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Abstract

The efficacy of protected areas is tied to the management of surrounding areas. Still, the importance of buffer zones for biodiversity conservation is overlooked. Manu Biosphere Reserve is one of the most biodiverse places on earth, yet destructive land-use practices are degrading the ecological integrity of its buffer zone. To better understand the importance of different land-uses within Manu's buffer zone for biodiversity conservation, we assessed amphibian communities across a land-use gradient in the buffer zone (immigrant agricultural land, forests used by three Indigenous communities, and a regenerating forest), in addition to a reference site in its core protected area. We surveyed six sites and sampled amphibian communities using visual encounter surveys and leaf litter searches over dry and wet seasons.

Overall, in 2249 ha surveyed of the buffer zone, we recorded 70 amphibian species (57% of the 124 species recorded in the Manu Biosphere Reserve from the same elevational range within our study). Species richness, evenness, and diversity of amphibians decreased with habitat degradation and were lowest in the agricultural land. Conversely, the richness and diversity of amphibians in the regenerating forest and the Indigenous communities' forests were similar to that of the core protected area, and each had a relatively unique community composition, whereas the agricultural land was dominated by generalist species. Our results suggest that increasing degradation through expanding agriculture traditionally adopted by immigrant communities could significantly threaten biodiversity within the buffer zone. However, our findings also underscore the high potential of buffer zones managed by Indigenous communities for biodiversity conservation. A combination of sustainable livelihood

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activities, cultural practices, and forest protection, as observed in many Indigenous communities, is critical to fulfilling the role of a Biosphere Reserve—to reconcile the conservation of biological and cultural diversity while improving social and economic development.

Keywords

buffer zone, protected areas, conservation, native communities, immigration

Introduction

Biosphere reserves around the world have been created to contribute to the conservation of biological and cultural diversity, ecosystem services, and sustainable development (Bridgewater, 2002). Their core areas are devoted to long-term conservation objectives, while their buffer zones aim to promote human and economic development in an ecologically sustainable manner, and to ensure the protection of the core zones (Bridgewater, 2002; Lasserre & Hadley, 1997). Despite the importance of buffer zones as an extra layer of protection to protected areas, the lack of political will, inadequate budget for enforcement and uncertain institutional responsibilities have limited their conservation effectiveness worldwide (Kintz et al., 2006; Lynagh & Urich, 2002; Mehring & Stoll-Kleemann, 2011; Weisse & Naughton-Treves, 2016). Intensive land-use has increased outside the administrative boundaries of existing protected areas causing, in many cases, habitat degradation and fragmentation (DeFries et al., 2005; Griffith et al., 2009; Joppa et al., 2008; Radeloff et al., 2010; Weisse & Naughton-Treves, 2016). Even though buffer zones influence the effectiveness of protected areas for conserving biodiversity (Hamilton et al., 2013), studies comparing biodiversity inside and outside protected areas are limited (Rada et al., 2019). A previous study by Whitworth et al., (2016) found that an intensively studied 40-year-old regenerating forest site, situated within the globally significant Manu National Park buffer zone, contains high biodiversity and conservation value, with 87% of alpha diversity detected within nearby old-growth primary forest. Understanding how much biodiversity buffer zones harbor and their contribution to the overall biodiversity conservation of a Biosphere Reserve is essential, especially for tropical protected areas that are becoming increasingly isolated as deforestation along the borders increases (DeFries et al., 2005).

The Manu Biosphere Reserve, in the Peruvian Amazon, is a UNESCO World Heritage Site designated to protect the globally important Amazon rainforest and its biodiversity (Myers et al., 2000). Its high levels of biodiversity can be in part explained by a remarkable elevation gradient of over 4000 m, extending from the lowland Amazon rainforest to the Puna grasslands of the high Andes (Boehm et al., 2018; Catenazzi et al., 2013; Shepard et al., 2010). The Manu National Park, the core protected area of the Manu Biosphere Reserve, is an area with one of the world's highest rates of

species endemism and richness (Myers et al., 2000). It has one of the highest herpetofaunal diversity on the planet, with over 155 known amphibian species (Catenazzi et al., 2013; Shepack et al., 2016), and many more amphibians species that are being continuously discovered (Chaparro et al., 2015; Serrano-Rojas et al., 2017; Shepack et al., 2016).

Amphibians are one of the key taxonomic groups used to assess the impacts of habitat disturbance in tropical rainforest (Whitworth et al., 2017), chosen because they are considered sensitive indicators within their ecosystems (Ficetola et al., 2014; Hocking & Babbitt, 2014) and because of their conservation importance—as nearly one-third of amphibians species are formally classified as threatened (IUCN 2021). Amphibians display a high level of sensitivity to disturbance due to complex life cycles, low mobility, limited dispersal capacity, and narrow ecological requirements (Lawler et al., 2010). These characteristics make amphibians especially vulnerable to land-use change and habitat loss compared to other terrestrial vertebrates (Ficetola et al., 2014). A better understanding of the amphibian diversity within the different land-use types in the Manu Biosphere Reserve can provide critical information for conservation management regarding how effective both National Park and the buffer zone are in conserving biodiversity and maintaining a healthy ecosystem.

Biodiversity within the Manu Biosphere Reserve is being threatened by intensive land-use in the surroundings of its core area (Catenazzi et al., 2013; Gallice et al., 2019; Shepard et al., 2010). Historically, Manu National Park has been kept relatively protected from uncontrolled deforestation, mining, and colonization because it is surrounded on one side by protected areas, including the Alto Purus National Park to the northeast and the Amarakaeri Communal Reserve to the southeast. On the other side, its southeastern border comprises the buffer zone where limited resource extraction and colonization are allowed. However, the construction of a road that cuts through the buffer zones of the Manu National Park and the Amarakaeri Communal Reserve has facilitated access to the Manu region since 1960 (Shepard et al., 2010). As a result, the buffer zone of the Manu Biosphere Reserve is subject to a diverse range of land-uses spanning intensive agriculture, logging activities, secondary forest regeneration, and native community territories, where hunting and selective logging is taking place. Moreover, a proposed expansion of the existing road is threatening Manu's Biodiversity even more, as this road aims to connect Manu with the illegal gold mining hub in the southeast of Peru, specifically Puerto

Maldonado (Gallice et al., 2019; Larrea-Gallegos et al., 2017). Therefore, it is essential to understand how biodiversity is responding to the changing and current land-use types, to be able to illustrate the potential future for increased human disturbance.

In this study, we carried out an amphibian biodiversity assessment within the Manu Biosphere Reserve. We chose sites to reflect the diverse land-use types within Manu and assess the changes in amphibian communities across a typical buffer zone transition, from clearance for agriculture, protected regenerating secondary forest, native community lands subjected to hunting and selective logging, and old-growth forest from within the National Park. We apply the MacKinnon list technique (Bach et al., 2020; Herzog et al., 2002; Macleod et al., 2011; Muir & Muir, 2011), a rapid assessment survey methodology specially designed for use in species-rich environments. This technique is a time-efficient and cost-effective sampling method that effectively detects changes in species richness, abundance, and community composition. Specifically, we set out to ask the following questions: (1) Do sites under different land-use types within the Manu Biosphere Reserve contain similar biodiversity levels of amphibians in terms of richness levels, abundance, and community structure? (2) To what extent can land-uses typical within indigenous lands in Manu's buffer zone conserve amphibian biodiversity compared with the National Park and more intensive agricultural use expanding in the region? (3) To what extent could the conservation of such

land-use type contribute to maintaining extreme biodiversity levels within the overall biosphere reserve? We predict: (1) different levels of biodiversity within the study sites and land-uses, with (2) the highest species richness and diversity in the old-growth forest, followed by the regenerating forest and the native community forests, and the lowest species richness and diversity in the site used more intensively for agriculture.

