ma, X., Stauffer, K., Chipman, R. B., & Olson, V. (2019). Vital Signs: Trends in Human Rabies Deaths and Exposures — United States, 1938–2018. *Morbidity and Mortality Weekly Report*, *68*(23), 524–528. https://doi.org/10.15585/mmwr.mm6823e1

Post, D. M. (2002). Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. *Ecology*, *83*(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Rocha, F., Ulloa-Stanojlovic, F. M., Rabaquim, V. C. V., Fadil, P., Pompei, J. C., Brandão, P. E., & Dias, R. A. (2020). Relations between topography, feeding sites, and foraging behavior of the vampire bat, Desmodus rotundus. *Journal of Mammalogy*, *101*(1), 164–171. https://doi.org/10.1093/jmammal/gyz177

Streicker, D. G., & Allgeier, J. E. (2016). Foraging choices of vampire bats in diverse landscapes: Potential implications for land-use change and disease transmission. *Journal of Applied Ecology*, *53*(4), 1280–1288. https://doi.org/10.1111/1365-2664.12690

Streicker, D. G., Fallas González, S. L., Luconi, G., Barrientos, R. G., & Leon, B. (2019). Phylodynamics reveals extinction–recolonization dynamics underpin apparently endemic vampire bat rabies in Costa Rica. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1912), 20191527. https://doi.org/10.1098/rspb.2019.1527

Vigilato, M. A. N., Clavijo, A., Knobl, T., Silva, H. M. T., Cosivi, O., Schneider, M. C., Leanes, L. F., Belotto, A. J., & Espinal, M. A. (2013). Progress towards eliminating canine rabies: Policies and perspectives from Latin America and the Caribbean. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1623), 20120143. https://doi.org/10.1098/rstb.2012.0143

Voigt, C. C., & Kelm, D. H. (2006). HOST PREFERENCE OF THE COMMON VAMPIRE BAT (DESMODUS ROTUNDUS; CHIROPTERA) ASSESSED BY STABLE ISOTOPES. *Journal of Mammalogy*, *87*(1), 1–6. https://doi.org/10.1644/05-MAMM-F-276R1.1

WHO. (2020). *Rabies Fact Sheet* [World Health Organization]. https://www.who.int/news-room/fact-sheets/detail/rabies

World Bank, CIAT, & CATIE. (2014). *Climate-Smart Agriculture in Costa Rica* (CSA Country Profiles for Latin America Series, p. 12). https://climateknowledgeportal.worldbank.org/sites/default/files/2019-06/CSA-in-Costa-Rica.pdf

World Health Organization (Ed.). (2018a). *WHO Expert Consultation on Rabies: Third report*. World Health Organization.

World Health Organization. (2018b). Rabies vaccines: WHO position paper, April 2018 - Recommendations. *Vaccine*, *36*(37), 5500–5503. https://doi.org/10.1016/j.vaccine.2018.06.061

