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Shade Cacao Plantations Provide Little Conservation Benefit for Forest-Dependent Amphibians in the Ecuadorian Chocó

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ABSTRACT

Shade cacao has been empirically shown to promote higher levels of biodiversity than sun cacao, due to conditions that resemble forest habitat. Understanding the conservation value of cacao plantations is important given the widespread transition from shade to sun cacao in the Ecuadorian Chocó, a global biodiversity hotspot. We sampled amphibian communities at 32 sites surrounding a private forest reserve in Esmeraldas, Ecuador, and assessed abundance, richness, Simpson diversity, and presence of endangered species in shade- and sun-grown cacao plantations, abandoned cacao plantations, and remnant forests. We also determined if local (leaf litter, canopy, and herbaceous vegetation cover) and landscape (surrounding forest cover, proximity to water, elevation) variables help predict amphibian abundance and richness to inform better management practices for amphibian communities in this agricultural landscape. We captured 1831 individuals representing 30 species across the wet and dry seasons of 2022–2023. We found no differences in the abundance, richness, or Simpson diversity among the four land use types. However, individual-based extrapolation indicated that abandoned cacao plantations and remnant forest were more diverse. Additionally, we found four species of conservation priority, which almost exclusively inhabited abandoned cacao plantations and remnant forests. We found limited evidence that measured local environmental variables affect amphibian abundance or richness, while elevation had a consistent negative effect on richness and Simpson diversity. Results suggest that the preservation of forest cover would be of greatest benefit to conservation-priority amphibians, unless substantial changes are made to cacao plantation management practices in the region.

RESUMEN

Se ha demostrado empíricamente que el cacao de sombra promueve mayores niveles de biodiversidad que el cacao de sol. Comprender el valor de conservación de los cultivos de cacao es importante, dada la transición generalizada del cacao en el Chocó ecuatoriano, un hotspot de biodiversidad a nivel mundial. Tomamos muestras de anfibios en 32 sitios alrededor de una reserva privada en Esmeraldas, Ecuador. Comparamos la abundancia, riqueza y la presencia de especies en peligro de extinción

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de anfibios en plantaciones de cacao cultivadas a la sombra y al sol, plantaciones de cacao abandonadas y bosques remanentes. Evaluamos si las variables locales (vegetación del sotobosque, hojarasca, cobertura del dosel) y del paisaje (cobertura forestal circundante, proximidad al agua, elevación) podían predecir la abundancia y riqueza de anfibios para comprender cómo las prácticas de gestión podrían influir en las comunidades en este paisaje agrícola. Capturamos 1831 individuos que representaban 30 especies durante las estaciones húmeda y seca de 2022–2023. No encontramos diferencias en la abundancia, riqueza o diversidad de Simpson entre los cuatro tipos de uso del suelo. Sin embargo, la extrapolación basada en individuos sugiere que las plantaciones de cacao abandonadas y los bosques remanentes contienen una mayor diversidad. Además, encontramos cuatro especies de prioridad para la conservación, las cuales habitaron casi exclusivamente en plantaciones de cacao abandonadas y bosques remanentes. Encontramos evidencia limitada de que las variables ambientales locales medidas afecten la abundancia o la riqueza de anfibios, mientras que la elevación tuvo un efecto negativo sobre la riqueza y la diversidad de Simpson. Resultados sugieren que el mantener la cobertura de bosque remanente sería muy beneficiosa para los anfibios de prioridad para la conservación, a menos que se produzcan cambios sustanciales en las prácticas actuales de gestión de los cultivos de cacao en la región.

1 | Introduction

Agriculture drives approximately 80% of deforestation globally (Kremen and Merenlender 2018; Ramankutty et al. 2018). In tropical regions that harbor the majority of global biodiversity, balancing conservation and agricultural productivity is especially challenging, and two contrasting paradigms exist. *Land sparing* argues that agricultural and natural areas should be separated to maximize the efficiency and integrity of each (Phalan et al. 2011), and that the conservation value of agricultural land is minimal (Williams et al. 2017). In contrast, *land sharing* advocates for the integration of agricultural and natural systems to improve the ecological resilience of both (Mendenhall et al. 2013; Kremen and Merenlender 2018). Evaluating the conservation value of agricultural spaces under different practices is critical to the land sharing-sparing debate and for informing local and regional policy and management (DeClerck et al. 2010; Ríos-Touma et al. 2023).

The Ecuadorian Chocó is a global conservation hotspot due to its exceptional biodiversity and endemism (Myers et al. 2000; Coloma and Duellman 2025), including many threatened amphibian species (Ortega-Andrade et al. 2021). It is also a highly fragmented ecosystem, with less than 10% of native forest cover remaining, primarily due to agricultural expansion (Fagua et al. 2019). The region is a leading global exporter of several crops, including cacao (Perlin and Leguizamón 2024).

Cacao is traditionally grown in shaded agroforests (Faria et al. 2007; Ríos-Orjuela et al. 2024) which can provide habitat for birds, bats, reptiles, and amphibians (Wanger et al. 2009; Palacios et al. 2013; Gras et al. 2016). Less is known about the conservation value of sun-grown cacao although it is assumed to be lower (Belsky and Siebert 2003; Cervantes-López et al. 2022). Over the past decade, a genetically-modified and intensively-managed sun-grown cacao variety, *Colección Castro Naranjal 51* (hereafter sun cacao), has been widely adopted in the Ecuadorian Chocó, replacing *Nacional* (hereafter shade cacao), which is typically grown below canopies of native and fruit-producing trees (Perlin and Leguizamón 2024). Given this widespread shift in cacao cultivation (Perlin and Leguizamón 2024), assessing the conservation value of these two varieties is important to determine potential consequences for biodiversity.

Amphibians are frequently used as an indicator group to assess habitat quality (Wanger et al. 2009; Urbina-Cardona et al. 2015). Furthermore, their sensitivity to environmental change, habitat loss, and pesticides makes them especially useful indicators in agricultural landscapes (Pfeifer et al. 2017; Davis et al. 2020). Amphibians are considered the most threatened vertebrate taxa globally (Luedtke et al. 2023) and remain understudied compared to other groups (Munstermann et al. 2022).

