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Jamieson Botsch April, 14 2016

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
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Abstract

Impacts of Forest Fragmentation on Species Diversity of Orchid Bees (Apidae: Euglossini) in the Chocó Biodiversity Hotspot of Northwest Ecuador

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Forest fragmentation is occurring throughout the tropics at a high rate. This study addresses how fragmentation impacts orchid bee species diversity, because orchid bees are especially important ecologically and live almost exclusively in forests. Unlike previous studies on the impact of forest fragmentation on orchid bees, this study examined beta diversity, along with alpha diversity. We sampled Male Euglossine bees in 18 forest fragments (ranging in size from 2.5 to 33.5 ha) and one large (2700 ha) continuous forest in the Chocó Biodiversity hotspot of Ecuador during the dry season in 2014. We compared alpha species diversity (as measured by abundance, richness, and evenness) in sites between fragments. We also compared alpha species diversity and beta diversity (as measured by turnover in community similarity over space) between sites in the continuous forest and in fragments. We found no significant result of fragment characteristics (size, shape, or distance to continuous forest) on abundance, richness, or evenness between sites within fragments. Sites within continuous forest did not differ significantly in abundance or richness, but were significantly more even. Species composition between sites in continuous forest appeared to have a faster rate of turnover over space than those in fragments. These results demonstrate the conservation value of continuous forest for orchid bee diversity.

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INTRODUCTION

Despite advances in our understanding of the impact of human activities on bee communities, bee responses to anthropogenic changes have been relatively understudied in the tropics (Mark & Robert, 2009; Potts et al., 2010; Winfree, et al., 2009). The tropics are currently experiencing widespread habitat changes, considered by many to be the most important driver of pollinator declines (Mark & Robert, 2009; Skole & Tucker, 1993; Winfree et al., 2009). Given that bee declines could potentially have serious impacts for plants that rely on their pollination services, the high diversity of flowering plants in these regions, and the fact that nearly all Neotropical angiosperms rely on pollinators for their pollen dispersal, these pollinator declines may have more serious impacts in the tropics than in other parts of the world (Bawa, 1990; Pauw, 2007; Potts et al., 2010; Vamosi et al., 2006; Wilcock & Neiland, 2002). In spite of the concern about bee decline and their importance in the tropics, few studies have evaluated the impact of forest fragmentation on native bees in these regions.

Orchid bees (Apidae: Euglossini) are an especially important group of tropical pollinators because they possess a number of unique characteristics. They pollinate both orchids, through the collection of essential oils, and other plants in the pursuit of nectar (families Apocynaceae, Rubiaceae, some Zingeberaceae, and some Verbenaceae) (Dressler, 1982). They fly long distances (up to 23 km), and pollinate plants with low population density (Bawa, 1990; Dressler, 1968; Janzen, 1971; Tonhasca, et al., 2003; Wikelski et al., 2010). They also pollinate across all vertical vegetation strata (Bawa, 1990). Because they depend on plants at all vegetation strata, they live almost exclusively in forests (R. L. Dressler, 1968; R. Dressler, 1982; Roubik, David

W., Hanson, 2004). Thus, they may be more susceptible to habitat fragmentation than other bees (Brosi, et al., 2007; Brosi, 2009; Dressler, 1982; Roubik, & Hanson, 2004).

Despite a growing body of literature on the impacts of forest fragmentation on orchid bees, there is still a lack of consensus on those impacts. Different characteristics of forest fragments, such as size, degree of isolation, and fragment shape, can impact organisms differently (Saunders & Hobbs, 1991). These fragment characteristics may impact orchid bee communities (Brosi et al., 2007; Brosi, 2009; A. Nemésio & Silveira, 2010; A. Nemésio & Vasconcelos, 2013; Otero & Sandino, 2003; Vasconcelos & Bruna, 2012). While, some studies have found no impact of fragment size on abundance or diversity of euglossines (Storck-tonon, et al., 2013; Tonhasca, et al., 2002), other studies suggest that a relationship exists (Brosi, 2009; A. Nemésio & Silveira, 2010). There are also conflicting results regarding the impact of amount of edge relative to the interior or shape. Brosi (2009) found higher abundance and diversity in fragments with more edge, while Nemesio and Silveira (2010) found the opposite result. Differences in the conclusions of these studies may result from a number of different factors including but not limited to confounding variables, such as elevation, lack of statistical power due to low sample size, different environmental conditions not examined, or the multifaceted aspect of the landscape. The present study is the first that we are aware of that examines the impact of forest fragmentation in Ecuador. The majority of other studies have taken place in Brazil (Aguiar & Gaglianone, 2012; A. Nemésio & Silveira, 2010; A. Nemésio & Vasconcelos, 2013; Storck-tonon et al., 2013; Tonhasca, Blackmer, & Albuquerque, 2002b) and Costa Rica (Brosi, 2009; Suni, Bronstein, & Brosi, 2014). A notable exception is Otero and Sandino (2003), which took place in the Chocó biodiversity hotspot in Colombia.

Although much work has been done to understand how forest fragmentation impacts species diversity, few studies have examined fragmentation's impact on β-diversity (Briggs, Perfecto, & Brosi, 2013; Brosi et al., 2007). Most of the previous studies focused on species diversity within a site (α diversity). β-diversity refers to the change in community composition over space and time (Anderson et al., 2011; Whittaker, 1972). Although originally intended to be used across gradients (Whittaker, 1972), it has been applied more generally to landscape heterogeneity (Anderson et al., 2011). It is more consistent across taxonomic groups (Kessler et al., 2009). In birds, β-diversity has been shown to decrease as a result of land use change (Karp et al., 2012). The studies that have characterized β-diversity of euglossine bees have focused on either small fragments (<20 ha) or large patches of continuous forest (>1,000 ha), and have not examined across fragment characteristics or compared these treatments (Aguiar & Gaglianone, 2012; A. Nemésio & Vasconcelos, 2013).

To address these gaps, we sampled orchid bees in 18 forest fragments and at eight sites within a large (2700 ha), continuous forest in the Chocó Biodiversity Hotspot in northwestern Ecuador. Our analyses focused on two main categories: (1) among fragments and (2) between fragments and continuous forest. We predicted that we would find higher euglossine α-diversity among fragments that were (1) larger, (2) nearer to continuous forest, and (3) had lower perimeter:area ratios. We also expected higher euglossine α and β-diversity in continuous forest than in fragments.

MATERIALS AND METHODS

Study Sites

We sampled male euglossines at 26 total sites within the Chocó Biodiversity Hotspot of northwestern Ecuador from August to December 2014 (Figure 1). Of these sites, 18 were in forest fragments that ranged in size from 2.5 to 33.0 ha and were 115 to 647 m asl (above sea level) along an altitudinal gradient (Table 1). Fragments were 0.3 to 17.4 km from the nearby Bilsa Biological Reserve, a large undisturbed old growth forest of 2700 ha at 500 m asl (hereafter referred to as "Bilsa"; Figure 1,Table 1). We also sampled within Bilsa; two sites were located at the forest edge and six sites were within the forest interior. We sampled all sites surrounding each of the five villages we stayed at in random order. The surrounding landscape is primarily agricultural land that was converted from continuous forest within the last 50 years (Durães, Carrasco, Smith, & Karubian, 2013).

