

Are orchid bees useful indicators of the impacts of human disturbance?

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12 **Abstract**

13 Biodiversity and ecosystem functions are threatened by human disturbance, and tropical
14 forests are one the most vulnerable habitats. Monitoring the impacts of disturbance and
15 the success of conservation projects is crucial, and to do this effectively it is important to
16 identify suitable measures that are sensitive to ecosystem disturbance. Orchid bees
17 (Euglossini) are a specialist group with mutualistic relationships with many plant species
18 and can fly long distances, making them important pollinators of widely dispersed plant
19 species. A loss of specialist pollinators such as these could have severe consequences for
20 the plants that rely on their services. We therefore aimed to answer the following
21 question: are orchid bees useful indicators of the impacts of human disturbance? If so,
22 what measures of orchid bee diversity are most sensitive? And do orchid bees provide any
23 indication of changes in pollination services along a disturbance gradient? Orchid bees
24 were collected from 18 sites across a gradient of disturbance in a tropical forest region in
25 southeast Peru. Alpha diversity across the gradient was compared using Hills numbers.
26 Beta diversity was assessed using community composition, species contributions to beta
27 diversity, beta diversity partitioning and novel measures of redundancy and
28 representativeness. The potential pollination services available at each site were
29 measured using artificial flowers and counts of pollinator visits. Alpha diversity of orchid
30 bees showed low sensitivity to disturbance. Beta diversity measures were more
31 informative, with disturbed sites found to be highly redundant in the ecosystem compared
32 to the less disturbed sites. However, the most sensitive measure across the gradient was
33 abundance - there was a significant decrease in the number of bees caught as disturbance
34 increased, with likely consequences for pollination services. These results suggest that
35 orchid bees may be useful indicators of the impacts of human disturbance, but alpha
36 diversity is a poor metric for this purpose. In order to understand how human disturbance
37 is affecting biodiversity, multiple diversity indices should be considered, and in the case of
38 orchid bees, redundancy and abundance could be useful for detecting sensitive responses
39 to forest disturbance.

40 **Keywords**

41 Biodiversity indices, indicator, Euglossini, disturbance, agriculture, rainforest.

1.1 Introduction

Covering only a small percentage of the world's surface, tropical forest harbours over 50% of terrestrial biodiversity. Yet this cover is decreasing globally, with forest being cut down for timber and conversion to agriculture (FAO, 2016), with severe consequences for biodiversity (Alroy, 2017; Gibson et al., 2011) and ecosystem functions (DeFries et al., 2004). It is therefore of the utmost importance that we have effective tools for detecting changes in biodiversity and ecosystem function in response to anthropogenic disturbance (Feest et al., 2010; Hill et al., 2016; Kremen, 2005; Pimm and Raven, 2000). Indicator groups have been widely used for this purpose (Cleary, 2004; Krug et al., 2017; Schulze et al., 2004), however choosing suitable indicator groups can be challenging (Broszeit et al., 2017; Fleishman and Murphy, 2009; Gao et al., 2015). Ideally they should be efficient to survey, show a predictable, sensitive response to environmental change, correlate well with overall biodiversity responses and play an important role in the ecosystem (Brown, 1997; Hilty and Merenlender, 2000).

Bees are the most important group of pollinators (Bawa, 1990) but have been declining globally (Allen-Wardell et al., 1998; Potts et al., 2010), with land conversion and habitat loss the leading causes (Winfree et al., 2009). Orchid bees (Hymenoptera: Apidae: Euglossini) are found throughout the Neotropics and are one of the best studied groups of bees in the region. They exhibit many of the recommended features that could make them suitable indicators of disturbance impacts (Brown 1997; Favila & Halffter 1997; Gardner et al. 2008a; Goodsell, Underwood & Chapman 2009), including being widespread, common, cost-effective, easy to sample using standardised methods (Pearson and Dressler, 1985) and having well developed taxonomic literature (eg. Dressler 1982a; Roubik 2004; Nemésio & Silveira 2007a). They have close associations with plant species and play important ecological roles as pollinators, able to access flowers that are unavailable to many other insects (Dressler, 1982a), due to their long tongues and also the large body size of some species. They can fly long distances, making them valuable long-distance pollinators (Janzen, 1971) for many widely-spaced plant species and many orchids are entirely dependent on orchid bees for their pollination (Dressler, 1982a). Another key feature of a good indicator is that they should display a clear, graded response to environmental change. There is some evidence that orchid bees display graded responses to human disturbance, but the results have been mixed and part of our motivation is to study this in

greater detail. Orchid bees can persist in heavily degraded ecosystems in some cases, including forest fragments (Storck-Tonon et al., 2013) and farmland (Otero and Sandino, 2003; Sandino, 2004), although this may depend on the proximity to intact forest (Briggs et al., 2013). At the same time, there is concern that orchid bees are sensitive to forest loss (Roubik and Hanson, 2004) and some species are thought to be at risk of extinction (Nemesio, 2013), which would have major consequences for the plants they pollinate. The loss of old-growth forest and canopy disruption is likely to result in a loss of orchid bee resources (Hietz et al., 2006; Nöske et al., 2008), such as orchids, other epiphytes and specific tree saps (Dressler, 1982a; Roubik and Hanson, 2004), with probable negative consequences for the orchid bee species that depend on them. Together these features suggest that orchid bees could be an excellent candidate for use as an indicator of faunal and floral responses to tropical forest disturbance.

So far, studies on orchid bee responses to forest disturbance have primarily focused on the effects of forest fragmentation, with mixed patterns identified. The abundance and diversity of euglossine bees have been suggested to decline with decreased forest fragment size (Brosi, 2009; Brosi et al., 2008), but other studies found no effect of fragmentation on abundance or richness (Storck-Tonon et al., 2013). A few studies have compared agricultural land to intact forest but these have mostly been focused on less diverse Central American habitats and generally only compared two or three land uses. Briggs *et al.* (2013) found that polyculture could sustain orchid bee communities similar to forest habitats in composition, but abundance was higher in the polyculture than in either forest or monoculture, although abundance did decline with increasing distance from the forest. Abundance and richness of orchid bees have been found to decrease in some agricultural matrix habitats compared to forests (Aguiar et al., 2015), although others have been found to support similar communities to forest (Rosa et al., 2015) and some found higher capture rates in agricultural areas than in forest (Otero and Sandino, 2003; Sandino, 2004). However, studies of forest disturbance gradients are relatively uncommon, and none have covered the response of orchid bees across a continuous, multi-level gradient from monoculture agriculture to intact forest. There are several studies on the mutualistic relationships between orchid bees and orchids (Dressler 1967; Ackerman 1983), but again there is a lack of studies relating orchid bee diversity to overall pollination services available in the ecosystem.

107 As well as the challenge of selecting suitable indicator groups, another difficulty is
108 deciding how to quantify biodiversity. There are many indices available to measure the
109 alpha, beta and gamma components of diversity. Alpha diversity is usually the main focus
110 of diversity studies, and species richness the most commonly used measure because it is
111 intuitive, simple and often sensitive. However, species richness lends a lot of weight to
112 rare species, while other indices such as Shannon and Simpson diversity place more weight
113 on the more dominant species in the community. Recent discussions on diversity
114 partitioning have concluded that diversity profiles (a set of multiple diversity measures
115 that differ in the relative weight given to rare and common species, and are particularly
116 powerful as a graphical tool) based on Hill numbers (Hill, 1973) could help avoid a narrow
117 focus on a single result (Chao et al., 2012; Jost, 2006). Beta diversity is also often of
118 interest in ecological studies but has an even wider array of indices to choose from than
119 alpha diversity (Anderson et al., 2011; Tuomisto, 2010). Beta diversity measures differ in
120 their approaches to quantifying similarity or dissimilarity between sites, and the
121 importance of species abundance differences and the relative importance of species
122 turnover or richness differences (Socolar et al., 2016). Beta diversity can also be placed
123 into a unified framework for partitioning diversity effectively using diversity profiles
124 (Reeve et al., 2016). The large number of diversity measures available have been
125 developed to fit a broad range of questions and few perform consistently well under
126 different conditions, which means that focusing on a single index may risk overlooking
127 important ecological patterns (Santini et al., 2017). We are therefore proponents of using
128 multiple measures of alpha and beta diversity to maximise our understanding of
129 biodiversity responses to anthropogenic disturbance.

130 In this study, we investigated how orchid bee communities respond to habitat conversion
131 across a gradient of human disturbance, from minimally disturbed tropical forest to
132 banana plantations. We aimed to study how alpha and beta diversity changed across this
133 disturbance gradient and aimed to investigate which measures were most sensitive for
134 detecting orchid bee responses to disturbance. Identifying the aspects of the orchid bee
135 community most sensitive to disturbance can inform which metrics to use when applying
136 orchid bees as indicators of environmental change. Identifying which components of the
137 community respond to disturbance may also help us better understand what they are
138 responding to and pave the way for further investigation into the mechanisms involved.

We assessed alpha diversity using Hill numbers and used several approaches to quantify beta diversity, including two recently developed measures, redundancy and representativeness (Reeve et al., 2016). Finally, we studied the activity of general pollinators across the gradient using artificial flowers. We aimed to identify if there was any change in pollinator activity across the disturbance gradient, and if orchid bee diversity provided any indication of the potential general pollination services available in the ecosystem (Engel and Irwin, 2003). We conducted our study in the highly biodiverse Manu Biosphere Reserve, Peru, where there has been limited work on the orchid bee communities, and none to our knowledge on their responses to disturbance.

1.2 Materials and methods

1.2.1 Study area

This study was based around the Manu Learning Centre (-12.789882, -71.391753, 470 m above sea level), a research station run by the Crees Foundation, in the cultural zone of the Manu Biosphere Reserve in southeast Peru, a UNESCO World Heritage Site. This zone contains a mixture of protected areas of lowland tropical forest interspersed with areas of high human impact, including logging and agriculture, and is intended as a buffer for Manu National Park. Eighteen sites were chosen to represent a gradient of human disturbance from banana monoculture to minimally disturbed tropical forest. A stratified site selection approach ensured sampling was not dominated by a single disturbance history, with three sites chosen for each of the six of major land uses in the local area, covering a gradient of human disturbance (supplementary materials Figure S.1). These were ranked from highest to lowest disturbance intensity based on land use (Beck et al., 2002; Eggleton et al., 2002), with 1 being the most disturbed and 6 the least disturbed (details in Table 1). For each land use type, one of the replicates was named 'A', 'B' or 'C', semi-randomly, taking into consideration suitable site groupings for access and analysis. We sampled all 'A' sites in the first week, then the 'B' and 'C' sites in the following weeks, so that one of each habitat types would be sampled concurrently.