Figures

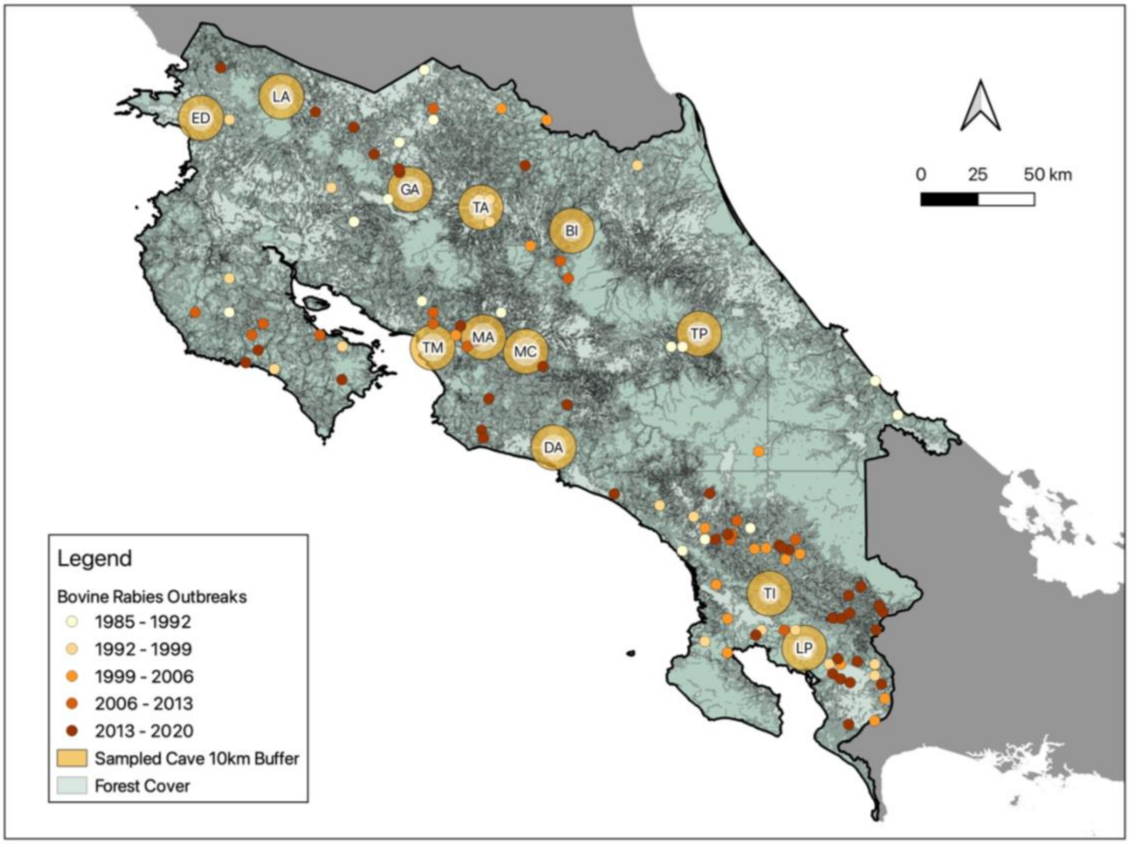

Figure 1: Cave sampling locations (n=12) of vampire bats (n=205) across Costa Rica. Bovine rabies outbreaks from 1985 to 2020 (n=109) provided by year for context.

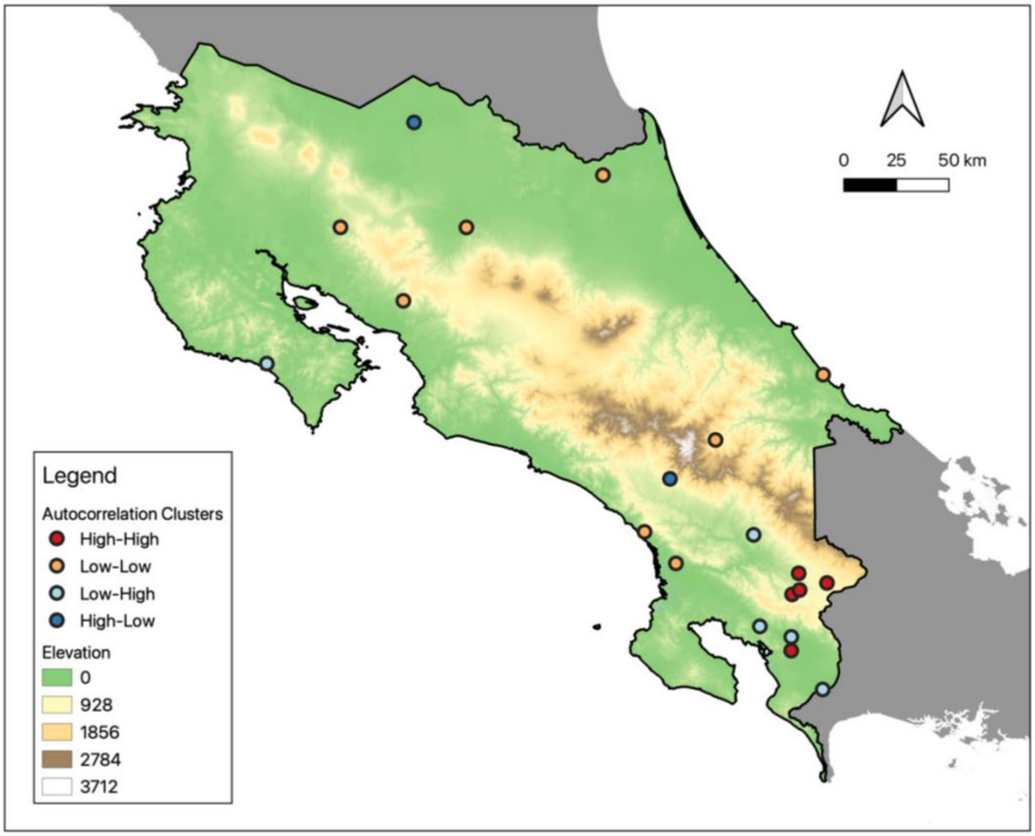

Figure 2: Spatial Autocorrelation of Bovine Rabies Outbreaks from 1985 to 2020 (n=109). Local univariate Moran's I (LISA analysis) highlighted four clusters of autocorrelation with a Moran's I of 0.138 ($p < 0.05$). Elevation provided for context.