Methodology

Study Location

This study was conducted in the Manu Biosphere Reserve, a UNESCO and IUCN World Heritage Site designated to protect the globally important Amazon rainforest in southeastern Peru. As part of the Western Amazon, an area that holds the highest levels of biodiversity throughout Amazonia, the Manu Biosphere Reserve consists of a network of core protected areas surrounded by areas designated as buffer zones. Six key survey sites were chosen to best represent typical current land-uses present within the Biosphere (Figure 1). Two sites were within core protected sites (an old-growth primary forest within the Manu National Park and one privately owned regenerating forest area in the buffer zone), both strictly protected from logging, hunting and agricultural activities. The other four locations were located in the buffer zone and lack strict formal protection (three native communities that have divided their land into different zones

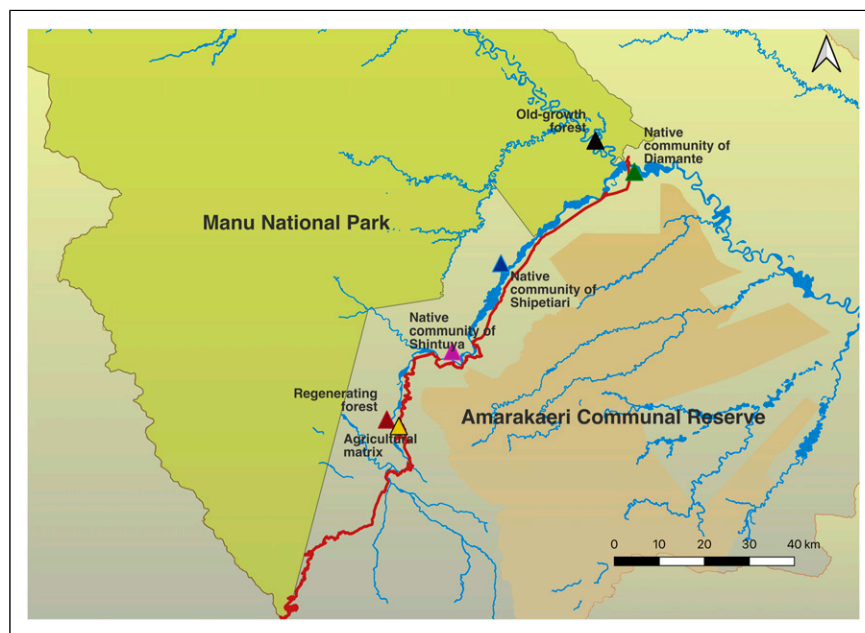


Figure 1. Map of the survey sites within the Manu Biosphere Reserve (with a black triangle for the old-growth forest, dark green for the native community of Diamante, dark blue for the Native community of Shipetari, magenta for the Native community of Shintuya, red for the regenerating forest and yellow for the Agricultural matrix). Major rivers are represented by blue, and the two major protected areas (Manu NP and Amarakaeri CR) indicated. The existing road network currently traversing the buffer zones of the Manu National Park and the Amarakaeri Communal Reserve is represented by a red solid line.

including areas for conservation and tourism, small-scale agriculture, hunting, and subsistence logging, and one community of non-indigenous settlers that use the land mostly for intensive agriculture). In all sites, we chose to survey the areas with the predominant use type; therefore, for the native communities, we surveyed areas designated for conservation and tourism. See [Supplemental Appendix A](#) for the comprehensive site descriptions and [Figure 1](#) for a map of the study sites.

Sampling

We sampled amphibian communities through both dry (Apr–Jun 2016) and wet (Sep–Nov 2016) seasons along 20 transects per site to better represent the amphibian community composition in the Manu Biosphere Reserve. The samples were taken between 1900 h and 2400 h, corresponding with the period of greatest anuran activity ([Duellman & Trueb, 1986](#); [Whitworth et al., 2017](#)). The transects were time constrained. Each sampling survey consisted of two trained surveyors carrying out a five-minute leaf litter search at the beginning of the transect, followed by a 25-minute transect visual encounter survey ([Bell & Donnelly, 2006](#); [Doan, 2003](#)). During the leaf litter search, leaves, branches and logs were moved to search for terrestrial amphibians—using thick leather gloves for protection. During the visual encounter survey, an area of four meters wide and up to two meters in height was searched by the observer along each survey sampling site. These sites were set off-trails to avoid known detection biases associated with pre-existing trails ([von May & Donnelly, 2009](#)). The direction off-trail was chosen based on selecting the less-dense route that allow us to freely walk without disturbing the vegetation of the area, generally following a perpendicular or diagonal direction. A Walktax measuring device was used to measure the distance covered by each observer per survey (mean, 161 m; range, 101–225 m). The Walktax incorporated a plastic box with a cotton string roll that observers attached to a trunk at the starting point of the survey. As observers walked, the string was pulled outside of the box, and the Walktax counter recorded the distance with an accuracy of 0.2%. The use of the Walktax avoided the need for any prior disturbance to the forest by the surveyors since there were no paths cut to establish transects. This design allowed the observers freedom to walk into the forest, helped by the string of the distance measurer to get back to the starting point. In addition, possible pseudo-replication of detection that could occur by the observer walking the same path twice was avoided by visualizing the string of the Walktax. The sampling effort was the same for all land-use types ($n = 40$; 20 surveys per season), except for the old-growth forest ($n = 32$; 16 surveys per season) due to limited accessibility in the area. All survey locations were separated at least 200 m from each other to assure statistical spatial independence of samples for amphibians ([Veith et al., 2004](#)). We

complemented this data with incidental amphibian records throughout the sampling period.

Forest Structure, Daily Factors, and Site Factors

In order to characterize the physical forest structure at each of the sampling locations, we measured a set of six structural variables, which are known to vary with anthropogenic or natural disturbance ([Whitworth, Villacampa, et al., 2016](#); [Whitworth et al., 2017, 2019](#)). These variables included average leaf litter cover, average leaf litter depth, shrub density, average canopy cover, canopy height, and density of trees (>5 cm DBH). See supplementary information in [Supplemental Appendix B](#) for details on how each of the variables was calculated.

Data Analysis

Amphibian Species Comparison Between the Manu Biosphere Reserve and its Buffer Zone. We compiled a list of all the species ever recorded in the Manu Biosphere Reserve, distributed within the same elevational gradient where this study took place (265–792 m asl), using the most recent published herpetofauna datasets from Manu ([Catenazzi et al., 2013](#); [Whitworth, Downie, et al., 2016](#)). We compared this list against (1) the total number of amphibian species recorded in our study sites to understand the importance of our sites for the whole amphibian diversity and (2) the number of species found only within the buffer zone sites to understand the contribution of the buffer zone for the whole amphibian diversity. Additionally, we compiled a list of all amphibians that have been found uniquely in the buffer zone in our study sites and were never recorded inside the Manu core protected area, this to highlight the importance of buffer zones as areas that lack formal protection but still support rare, endemic, and/or threatened species.

