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The effects of agroforestry and conventional banana plantations on multiple dimensions of butterfly diversity in the Atlantic Forest

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Abstract

Environmental changes, particularly in agriculture, contribute significantly to biodiversity loss, with habitat fragmentation hindering dispersal and reducing biological diversity. Specific land uses can confine evolutionary groups to certain areas, decreasing local genetic and phylogenetic diversity but potentially increasing them at a larger spatial scale. Utilising genetic information at the population level, along with richness, phylogenetic and composition data at the community level, offers a comprehensive understanding of agriculture's impact on biodiversity. In this study, we compared the effects of conventional and agroforestry banana plantations on butterflies' dispersal and diversity relative to native forests. Analysing ddRAD genomic data from *Heliconius ethilla narcaea* at the population level and assessing richness, abundance, phylogenetic and species diversity of the Nymphalidae family at the community level, we found that agroforestry plantations exhibited the highest butterfly abundance and maintained the rarest genetic groups from *H. ethilla narcaea*. These genetic groups appeared in both native forests and agroforestry areas, more often in native forests, but were absent in conventional plantations despite evidence for extensive genetic dispersal. Regarding species composition, both banana plantations shared similar species but differed from the native forest, which contained a distinct and phylogenetically clustered group of species, possibly due to its unique microhabitat conditions and more complex structure. The presence of the rarest genetic groups in the population and the occurrence of distinct biological species emphasise the critical role of native forests within an anthropogenic landscape. Agroforestry demonstrates the potential to sustain biodiversity alongside food production.

Keywords Agriculture · ddRAD · Microevolution · Phylogenetic relationship · Pesticide use

Communicated by Louise Ashton.

Extended author information available on the last page of the article

Introduction

Agriculture has transformed human life and played a pivotal role in our evolution. However, anthropogenically induced changes related to agricultural intensification result in habitat loss, fragmentation, and environmental homogenization, contributing to biodiversity loss (Tilman et al. 2002). Particularly, chemical pollution, such as pesticides, is identified as the second most significant driver for the global decline of insects (Sánchez-Bayo and Wyckhuys 2019). Since the ‘Green Revolution’ marked by the development of synthetic chemicals with the idea of feeding the growing global human population, there has been a notable increase in pesticide use, further exacerbating the decrease in biodiversity (Tilman et al. 2001). As a result, urgent large-scale planning is needed to prevent spiralling costs (Varah et al. 2020) and to integrate the preservation of yield with biodiversity (Wurz et al. 2022).

Numerous scientists have warned about insect extinctions due to pesticide use (Cardoso et al. 2020). Pesticide use has been linked to a reduction in species richness (Braak et al. 2018), and organic farms have been found to host a greater diversity of butterfly species than conventional farms (Feber et al. 2007). In addition, several studies have found that organic practices also increase the functional diversity of insect communities, supporting important functional groups like predators and pollinators (Letourneau and Bothwell 2008; Krauss et al. 2011). Herbicide use may also indirectly impact species like butterflies that rely on herbs as host plants during their life cycle (Mallick et al. 2023). Saunders et al. (2017) evidenced an association between glyphosate levels and local reductions in adult monarch (*Danaus* spp.) populations. At the microevolution level, pesticides select specific genetic variants that confer resistance to these chemicals (Fouet et al. 2018). This intense selection pressure can potentially eradicate genetic variation from the population, posing a potential threat to non-pest species by reducing their ability to adapt to other environmental challenges. In an agricultural context, balancing biodiversity and yields represents a significant challenge in addressing the biodiversity crisis, particularly in tropical agricultural landscapes (Wurz et al. 2022).

Banana plantations are one of the main crops replacing natural South Atlantic Forest habitats, one of Brazil’s most threatened and impacted biomes. Only 14% of this biodiversity hotspot remains (Myers et al. 2000; Ribeiro et al. 2009). Large-scale banana production using pesticides started in the 1960s. In 1991 several banana producers adopted organic agroforestry methods where the banana crop is cultivated among trees and natural vegetation without pesticides or other synthetic chemicals (Gonçalves 2008). Conventional and agroforestry areas are cultivated in small holds, keeping some native forest patches inside farms. A previous study indicated that agroforestry banana production systems exhibited superior economic performance compared to conventional farms when considering net income per hectare and labour productivity (Gonçalves 2008). Given this context and acknowledging the high biodiversity present in the Atlantic Forest (Brown and Freitas 2000; Santos et al. 2020), it is crucial to evaluate the impact of pesticide use on biodiversity at the landscape scale.

Although dispersal and diversity are interconnected metrics (Albright and Martiny 2018) as well as genetic and species diversity (Vellend et al. 2014), the combined use of these approaches allows us to assess distinct dimensions of biodiversity in an agricultural context, with or without the use of pesticides. Dispersal plays a crucial role in shaping the genetic diversity of populations, serving as a key factor for evaluating different biological levels,

connecting populations and communities, and can be assessed indirectly, for example, by determining whether individuals from distinct populations share similar genetic information, or whether communities share similar species. Additionally, dispersal contributes to greater local diversity by introducing variants from neighbouring demes and communities while leading to a homogenisation of diversity at the landscape scale (Ronce 2007).

Due to their rapid response to both biotic and abiotic conditions (Diekötter et al. 2007), well-resolved phylogenetic trees and reference genomes (Chazot et al. 2019), butterflies provide an excellent system for assessing anthropogenic effects using eco-evolutionary information. Among butterfly families, Nymphalidae stand out as one of the most diverse and extensively studied groups. Within the Nymphalidae, the genus *Heliconius* offers extensive information about ecology, behaviour, genetics, and phylogenetics, with sequenced genomes available for several species (Jiggins 2018).

Heliconius ethilla ranks among the most abundant butterfly species in the Atlantic Forest, spanning the Neotropics from Panamá to South Brazil (Zhang et al. 2016). In the southernmost part of its distribution, the species coexists with banana plantations, making it valuable for investigating the population and community eco-evolution effects of land use in this region. *Heliconius ethilla* adults exhibit relatively long lifespans, with a substantial mark-recapture study in Trinidad reporting a maximum age of 161 days and a mean of 50 days (Ehrlich and Gilbert 1973). While this study suggested high site fidelity among adult butterflies, subsequent research in other *Heliconius* revealed long-distance early adult pre-breeding dispersal (Mallet 1986). Population genetic studies generally find limited genetic differentiation between populations across large distances (Jiggins 2018). Despite fluctuations in the number of individuals throughout the year, *H. ethilla* has a constant presence (Andrade and Freitas 2005), possibly due to its high ecological plasticity. For instance, in terms of host plant use, *H. ethilla* prefers *Passiflora alata* and *P. edulis*, however, in the absence of these plants, it demonstrates the flexibility to explore other Passifloraceae (Silva et al. 2014). In southern Brazil, we encountered *H. ethilla narcaea*, a subspecies more restricted to the Atlantic Forest and related habitats, and observed its abundance across various areas in this biome, ranging from lowlands to highland forests (Iserhard and Romanowski 2004; Iserhard et al. 2010; Bellaver et al. 2012).