At each site, data were collected on the vegetation structure to assess how this changed across the disturbance gradient. Three vegetation points were randomly selected within each site, and a 25m² plot marked out at each. Within this plot, canopy cover was

quantified using a quadrat held above the sampler's head and the quadrat used to estimate the percentage of canopy cover at five points within the circle. Understory vegetation density was estimated at the four corners of the sampling plot using the modified Braun-Blanquet scale as described in Hurst and Allen (2007). We counted the number of trees with a diameter >5cm at breast height and measured the diameters of the three largest trees within each vegetation plot, and measured leaf litter depth at 16 random points within the plot. These followed the protocols for vegetation assessment used by Whitworth et al., (2016). Weather data were collected at the research station, with temperature, rainfall and humidity data collected daily at 7am, manually checking a thermometer, humidity meter and rain gauge at the weather station located at the research centre each morning. Elevation data for each site were obtained from Google Earth Pro 7.3.2.5491, and distance to the main river, the Alto Madre de Dios, was measured in QGIS 2.18.7 (QGIS Development Team, 2017), measuring the straight line distance from the centre of each site to the nearest edge of the river using the 'measure line' tool and manually comparing the distances to the edges of the river to find the shortest.

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Table 1 - Disturbance gradient description

Disturbance Rank	Disturbance type	Description
1	Banana plantation	Primarily banana monocultures, but occasionally including a few other crops; generally open habitat with low vegetation.
2	Agroforestry	Banana plantations interspersed with native tree species, providing more shade and habitat complexity.
3	Disturbed secondary forest	Uncultivated but heavily disturbed forest lying in between plantations, with more canopy cover and thick understory vegetation.
4	Cleared regenerating forest	Forest once cleared for agriculture and grazing, now regenerating under protection for over 30 years to form a closed canopy with thick understory vegetation.
5	Mixed history forest	Selectively logged forest with small patches cleared for cultivation, regenerating under protection for over 30 years. The canopy is well defined and the understory less dense, but large trees are absent.
6	Minimally disturbed primary forest	Primary forest protected for over 30 years, with little evidence of previous disturbance. The canopy is high and well defined, large trees are present and the understory is more open.

187

188 Sites of the different disturbance types were interspersed in space as much as possible.
 189 The potentially confounding effects of large landscape and climatic differences were
 190 minimised by selecting sites within a small area (20 km²), while ensuring sites of the same
 191 disturbance type were far enough apart (>500 m) to avoid sampling pseudo-replication
 192 (Ramage et al., 2013). To minimise spatial effects on the results we kept groups of sites of
 193 different disturbance levels as close together as possible given other constraints and the

194 locations of these habitats. We used linear models to validate the results of our
 195 correlation tests and to check for any significant effect of these other environmental
 196 variables, including them as explanatory variables along with disturbance if they had any
 197 significant effect on the response.

198 This project was conducted in two parts, the primary study looking at changes in the
 199 orchid bee community along the gradient and then a second study exploring potential
 200 pollination services across the same sites. We cover the methods and results of the main
 201 study first, followed by the pollination study.

202

203 **1.2.2 Orchid bee diversity**

204 **1.2.2.1 Data collection**

205 Orchid bees were sampled in the morning between 09:00-12:00 and in the afternoon
 206 between 12:30-15:30 (\pm 15 mins). Each site was sampled for two morning and two
 207 afternoon sessions (a total of 12 hours per site) and these four sampling sessions were
 208 pooled to form a single sample for each site (a total of 18 samples). To reduce potential
 209 biases from weather or other potential temporal sampling biases, two sites were sampled
 210 simultaneously - one more disturbed (rank 1-3) and one less (rank 4-6), and on each day
 211 different disturbance types were sampled in the mornings and afternoons.

212 At each site, two sampling stations were set up 50 m apart to reduce any bias from a
 213 single within-site location choice. At each of these stations, eight balls of cotton were
 214 hung from branches at a height of 1.5 m, with 2 m between cotton balls. Each cotton ball
 215 was baited with two drops of one of the following eight attractants: wintergreen oil,
 216 methyl salicylate, eucalyptus oil, eucalyptol, vanillin (3 tsp vanillin dissolved in 50 ml 96%
 217 ethanol), benzyl acetate, clove oil, eugenol. The bait stations were monitored over the
 218 three-hour sampling period, and orchid bees attracted to the baits were caught using hand
 219 nets then killed and preserved in 70% ethanol. Orchid bees attracted to the survey area
 220 that did not settle at a specific bait but came within 1m of a bait and flitted between
 221 baits, were also captured. Multiple researchers conducted the hand netting, three per site
 222 each day. This included trained researchers experienced with capturing insects with nets,
 223 and less experienced volunteers. To reduce biases from experience, we ensured that the

224 teams surveying the different sites were as balanced as possible, with less experienced
 225 people paired with more experienced people and the teams rotated between the sites.
 226 Some of the potential issues associated with capture rates are explored in the discussion.

227 Preserved orchid bees were identified in Cusco, using a stereo microscope, published keys,
 228 checklists and descriptions (Bonilla-Gomez and Nates-Parra, 1992; Dressler, 1978, 1979,
 229 1982b, 1982c, 1982d, 1984; Faria and Melo, 2007; Hinojosa-Díaz and Engel, 2011, 2012,
 230 2014; Kimsey, 1979, 1982; Melo, 2014; Moure, 1965; Nemésio, 2011, 2009; Nemésio and
 231 Silveira, 2007b; Niemack et al., 2012; Roubik, 2004; Roubik and Hanson, 2004). For as
 232 many species as possible, identification was verified by consulting the collections at the
 233 Department of Entomology at the Universidad de San Antonio Abad de Cusco, and at the
 234 Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima. Reference
 235 specimens were deposited in both these collections in September 2016.

236 **1.2.2.2 Analysis**

237 A principal component analysis (PCA) of the vegetation structure data across the gradient
 238 was performed using the vegan package v.2.4-6 in R (Oksanen et al., 2018), and we used a
 239 Spearman rank correlation test (Spearman, 1904) to check the correlation between
 240 disturbance rank and the first principal component of the vegetation PCA.

241 To test if alpha diversity varied with human disturbance, orchid bee alpha diversity was
 242 calculated for each site using Hill numbers, a family of diversity measures parameterised
 243 by a viewpoint parameter, q , with increasing emphasis on dominant species as the value of
 244 q increases (Hill, 1973; Reeve et al., 2016). We calculated diversity at $q = 0, 1, 2$ and ∞ ,
 245 as these correspond to the following commonly used diversity measures: species richness,
 246 Shannon entropy, Simpson diversity and Berger Parker diversity respectively, so our
 247 measures can be easily compared to previous studies.

248 All analyses were conducted using R version 3.3.2 (R Core Team, 2017) in RStudio version
 249 1.0.136 (RStudio Team, 2016). Observed alpha diversity was calculated using the package
 250 rdiversity v.0.4.3 (Mitchell and Reeve, 2016). Estimates of species richness, Shannon
 251 entropy and Simpson diversity were calculated using the package iNEXT v.2.0.14 (Hsieh et
 252 al., 2016) to rarefy or extrapolate estimates to a standard sample size of 50 individuals at

each site (Chao et al., 2014; Colwell et al., 2012). This sample size represented approximately double the smallest sample size, the maximum that can be reliably extrapolated at $q = 0$ with high confidence (Hsieh et al., 2016). This provided approximately 90% sample coverage for all sites (supplementary materials Figure S.3). The diversity estimates at each value of q were bootstrapped 1000 times using the iNEXT function in R, to calculate a confidence interval around our mean estimates. This allowed us to test for consistency in the direction of change in the diversity estimates while accounting for the uncertainty in the diversity estimates. We did this by extracting the 1000 raw estimates of the diversity of each site, and then calculating the proportion of times a less disturbed site was more diverse than a more disturbed site. All disturbance type pairs were compared, and a Spearman rank correlation test (Spearman, 1904) was used to test the correlation between the proportion of times that the less disturbed site was the more diverse of the pair and the difference in disturbance rank between the sites.

To understand how beta diversity was affected by human disturbance, we quantified the change between sites along the gradient using several methods, including community composition, beta diversity partitioning and two recently developed beta diversity measures, redundancy and representativeness (Reeve et al., 2016). The total beta diversity of the gradient, measured as the total variance of the community matrix, was calculated using the `beta.div.comp` function in the R package `adespatial` v.0.1-1 (Dray et al., 2018) along with the partitioning of the total beta diversity into nestedness and turnover. A Hellinger transformation of the species x site abundance matrix was used, as the Hellinger distance provides a good compromise between linearity and resolution and has been found to correlate better with ‘true’ distances in simulations than many alternatives (Legendre and Gallagher, 2001). Beta diversity decomposition was then calculated for this matrix using the quantitative form of Sorensen’s dissimilarity coefficient, in order to account for differences in relative abundances as well as species identity (Legendre and De Cáceres, 2013). A redundancy analysis (RDA) was used to examine the change in species composition across the gradient, applied to the Hellinger transformed community matrix and constrained by disturbance rank, elevation and distance to the river, using the `vegan` package in R (Oksanen et al., 2018). The use of this transformation overcomes many of the issues associated with raw Euclidean distances, including many zeros and large differences in abundances, which may lend

disproportionate weight to rare species (Borcard et al., 2011; Legendre and Gallagher, 2001). Species contributions to beta diversity (SCBD index), which is the relative degree of variation in the abundance of individual species across the study gradient (Legendre and De Cáceres, 2013), were calculated with the `beta.div` function in the `adespatial` package, using the Hellinger dissimilarity coefficient (Legendre and De Cáceres, 2013). The abundances of the species with the highest SCBD values were compared across the gradient, with some low SCBD species included for contrast.

The redundancy of the communities at each site was calculated using the redundancy (ρ) measure, available in the package `rdiversity` (Mitchell and Reeve, 2016). This is a measure of beta diversity that represents the extent to which the diversity of the overall metacommunity (the diversity of the whole gradient in this case) would be preserved if a single community or site was lost (Reeve et al., 2016). We also calculated the representativeness ($\bar{\rho}$) of the sites, which is a measure of how well a single site represents the overall metacommunity. Representativeness considers how much of the metacommunity diversity a site holds (i.e. the redundancy) relative to the size of the community at that site, providing a correction for the different sample sizes across the gradient. Both redundancy and representativeness were calculated at $q = 1$, as this provides an intermediate level of conservatism and is a key value of q due to its correspondence to many measures of beta diversity through relative entropy and K-L divergence (Reeve et al., 2016).