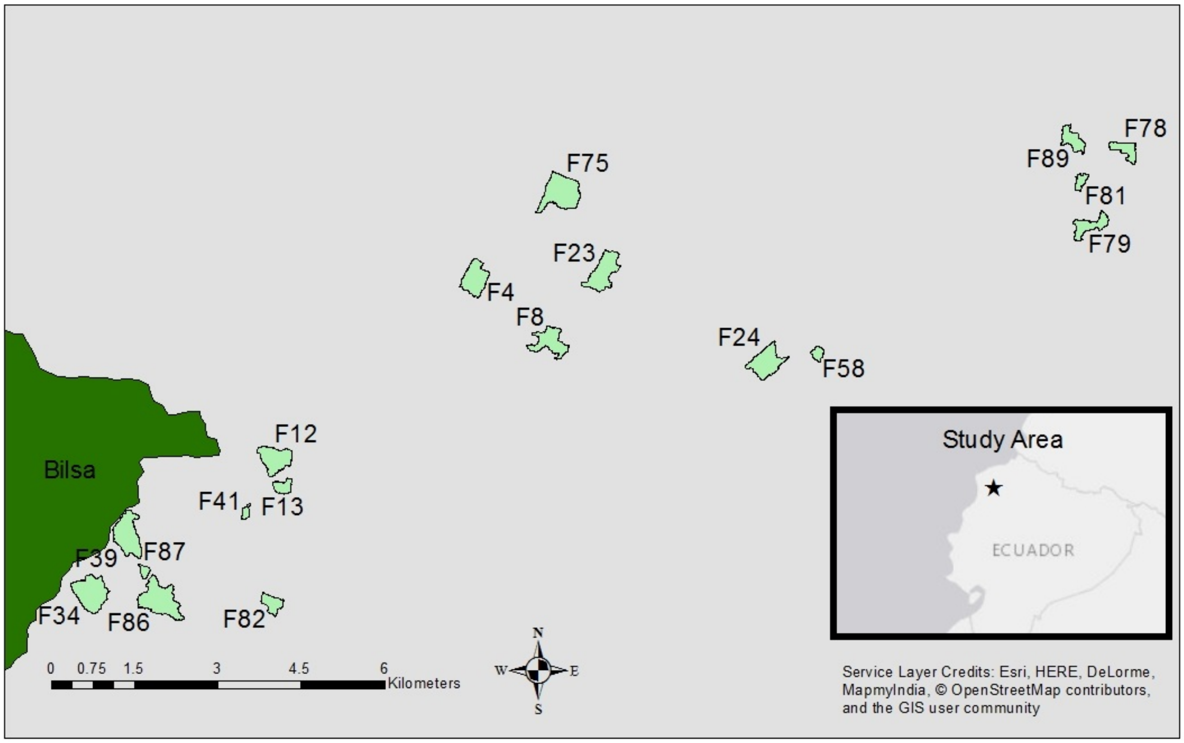

Figure 1: Map of fragments where bees were sampled between August and December 2014. Bilsa, depicted in dark green, is a large continuous forest.

Table 1: Site Attributes. Perimeter^2:Area is a measure of shape. A higher value indicates a greater amount of edge per area.

Site	Area (ha)	Perimeter (m)	Perimeter^2:Area	Distance to Bilsa (m)	Elevation (m asl)
4	21.31	2130.64	213.03	5467.00	376
8	20.51	3036.70	449.61	6243.00	345
12	21.40	2221.49	230.61	1001.20	601
13	6.75	1147.73	195.15	1287.50	598
23	24.85	2758.51	306.21	7576.20	309
24	23.37	2475.62	262.25	9955.40	264
34	29.70	2577.16	223.63	561.60	523
39	27.76	2791.47	280.70	323.20	601
41	2.67	810.62	246.11	1141.00	543
58	4.23	876.23	181.51	10895.30	248
75	33.60	2961.25	260.98	7606.50	394
78	9.92	1859.51	348.57	17164.70	162
79	13.57	2569.78	486.64	16161.20	192
81	4.55	1132.35	281.81	16224.70	120
82	9.22	1469.25	234.13	2836.60	320
86	33.62	3337.61	331.34	1371.20	418
87	3.11	805.93	208.85	834.10	430
89	11.58	1961.13	332.13	16315.30	126
Bilsa	2700.00	NA	NA	0	553

Bee Sampling and Identification

We sampled each site in two different trapping stations separated by approximately 200m. Paired trapping stations were each sampled once within 1 and 11 days of each other (avg. = 5 d, SD = 3.5 d). At each trapping station we set 3 ISCA Ball Traps™ (i.e. McPhail traps) with 1.5 mL each of the baits cineole, eugenol, and methyl salicylate in separate traps. In fragments and at the Bilsa ‘edge’ sites, we established trapping stations 20 m from the forest edge. At each station we spaced the three traps 3 m apart from each other by hanging them 1.5 m off the ground on a line tied between trees. We measured rainfall with a rain gauge at each station during trapping. As our study was conducted during the dry season no site experienced more than 0.5 mL of rain during sampling (max = 0.5 mL, mean = 0.1 mL, SD = 0.12 mL). Traps were open for 24 to 25.1 hours (mean 24.10, SD = 0.189 hours). We pinned captured bees the same day and stored them in a dehumidified box over the duration of the field sampling period to prevent spoiling. In 2015 the bees were imported to the U.S for identification to species following Roubik and Hanson (2004).

Site Characteristics and Environmental Variables

We calculated the area of each fragment by walking the perimeter of the fragment with a handheld GPS unit (Garmin eTrex 30x). In order to confirm our fragments had not been recently deforested, we used the Global Forest Change dataset created by Hansen et al. (2013). Due to cloud cover, this dataset may underestimate the scale of deforestation (Tropek et al., 2014). We used this dataset to calculate the percent forest cover and loss between 2000 and 2013, by creating 30 × 30 m cell rasters. A grid was classified as forest if it had >95% forest cover. We used this method to locate and select fragments to sample and confirmed the calculated values through observation upon arrival. For our study site, the estimates matched up with the conditions on the ground. We recorded elevation at each of the trapping stations with a handheld GPS Unit. To calculate a metric of perimeter:area ratio that does not scale with fragment area, we used $\text{perimeter}^2/\text{area}$, following Brosi (2009).

There was no correlation between area and distance to Bilsa, or our perimeter:area ratio. However, sites had higher perimeter area ratios the further they were from Bilsa (Pearson's Correlation Coefficient = 0.48, $p = 0.04$). Because the data were collected linearly from Bilsa (Table 1; Figure 1), elevation was highly significantly correlated with distance (Pearson's Correlation Coefficient = -0.92, $p < 0.0001$).

Data Analysis

We compared the impact of forest fragment characteristics (area, perimeter:area ratio, and distance from Bilsa) on bee abundance (raw count of bees collected at each site) and diversity (measured through its two components: species richness and evenness) in sites between fragments using Generalized Linear Models (GLM) for observed richness and abundance and linear models for evenness. We used a quasi-Poisson distribution for the GLMs. As a metric of evenness, we calculated Pielou's *J* (Pielou, 1975). We did this by using the diversity function to compute the Shannon diversity index and dividing it by the log of the species number, calculated using the specnumber function in the "vegan" package in R(Oksanen et al., 2015). We compared the evenness between fragment sites through the use of linear models.

We also quantified the impact of fragmentation on the species diversity by comparing species richness, evenness, and spatial turnover between sites within fragments and continuous forest. To account for differential sampling effort in the fragments and continuous forest, we compared species richness through the use of species estimators and individual based rarefaction. We estimated richness by pooling all sites within the two treatments (continuous and fragments) and calculating individual-based rarefaction and the Chao, first order jackknife, and bootstrapped species richness estimators using the specpool function in the "vegan" package. We also used individual based rarefaction using the specaccum function in "vegan." These allowed us to estimate the number of species and 95% confidence intervals and to compare number of species given our differential sampling effort. To quantify impacts of fragmentation on evenness, we calculated Pielou's *J*, using "vegan." We ran linear models to compare evenness using type of site (either fragment or Bilsa) and site location type (e.g. fragment edge) for all data. To quantify β-diversity, we estimated turnover in community composition over space. We did this through creating a dissimilarity matrix using the Morisita-Horn index (Horn, 1966), and running a mantel test using the pairwise site distances in our sites within continuous forest, fragmented forest, and across the entire study area in “vegan”. We used 9,999 permutations to create the null models that the data were compared to. This allowed us to see spatial turnover. To compare rates of turnover over distance between sites, we ran two separate mantel tests one for fragments, and the other for Bilsa sites. We also ran a permutational multivariate ANOVA (Adonis test in “vegan”) to compare the differences in community composition between sites within continuous forest and fragments. All other analyses were calculated using the statistical software program R (R Core Team, 2015).