Our general goal was to assess whether different farming techniques, namely agroforestry (cultivation amongst native plants and without synthetic chemicals) and conventional banana plantations (treated with pesticides, fungicides, and herbicides), acted as barriers to dispersal and influenced diversity at the population level within *H. ethilla narcaea* and the community level within Nymphalidae, in comparison to native Atlantic Forest (Fig. 1). Specifically, we examined: (i) the effect of treatment on population genetic structure in *H. ethilla narcaea*; (ii) the impact of treatments on butterfly abundance and richness, plus diversity, at the population level using genetic information from *H. ethilla narcaea*, and at the community level by examining phylogenetic and species composition of Nymphalidae; (iii) the effect of treatment on dispersal by examining between-site differences, at both the population and community levels; finally, (iv) the effect of treatment on phylogenetic diversity at the population and community level.

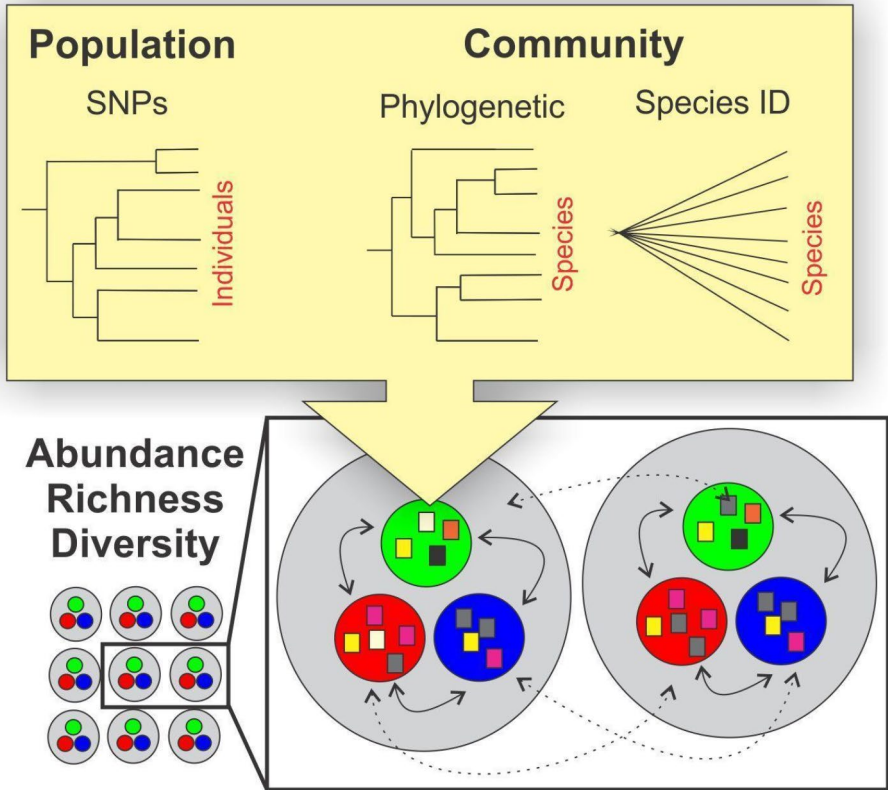


Fig. 1 Eco-evolutionary approaches used to assess the effect of agriculture compared to native forest. Abundance, richness and diversity were assessed using data from population and community levels, respectively, genetic distance between individuals using single nucleotide polymorphisms (SNPs), as well as phylogenetic and species composition distances within and between sites. A total of nine distinct blocks were evaluated (grey circles in left below). The scheme in the centre represents a zoom-in of two blocks containing three different types of treatments in each block (red: conventional agriculture, blue: agroforestry agriculture, green: native forest). Coloured squares denote individuals belonging to distinct species or genetic groups. Continuous arrows indicate dispersal between different treatments, while dashed arrows represent dispersal within the same type of treatment

Materials and methods

Sample collection

Butterfly sampling occurred from January to April 2016 in the northeast of the southernmost state of Brazil, Rio Grande do Sul (Fig. 2), under permit (SisBio permanent permit no. 45673-1). We collected butterflies in 27 sites designed in nine blocks. Each block was composed of three treatments: conventional and agroforestry banana plantations, and native forest (Fig. 2). The conventional banana plantations were sites where the banana crop was treated with the insecticide Furadan, fungicides Manzate and Tilt, and herbicides Tordon and Roundup-Glyphosate, while in the agroforestry no synthetic chemical treatment was

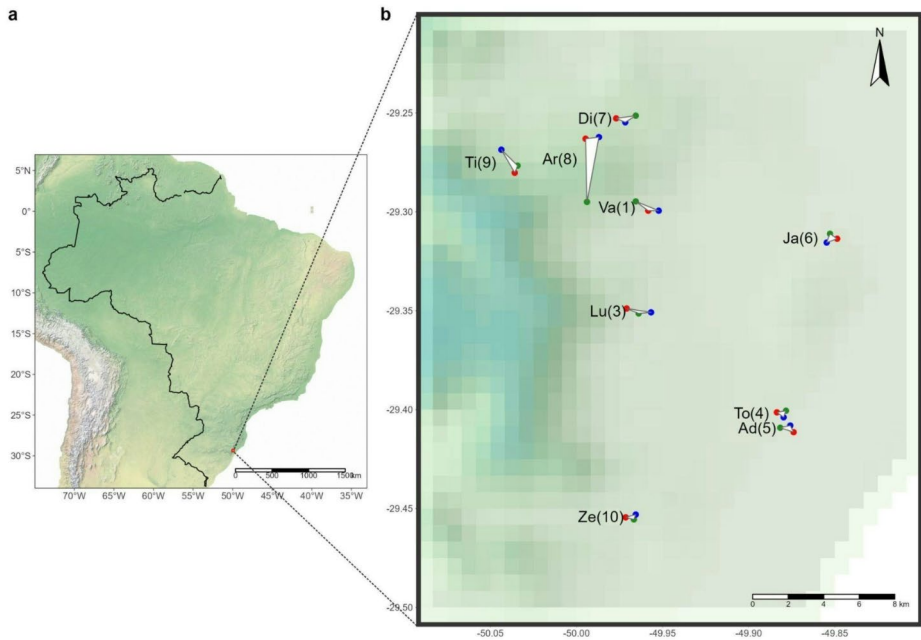


Fig. 2 Location of the sampling sites in the northeast of the Rio Grande do Sul state (Brazil). **(a)** Geographic location of the sampling sites in Brazil is represented in the red square in the South portion of the country. **(b)** The 9 polygons connecting three distinct treatments (Native Atlantic Forest (green), agroforestry (blue) and conventional (red) banana farms) correspond to each sample block. Their respective names are written on the left side of each polygon using 2-letters code and a number in parenthesis; this number was used to identify the site in the individuals used for genetic analysis

used and the bananas were cultivated amongst other native plants, maintaining, in part, the forest structure. Finally, the native forest consisted of areas with native Atlantic Forest vegetation, without plantations or farming.