We used disturbance as a continuous explanatory variable since this allowed us to consider land-use on a continuous spectrum of disturbance intensity, with the possibility of other land-uses falling at intermediate intensities. We could have used an ordered discrete variable, which would have been better able to detect a signal in the presence of unevenness in disturbance differences, but this would have required more data to fit. Our simpler approach is supported by the strong correlation of our continuous disturbance rank with vegetation structure data. Nonetheless, because we could not be sure of the exact difference in disturbance between each level, where possible we used a Spearman rank correlation test to assess the patterns of diversity along this gradient. This approach makes no assumptions regarding the shape of the relationship between the variables, only that the pattern is monotonic along the gradient, and is therefore a conservative

316 approach for these analyses. Spearman rank correlation tests were used to test for
 317 correlations of disturbance rank with abundance, observed alpha diversity at $q = 0, 1, 2$
 318 and ∞ , estimated alpha diversity at $q = 0, 1$ and 2 , and representativeness and
 319 redundancy at $q = 1$. In addition to checking each value of q independently, we tested if
 320 the overall pattern of alpha diversity change across the disturbance gradient was
 321 significant at $\alpha = 0.05$. To overcome the non-independence of the q values from one
 322 another, we used a permutation test to calculate the combined p value for the correlation
 323 between disturbance rank and observed alpha diversity at $q = 0, 0.5, 1, 2$ and ∞ and
 324 estimated alpha diversity at $q = 0, 0.5, 1$ and 2 ($q = \infty$ could not be included because the
 325 estimates all converged at 1 , so there was no variation; we included $q = 0.5$ because q is
 326 on a logarithmic scale, so this prevents biased weighting of rare species when calculating
 327 the overall pattern of alpha diversity across multiple values of q). The permutation test
 328 involved randomly re-labelling the study sites, and then re-calculating the significance of
 329 the correlation between disturbance rank (now randomly re-assigned) and alpha diversity
 330 at each value of q (Good, 2000). These permuted p -values for each value of q were
 331 combined to determine the significance of the overall alpha diversity change across the
 332 gradient (Fisher, 1925). This was repeated for 10000 permutations, and the permuted p -
 333 values compared to those obtained with the original data, to determine the probability
 334 that the observed correlation of diversity could have occurred by chance. To check that
 335 the patterns of change in redundancy and representativeness across the gradient were not
 336 specific to our chosen value of $q = 1$, we also used a permutation test to obtain the
 337 combined p value for the correlation of disturbance rank with redundancy and
 338 representativeness calculated at $q = 0, 0.5, 1, 2$ and ∞ .

339 As well as testing for the effect of disturbance on diversity and abundance, the potential
 340 effects of altitude, rainfall and distance to river were investigated using linear models, to
 341 confirm whether the effects of disturbance identified with the correlation tests remained
 342 significant when accounting for these variables. The diversity and abundance response
 343 variables were log-transformed and modelled with a gaussian distribution. The model
 344 summaries and residuals were inspected to evaluate model fit. None of the additional
 345 environmental variables resulted in a significant improvement compared the model that
 346 included only disturbance rank. Full details of the models tested can be found in Table S.2
 347 of the supplementary materials. In the case of the relationship between abundance and

disturbance rank, visual inspection of the data prompted us to also test a quadratic polynomial, but the AIC values indicated that a linear relationship was a better fit ($\Delta AIC = 1.42$). Throughout the results section, the statistics reported are the results of the Spearman rank correlation test unless stated otherwise.

1.2.3 Pollination services

1.2.3.1 Data collection

The potential for the provision of pollination services (from any pollinators) across the gradient was tested using artificial flowers filled with sugar solution (Internicola et al., 2007; Real, 1981). The flowers were 5 cm in diameter and constructed from thin craft foam with a central well containing 1.5 ml of the sugar solution (1:1 sugar and water). Red, blue and yellow flowers were used, with five of each colour on an array that was suspended at a height of 1.3 m at the survey site. Two flower arrays (30 flowers in total) were used at each site, separated by approximately 30 m, and each monitored by a member of the research team. All insects (of any Order) that arrived at the flower array were counted as potential pollination events, apart from individuals that simply moved from one location on the array to another. The flowers were monitored for three periods of 45 minutes separated by 15-minute intervals during which the flowers were covered. This was repeated for four mornings, between 08:45 and 13:00, at each of the 18 sites.

1.2.3.2 Analysis

Spearman rank correlation tests were used to test the correlation between the number of potential pollination events (flower visits) with disturbance rank and with observed orchid bee species richness.

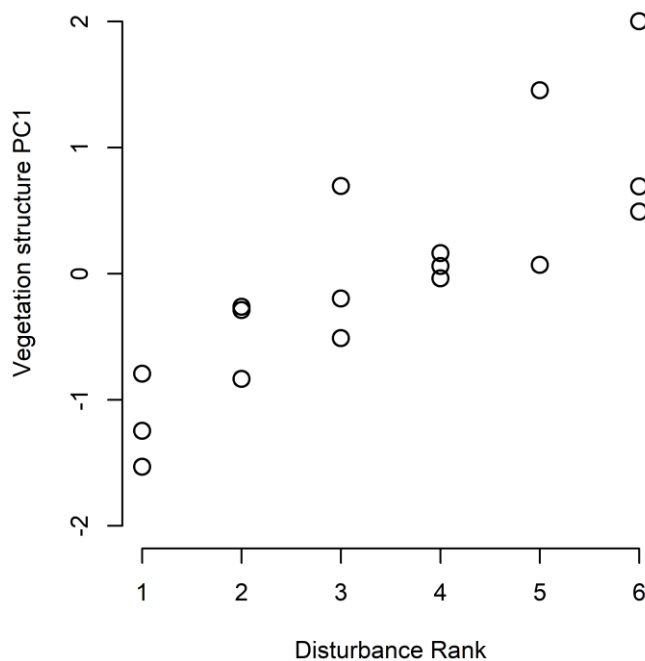
1.3 Results

1.3.1 Orchid bee diversity

1.3.1.1 Vegetation structure

The first component of the vegetation structure PCA (PC1) explained 33% of the variation in vegetation structure, with higher rankings (lower disturbance) correlated positively with

canopy cover, tree count and diameter, and negatively with leaf litter depth and understory herb abundance (Figure S.2). There was a strong correlation between vegetation structure (PC1) and disturbance rank, supporting the disturbance ranking used to represent human disturbance intensity along the gradient ($p < 0.00001$, $\rho = 0.86$; Figure 1). The results of all correlation tests are provided in Table S.1 in the supplementary materials.



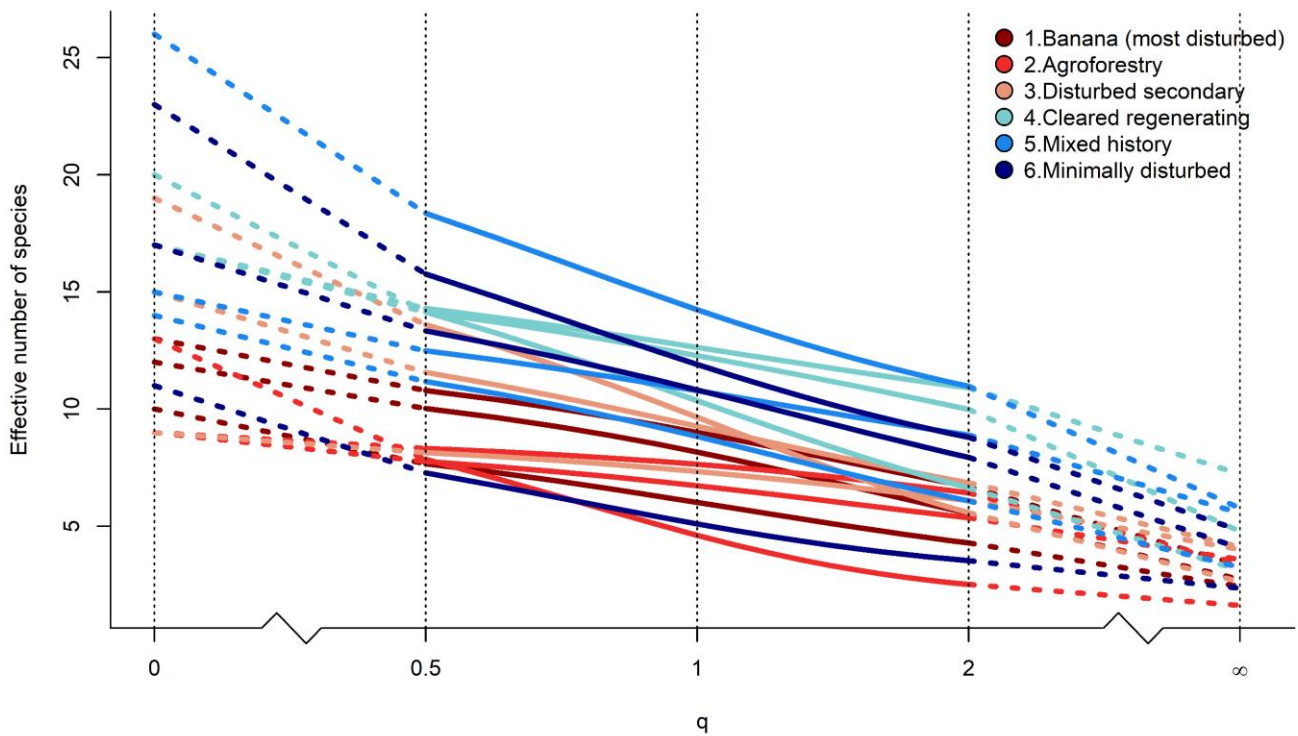
381

382 Figure 1 - Correlation between vegetation structure and disturbance rank ($p < 0.001$, $\rho = 0.86$). Disturbance
 383 rank runs from the most disturbed (rank 1) to the least disturbed habitat (rank 6). The first axis of the principal
 384 component analysis of vegetation structure (PC1) was positively correlated with canopy cover, tree count and
 385 diameter, and negatively with leaf litter depth and understory herb abundance.

386 1.3.1.2 Alpha diversity

387 We collected 1783 individuals of 31 species of orchid bee. Overall observed alpha diversity
 388 decreased across the disturbance gradient (permuted combined p value for $q = 0, 0.5, 1, 2$
 389 and ∞ : $p = 0.025$; Figure 2). This was mostly driven by the patterns of alpha diversity at
 390 low values of q , with observed species richness ($q = 0$) and observed Shannon diversity ($q =$
 391 1) both decreasing with increased disturbance across the gradient ($\rho = 0.57$, $p = 0.01$;
 392 $\rho = 0.51$, $p = 0.03$). There was no significant change in alpha diversity across the
 393 gradient at higher values of q . However, when estimated diversity was examined,

394 correcting for sample size ($n = 50$), there were no significant differences in estimated
 395 diversity across the disturbance gradient, either overall (permuted combined p value for q
 396 $= 0, 0.5, 1$ and 2 : $p = 0.330$) or for any individual value of q , due to the wide confidence
 397 intervals around the mean estimates (Figure 3 and Figure S.4).



