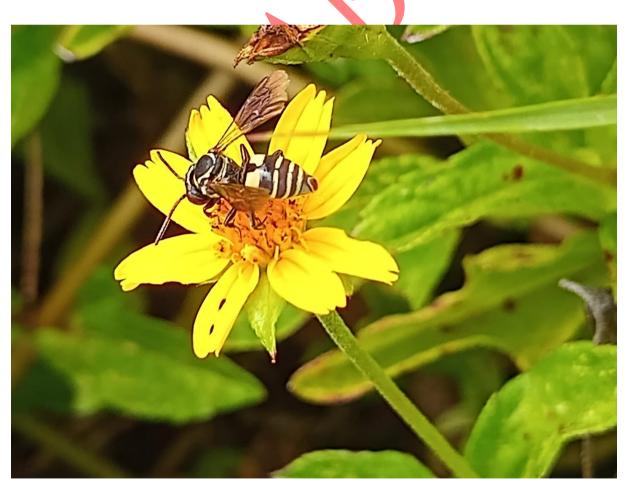
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Eric van den Berghe, Giuliana Caprotti & José Fernando Tercero Iglesias



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Bee richness and abundance at seven sites in San Antonio de Oriente, Honduras

Eric van den Berghe¹, Giuliana Caprotti² & José Fernando Tercero Iglesias³

RESUMEN

Las abejas son de importancia crítica por los servicios de polinización que prestan, Sin embargo, se ha producido un grave declive de sus poblaciones, que puede atribuirse a actividades antropogénicas como el uso de agroquímicos y puede verse agravado por los efectos del cambio climático. La presente investigación utiliza gradientes altitudinales para evaluar el efecto del clima en la riqueza y abundancia de las especies de abejas. Para ello se tomaron datos durante un periodo de 11 meses, obteniendo un total de 393 abejas pertenecientes a 64 morfoespecies. Se halló una correlación directa entre riqueza y abundancia, y una relación inversa entre elevación, riqueza y abundancia. Se observó que las estaciones influían más en la abundancia de abejas que en diversidad (p<0.05). Los resultados obtenidos sugieren que el principal factor que afecta a la diversidad y abundancia de las abejas no es la elevación, sino otros factores como la agricultura convencional y el hábitat. Se requieren medidas adicionales para la conservación de la biodiversidad en paisajes agrícolas, específicamente, los refugios o corredores tienen que ser suficientemente amplios para amortiguar la exposición de abejas de toxinas, o mejor aún, no usar químicos que afectan abejas.

Palabras clave: Servicios de polinización, cambio climático, gradientes altitudinales, trampas malaise, biodiversidad, estacionalidad, abejas silvestres.

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ABSTRACT

Bees are an essential component in any community with flowering plants which makes their global decline a concern. Moreover, this decline can be attributed to anthropogenic activity like the use of agrochemicals and may be exacerbated by the effects of climate change. The present investigation was designed to use altitudinal gradients to evaluate the potential effect of elevation, and seasonality in the richness and abundance of bee species. Malaise traps were run over 11 months with a total of 393 bees collected, belonging to 64 morphospecies. A direct correlation between richness and abundance was found, and an inverse relation was found between richness. and abundance. Precipitation influenced abundance of bees (p<0.05), more than their richness (p > 0.05). The main factor affecting bee richness and abundance wasn't elevation, but agricultural intervention and habitat. Further measures are required for the preservation of biodiversity in agricultural landscapes, specifically refuge areas need to be large enough to provide effective buffers to prevent exposure of bees to toxins, or better yet, abstain from using pesticides that affect bees.

Keywords: Pollination services, climate change, altitudinal gradient, malaise traps, biodiversity, seasonality, wild bees.

INTRODUCTION

Bees are coevolved to pollinate angiosperm ovules (Arindam Das *et al.*, 2018) rendering them essential for many flowering plants. The domestication and commercial production, of honey, pollen, wax as well as pollination services encouraged the transport of *Apis mellifera* to their current global distribution (Hung *et al.*, 2018). Wild bees also play a vital role in agriculture and ecosystem health, due to their ability to pollinate many specialized angiosperms. Serious concerns and economic losses have resulted from recently documented global bee declines (Widhiono *et al.*, 2017).

