

1 HERPETOFAUNA RICHNESS, COMMUNITY COMPOSITION AND ABUNDANCE IN  
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**Herpetofauna richness, community composition and abundance in forested and agricultural habitats of the Mache Chindul Reserve, northwest Ecuador**

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44

45 **Abstract:** Amphibians and reptiles worldwide are experiencing some of the highest rates of  
46 population decline of any taxonomic groups. This population loss is often attributed to habitat  
47 loss and fragmentation by humans for agriculture and development. We investigated how  
48 conversion of natural forests into agriculture affects amphibians and reptile assemblages in the  
49 Mache-Chindul Reserve of northwest Ecuador, an understudied biodiversity ‘hotspot’.  
50 Herpetofauna species richness, community composition, and abundance were estimated via 320  
51 total surveys at 40 points in agricultural and forest areas over a 6-month period. We observed a  
52 significant decline in species richness in agricultural areas relative to forest areas, and our  
53 analyses suggest that changes in canopy openness and leaf litter depth may be important habitat  
54 determinants of richness. We also observed significant differences in community composition  
55 between the two habitat types, with threatened and endemic species occurring in forested but not  
56 agricultural sites. In contrast, we did not observe any significant differences in abundance based  
57 on land cover type. These findings highlight the importance of intact forests and maintenance of  
58 complex habitat in promoting the persistence of amphibians and reptiles in the Chocó  
59 biogeographic zone.

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61 Key Words: herpetofauna, disturbed habitat, amphibian communities, species richness,  
62 Neotropics, Ecuador

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65 INTRODUCTION

66 Habitat destruction and fragmentation are two of the foremost reasons for contemporary global  
67 wildlife decline (Pimm and Raven 2000). Much of this habitat loss is due to conversion of  
68 natural forest to human-based land use types, particularly agriculture (Newbold, Hudson et al.  
69 2015). This decline in wildlife is especially prevalent in the tropics. Researchers have estimated  
70 an average decline of 68% in global abundance of mammals, birds, amphibians, reptiles, and  
71 fish, and a staggering 94% abundance decline in the subtropics of the Americas over the past 50  
72 years (Almond, Grooten et al. 2020). Shifts in community structure have also been observed  
73 worldwide, although in many cases species richness often appears to remain stable (Blowes,  
74 Supp et al. 2019). South America has also experienced some of the highest rates of forest loss in  
75 the last 20 years, totaling 39 million hectares lost (Nations 2020). Obtaining quantifiable data on  
76 how land conversion affects richness, abundance and community composition of various  
77 taxonomic groups is a critical component of conservation management as these changes  
78 continue.

79         Using a taxonomic group as a representative of an ecosystem's health has often resulted  
80 in useful insights into issues around conservation of biodiversity that an area may be facing  
81 (Zakaria, Leong et al. 2005) because of the interactive nature of trophic levels. Amphibians and  
82 reptiles, collectively herpetofauna, are two of the taxonomic groups that are most in need of  
83 conservation measures. Amphibians are a particularly important indicator group due to their high  
84 degree of sensitivity to changes in their environment (Blaustein, Walls et al. 2010). Moreover,  
85 elimination of a critical component of an ecosystem's food web, like amphibians, can often result  
86 in cascading effects through numerous trophic levels (Zipkin, DiRenzo et al. 2020).

87 One-third of the world's 8000 known species of amphibians are listed as threatened and 43%  
88 have populations in decline (Zedan 2004). In the Neotropics, where about 35% of the worlds'  
89 species of amphibians are found (Duellman 1999), deforestation and disease have led to the  
90 extinction of over 30 species of amphibians and more than 2000 being listed as threatened  
91 (Almond, Grooten et al. 2020).

92 Site-specific studies on how habitat alteration in tropical forests impacts amphibian  
93 species richness and abundance have produced variable results, highlighting the need for a better  
94 understanding of the factors that shape patterns of diversity in this group. Many studies have  
95 found that habitat alteration results in a decline in amphibian diversity, with sensitive (specialist)  
96 species disproportionately affected (Pearman 1997, Steininger, Tucker et al. 2001,  
97 Krishnamurthy 2003, Hamer and McDonnell 2008). Conversely, others have found that when  
98 habitat perturbation is mild to moderate, it may actually result in an increase in amphibian  
99 diversity (Toral, Feinsinger et al. 2002, Urbina-Cardona, Olivares-Pérez et al. 2006), particularly  
100 in edge habitats of connecting forest and agriculture, where the combination of habitat types  
101 allows both specialist and generalist to persist. More data on a larger range of systems is required  
102 to better understand these relationships.

103 Amphibians are a diverse group whose lifestyles range from purely aquatic, to aquatic  
104 and terrestrial, solely terrestrial, arboreal, and fossorial (Crump 2009). Research has shown that  
105 amphibian diversity and abundance are often related to some critical components of habitat such  
106 as leaf litter, understory, or canopy structure (Stephens, Berven et al. 2013, Gallmetzer and  
107 Schulze 2015). For example, Stephens et al. (2013) found that changes to leaf litter composition  
108 as a result of human activities, invasive species, and climate change, had strong effects on the  
109 fitness of larval frogs. Gallmetzer (2015) found a strong relationship between understory density

110 and amphibian composition, in which the absence of understory vegetation resulted in a dramatic  
111 shift from endemic species and high functional diversity to disturbance tolerant species and a  
112 more homogeneous community. Topographical properties of habitat such as elevation and slope  
113 have also been shown to impact amphibian diversity (Ribeiro Jr, Siqueira et al. 2018, Khatiwada,  
114 Zhao et al. 2019). Species belonging to more aquatic genera are commonly found at lower  
115 elevation, corresponding with proximity to water and warmer air temperatures, whereas more  
116 terrestrial species remain at higher elevation and cooler temperatures.

117         Like amphibians, reptiles have experienced drastic global populations decline. Moreover,  
118 because they are the most species-rich and widespread terrestrial group of vertebrates, only 45%  
119 of their species have been assessed by the IUCN. Of those that have been assessed, 20% are  
120 listed as threatened or endangered and 19% are lacking sufficient data (Tingley, Meiri et al. 2016).  
121 This lack of data because of the widespread nature of the group has resulted in a deficit of  
122 directed conservation planning for reptiles compared to other taxonomic groups (Roll, Feldman  
123 et al. 2017). Although taxonomically different, reptiles are often analyzed alongside amphibians  
124 because of their similarities as ectotherms and habitat needs. Studies have shown that reptile  
125 species richness and abundance exhibit the same positive correlation with leaf litter depth, as  
126 well as the same negative relationship with elevation as amphibians (Fauth, Crother et al. 1989).  
127 Reptile assemblages tend to respond to human modified habitat, such as agricultural land, with  
128 significantly decreased abundances and moderately decreased species richness (Doherty,  
129 Balouch et al. 2020).