398
 399 Figure 2 – Orchid bee observed alpha diversity across the disturbance gradient. Increasing values of q indicate
 400 increased emphasis on dominant species ($q = 0$ = species richness, 1 = Shannon diversity, 2 = Simpson
 401 diversity, ∞ = Berger Parker diversity). Disturbance rank is shown by a colour gradient, dark red for the most
 402 disturbed sites, dark blue for the least disturbed. Overall diversity was significantly higher in less disturbed sites
 403 (permuted combined p value = 0.025), and at $q = 0$ ($\rho = 0.57$, $p = 0.01$) and $q = 1$ ($\rho = 0.51$, $p = 0.03$), but
 404 not significantly different at or above $q = 2$ ($\rho = 0.45$, $p = 0.06$). Because q is on a log scale, the broken axis
 405 and dashed lines indicate inferred values as diversity was calculated only for the values at either side of the
 406 break (0 and ∞); along the solid line, q was calculated at intervals of 0.1 .

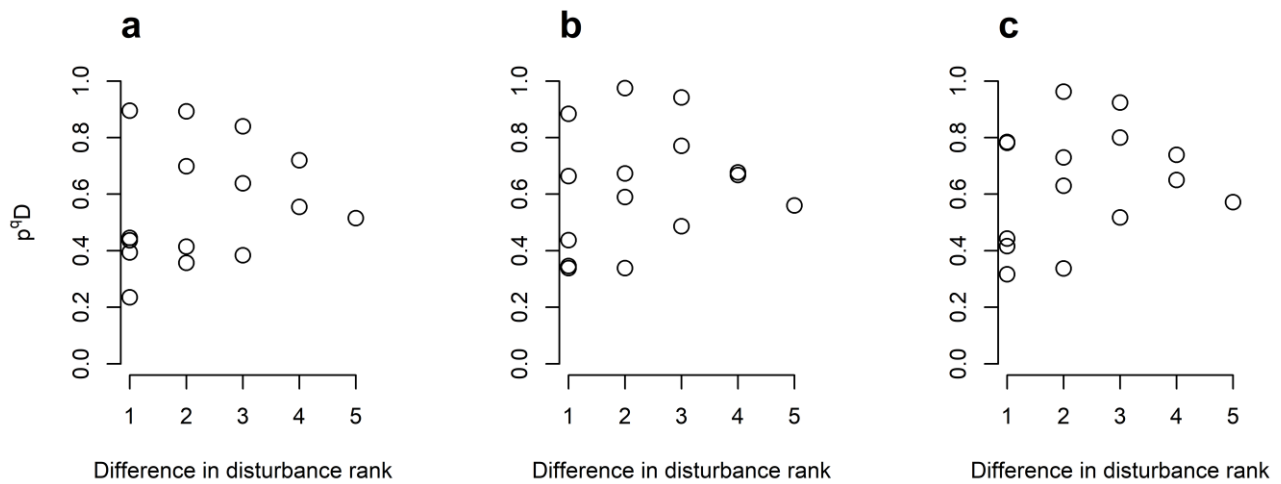


Figure 3 – The correlation between diversity estimates and disturbance ranking. Using the iNEXT package in R, we generated 1000 estimates for the diversity of each site. We then compared all possible habitat pairs to calculate the proportion of times that the less disturbed habitat was estimated to have a higher diversity than the more disturbed habitat, shown on the y axis (p^qD). This was calculated for diversity at a) $q = 0$ ($p = 0.33$; $\rho = 0.27$), b) $q = 1$ ($p = 0.37$; $\rho = 0.25$), and c) $q = 2$ ($p = 0.42$, $\rho = 0.22$); $n = 50$. The difference in disturbance ranking between the sites is shown along the x-axis – high values mean the sites are further apart along the disturbance gradient.

1.3.1.3 Abundance

Abundance of orchid bees declined across the disturbance gradient ($\rho = 0.63$, $p = 0.005$), with less than a quarter of the number of bees found in the most disturbed habitat compared to the best of the less disturbed forest sites (Figure 4).

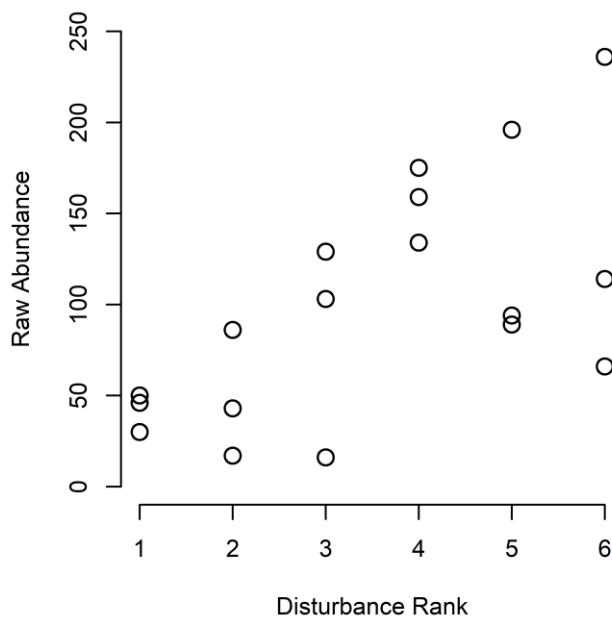


Figure 4 – Change in orchid bee abundance across the disturbance gradient. Abundance is the number of orchid bees captured at each site, plotted against the disturbance rank of the site ($\rho = 0.63$, $p = 0.005$). Disturbance rank runs from 1 (most disturbed) to 6 (least disturbed).

1.3.1.4 Beta diversity

Although there was some change in the number of orchid bee species found across the disturbance gradient, beta diversity was driven primarily by a turnover of species, rather than a change in richness. The results of the beta diversity partitioning were a total beta diversity of 0.18 across the gradient (a total beta diversity of 1 would indicate that the sites had completely distinct communities; Legendre & De Cáceres 2013), with 77% of this due to species turnover and 22% due to differences in richness (nestedness).

The composition of the orchid bee community changed across the gradient, with different communities found in the more and less disturbed sites, as demonstrated by a separation along the RDA1 axis (Figure 5), which captured 37% of the variation in community composition. The Species Contributions to Beta Diversity (SCBD) index identified the

species that changed most in abundance along the gradient (Figure 6). *Euglossa chalybeata* and *Euglossa orellana* appear to be forest specialists that are lost as forest disturbance increases, whereas *Euglossa despecta* appears to favour intermediate levels of disturbance.

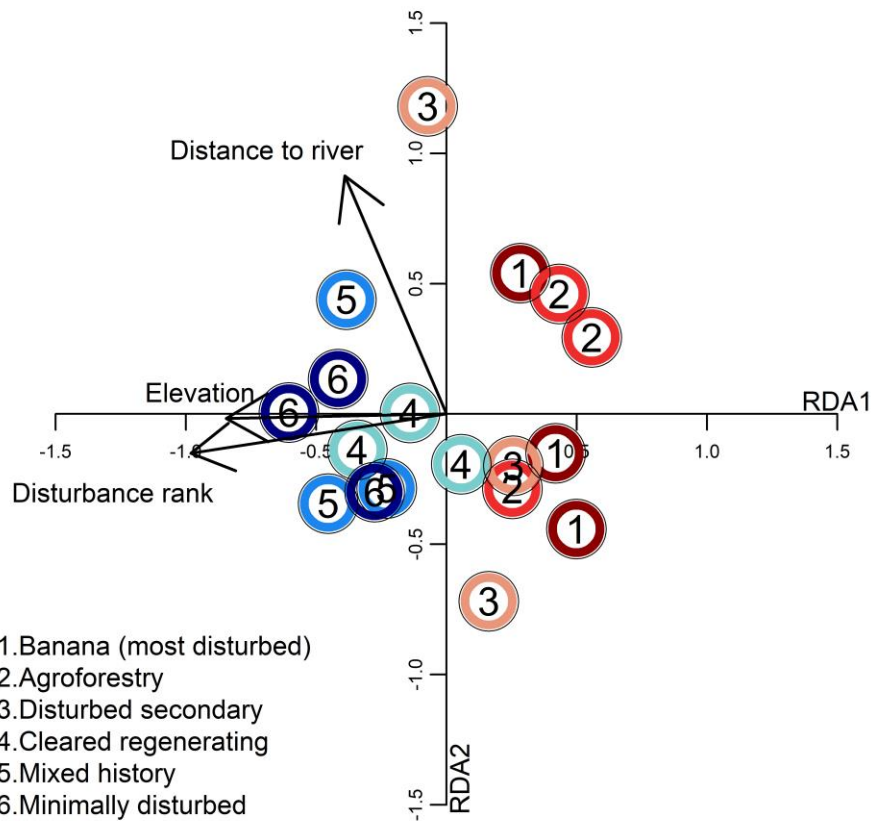


Figure 5 – RDA of orchid bee community composition across the disturbance gradient, constrained by disturbance rank, elevation and distance to the river. Disturbance rank is represented by a colour gradient from dark red (most disturbed) to dark blue (least disturbed), with the rank of each site also shown numerically.

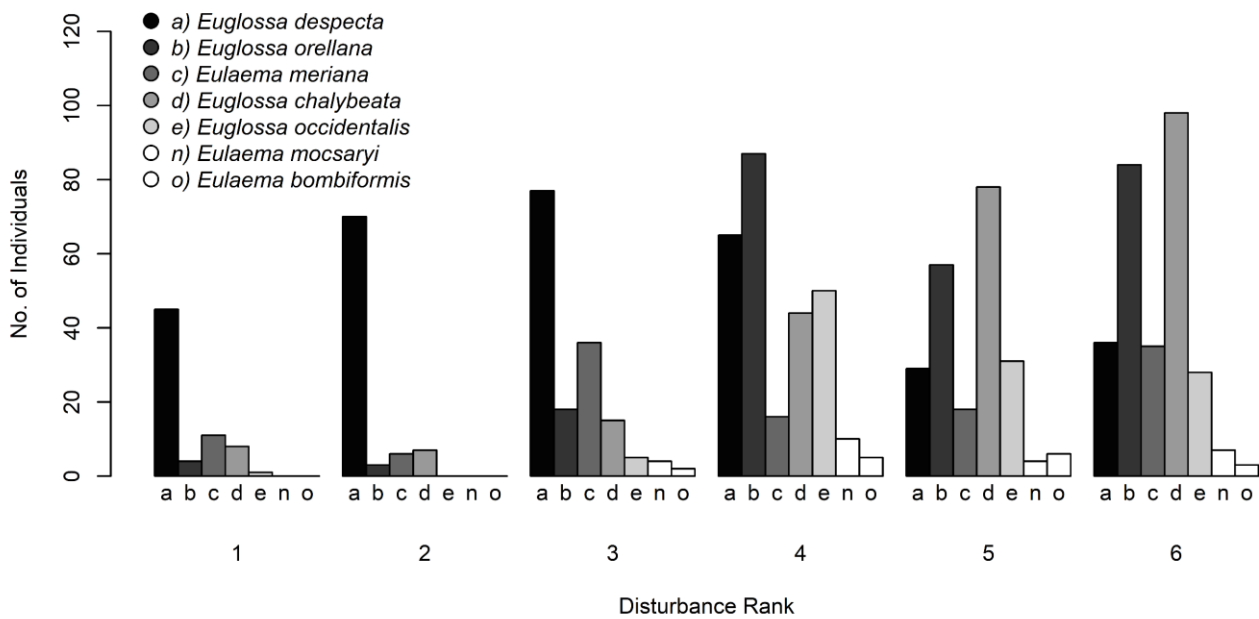


Figure 6 – Differences in abundance across the gradient of the species that contribute most to beta diversity. A total of 31 species were found. The grey bars (a-e) show the top five contributors to beta diversity, in order of contribution. Two examples of low contributors to beta diversity are shown in white (n and o) for comparison. Disturbance rank runs from 1-6, with 1 representing the most disturbed sites.