RESULTS

Overview

We caught 2,305 orchid bees comprising 24 species in the genera *Euglossa*, *Eufriesea*, and *Eulaema* (Table 2). The species accumulation curve suggests that we likely sampled the majority of species in the area that were attracted to these baits (Figure 3). There was high variability between paired trapping stations of the same site. We found that abundance and evenness were significantly correlated between trapping stations of the same site (Pearson’s correlation coefficient = 0.57, p = 0.0027; Pearson’s correlation coefficient = 0.49, p = 0.017 respectively), but richness was not (Pearson’s correlation coefficient = 0.22, p = 0.28). The majority of the bees collected were collected in cineole-baited traps and the most common bee was *Euglossa tridentata*, which comprised around two-thirds (66.45%) of our total sample (Table 2; Figure 2). *Eg. tridentata* the most common bee species captured in fragments (comprising 77.79% of the bees collected in fragments), but was not as common as *Eg. sp. A* (comprising 41.07% of the bees collected in continuous forest) in continuous forest regardless, of the location (Table 3). *Eg. tridentata* made up only 25.99% of the bees captured in continuous forest (Table 3; Figure 9).

Comparing Sites Among Fragments

Fragment characteristics (area, perimeter:area ratio, and distance to continuous forest) poorly predicted the α-diversity of sites in fragments. Distance to continuous forest was associated with a marginally significant increase in evenness ($p = 0.063$; Table 4), but not with abundance or species richness. Neither fragment size, nor perimeter:area ratio had any detectable relationship with abundance, richness or evenness.

Comparing Sites within Fragments and Continuous Forest

We found no significant difference between euglossine abundance (Table 4) or species richness in continuous forest and fragments using either individual based rarefaction or species richness estimators (Figure 4; Figure 5). Sites within continuous forest were significantly more even euglossine communities (as measured by Pielou's *J*) than sites within fragments ($p = 0.00085$; Figure 9; Table 4). When we included location within the continuous forest (i.e., edge vs. interior) into the model, however, this relationship was no longer significant (Table 4). Euglossine community composition differed significantly between continuous and fragmented sites ($R^2 = 0.90$, $p = 0.0001$). This relationship remained significant when location was included into the model ($R^2 = 0.92$, $p = 0.0001$).

β-Diversity

Euglossine community similarity was significantly correlated with distance for fragmented sites (Mantel R = 0.24, p = 0.022; Figure 6), but not for sites in continuous forest. Rates of spatial turnover, however, showed a non-significant trend toward being higher in continuous forest relative to fragments (Figure 6; Figure 8). Distance was not a better predictor of species similarity in continuous, fragments, or both combined (Figure 6).

Table 2: Species (in alphabetical order) captures as a function of essential oil baits. Proportion indicates percentage of all bees collected across all sites. Number of sites in fragments or continuous indicates how many sites within each site type the species was found.

Species	Cineole	Eugenol	Methyl salicylate	Proportion (%)	Number of Sites in Fragments	Number of Sites in Continuous Forest
Eufriesea sp.	3	1	0	0.17	1	2
Euglossa allosticta	32	0	0	1.39	8	4
Euglossa asarophora	14	0	0	0.61	0	4
Euglossa azureoviridis	24	0	0	1.04	9	0
Euglossa cyanura	2	0	0	0.09	1	0
Euglossa deceptrix	4	0	0	0.17	3	0
Euglossa despecta	8	0	0	0.35	5	0
Euglossa dissimula	12	0	0	0.52	5	3
Euglossa dodsoni	65	0	0	2.82	17	5
Euglossa dressleri	18	0	0	0.78	5	4
Euglossa flammea	28	3	0	1.34	3	6
Euglossa gorgonensis	153	0	0	6.64	13	8
Euglossa hansoni	15	0	0	0.65	6	4
Euglossa heterosticta	27	0	0	1.17	9	2
Euglossa ignita	63	0	14	3.34	10	6
Euglossa maculilabris	16	0	1	0.74	5	5
Euglossa mixta	4	0	7	0.48	8	0
Euglossa sp. A	226	0	0	9.80	3	8
Euglossa sp. B	9	1	0	0.43	8	1
Euglossa sp. C	8	0	0	0.35	3	1
Euglossa sp. D	2	0	0	0.09	1	0
Euglossa tridentata	1523	9	0	66.46	18	8
Eulaema speciosa	1	0	0	0.04	0	1
Eulaema sp.	12	0	0	0.52	2	1

Table 3: Species (in alphabetical order) captures as a function of site type. Proportion indicates percentage of all bees collected from that particular site type.

Species	**Continuous Interior**		**Continuous edge**		**Fragment edge**	
	Count	Proportion	Count	Proportion	Count	Proportion
Eufriesea sp.	2	0.52	0	0.00	2	0.11
Euglossa allosticta	6	1.55	1	0.85	25	1.39
Euglossa asarophora	14	3.62	0	0.00	0	0.00
Euglossa azureoviridis	0	0.00	0	0.00	24	1.33
Euglossa cyanura	0	0.00	0	0.00	2	0.11
Euglossa deceptrix	0	0.00	0	0.00	4	0.22
Euglossa despecta	0	0.00	0	0.00	8	0.44
Euglossa dissimula	3	0.78	0	0.00	9	0.50
Euglossa dodsoni	6	1.55	0	0.00	59	3.28
Euglossa dressleri	9	2.33	1	0.85	8	0.44
Euglossa flammea	22	5.68	2	1.71	7	0.39
Euglossa gorgonensis	44	11.37	6	5.13	103	5.72
Euglossa hansoni	9	2.33	0	0.00	6	0.33
Euglossa heterosticta	4	1.03	0	0.00	23	1.28
Euglossa ignita	19	4.91	2	1.71	56	3.11
Euglossa maculilabris	9	2.33	1	0.85	7	0.39
Euglossa mixta	0	0.00	0	0.00	11	0.61
Euglossa sp. A	151	39.02	56	47.86	19	1.05
Euglossa sp. B	1	0.26	0	0.00	9	0.50
Euglossa sp. C	1	0.26	0	0.00	7	0.39
Euglossa sp. D	0	0.00	0	0.00	2	0.11
Euglossa tridentata	84	21.71	47	40.17	1401	77.79
Eulaema speciosa	0	0.00	1	0.85	0	0.00
Eulaema sp.	3	0.78	0	0.00	9	0.50
Total	387	100	117	100	1801	100