130         South America's Chocó biogeographic zone is a biodiversity hotspot, containing some of  
131 the highest levels of diversity and endemism in the world, including many threatened and  
132 endangered species (Fund 1995). Stretching from northwest Ecuador into the Pacific coast of

133 Colombia and Panama, much of the natural forest in this area has been converted into agriculture  
134 over the past few decades (Dodson and Gentry 1991, Sierra 1996, Sierra, Campos et al. 2002).  
135 One of the largest ecological reserves in the Chocó area is the Mache Chindul Ecological  
136 Reserve (REMACH), covering 120,000 hectares. Despite being an ecological reserve, logging  
137 and agricultural development over the last 50 years have left the forests highly fragmented and  
138 experiencing the second highest rates of deforestation among protected lands in Ecuador (van  
139 Der Hoek 2017). REMACH has been the site of two recent studies that share similar research  
140 questions to the current project. Jongsma (Jongsma 2014) looked at amphibian diversity and  
141 species composition in relation to primary forest habitats and altered habitat along road edges,  
142 and Ortega (Ortega-Andrade, Bermingham et al. 2010) likewise looked at amphibians as well as  
143 reptiles in an effort to construct an inventory on another research station near to FCAT, Bilsa  
144 Biological Station. In addition to studies on amphibians and reptiles, FCAT has been the site of a  
145 suite of projects on impacts of habitat fragmentation on various taxa (Cook, Ramirez-Parada et  
146 al. 2020, Lueder, Narasimhan et al. 2022). Jongsma and Ortega-Andrade documented some of  
147 the effects that human activities in this area have had on amphibians, but little work to date has  
148 assessed amphibian and reptile species richness, composition, and abundance in agricultural  
149 (e.g., pasture or cacao plantations) habitat types, and how it may compare to forested areas in the  
150 region.

151 In the current study, we established survey points across forest and agricultural areas in  
152 and around the Mache Chindul Reserve, quantified relevant habitat attributes, and surveyed each  
153 point for herpetofauna species richness, community composition and individual abundance. In  
154 doing so, our goal was to understand how amphibian and reptile species richness, composition  
155 and abundance varies in relation to land cover type and habitat across a representative spectrum

156 of land cover types. These data can be used to inform management strategies in this diversity  
157 hotspot as well as our broader understanding of the biotic and abiotic factors that shape  
158 herpetofauna communities. We hypothesized that species richness would be higher in forest, that  
159 community composition would differ between the two habitat types, and that abundance would  
160 be higher in agricultural areas.

161

## 162 METHODS

### 163 **Study site**

164 We conducted our study in and around the 120,000 ha Mache-Chindul Ecological  
165 Reserve (REMACH) in Esmeraldas Province, Ecuador in the Chocó Biogeographic zone. The  
166 reserve has experienced extensive conversion of forest to agricultural land cover types in the past  
167 50 years and deforestation continues at a rapid rate (van Der Hoek 2017). REMACH contains a  
168 coastal mountain range separated from the Andes by a coastal plain; elevations range from sea  
169 level to 700 m a.s.l. Rainfall varies from 2500–3500 mm annually, with the rainy season  
170 typically occurring between January and June (Clark, Neill et al. 2006). The area experiences a  
171 temperate climate year-round, with average monthly temperatures ranging from 21 to 24°C  
172 (Charlat, Thatcher et al. 2000). Within REMACH, is the Fundación para la Conservación de los  
173 Andes Tropicales (FCAT) research station, a 550 ha privately owned reserve which contains  
174 large patches of intact forest that were used as forest sampling sites. These forests are  
175 categorized as Humid Premontane and Humid Tropical Forest life zones (Holdridge 1967). The  
176 study areas outside of the station’s forest sites composed primarily of pasture, cacao and other  
177 crops that were used as agricultural sampling sites.



178 **Habitat sampling**

179 Field sampling surveys were conducted from March – July 2021, which allowed  
180 sampling from both the later part of the wet season and the transition to the dry season. We  
181 surveyed two distinct land cover types, forest and agriculture. Points in each land cover type  
182 were selected according to accessibility, logistics (i.e., permission for land access) and to ensure  
183 representativeness of agricultural and forest land cover types in the Mache Chindul Reserve. All  
184 points, regardless of type were placed at least 10-meters from any walking path, and 250 meters  
185 from any other points. Each point was located entirely within its representative habitat type at  
186 least 30 meters from adjoining forest or agriculture respectively. After reaching a distance of 250  
187 meters from a previous point, ideal habitat for herpetofauna was identified within visual range  
188 and established as the survey point. Each point was centered around a single GPS location,  
189 surrounded by a 20-meter diameter survey circle. Forest sites included both primary and  
190 secondary forest habitats, ranging in elevation from 308 to 556 meters. Agriculture sites included  
191 three of the most common land uses for farming in the area: cattle pasture (18), cacao plantations  
192 (3), and banana plantations (3).

193 Six predictor variables of habitat structure were measured based on previous studies in  
194 the region (Ortega-Andrade, Bermingham et al. 2010, Jongsma 2014, Gallmetzer and Schulze  
195 2015), and previous studies on patterns of diversity conducted in the region (Walter, Browne et  
196 al. 2017, Cook, Ramirez-Parada et al. 2020). These six attributes were canopy openness, canopy  
197 height, elevation, leaf litter depth, slope, and understory density. Elevation at the center point  
198 was recorded using a handheld GPS. Openness of the canopy was measured using a densiometer,  
199 which gives a percent cover of the canopy using sunlight penetration. Canopy height was  
200 collected by using a range finder from the base of the tallest tree measured to the top canopy.

201 The maximum slope was taken by using a clinometer looking towards a secondary surveyor  
202 down the steepest portion of the 20-diameter site at a distance of 15 meters. The clinometer  
203 displays the vertical difference from the top surveyor to the secondary surveyor giving a vertical  
204 distance of the drop in feet. Leaf litter depth was taken by inserting a metal file into the litter  
205 until the tip penetrated the soil, then placing a finger at the base of the exposed file and  
206 measuring the distance with a ruler. This process was done three times at each site and then  
207 averaged to give a representation of the litter as a whole. Lastly, we measured understory density  
208 using a two-meter-long pole with neon flagging every six inches along its length. This pole was  
209 placed 10 meters from the point center in each cardinal direction and looking from the point  
210 center, the number of visible flagging was recorded and averaged among the four observations.