Mackinnon List Technique

All the opportunistic amphibian observations found outside the limits of our survey area (four meters wide and two meters height) while on survey, and all the amphibians encounter during our walks between sampling locations were recorded as incidental records. These incidental records were combined with the records from nocturnal transects to maximize the number of encounters per site. Both were included in the analysis creating samples using the Mackinnon Species List Technique (MLT; ([Bach et al., 2020](#); [Herzog et al., 2002](#); [Macleod et al., 2011](#); [Muir & Muir, 2011](#))). This technique has been successfully used for birds ([Herzog et al., 2002](#); [Macleod et al., 2011](#)), marine ecosystems ([Bach et al., 2020](#)) and amphibians ([Muir & Muir, 2011](#)) as a rapid assessment technique. To create lists, we generated a chronologically ordered master list by recording a list of all individuals seen during the nocturnal transects and incidental records. Once the data were assembled into this chronologically ordered

master list, we separated it into list samples consisting of five species each. Most bird community studies use lists of 10 species instead of five; however, the avian community is generally greater (between 150 and 300 species) in comparison to the amphibian community (with fewer than 150 amphibian species found in previous work conducted nearby; Russell et al., 2019; Von May et al., 2010; Whitworth, Downie, et al., 2016). A recent marine ecosystem study with 90 fish species also utilized Mackinnon lists of five species (Bach et al., 2020). The created lists were used in the subsequent analysis for species richness, diversity, relative abundance, and community composition.

Species Richness and Diversity Profiles Across Land-Use Types

We compared amphibian species richness between land-use types using rarefaction and extrapolation of richness following Hsieh et al. (2016). We generated sample-based rarefaction curves along with confidence intervals using the *iNEXT* R package (Hsieh et al., 2016). We calculated the 84% confidence intervals for the average estimated species richness for each land-use type following Altman & Bland (2011)—where non-overlapping 84% intervals suggest a significant difference at $p < 0.05$ (MacGregor-Fors & Payton, 2013). To have a clear comparison of where our observed species richness values would have projected given the detection of an even number of individuals, we extrapolated the lower-lying curves towards an equal number of individuals when sampling effort detected fewer individuals. Moreover, a variety of species richness estimators, including ACE, ICE, Chao 1 and 2, Jackknife 1 and 2, Bootstrap, and MMEans, were calculated and averaged for each land-use type, as recommended by Veith et al. (2004) and Whitworth et al. (2017), using Estimate S (Colwell, 2013). Sampling completeness in each habitat was calculated as the percentage proportion of observed species richness compared to the estimated species richness (Kudavidanage et al., 2012).

Additionally, we generated diversity profiles for each land-use type using the function *div_profile* from the R package *hilldiv* (Alberdi & Gilbert, 2019). Diversity profiles are a plotted series of Hill numbers (Hill, 1973) as a function of the impact of rare species on the measure of diversity (q). The sensitivity of the diversity measure depends on the order of the parameter q , where the parameter q indicates the weight given toward rare or common species. Where $q = 0$ (species richness) is weight towards rare species, $q = 1$ (exponential of Shannon) is weighted toward common species, and $q = 2$ (inverse Simpson) is weighted toward abundant species. The shape of the diversity profile curves informs us about the evenness of a community, where the more uneven a community is, the faster the curve declines as the coefficient q increases. We conducted all analyses in the R statistical software version 4.1.2 (R Core Team, 2021).

Community Composition, Evenness, and Structure

Community composition differences between land-use types were assessed using non-metric multidimensional scaling (NMDS; using the Bray-Curtis similarity measure). The stress value was relatively low (0.10), so it was displayed within two dimensions. To assess the statistical significance of observed differences in assemblage composition between land-use types, we conducted an analysis of similarities tests (ANOSIM; using 999 permutations, see Helbig-Bonitz et al., 2015).

The dominance-diversity Whittaker plots compared the community evenness and structure between land-use types. Significant differences in community evenness were assessed using a linear model with the log relative abundance as the response variable and the interaction between species rank and study site as continuous and categorical fixed effects, respectively (Beirne et al., 2013; Whitworth, Villacampa, et al., 2016). Absolute change in gradient between disturbance areas is reported as ΔG , where more negative values denote a less even assemblage (displayed by steeper curves). NMDS ordinations, ANOSIM tests and Whittaker plots were carried out using the *vegan* R package (Oksanen et al., 2019).

Analysis of Environmental and Forest Structure Variables to Explain Species Richness and Abundance

Multi-collinearity among our environmental and forest structure variables was examined with the variance inflation factor (VIF; Zuur et al. (2009)). Our VIF values were all below 4 indicating that there was not collinearity between our predictor variables. Amphibian species richness and abundance were compared between land-use types using generalized linear mixed-effects models. We assessed whether forest structural variables and altitude could predict: (1) amphibian species richness and (2) abundance using a zero-inflated negative binomial GLMM type 2 (family *nbinom2*) (Blasco-Moreno et al., 2019). In both models, season was used as a random effect, and they were built using the function *glmmTMB* from the *glmmTMB* R package (Brooks et al., 2017). Overdispersion for count data was checked using the function *dispersion_glm* from the *blme* R package (Korner-Nievergelt et al., 2015). Zero inflation was checked using the function *check_zero_inflation* implemented in the *glmmTMB* R package (Brooks et al., 2017). For each model, using the dredge function within the *MuMin* R package (Bartoń, 2019), we generated and evaluated the small-sample corrected Akaike information criterion (AICc), $\Delta AICc$, and AICc model weights (w_i). We followed this with a top model averaging approach (on models where $\Delta AICc < 2$), to determine predictor relative importance (Anderson, 2008; Grueber et al., 2011). We used the function *plot_model* in the *sjPlot* R package (Lüdtke, 2021) to visualize the marginal effect of each fixed predictor.

Results

Amphibian Species Comparison Between the Manu Biosphere Reserve and its Buffer Zone

A total of 1051 individuals belonging to 74 amphibian species were recorded during our surveys (Figure 2). This includes 886 individuals of 70 species within our sites in the buffer zone and 165 individuals of 33 species in our site inside the protected area. Our numbers represent 60% of the total number of amphibian species ever recorded in the Manu Biosphere Reserve (124 species) from the same altitudinal range covered in this study. Considering only the sites within the buffer zone we registered 57% of amphibian species from the Manu Biosphere Reserve within the same elevational range. Out of this last number, ten amphibian species recorded were unique to the buffer zone. See Supplemental Appendix C for a full list of all amphibians found at each study site.