These losses affect both wild and domesticated pollinators and the plants that depend on their services (Potts *et al.*, 2010; Ramos-Jiliberto *et al.*, 2020). The conservation of bees continues to be a challenging task due primarily to anthropogenic activities and may be exacerbated by climate change. Anthropogenic threats to bee species include: habitat loss, introduced parasites/pathogens, emergent viral diseases, invasive plants, and agrochemicals (Grünewald, 2010).

In fact, the use of agrochemicals is one of the gravest factors related to bee mortality (Siviter *et al.*, 2021). This is caused by the effects of lipophilic compounds like pyrethroids and organophosphates, which are associated with fungicides and herbicides (Belsky y Joshi, 2020).

This not only threatens the health of bees but also contaminates the pollen and nectar that they produce (Mullin *et al.*, 2010). In the U.S. alone, the number of commercial bee colonies dropped from 5.9 million to 2.7 million from the late 1940s to 1995. Additionally, there's an estimated 10 percent loss of production in the U.S. bee industry annually, caused solely by pesticides (Arindam Das *et al.*, 2018). Moreover, throughout the past 70 years, bee occupancy has decreased by approximately 33 percent, which can be attributed to the intensification of agriculture (Duchenne *et al.*, 2020).

While cultivated bees are widely investigated, wild bees suffer from a lack of research regarding the state of individual species and the trends for their decline, which can be attributed to their high diversity and habitat variations. Moreover, honeybees cannot be used as a model species to determine wild bee susceptibility to threats, despite similarities between them, many of their responses vary, highlighting the need to further expand research on wild bees, and monitoring directed solely at them (Wood et al., 2020). Moreover, A. mellifera could have a negative effect on native species, as it is an introduced species that competes for resources, especially considering its massive colonies compared to many solitary wild species (Cunningham et al., 2022). Altitudinal gradients can help predict the impact of global temperatures on species abundance and distributions (Hoiss et al., 2012). The decline of pollinators, in impacts ecosystem services valued at EUR 153 billion worldwide, which is about 9.5% of the world's agricultural economy in 2005 (Gallai et al., 2009). Altitudinal gradients can have a direct or indirect effect on speciation and extinction. Diversification rates in species are likely to be driven by hostplants which are subject to specific climatic conditions (Willig v Presley, 2018). Bees, like all animals, seek to adapt and survive. Therefore, they will likely migrate vertically, tracking commensal plants in the case of specialized species. Nonetheless, it is not the altitude itself species adapt to, but rather the change in conditions (Montesinos-Navarro et al., 2011). On top of that, seasonality, can have an effect on richness of bees, since precipitation alters the availability of resources and the foraging activities of bees (Escobedo-Kenefic et al., 2020).

Temperatures in Honduras are predicted to increase by 1.0-1.5°C by 2030. and up to 2.5°C by 2050 (Perez & Klein 2020). This could impact bee populations in the region, bearing consequences in agricultural landscapes which could also affect crop production because of the pollination services. On top of this, changes in temperature can also affect plant diversity causing breakdown in mutualisms in the native community (Jump y Peñuelas, 2005).

The main objective of this study was to estimate how elevation, seasonality, or factors such as agricultural intervention affects richness and abundance of bee species in the region.

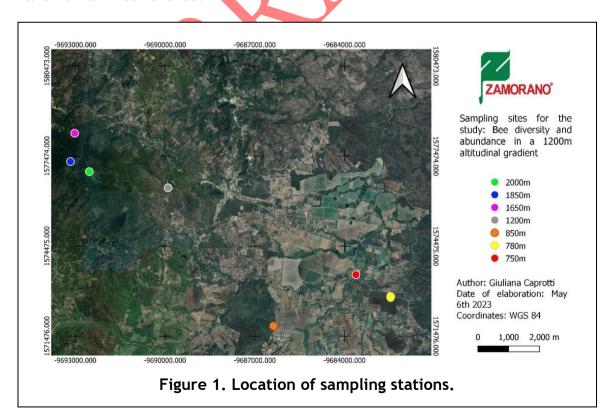
Moreover, the secondary objective was to verify that very low bee abundance documented by Callejas (2022), may be attributable to low elevation on Zamorano campus and not the product of other factors.