211

## 212 **Species sampling**

213 After all sites had received their habitat inventory, sampling for species began. Each site  
214 was sampled four times diurnally and 4 times nocturnally in a random order over the five-month  
215 sampling period. Each site was given at least 10 days between a visit of either treatment. Surveys  
216 were conducted by JO and TM. Before a survey of a point began, climate conditions at the site  
217 were recorded including temperature, wind speed, cloud cover percentage, presence of rain, and  
218 beginning of survey time. A single survey of a site consisted of a 10-minute visual encounter  
219 survey (VES) within the 20-meter diameter sampling area. This VES involved actively moving,  
220 listening, and searching over any and all substrate, as well as lifting and moving of any cover  
221 that the herpetofauna may shelter under. VES, while unreliable when trying to assess species  
222 density without a mark-recapture component, are ideal for conducting inventories of  
223 herpetofauna species richness and relative abundance in understory habitats (Heyer, Donnelly et

224 al. 2014). Diurnal surveys were conducted in the morning from 0800 to 1300 hours, while  
225 nocturnal surveys were conducted from 2000 to 0200 hours. All amphibians and reptiles  
226 encountered during a survey were captured when possible, and identified on site before being  
227 released. For accurate identification of species three main resources were used; previous  
228 literature comparison that provided photo vouchers, assistance for local experts with decades of  
229 experience in local wildlife identification, and examining online academic resources from  
230 BioWeb Ecuador (<https://bioweb.bio/>)(Orozco) and Museo de Zoología QCAZ  
231 (<https://bioweb.puce.edu.ec/QCAZ/inicio>)(Orozco N.D.). How the individual was encountered,  
232 via sound, flushed or unmoving, as well as on which substrate and estimated age (juvenile or  
233 adult) was also recorded for each individual encountered. Each of the two surveyors maintained  
234 a distance of 5m and communication about encounters in order to limited double counts of  
235 individuals during sampling bouts. Individuals were not marked or tagged in any mark-recapture  
236 capacity.

### 237 **Statistical analysis**

238         There was no statistical difference in species richness ( $3.5 \pm 1.65$  vs.  $2.6 \pm 0.84$ ;  
239  $t_{22}=1.33$ ,  $P = 0.197$ ) between pasture and non-pasture non-forest sites, so we combined both land  
240 use types into a single ‘agriculture’ category for subsequent analyses. Expected species  
241 accumulation curves (e.g., sample-based rarefaction curves), which allow for comparison of  
242 richness levels while controlling for different sample sizes, were computed independently for  
243 each habitat type.

244         We used t-tests to assess whether species richness and abundance varied between forest  
245 vs. agriculture sites. Next, we conducted a principal component analysis (PCA) with our six  
246 habitat attributes (i.e., canopy openness, canopy height, elevation, leaf litter depth, slope, and

247 understory density, above) to characterize habitat differences between agriculture vs. forest sites,  
248 followed by Spearman rank correlations to assess the effect of the top two PCs on site species  
249 richness or abundance.

250 A nonmetric multidimensional scaling (NMDS) ordination based on a Bray-Curtis  
251 similarity matrix was conducted to compare community compositions across habitats. An  
252 analysis of variance was used to identify if the variance in agriculture was the same as the  
253 variance in forest sites to test for the validity of the NMDS. We conducted an indicator species  
254 analysis (Dufrière and Legendre 1997) in which information on both species presence and  
255 abundance was combined to identify species especially associated with a particular habitat type.

256 Statistical analyses were conducted in R v 4.1.1 using the package *vegan* (Team 2013).

257

## 258 RESULTS

### 259 **Sampling overview**

260 In total, 40 sampling sites over an area of approximately 30 km<sup>2</sup> were established and  
261 sampled: 16 forest sites and 24 agriculture sites (Figure 1). Forest sites averaged higher canopy  
262 height, leaf litter depth, slope, and understory density (Supplementary table 1). Each of the 40  
263 sites was visited 8 times for sampling, 4 times diurnally and 4 times nocturnally, for a total of  
264 320 surveys. Over all surveys, 784 individuals were recorded, representing 37 species, 28 genera,  
265 and 13 families. Amphibians accounted for 96% of total observations and reptiles the remaining  
266 4%. Because of the low sample size for reptiles precluded most statistical analyses, we limited  
267 most analyses below to amphibians.

268           The most abundant species was *Pristimantis achatinus*, which accounted for almost 70%  
269 of all individuals encountered; most other species were encountered < 5 times (Table 1). The  
270 species accumulation curves indicated that both habitats were under-sampled, as encountered  
271 species totals were still increasing at our maximum site totals (Figure 2).

272

### 273 **Species richness and individual abundances**

274           A total of 13 species were encountered in our 16 forested sites versus 16 species  
275 in our 24 agricultural habitat sites. Forest sites had significantly more species per site than did  
276 agricultural sites (4.2 +/- 1.27 vs. 2.9 +/- 1.15 species per site;  $t_{38} = 3.367$ ;  $P = <0.002$ ; Figure 3a).  
277 In terms of abundance, 445 individuals were encountered in agricultural habitats versus 341  
278 individuals in forest habitats (Table 1). There was no significant difference in the number of  
279 individuals recorded per site for forest vs. agricultural sites (20.3 +/- 7.7 vs. 18 +/- 16.6  
280 individuals per site;  $t_{38} = 0.512$ ,  $P = 0.612$ ; Figure 3b).

281

282

### 283 **Habitat correlates**

284           Our top two principal components for habitat attributes collectively accounted for 60% of  
285 site variance. PC1 was most strongly associated with canopy openness and leaf litter depth, while  
286 PC 2 was most strongly associated with understory density and slope height (Table 2). The  
287 Spearman rank correlations revealed a significant relationship between PC1 and site species  
288 richness ( $S = 16015$ ,  $P < 0.001$ ,  $\rho = -0.502$ ) and a near-significant relationship between PC1

289 and site abundance ( $S = 13892$ ,  $P = 0.057$ ,  $\rho = -0.303$ ), suggesting that both richness and  
290 abundance may increase with more closed canopies and deeper leaf litter. The Spearman rank  
291 correlations for PC2 showed no significant relationship between PC2 and site species richness ( $S$   
292  $= 13381$ ,  $P = 0.111$ ,  $\rho = -0.256$ ) and a significant relationship between PC2 and site abundance  
293 ( $S = 15603$ ,  $P = 0.003$ ,  $\rho = -0.464$ ), suggesting abundances increased with denser understory.

294

### 295 **Community composition**

296 Agriculture and forest points hosted distinct communities (Figure 4,  $P = 0.006$ ) and the  
297 variation inside of each category was equal (test for assumption of homogeneity of multivariate  
298 dispersion; ( $F_{38} = .0.255$ ,  $P = 0.617$ ). The indicator species analysis identified four species  
299 indicative of forest (Table 3). Of the four species, *Oophaga sylvatica* had the strongest relation  
300 with forest, followed by *Hyloxalus awa*, *Epipedobates boulengeri*, and *Pristimantis latidiscus*  
301 respectively. All four indicator species are endemic to the Chocó region with *O. sylvatica* and *P.*  
302 *latidiscus* having declining population trends and *O. sylvatica* considered Near Threatened  
303 (IUCN 2022). There were no indicator species for agriculture.

304

305

### 306 **DISCUSSION**

307 This study's goal was to identify relationships between habitat attributes and  
308 herpetofauna richness, community composition and abundance in a biodiversity hotspot, the  
309 Chocó biogeographic zone. Based on visual encounter surveys and measurements of habitat

310 metrics, we found evidence for loss of amphibian species richness and changes in community  
311 composition in agriculture relative to forest, but no impact on abundance.