Hand nets were used for butterfly collection, a method proven to sample more butterfly species in the Neotropics compared to alternatives like bait traps (Checa et al. 2019). This approach is particularly efficient for sampling nectar-feeding butterflies, which constitute 85% of the species found in the Atlantic Forest. It is an effective method for comparing biodiversity in different areas (Iserhard et al. 2013). The three sites corresponding to each treatment (conventional, agroforestry farms, and native) within the same block were sampled on the same day by the same three researchers using butterfly nets with similar catching abilities. They walked at a constant pace through a transect in each of the 27 sites, minimising collector bias (Freitas et al. 2021). Sample effort was standardised at 1 h and 30 min of net catching per person per site (totalling 4 h and 30 min of sampling in each site per occasion), conducted between 9 am and 4 pm, exclusively under sunny weather conditions with less than 50% cloud cover and no rain (Iserhard et al. 2013, 2017; Freitas et al. 2021). This time period aligns with the peak activity for most butterfly species sampled with this technique in subtropical regions (Iserhard et al. 2017). The sampling order was randomised, and the treatments were sampled at the beginning, middle, and end of each sampling period. Each site underwent three rounds of sampling, resulting in a total of 13 h and 30 min of sampling

time for each site and 121 h and 30 min for each treatment. Each selected treatment had an area of at least 2 hectares and a distance of 200 m between each transect and block (Fig. 2). One exception was block “Ar” (Fig. 2), where no representative native area was found nearby the agriculture treatments, resulting in a more spread block compared with others. Butterfly species and numbers were recorded, and individuals were collected and stored in entomological envelopes for species identification and subsequent genetic analysis in the laboratory. *Heliconius ethilla narcaea*, a species with a relatively equal distribution among treatments, was collected in 21 of 27 sites (Tables S1 and S2) and eight of nine blocks. Therefore, it was chosen for the population-level genetic analysis.

Access to genetic heritage was registered in SISGEN (National System for the Management of Genetic Heritage and Associated Traditional Knowledge) under the ADFC306 number, and DNA extracted from these individuals was transported to the UK in 2017 under a Material Transport Declaration from the Universidade Federal do Rio Grande do Sul to comply with Brazilian law. The *rnatuarearth* (Massicotte and South 2024) package was used to create a map in R statistical software (v4.3.2, R Core Team 2023).

Population structure

Population structure was assessed using genetic information obtained through double-digest restriction-associated DNA sequencing (ddRADseq) in *H. ethilla narcaea* (refer to Appendix S1 for details on genomic library preparation, sequencing, and analysis of *H. ethilla narcaea*). Sequence reads were aligned to the *H. melpomene* (v2) reference genome (The Heliconius Genome Consortium 2012) (Davey et al. 2016) and variants called using UnifiedGenotyper in GATK (v2.5.2). After filtering, a total of 6213 single nucleotide polymorphisms (SNPs) genotyped in 65 *H. ethilla* individuals were used to analyse population structure (Fig. S1).

Firstly, the admixture coefficients from the genotype matrix were assessed (Pritchard et al. 2000; François and Durand 2010) in the landscape and ecological association studies (LEA) package (Frichot and Francois 2018). LEA has a similar Bayesian clustering algorithm to STRUCTURE (Pritchard et al. 2000) and uses an entropy criterion to assess the number of ancestral populations that best explains the genotypic data, determining the number of principal components to predict the ancestral population number (Alexander and Lange 2011; Frichot et al. 2014). The fit of the statistical model to the genetic data was assessed using a cross-entropy technique, where a lower cross-entropy value indicates better model performance.

Afterwards, discriminant analysis of principal components (DAPC) (Jombart 2012) was performed to assess the genomic clusters in the genetic data. DAPC optimises the variance between groups and minimises the variance within groups. In a high dispersal environment, this method allows for better distinguishing of the effect of a specific variable among highly similar groups. For the DAPC we selected the maximum number of PCs that could explain the cumulative variance while suggesting more than one cluster based on the BIC result. Under this assumption the total number of PCs that resulted in more than one cluster was 15, explaining 37% of cumulative variance. We applied this approach ten times to ensure consistency of the results. To quantify the level of genetic differentiation between the clusters identified in the first run, we calculated the pairwise F_{ST} between clusters, using the Weir

and Cockerham (1984) method implemented using *genet.dist* in the *hierfstat* R package (Goudet et al. 2022).

To verify whether the treatments affected the genetic groupings and determine which genetic groupings were affected, we used the probability of each treatment presenting distinct genetic groupings. We calculated the probability of each treatment presenting distinct genetic groupings as the average probability of individuals showing genetic groupings from each treatment. These membership probabilities for each of the genetic groups being found in the three distinct treatments were compared using a beta distribution in a generalised linear model (GLM) with a logit link function in template model builder (TMB) *glmmTMB* package (Brooks et al. 2017). Considering there is zero probability of some genetic groups being present in a particular treatment, we included zero inflation in the analysis. Finally, the model adequacy was tested by assessing the homogeneity of residuals in *qq-plots* using the *DHARMA* package (Hartig 2022).

Treatment effects on within-site population and community diversity

The abundance of individuals and species within sites was compared according to their treatment type employing an ANOVA test. Subsequently, Tukey tests from the *stats* package were utilised to discern specific differences between pairs using R statistical software (v4.3.2, R Core Team 2023).

Diversity was evaluated using Rao's quadratic entropy at each site. This metric assesses diversity-based dissimilarity matrices, whether or not they include evolutionary distances. In cases where phylogenetic information is absent, as in species composition data used here, the diversity measured using Rao's quadratic entropy is equivalent to that predicted using the Simpson index. The *H. ethilla narcaea* dissimilarity matrix was constructed using genetic distance measured as 1 minus IBS (Identity By State) base on the SNP data in TASSEL (Trait Analysis by aSSociation, Evolution and Linkage v. 5.2.43) (Bradbury et al. 2007). Specifically, IBS calculates the probability that alleles drawn randomly from two individuals at the same locus are identical.

The phylogenetic composition of each site was assessed using an existing genus-level phylogeny for Nymphalidae (Wahlberg et al. 2009), refined to the species level where necessary (Appendix S2: Nymphalidae phylogenetic analysis). A phylogenetic fuzzy weighting approach (Pillar and Duarte 2010; Duarte et al. 2016) was applied, wherein species composition at each site was weighted by pairwise phylogenetic distances between species. This was performed using the *matrix.p* function of the *SYNCSA* R package (Debastiani and Pillar 2012).

Treatment effects on between-site population and community diversity

We assessed how similar or dissimilar sites were to each other to determine whether treatment affected site similarity. The same dissimilarity matrix used previously for *H. ethilla narcaea* and at the community level (both including and excluding phylogeny) were used here to assess dissimilarity between sites.

The distance between two sites regarding genetic and species composition was calculated using Bray-Curtis metric with the *vegan* package (Oksanen et al. 2017). We did this both including phylogenetic community composition, and considering all species to have

the same distance from each other (Fig. 1). To determine whether the treatment type had an effect on the difference between sites, the effect of the treatment on the distance matrices, both at population and community levels, within each experimental block (each containing the three treatment types) was evaluated using the *adonis2* function in a PERMANOVA analysis (Oksanen et al. 2017) with 999 permutations. Tukey tests were employed to identify pairwise differences when there was a treatment effect between groups.