The less disturbed sites hold communities that are less redundant (Figure 7a; at $q = 1$: $p = 0.02$, $\rho = -0.55$; permuted combined p value for $q = 0, 0.5, 1, 2$ and ∞ : $p = 0.016$) and more representative of the overall metacommunity (Figure 7b; at $q = 1$: $p = 0.04$, $\rho = 0.4922$; permuted combined p value for $q = 0, 0.5, 1, 2$ and ∞ : $p = 0.024$) than the more disturbed sites. This suggests that the overall diversity of the region (all sites across the gradient) would suffer a greater loss should the community of one of the less disturbed sites be lost, than if the community of a highly disturbed site was lost.

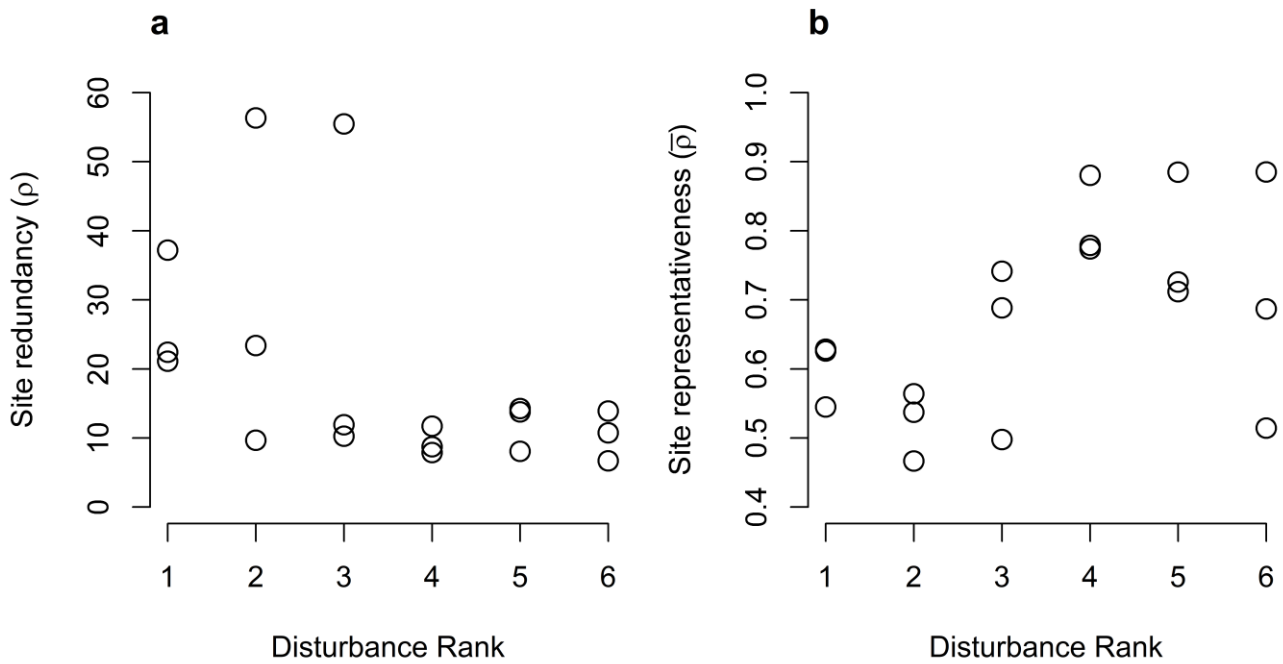


Figure 7 – Change in the a) redundancy (ρ) of sites ($p = 0.02$, $\rho = -0.55$) and b) representativeness ($\bar{\rho}$) of sites ($p = 0.04$, $\rho = 0.49$) across the disturbance gradient at $q = 1$. The disturbance rank runs from 1 (most disturbed) to 6 (least disturbed).

1.3.2 Pollination services

There was no relationship between the number of visits from potential pollinator insects and disturbance rank (Figure 8a; $\rho = 0.1160$, $p = 0.6467$) or orchid bee diversity (Figure 8b; $\rho = -0.19$, $p > 0.4$ at $q = 0, 1$ and 2). The artificial flowers received similar numbers of visits across the disturbance gradient, with slightly more visits occurring in lower disturbance sites but with a large amount of variance within each disturbance level. We found no evidence that orchid bee diversity (specialised pollinators) indicated any trend in the activity of pollinators in general.

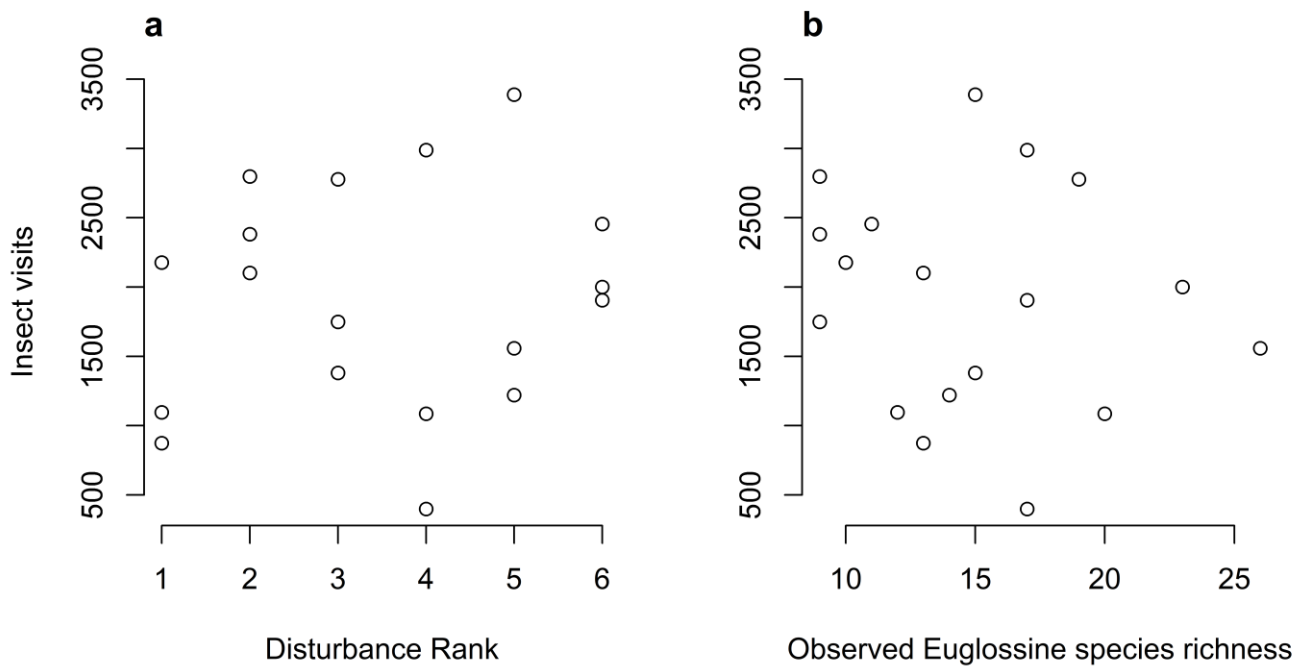


Figure 8 – Potential pollination events. Number of insect visits to artificial flower arrays correlated with (a) disturbance rank ($\rho = 0.12$, $p = 0.65$) and (b) observed orchid bee species richness ($q = 0$; $\rho = -0.20$, $p = 0.44$). Disturbance ranking runs from 1 (most disturbed) to 6 (least disturbed).

1.4 Discussion

Orchid bees declined in abundance as disturbance increased, and community composition changed across the gradient, suggesting that orchid bees can be useful as indicators of the impacts of human disturbance. Redundancy of the orchid bee community at a site increased with increased disturbance and representativeness of the sites decreased. No changes in species richness or higher order alpha diversity could be detected after controlling for sample sizes, suggesting that, unlike many other groups, alpha diversity of orchid bees is unlikely to be a strong indicator of human disturbance impacts. We found no change in pollinator visits in response to disturbance nor any correlation between pollinator visits and orchid bee species richness.

Previous studies that suggested orchid bees show little response to human disturbance focused on different disturbance types and fewer sites with less replication than covered in our study (Nemésio and Silveira, 2006; Rasmussen, 2009). The first of these studies sampled a similar number of bees as in our study over a longer period in only six sites, focusing on the effect of distance to the forest edge. They found no change in observed species richness, and erratic differences in abundance, uncorrelated with distance to edge, but they did find some change in community composition, though many species were

489 shared across sites (Nemésio and Silveira, 2006). The second study considered alpha
 490 diversity at $q = 0, 1$ and 2 as well as abundance and community similarity between sites
 491 and found no significant relationship between any of these with disturbance level
 492 (Rasmussen, 2009). However, only three sites were compared, one of each disturbance
 493 type: primary forest, a reforested site and one heavily disturbed forest. Like these studies,
 494 we also found no significant change in alpha diversity after controlling for sample size.
 495 However, it should be noted that this lack of a significant response appeared to be
 496 because there were many more bees caught in the less disturbed sites, consistent with the
 497 marked decrease in orchid bee abundance with increased disturbance we detected. When
 498 comparing observed alpha diversity, without controlling for the number of bees caught,
 499 there was a decrease in species richness and Shannon diversity as disturbance increased.
 500 Since standardised sampling effort was used there is no reason to suspect the observed
 501 differences were caused by any bias in the sampling design, and so this difference should
 502 not be dismissed. That there are fewer bees in the disturbed sites means that the
 503 confidence interval around the estimated true number of species present is large, however
 504 if sampling time was extended to catch the same number of bees as found in the least
 505 disturbed sites, it would be unsurprising if there were still fewer species (supplementary
 506 materials Figure S.4). In any case, the fact that during a set time period, fewer individuals
 507 of fewer species are likely to visit plants in disturbed habitats is an ecologically
 508 meaningful result, as it is likely to impact plant pollination, even if similar total numbers
 509 of species could eventually be detected over a longer time.