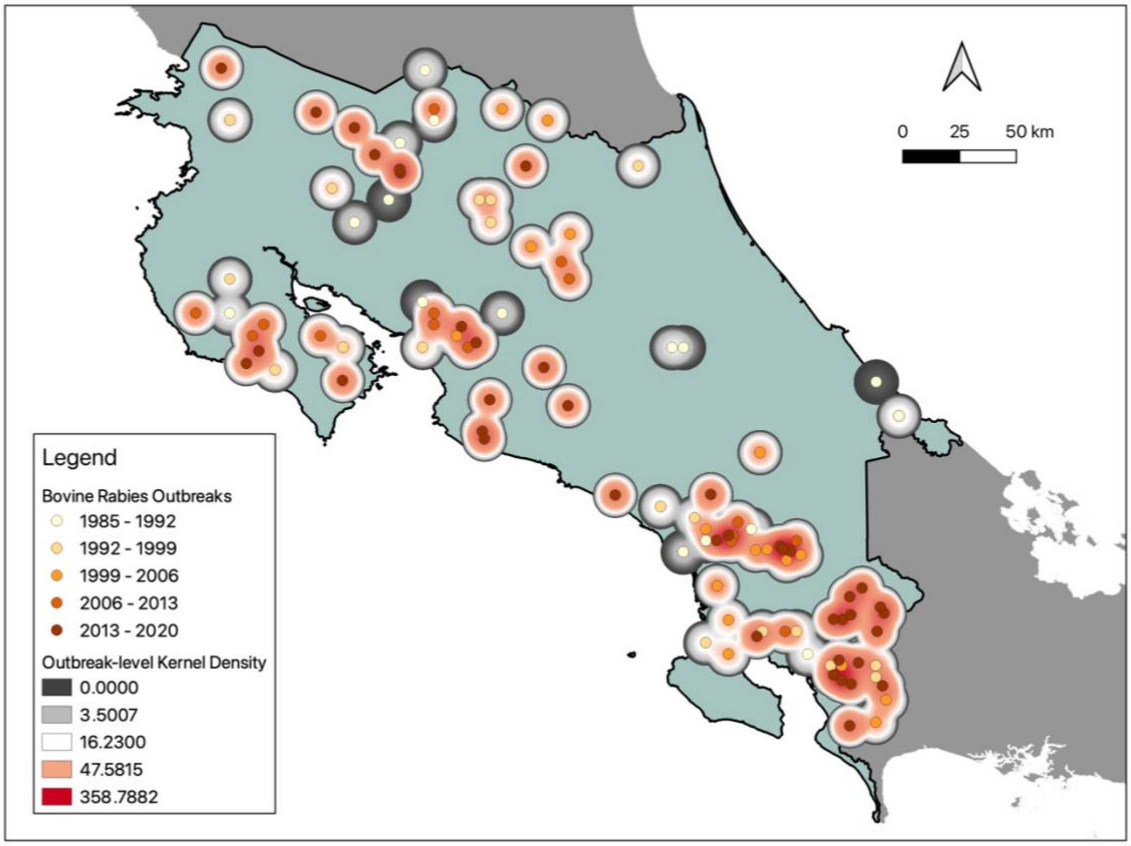

Figure 3: Kernel Density Estimations of Bovine Rabies Cases in Costa Rica from 1985 to 2020 (n=109). Kernel radius set at 10km to interpolate risk within bat foraging range.