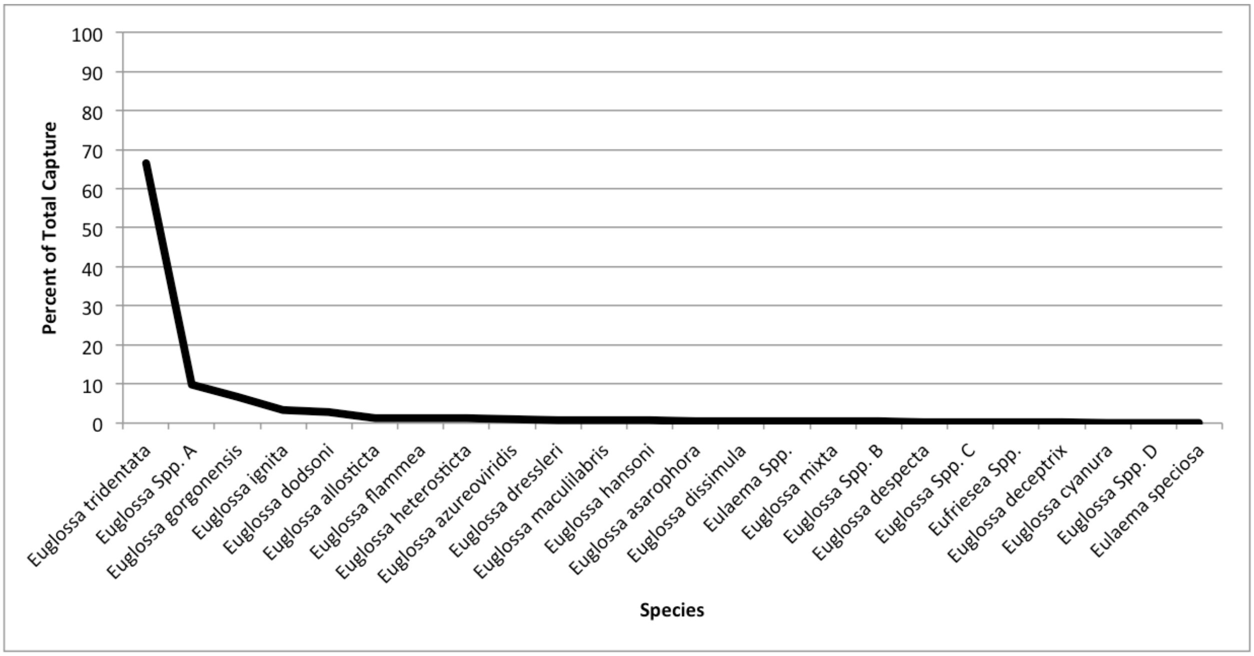

Figure 2: Rank abundance curve of species captured from all sites

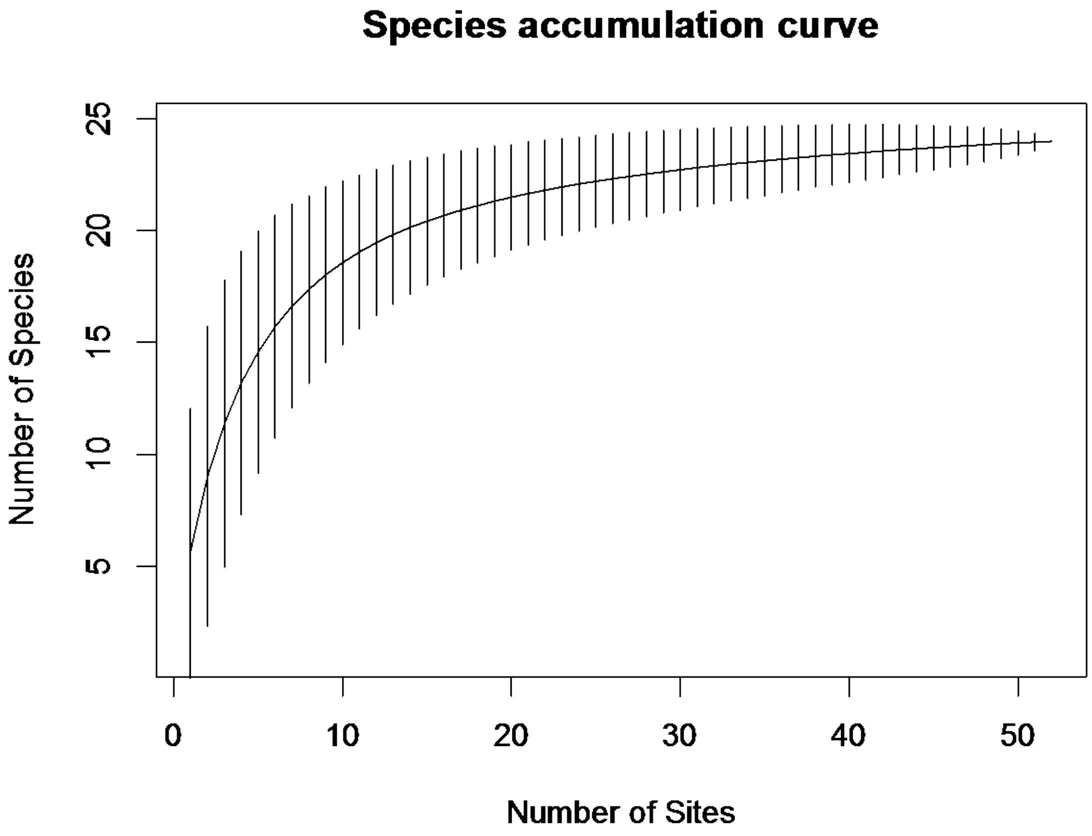

Figure 3: Species Accumulation Curve, based on randomizing sample days 9999 times without replacement with 95% confidence intervals.

Table 4: The impact of explanatory variables as described by GLMs for abundance, richness, and type. Linear models were used for evenness. a indicates that the analyses were done for fragments only with the values for trapping stations pooled per fragment. b indicates that both Bilsa edge and interior sites were both categorized as continuous, regardless of location within Bilsa.

	Abundance		**Richness**		**Evenness**	
Variable	**Coefficient**	**P**	**Coefficient**	**P**	**Coefficient**	**P**
Area[a]	0.025	0.22	-0.0028	0.82	-0.0041	0.22
Distance to Bilsa[a]	-0.000075	0.11	-0.000030	0.24	0.000013	0.063
Perimeter: area[a]	-0.0025	0.39	0.0020	0.25	-0.00042	0.37
Continuous[b]→ fragmented	0.46	0.29	-0.14	0.45	-0.23	0.00085
Continuous edge→ continuous interior	0.10	0.92	0.43	0.27	0.07	0.53
Continuous edge→ fragmented edge	0.54	0.52	0.20	0.58	-0.17	0.12