510 This kind of study is prone to problems with biases arising from study design and the
 511 assistance of volunteers. As well as training, and pairing experienced staff with
 512 inexperienced samplers, we randomised the location of the teams to avoid systematic
 513 biases. The high turnover of volunteers allowed us to keep this strategy constant over the
 514 sampling period. Capture success was high, and although there were some escapes, many
 515 of these individuals could be seen leaving and returning to the bait, allowing a second
 516 capture attempt. We did not record miss rates but there was no noticeable difference
 517 observed between genera or species identifiable in flight, although this would be valuable
 518 to investigate in future studies. Another known issue in studies like this is the potential for
 519 differences in the attractive radius of the baits between sites to affect capture rates,
 520 because differences in vegetation structure and shade between the sites could affect the

521 evaporation rates and how much the baits might be carried on the wind. Further research
 522 is needed to fully address this (Nemésio, 2012), but we followed standard practice from
 523 previous studies in using standardised bait protocols across all sites, including regularly
 524 refreshing the bait to avoid evaporation issues. Since capture rate was higher in the less
 525 open forest, there was also no evidence for sampling issues related to scent dispersal
 526 being inhibited by vegetation. Due to this study region consisting of a matrix of different
 527 habitat types, it was unavoidable that some sampling sites would not be far from other
 528 disturbance types. We set up the bait stations near the middle of a habitat type to ensure
 529 that the majority of bees were likely to be attracted from within that site, with a
 530 minimum of 100m to the neighbouring habitat. It is plausible that some bees may have
 531 been attracted from neighbouring habitats, yet this should theoretically weaken our power
 532 to detect a correlation between the orchid bee community and habitat type, lending
 533 confidence to the patterns that we have detected, as discussed in Brosi (2009).
 534 Furthermore, for ecological purposes we care about the bees that visit a site to provide
 535 pollination services, so it is still relevant if some bees visit from neighbouring habitats, as
 536 we are interested in the disturbance impacts on orchid bees in the context of this matrix
 537 landscape.

538 Regarding the value of orchid bees as indicators, in this study we have shown that changes
 539 in the orchid bee population are strongly correlated with intensity of forest disturbance,
 540 and identified which measures are most useful for detecting these responses. The results
 541 of the vegetation surveys showed a loss of canopy cover and number of large trees as
 542 disturbance increased (Figure S.2). This may also result in a change in microclimate and a
 543 loss of epiphytes. A loss of resources such as nectar and nesting habitats may have
 544 contributed to the decline in orchid bee abundance, and a change in the plant species
 545 composition could have influenced the change in orchid bee species along the gradient. A
 546 more detailed exploration of the mechanisms behind the orchid bee responses are
 547 important in order to understand what exactly they are indicative of: is it a change in
 548 vegetation structure (shown to be closely correlated with disturbance in our study), a
 549 decline in overall biodiversity, a loss of important nesting habitats, a loss of epiphytes or
 550 particular flower species? These are important points for further investigation, as there is
 551 a general lack of information about the relationship between ecological indicators and
 552 indicandum (Gao et al., 2015). It would also be worth expanding on research assessing how

far these disturbance effects penetrate into forest edges (Nemesio and Silveira, 2006), considering the different indices used in this study and comparing different land uses and regions. For basic assessments, orchid bee abundance is a simple measure that can be monitored by conservation managers without the need for much analytical expertise or even species identification. Although this should not be used in isolation, it could serve as a useful warning flag of negative impacts of disturbance. The Hill numbers diversity measures in the rdiversity package require more technical skills but allow efficient calculation of a broad suite of indices, including alpha and beta diversity (redundancy and representativeness) at multiple values of q , using a single input of the species x site data matrix and only simple code modifications to calculate multiple indices.

Species richness has been recognised as a poor index for detecting the effect of disturbance on some other taxonomic groups, and it has been suggested that community composition may be more sensitive (Stork et al., 2017). For example, Samejima *et al.* (2004) found that stingless bees (Meliponini) showed a change in community composition in response to human disturbance. We also found that community composition changed across the disturbance gradient, but community composition can be expected to change for many reasons, including non-disturbance related changes in vegetation and other habitat features. Due to the small spatial scale of this study and the history of the area, we expect that the habitats of the study sites would have been very similar prior to the anthropogenic disturbance; this assumption is less likely to hold true across larger spatial scales, making it harder to link changes in species composition to habitat disturbance across space. However, as a potential indicator, identifying shifts in orchid bee community composition could be useful for monitoring a site over time, to provide an indication of whether the site is being disturbed to a degree that is negatively impacting the ecosystem (Santini et al., 2017).

In ecological terms, reduced orchid bee abundance in highly disturbed habitats is of concern, as it is indicative of a potential cascade effect resulting from the loss of forest canopy, along with habitat complexity and epiphytic diversity (Barthlott et al., 2001). This could result in a loss of specialist pollinators that are crucial for the persistence of many plant species, which may reduce the resilience of the remaining degraded forest, as the ecological networks have been weakened. However, the services provided by orchid bees may be partially maintained by a well-connected patchwork of habitats including high

quality forest, as the dispersal distances of this group (Janzen, 1971) enables them to make opportunistic visits to degraded habitats when resources are available. This way, they may be able to provide pollination services to habitats that possibly lack the resources to support viable orchid bee populations independently, but this will require a substantial area of intact forest to be maintained nearby.

We found that the redundancy (ρ) of the sites increased with increased disturbance. Redundancy is therefore likely to be a useful measure of disturbance impacts, because it considers both the species present and their abundance, so gives a more complete picture of how the sites differ. From these results, we can see that the impact of losing a minimally disturbed site would more severely impact the overall diversity of the region than the loss of a highly disturbed site. Similarly, when we considered the representativeness ($\bar{\rho}$) of the sites at $q = 1$, we found that in each of the less disturbed sites (ranks 4-6) about 80% of the overall biodiversity of the study area could be found, on average, whereas the more disturbed sites (ranks 1-3) only held an average of about 60%.

Pollination potential showed no correlation with orchid bee abundance or alpha diversity (Figure 8; supplementary materials Table S.1). This is probably because visitors to the flower arrays were mostly sweat bees (family Halictidae) and these visited the flowers in high abundance. Other visitors included flies, wasps, butterflies, a few orchid bees, and even a hummingbird on one occasion. An important caveat in interpreting this experiment was that we were only able to measure the potential opportunities for pollination (the number of visits the flower received), and were not able to account for the fact that insects differ widely in their effectiveness as pollinators (Ivey et al., 2003; King et al., 2013; Primack and Silander, 1975; Ramsey, 1988; Schemske and Horvitz, 1984). Turnover of species between sites also means that higher bee diversity than expected is likely to be required to deliver pollination services over large spatial scales (Winfree et al., 2018). Halictid bees are considered valuable pollinators and visit many different plant species (Lindsey, 1984), although they do not show such distinctive host adaptations as the orchid bees. Orchid bees do pollinate a wide range of plants; however, they are particularly important due to their many species-specific relationships and cannot be easily substituted by more generalist pollinators. Further research into the pollination networks of orchid bees and the plants they visit would be helpful to understand how a loss or change in the

orchid bee community might impact the plant community. Exploring functional differences (e.g. tongue length and body size) between the orchid bee species affected by disturbance would also add valuable information on the contributions of specific orchid bee species as specialist pollinators.

1.5 Conclusion

Orchid bees show a clear negative response to human disturbance across a tropical forest-agricultural gradient. They are also efficient to sample and play a key role in pollination services. We therefore suggest they can be a useful addition to the indicator groups available for studying the impacts of forest loss on biodiversity and ecosystem functioning. In choosing whether to use orchid bees in future biodiversity studies it should be noted that it is important to consider more than one indicator group in any assessment (Hilty and Merenlender, 2000; Lawton et al., 1998). When orchid bees are selected as a suitable indicator group, our results show that abundance, redundancy (ρ) and representativeness ($\bar{\rho}$) provide the most sensitive measures for detecting the response of orchid bees to human disturbance. In order to understand the response of biodiversity to human disturbance, it is essential to consider the response measures carefully, as a measure that works well for one group may not always be the best for another, and often multiple indices are necessary.

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1.8 Competing interests

The authors have no competing interests to declare.

1.9 Figures

Figure 2 to be printed in colour.

1.10 Data Access

The data have been made publicly available and can be accessed from the University of Glasgow Enlighten repository: <http://dx.doi.org/10.5525/gla.researchdata.589>

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1.12 Supplementary materials

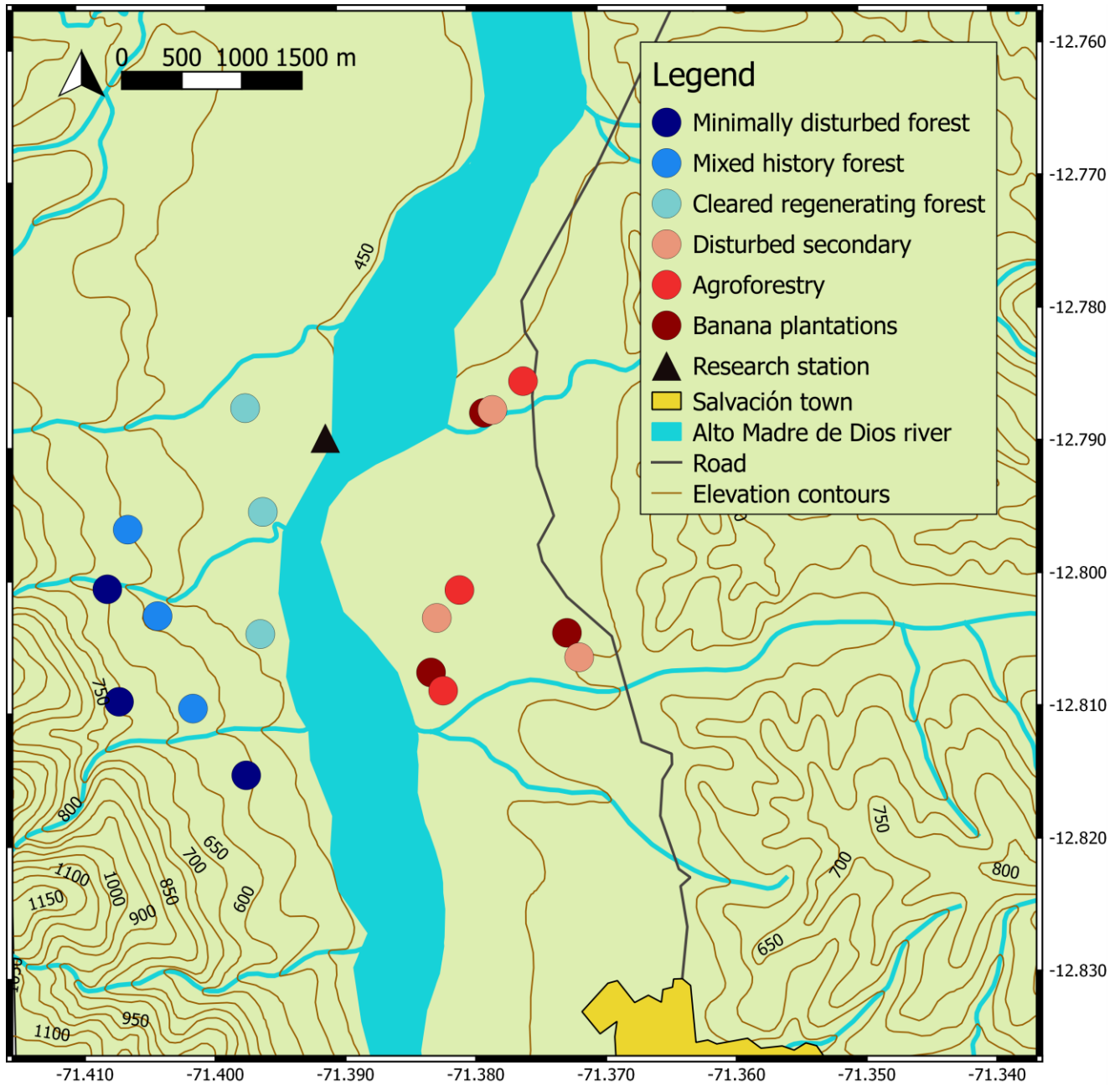


Figure S.1 – Map of our study sites in Manu, Peru. Colours represent disturbance gradient, dark red = 1. Most disturbed, dark blue = 6. Least disturbed. The map also shows: elevation contour lines; the Manu Learning Centre research station; the nearest local town, Salvación; the local road; the main river, Alto Madre de Dios, and its tributaries.