312         This study is the first to evaluate herpetofaunal diversity in agricultural (i.e., pasture and  
313 cacao) landscapes in the Ecuadorian portion of the Chocó biogeographic zone. Our finding that  
314 species richness was lower in agricultural vs. forest sites corroborates the results of two previous  
315 studies by Jongsma et al. (2014) and Ortega et al. (2010) that evaluated other types of disturbed  
316 habitats relative to forest in our project area. All three studies found declines in species richness  
317 in “disturbed habitats” relative to forest, with Jongsma et al. (2014) encountering only 9 out of  
318 25 species in road edge and the remainder in forest, Ortega encountering 19 of 58 species in  
319 ‘disturbed’ sites and the remainder in forest, and this study encountering 16 of 21 total species in  
320 agriculture vs. 13 in forest, despite higher sampling effort in agriculture. Taken together, this  
321 body of work points to a robust effect of deforestation on reduced species richness in  
322 amphibians. These findings are common results of studies looking to identify drivers of reduced  
323 amphibian communities in tropical study sites around the world. Studies from Sri Lanka  
324 (Kudavidanage, Wanger et al. 2012) to Nigeria (Akani, Politano et al. 2004) all found a  
325 reduction in amphibian species richness from pristine forest to human altered habitats.

326         Our exploration of the effects of principal components on species richness suggested that  
327 canopy openness and leaf litter depth are important determinants of alpha diversity (PC1). Forest  
328 sites averaged 4x more closed canopy and 90% more leaf litter depth than did agricultural sites.  
329 This suggests that verticality to habitat is an important component in maintaining diversity, and  
330 also suggests that we may have under-estimated richness in forested sites by missing arboreal  
331 species, as indicated by the species accumulation curves (DeMaynadier and Hunter Jr 1999). The  
332 relationship with leaf litter depth and richness points to findings that reduced leaf litter results in

333 higher soil temperatures and therefore higher rates of desiccation for amphibians may be at play,  
334 as well as greater risk of predation because of the reduced forest floor cover (Schurbon and Fauth  
335 2003). Much like Gallmetzer (2015) we found shifts in communities and richness with changes  
336 to understory, with sites lacking connective understory foliage corresponding with low diversity  
337 and high abundance of disturbance tolerant species such as *P. achatinus*. Taken together, these  
338 findings suggest that healthy, diverse, habitat seems to have multiple tiers of habitat: leaf litter,  
339 understory, and canopy. These findings are congruent with Jongsma et al (2014) and other  
340 studies on amphibians, that structural habitat alteration cause by humans can impoverish species  
341 richness and diversity.

342         Interestingly, unlike numerous other studies on other taxonomic groups in our project  
343 area (e.g., Walter et al. 2017, Cook et al. 2020), we did not find a significant relationship  
344 between elevation and species richness, perhaps because the elevational range in our study was  
345 relatively small compared to these previous studies (248m separated our highest and lowest  
346 sampling sites); additional work is required to better understand this discrepancy. Another future  
347 research direction concerns proximity to water. Jongsma et al (2014) suggested that access to  
348 water may be higher in forested areas and may be a causal factor in driving observed differences  
349 in species diversity between forested vs. disturbed sites. While we did not directly evaluate  
350 distance to water in our study, we note that our forested sites were located nearer (< 15m) to  
351 streams or rivers at a much higher rate than agriculture sites, 5/16 for forest and 4/24 for  
352 agriculture. Additionally, agriculture runoff is commonly linked with pollutions of water nearby  
353 sources (Wiens 1980), and many amphibians have an aquatic development stage as well as  
354 permeable eggs, skin, and gill, that have been shown to absorb agricultural pollutants during  
355 development (Jung 1996).



356 As with species richness, we also found strong differences in community composition  
357 between forested vs. agriculture sites. One species encountered mainly in forested sites, *O.*  
358 *sylvatica*, is listed as Near Threatened with 85% of specimens encountered in forest habitats. The  
359 growing conversion of natural forested areas into agricultural production, is cited as a reason  
360 why *O. sylvatica* populations are under threat in the region (IUCN 2022). Moreover, all four of  
361 the indicator species for forest sites were Chocó endemics. Interestingly, three of the four  
362 indicator species were members of the family Dendrobatidae which is commonly identified as a  
363 specialized family because of their alkaloid skin defense. These findings suggest a difficulty for  
364 specialist species to adapt to agricultural habitats. Although another species, *L. peritoaktites*,  
365 though not an indicator species, is also considered conservation concern (VU), was only  
366 encountered in agriculture. These findings are encouraging for the future of this particular  
367 species to adapt to changes in their environment, though more analysis would need to be done as  
368 there were only two total specimens encountered. Studies in the Ecuadorian Amazon by  
369 (Pearman 1997) found that species richness of the genus *Pristimantis* declined with proximity to  
370 pasture, suggesting that *Pristimantis* may be a useful indicator assemblage for assessing habitat  
371 disturbance. However, *Pristimantis* seen in our assessment had similar richness and abundance in  
372 both forest and agriculture and all but one species (*P. achatinus*) was very rare, suggesting that  
373 *Pristimantis* has little value as an indicator assemblage for assessing the disturbance in  
374 premontane or humid tropical forest in the Mache Chindul Reserve. On the other hand, the sheer  
375 abundance of *achcatinus* across a wide range of habitat types suggests that it would be useful for  
376 ecological studies, for example assessing how rates of Chytrid fungus infection vary in relation  
377 to habitat quality.

378 Without the inclusion of a mark-recapture effort, there is a potential for pseudo  
379 replication on encounter efforts and abundance estimates. We ensured that there was at least a  
380 10-day period between sampling bouts at each site to mitigate double counts of individuals as  
381 has been suggested by previous studies (Petitot, Manceau et al. 2014) but recognize pseudo-  
382 replication could be a potential confounding factor (Ramage, Sheil et al. 2013) and for this  
383 reason do not present results of abundance-based diversity indicators. With these caveats in  
384 consideration, our results suggest that agricultural and forested areas host similar abundances of  
385 herpetofauna, with *P. achcatinus* being present in nearly all sites. The findings that abundance is  
386 significantly affected by understory density and slope (PC2) suggests that agricultural areas that  
387 maintain an intermediate vegetation structure can create opportunities for a high number of  
388 amphibians despite habitat conversion. Additionally, it is possible that both increased slope made  
389 it more difficult for researchers to navigate, potentially decreasing surveyor proficiency.