Cluster dendrograms were constructed using complete agglomeration method in *hclust* function in the *stat* package in R statistical software (v4.3.2, R Core Team 2023). This visualisation aims to represent the distances between individuals (genetic distances) in *H. ethilla narcaea* and between communities at each site in Nymphalidae (phylogenetic and species composition), focusing on the treatment type.

Additionally, we assessed the extent to which populations and communities from different treatment groups differed, using a multivariate homogeneity of group dispersions (variances) analysis with the *betadisper* function in the *vegan* package (Oksanen et al. 2017). We utilised the previously generated distance matrices: genetic distance between individuals from *H. ethilla narcaea* and site distance considering phylogenetic and species composition within Nymphalidae (Fig. 1). In this context, distances among group centroids were reduced to principal coordinates, and the residuals from a statistical model including the effect of treatment were permuted to generate a random distribution of F under the null hypothesis, assuming no difference in dispersion between groups. Subsequently, pairwise comparisons of mean dispersions among groups were performed.

Treatment effects on phylogenetic diversity at the population and community level

We evaluated phylogenetic diversity within treatments by constructing genetic and phylogenetic distance matrices between sites at the population and community levels, respectively. To assess whether the treatment significantly affected phylogenetic diversity, we compared these matrices to a null model created by randomising the tip labels (Gotelli 2000). The distance matrices were constructed using the mean pairwise distance (MPD) and the mean nearest taxon distance (MNTD). MPD considers the entire phylogeny and more ancient relationships, while MNTD reflects phylogenetic structure closer to the tips and more recent divergence. Since MPD and MNTD utilise phylogenetic distance, species composition was not evaluated in this context (Fig. 1). For the community comparison, we also computed MPD.n and MNTD.n, which weight the distance measures by species abundance. We utilised the *raoD*, *ses.mpd*, and *ses.mntd* functions in the *picante* package (Kembel et al. 2010) to assess Rao's quadratic entropy, MPD, and MNTD, respectively.

Results

Nymphalidae sample composition

Within the Nymphalidae as a whole, the samples totalled 1518 individuals (Table S1). Some species that were difficult to identify precisely were grouped at the genus level, for example, *Actinote* spp., *Pteronymia* spp., *Tegosa* spp., *Junonia* spp., *Blepolenis* spp., *Opsiphanes* spp., *Ypthimoides* spp., *Hermeuptychia* spp., and *Catonephele* spp. Besides that, *Marpesia*

chiron (1 individual in conventional plantation), *Marpesia petreus* (1 individual in native and 1 individual in agroforestry) and *Marpesia zerynthia* (1 individual in conventional and 1 individual in agroforestry plantation) were grouped into *Marpesia* spp. because of undetermined phylogenetic relationships. The final data set comprised 89 Nymphalidae taxa (genus or species) (Table S1; Fig. S2).

The species with the most even distribution among the treatments share a remarkable tiger colour pattern. Belonging to a Müllerian mimicry system, *H. ethilla narcaea*, *Mechanitis lysimnia* and *Placidina eurynassa*, were considered potential candidates for population analysis as they were widely spread among the treatments. Other species within the same tiger mimicry ring, *Eueides isabella*, *Lycorea halia*, and *Consul fabrius*, were also recorded, but were more restricted in number and between treatments. While *Eueides isabella*, and *Lycorea halia* are not documented in the native forest areas, *Consul fabrius* was not found in the conventional plantations (Table S1). *Heliconius ethilla narcaea* was selected for the population genetic analysis, as reference genomes were not available for the other genera at the time.

Population structure

LEA analysis (Frichot and Francois 2018) suggested there was only one ancestral population within the genomic data (Fig. S3a), indicating that *H. ethilla narcaea* shows very little genetic population structure. The DAPC analysis (Jombart et al. 2010), which maximises the differences among genetic groups, suggested the genetic group number could vary between three and eight distinct clusters (Fig. 3; Fig. S7) across our ten runs, which is consistent with a lack of strong structure within the data. Our first run identified six genomic clusters (Fig. 3a; Fig. S3b) three of which were common (clusters one, four, and six) and three that were less common (clusters two, three, and five). Clusters two and five were the most genetically distinct in the PCA (Fig. 3; Fig. S4; Table S4), and based on between-cluster F_{ST} (Table S5), with the highest pairwise F_{ST} between these two clusters (0.186). Across the additional nine runs of the DAPC analysis, although the number of clusters varied, certain rarer clusters were always identified and there was some consistency in the individuals identified as belonging to these clusters (Fig. S7). In all cases, rare and distinct genetic clusters were found in the native forest and agroforestry that were completely absent in the conventional plantations (Fig. 3; Fig. S7). For example, in our first run clusters two, three, and five occurred only in native and agroforestry sites (Fig. 3b; Fig. S4; Table S4).

Across most of our runs, the rarer genetic clusters that were absent from the conventional plantations were not otherwise spatially restricted, and were sampled in plots belonging to different sampling blocks (e.g. in our first run, cluster two is present in blocks Ja and Lu, while cluster three is present in blocks Ad and Lu, Fig. 3c). This suggests that their absence from conventional plantations is not due to limited spatial distribution of these genotypes. On the other hand, across most of our runs (8 of 10) we found one cluster (cluster five in Fig. 3) that was present in only the most distant sampling block (Ze), suggesting this may be spatially restricted, but it was consistently absent from the conventional plantation plot in this block (Table S4).

Despite the continuous genetic landscape indicated by our LEA analysis, we find an impact of treatment on the likelihood of affiliation with distinct genetic groups when using the first 15 PCs (Fig. 3b; Fig. S7c). However, its significance is not completely consistent