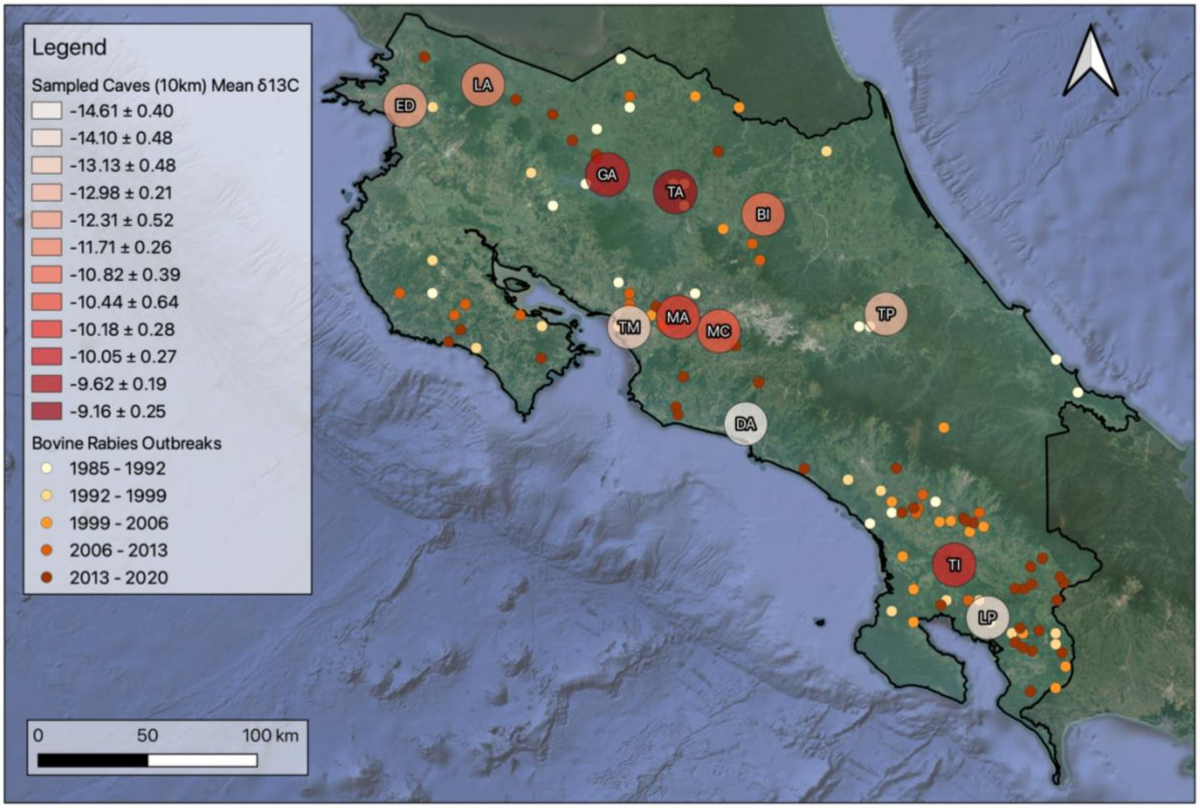

Figure 4: δ^{13}C Variation by Cave (n=12). DA denotes Damas (δ^{13}C = -14.61 ± 0.40‰), LP denotes Laguna Perdida (δ^{13}C = -14.10 ± 0.48‰), TM denotes Túnel Miraflores (δ^{13}C = -13.13 ± 0.48‰), TP denotes Túnel Peralta (δ^{13}C = -12.98 ± 0.21‰), ED denotes El Duende (δ^{13}C = -12.31 ± 0.52‰), LA denotes Los Araya (δ^{13}C = -11.71 ± 0.26‰), BI denotes Bijagual (δ^{13}C = -10.82 ± 0.39‰), MC denotes Mina Ciudad Colón (δ^{13}C = -10.44 ± 0.64‰), MA denotes Mina del Aguacate (δ^{13}C = -10.18 ± 0.28‰), TI denotes Túnel ICE Terraba (δ^{13}C = -10.05 ± 0.27‰), GA denotes Gabinarraca (δ^{13}C = -9.62 ± 0.19‰), and TA denotes Túnel Arenal (δ^{13}C = -9.16 ± 0.25‰).