Cacao agroforests can sometimes harbor more diverse amphibian communities than remnant forests (Faria et al. 2007; Cervantes-López et al. 2022). Nonetheless, agroforest management varies substantially across regions, and even between neighboring landowners (Wanger et al. 2009; Jithin et al. 2025). Slight variations in leaf litter depth and cover have been found to increase amphibian abundance in cacao (Wanger et al. 2009; Cervantes-López et al. 2022) and coffee plantations (Ríos-Orjuela et al. 2024). Canopy cover is also positively correlated with amphibian abundance and richness in tropical agroforests (Cervantes-López and Morante-Filho 2024), coffee plantations (Ríos-Orjuela et al. 2024), and restoration sites (Díaz-García et al. 2020). In turn, streams, ponds, and forest remnants can act as population sources for amphibians in fragmented tropical landscapes, correlating with greater amphibian diversity and abundance in nearby agroforests (Sankararaman et al. 2021; Cervantes-López et al. 2022) and recovering forests (Ribeiro et al. 2018; Díaz-García et al. 2020).

We sought to assess the conservation value of cacao agroforests in the context of the widespread transition of shade to sun cacao, and to contextualize them against recovering and primary forest conditions. To do so, we sampled the amphibian community in active shade and sun cacao plantations, abandoned shade cacao (hereafter abandoned) plantations, and remnant forest sites in and near the Mache-Chindul Reserve in Esmeraldas, Ecuador. Abandoned cacao plantations share secondary forest characteristics and are becoming more common in the landscape as some farmers transition away from cacao farming entirely due to price volatility (Perlin and Leguizamón 2024).

We compared the abundance, richness, diversity, and community composition of amphibians across these four habitat types. We predicted that shade cacao plantations would harbor higher amphibian abundance, richness, and diversity than sun cacao due to greater structural complexity and diversity

of microhabitats found in shaded agroforests, and the lower management intensity of shade cacao, which should allow for more disturbance-sensitive species to persist (Cervantes-López et al. 2022). We expected all response variables to respond negatively to increasing land management intensity, with shade cacao and abandoned plantations showing intermediate values between sun cacao and remnant forest sites. We also sought to compare the community composition found across land use types, with particular emphasis on species of conservation concern. We expected the amphibian community composition of abandoned cacao plantations to most closely resemble remnant forest, followed by shade and lastly sun cacao plantations due to decreasing land use intensity across these sites (Mendenhall et al. 2014).

We assessed the effect of local and landscape factors on amphibian communities in active cacao plantations by measuring three local—(leaf litter, canopy, and herbaceous vegetation cover) and three landscape—(percent surrounding forest cover, proximity to water, elevation) level variables that influence amphibian abundance, richness, and diversity elsewhere (Wanger et al. 2009; Ribeiro et al. 2018). Of these variables, we predicted that leaf litter, canopy, and surrounding forest cover would have the strongest effects on abundance, richness, and diversity. We expected leaf litter and canopy cover to provide important microhabitats for terrestrial and arboreal amphibians, and to lower understory temperatures and desiccation risk, thereby benefiting forest-dependent and sensitive species (Wanger et al. 2009). We expected leaf litter and canopy cover to be higher in shade cacao plantations (Cervantes-López et al. 2022), thereby supporting our first hypothesis that these sites would support robust amphibian communities. We hypothesized that surrounding forest cover would provide important resources for many species and act as population sources, thereby increasing the abundance, richness, and diversity of amphibian communities in adjacent plantations of both cacao types (Ribeiro et al. 2018; Roach et al. 2020).

2 | Methods

2.1 | Study Area

We surveyed the amphibian community at 32 sites in and around the Fundación para la Conservación de los Andes Tropicales (FCAT) Reserve, located within the Mache-Chindul national reserve (between 0°23′33″ N, 79°38′50″ W, 300 m a.s.l. and 0°21′20″ N, 79°41′22″ W, 500 m a.s.l.), Esmeraldas, Ecuador (Figure 1). Native vegetation cover is tropical rainforest (Jongsma et al. 2014), with average annual precipitation of 2500–3500 mm