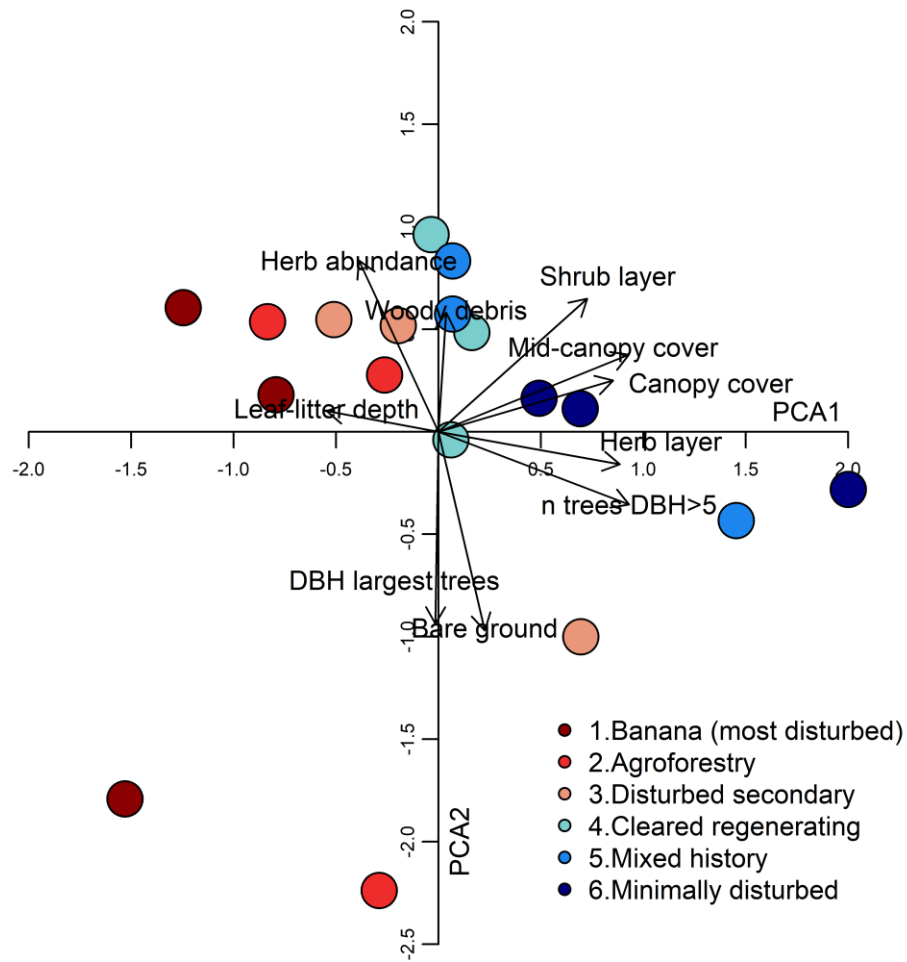


Figure S.2 – PCA of vegetation structure across the gradient, with sites colour coded by their disturbance level (dark blue least disturbed – dark red most disturbed).

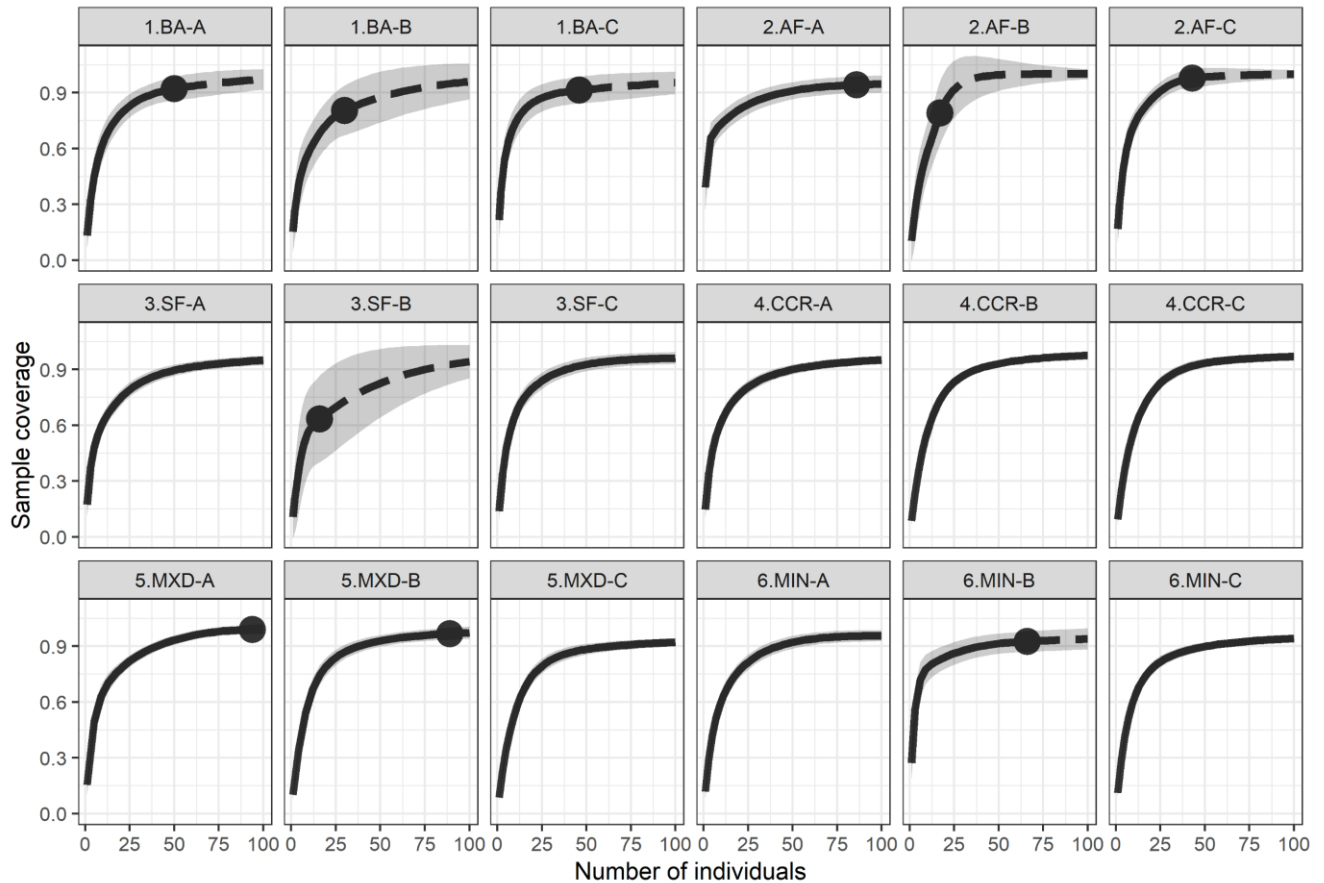


Figure S.3 – Sample completeness at $q = 0$. The sample completeness for each site is shown in a separate panel. The labels above each panel describe the disturbance rank (1-6, 1 is most disturbed) and habitat type of each site (BA: Banana, AF: Agroforestry, SF: Disturbed secondary forest, CCR: Cleared regenerating forest, MXD: Mixed history regenerating forest and MIN: minimally disturbed primary forest), as well as which of the replicates it was (A, B or C). The solid black line shows the rarefied estimate of sample coverage, and the dashed line is the extrapolated estimate, with the circle indicating the sample size collected. The grey shading indicates the 95% confidence interval around the coverage estimate. Coverage is shown up to 100 individuals for easy comparison between sites, but some sites did exceed this number; estimated diversity was compared at $n = 50$.