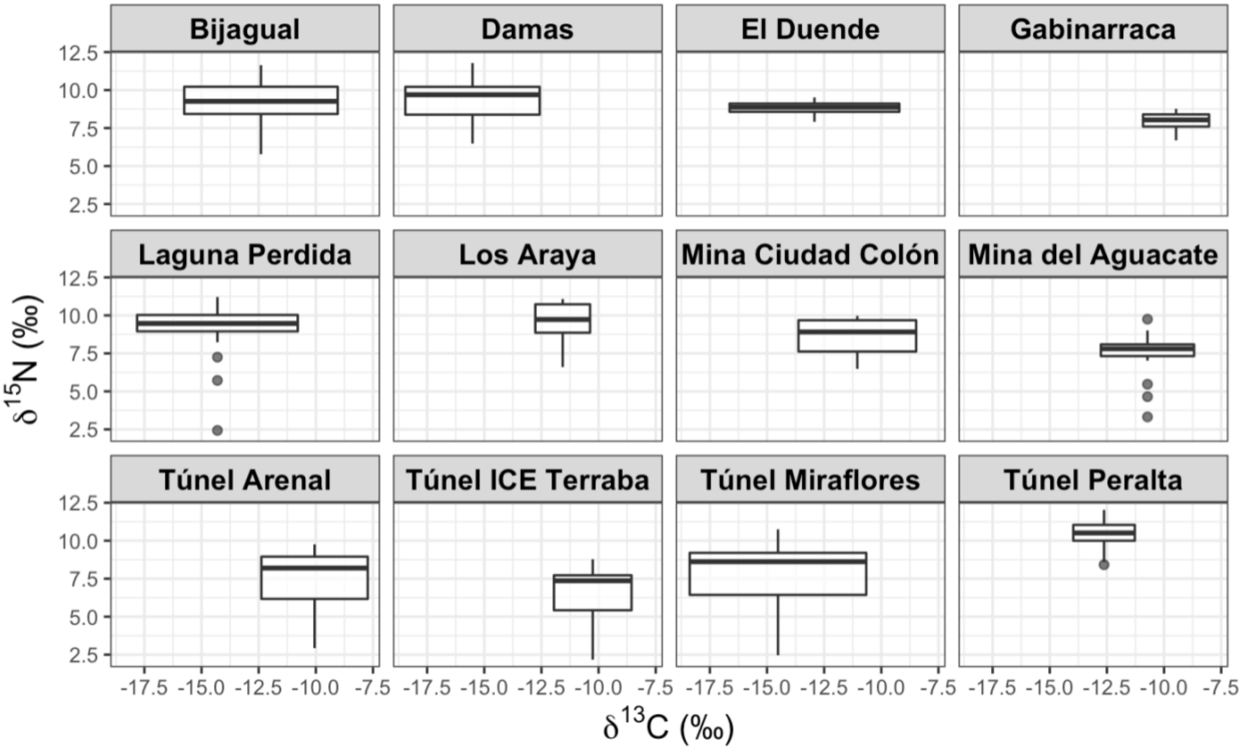

Figure 5: Biplot of $\delta^{13}C$ and $\delta^{15}N$ levels for bats sampled at 12 cave sites across Costa Rica.