METHODS

Description of the Study Site

The investigation took place in seven sampling sites, spread over a 1,200 masl elevational gradient, starting at the Zamorano Agroecological farm 780 masl, up to Uyuca biological reserve 2,000 masl (Figure 1). Sampling sites were selected to meet three criteria: 1) roughly 200 masl elevation intervals and 2) an open area/ clearing suitable for flowers and bee activity to place the Malaise trap, which was emptied monthly, 3) the specific sites were not used for conventional agriculture in the last decade. Bees were sorted manually from the bycatch in a tray with enough alcohol to suspend the material; both bees and bycatch were saved separately for the Zamorano entomological collections.

The sites chosen presented no agricultural intervention, since both Masicarán and Uyuca are conservation areas. The Zamorano agroecological farm, does not use pesticides or chemical fertilizers, but rather organic alternatives (Gliessman, 2018). Traps that were molested were excluded from analyses; this included mammal damage, resulting in tears or collapse and human interference.



All results were standardized to captures per trap per day to compensate for variation in length of set. Traps were also rotated among sites to compensate for trap effects as not all were identical. Also, data from different sized traps were adjusted to compensate for disparities.

Malaise Traps

Malaise traps (Figure 2) are widely used in biodiversity surveys, especially in the case of flying insects like Diptera and Hymenoptera. They work by intercepting insects with their fine mesh netting walls (Skvarla *et al.*, 2021). Once caught within the panels, insects then fly up the sides of the trap where they are funneled into alcohol filled jars. The primary benefits of these traps is low maintenance, and randomized captures, since they can be left in the field unattended for long periods of time (Fraser *et al.*, 2008).



Figure 2. Malaise traps photographed at 1,650 masl, 2,000 masl and 800 masl, respectively.

An advantage when using malaise traps is that they are relatively impervious to the vagrancies of weather (Matthews y Matthews, 2017). This methodology was adapted from Luis Calleja's study, which ended in May 2022 in Zamorano University, and the same statistical analysis was applied. Colored pan traps were discarded for this investigation since Calleja's study (2022), found that these were not as effective as malaise traps in obtaining clean utilizable samples, due to several externalities that affected the proper function of the pan traps, such as wind, rainfall, and frequent molestation by animals. Daily visits were not an option.

Identification of Captures.

The bees were sorted manually from by-catch, pinned, labelled, and photographed using a Cannon EOS Rebel 350-camera mounted on a Leica EZ4 stereoscopic microscope. The images of taxa, and data on captures, are in Appendices A, B. respectively. Photographs were also uploaded to the iNaturalist platform to aid with their identification. Additionally, dichotomous keys were used to support identification by reviewers on the iNaturalist platform. Most were separated into morphospecies, according to the genus or tribe (Appendix A). Precipitation data was used from weather stations located on Uyuca and in the valley recorded daily by workers and forest rangers.

The statistical analyses included Spearman's correlation coefficient, to identify a possible relation between abundance, richness, and altitude. We conducted Linear regressions; Qq-plots with use of residuals followed by Varldents 'transformation to change the residuals' scales when there was a lack of homogeneity; an Analysis of Covariance (ANCOVA) using General Linear Models to identify the possibility of significant differences between the altitudes; as well as LSD (Least Significant Difference) fisher's test, to identify the optimal site for bee richness and abundance, with the use of precipitation as a covariable (Dowdy *et al.*, 2004). The data was analyzed using the program Infostat 2020 with a 95% confidence interval.

The data was standardized to captures per trap per day. Since two sizes of Malaise traps were used for the study, the data was standardized for trap size, small traps run side by side with large traps at the agroecological farm traps, yielded a 1:5 capture relation with big traps.

Table 3. Distribution of morphospecies along the altitudinal gradient.