Species Richness and Diversity Profiles Across Land-Use Types

Records from nocturnal transect ($n = 434$) and incidental records ($n = 617$) were combined to maximize the number of encounters ($n = 1051$). We created 146 lists of five species using the Mackinnon List Technique. The highest average estimated species richness was found in the native community of Diamante (51 ± 1.76 species: Table 1 and Figure 3), followed by the native community of Shipetiari

(40 ± 2.20 species) and the regenerating forest (39 ± 2.92 species). The lowest estimated species richness was found in the agricultural matrix (19 ± 0.88 species). All sites but the agricultural matrix showed no significant difference between them ($p > 0.05$) (Figure 3). Overall, our sites estimated a total of 79 species for the buffer zone, and 83 species including the protected forest site from the National Park, with sampling completeness of 89% (Table 1).

According to the diversity profiles (Figure 4), the species richness ($q = 0$) and community evenness ($q = 1$ and $q = 2$) declined dramatically when an area is used for intensive agriculture as in our agricultural matrix. The native community of Shipetiari, Diamante, and the regenerating forest supported the highest amphibian diversity, whereas the agricultural matrix showed the lowest levels of amphibian diversity. The native community of Shintuya had higher values of diversity when $q < 1.5$ (weighted toward rarer species), but this diversity is reduced when $q > 2$ (more weighting towards common species). Our results show that land-use types used for ecotourism and conservation harbor a higher number of rare species than common or dominant species, whereas areas used for intensive agriculture harbor only few abundant generalist species.

Community Composition, Evenness, and Structure

The community composition analysis from NMDS plots and the associated ANOSIM analysis showed that the community composition between forest types was significantly different ($R = 0.43$, $p = 0.001$). However, the native communities, the

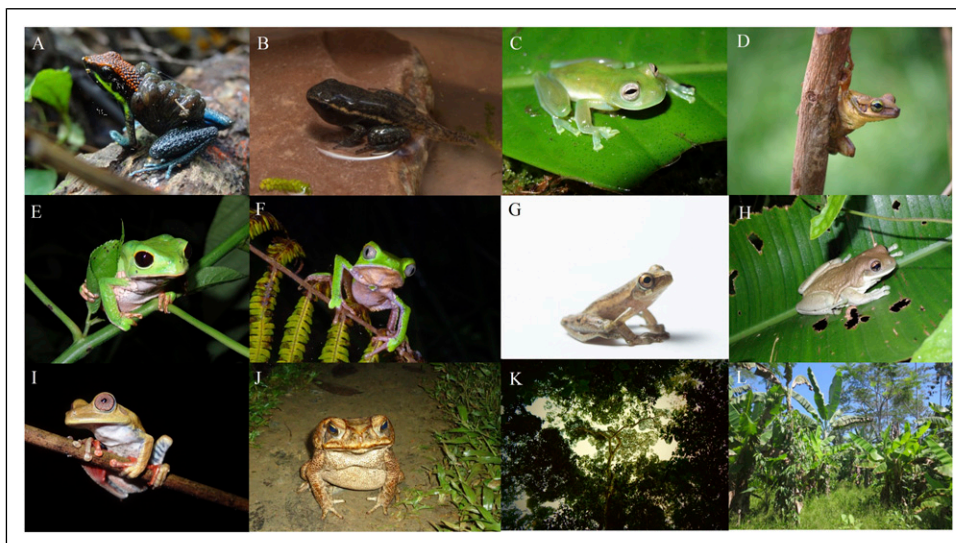


Figure 2. (a) *Ameerega macero*, (b) *Ameerega shihuemoy* and (c) *Cochranella nola* are found in the regenerating forest and the Native community of Shintuya. (d) *Osteocephalus taurinus*, (e) *Phyllomedusa camba* and (f) *Phyllomedusa vaillantii* are found in the regenerating forest, the Native community of Shipetiari and the Native community of Diamante. (g) *Dendropsophus schubarti* and (h) *Trachycephalus typhonius* are found in the Native community of Diamante. (i) *Boana geographica* and (j) *Rhinella marina* are generalist species found in the agricultural matrix and all the other sites. (k) close canopy from the Native community of Shipetiari. (l) Banana plantations in the agricultural matrix. Photo credits: AW (a, c–f, h–j), Marcus Brent – Smith (b and g), SJSR (k and l).

Table 1. Observed, Extrapolated, and Estimated Species Richness Patterns in Six Different Land-Use Types in the Manu Biosphere Reserve. The Number of Lists was Built Using Mackinnon Lists of Five Species Per Site With the Data From the Herpetological Transects and the Incidentals Recorded During the Survey Period. Survey Effort is the Total Distance Walked in Meters Through the Herpetological Transects Per Site.

Site	n ^a	Number of lists	Survey effort (distance m)	Observed species	Extrapolated species ^b	Species richness estimates										Sampling completeness (%) ^d		
						ACE		ICE		Chao		Jackknife		Bootstrap			MMMean	Average ^c
						1	2	1	2	1	2	1	2	1	2			
Agricultural matrix	59	12	6623	17	24	20	22	22	21	17	17	18	15	19	19	89		
Native community of Shipetiari	192	39	6792	38	38	40	42	39	38	38	42	33	48	40	95			
Native community of Diamante	199	40	6254	48	48	53	56	49	49	48	54	41	56	51	95			
Native community of Shintuya	47	10	6227	24	43	37	43	40	40	24	28	19	37	33	72			
Regenerating forest	115	23	5154	35	38	40	43	37	36	35	38	30	50	39	90			
Old-growth forest	110	23	6287	33	41	43	46	37	36	33	39	27	36	37	89			
Buffer zone	612	124	31049	69	75	75	75	79	79	81	87	75	77	79	87			
All	722	144	37336	74	81	81	81	83	83	86	92	80	80	83	89			

^aNumber of individual records.

^bNumber of species estimated when curves extrapolated to the same number of encounters.

^cMean estimated species richness – classic Chao values were used in cases where CV > 0.5.

^dSampling completeness defined as: Observed species/mean estimated species richness*100.

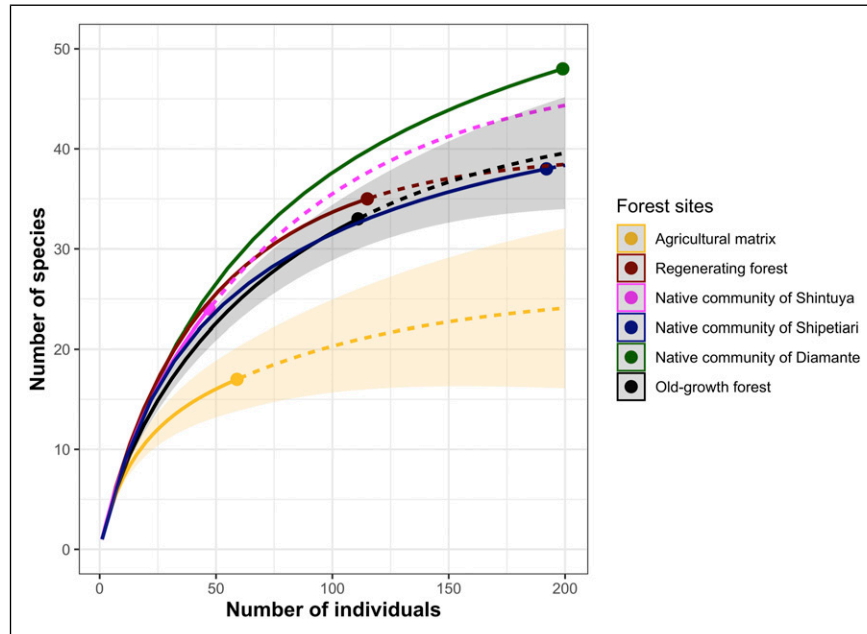


Figure 3. Amphibian species richness accumulation curves in six different land-use types in the Manu Biosphere Reserve created using the Mackinnon species list technique. Solid dots represent the observed number of individuals recorded and dashed lines represent predicted species richness based on extrapolated rarefaction curves. The gray and yellow shades represent 84% confidence intervals for the old-growth forest and the agricultural matrix, respectively.