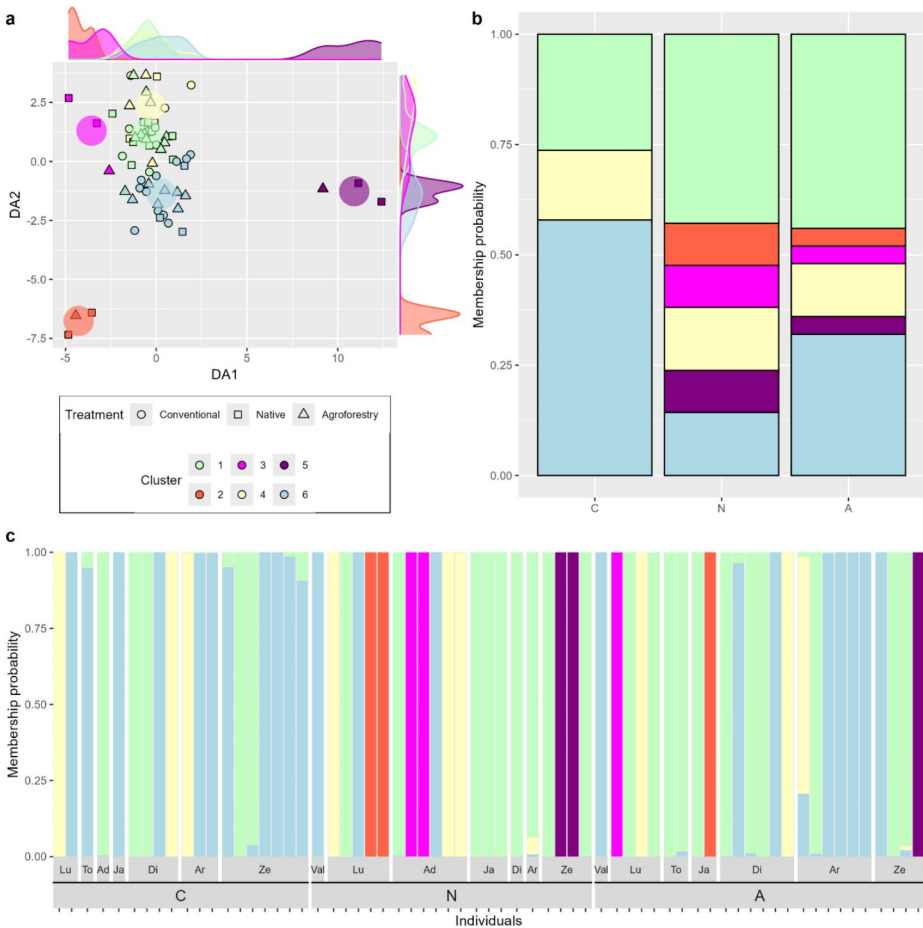


Fig. 3 Genetic clusters from *Heliconius ethilla narcaea*. **(a)** The discriminant analysis of principal components (DAPC) used a total of 15 PCs which represents 37% of the cumulative variation. Individuals are coloured based on their genetic group, while the treatments are represented per distinct shape and the group centroid as a big circle. **(b)** The membership probability proportion for each treatment type according to the six genetic groups from the DAPC analysis. Each column represents the summed membership probabilities of all individuals from each of the 3 treatments. **(c)** The individual membership probability to each of the six clusters identified from the DAPC analysis. Their respective sites are named below, according to the block name (Fig. 2), and grouped by treatment type: conventional plantation (C), native forest (N), and agroforestry (A)

between all runs we performed. Generalised linear mixed models showed good fit (Fig. S5), and confirmed the significance of the interaction between native forest habitat and genetic composition in 3 runs, as shown in Table 1, ($p=0.025$), and Table S6 for runs 05 and 06, suggesting that membership probability to the different genetic groups differed significantly from chance in the native forest. Furthermore, we find significant disparities in the estimated log odds for native forest in three of our runs (Table 1 and Table S6) and conventional agriculture in run one (Table 1). The native forest had the highest membership probability of the rarest genetic groups in all analyses performed and less of the common genetic groups. Spe-

Table 1 Assessing the impact of treatment on the probability of membership to distinct genetic groups in *H. ethilla narcaea* using a generalized linear mixed model. Corresponding standard errors (SE) and significance (*) are provided for each parameter, based on a total six genetic groups across three treatments

Parameter	Estimate	SE	p - value
Conventional farm	-1.568	0.799	0.0497*
Native area	2.394	0.983	0.0148**
Agroforestry farm	0.093	0.979	0.3402
Conventional: Genetic group	0.025	0.180	0.1749
Native: Genetic group	-0.512	0.229	0.0256**
Agroforestry: Genetic group	-0.245	0.232	0.2922
Zero-inflation model	-1.609	0.632	0.0109**

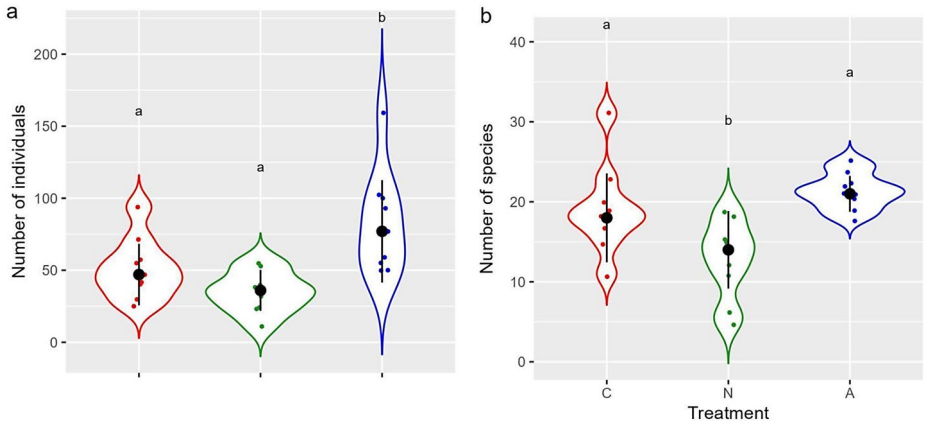


Fig. 4 Abundance and richness by treatment. (a) The number of Nymphalidae individuals and (b) Species captured per site in each treatment. Conventional plantation (C: red), native forest (N: green), agroforestry (A: blue). Different letters above each boxplot indicate differences among treatments based on Tukey tests

cifically, rarer genetic variants exhibit a pronounced preference for native habitats (genetic clusters two, three and five, Fig. S6), contrasting with more ubiquitous genetic clusters that span across various treatment conditions (Fig. 3). This result is consistent when we look at the quantitative data (Fig. S7c), but its significance varied between runs (Table S6).

This statistical analysis does not consider that our sample size is relatively small and slightly skewed towards the native and agroforestry areas (19 individuals in the conventional plantations versus 21 and 25 in native forest and agroforestry respectively). Therefore, it is possible that with further sampling we may have found the rarer genotypes in the conventional banana plantations, and a more even distribution of genotypes across the treatment types.

Treatment effects on within-site abundance, richness and diversity

We observed differences in the number of individuals among the treatments ($F_{2,24} = 8.41$, $p < 0.001$, Fig. 4a). The mean, standard deviation and total number of individuals was largest in agroforestry area (82.78 ± 35.53 , 745 individuals), as compared to conventional area (51.22 ± 21.32 , 461 individuals), and native forest (34.67 ± 14.18 , 312 individuals). The number of species also varied between treatments ($F_{2,24} = 8.97$, $p = 0.001$, Fig. 4b), with both banana plantations, agroforestry (21.33 ± 2.24) and conventional (19.11 ± 5.55), host-

ing more species than native forest (12.78 ± 4.84). The highest number (31 species) was recorded in a conventional area (Ti block), while the smallest numbers (5 and 6 species) were observed in native areas (To and Di blocks, respectively).

We further assessed the effect of treatment on diversity by Rao's quadratic entropy. In *H. ethilla narcaea* populations it was marginally non-significant ($F_{2,18} = 0.55$, $R^2 = -0.05$, $p = 0.059$; Fig. 5a; Table S7) but did follow the trend identified in the population analysis of reduced diversity in the conventional plantation populations and higher diversity in the native forest and agroforestry. Similarly, Nymphalidae phylogenetic diversity ($F_{2,24} = 2.80$, $R^2 = 0.12$, $p = 0.08$; Fig. 5b; Table S7) and species diversity ($F_{2,24} = 0.38$, $R^2 = 0.05$, $p = 0.68$; Fig. 5c; Table S7) did not exhibit significant differences between treatments, but sites appear to be more similar in diversity in conventional plantations compared with agroforestry and native forest.