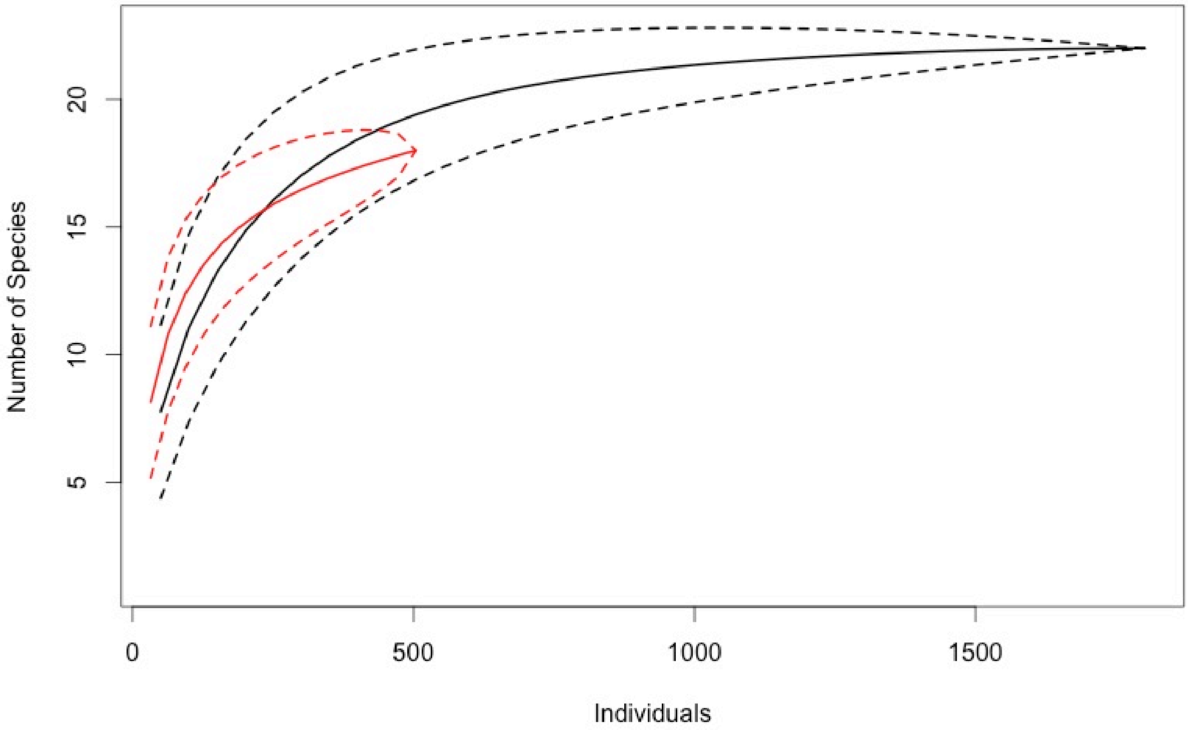

Figure 4: Individual based rarefaction for sites within continuous forest and fragments. This estimates number of new species for each individual collected. Red indicates continuous, black indicates fragmented. Dashed lines are 95% confidence intervals.

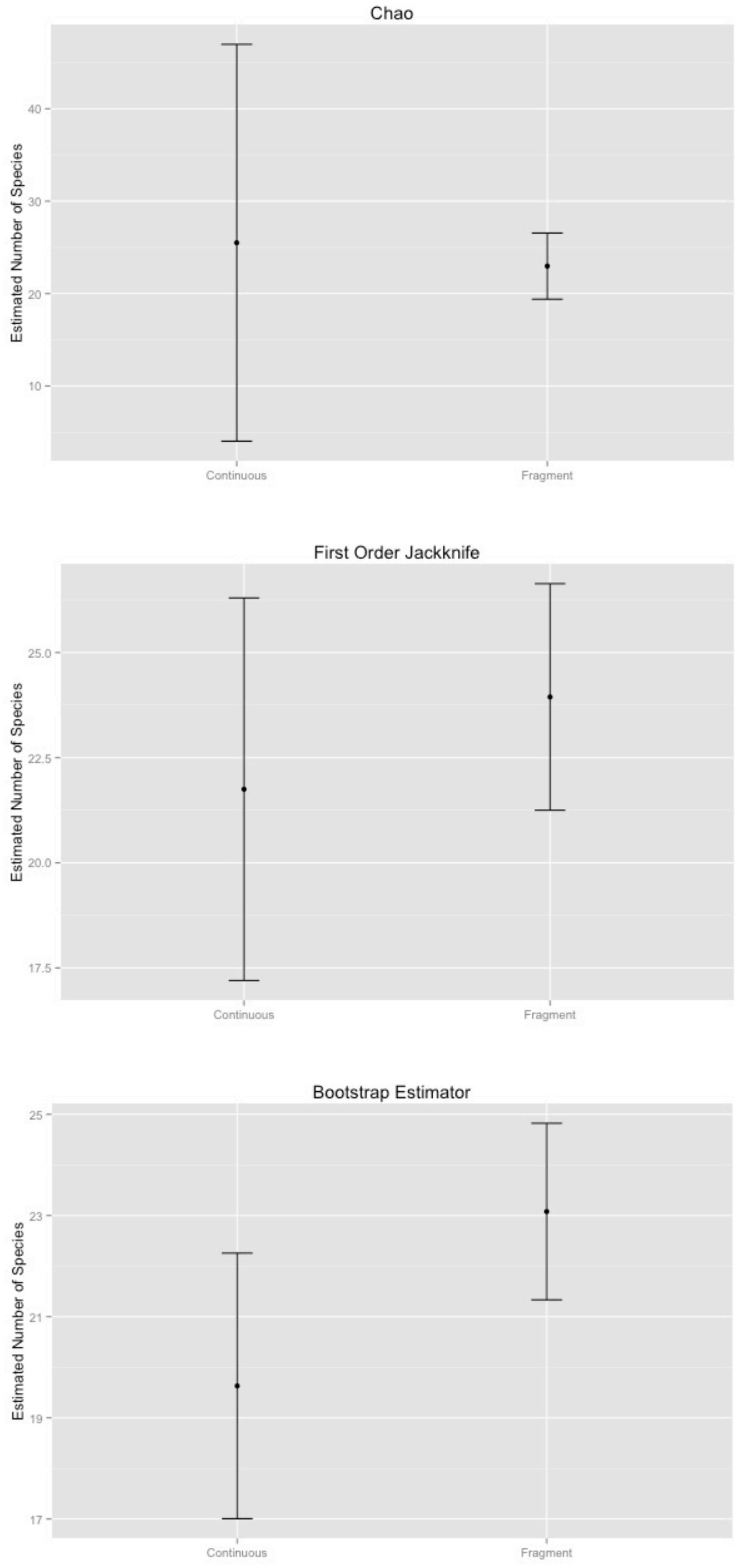

Figure 5: Species Richness Estimators.

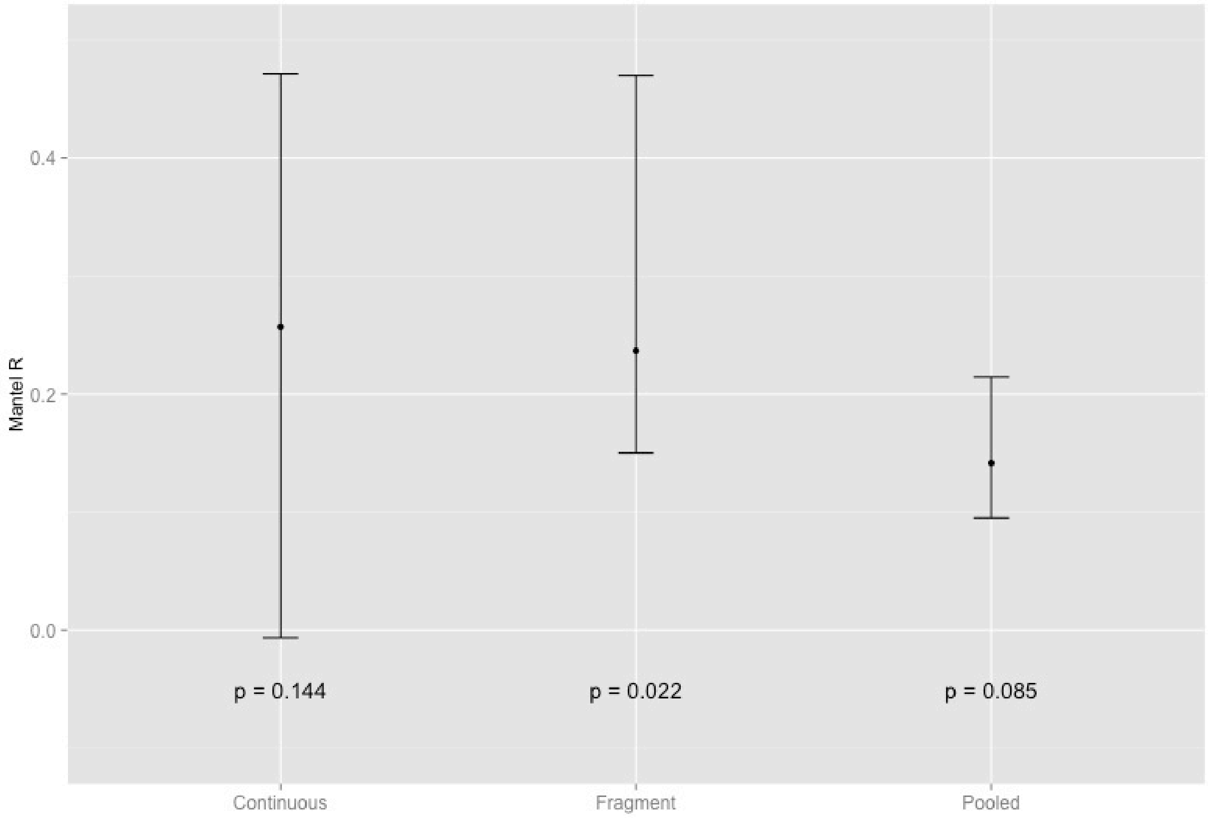

Figure 6: 95% confidence intervals for the Mantel R value comparing pairwise distance and species dissimilarity in community composition in continuous forest, in the fragments only and pooled across both treatments. P values are labeled underneath each.