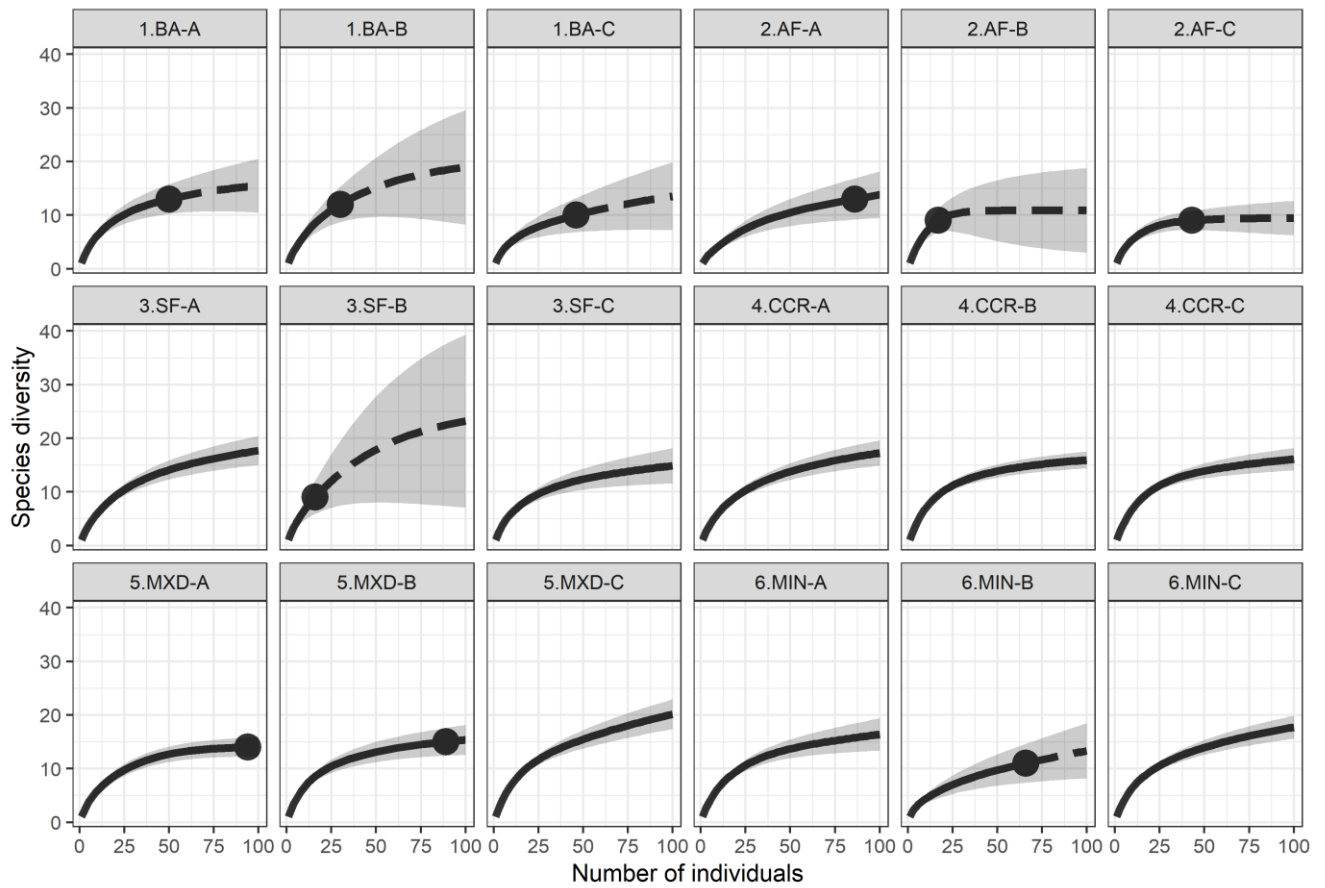


Figure S.4 – Estimated richness at each site extrapolated to a sample size of 250 individuals. Solid line shows rarefied estimate, dashed line shows extrapolation, and filled circle indicates the collected sample size. Shaded area indicates 95% confidence interval with 1000 bootstraps.

Table S.1 – Results of the Spearman rank correlation tests.

The p and rho values of the results of correlations between variables 1 and 2 are shown for all tests, along with the 95% confidence interval for the rho values.

Variable 1	Variable 2	p	rho	rho 95% CI
Abundance	Disturbance rank	0.0051	0.6301	0.2534 - 0.8303
Observed diversity $q = 0$	Disturbance rank	0.0141	0.5672	0.1538 - 0.808
Observed diversity $q = 1$	Disturbance rank	0.0302	0.5110	0.0105 - 0.8576
Observed diversity $q = 2$	Disturbance rank	0.0581	0.4546	-0.0517 - 0.817
Observed diversity $q = \infty$	Disturbance rank	0.0801	0.4232	-0.0701 - 0.7895
Vegetation structure PC1	Disturbance rank	0.0000	0.8621	0.6124 - 0.9679
Estimated diversity $q = 0$ (mean)	Disturbance rank	0.4472	0.1912	-0.3512 - 0.6686
Estimated diversity $q = 1$ (mean)	Disturbance rank	0.3747	0.2226	-0.2824 - 0.622
Estimated diversity $q = 2$ (mean)	Disturbance rank	0.2300	0.2978	-0.1826 - 0.6794
$q = 0$ raw iNEXT diversity estimates (x1000)	Difference in disturbance rank	0.3318	0.2693	-0.2551 - 0.6685
$q = 1$ raw iNEXT diversity estimates (x1000)	Difference in disturbance rank	0.3782	0.2453	-0.295 - 0.6639
$q = 2$ raw iNEXT diversity estimates (x1000)	Difference in disturbance rank	0.4201	0.2250	-0.3067 - 0.6463
Redundancy (ρ) $q = 1$	Disturbance rank	0.0168	-0.5549	-0.7938 - -0.1434
Representativeness (ρ) $q = 1$	Disturbance rank	0.0380	0.4922	0.0075 - 0.8126
Pollinator visits	Disturbance rank	0.6467	0.1160	-0.3499 - 0.5397
Pollinator visits	Observed diversity $q = 0$	0.4380	-0.1950	-0.5965 - 0.2459
Pollinator visits	Observed diversity $q = 1$	0.4331	-0.1971	-0.6293 - 0.3067
Pollinator visits	Observed diversity $q = 2$	0.4429	-0.1930	-0.6426 - 0.3347

Table S.2 – Model selection.

Where the correlations tested in Table S.1 were significant, linear models were used to control for the effect of other environmental variables. The log likelihoods and degrees of freedom for all models tested are presented here.

Model	Log Likelihood (Max. Likelihood)	Degrees of freedom
log(Abundance)~ Disturbance rank	-16.6356	3
log(Abundance)~ Disturbance rank + Elevation	-16.2785	4
log(Abundance)~ Disturbance rank + Distance to river	-14.0386	5
log(Obs. Diversity q = 0) ~ Disturbance rank	-1.1849	3
log(Obs. Diversity q = 0) ~ Disturbance rank + Elevation	-1.0606	4
log(Obs. Diversity q = 0) ~ Disturbance rank + Distance to river	0.4615	4
log(Obs. Diversity q = 1) ~ Disturbance rank	-2.3260	3
log(Obs. Diversity q = 1) ~ Disturbance rank + Elevation	-2.1954	4
log(Obs. Diversity q = 1) ~ Disturbance rank + Distance to river	-1.3630	4
log(Obs. Diversity q = 2)~ Disturbance rank	-6.2357	3
log(Obs. Diversity q = 2) ~ Disturbance rank + Elevation	-5.9173	4
log(Obs. Diversity q = 2) ~ Disturbance rank + Distance to river	-5.6520	4
log(Obs. Diversity q = ∞) ~ Disturbance rank	-5.0875	3
log(Obs. Diversity q = ∞) ~ Disturbance rank + Elevation	-4.6954	4
log(Obs. Diversity q = ∞) ~ Disturbance rank + Distance to river	-4.8372	4
Vegetation structure PC1 ~ Disturbance rank	-6.4559	3
Vegetation structure PC1 ~ Disturbance rank + Elevation	-6.2303	4
Vegetation structure PC1 ~ Disturbance rank + Distance to river	-5.7609	4
log(Est. diversity q = 0) ~ Disturbance rank	6.70	3
log(Est. diversity q = 0) ~ Disturbance rank + Elevation	6.82	4
log(Est. diversity q = 0) ~ Disturbance rank + Distance to river	6.83	4
log(Est. diversity q = 1) ~ Disturbance rank	-2.15	3
log(Est. diversity q = 1) ~ Disturbance rank + Elevation	-1.86	4
log(Est. diversity q = 1) ~ Disturbance rank + Distance to river	-2.10	4
log(Est. diversity q = 2) ~ Disturbance rank	-5.87	3
log(Est. diversity q = 2) ~ Disturbance rank + Elevation	-5.50	4

Model	Log Likelihood (Max. Likelihood)	Degrees of freedom
log(Est. diversity $q = 2$) ~ Disturbance rank + Distance to river	-5.70	4
log(ρ Redundancy $q = 1$) ~ Disturbance rank	-13.3025	3
log(ρ Redundancy $q = 1$) ~ Disturbance rank + Elevation	-12.7122	4
log(ρ Redundancy $q = 1$) ~ Disturbance rank + Distance to river	-11.5194	4
log(ρ Representativeness $q = 1$) ~ Disturbance rank	6.1746	3
log(ρ Representativeness $q = 1$) ~ Disturbance rank + Elevation	6.1913	4
log(ρ Representativeness $q = 1$) ~ Disturbance rank + Distance to river	9.1013	4

Table S.3 – Results of the best fitting models.

The estimated coefficients and significance are presented for the explanatory variables found to produce the best fitting models identified in Table S.2.

Model	Variable	Est. coefficient	SE	t	p
log(Abundance) ~ Disturbance rank + Distance to River	Rank	0.317	0.082	3.871	0.002
	Distance to river	-0.001	0.000	-2.240	0.041
log(Obs. Diversity $q = 0$) ~ Disturbance rank	Rank	0.103	0.038	2.724	0.015
log(Obs. Diversity $q = 1$) ~ Disturbance rank	Rank	0.077	0.040	1.914	0.074
log(Obs. Diversity $q = 2$) ~ Disturbance rank	Rank	0.087	0.050	1.730	0.103
log(Obs. Diversity $q = \infty$) ~ Disturbance rank	Rank	0.091	0.047	1.946	0.069
Vegetation structure PC1 ~ Disturbance rank	Rank	0.492	0.051	9.703	0.000
log(Est. diversity $q = 0$) ~ Disturbance rank	Rank	0.020	0.024	0.813	0.428
log(Est. diversity $q = 1$) ~ Disturbance rank	Rank	0.031	0.040	0.772	0.451
log(Est. diversity $q = 2$) ~ Disturbance rank	Rank	0.055	0.049	1.112	0.282
log(ρ Redundancy $q = 1$) ~ Disturbance rank	Rank	-0.217	0.074	-2.920	0.010
log(ρ Representativeness $q = 1$) ~ Disturbance rank + Distance to river	Rank	0.0711	0.0227	3.136	0.0068
	Distance to river	-0.0002	<0.0001	-2.401	0.0298

Table S.4 – Moran's I

Results of the tests for spatial autocorrelation performed on the residuals of the best fitting models (Table S.3), for all models where a significant correlation was found.

Model	Observed	Expected	SD	p	Observed - Expected
log(Abundance) ~ Disturbance rank + Distance to river	-0.1074	-0.0588	0.0916	0.5950	-0.0487
log(Obs. Diversity $q = 0$) ~ Disturbance rank	-0.1237	-0.0588	0.0940	0.4901	-0.0649
log(Obs. Diversity $q = 1$) ~ Disturbance rank	-0.1346	-0.0588	0.0879	0.3883	-0.0757
log(Obs. Diversity $q = 2$) ~ Disturbance rank	-0.0950	-0.0588	0.0879	0.6806	-0.0362
log(Obs. Diversity $q = \infty$) ~ Disturbance rank	-0.0788	-0.0588	0.0912	0.8270	-0.0199
Vegetation structure PC1 ~ Disturbance rank	-0.0176	-0.0588	0.0958	0.6669	0.0412
log(Est. diversity $q = 0$) ~ Disturbance rank	-0.0403	-0.0588	0.0920	0.8401	0.0186
log(Est. diversity $q = 1$) ~ Disturbance rank	-0.0492	-0.0588	0.0889	0.9140	0.0096
log(Est. diversity $q = 2$) ~ Disturbance rank	-0.0648	-0.0588	0.0883	0.9464	-0.0059
log(ρ Redundancy $q = 1$) ~ Disturbance rank	0.00095	-0.0588	0.0914	0.5130	0.0598
log(ρ Representativeness $q = 1$) ~ Disturbance rank	-0.1439	-0.0588	0.0924	0.3570	-0.0851