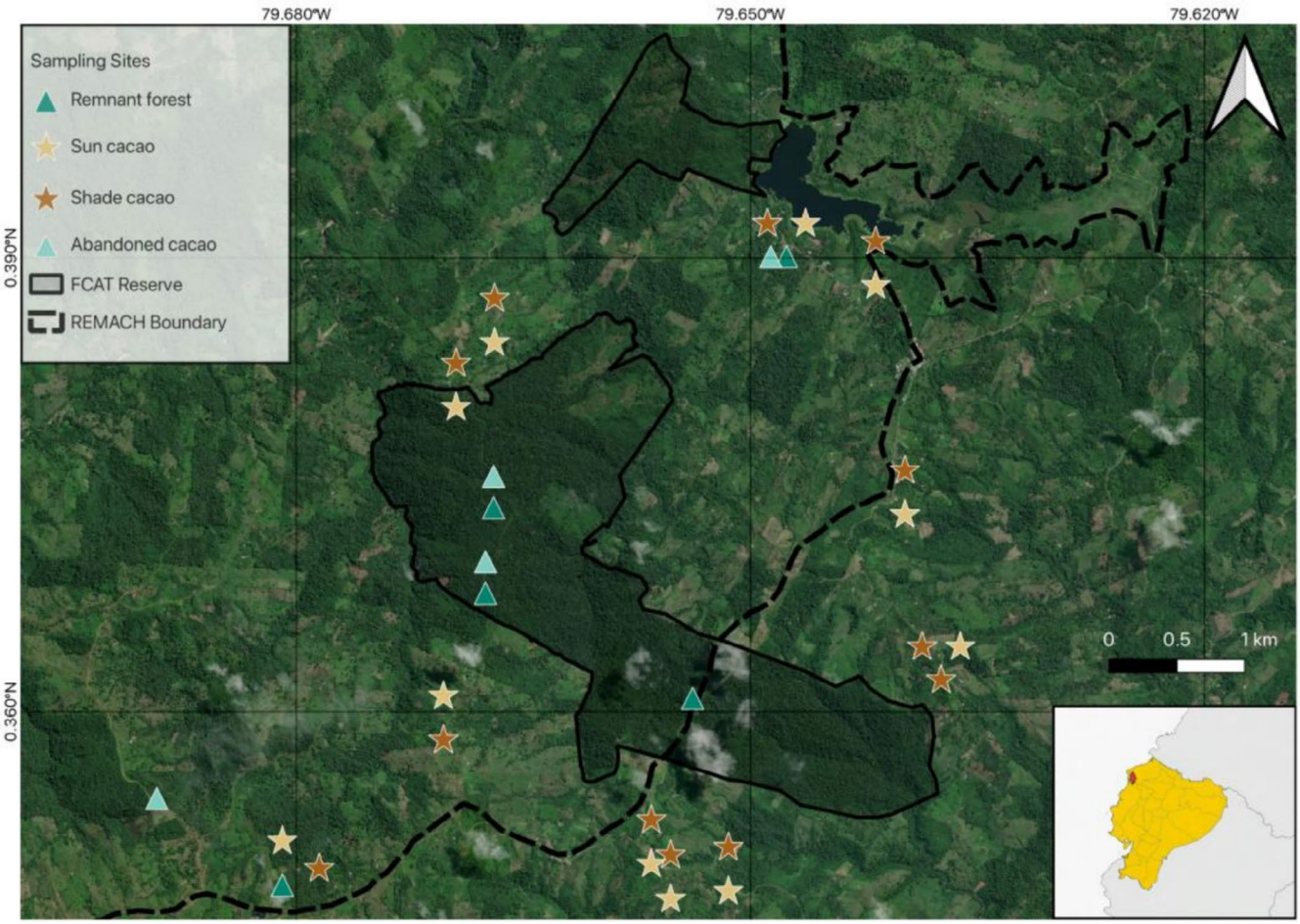

FIGURE 1 | Field study site indicating the location of active sun and shade cacao plantations, abandoned cacao plantations, and mature forest reference sampling sites in relation to the private Fundación para la Conservación de los Andes Tropicales (FCAT) Reserve, and the Reserva Ecológica Mache Chindul (REMACH) in Esmeraldas, Ecuador. Inset shows the location of the REMACH Reserve in mainland Ecuador.

and average temperature of 23°C–25.5°C (Clark et al. 2006). Surrounding land use is primarily agricultural, dominated by cacao and cattle pasture (Perlin and Leguizamón 2024).

We selected sampling sites after consultation with farmers and landowners, who granted us land access. We sampled 12 shade cacao and 11 sun cacao plantations ranging between 0.5 to 5 ha, five remnant forest sites, and four abandoned cacao plantations (1–5 years post-abandonment) (Figure 1). At each site, we established four parallel transects (4 × 20 m), spaced 4 m apart on navigable terrain, avoiding walking paths and water sources (Figure 2). Each transect was divided into four 4 × 5 m quadrats to facilitate leaf litter and canopy cover (Figure S1).

2.2 | Amphibian Community Sampling

We surveyed each site every other night across three visits in the dry season (September–December 2022) and three visits every other night in the wet season (January–March 2023), totaling six sampling visits per site. During each visit, two samplers captured as many amphibians as possible within each transect and up to 2 m in height for 12 min intervals during peak amphibian activity (2000h–0200 h) (Ortega-Andrade et al. 2010). We used latex gloves to reduce stress and infectious disease transmission. We sampled two sites each night, alternating sampling order to minimize the effect of sampling time on amphibian activity.

We placed captured amphibians in individual plastic bags with fresh leaves and rainwater to reduce stress before transport to the FCAT station where we classified each individual as a novel- or re-capture. We toe-clipped all novel captures and photographed the ventral, dorsal, and lateral sides of each phenotype collected per sampling night to facilitate identification to species level. We cleaned latex gloves and toe-clipping scissors with alcohol and water after processing each individual to minimize the likelihood of cross-contamination during handling. We returned all individuals to the exact quadrat where they were captured within 24 h. Nine of 2262 total captures were deceased upon release.

We used DNA sequencing (Appendix 1) to identify twenty individuals. All field research was conducted under permit MAATE-CMARG-2022-0575, issued by the Ministerio del Ambiente, Agua y Transición Ecológica (MAATE) of Ecuador, and protocol #22-3800-1 issued by the University of Hawai'i Institutional Animal Care and Use Committee (IACUC).

FIGURE 2 | Representative sun (a), abandoned (b), and shade (c) cacao plantation, and remnant forest (d) sampling sites.

2.3 | Local and Landscape Variables

We assessed three local—(leaf litter, canopy, and herbaceous vegetation cover) and three landscape—(forest cover, proximity to fresh water, elevation) level environmental variables to investigate drivers of amphibian communities across sampling sites. We estimated canopy cover using a spherical densiometer (Forestry Suppliers, Jackson, Mississippi, Model C), averaging four measurements taken in each cardinal direction in the center of each quadrat during transect establishment. We estimated leaf litter and herbaceous vegetation cover above knee-height in each sampling season at the quadrat level.

We used QGIS version 3.30.2 (QGIS.org 2024) to measure percent forest cover within a 400 m radius of each sampling site using a 3 m resolution regional forest cover map produced by FCAT in 2022. We measured proximity to fresh water using a map of year-round water sources provided by the Aquatic Ecology Laboratory at the Universidad San Francisco de Quito. We recorded the elevation of each site using a handheld GPS.

2.4 | Amphibian Abundance, Richness, and Diversity

We excluded recaptured individuals to calculate site-level amphibian abundance and species richness, summed across both seasons and all transects. We used the *vegan* package (Oksanen et al. 2024) to calculate Simpson diversity for each site because it emphasizes dominant species (Baselga 2017).

We used Generalized Linear Models (GLMs) with land use type as a fixed effect to compare amphibian abundance, richness, and Simpson diversity across 30 equally-sampled sites (11 shade cacao, 10 sun cacao, 4 abandoned cacao, 5 remnant forest). We used the *DHARMa* package (Hartig 2022) to assess all model residuals. We used the *MASS* package (Venables and Ripley 2002) to fit a negative binomial model for abundance to account for overdispersion (Smith et al. 2009). We used the Poisson distribution to fit richness as count data, and the beta regression distribution to fit Simpson diversity (Grün et al. 2012). We used Likelihood Ratio Tests to assess the effect of land use type on the response variable of each model, using the *lmtest* package for Simpson diversity with a beta regression distribution (Zeileis and Hothorn 2002). We performed Tukey's HSD post hoc tests with the *multcomp* package (Hothorn et al. 2008) to assess pairwise differences between site types as necessary. We used R version 4.4.1 for all statistical analyses (R Core Team 2024), and *ggplot2* for visualizations (Wickham 2016).