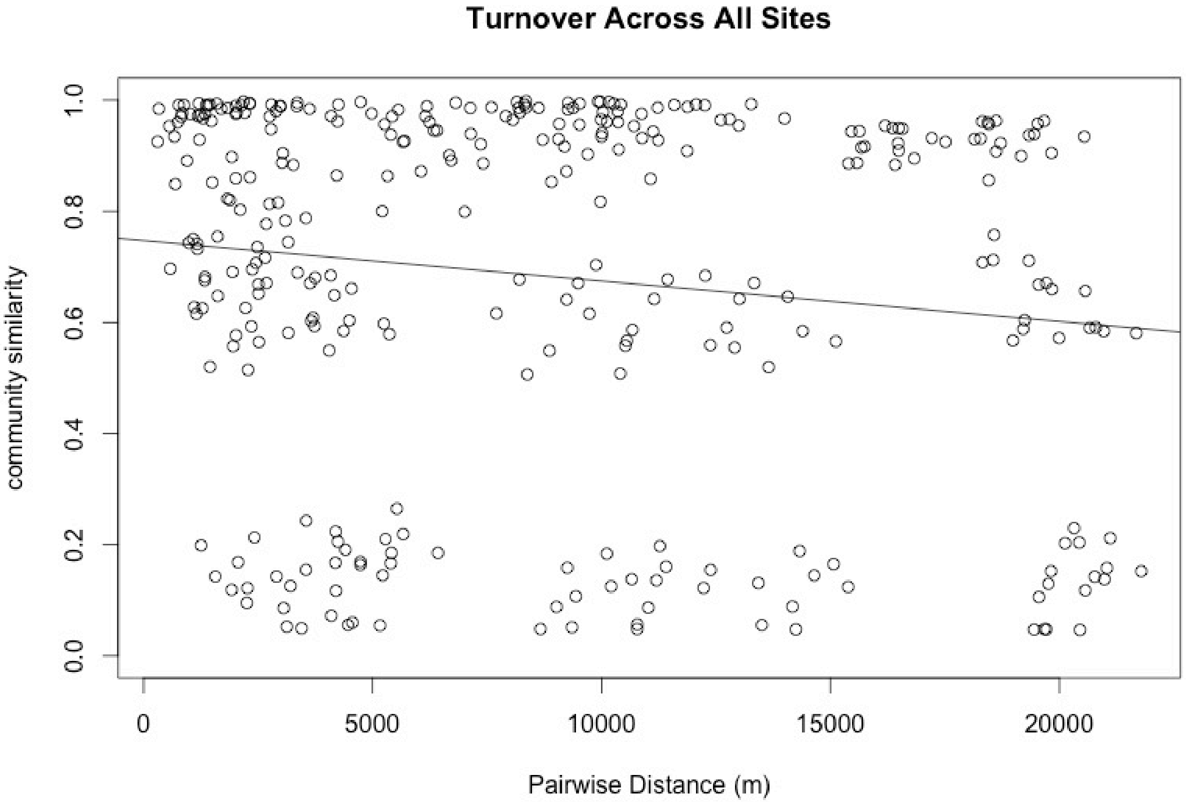

Figure 7: Community similarity between each site pair plotted by distance.

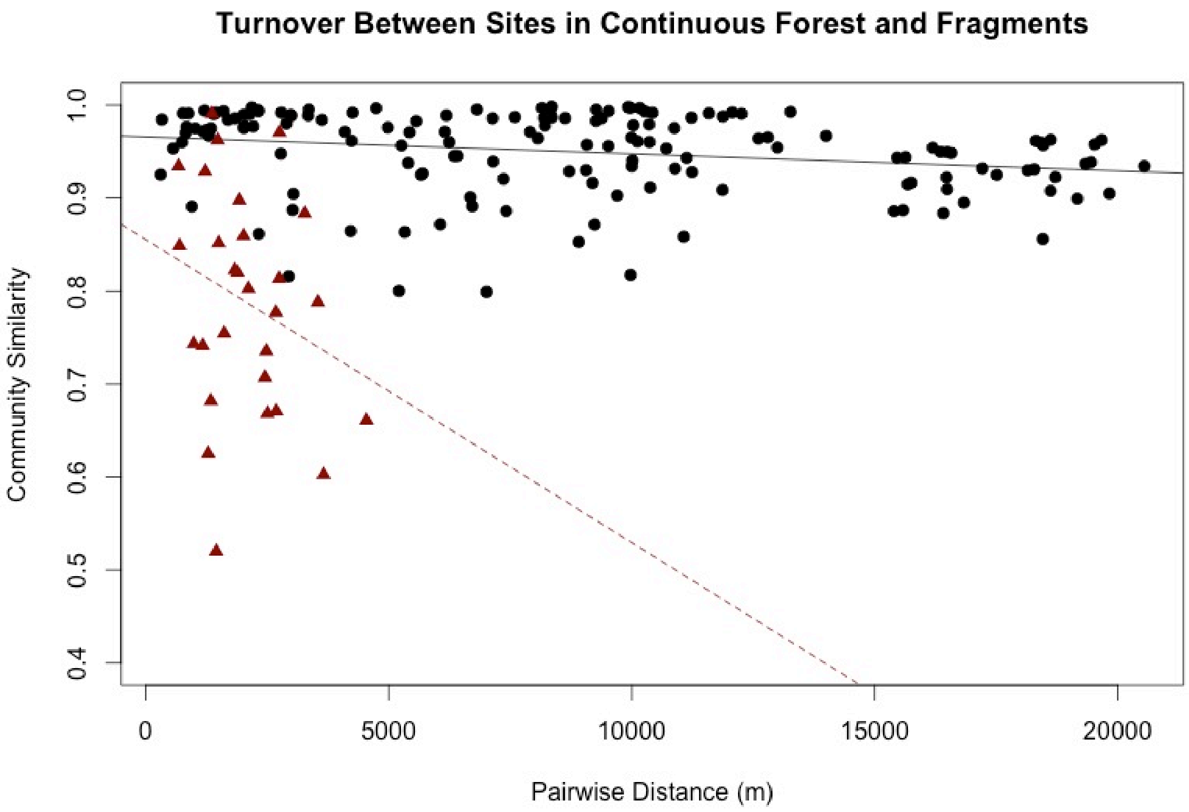

Figure 8: Community similarity between sites in fragments and continuous. Red triangles indicate each pair of sites within continuous forest; black circles indicate each pairwise combination of sites within fragments. The red line indicates the best-fit line for pairwise similarity and distance between sites within the continuous forest. The black line is the best-fit line for pairwise similarity and distance between sites in different fragments. Note there is a change in the Y axis scale from Figure 7.