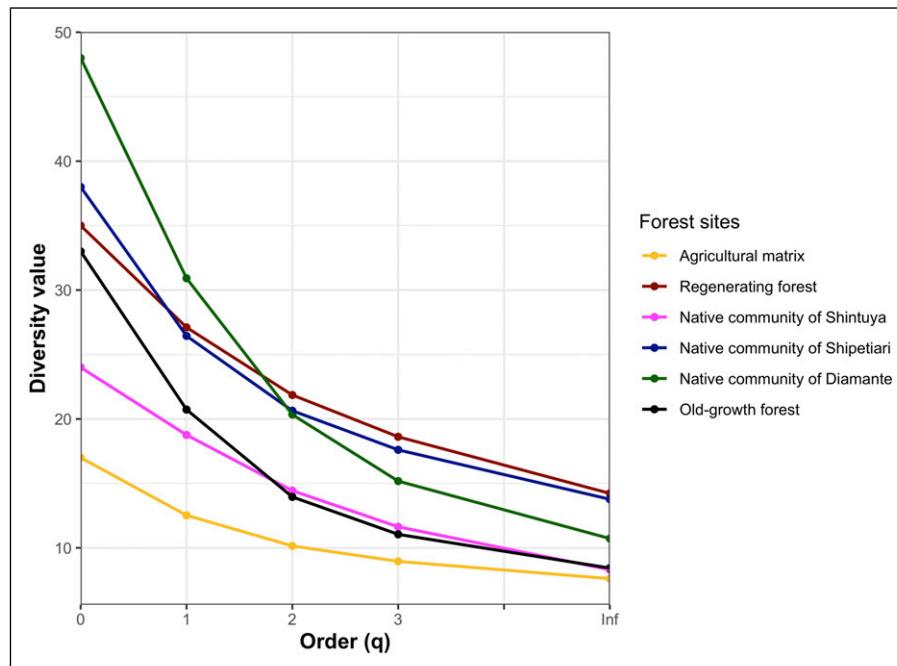


Figure 4. Amphibian diversity profile of the orders $q = 0$ to $q = \text{inf}$ of different sites (different land-use types) in the Manu Biosphere Reserve created using the Mackinnon species list technique. Where diversity order: $q = 0$ is the species richness in each site, $q = 1$ is the exponential of Shannon's entropy index, $q = 2$ is the inverse of Simpson's concentration index, and $q = \text{infinite}$ is the inverse of Berger Parker index.

regenerating forest, and the old-growth forest amphibian communities were more similar than to the agricultural matrix (Figure 5). Known habitat generalists, *Hypsiboas geographicus*, *Pristimantis fenestratus*, and *Scinax ruber*, were

detected in increased abundance in the most intensely disturbed habitat. *Cochranella nola*, a near-threatened glass frog species, was found only in the native community of Shipetari and the regenerating forest site. Species of the genus

Pristimantis, known ecological indicators, were abundant in the native communities and the regenerating forest.

Dominance–diversity or Whittaker plots demonstrated that the amphibian community in the agricultural matrix supports a significantly less even assemblage ($p < 0.001$) than all the other five sites (Figure 6). The community evenness of the native community of Diamante was the most even, with a curve of few dominant species followed by a long tail of increasingly rarely encountered species. When comparing the old-growth forest to the native community of Shipetiari ($t = -0.13$, $p = 0.89$, $\Delta G = 0.00$) and the native community of Shintuya ($t = 0.60$, $p = 0.55$, $\Delta G = 0.01$) we detected no significant difference, but similar evenness. However, there was a significant difference when compared to the curve of the regenerating forest ($t = 2.04$, $p < 0.05$, $\Delta G = -0.01$), where the last one showed a more even community. A full comparison of community evenness between sites is provided in Supplemental Appendix D.

Analysis of Environmental and Forest Structure Variables to Explain Species Richness and Abundance

We found no effect of the distance covered during sampling on the amphibian species richness and abundance. At the sample level, there was strong support for altitude, canopy cover, leaf litter depth, and shrub density in predicting

amphibian species abundance (R^2 of best-supported model = 30%) and richness (R^2 of best-supported model = 20%) calculated from zero-inflated generalized linear models (Figure 7, Table 2), with altitude having a significant negative effect on both (species richness and abundance), leaf litter depth and canopy cover having a significant negative effect in abundance, whereas shrub density showed to have a significant positive effect in species richness (See Supplemental Appendices E and F for full model selection).

Discussion

We find that the Manu National Park buffer zone holds a high proportion of the amphibian community from the Manu Biosphere Reserve as a whole. Yet each of the different survey locations that we surveyed hosts a distinctive community that contributes to the high levels of amphibian diversity in the overall region. However, the agricultural matrix is species-poor, dominated by just three generalist amphibian species, each encountered at other sites. This agricultural matrix has the lowest species richness and diversity, supporting our initial predictions. The predominance of monoculture in this agricultural matrix, should it continue to expand throughout the region's buffer zone, has the potential to threaten the