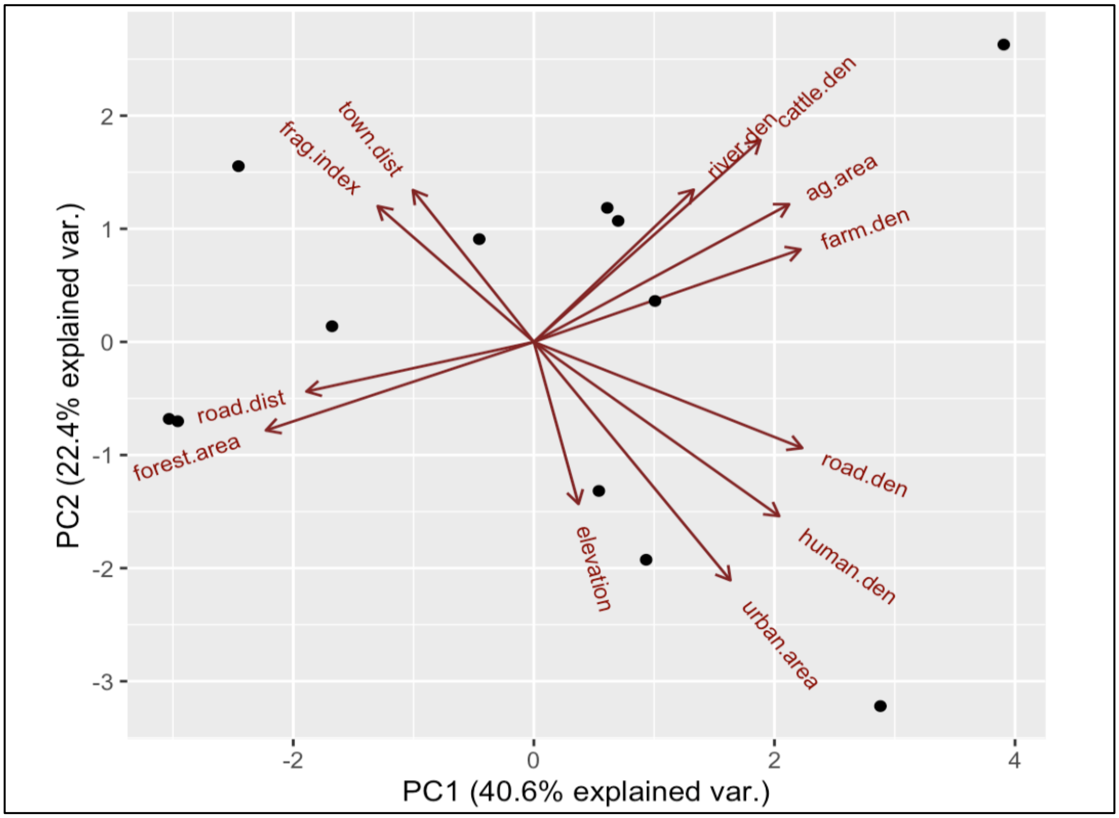

Figure 6: Biplot of the first two PCs on twelve measures of land-use, with arrows indicating variable contribution to PC loadings and points scaled by variance.

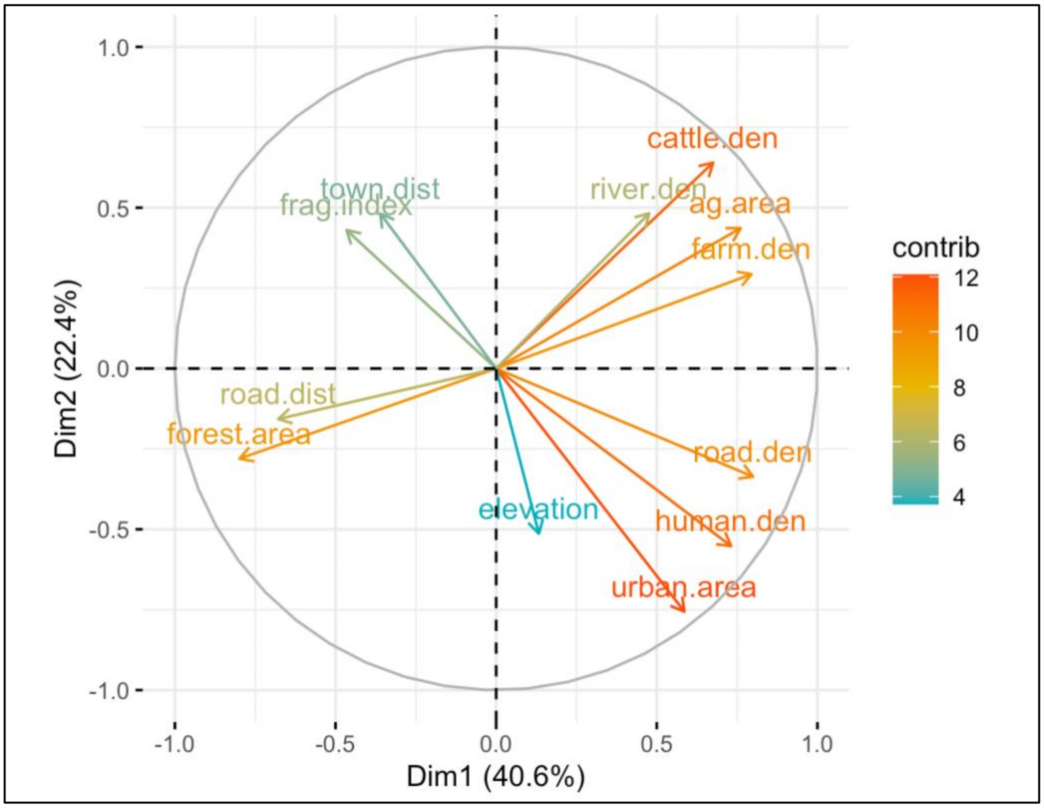

Figure 7: Contributions of twelve land-use predictors to PC variables. Level of contribution to PCs is depicted in a color gradient.