390 Taken together, our results suggest that amphibian richness, community composition, and  
391 abundance in our project area may all be influenced by habitat structure, as has been previously  
392 suggested (Gallmetzer and Schulze 2015). Leaf litter, canopy openness, and understory density  
393 all had significant or near-significant relationships with at least one of these indices of diversity,  
394 suggesting that habitat verticality and/or complexity is a key variable for understanding  
395 herpetofauna communities in our area. These findings also suggest that maintaining at least  
396 some canopy and understory vegetation in agriculture could help reduce the impact of  
397 conversion to agriculture on species diversity. Given the ongoing spread of agriculture in our  
398 project area, a better understanding of how different types of agricultural land management  
399 practices influence richness is a clear priority for future research in this area. Similarly,  
400 effectively sharing these results with local communities with the help of local NGOs like FCAT

401 ([www.fcat-ec.org](http://www.fcat-ec.org)) will help to support grassroots conservation activities by farmers in this  
402 priority area.

403

404

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412 Environment.

413

#### 414 LITERATURE CITED

415 Akani, G. C., et al. (2004). "Amphibians recorded in forest swamp areas of the River Niger Delta  
416 (southeastern Nigeria), and the effects of habitat alteration from oil industry development on  
417 species richness and diversity." *Applied Herpetology* 2: 1-22.

418

419 Almond, R. E., et al. (2020). *Living Planet Report 2020-Bending the curve of biodiversity loss*,  
420 World Wildlife Fund.

421

422 Blaustein, A. R., et al. (2010). "Direct and indirect effects of climate change on amphibian  
423 populations." *Diversity* 2(2): 281-313.

424

425 Blowes, S. A., et al. (2019). "The geography of biodiversity change in marine and terrestrial  
426 assemblages." *Science* 366(6463): 339-345.

427

428 Charlat, S., et al. (2000). "Survey of *Alouatta palliata* at the Bilsa biological reserve north-west  
429 Ecuador." *Neotropical Primates* 8: 40-44.

430

431 Clark, J. L., et al. (2006). "Floristic checklist of the Mache-Chindul mountains of Northwestern  
432 Ecuador." *Contributions from the United States National Herbarium*: 1-180.

433

434 Cook, R. N., et al. (2020). "Environmental correlates of richness, community composition, and  
435 functional traits of terrestrial birds and mammals in a fragmented tropical landscape." *Landscape  
436 Ecology* 35(12): 2825-2841.

437

438 Crump, M. L. (2009). "Amphibian diversity and life history." *Amphibian ecology and  
439 conservation. A handbook of techniques*: 3-20.

440

441 DeMaynadier, P. G. and M. L. Hunter Jr (1999). "Forest canopy closure and juvenile emigration  
442 by pool-breeding amphibians in Maine." *The Journal of Wildlife Management*: 441-450.

443

444 Dodson, C. H. and A. H. Gentry (1991). "Biological extinction in western Ecuador." *Annals of  
445 the Missouri Botanical Garden*: 273-295.

446

447 Doherty, T. S., et al. (2020). "Reptile responses to anthropogenic habitat modification: A global  
448 meta-analysis." *Global Ecology and Biogeography* 29(7): 1265-1279.

449

450 Duellman, W. E. (1999). *Patterns of distribution of amphibians: a global perspective*, JHU Press.

451

452 Dufrêne, M. and P. Legendre (1997). "Species assemblages and indicator species: the need for a  
453 flexible asymmetrical approach." *Ecological monographs* 67(3): 345-366.

454

455 Fauth, J. E., et al. (1989). "Elevational patterns of species richness, evenness, and abundance of  
456 the Costa Rican leaf-litter herpetofauna." *Biotropica*: 178-185.

457

458 Fund, W. W. (1995). A conservation assessment of the terrestrial ecoregions of Latin America  
459 and the Caribbean, The World Bank.

460

461 Gallmetzer, N. and C. H. Schulze (2015). "Impact of oil palm agriculture on understory  
462 amphibians and reptiles: A Mesoamerican perspective." *Global Ecology and Conservation* 4: 95-  
463 109.

464

465 Hamer, A. J. and M. J. McDonnell (2008). "Amphibian ecology and conservation in the  
466 urbanising world: a review." *Biological conservation* 141(10): 2432-2449.

467

468 Heyer, R., et al. (2014). *Measuring and monitoring biological diversity: standard methods for*  
469 *amphibians*, Smithsonian Institution.

470

471 Holdridge, L. (1967). "Life Zone Ecology (Tropical Science Center, San Jose, Costa Rica,  
472 1967)." See (37-40) for more complex biome models.

473

474 IUCN (2022). *The IUCN Red List of Threatened Species. Version 2022-1.*, IUCN.

475

476 Jongsma, G. (2014). "Amphibian diversity and species composition in relation to habitat type  
477 and alteration in the Mache–Chindul Reserve, Northwest Ecuador." *Herpetologica* 70.1: 34-46.

478

479 Jung, R. E. (1996). *The potential influence of environmental pollution on amphibian*  
480 *development and decline*, The University of Wisconsin-Madison.

481

482 Khatiwada, J. R., et al. (2019). "Amphibian community structure along elevation gradients in  
483 eastern Nepal Himalaya." *BMC ecology* 19(1): 1-11.

484

485 Krishnamurthy, S. (2003). "Amphibian assemblages in undisturbed and disturbed areas of  
486 Kudremukh National Park, central Western Ghats, India." *Environmental Conservation* 30(3):  
487 274-282.

488

489 Kudavidanage, E. P., et al. (2012). "Amphibian and butterfly diversity across a tropical land-use  
490 gradient in Sri Lanka; implications for conservation decision making." *Animal Conservation*  
491 15(3): 253-265.

492

493 Lueder, S., et al. (2022). "Functional Traits, Species Diversity and Species Composition of a  
494 Neotropical Palm Community Vary in Relation to Forest Age." *Frontiers in Ecology and*  
495 *Evolution* 10.

496

497 Nations, U. (2020). "Global Forest Resources Assessment 2020: Main report." from  
498 <https://www.fao.org/forest-resources-assessment/2020/en/>.

499

500 Newbold, T., et al. (2015). "Global effects of land use on local terrestrial biodiversity." *Nature*  
501 520(7545): 45-50.

502

503 Orozco, J. "Bioweb Ecuador." from Bioweb.<https://bioweb.bio>.

504

505 Orozco, J. (N.D.). "Museo Qcaz." from <https://bioweb.puce.edu.ec/QCAZ/inicio>.

506

507 Ortega-Andrade, H. M., et al. (2010). "Herpetofauna of the Bilsa Biological Station, province of  
508 Esmeraldas, Ecuador." *Check List* 6(1): 119-154.

509

510 Pearman, P. B. (1997). "Correlates of Amphibian Diversity in an Altered Landscape of  
511 Amazonian Ecuador: Correlaciones de la Diversidad de Anfibios en un Paisaje Alterado de la  
512 Amazonía Ecuatoriana." *Conservation Biology* 11(5): 1211-1225.

513

514 Petitot, M., et al. (2014). "Optimizing occupancy surveys by maximizing detection probability:  
515 application to amphibian monitoring in the Mediterranean region." *Ecology and evolution* 4(18):  
516 3538-3549.