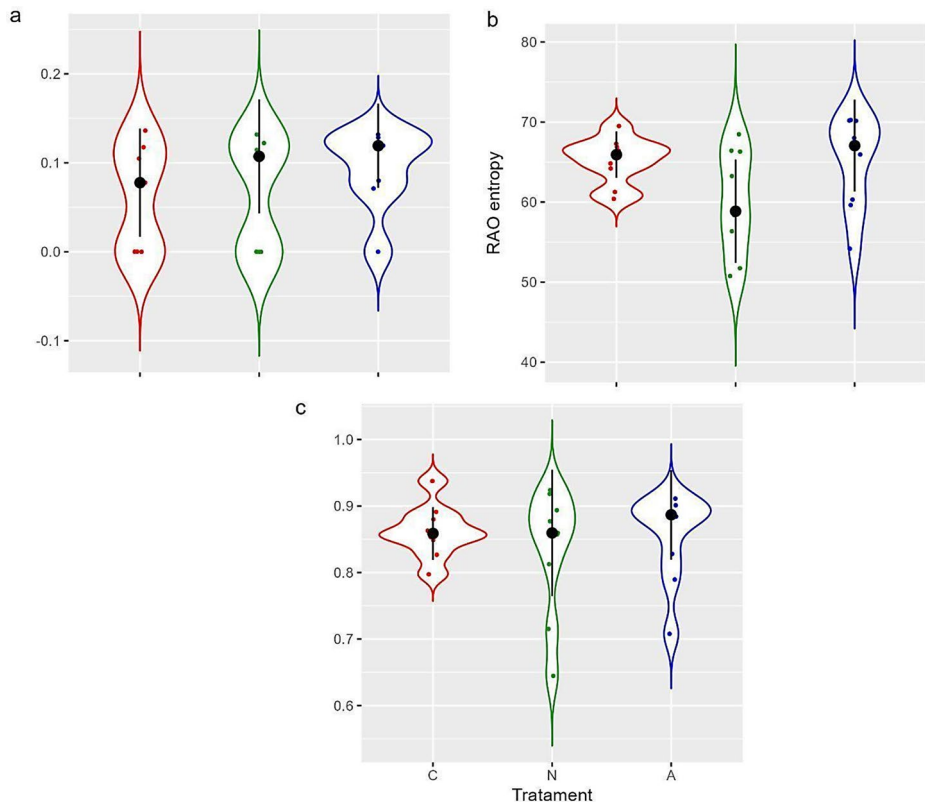


Fig. 5 Rao's quadratic entropy diversity in each treatment. The conventional plantation (C), native forest (N), and agroforestry plantation (A) considering: **(a)** genetic distance within *H. ethilla narcaea*, **(b)** phylogenetic distance between Nymphalidae communities at each site, and, **(c)** species composition of Nymphalidae communities at each site. The violin plots indicate the variation found across the 9 sampled sites (7 for the genetic data) within each treatment. Black dots represent the median values, and no significant differences were found among the treatments

Treatment effects on between-site diversity and dispersal

In contrast to our population structure analysis, we did not find any evidence for genetic differences between treatment types using PERMANOVA to assess genetic distance ($F_{2,62} = 1.02, R^2=0.03, p=0.43$) or using dispersion to assess genetic diversity ($F_{2,62} = 0.28, p=0.75$, Fig. 6a), although there is a visible trend towards reduced dispersion (diversity) in the conventional plantations. These results also support that dispersal between sites is high, leading to a lack of strong differentiation between them.

Similarly, at the phylogenetic levels, there was no effect of treatment, be it conventional and agroforestry banana plantations or native forest, PERMANOVA revealed neither significant differences on the Nymphalidae phylogenetic community distances among sites ($F_{2,24} = 1.13, R^2=0.08, p=0.23$) (Fig. S5B), nor in variability among sites, using dispersion ($F_{2,24} = 1.22, p=0.31$, Fig. 6b). However, the species composition of the Nymphalidae community yielded different results, with a significant difference in the community composition

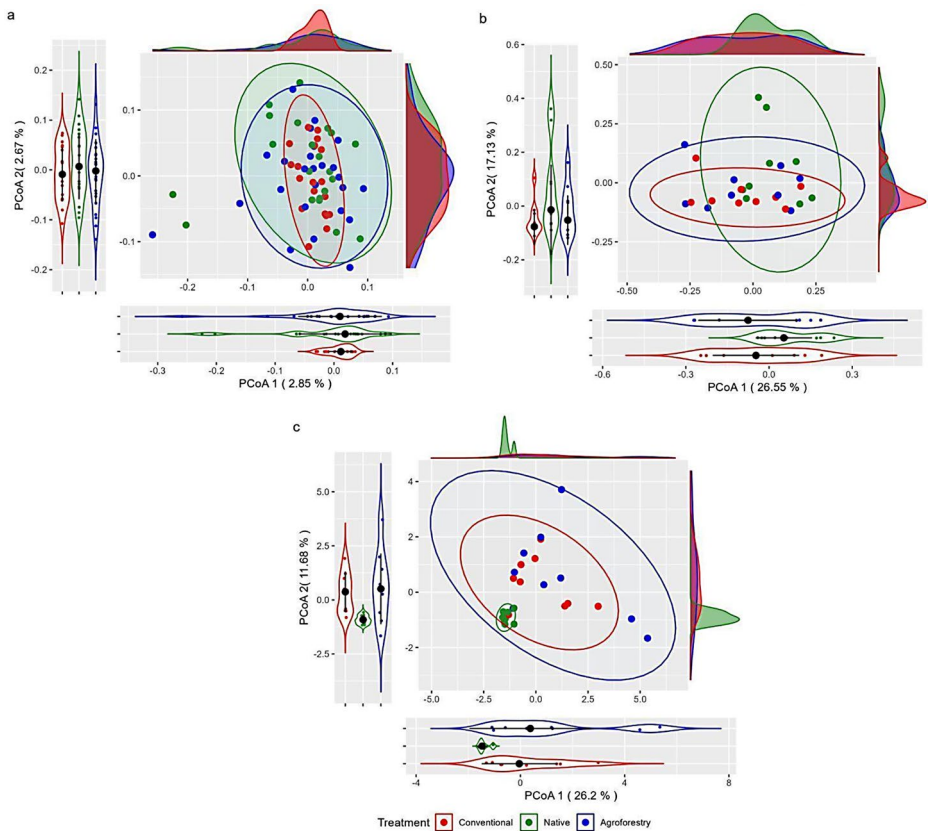


Fig. 6 Diversity between-sites according to treatment, assessed using multivariate homogeneity of group dispersions. The analysis was conducted on: (a) Genetic distance between *H. ethilla narcaea* individuals; (b) Phylogenetic distance between Nymphalidae communities at each site; and, (c) Difference in species composition of Nymphalidae at each site. The density of dots per axes is represented in the right and left part of the main plot, while the violin boxplot shows the median (black dot) of each axis considering the treatment: conventional plantation (red), native forest (green), agroforestry (blue)