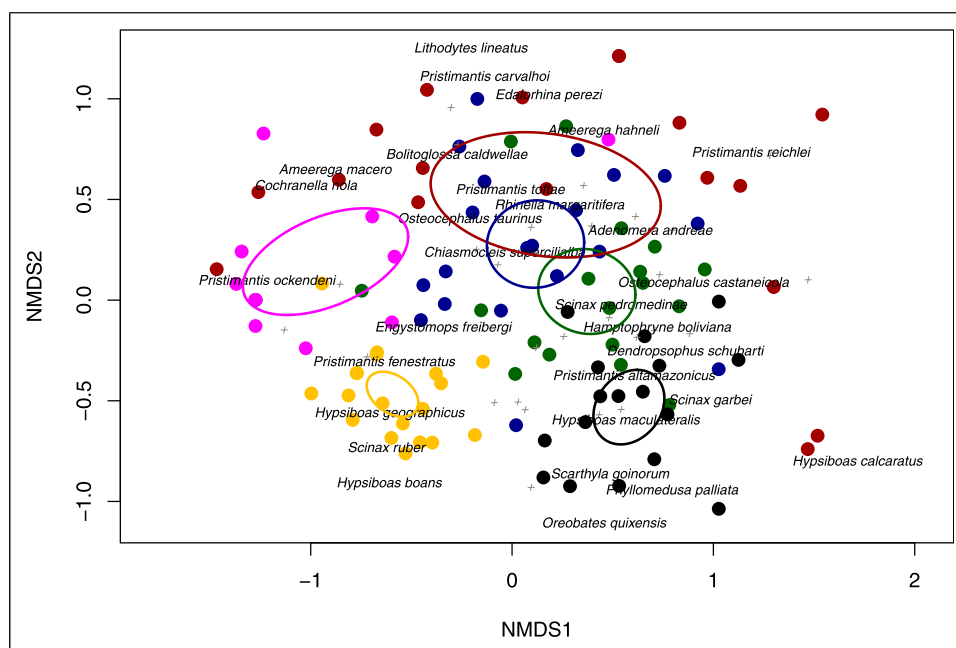


Figure 5. Community composition NMDS plot for amphibian communities in six different land-uses in the Manu Biosphere Reserve using data from nocturnal transects. For each sampling location, it shows the name of the most abundant species at that locations in comparison to others. The yellow circles represent the agricultural matrix sampling locations, the magenta circles represent the native community of Shintuya, the dark-red circles represent the regenerating forest, the dark blue circles represent the native community of Shipetiari, the dark green represent the native community of Diamante, the black circles represent the old-growth forest. Species names and the 95% confidence intervals for land-use classifications assigned to sampling locations were plotted using the functions *orditorp* and *ordiellipse*, respectively, in the Vegan package (Oksanen et al., 2019). The stress value of the NMDS for two dimensions is equal to 0.10, with an R statistic equal to 0.43 and a p -value of 0.001.

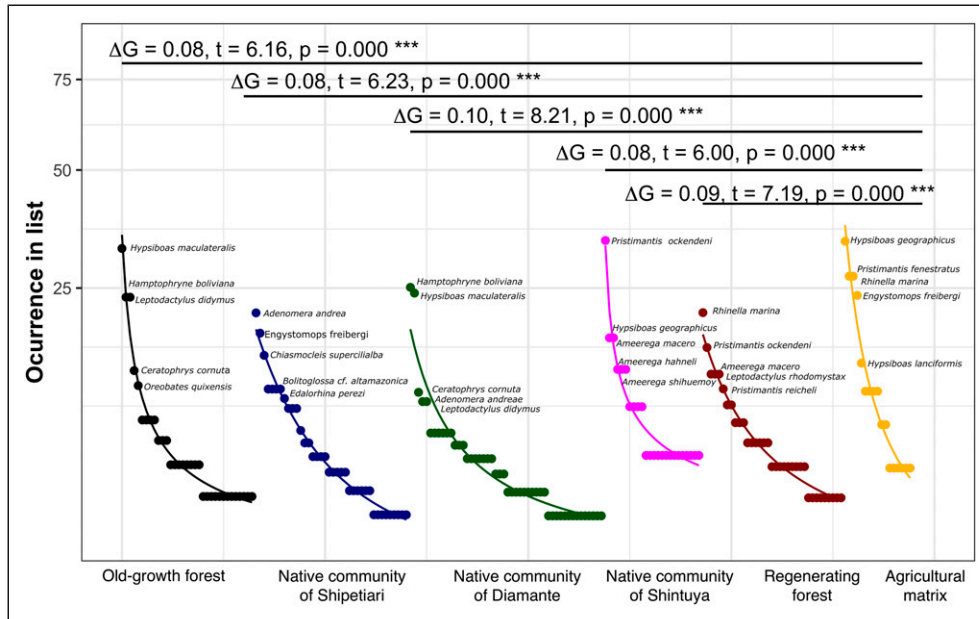


Figure 6. Dominance-diversity (Whittaker) plots for amphibian communities in six different land-uses in the Manu Biosphere Reserve using the Mackinnon species lists technique. For each land-use, the relative abundance of each species (number of lists on which each species appears/total number of lists) was plotted on a logarithmic scale against the species rank-ordered from most to least abundant. We are labeling the five most abundant species at each site. This plot shows the comparison between the agricultural matrix against the other sites. Linear models were used to determine if the slopes of the sites were significantly different, where ΔG denotes an absolute change in gradient from the comparative gradient, and the * symbol indicates the level of significance where *** = $p < 0.01$ is highly significant.

biodiversity conservation value of the buffer zone itself, but also the protected area in its own mission to provide effective biodiversity conservation.

Of the total amphibian species found in this study, 93% were recorded within the three native communities. The native communities in this study are formed by indigenous people (Matsigenka, Harakmbut, and Yine), who through their traditional knowledge, show enhanced ecosystem management via some sustainable practices. These involve a combination of ecotourism, small-scale agriculture, selective logging, and the protection of large extensions of preserved forest, allowing these Native communities to play an important role in biodiversity conservation (Dudley et al., 2018; Garnett et al., 2018; Maxwell et al., 2020; Schleicher et al., 2017). Our study site at Shintuya (Harakmbut people), for example, although once cleared in the lower regions, is now regenerating and hosts tourists that visit the hot springs and hike up toward a waterfall in the higher elevations, where the forest is largely intact and only selectively logged for local use. The study forest in Shipetari consists mostly of old-growth forest set aside for conservation and tourism, supported by a small community lodge named “Pankotsi,” which means “home” in the native Matsigenka tongue. Diamante (Yine people) is probably the community with the greatest current impact, undergoing significant selective logging, and with a community growing with an influx of outsiders, many of whom are in favor of the road reaching the community. The forest we surveyed at Diamante was the

area set aside for conservation and tourism, a mix between an old-growth and a well conserved regenerating forest. Unfortunately, the sound of chainsaws was heard daily in the surrounding area.

Strong evidence from another region in the Western Amazon (see Pitman et al., 2021), in Loreto of Peru, found that conservation-friendly land-use categories (indigenous territories, conservation concessions, ecotourism concessions, inactive forestry concessions, and private conservation areas) exceeds that in its protected areas, accounting for roughly 50% of Loreto’s territory. The authors suggest that with continuing investment and coordination, these lands can provide an effective defense against threats such as road building and plantation farming; two of the major threats to Manu’s lowland buffer zone. We agree and suggest that effort to invest in such designations in this region are imperative. The agricultural matrix study site is inhabited primarily by Andean migrants, most of whom are based at the major towns along the road (Salvacion, Pilcopata, and Patria) arriving to exploit natural resources along an unpaved road that opened accessibility to the remote areas in the 1960s (Gallice et al., 2019; Larrea-Gallegos et al., 2017). This increased access to the Manu region has facilitated illegal activities that are continually eroding the ecological and cultural integrity of the lowlands of the Biosphere Reserve. Evidence summarized from government population census data from 2015 (see Supplemental Appendix G) suggests that the Manu