2.5 | Amphibian Community Composition and Sampling Coverage

We used the *vegan* package (Oksanen et al. 2024) to create a Bray–Curtis dissimilarity matrix for the amphibian community at each site and to assess differences in community composition across land use types. Bray–Curtis dissimilarity indices range from zero to one for each site pair, with values close to zero indicating similar communities and values close to one indicating dissimilar communities. We visualized this abundance matrix in two-dimensional space using a Non-Metric Multidimensional Scaling (NMDS) ordination plot and drew ellipsoids bounding the 95% confidence interval of the centroid for each land use group. We compared the ordination's stress level against a null hypothesis of random assortment following the methods of Dexter et al. (2018). We ensured that dispersion was equal across land use groups using the *betadisper* function before assessing differences in community assemblage using a Permutational Multivariate Analysis of Variance (PERMANOVA) with the *adonis2* function (Oksanen et al. 2024).

Next, we created rank-abundance curves to visualize community structure and compare the identity of dominant and subordinate species across land use types. We ranked species abundance in each land use type, with values $\log_{10}$ transformed to facilitate visualization. We then compared the abundance of species listed as Data Deficient, Near Threatened, Vulnerable, Endangered, or Critically Endangered (DD, NT, VU, EN, CR) by the International Union for the Conservation of Nature (IUCN 2024) across land use types.

We used the *iNEXT* package (Hsieh et al. 2016) to extrapolate Hill numbers to an individual sample size of 1000 for each land use type (Roswell et al. 2021). We used Hill numbers of orders $q = 0$, 1, and 2, corresponding to species richness, the exponential of Shannon diversity, and the inverse of Simpson diversity. This allowed us to compare amphibian communities across land use types while accounting for differences in our ability to completely sample each community (Chao and Jost 2012).

2.6 | Influence of Environmental Variables on Amphibian Communities in Cacao Plantations

We calculated the means of each environmental variable for each land use type and assessed the normality of distributions using Shapiro-Wilks tests. We used ANOVA to test for differences among land use types for each variable, followed by Tukey's HSD post hoc tests when appropriate. For variables that violated ANOVA assumptions, we used Kruskal–Wallis tests with post hoc pairwise comparisons. We used Principal Components Analysis (PCA) to ordinate site-level response variables (abundance, richness, Shannon and Simpson diversity) in multi-dimensional space. We used the *factoextra* package (Kassambara and Mundt 2020) to create a biplot to visualize this ordination in two dimensions.

Finally, we built a series of additive GLMs to explore the effect of the local- and landscape-level environmental variables on amphibian abundance, richness, and Simpson diversity as response variables in 21 equally-sampled cacao plantations (11 shade, 10 sun). To do so, we scaled the values of all variables to facilitate model convergence and interpretation and removed predictor terms with Pearson Correlation Coefficients (PCC) > 0.7 (Tredennick et al. 2021). For each response variable, we started with a full model including all predictors and used the *MuMIn* package (Bartoń 2025) to select simplified models using Akaike Information Criterion corrected for small sample sizes (AICc). We interpreted all models with delta AICc scores < 2.0 as equally plausible. We used the negative

binomial distribution to model abundance and account for overdispersed count data (Lindén and Mäntyniemi 2011) and the Poisson distribution to model richness. We used the beta regression distribution from the *betareg* package (Cribari-Neto and Zeileis 2010) to model Simpson diversity.

3 | Results

3.1 | Field Survey

We captured and recorded 1831 individuals representing 30 species (Table S2, Appendix 2) over 298 sampling hours. *Pristimantis achatinus* accounted for 78% (1748) of total captures, 76% (1389) of total unique individuals, and was found in all sites.

3.2 | Amphibian Abundance, Richness, and Diversity

We hypothesized that amphibian abundance, richness, and diversity would be highest in remnant forest sites, followed by abandoned cacao plantations, and shade and sun cacao plantations, respectively. Using GLMs, we found no effect of land use type on amphibian abundance ($\chi^2 = 1.11$, df = 3, $p = 0.77$), species richness ($\chi^2 = 3.82$, df = 3, $p = 0.28$), or Simpson diversity ($\chi^2 = 5.60$, df = 3, $p = 0.13$) across 30 sites (Figure 3).

3.3 | Amphibian Community Composition and Sampling Coverage

We hypothesized that the amphibian communities of abandoned cacao plantations would most closely resemble remnant forest sites, followed by shade and sun cacao plantations, respectively. We compared amphibian community composition across land use types via NMDS ordination and PERMANOVA analysis of a Bray–Curtis dissimilarity matrix, by comparing the shape and species identities of rank-abundance curves, and via distributions of species of conservation concern. NMDS ordination of the Bray–Curtis dissimilarity matrix produced low stress (Figure S2, stress = 0.08), although it did not significantly differ from the mean stress level of 1000 simulated ordinations derived from an ecological null model of random assortment (mean = 0.08, $z = -0.10$, $p = 0.15$). Amphibian community composition did not significantly differ between land use types (PERMANOVA; $R^2 = 0.11$, df = 3, $F = 1.03$, $p = 0.39$).

Rank-abundance curves for each land use type show the universal dominance of *P. achatinus* and similar overall shapes, with variation in subordinate species rankings (Figure S3). We found four species of conservation concern, and one listed as DD by the IUCN (2024). All were found in remnant forest, while two were also found in active cacao plantations (Table 1). Although *Epipedobates espinosai* is classified as DD, it appears to be widespread in our study area (López-Hervas et al. 2024), and was found across all four land use types.

We extrapolated Hill numbers to standardize and compare species diversity at equal sampling coverage across land use types. Hill number estimates of all orders ($q = 0, 1, 2$) show remnant forest sites with higher diversity, followed by abandoned plantations, and shade and sun cacao plantations, although 95% confidence intervals overlap (Figure 4).

3.4 | Influence of Environmental Variables on Amphibian Communities in Cacao Plantations

Leaf litter and canopy cover were highest in remnant forest and abandoned cacao plantation sites, intermediate in shade cacao, and lowest in sun cacao (leaf litter: $F = 17.81$, df = 3, $p \leq 0.001$, canopy cover: $F = 26.04$, df = 3, $p \leq 0.001$, Table 2). Surrounding forest cover was higher in remnant forest and abandoned cacao plantations, and lower in shade and sun cacao plantations ($F = 10.08$, df = 3, $p = 0.001$, Table S3). All other local and landscape variables did not show differences across land use types (Table 2).