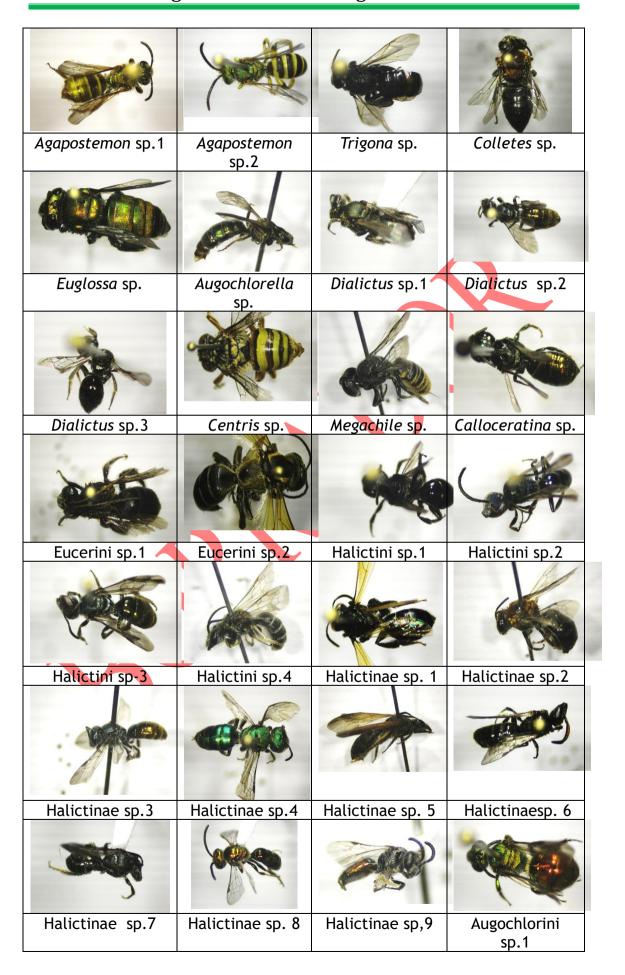
Chasias	2000~	1050m	16E0m	1200m	0E0m	7F0m	790m
Species	2000m	1850m	1650m	1200m	850m		780m
Tris sp.	U	/	37	0	6	0	26
Apis mellifera	1	5	49	2	3	0	5
Centris varia	0	0	0	0	0	0	1
Nannotrigona perilampoides	0	0	0	0	0	0	1
Cephalotrigona zexmeniae	0	0	0	0	0	0	1
Partamona sp.	0	0	8	0	1	0	3
Paratetrapedia sp.	0	0	0	0	0	0	4
Neocorynura sp.	0	, 0	11	0	2	0	0
Lasioglossum sp.	1	0	5	0	1	0	0
Bombus sp.1	0	16	10	0	3	0	0
Bombus sp.2	0	1	0	0	0	0	0
Augochlora sp.1	0	0	1	0	0	0	0
Augochlora sp.2	0	0	2	0	1	0	2
Augochlora sp.3	0	0	1	0	0	0	0
Ceratina sp.1	0	3	4	0	1	0	0
Ceratina sp. 2	0	0	1	0	0	0	0
Ceratina sp.3	0	0	0	0	0	0	1
Sphecodes sp.	0	0	2	0	0	0	0
Dinagapostemon sp.	0	0	0	0	1	0	0
Thygater sp.	0	0	1	0	0	0	0
Halictus sp.1	0	0	1	0	1	0	0
Halictus sp.2	0	0	0	0	0	0	1
Augochloropsis sp.1	0	1	2	0	0	0	1
Augochloropsis sp.2	0	0	1	0	0	0	0
Agapostemon sp.1	0	0	2	0	0	0	0
Agapostemon sp.2	0	0	_ 1	Ô	0	0	0
Trigona sp.	Õ	0	0	0	0	0	2
Colletes sp.	Ö	0	1	Õ	0	0	0

