

# The road of >1000 corpses: landscape and roadrelated features that promote mortality in the Amazon

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#### **Abstract**

Roads impact wildlife around the world; however, dedicated studies are lacking in many biodiverse areas such as the Amazon. Identifying which species are more often hit by vehicles and which landscape and road-related features promote roadkill is essential to guide future development and ensure adequate mitigation actions. For six months, we monitored 240 km of roads in the Ecuadorian Amazon and recorded 1125 dead vertebrates (148 species). Reptiles were the most affected class with 380 individuals (56 species), followed by amphibians with 278 individuals (11 species), birds with 259 individuals (62 species), and mammals with 208 individuals (20 species). We used Random Forest models to explore the role of various land cover types and road sinuosity on the observed mortality. Additionally, we created heatmaps to visualize the road segments where roadkills were more frequent. For all vertebrates, mortality was more likely in straight road sections near rivers. The effect of other variables was taxaspecific. Amphibian mortality was more likely near bare soil or forest, birds and mammals died more often near herbaceous-shrubby vegetation, whereas reptile mortality occurred more often further from herbaceous-shrubby vegetation. Road segments with a high mortality (roadkill hotspots) varied across taxa. These hotspots identify areas where further research is needed to assess road impacts and where mitigation could prevent collisions. Among records, we found rare and threatened species, including some that may be new to science. Roadkill surveys not only aid in quantifying threats and informing future planning but can also provide insight into local biodiversity.

## Introduction

Roads are an important mortality source for wildlife around the world (Kioko et al. 2015; Grilo et al. 2020; Medrano-Vizcaíno et al. 2022); however, some regions with high biodiversity and expanding road networks are understudied (Silva et al. 2021). For example, the Amazon is the most biodiverse wilderness area in the world (Sangermano et al. 2012) but is threatened by growing human activities such as hunting, urbanization, agriculture, and roads (Viteri-Salazar and Toledo 2020). The addition of over 12,000 km of new roads planned in this region for the next five years (Vilela et al. 2020) may increase the risk of local extinction of several animal populations (Grilo et al. 2021) and could lead to the disappearance of many undescribed species (Funk et al. 2012).

Lack of local and regional information limits our understanding of how wildlife populations are affected by infrastructure and hinders sustainable development of new roads and effective mitigation plans. Conducting local studies is essential to better understand how species' traits and their habitat preferences, the natural and anthropogenic features of the landscape, the configuration of roads, and driver behaviour influence the incidence of road mortality and therefore guide future work and mitigation measures (Van Der Ree et al. 2015). For example, in Latin America, generalists, and short-lived birds and mammals with faster reproductive rates and living at higher population densities have been found to be more vulnerable to road mortality in general but distribution of these species varies regionally (Medrano-Vizcaíno et al. 2022). Road mortality has also been linked to neighboring habitat with distinct effects across vertebrate groups. For example, higher mortality occurs near water bodies in amphibians (Coelho

et al. 2012), near shrubs in birds (Carvalho et al. 2014), near forests in mammals (Freitas et al. 2015), and near grassland in reptiles (Braz and Rodrigues 2016). Finally, the size and configuration of the road can also influence how animals use it (Mulero-Pázmány et al. 2022) and their risk of collision leading to mortality (Ciocheti et al., 2017).

Here we present an assessment of mortality among terrestrial vertebrates on roads in the Ecuadorian Amazon. First, we describe the species found as roadkill and calculate standardized roadkill rates. Second, we evaluated the role of distance and coverage of different land covers and road configuration on roadkill incidence and identified hotspots of mortality. We hypothesized different land covers would influence roadkill events differently based on how they affect animal abundance and movement and road traffic. In particular, we predicted a high incidence of roadkill closer to natural land types (forest and herbaceous-shrubby vegetation) where vertebrates are more abundant (Carrete et al. 2009). We also predicted more roadkill of species that use human-dominated ecosystems near agriculture and urban areas where more road traffic should occur (Weisbrod et al. 2003). In addition, because rivers provide water resources and individuals may approach them regularly (Seo et al. 2013; Medrano-Vizcaíno 2018), we expected higher animal movement in these areas leading to higher roadkill near rivers. We also predicted more roadkill in straighter roads particularly for species that do not show behavioural avoidance towards traffic (e.g., herpetofauna, Jacobson et al., 2016). Vehicles generally circulate faster in straight (less sinuous) roads (Kang et al., 2019) which may increase roadkill likelihood (Tejera et al. 2018). On the other hand, sinuosity affects visibility which decreases the ability of drivers to prevent collision on the roads and therefore we may find more roadkills in sinuous road sections. To test these predictions, we collected roadkill data over a period of six months along 240 km of roads in the Napo province of Ecuador, an area of high endemism within the Amazon (Ribas et al., 2022) and analysed the records at different taxonomic levels.