517

518 Pimm, S. L. and P. Raven (2000). "Biodiversity. Extinction by numbers." *Nature* 403(6772):  
519 843-845.

520

521 Ramage, B. S., et al. (2013). "Pseudoreplication in tropical forests and the resulting effects on  
522 biodiversity conservation." *Conserv Biol* 27(2): 364-372.

523

524 Ribeiro Jr, J. W., et al. (2018). "Effects of agriculture and topography on tropical amphibian  
525 species and communities." *Ecological Applications* 28(6): 1554-1564.

526

527 Roll, U., et al. (2017). "The global distribution of tetrapods reveals a need for targeted reptile  
528 conservation." *Nature ecology & evolution* 1(11): 1677-1682.

529

530 Schurbon, J. M. and J. E. Fauth (2003). "Effects of prescribed burning on amphibian diversity in  
531 a southeastern US national forest." *Conservation Biology* 17(5): 1338-1349.

532

533 Sierra, M. (1996). *La deforestación en el noroccidente del Ecuador 1983-1993*, Fundación  
534 Ecuatoriana de Estudios Ecológicos, Quito (Ecuador).

535

536 Sierra, R., et al. (2002). "Assessing biodiversity conservation priorities: ecosystem risk and  
537 representativeness in continental Ecuador." *Landscape and Urban Planning* 59(2): 95-110.

538

539 Steininger, M. K., et al. (2001). "Tropical deforestation in the Bolivian Amazon." *Environmental*  
540 *Conservation* 28(2): 127-134.

541

542 Stephens, J. P., et al. (2013). "Anthropogenic changes to leaf litter input affect the fitness of a  
543 larval amphibian." *Freshwater Biology* 58(8): 1631-1646.

544

545 Team, R. C. (2013). "R: A language and environment for statistical computing."

546

547 Tingley, R., et al. (2016). *Addressing knowledge gaps in reptile conservation*, Elsevier. 204: 1-5.

548

549 Toral, E., et al. (2002). "Frogs and a cloud-forest edge in Ecuador." *Conservation Biology*: 735-  
550 744.

551

552 Urbina-Cardona, J. N., et al. (2006). "Herpetofauna diversity and microenvironment correlates  
553 across a pasture–edge–interior ecotone in tropical rainforest fragments in the Los Tuxtlas  
554 Biosphere Reserve of Veracruz, Mexico." *Biological conservation* 132(1): 61-75.

555

556 van Der Hoek, Y. (2017). "The potential of protected areas to halt deforestation in Ecuador."  
557 *Environmental Conservation* 44(2): 124-130.

558

559 Walter, S. T., et al. (2017). "Landscape-level tree cover predicts species richness of large-bodied  
560 frugivorous birds in forest fragments." *Biotropica* 49(6): 838-847.

561

562 Wiens, J. (1980). "Agricultural runoff and water pollution." *Canadian Water Resources Journal*  
563 5(3): 78-89.

564

565 Zakaria, M., et al. (2005). "Comparison of species composition in three forest types: Towards  
566 using bird as indicator of forest ecosystem health." *Journal of biological sciences* 5(6): 734-737.

567

568 Zedan, H. (2004). 2004 IUCN red list of threatened species: a global species assessment, Iucn.

569

570 Zipkin, E. F., et al. (2020). "Tropical snake diversity collapses after widespread amphibian loss."  
571 *Science* 367(6479): 814-816.



Family	Species	Habitat		Total Encounters	Relative Percentage
		Agriculture	Forest		
<b>Amphibians</b>					
Craugastoridae	<i>Barycholos pulcher</i>	14	9	23	3%
	<i>Craugastor longirostris</i>	1		1	0.1%
	<i>Pristimantis achatinus</i>	365	175	540	69%
	<i>Pristimantis latidiscus</i>		5	5	1%
	<i>Pristimantis subsigillatus</i>	7	4	11	1%
	<i>Pristimantis walkeri</i>		1	1	0.1%
Bufonidae	<i>Rhaebo haematiticus</i>		2	2	0.3%
	<i>Rhinella alata</i>	10	22	32	4%
	<i>Rhinella horribilis</i>	4		4	0.5%
	<i>Rhinella marina</i>	1		1	0.1%
Dendrobatidae	<i>Epipedobates boulengeri</i>	3	19	22	3%
	<i>Hyloxalus awa</i>	3	41	44	6%
	<i>Oophaga sylvatica</i>	5	37	42	5%
Hylidae	<i>Boana pellucens</i>		1	1	0.1%
	<i>Boana picturatus</i>		3	3	0.4%
	<i>Hypsiboas rosenbergi</i>	3	6	9	1%
	<i>Scinax quinquefasciatus</i>	1		1	0.1%
	<i>Smilisca phaeota</i>	4		4	1%
Leptodactylidae	<i>Engystomops pustulatus</i>	2		2	0.3%
	<i>Leptodactylus labrosus</i>	7		5	1%
	<i>Leptodactylus peritoaktites</i>	2		2	0.3%
<b>Reptiles</b>					
Colubridae	<i>Clelia clelia</i>		1	1	0.1%
	<i>Dendrophidion clarkii</i>		1	1	0.1%
	<i>Leptodeira ornata</i>	2		2	0.3%
	<i>Oxybelis brevirostris</i>		1	1	0.1%
	<i>Rhinobothryum bovallii</i>	1		1	0.1%
Dactyloidae	<i>Anolis gracilipes</i>	1	2	3	0.4%
	<i>Anolis lyra</i>	3		3	0.4%
	<i>Anolis parvauritus</i>	1		1	0.1%
Elapidae	<i>Micrurus mipartitus</i>		1	1	0.1%
Gymnophthalmidae	<i>Echinosaura horrida</i>		1	1	0.1%
Hoplocercidae	<i>Enyalioides heterolepis</i>		2	2	0.3%
Phyllodactylidae	<i>Thecadactylus rapicauda</i>	4	1	5	1%
Sphaerodactylidae	<i>Lepidoblepharis buchwaldi</i>		4	4	1%
Viperidae	<i>Bothriechis schlegelii</i>		1	1	0.1%
	<i>Bothrops asper</i>		1	1	0.1%
	<i>Lachesis acrochorda</i>	1		1	0.1%
<b>Grand Total</b>		<b>445</b>	<b>341</b>	<b>784</b>	

572

573 **Table 1:** Amphibian and reptile species recorded during sampling of forested and agricultural

574 habitats in the Mache Chindul Reserve, northwest Ecuador.