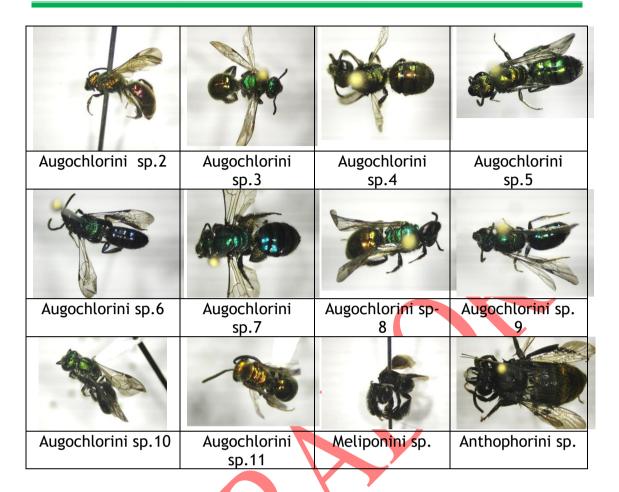
Species	2000m	1850m	1650m	1200m	850m	750m	780m
Euglossa sp.	0	0	1	0	0	0	0
Augochlorella sp.	9	0	1	0	0	0	0
Dialictus sp.1	0	0	58	0	1	0	4
Dialictus sp.2	0	1	4	0	1	0	2
Dialictus sp.3	0	0	1	0	0	0	0
Centris sp.	0	0	0	0	0	0	1
Megachile sp.	0	0	0	0	0	0	1
Calloceratina sp.	0	0	2	0	0	0	0
Eucerini sp.1	0	0	2	0	0	0	0
Eucerini sp.2	0	1	1	0	0	0	0
Halictini sp.1	0	0	1	0	0	0	0
Halictini sp.2	0	1	0	0	0	0	0
Halictini sp.3	0	1	1	0	0	0	0
Halictini sp.4	0	0	1	0	0	0	0
Halictinae sp.1	1	0	5	0	1	0	0
Halictinae sp.2	0	0	0	0	1	0	0
Halictinae sp.3	0	0	1	0	0	0	0
Halictinae sp.4	0	1	0	0	0	0	0
Halictinae sp.5	0	0	1	0	0	0	0
Halictinae sp.6	0	0	1	0	0	0	0
Halictinae sp.7	0	0	2	0	0	0	0
Halictinae sp.8	0	0	0	0	0	0	1
Halictinae sp.9	0	0	0	0	0	0	1
Augochlorini sp.1	0	0	1	0	0	0	0
Augochlorini sp.2	0	0	1	0	0	0	0
Augochlorini sp.3	0	1	16	0	0	0	7
Augochlorini sp.4	0	0	1	0	0	0	0
Augochlorini sp.5	0	0	2	0	0	0	2
Augochlorini sp.6	0	2	1	0	0	0	0
Augochlorini sp,7	0	0	1	0	0	0	0
Augochlorini sp.8	0	0	4	0	0	0	0
Augochlorini sp.9	0	0	2	0	0	0	0
Augochlorini sp.10	0	0	0	0	0	0	1
Augochlorini sp.11	0	0	0	0	0	0	1
Meliponini sp.	0	0	1	0	0	0	0
Anthophorini sp.	0	1	0	0	0	0	0

[Revisar el orden de las especies, *Centris* aparece dos veces. Cuando se pone una tribu o una subfamilia eberia luego decir "g. sp." No solo "sp."]

Bee species and morphospecies from all traps.

Dec specie	and morpho	species from a	tt traps.
Trigona fulviventris	Apis mellifera	Centris varia (Erichson, 1848)	Nannotrigona perilampoides (Cresson, 1878)
- Company of the Comp			
Cephalotrigona zexmeniae Cockerell, 1912	Partamona sp.	Paratetrapedia sp.	Neocorynura sp.
Lassioglossum sp.	Bombus sp.1	Bombus sp.2	Augochlora sp.1
Augochlora sp.2	Augochlora sp.3	Ceratina sp.1	Ceratina sp.2
Ceratina sp.3	Sphecodes sp.	Dinagapostemon sp.	Thygater sp.





RESULTS

A total of 393 wild bees were captured, which were classified into 64 morphospecies. These belonged to two families: Halictidae and Apidae, the latter being the most dominant. The most abundant species were *Trigona fulviventris*, Guerin 1835 and the honey bee, and the most common Morphospecies were *Bombus* sp.1, *Diolictus* sp.1 and *Augochlorini* sp.3, (Appendix B).