Table 2 Standardised effect size of mean pairwise distance (MPD) and mean nearest taxon distance (MNTD) within each treatment in *H. ethilla Narcaea* populations and Nymphalidae communities compared to a null model. The corresponding number of individuals or species in each case are represented as n. Standardised effect size in MPD.n and MNTD.n considering distance weighted by species abundance. The bold values represent a p -value < 0.05 when compared to a null distribution

		Treatment		
		Conventional	Native	Agroforestry
<i>H. ethilla narcaea</i>				
	n	19	21	25
MPD	z	-0.24	-0.17	-0.75
	<i>p</i>	0.35	0.37	0.19
MNTD	z	-0.25	-1.95	-0.14
	<i>p</i>	0.34	0.04	0.41
Nymphalidae				
	n	61	50	64
MPD	z	-2.98	-0.12	-1.29
	<i>p</i>	0.01	0.51	0.10
MPD.n	z	-0.58	-2.43	-0.33
	<i>p</i>	0.23	0.03	0.25
MNTD	z	2.36	0.11	-0.96
	<i>p</i>	1.00	0.59	0.14
MNTD.n	z	0.44	-0.51	-0.32
	<i>p</i>	0.71	0.31	0.40

between treatment types in the PERMANOVA analysis ($F_{2,24} = 2.44$, $R^2 = 0.17$, $p = 0.002$) (Fig. 6c). This is likely driven by certain species that were abundant in the native forest but almost absent from the plantations, such as *Aeria olena*, and other rarely caught species that were only caught in the native forest (Table S1). We also found a significant effect of treatment on diversity in the dispersion analysis ($F_{2,24} = 5.74$, $p = 0.01$), with the native forest cluster being less diverse compared to the banana plantation clusters (Fig. 6c).

Treatment effects on phylogenetic diversity at the population and community level

We assessed whether treatment had an effect on clustering within the phylogeny, using the SNP tree for *H. ethilla narcaea* and the Nymphalidae phylogeny. The mean nearest taxon distance (MNTD) between *H. ethilla narcaea* individuals in the native forest was found to be lower than expected by chance ($z = -1.95$, $p = 0.04$, Table 2; Fig. S8a). Consequently, individuals in the native forest tended to be over-represented in certain clusters at the tips, potentially a result of the rarest genetic groups found in this area (Fig. S6a). However, these individuals were otherwise distributed randomly across the entire tree, as indicated by the mean pairwise distance (MPD) result (Table 2). This pattern was not observed in either of the plantations, where MNTD did not significantly differ from the null distribution (Table 2). Clustering at the tips could also result from the sampling of genetically related individuals, possibly indicating breeding within the native forest but not in either of the plantations.

In terms of community composition, Nymphalidae species demonstrated a higher phylogenetic clustering in the conventional plantation than expected by chance, indicated by lower MPD values in the conventional plantation when species abundance was not consid-

ered ($z = -2.98$, $p = 0.01$, Table 2). This suggests that the conventional plantation may provide specific conditions or resources that favour the prevalence of particular phylogenetic groups or exclude others. Conversely, when considering the abundance of each species (MPD.n), greater clustering was observed in the native forest ($z = -2.43$, $p = 0.03$, Table 2; Fig. S8b). All native sites presented a similar phylogenetic composition with the exception of Ja and Ad sites (Fig. S8b). The MPD.n result implies that besides the phylogenetic proximity found between native sites, the abundance of the species is crucial for clustering the native sites. Similar grouping was found using species composition (Fig. S8c), which is consistent with our dispersion analysis. In the agroforestry system, a neutral process was identified, considering both MPD and MNTD, where no clustering or overdispersion occurred. The lack of a clear pattern in this case may suggest a broader distribution of species within the agroforestry system, perhaps representing an intermediate habitat type where the conventional plantation and forest species are both found. This is reminiscent of the genetic results, which also suggested a more even distribution of genotypes within the agroforestry plantation.

Discussion

Effects of banana plantation and native forestry at the population level

Comparing the impacts of both banana plantations on microevolution, our findings suggest that agroforestry systems effectively preserve the rarest genetic groups in *H. ethilla narcaea* populations, a feature apparently absent in conventional plantations, and does not seem to be attributed to limited dispersal or spatial constraints, as they are present across multiple, spatially distant sampling blocks (Figs. 2 and 3; Fig. S7). This indicates that the distinct distribution of these genetic groups is likely attributed to other selective forces filtering particular genotypes from the conventional plantations. One explanation could be the selection pressure exerted by pesticides, leading to the elimination of certain genotypes and favouring individuals with greater resistance to these chemicals (Fouet et al. 2018). While other selective forces and phenotypic differences are possible, pesticides seem the most plausible factor, given the need for strong selective pressures to eliminate distinct genetic groups differentiated at a genome-wide scale (Burke et al. 2010; Prezeworski et al. 2005). This finding is particularly striking given the small spatial scales involved and the high gene flow detected between sites. It implies that selection is potent enough to consistently eliminate non-adapted migrants into the conventional banana plantations from the adjacent forest and agroforestry land.

Admittedly our sample size is small (64) and we cannot rule out the possibility that further sampling would eliminate this trend, making it advisable to validate these findings with a larger sample size. The small sample size may also be why the diversity metrics do not support a significant difference in genetic diversity between treatment types. Nevertheless, this potentially large effect of land use on genetic diversity within non-target insect populations, is not something that has been widely investigated and is certainly worthy of further investigation. If verified, this reduction in genetic diversity may compromise the population's ability to respond to environmental change (Jump et al. 2009), posing a threat to long-term persistence in space and time. A recent study identified reductions in genetic diversity in aquatic insect populations associated with recent farming (Crossley et al. 2023),

but suggested these were most likely due to reductions in overall population sizes in these areas, which were distantly spaced, likely with low dispersal between them. In contrast, our results suggest that reductions in genetic diversity may be observed without obvious changes in population size across a small spatial scale within a high dispersal species.

Although the agroforestry areas demonstrate the potential to preserve biodiversity at the microevolutionary level, the identified genotypes are rare even within these areas. This scarcity may suggest that, over time, the genetic diversity of the entire population may undergo erosion. Consequently, we propose that the forest, including small fragments near larger and well-structured areas, may serve as a source (Vedeller et al. 2005; Iserhard et al. 2019; Bellaver et al. 2022) helping the movement of butterflies and other animals in the landscape, depending on its spatial variation, configuration, and connectivity (Brown and Freitas 2002; Hanski et al. 2004; Iserhard et al. 2019; Melo et al. 2019). On the other hand, the banana plantations, particularly conventional plantations, might act as a final sink (Pulliam 1988) for the smallest microevolution unit: genetic diversity. This assertion gained further support from an analysis utilising the mean nearest taxon distance (MNTD) that compares the genetic diversity between treatments. The results revealed that *H. ethilla narcaea* individuals from the native forest tend to cluster more closely in terms of genetic relatedness than expected by chance. This is consistent with our results suggesting that the native forest has a higher likelihood of harbouring the rarest genetic groups, while the most common genetic groups exhibit a similar probability of belonging to any area, irrespective of the treatment. This underscores the idea that native forest supports individuals with specific genotypes, suggesting that it could be a source of genetic diversity, perhaps to a greater extent than agroforestry. In addition to further sampling within *H. ethilla*, integrating population genetic studies across other species could enhance our understanding of the land use impact on less dispersive species. Our choice to focus on a species abundant across all sites in the population genomic analysis implies that the observed effects may be even more pronounced in other species, potentially more susceptible. Moreover, this impact is likely to be more substantial in areas where bananas are cultivated as monoculture across the landscape (Bellamy et al. 2018), as opposed to our current study area where both types of banana plantation occupy small areas with remaining patches of native forest.