575

576

577

<b>Predictor Variable</b>	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>	<b>PC4</b>	<b>PC5</b>	<b>PC6</b>
Elevation	0.41	-0.19	0.73	-0.19	0.43	-0.20
Canopy Openness	0.61	-0.13	-0.22	0.02	0.09	0.74
Canopy Height	-0.35	-0.47	0.49	0.13	-0.48	0.41
Slope	-0.27	-0.51	-0.30	-0.73	0.23	0.03
Leaf litter Depth	-0.52	0.25	0.13	0.19	0.66	0.42
Average Understory Density	0.02	-0.64	-0.25	0.62	0.29	-0.26
Standard Deviation	1.44	1.25	0.92	0.80	0.76	0.53
Propotion of Variance	0.35	0.26	0.14	0.11	0.09	0.05

578

579 **Table 2:** Loadings, standard deviation, and variance outputs for principal component analysis.

580 PC1 and PC2 were chosen as the representative models as together they supplied the greatest

581 proportion of variance, 60%. PC1 was most strongly associated with canopy openness and leaf

582 litter depth and PC2 was most strongly associated with slope and understory density.

583

<b>Species</b>	<b>Type</b>	<b>Indicator value</b>	<b>P value</b>	<b>Frequency</b>
<i>Oophaga sylvatica</i>	Forest	0.631	0.001	14
<i>Hyloxalus awa</i>	Forest	0.417	0.003	9
<i>Epipedobates boulengeri</i>	Forest	0.338	0.006	7
<i>Pristimantis latidiscus</i>	Forest	0.313	0.004	5

584

585 **Table 3:** Indicator species values and significance for four species presented as indicative of a

586 habitat type. All indicator species were indicative of forest habitat with *O.sylvatica* having the

587 strongest preference for forest.

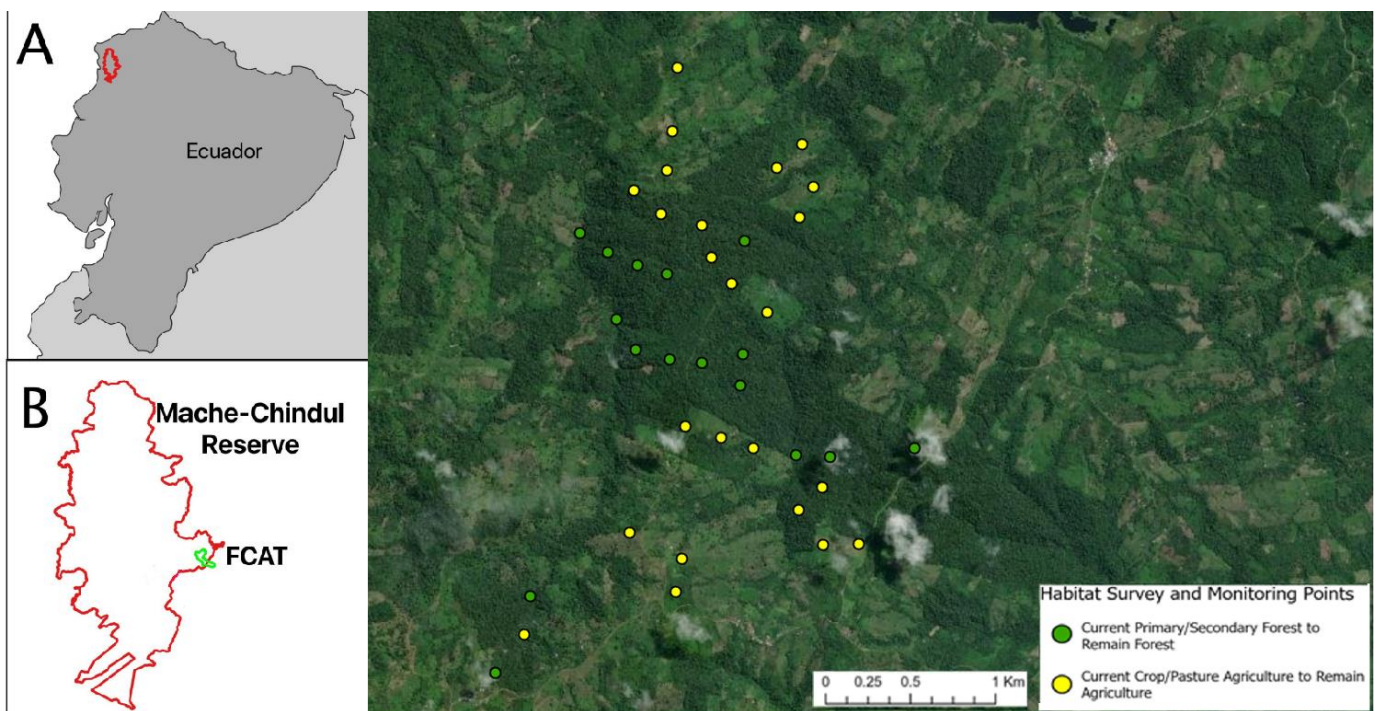
<b>Predictor variable</b>	<b>Mean <math>\pm</math> SD</b>	
	<b>Agriculture</b>	<b>Forest</b>
Canopy height (m)	15.3 $\pm$ 12.4	29.3 $\pm$ 6.9
Canopy openness (%)	67.2 $\pm$ 22.6	18.8 $\pm$ 5.2

588	Elevation (m)	485 ± 50.8	454.3 ± 65.6
	Leaf litter depth (cm)	2.2 ± 1.4	4.2 ± 2.1
589	Slope height (ft)	-5.7 ± 2.4	-6.2 ± 2.8
	Understory density	6.6 ± 3.8	5.3 ± 3.5

590

591 **Supplementary Table 1:** Averages and standard deviations of the six habitat attributes for each

592 habitat type.



593

594

595 **Figure 1:** (A)- Map of Ecuador with the Mache-Chindul Ecological Reserve (REMACH,

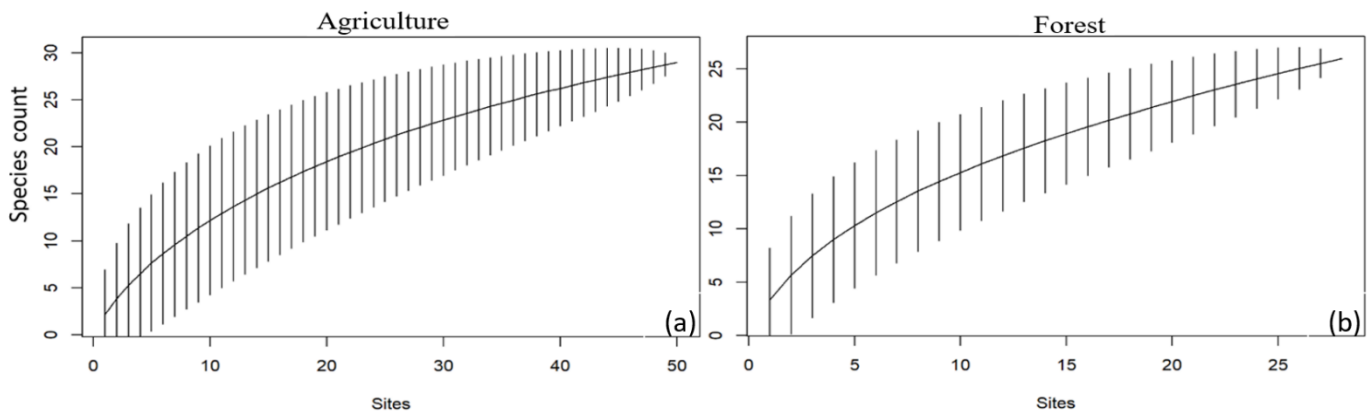
596 119,172 ha) outlined in red. (B)- A close up of REMACH with the Fundación para la