The most diverse and species rich site was the Zamorano Agroecological farm. The second site with high richness and abundance was the Uyuca Biological Station. The species with presence on most altitudinal gradients were honey bees, *Trigona* sp., *Fulviventis* sp. and *Dialictus* sp.2 (Appendix B).

Monthly precipitation data was used to establish wet and dry seasons. According to precipitation data, the wet season lasted from June to November, while the dry season lasted from December through March (Figure 3).

The only significant correlation between variables found was between richness and abundance, which are both indicators of healthy ecosystems. This yielded an inversely proportional relation between richness, abundance, and elevation.

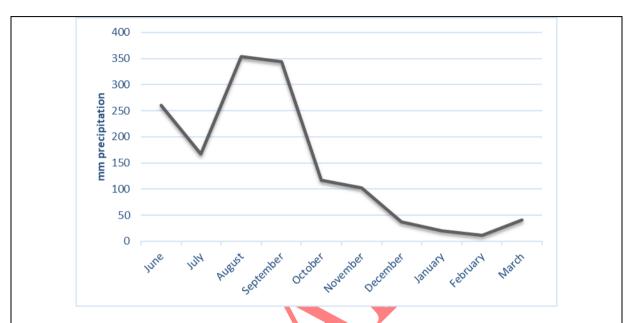


Figure 3 A. Precipitation data from study sites. Monthly precipitation in Uyuca reserve in mm (2022-2023).



Figure 3 B. Precipitation data from study sites. Monthly precipitation in Agroecological farm in mm (2022-2023).

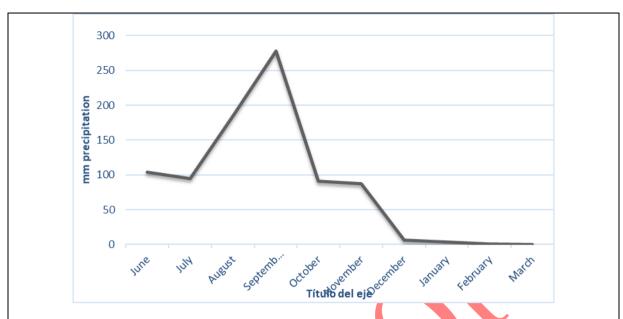


Figure 3 C. Precipitation data from study sites. Monthly precipitation in Masicarán and campus in mm (2022-2023). [quitar "titulo del eje"]

Table 1. Spearman's correlation coefficient for richness and abundance.

Elevation	Precipitation	Richness	Abundance
1.000	0.089	0.216	0.325
0.301	1.000	0.297	0.242
-0.221	0.187	1.000	0.000
-0.177	0.21	0.974	1.000
	1.000 0.301 -0.221	1.000 0.089 0.301 1.000 -0.221 0.187	0.301 1.000 0.297 -0.221 0.187 1.000

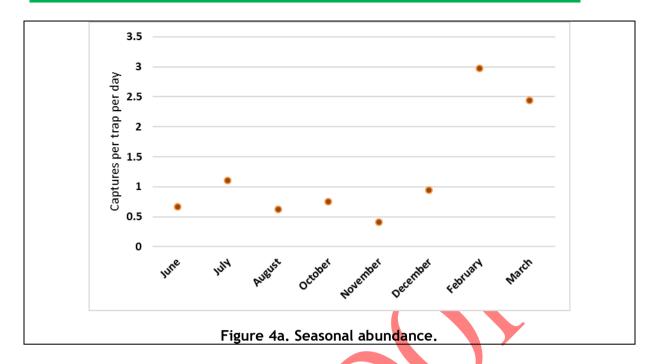
Seasonal abundance and diversity of bees at 1650 m.