## **Methods**

# Roadkill surveys

Our work covered almost the entire network of primary and secondary roads in the province of Napo-Ecuador. These roads link main towns in the region and are near four protected areas: Antisana Ecological Reserve, Sumaco-Napo-Galeras National Park, Cayambe-Coca National Park, and Colonso Chalupas Biological Reserve, where the dominant ecosystems are: evergreen high montane forest of the north of the eastern Andes mountain range, evergreen montane forest of the north of the eastern Andes mountain range, and evergreen piemontane forest of the north of the eastern Andes mountain range (MAE 2013). The sampled areas covered an altitudinal range between 300 and 3000 m a.s.l., with annual average temperatures ranging from 4.63 to 23.7°C, and annual precipitation from 1100 to 3400 mm (GADPN 2018; FONAG - EPMAPS 2021). All roads are paved, most of them have two lanes with a similar width and are mainly surrounded by agricultural land and forest.

From the 19th of September 2020 to 23rd March 2021 two people conducted roadkill surveys four-five days per week between 08:00 and 17:00 from a car driving at an average speed of 40 km/h (total 100 survey days). While monitoring, one member of the team was the designated driver while the passenger (always PMV) continuously scanned the road to locate carcasses on the pavement (roadkilled animals). Total study area covered 240 kms of primary and secondary paved roads. We monitored approximately half of this area in each survey day and each road section was surveyed approximately every three days. When a carcass was detected, the vehicle stopped on the side of the road on a safe area (both observers wore high-vis vests for safety). All carcasses were photographed onsite and georeferenced with a GPS unit (Garmin Etrex 22X). Then carcasses were either collected for a separate study or removed from the pavement to avoid doble counting. We aimed to identify all carcasses to species but in some cases, the specimen was too deteriorated or differed from recognized species (potentially undescribed fauna). Taxonomic identification was based on observer knowledge, specialized guides (e.g. Ridgely, R.; Greenfield, 2006; Tirira, 2017; Valencia et al., 2016; Wallach et al., 2014). In some cases, further taxonomic evaluation was done in the laboratory at Universidad Regional Amazónica IKIAM.

# Land cover and road configuration

We used available land cover data (Ministerio del Ambiente del Ecuador 2018) in QGIS software v.3.18.2-Zürich (https://qgis.org/en/site/) to calculate proximity and percentage of six different land cover types (agricultural lands, bare soil, herbaceous-shrubby vegetation, human settlements, forest, rivers) for each roadkill record and control sites (geographical coordinates of random points across the surveyed road whose quantity was the same as the number of roadkill records). Control sites were at least 200 m from a recorded roadkill and a minimum of 50 m from another control site. We estimated Euclidean distances to the nearest feature (e.g., closest distance to a river) for all land covers and percentages of the dominant land covers (forest and agriculture) at two scales: circular areas centered on each roadkill and control site with radius of 100 m and 500 m.

We also evaluated how road configuration influenced roadkill, using road data from the Instituto Geográfico Militar (2017). For this, we calculated a sinuosity score for each roadkill and control site considering a section of 100 m of road with the site located in the center (as we did not know the travelling direction of the vehicle that caused the collision, we had to consider sinuosity in both directions). We then calculated the score as the actual length of the road divided by the Euclidian distance between the two ends. A straight road had a sinuosity score of 1. Other road features such as road width and number of lanes were not evaluated because surveyed roads were mostly identical.

# Data analyses

First, we calculated the total number of roadkill (per class and for all identified species) and estimated standardized roadkill rates per species (ind./km/year) calculated as (total number of detected carcasses/240 km of road surveyed/185 days of survey period) \*365 days. We applied machine learning Random Forest classification methods to identify features differentiating roadkill from control sites. This method ensembles multiple classification trees from a bootstrap sample of the original data, and shows

higher classification accuracy than other methods such as logistic regression and linear discriminant analysis (Cutler et al. 2007).