597 Conservación de los Andes Tropicales (FCAT, green) outlined. (C)- Map of the study area.

598 Indicated are points of monitoring for this study. Green points correspond to forest habitat and

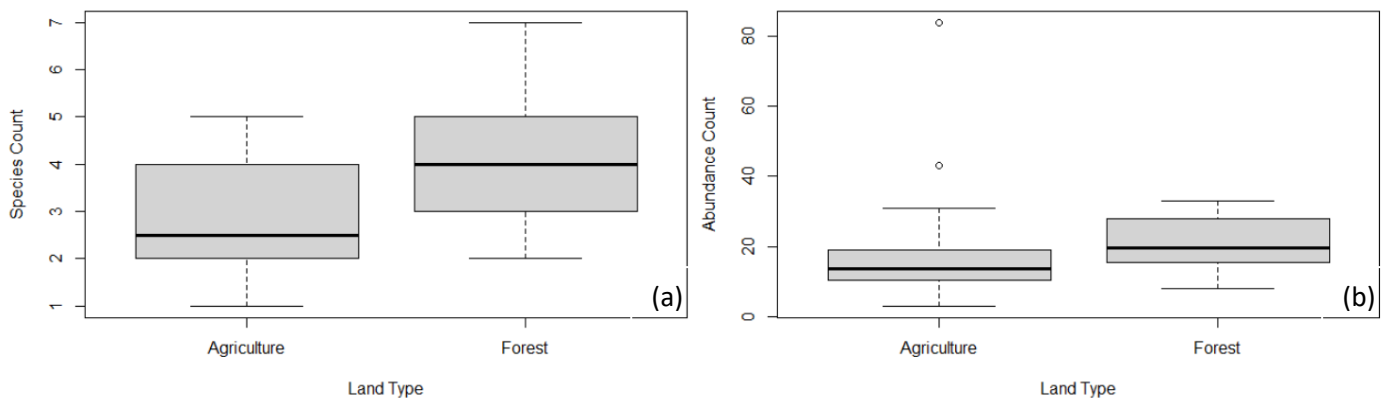
599 yellow points correspond to agricultural habitat.

600



601

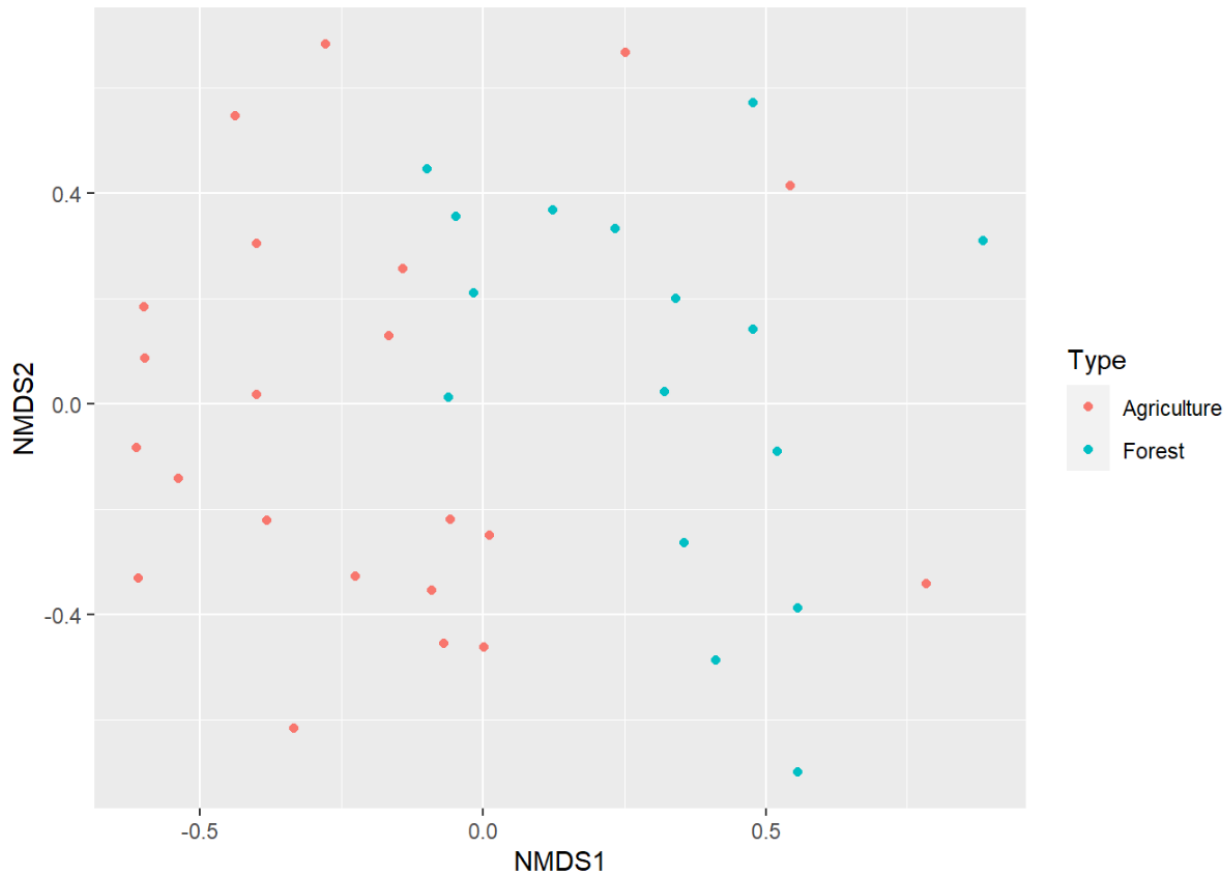
602 **Figure 2:** Amphibian species accumulation curve for land cover types agriculture (a) and forest  
603 (b). With forest sites being 16 and agriculture being 24, we see that our species encounter rate  
604 was below the estimated species richness.



605

606 **Figure 3:** Boxplots of site averages for amphibian species richness (a) and abundance (b) for  
607 forested and agricultural habitat types in the Mache Chindul Reserve, northwest Ecuador. Forest  
608 sites had significantly higher species richness than agriculture sites, but abundance did not differ  
609 statistically.

610



611

612 **Figure 4** Nonmetric multidimensional scaling ordination based on a Bray-Curtis similarity  
613 matrix comparing community composition by habitat type (forest or agriculture). Two distinct  
614 groups were identified as shown by the distribution of forest points (blue) and agriculture points  
615 (red).