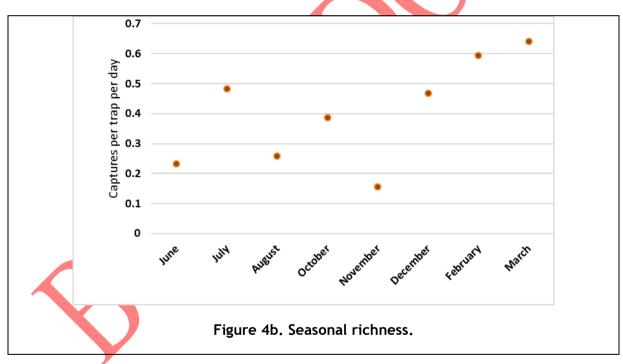
Dry vs. wet season data showed significant differences in abundance with greater abundance in dry season samples, however diversity did not present a significant tendency.

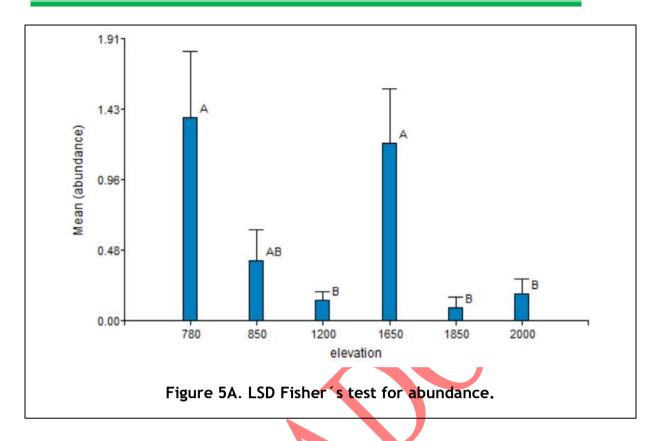
Table 2 Sequential test: Abundance and Richness

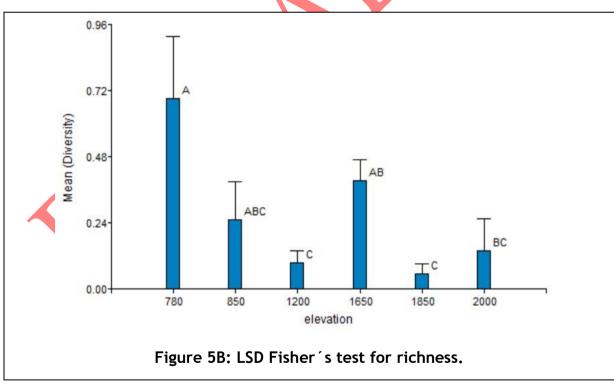
Abundance	numDF	F-value	p-value	Richness	numDF	F-value	p-value
Elevation	5	3.59	0.01	Elevation	5	4.82	0.003
Precipitation	1	8.75	0.01	Precipitation	1	2.35	0.138

A Fischer exact test (figure 5a) yielded two sites that clearly have high abundance, one with intermediate abundance and the remainder with low abundance. The same test (figure 5b) for diversity, followed a similar pattern although some of the differences were not as clear cut.









DISCUSSION

The low abundance at Zamorano in the earlier study (Callejas 2022) was responsible for a very tight relationship reported for abundance, diversity, and elevation. The present study sought to corroborate this and to determine its hold outside the dry season. We also set out to test if the pattern holds for areas outside the influence of conventional agriculture. Our results show that Callejas probably had a spurious correlation with pesticide effects, as the altitudinal relationship disappeared when we included sites that are buffered from conventional agriculture. While Callejas's sites were not directly in fields, they were in a narrow corridor with pesticide use on either side. Neither we, nor Callejas quantified pesticides, nor investigated the specific chemicals in use, so the relationship is circumstantial but the trend is consistent with the negative effects of pesticides on wild bee communities, shown by Park et al. (2015), even a year after their application (Parke et al. 2015).

The present results clearly reveal the agroecological site (750 masl) to have much richer diversity and greater abundance on parr with the Uyuca Biological Reserve (1650 masl (Fig 5) with values comparable to those reported by Callejas for the reserve. Callejas' lower elevation sites (such as the Zamorano eco-trail) were all directly adjacent to areas with conventional agricultural presence, and this proximity is likely to have influenced his findings.