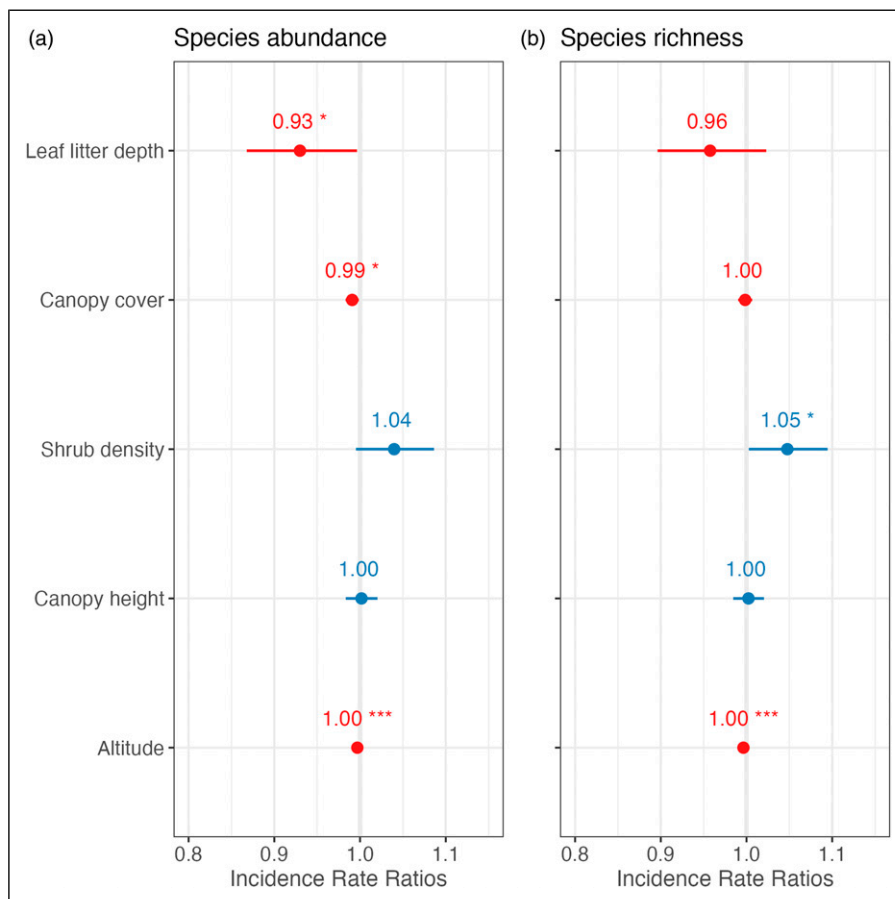


Figure 7. Beta estimates for fixed effects in zero-inflated models for (a) species abundance and (b) species richness, with 95% CIs, testing the effect of forest structural characteristics and altitude. The * symbol denotes the level of significance, where “***” = < 0.05.

Table 2. Model Selection Table for the Forest Structural Factors and Altitude Influencing Species Richness and Abundance of Amphibians at Each Survey Site.

Response term	Intercept (Cond)	Altitude	Canopy cover	Canopy height	Leaf litter depth	Shrub density	df	logLik	AICc	ΔAIC	weight
Species richness	1.679	-0.004			-0.046	0.046	6	-314.928	642.230	0.000	0.205
	1.442	-0.004				0.049	5	-315.996	642.257	0.027	0.202
	1.624	-0.004	-0.003			0.050	6	-315.728	643.830	1.600	0.092
	1.743	-0.004	-0.001		-0.043	0.047	7	-314.884	644.268	2.038	0.074
	1.480	-0.004		-0.001		0.049	6	-315.979	644.331	2.101	0.072
	1.663	-0.004		0.001	-0.047	0.046	7	-314.925	644.349	2.119	0.071
...
...	0.329						3	-343.852	693.810	51.580	0.000
Species abundance	2.494	-0.003	-0.009		-0.072	0.039	8	-382.129	780.904	0.000	0.301
	2.720	-0.003	-0.008		-0.081		7	-383.660	781.820	0.916	0.191
	2.475	-0.003	-0.009	0.002	-0.073	0.039	9	-382.113	783.036	2.133	0.104
	2.323	-0.003	-0.012			0.046	7	-384.300	783.099	2.196	0.100
	2.684	-0.003	-0.008	0.003	-0.081		8	-383.611	783.868	2.964	0.068
	2.269	-0.003			-0.112		6	-385.977	784.327	3.423	0.054

...	0.598						4	-404.710	817.596	36.693	0.000

Where all models above “...” were included in the top model set; the model below the “...” is the null model (for all models in between, see Supporting Information in Supplemental Appendices E and F); df = degrees of freedom; AIC = Akaike information criterion; ΔAIC = the change in AIC relative to the best-supported model; weight = model weight

Province has seen a 212% increase in population since 2000, and 628% increase since 1990: now with over 5000 inhabitants. Although these densities remain low, small numbers of inhabitants carrying out extractive or intensive activities can quickly deplete natural rainforest resources if sustainable plans and strategies are not implemented.

Degraded areas that have been safeguarded to regenerate for more than 20 years also offer promising opportunities for biodiversity recovery (Edwards et al., 2017; Raub et al., 2014; Whitworth, Downie, et al., 2016), as is the case of the regenerating site in this study. Half of all the species recorded in this study were found in the regenerating forest study site, where amphibian indicator families such as Craugastoridae (López-Rojas et al., 2015), Dendrobatidae, and Centrolenidae (Palacios-Rodríguez et al., 2019) were all detected. This regenerating forest historically experienced two major types of anthropogenic disturbances, selective logging and complete clearance for agriculture. After more than 40 years of regeneration and protection, this forest is considered to have a high biodiversity and conservation value (Whitworth, Downie, et al., 2016). However, another study from the same site showed that the recovering forest in areas once cleared for agriculture still held lower levels of amphibians diversity and greater disruption to community evenness, compared with forest once subjected to selective logging (Whitworth et al., 2017); suggesting that impacts from intensive land-use are not only notable in the immediate, but are also long lasting, despite recovery.

In addition to understanding the overall difference between study sites in the landscape, we included altitude as a predictor in our sample-level models to account for altitude variation. Two of our sites (the regenerating forest and the native community of Shintuya) have sampling locations at elevations ranging from 400 to 792 m asl (some slightly higher than the other sites). Altitude was found to be a strong predictor of species richness and abundance at the sample level, with fewer species and fewer individuals detected per sample at higher elevations; notably observed in some of the highest sampling locations in the regenerating forest, and especially in the native community of Shintuya. This pattern of species richness and abundance decline with increasing elevation has been shown in other amphibian studies (Khatiwada et al., 2019; Villacampa et al., 2019). Despite the role of altitude in the amphibians found in our nocturnal transects, it is important to note that this effect of altitude was found only at the sample level, as the models are trying to explain the species richness and abundance found within each survey carried out across study locations. Although the agricultural site had locations at mid elevation range (~520 m asl), overall, the site had the lowest diversity in comparison to all other sites.

Among the forest structural variables, we also identified forest characteristics, such as leaf litter, canopy cover, and shrub density, as predictors to explain general patterns of amphibian species richness and abundance at the sample

level. Surprisingly, deeper leaf litter depth negatively impacts sample-level counts of both species richness and abundance, despite leaf litter depth impacting biodiversity positively in other studies (Van Sluys et al., 2007; Yu et al., 2006; Ziesche & Roth, 2008). This might be explained due to the terrestrial amphibians being harder to detect by observers within survey areas with deeper leaf litter. Canopy cover has been shown to have a positive effect on biodiversity in other studies (Armbrecht & Perfecto, 2003; Lemenih et al., 2004; Surendran & Vasudevan, 2015). The negative effect of forest canopy cover we detect on sample-level richness and abundance could be related to dense coverage observed within plantation or second-growth areas, and alone is unlikely a good measure of habitat integrity or quality for this study. The positive correlation between shrub density and amphibian richness and abundance might relate to the availability of understory perching sites for species as shown by Lescano et al. (2015) and Pikacha et al. (2017). But once again as with the leaf litter effect, this might be a sample-level detectability effect by observers, as the shrub layer is directly within eye-line—although we did attempt to overcome this by searching both the leaf litter and general habitat with both leaf litter searches and active searches over distance.