We created Random Forest models based on 1500 classification trees using the R package "randomForest" and applying the function "randomForest" (Liaw and Wiener 2002). We fitted a general model including data from all vertebrates, class-specific models (i.e., amphibians, reptiles, birds, and mammals). We also fitted a model for fossorial species that included Caecilians and Amphisbaenians due to a previously known high prevalence of road mortality across this province (see Filius et al., 2020; Medrano-Vizcaíno & Espinosa, 2021). In addition, for any group in which any single species or related species were overrepresented (> 50% of the data within a taxonomic class), we also completed two additional analyses: 1) for the overrepresented taxa and 2) for all others in the taxonomic class. Models included 11 variables as predictors (proximity for each of the six land covers, percentage cover at 100 and 500 m for forest and agricultural land, and road sinuosity). We determined variables importance considering two measures: mean decrease accuracy and mean decrease gini index (means calculated across all fitted trees). Mean decrease accuracy is calculated as the loss of accuracy in the model when a variable (feature) is removed, while the mean decrease gini measures the impurity of nodes in split based on a variable (feature). We used the functions "important\_variables" and "plot\_multi\_way\_importance" from the R package "randomForestExplainer" (Paluszynska et al. 2020) to calculate and plot these metrics. Additionally, we generated dependence plots to represent the probability of roadkill according to changes in the value of each predictor using the function "partial" from the R package "pdp" (Greenwell 2017). This function allows visualization of the relationship between the response variable and a given feature including the interaction with the rest of features in the model.

In addition, we created heatmaps of roadkill based on kernel estimation density using QGIS software v.3.18.2-Zürich (https://qgis.org/en/site/) to visualize the areas where roadkills were more frequent in general (all vertebrates) and for each taxonomic class. This was done across the road network at a scale of 2 km radius and aggregated at two resolutions: 100 and 500 m. Then, we classified the heatmap data using a discrete interpolation into three classes of equal intervals, where the top tercile were identified as roadkill hotspots.

#### Results

We found 1125 wildlife vertebrate carcasses representing at least 148 species (308 carcasses could not be identified to species level mainly because of their poor state). Reptiles were the most common with 380 individuals from 56 species (89.74% of individuals and 91.5% of species were snakes), followed by amphibians with 278 individuals (11 species), birds with 259 individuals (62 species), and mammals with 208 individuals (20 species). The most roadkilled species was the cane toad *Rhinella marina* (208 carcasses: 1.71 ind./km/year), followed by the Andean opossum *Didelphis pernigra* (87 roadkills: 0.715 ind./km/year), the common opossum *Didelphis marsupialis* (48 individuals: 0.385 (ind./km/year), and the klebba's snail-eater *Dipsas klebbai* (47 individuals: 0.386 ind./km/year). The smooth-billed ani *Crotophaga ani* (23 individuals: 0.189 ind./km/year) was the most common roadkilled bird. Interestingly, we found numerous carcasses from fossorial organisms including Caecilians (40 individuals: 0.239

ind./km/year) and the speckled worm lizard *Amphisbaena bassleri* (29 individuals: 0.238 ind./km/year) (Table S1) (Figs. S11a and S11c).

Although most species found in this study (93.4%) are listed as Least Concern according to the IUCN red list (IUCN 2022), we recorded carcasses from two species catalogued as Vulnerable: the bailey's blind snake *Trilepida anthracina* (one roadkill) and the giant armadillo *Priodontes maximus* (one roadkill); one catalogued as Near Threatened: the grey ground snake *Atractus occipitoalbus* (one roadkill); two listed as Data Deficient: the touzet's ground snake *Atractus touzeti* (two roadkills), and the Andean cottontail *Sylvilagus andinus* (one roadkill), and three not yet evaluated: *Dipsas klebbai* (47 roadkills), the giant Ecuadorian toad *Rhaebo ecuadorensis* (two roadkills), and the white-striped eyed lizard *Cercosaura oshaughnessyi* (one roadkill).

# How Land Cover And Road Configuration Influence Roadkill Likelihood

The general and class-specific models revealed consistency regarding some factors that explain road mortality but also considerable variation among groups (Figs. 1 and 2). In all models, roadkill sites were more likely to be in straight roads (lower sinuosity) and near rivers than control sites. In general, distances to land cover were more important than percentages of cover to explain roadkill. However, the role of distance to land cover varied among taxa. While not always found to be a very important variable, we noted a general trend towards an increase in the likelihood of roadkill in areas with greater agricultural cover (Fig. 1).