The first two axes of the PCA, including all continuous response and predictor variables, explained 57% of variance (Figure S5). On the first axis, elevation and vegetation cover showed strong positive loadings, while richness and Simpson diversity all had strong negative loadings. On the second axis, distance to water had a strong positive loading, while leaf litter, canopy, surrounding forest cover, and amphibian abundance all had strong negative loadings. Because leaf litter and canopy cover were highly correlated (PCC = 0.79), we used canopy cover in all models.

We used GLMs and model selection via AICc to explore the relative influence of environmental variables on amphibian abundance, richness, and Simpson diversity in 21 active cacao plantations. The best fit model for abundance contained only the intercept, although three other models containing canopy cover, forest cover, and water distance, respectively, were equally plausible (Table 3). An intercept-only model produced the best fit for richness, although a model containing only a negative effect for elevation was equally plausible. The two best models for Simpson Index both contained a negative effect for elevation (Table 3).

4 | Discussion

We assessed the conservation value of active shade and sun cacao plantations by comparing their amphibian communities to remnant forest and abandoned cacao plantations in and around the Mache-Chindul Reserve in Esmeraldas, Ecuador. We hypothesized that remnant forest sites would have the highest abundance, richness, and diversity, followed by abandoned, shade, and sun cacao plantations, but found no support for this hypothesis. Similarly, we expected that amphibian community composition would most closely resemble that of remnant forest sites in abandoned cacao plantations, followed by shade and sun cacao plantations. We found some support for this hypothesis, although evidence was variable and method-dependent. We also collected a series of environmental variables in active cacao plantations of both types to determine the impact of local plantation management on amphibian communities. We hypothesized that local-level leaf litter and canopy cover and

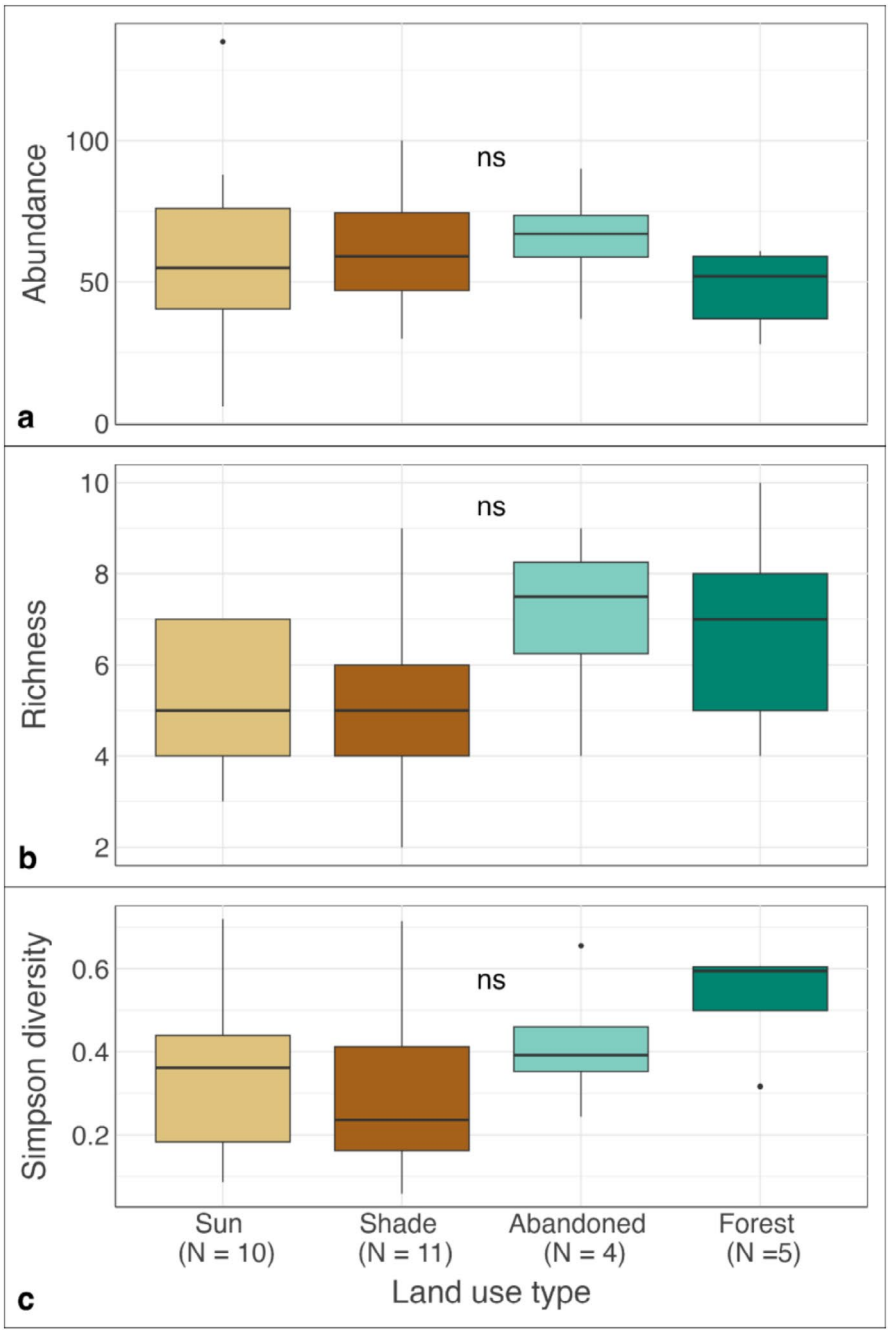

FIGURE 3 | Generalized Linear Models with land use type as a fixed effect found no significant difference in the abundance (a), species richness (b), or Simpson diversity (c) of amphibians across sun cacao, shade cacao, abandoned cacao, and remnant forest sites.

TABLE 1 | Number of individuals captured in each land use type for each of the five species of conservation concern. Species are listed along with their IUCN (2024; DD = data deficient, NT = near threatened, VU = vulnerable, EN = endangered, CR = critically endangered).