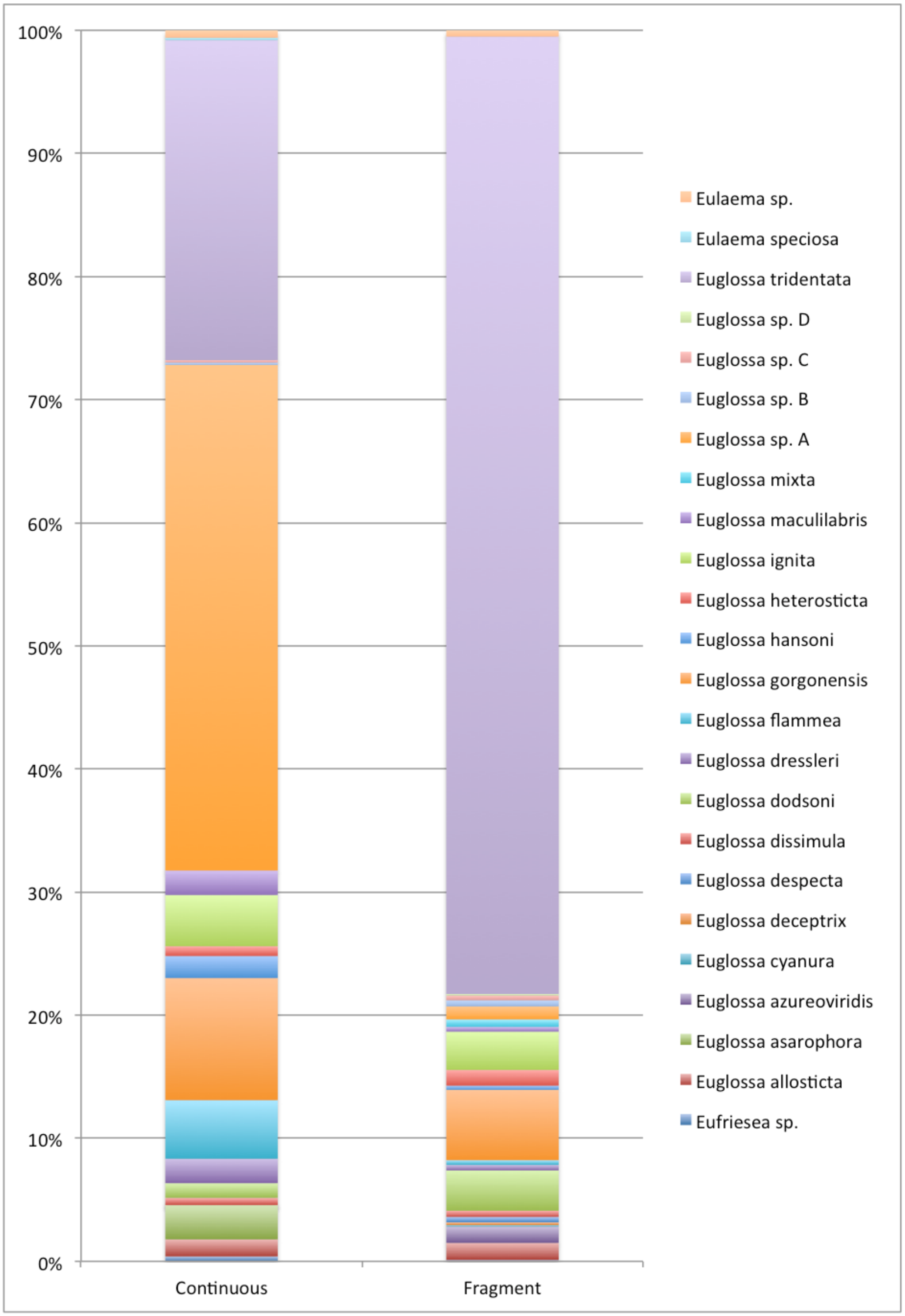

Figure 9: Community composition of bees captured in Bilsa and the fragments

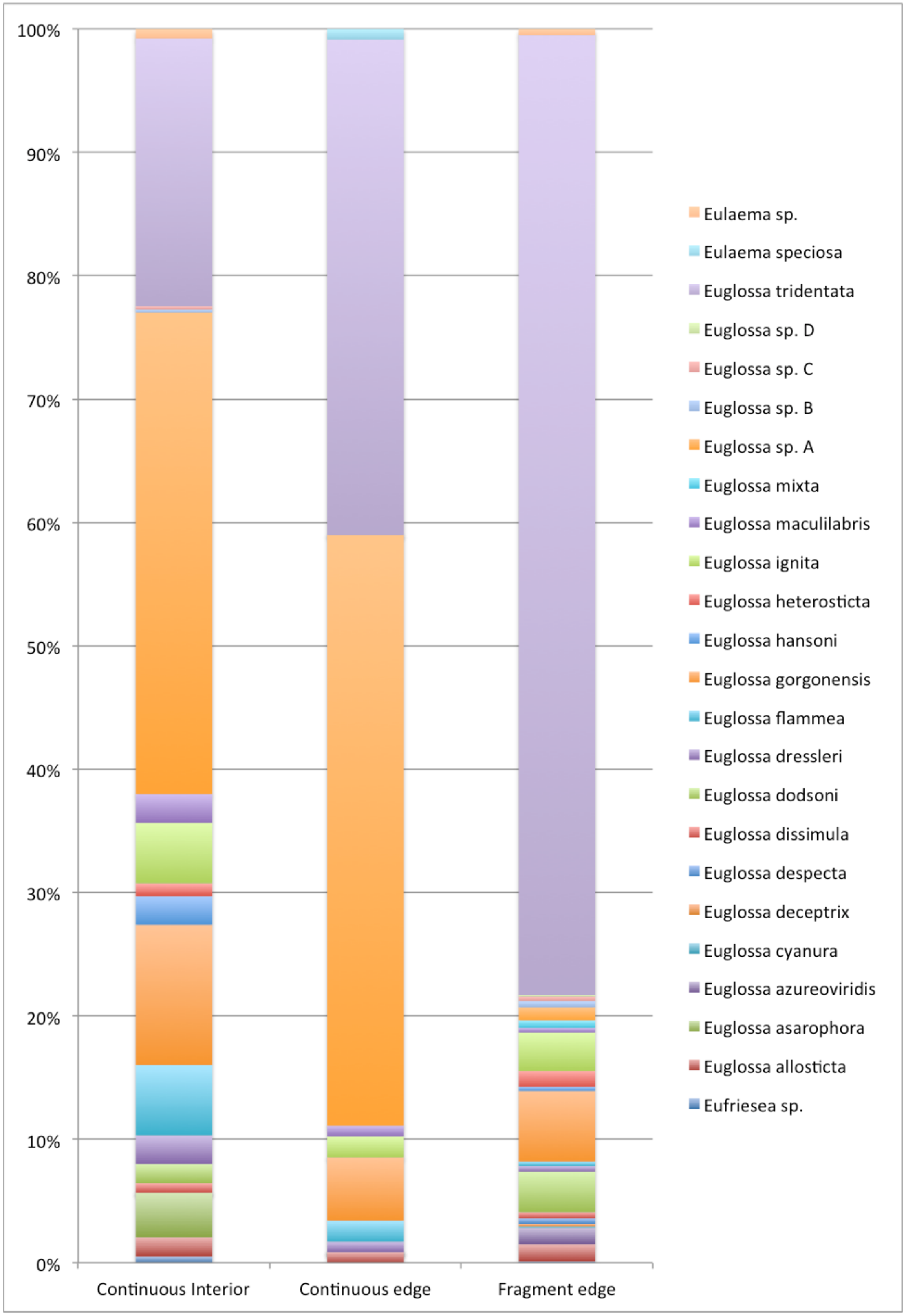

Figure 10: Community composition of bees captured in each location within the different types.