Class-specific models revealed that amphibians were more likely to be roadkilled on straight roads located near rivers, bare soil, or forest, and at intermediate distances from herbaceous-shrubby vegetation (Figs. 1 and 2). Reptile mortality was higher on straight roads away from herbaceous-shrubby vegetation, and near rivers. Roadkilled birds were more often found across straight roads near herbaceous-shrubby vegetation, with less clear effects for other variables. Mammals were more likely to die from collisions on straight roads at low to intermediate distances from herbaceous-shrubby vegetation.

For amphibians we also separately analysed data from the common cane toad (very common species) and other taxa. Distance to river was the most important variable in both models, and cane toads were more likely to be found as roadkill away from herbaceous-shrubby vegetation (Figs. S1, S2). Because of the high abundance of opossums (*Didelphis pernigra* and *Didelphis marsupialis*) also mammals, we also analysed these and all others separately, but relevant factors were largely consistent in both models (Figs. S1, S3).

Fossorial species that included caecilians and amphisbaenians were more likely roadkilled on straight roads located near herbaceous-shrubby vegetation, and rivers. Additionally, mortality likelihood was higher on roads near and with high cover of agricultural land and forest (Figs. S1, S4).

## Roadkill Hotspots (Dup: Abstract?)

We identified several general roadkill hotspots (data from all vertebrates) particularly located around urban areas in Sala honda, Baeza, Oritoyacu, Puerto Napo, El Ansu, Santa Rosa and Buena Esperanza (Fig. 3). At the lowest scale these areas had more than 3.7 detected carcasses in 100 m, with the larger scale reflecting over 25 vertebrates killed in the 2 km stretch over a 6-month period (~ 1 vertebrate killed per week). Some of these areas were also detected as hotspots when analysing data for each taxonomic class separately, but also new taxa-specific hotspots were detected. For amphibians (excluding R. marina), hotspots were near Oritoyacu, Urcusiqui, and Sarayacu towns ( $\geq 1.92$  roadkills/100 m,  $\geq 2.81$ roadkills/500 m, and  $\geq$  4.45 roadkills/2 km. Fig. S5). Hotspots for *R. marina* were distinct and found at low altitude (between 373 and 569 m a.s.l.) near Puerto Napo, Sindy, Pucaurcu, Buena Esperanza, and Santa Rosa towns ( $\geq 2.37$  roadkills/100 m,  $\geq 5.58$  roadkills/500 m, and  $\geq 10.24$  roadkills/2 km. Fig. S6). We identified three reptile hotspots near Sala honda, Baeza, and Santa Rosa (≥ 3.21 roadkills/100 m, ≥ 7.46 roadkills/500 m, and  $\geq$  12 roadkills/2 km. Fig. S7). There were four hotspots of bird roadkill, one near San Francisco de Borja and Baeza and the other three near Las Cavernas, Calmituyacu, El Ansu, Buena Esperanza, and Santa Rosa ( $\geq 2.23$  roadkills/100 m,  $\geq 2.83$  roadkills/500 m, and  $\geq 6.38$ roadkills/2 km. Fig. S8). We found one hotspot of mammal roadkill (excluding opossums) near Baeza (≥ 1.29 roadkills/100 m,  $\geq$  1.81 roadkills/500 m, and  $\geq$  4.19 roadkills/2 km) which was near one of the four hotspots identified for opossums (the other three hotspots for opossums were near Sardinas, Sala honda, Oritoyacu, and Cuyuja. Figs. S9 and S10).

#### **Discussion**

Our study in the Ecuadorian Amazon revealed a great diversity of wild tetrapods being killed on roads (149 species from 1125 carcasses). This mortality followed some general spatial patterns for all vertebrates but also showed variation between classes and in some cases within classes. Consistently roadkill occurred more often on straight roads (lower sinuosity) near rivers for all vertebrates. Distances to herbaceous-shrubby vegetation, forest, and bare soil were also important predictors but their association with mortality were taxa-dependent. We additionally identified that the location of mortality hotspots varied among taxa but were mainly located near urban areas.