	Epipedobates espinosai **(DD)**	***Oophaga sylvatica*** **(NT)**	***Pristimantis laticlavius*** **(VU)**	***Leptodactylus peritoaktites*** **(EN)**	***Leucostethus bilsa*** **(CR)**
Sun Cacao ($N = 11$)	23	—	—	—	—
Shade Cacao ($N = 12$)	29	—	2	—	—
Abandoned Cacao ($N = 4$)	13	13	1	—	—
Remnant Forest ($N = 5$)	21	7	3	3	1

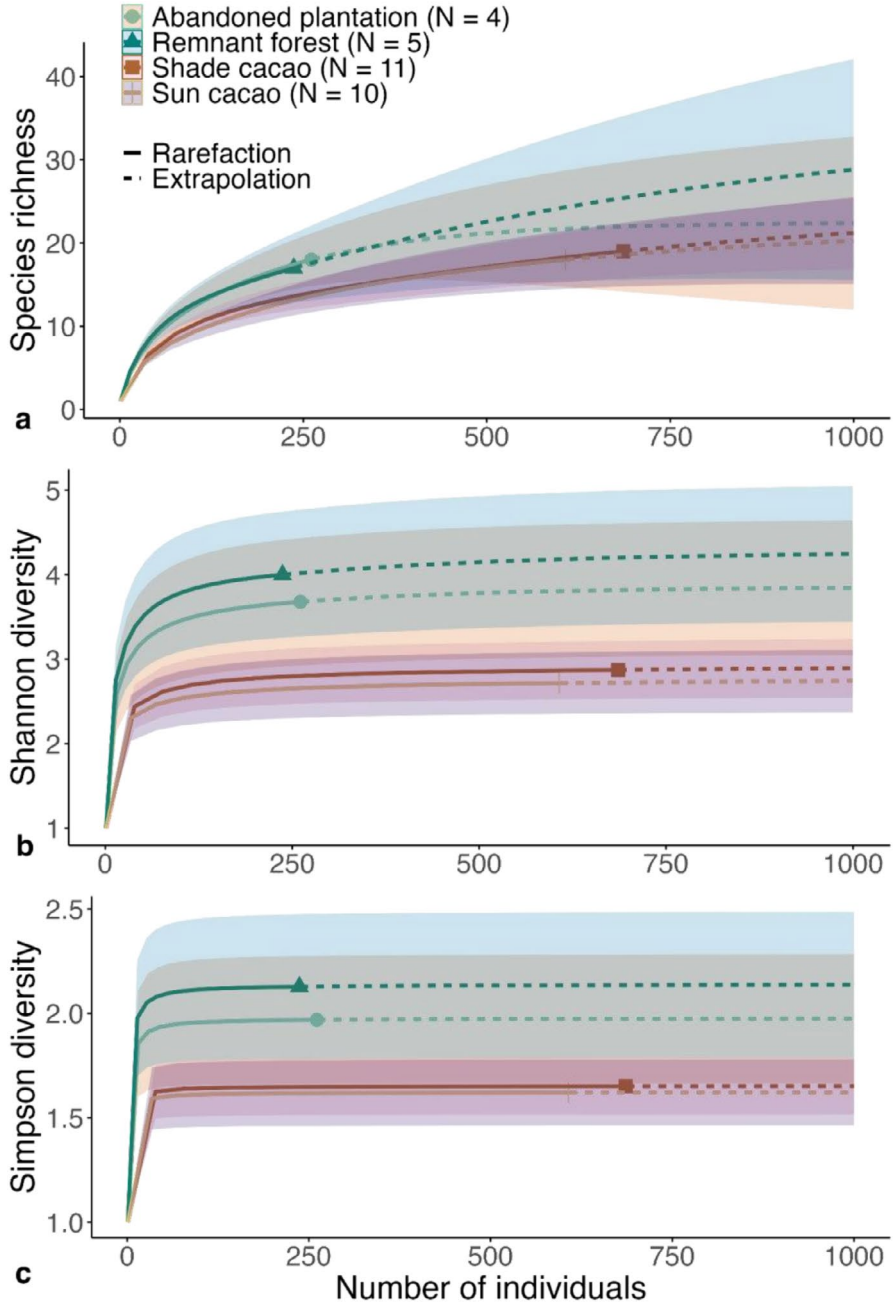

FIGURE 4 | Extrapolated individual-based Hill numbers using the *iNEXT* package (Hsieh et al. 2016) suggest that with additional sampling effort, remnant forest sites would produce higher species richness (a, $q = 0$), Shannon diversity (b, $q = 1$), and Simpson diversity (c, $q = 2$) than abandoned cacao plantations, and that both habitat types would produce higher Hill diversity than shade and sun cacao plantations.

landscape-level surrounding forest cover would have positive effects on amphibian abundance, richness, and diversity, but found no evidence to support this hypothesis.

4.1 | No Differences in Observed Abundance, Richness, or Diversity Across Land Use Types

We were unable to detect differences in the abundance, richness, or diversity of amphibians between active sun and shade cacao plantation types. We also compared amphibian communities of active cacao plantations to abandoned cacao plantations and remnant forests to contextualize the conservation value of these sites against recovering and primary forest conditions. Although we expected to find marked differences due to greater structural complexity and the absence of management interventions in the latter, we found no differences for any response variable across the four land use types (Figure 3). While this may suggest that amphibian communities across sites are equivalent, species richness and diversity values do not necessarily equate to conservation value (Roswell et al. 2021).

TABLE 2 | Environmental variable comparisons across land use types.

Variable	Sun cacao	Shade cacao	Abandoned cacao	Remnant forest	df	Test statistic	*p*
Leaf litter cover	33.73[a] (17.48)	61.59[b] (17.54)	87.15[c] (4.63)	81.81[c] (3.89)	**3**	**17.81**	**<0.001**
Canopy cover	44.93[a] (14.49)	68.94[b] (11.42)	89.70[c] (2.67)	90.70[c] (3.10)	**3**	**26.04**	**<0.001**
Vegetation cover	29.94 (18.50)	34.49 (22.14)	48.87 (9.26)	54.44 (7.79)	3	2.66	0.07
Forest cover	0.37[a] (0.11)	0.37[a] (0.11)	0.70[b] (0.26)	0.75[b] (0.26)	**3**	**10.08**	**0.001**
Water distance	220.00 (107.38)	189.45 (121.58)	198.75 (137.58)	164.60 (103.56)	3	0.28	0.84
Elevation	435.00 (78.25)	425.82 (79.73)	470.25 (80.69)	455.00 (72.28)	3	1.06	0.79

Note: Mean and standard deviation values (in parentheses), as well as degrees of freedom (df) and *F*-value test statistics are reported from ANOVA for all variables except elevation which did not meet normality assumptions from a Shapiro-Wilks test. Elevation was analyzed using a Kruskal-Wallis test, with the χ^2 test statistic reported. Superscript letters denote significantly different groupings of land use types for each variable, from Tukey HSD post hoc tests where applicable. Bold denotes statistically significant differences across land use types from ANOVA at $p = 0.05$ threshold.