Some areas called "ecological buffers" pose safe havens for animals and insects alike when surrounding areas are inhospitable for them due to factors like agricultural intervention or pesticide use. However, it's important to evaluate how large these areas need to be so that they are buffered from effects of pesticides carried by wind or bodies of water. An example of that is the Zamorano Eco-trail, which has been sampled for bee communities by both Callejas (2022) and Mazariegos (2022), Mazariegos in fact sampled directly for differences in the ecotrail and adjacent agricultural areas across a variety of crops.

Mazariegos found marginally higher, albeit not significantly higher, abundance and diversity on the eco-trail sites compared to the adjacent conventional crops. This is in marked contrast to the result from a study of spider diversity and abundance in adjacent areas on the eco-trail (Navarro 2019). Navarro found much higher abundance and diversity on the narrow strip that is the eco trail. The difference with the bee studies may be explained by the relatively sedentary nature of spiders whereas, bees forage over significant distances and are hence more vulnerable to practices in adjacent areas.

Influence of Precipitation on Richness and Abundance.

Sequential tests were applied for both richness and abundance (Table 2), with the use of precipitation as a covariable. This, in turn, showed that precipitation influenced abundance (P<0.05) of bee species, though it had no effect on the richness of these (P>0.05). The results of a study published in 2021 by the United States Geological Survey and Native Bee Inventory showed that weather conditions are effective at predicting diversity and abundance of bees, compared to landscape conditions and topography of the site. Wild bee abundance in their study suffered a decrease in summer. where precipitation was low (Kammerer et al., 2021). Our results, showed the opposite pattern: we found fewer bees during the wet season. We surmise that our lower capture rates, but not lower diversity, during rainy periods, indicate that the bees are present, but simply less active under rainy and overcast conditions. This also explains the abundance pattern found inside the reserve where the highest elevation sites in cloud forest had few bees consisting primarily of large bodied bumblebees (Bombus sp.) which have an advantage in maintaining core temperature under colder conditions.

In the case of richness, the relationship was not as tight which is consistent with Classen *et al.* (2015). There are other factors that come into play, like hostplant diversity, which may result in an accelerated evolutionary radiation (Classen *et al.*, 2015).

Comparison of Richness and Abundance along the Altitudinal Gradient.

The Fischer exact test for abundance and richness (Figure 5) showed that in terms of abundance, 780 masl and 1650 masl had no statistically significant differences, though differences were found along the rest of the gradient. On the other hand, with richness, statistically significant differences were found between 780 masl and 1650 masl, the first being the richest site. The common factor for the high abundance sites is distance from areas where agrochemicals are used, one being the biological reserve and the other, the core of the Agroecological farm.

Seasonality

For the Uyuca Biological Station seasonality analysis, no statistically significant differences were observed for the variable abundance (p = 0.06), unlike richness, where significant differences were found (p < 0.01), resulting in higher richness for the dry season compared to wet season (figure 4 A. B). Previous studies on seasonality and bees have proven that seasonal changes affect both the presence of bees and flowering plants. A study on climate and seasonal bee richness along a tropical elevational gradient showed that bee richness decreased with an increase in precipitation, and that it decreased linearly and significantly with elevation (Dzekashu *et al.*, 2022). However, habitat, forest types, and anthropogenic activity may also interact with richness and abundance (Quintero *et al.*, 2010).

CONCLUSIONS

The best site in terms of both richness and abundance for this study was 780 masl, also known as the Zamorano agroecological farm. Elevation was found not to be the most influential variable for bee richness and abundance, whereas other factors such as habitat and pesticide use associated with conventional agriculture appears to exert a greater impact on the presence and richness of bees. Low bee abundance for lower elevation sites found by Callejas in 2022 is likely due to the proximity of his sites with areas using conventional agriculture. In order to boost bee abundance in depauperate areas, the ecological corridors will require significant expansion of pesticide free zones beyond narrow wooded corridors which work for connectivity, but do not provide a safe haven for bees.

Seasonality was found to influence abundance of species, with higher abundance during the dry season, but had no effect on richness of wild bee species.

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