DISCUSSION

We found that forest fragmentation impacts orchid bee communities. Although there was no significant change in abundance, richness, or evenness between sites within fragments based on patch-level fragment characteristics, we observed differences between sites in fragments and sites in continuous forest. Abundance and richness did not significantly differ between fragments and continuous forest, but sites in continuous forests were significantly more even than sites in fragments. Sites in continuous forests also had different community composition (Adonis test, $p = 0.0001$; Table 3; Figure 9). When examining β-diversity, formulated as the change in community composition over geographic space, sites within continuous forest showed a non-significant trend toward having greater turnover rates in space relative to fragmented sites (Figure 8). For continuous forest sites, this pattern was also not statistically significant in terms of the relationship between similarity and geographic distance *per se*, though there was a significant pattern for fragmented sites (likely due to greater statistical power; $p = 0.022$, Figure 6). Different species of orchid bees responded differently to landscape characteristics. *Euglossa tridentata* in particular were highly abundant in fragmented sites (Table 3), while other species (including, e.g. *Euglossa asarophora* and *Eulaema speciosa*) were found exclusively in continuous forest (Table 2; Table 3).

Unlike previous work (Brosi, 2009; A. Nemésio & Silveira, 2010), we did not find sufficient evidence to conclude that patch-level fragment characteristics (size, perimeter:area ratio, and distance to continuous forest) alter α diversity (as measured by abundance, richness, and evenness). Both Brosi (2009) and Nemésio and Silveira (2010) found significant increase in abundance and richness as a result of fragment size. Both studies also found significant differences in abundance and richness as a result of fragment perimeter:area ratio. The results we report here could be due to low power, less variation in fragment size (Brosi's fragments ranged in size between 0.24 ha and 296 ha and Nemesio and Silveira's fragments were between 1 and 354 ha) or location of sites within fragments. Because our sites were near the edge, we likely encountered more bees that utilize edge habitat than may be observed in the interior. Like other fragmentation studies (Brosi, 2009), we found no significant impact of isolation on species abundance and richness. This may be due to the ability for long distance flight (Janzen, 1971; Wikelski et al., 2010), especially given that orchid bees seem to be able to move across deforested land (Suni & Brosi, 2012; Tonhasca, et al. 2003b). Another possible explanation for these results is that other habitat factors, such as vegetation structure or surrounding land use, play a larger role in defining euglossine communities. Some studies have found that the land use surrounding fragments may be more important than the characteristics of the fragments themselves (Briggs et al., 2013; Storck-tonon et al., 2013).

We found that evenness (Table 4) and community composition significantly differed between sites in fragments and sites in continuous forest. These results are consistent with the literature. Although no other study has compared fragments to continuous forest, many studies have found changes in community composition of orchid bees resulting from increased disturbance (Brosi, 2009; Otero & Sandino, 2003). Abundance and richness did not significantly differ between sites in fragments and sites in continuous forest. This could be driven by species that are especially tolerant of fragmentation. Some species of euglossine bees appear to be more successful in fragments, while others are more successful in continuous forest (Table 3; Figure

9). This is consistent with the findings of Powell and Powell (1987), Aguiar (2012), Suni, Bronstein, and Brosi (2014), and Nemésio (2013).

There was a statistically significant relationship between community composition and geographic distance in the fragmented sites (Figure 6). This was not the case for our sites within continuous forests and across both types of sites. This is likely the result of low power for sites exclusively in continuous forest, because there were less than half as many sites (8) in the continuous forest as were in fragments (18), and a result of the differences in community composition between sites within the continuous forest and fragments. These results would be strengthened with further distances between sites. Sites in continuous forest appear to have a higher rate of turnover than sites in fragments (Figure 8). This could be the result of more varied habitat within continuous forest or climatic variation resulting from altitudinal or structural changes within the continuous forest compared to those habitats in fragments. Nemésio and Vasconcelos (2013) found climatic changes had a bigger impact on species similarity than geographic distance, but had a much larger spatial scale, with some sites nearly 600km apart.

As with all field studies there are a number of limitations. First, the strong correlation between distance to Bilsa and elevation prohibits us from being able to determine whether the effect we are seeing is caused by distance from continuous forest or elevation. Environmental gradients such as elevation are considered important for orchid bee communities (Nemésio & Vasconcelos, 2013) and Brosi (2009) found that elevation better explained community similarity than distance. Another potential confounding variable is time. Because of the distribution of our sites, the sites further from Bilsa were all sampled within a period of a month and sites nearer to

Bilsa were sampled in another month. As euglossine communities are known to shift with season, this sampling scheme could have potentially biased the results, particularly in terms of community composition. By sampling fragments only at edge sites and sampling the continuous forest largely in the interior, the observed differences in community composition between treatments may be driven by edge effects. There were some non-significant differences between the species composition sites in the edge and interior of the continuous forest; however, sites in the edge and interior of forests both have much higher proportions of *Eg. sp. A* than fragments (Table 3; Figure 10). Nemésio and Silveira (2006) found that edge effects on orchid bees can occur up to 50m from the perimeter of a forest, so including sites in the interior of fragments, as well as the edge, would better characterize those euglossine communities. Finally, in our study design we maximized site-level replication, at the expense of sampling each site effectively only once. Using a more in-depth characterization of euglossine communities, as well as a greater number of sites, we would have likely increased our power to detect differences generated by landscape patterns. These design trade-offs are a central issue in landscape ecology field studies (McGarigal & Cushman, 2002).

Despite our species accumulation curve (Figure 3), our results do not provide a complete estimate for Ecuadorian orchid bee diversity. Because our samples were taken during the dry season, we can make no inference about the diversity of orchid bees during the wet season, when there may be the highest orchid bee diversity (Becker, Moure, & Peralta, 1991). We only used three essential oils, and therefore did not sample for bees that are exclusively attracted to other essential oils. Furthermore, even using the same essential oils, our species captured may have differed if we sampled in the wet season, as there is some evidence that essential oil preference changes with the seasons for some species of orchid bee (Abrahamczyk, Gottleuber, & Kessler, 2012). Furthermore the lack of correlation between richness between trapping stations within the same fragments suggest either heterogeneity in the habitat, as was suggested by Armbruster (1993); insufficient sampling; and/or high sample variability (as was noted in Roubik, 2001 and Nemesio and Silveira, 2006).

Future studies should be conducted at a larger spatial and temporal scale. Few studies (Nemésio & Vasconcelos, 2013; Suni et al., 2014; Tonhasca et al., 2002a) have lasted for longer than a single year, and of these, only one (Nemésio & Vasconcelos, 2013) has more than ten sites. Inclusion of more sites will also increase the ability to discern patterns. The largest number of sites used in a forest fragmentation study has been 22 (Brosi, 2009), and many have fewer than ten. The differences found between fragments and continuous forest in our study, suggest that to better understand fragmentation, more studies should include sites within continuous forest as a point of comparison. Our results also demonstrate the value of β-diversity in the study of forest fragmentation. Finally, more studies should be conducted across other tropical regions. Most previous studies have been located in Brazil. For more generalizable conclusions of the impact of fragmentation on orchid bees, more studies should be conducted across differing biogeographic and latitudinal regions throughout the tropics.

It is of vital importance that we continue to study the impacts of human activities on these important pollinators. In the face of widespread habitat loss and land use change, understanding the implications of these changes for pollinators is imperative. This is particularly important in the tropics, where relatively little research has been conducted on bee responses to habitat loss.

Since euglossines are thought to require tropical forest for survival, they are likely more susceptible to these changes. Better studies on forest fragmentation, especially those at larger spatial and temporal scales, with high sample size, and including β-diversity, are necessary for the conservation of euglossines and the plants that depend on them.

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