TABLE 3 | Model selection showing all models in ascending order of ΔAICc for each response variable, with the effect sign denoted in parentheses.

Response	Distribution	Parameters	*k*	ΔAICc	Weight
Abundance	Negative binomial	Intercept-only	2	0	0.38
		(+) canopy cover	3	0.60	0.28
		(+) forest cover	3	1.56	0.17
		(–) water distance	3	1.75	0.16
Richness	Poisson	Intercept-only	2	0	0.52
		(–) elevation	3	0.13	0.48
Simpson Index	Beta regression	(–) elevation	3	0	0.64
		(–) elevation (–) canopy cover	4	1.19	0.36

Note: Model selection was performed using the *MuMIn* package (Bartón 2025). Model fit and distribution were assessed using the *DHARMa* package (Hartig 2022).

4.2 | Forests Likely Contain Higher True Diversity, Including Conservation-Priority Species

NMDS ordination and PERMANOVA revealed no differences in community composition across land use types (Figure S2). Rank-abundance curves show the dominance of *Pristimantis achatinus* across all sites, with subtle differences in subordinate species rankings (Figure S3).

Forty-one percent of globally assessed amphibian species are listed as Threatened by the IUCN (2024), including 57% of Ecuador's 655 species (Coloma and Duellman 2025). In our study, species of conservation concern were predominantly found in remnant forest and abandoned cacao plantations (Table 1) and were largely absent from active cacao plantations, despite a highly unbalanced sampling effort. Indeed, extrapolated Hill numbers suggest that abandoned cacao plantations and remnant forest sites were undersampled and would show greater diversity with additional sampling (Figure 4). Nonetheless, we found 28 individuals belonging to four IUCN-listed species in nine remnant forest and abandoned cacao sites, compared to only two individuals of one species across 23 active cacao plantations.

Our findings suggest that some species may be good indicators of habitat quality in the Chocó. *Oophaga sylvatica* has previously been proposed as an indicator of forest habitat quality (Jongsma et al. 2014) and was only found in remnant forest and abandoned plantation sites in our study. These forest patches appear to provide key habitat for forest-dependent and threatened species such as *Pristimantis laticlavius* (VU), *Leptodactylus peritoaktites* (EN), and *Leucostethus bilsa* (CR). Indeed, this study led to a range expansion for the latter species (Jones et al. 2024).

4.3 | Limited Evidence for Drivers of Amphibian Communities in Active Cacao Plantations

We found limited evidence that any of the six environmental variables we collected were meaningfully driving amphibian abundance, as the four best fitting models each contained no or one unique predictor (Table 3). We interpret the consistent negative effect of elevation on richness and Simpson diversity (Table 3) as biologically meaningful, albeit unlikely to inform plantation-level management practices. This finding is somewhat curious, as temperatures are likely more extreme and restrictive at *lower* elevations in our study area, especially for forest-dependent and sensitive species (Frishkoff et al. 2015; Gunderson 2023). Regardless, our study region lies between two biogeographic regions in the Ecuadorian Chocó (Ortega-Andrade et al. 2021); lower elevation cacao sites may better

support a mix of amphibian species found in lowland and foothill forests.

Canopy cover has elsewhere been correlated with amphibian abundance and richness in restoration sites (Díaz-García et al. 2020) and coffee plantations (Ríos-Orjuela et al. 2024). Leaf litter depth and cover have been shown to increase amphibian abundance in cacao plantations (Faria et al. 2007; Wanger et al. 2009; Cervantes-López et al. 2022), coffee plantations (Ríos-Orjuela et al. 2024), and restoration sites (Hilje and Aide 2012). At a landscape level, the amount and proximity of surrounding forest and freshwater have a well-documented positive correlation with more diverse amphibian communities (Mendenhall et al. 2014; Ribeiro et al. 2018). Remnant forests likely act as sources for amphibian communities in nearby cacao plantations, especially for species capable of crossing agricultural matrices (Frishkoff et al. 2015; Nowakowski et al. 2018), and freshwater is critical to the life cycle of many amphibian species(Becker et al. 2007).

We were surprised to find limited support for the variables measured, especially given that most showed a wide range across active cacao sites (Figure S4). Although it is possible that abundance and richness patterns are driven by unmeasured local or landscape variables (Jithin et al. 2025), a more likely explanation is that the diversity of this amphibian community has already been reduced by disease, habitat loss, and pesticide use (Scheele et al. 2019; Ríos-Touma et al. 2023), leaving a depauperate assemblage of resilient species.

4.4 | The Pastures Rainfrog, *Pristimantis achatinus*

Pristimantis achatinus was recorded at all 32 sites and captured at similar rates across land use types. This generalist species, which is native to the region (Ortega-Andrade et al. 2010), has likely increased in abundance following decades of extensive forest degradation and clearing (Perlin and Leguizamón 2024). As a direct-developing species, *P. achatinus* does not require waterbodies for reproduction. The species is ubiquitous across our study area (Mazanec 2022), and has been shown to be resistant to chytridiomycosis infection (Perkins 2022).

Pristimantis achatinus likely has a large impact on the ecology of agricultural sites in the region. Amphibians connect trophic levels across aquatic and terrestrial systems, serving as both predator and prey to a wide range of taxa (Hopkins 2007). Trophic ecology studies have found *Pristimantis* to feed widely on multiple insect orders (Pedroso-Santos et al. 2024). Given its abundance, the impact of this generalist species on food webs of cacao plantations warrants further study.

4.5 | Limitations and Recommendations for Future Research

A primary limitation of our study was the low number of remnant forest and abandoned cacao plantation sites. These sites are inherently scarce due to the fragmented nature of our study region and are often restricted to inaccessible areas (Fagua et al. 2019). In turn, our capture-based sampling methodology likely underestimated amphibian diversity, especially in more structurally complex forest and abandoned cacao sites. For example, the lower height of cacao plantations facilitated arboreal amphibian community sampling. We found 32 individuals of four species of the primarily arboreal *Boana* genus in shade cacao, 38 in sun cacao, but only three in abandoned plantation, and two in remnant forest (Table S2), despite occasionally hearing them while sampling in the latter, and evidence that these species are common in the canopies of forest sites in our study area (Ortega-Andrade et al. 2010). This suggests that our results likely underestimate the diversity of remnant forest and abandoned cacao plantations. Future research should incorporate additional sampling methods, such as pitfall traps and acoustic recording to improve species detection (Pulsford et al. 2018; Anunciação et al. 2022).