## Wildlife Mortality

We estimate approximately 9.25 vertebrates die on each kilometer of road in this province each year. This number is likely an underestimate because individuals may be hit by vehicles but died away from the road and not all carcasses are detected during road surveys (Ogletree and Mead 2020). Moreover, factors such as weather conditions, survey method and vehicle speed, and the destruction or removal of carcasses by predators, scavengers, and circulating vehicles influence detectability (Santos et al. 2011).

Our roadkill list includes a great proportion of the species distributed in Napo province. The area hosts 85 amphibian species, 48 reptiles, 587 birds, and 134 mammals (Calles López 2008). Roadkill affected

12.94% of these amphibians, all reptiles (in fact, we found more species than those previously described as present in Napo), 10.56% of birds, and 14.9% of mammals. Although it is evident that Napo roads are a noticeable source of mortality for many vertebrates, the fact that we recorded more reptiles' species than those already reported in the literature reflects the great biological richness of this area where the description of new species and distribution ranges is frequent (e.g., Medrano-Vizcaíno & Brito-Zapata, 2021; Melo-Sampaio et al., 2021). However, it also reflects the major impact of roads on reptiles. Indeed, reptiles were the most commonly found in our study. This may relate to higher local abundance but also could reflect driver behaviour. Snakes (89.74% of roadkilled reptiles in our study) can be intentionally killed by drivers (de Resende Assis et al. 2020).

The mortality of threatened species was low, with single records for the giant armadillo and the bailey's blind snake, both catalogued as Vulnerable (IUCN 2022). However, *Atractus* spp snakes were the most roadkilled genus of reptiles (Fig. S9). This is a poorly studied genus of neotropical herpetofauna (Cisneros-Heredia 2005), with 23% of the species distributed in Ecuador listed as threatened, and 47% as Data Deficient or remaining unassessed (Torres-Carvajal et al. 2022). Additionally, caecilians, considered as the least known terrestrial vertebrates (Jared et al. 2019), with 56% of the species distributed in Ecuador classified as threatened, and 28% as Data Deficient or remaining unassessed (Ron et al. 2021), had the second highest mortality in amphibians. While detailed population assessments are needed to understand the impacts of road mortality, for threatened species even low roadkill rates can have major impacts on their populations (Grilo et al. 2021). Our study suggests that roads in Napo could be affecting the vertebrate community, including threatened, poorly studied, and even undescribed species. Research and conservation actions should focus on threatened and poorly-known species, but a special effort to quantify risk for snakes seems a priority as road mortality of even few individuals can lead to local extinctions (Row et al. 2007).

## How Land Cover And Road Configuration Influence Roadkill

Road configuration was an important predictor in all cases, supporting our hypothesis that because vehicles circulate faster on straight roads, drivers are likely be less able to detect and avoid collision on these areas leading to a higher wildlife mortality. Our alternative hypothesis, that sinuous roads could prevent early detection was not clearly supported, although in some groups roadkill increased slightly with higher sinuosity. This could reflect a trade-off between speed and visibility: mortality is low in areas where speed needs to be reduced due to curves and visibility is still enough to detect and avoid some animals crossing roads. Also as hypothesized, generally for all vertebrates roadkill occurred more often in areas closer to rivers (see also Bastos et al., 2019; Lala et al., 2021). The need for individuals to access this vital resource can increase movement near water sources leading to greater mortality (Newmark et al. 1996). Proximity to rivers was especially relevant for amphibians, which is not surprising given how many of these species breed and live in water (Ficetola and De Bernardi 2004). This association has been described in other studies in temperate regions (eg., D'Amico et al., 2015; Seo et al., 2013) and has led to

suggestions that the construction of artificial water bodies near roads is avoided as it can particularly affect amphibian populations (Coelho et al. 2012).