One of the limitations of our study to assess the biodiversity of different habitat uses in the Manu Biosphere Reserve could be the focus of only one taxonomic group, amphibians. The Manu National Park, however, located at the core of the Manu Biosphere Reserve, stands out as the most diverse area globally, not only for amphibians and reptiles (represents only 0.01 percent of the planet's land area, but, houses 2.2% of all amphibian species known worldwide (Catenazzi et al., 2013), but it also holds considerable diversity of other taxa such as birds (Socolar et al., 2013; Terborgh, 1977), mammals (Endo et al., 2010), beetles (Maveety et al., 2011), and plants (Gentry, 1988). We chose to focus on amphibians as they represent well-known indicator group (Böll et al., 2013; Whitworth et al., 2017), are relatively simple to sample and we had a comprehensive guide of the amphibian identification for the region (Villacampa et al., 2017), but most importantly, amphibian communities are known to be an excellent surrogate group to represent overall biodiversity health. As such, amphibians make an excellent group to carry out a rapid assessment and act as a strong indicator of overall habitat quality and health. If we had chosen a more vagile group, like birds or mammals, then we might have still detected the degraded community in the agricultural landscape but lost the evident levels of unique community composition as displayed by the less-vagile amphibian communities at each site.

Another limitation of our study was the lack of site replication for some of the land-use types. The restricted access to the old-growth forest and availability of only one strictly protected regenerating forest limited our study to have single sites representing these land-use types. Although there is a large extension of agricultural matrix in the buffer zone of the study,

factors such as owner's permission, closeness to our study sites, cost of fieldwork, and access by boat limited our study to have only one site representing the agricultural matrix. We chose to have more site replicates representing the native communities because of the lack of studies assessing their role for biodiversity conservation, and each one of those sites differed in terms of the current specific use of each forest's communities.

An important consideration might also be the differences in the physical aspects of the study sites and the significant distance between some of the study locations. But this was a strategy to cover as many areas of the Manu lowlands as possible. The design, however, was key to showing how both the regenerating study site, and the agricultural matrix differed significantly, but were almost adjacent, facing each other on either side of the river. Before any habitat change, we would have expected both locations to have very similar amphibian communities, whereas all other sites might show unique communities due to physical or climatic differences—as shown by the distinctiveness of communities.

As a biodiversity hotspot, effective biodiversity conservation measures for the Manu Biosphere Reserve are critical. In order to avoid the conversion of forests to an agricultural matrix, a combination of sustainable livelihood activities, cultural practices, and forest protection, as observed in the native communities in this study, can help to fulfill the role of a Biosphere Reserve—to reconcile the conservation of biological and cultural diversity improving social and economic development. However, the increasing pressure from the road network and incoming immigrants from the highland regions threaten the integrity of the Biosphere Reserve, which in time could bring increased pressure and will be reflected within the core National Park areas. Given the uniqueness of amphibian communities at each of the study sites, there is a strong likelihood that continued destruction and degradation could convert these species-rich biodiversity communities to species-poor groups of generalists, and some species could conceivably face extinction. Therefore, solutions to avoid extensive clearance and intensive forms of agriculture need to be available to local people to avoid biodiversity degradation that could end up reflected within the core protected area of a Biosphere Reserve in the longer term.

Implications for Conservation

This case study highlights the importance of identifying current land management practices that have the potential to contribute to biodiversity conservation and sustainable biodiversity services within Biosphere Reserves (as those identified by [Pitman et al., 2021](#)). We have seen how Indigenous community-managed forests around protected areas can promote biodiversity conservation while allowing sustainable output of ecosystem goods and services ([Edwards et al., 2019](#)), being aligned with the goals of Biosphere Reserves. Therefore, supporting indigenous communities' contributions and empowering their environmental stewardship is critical to conserving biodiversity worldwide

([Edwards et al., 2019](#); [Ellis et al., 2021](#)). In this study, we underscore the high conservation potential of buffer zones managed by Indigenous communities for amphibian biodiversity conservation. We used amphibians as a model system because of their sensitivity to environmental change and because they are considered the most threatened vertebrate class on the planet ([Catenazzi, 2015](#)). Currently, 41% of amphibians species are threatened with extinction ([IUCN 2021](#)), with habitat loss and fragmentation as one of the major threats ([Gardner et al., 2007](#); [Stuart et al., 2004](#)). Our findings, therefore, suggest that promoting a combination of sustainable livelihood activities, cultural practices, and forest protection, as observed in the Indigenous communities, and avoiding a shift towards intensive agriculture often applied by immigrant groups, is critical to fulfilling the conservation role of Biosphere Reserves' protected areas.

Although Native communities and forests in regeneration offer an opportunity to safeguard areas for biodiversity within Manu's buffer zone, the culmination of the proposed expansion of the Manu road, which is currently traversing the buffer zones of the Manu National Park and the Amarakaeri Communal Reserve, will give rise to deforestation, habitat degradation, illicit activities and threaten indigenous cultures ([Gallice et al., 2019](#); [Salazar Moreira & Palomino-Schalscha, 2020](#)). This proposed road was being illegally built until 2016 ([Gallice et al., 2019](#); [Larrea-Gallegos et al., 2017](#)) despite the disapproval by the National Park Service (SERNANP). Although the people leading this were first sued for illegal deforestation ([Gallice et al., 2019](#)), the proposed road received approval in October 2018 and continued to be built immediately with plans to complete the road all the way to Boca Manu in early 2019 ([John & Munro, 2019](#)). Boca Manu is located at the mouth of the Manu River and is the entrance to the core area of Manu National Park (see [Finer & Mamani, 2022](#) and [Gallice et al., 2019](#) for a detailed map of the road placement and the planned areas of expansion). The Manu Road project aims to connect Boca Manu with the illegal gold mining hub of Boca Colorado to the southeast ([Gallice et al., 2019](#)), which will eventually connect Manu to Puerto Maldonado city, a large city famed for its growth through illegal resource extraction. This new road highly threatens biodiversity and indigenous communities in one of the world's most species-rich and environmentally sensitive rainforest areas.

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Author Contributions

S.J.S-R., A.W., and R.M. conceived the idea and design the project. S.J.S-R., A.W., J.A.P-G., R.P-H., L.W., and K.H.H-L. conducted fieldwork. S.J.S-R., A.W., and J.A.P-G. analyzed the data. S.J.S-R. and A.W. wrote the original draft. All authors contributed to editing the manuscript. A.W. and R.M. supervised this work and were responsible for funding acquisition.

Declaration of Conflicting Interests

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Data Availability

The dataset underlying this study is deposited online with Figshare doi:[10.6084/m9.figshare.19624425](https://doi.org/10.6084/m9.figshare.19624425)

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Supplemental Material

Supplemental material for this article is available online.

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