Our study exclusively measured taxonomic diversity. Elsewhere, amphibian communities have been observed to segregate across a similar array of land use types according to phylogenetic (in Brazil: Almeida-Gomes and Rocha 2014) and functional (in Mexico: Hernández-Ordóñez et al. 2019) diversity. Compensatory dynamics may be masking the effect of land conversion on amphibian communities in our study landscape (Russildi et al. 2016), whereby more disturbed environments, such as sun cacao plantations, may show high richness and abundance due to the presence of generalist and open-area specialist species. Assessing phylogenetic diversity variability across habitat types is particularly relevant for this region of Ecuador, given the exceptional levels of amphibian diversity (Ortega-Andrade et al. 2021).

Unmeasured variables, including pesticide usage, may impact amphibian distribution in this highly agricultural landscape. A recent survey of cacao farmers in our study landscape revealed that agricultural chemicals are applied at comparable frequency and intensity across both shade and sun cacao plantations (Sánchez-Julia 2024). A multi-year Before-After-Control-Impact (BACI) study would help determine the conservation impact of pesticide application in the region.

4.6 | Implications for Amphibian Conservation in a Fragmented Tropical Landscape

Farmers in our study region have been widely transitioning to sun cacao over the past decade due to economic conditions prioritizing yield over quality (Perlin and Leguizamón 2024). Our results suggest that shade cacao cannot be universally endorsed as more environmentally-friendly than sun cacao as currently practiced, despite the fact that shade cacao sites in this study were generally older and had higher levels of leaf litter and canopy cover.

Although other studies have found that shade cacao plantations can provide habitat for forest-dependent amphibians (Faria et al. 2007; Cervantes-López et al. 2022), that does not appear to be the case in our study area. Pesticides application likely reduces amphibian species richness in all but the most forested habitats (Sánchez-Julia 2024). Minimizing pesticide use has a well-documented effect of improving outcomes for biodiversity at large (Brooker et al. 2016), including for amphibians within and adjacent to agricultural areas (Buck et al. 2015; Davis et al. 2020).

Our results underscore the importance of maintaining remnant forest cover in fragmented agricultural landscapes (Palacios et al. 2013; Kremen and Merenlender 2018; Rozendaal et al. 2019), and suggest that land sparing would be a more effective strategy for amphibian conservation in the region (Phalan et al. 2011; Williams et al. 2017). Abandoned cacao plantations could be prioritized for forest restoration (Arnold et al. 2021), as their structural complexity may promote bird visitation and subsequent seed deposition, thereby kickstarting forest recovery (Carlo et al. 2013; González-Varo et al. 2017). Furthermore, abandoned cacao plantations appear to have recovered some important remnant forest characteristics, noted by the census of several forest-dependent amphibians.

Environmental certification and conservation initiatives often promote the cultivation of slow-growing, shade cacao varieties and reduce the frequency and intensity of pesticide application (Robbins et al. 2015; Brako et al. 2020). Such initiatives have the potential to provide greater economic benefit and stability for farmers while increasing structural complexity within cacao plantations (Perfecto et al. 2005; Middendorp et al. 2018). Our results suggest that neither shade nor sun cacao plantations are likely to benefit forest-dependent and conservation priority amphibian species in the region without substantial changes to plantation management. In the context of the widespread transition from shade to sun cacao, the more immediate concern for regional amphibian conservation should be preserving and increasing native forest cover in the heavily fragmented Ecuadorian Chocó.

Author Contributions

S.H.J., J.M.G., and R.A.Z. conceptualized the study. S.H.J., D.Z.C., and J.C.L.V. collected the data. S.H.J., D.Z.C., J.C.L.V., D.F.-M., F.V.-E., and J.M.G. reviewed amphibian identifications. S.H.J. wrote the first draft. S.H.J., D.F.-M., F.V.-E., J.M.G., and R.A.Z. reviewed and edited the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and code that support the findings of this study are openly available in a public Github repository at https://github.com/sholdenjones/pub_cacao_ranas.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Typical sampling design demonstrating quadrat and transect placement and spacing within a larger cacao plantation. **Table S1:** Species, vouchers, and GenBank accession numbers for newly generated DNA sequences (16S) used in genetic analyses. All individuals were collected in the Fundación para la Conservación de los Andes Tropicales Reserve in Esmeraldas Ecuador (between 0°23′33″ N, 79°38′50″ W, 300 m a.s.l. and 0°21′20″ N, 79°41′22″ W, 500 m a.s.l.). **Table S2:** Amphibian abundance of all species captured, indicating IUCN threat status; Least Concern (LC), Data Deficient (DD), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), as of November 13th, 2024 (IUCN 2024). Total abundance and abundance across the four different land use types are shown for each species (Sun cacao, $N = 11$; Shade cacao, $N = 12$; Abandoned plantation, $N = 4$; Remnant forest, $N = 5$). **Figure S2:** NMDS ordination plot of Bray–Curtis dissimilarity matrix with sun cacao (light brown), shade cacao (dark brown), abandoned cacao (light green) and remnant forest (dark green) sites (stress level 0.08). Ellipsoids contain the 95% confidence interval surrounding the group centroid. **Figure S3:** Rank-abundance curves for each land use type showing species abundance on a logarithmic scale. All species with at least ten individuals in each land use type are annotated using their species code, with the first three letters corresponding to their genus name, and the last three corresponding to their species name. Refer to Table S1 for complete species inventory. **Figure S4:** Histogram showing the distribution of the mean for each of the six collected local and landscape variables across each land use type; (a) canopy cover, (b) leaf litter cover, (c) forest cover within 400 m radius, (d) distance to freshwater, (e) herbaceous vegetation cover, (f) and elevation. **Figure S5:**. Principal components analysis biplot for 21 active cacao plantation sites (sun and shade), including amphibian richness, abundance, Simpson and Shannon diversity, and local and landscape variables. The first two axes explained 57% of the variance.