The effect of other land cover types was taxa specific. Proximity to bare soil or forest predicted amphibian roadkill, potentially linked to higher abundance and movement within suitable habitats (forests) and to increased movement when crossing largely unsuitable areas to access other resources (bare soil). Bird and mammal mortality was greater near herbaceous-shrubby vegetation (see also Ferreguetti et al., 2020; Plante et al., 2019). For birds, collision risk may increase with low or medium high vegetation as birds that fly from low vegetation to low vegetation likely stay closer to the ground and thus in the path of circulating vehicles increasing collision risk (Santos et al. 2016). Likewise, shrubs near roads are used as nestling sites for some birds, and as refuge and foraging sites for birds and mammals, increasing their risk of mortality due to a close interaction with roads (Gunson et al. 2011; Bravo-Naranjo et al. 2019). Shrubby vegetation near road edges can also reduce visibility preventing drivers from noticing approaching birds or mammals in time to avoid collision and preventing animals from detecting travelling vehicles (Lala et al. 2021). Additionally, areas with herbaceous-shrubby vegetation may attract animals, leading to greater local abundance and movement and thus, higher roadkill likelihood. These effects would increase when vehicles circulate faster (as expected on straight roads). Reptiles roadkill occurred more often close to human settlements and in areas of higher agricultural land cover, potentially because these areas often hosts high prey abundances (e.g. rodents, Stenseth et al., 2003). Finally, a higher mortality of fossorial species (caecilians and amphisbaenians) associated to herbaceous-shrubby vegetation, rivers, agricultural land and forest could be due to soils rich in organic matter across these land covers. These soils offer shelter, and harbor a great diversity of invertebrates, which can be attractive for these fossorial species whose diet is mainly composed by earthworms and insects (Jared et al. 2019; Amorim et al. 2019; Kouete and Blackburn 2020). Nevertheless, soil compaction near roads could limit underground movements forcing fossorial animals to emerge to the surface, and consequently expose them to vehicular traffic (Maschio et al., 2016).

## **Roadkill Hotspots**

Identified hotspots were generally in the vicinity of towns, likely due to increased traffic. When hotspots were defined for different classes (and even for different taxa within class) we found that locations were not consistent. This can reflect differences in animal behaviour, movement and abundance, as well as traffic patterns and driver behaviour, factors that can lead to different hotspots for various taxa (Teixeira et al. 2013; Silveira Miranda et al. 2020). Two roadkill hotspots were consistent with hotspots identified in a study conducted in 2014 (see Medrano-Vizcaíno & Espinosa, 2021). These areas were located near Baeza and Sala honda towns and could be potential sites for further research to assess how roadkill impacts long term population viability.

Road ecology studies not only provide information on wildlife mortality but can also expand our knowledge of local biodiversity. Records from our survey have already contributed to extend the

geographic distribution ranges for six snake species rarely observed in the wild (*Anilius scytale, Drymarchon corais, Erythrolamprus breviceps, Micrurus lemniscatus, Oxyrhopus vanidicus, Trilepida anthracina*. Medrano-Vizcaíno & Brito-Zapata, 2021). Additionally, ongoing research could identify new species and extended geographic areas among roadkill specimens of caecilians and *Atractus* snakes, which are poorly-studied vertebrates with taxonomic uncertainties (Cisneros-Heredia 2005; Wilkinson 2012). A roadkilled specimen collected in a previous study in this area (Medrano-Vizcaíno and Espinosa 2021) was described as a new snake species by Melo-Sampaio et al., (2021).

Our work, besides revealing how certain landscape and road features can drive to a higher mortality across the biodiverse Amazonian province of Napo, also provides a relevant insight into taxonomic groups and areas that need special attention and dedicated research to assess the impact of road mortality on their populations. The replication of our approach across other biodiverse areas could be considered as a first step of a framework of evaluation of the impact of roadkill on local extinction risk. We hope that this work can be useful to guide future research and conservation initiatives to favor wildlife populations.

## **Declarations**

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#### **Competing interests**

The authors have no relevant financial or non-financial interests to disclose.

#### **Authors contributions**

PM-V, MG-S and CG conceived the ideas and designed methodology; PM-V and DB-Z collected the data; PM-V analysed the data; PM-V led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

#### **Data availability**

Datasets that include distances to landscape features, percentage of cover, and road sinuosity are available on figshare [https://figshare.com/s/b8ddfa99cef4a22698ee].

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## **Figures**

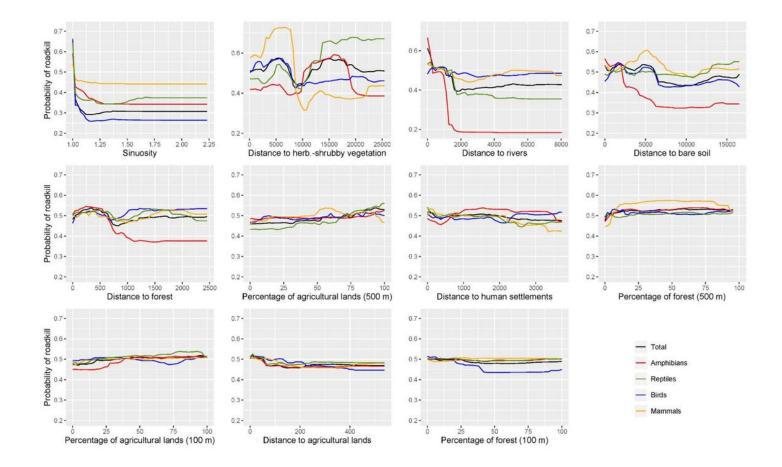


Figure 1

Dependence plots showing how land cover and road configuration influence probabilty of vertebrate roadkill in the Napo region of Ecuador. Plots are shown in descending order of variable importance for models fitted for all vertebrates (Total) and the separate taxonomic classes.

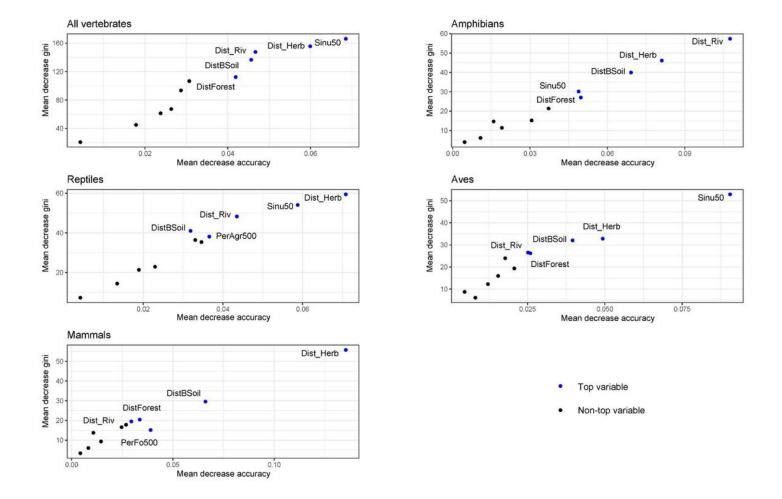


Figure 2

Variable importance from Random Forest models evaluating the effect of land cover and road configuration on vertebrate roadkill in the Napo region of Ecuador. Results are presented for models fitted for all vertebrates combined and for each taxonomic group. Variable importance is based on mean gini decrease and mean accuracy decrease. Labels indicate the five most important variables in blue dots (higher accuracy and gini decrease values). Sinu50=sinuosity (50 m), Dist\_Herb=distance to herbaceous-shrubby vegetation, Dist\_Riv=distance to rivers, DistForest=distance to forest, DistBsoil=Distance to bare soil, PerAgr500=Percentage of agricultural lands (500 m buffer), PerFo500=Percentage of forest (500 m buffer).

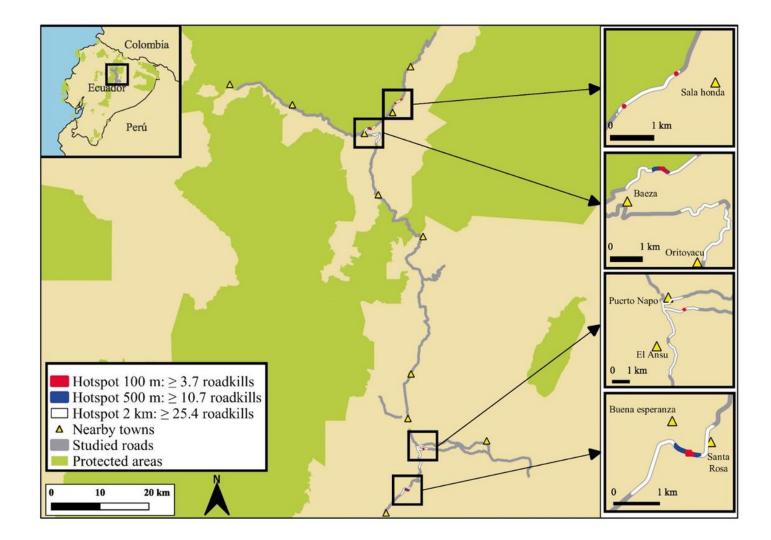


Figure 3

Roadkill hotspots across the 240 km of our study area in Napo when data for all vertebrates were combined (amphibians, reptiles, birds, and mammals).

## **Supplementary Files**

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• Supplementarymaterial.docx