Effects of banana plantation at the community level

Our community-level analysis showed either no difference in diversity between treatment types (Rao's quadratic entropy) or reduced diversity in the native forest (dispersion analysis). Species richness and abundance were highest in agroforestry and lowest in native forest. These differences may partly be an artefact of the native forest structure making it harder to observe and catch butterflies, but could also be due to many common, eurytopic, and sun-lover species with great vagility (Melo et al. 2019) preferring to fly in more open and less heterogeneous habitats (as found in S.E. Asian tropical forests, Hill et al. 2001), such as banana plantation, encompassing species of the regional pool able to use distinct perturbed habitats (Schulze et al. 2004). Additionally, most species collected with butterfly nets are recorded in the understory of forests and belong to the nectar-feeding guild (Freitas et al. 2021), a resource with low availability inside dense tropical forests.

Nevertheless, as with studies in other tropical forest systems, we found that the native forest sustains unique butterfly species within the communities (Vedeller et al. 2005; Benedick

et al. 2006; Schulze et al. 2010; Spaniol et al. 2019). The species composition observed in the native forest significantly differed from both banana plantation types (Fig. 4b-c). This may be attributed to the native forest providing a well-structured environment with distinct microhabitats compared to banana farms. These findings align with numerous prior studies demonstrating that native forest habitats of Atlantic Forest support distinct species composition, which is diminished by human modification, including fragmentation (Uehara-Prado et al. 2007; Melo et al. 2019), silviculture (Casas-Pinilla et al. 2022; Thomas et al. 2024), urbanisation (Iserhard et al. 2019), edge effects (Filgueiras et al. 2016; Lourenço et al. 2019; Bellaver et al. 2022), and environmental gradients with small scale disturbances (Uehara-Prado et al. 2009; Iserhard et al. 2024). Nevertheless, the forest community appeared more similar to the plantation community's composition in our analysis when considering phylogenetic relatedness, something that has also been found in previous studies of butterfly community composition (Graça et al. 2017; Iserhard et al. 2019), possibly suggesting that forest specialism is not phylogenetically conserved. However, when we consider individual abundance, our MPD analysis showed there are some phylogenetic clusters in native areas, so losing the native forest would impact the phylogenetic composition of the community.

The native forest likely supports species with distinct biological requirements, in part, due to the absence of the shaded first forest stratum in the evaluated banana farms. Variation in the retention and significance of this stratum in other agroforestry types and for different species could explain why some studies have found a similar species composition in agroforestry and native forest (Williams-Guillén et al. 2006; Harvey and González Villalobos 2007; Schroth and Harvey 2007). However, it is essential to acknowledge that our sampling, conducted using hand nets, predominantly targeted the lower forest strata. The observed differences between the native forest and agroforestry may diminish if sampling extends to higher strata because the species composition is stratum dependent (Graça et al. 2017). Nevertheless, the agroforestry banana plantations are dominated by a few plant species, despite the use of native trees to maintain this system. Thus, it is not surprising that there are differences in the species composition of butterflies when compared to Atlantic Forest habitats. On the other hand, native forest patches showed a similar species composition across the landscape, which was distinct from neighbouring agricultural patches (Fig. 3C), showing that even these small and disconnected areas of forested habitat have high biodiversity value (Fahrig et al. 2020) and that dispersive species like butterflies can colonise them, maintaining metacommunity dynamics in a regional context. Consequently, we advocate for the importance of preserving native forest areas as legal reserves in private properties (Lorandi et al. 2023) and as protected areas in the landscape. These areas offer optimal conditions and resource availability for biodiversity conservation.

It is also important to acknowledge that our sampling targeted only the adult stage, and that larval stages may show different habitat preferences depending on where host-plants are found (Janzen 1988), with the adults dispersing to other areas that are more favourable for adult food sources (flowers or fruit) or for courtship (open areas). For example, some species that are commonly found in the plantations may be breeding in the native forest (as possibly suggested by our genetic data). This dispersal effect likely explains why studies at smaller spatial scales (like ours) tend to find a smaller difference in diversity between disturbed and undisturbed habitat compared to studies at larger spatial scales (Hamer and Hill 2000). Therefore, care should be taken in extrapolating our results to the effects of removing

certain habitat types, as this could lead to the loss of larval host plants that support butterflies found elsewhere.

Conservation implications

The escalating global human population necessitates the optimization of farming practices, balancing economic performance for farmers and biodiversity conservation for ecosystem services (Wurz et al. 2022). Our study suggests there may be certain biodiversity advantages of banana agroforestry over conventional plantations, which complements previously documented economic advantages of agroforestry (Gonçalves 2008). Agroforestry practices linked with organic family farming in small holdings can help in the sustainability of ecosystems, food safety for human beings, and preserving biodiversity (Robertson and Swinton 2005; FAO and IFAD 2019; Lorandi et al. 2023). This practice aligns with certain Sustainable Development Goals (SDGs) outlined by the UN, notably SDG 12 (Responsible Consumption and Production) and SDG 15 (Life on Land). This alignment underscores the critical importance of adopting organic and agroecological approaches to uphold biodiversity in the Atlantic Forest. By doing so, we actively contribute to the broader global agenda of sustainable development, addressing key environmental and agricultural challenges.

Furthermore, our findings reinforce the significance of preserving native forest fragments for butterfly dispersal and movement in the Atlantic Forest landscape (Iserhard et al. 2019; Melo et al. 2019; Bellaver et al. 2022). Genetic analysis suggests that agroforestry patches may facilitate dispersal between these native patches by providing a more wildlife-friendly habitat without agrochemicals. Consequently, both environments can serve as stepping stones for insect movement in the landscape, offering shelter and diverse resources, consistent with findings in other systems (Lorandi et al. 2023). Therefore, the landscape management of tropical areas should consider the proximity of different types of natural and semi-natural habitats with structured and preserved forested sites (Schulze et al. 2004), creating a connected approach to conservation and sustainable land use (Wurz et al. 2022).

Conclusion

For the first time, we evaluated the effects of banana plantations on multiple dimensions of butterfly diversity using an eco-evolutionary approach at distinct ecological levels. In particular, the effect of agricultural practices on genetic diversity on non-target species, has rarely been assessed. We used both traditional and evolutionary metrics that can be applied at both the population and community levels for measuring the impact of agroforestry and conventional banana compared with native forest areas. Agroforestry appears to give a win-win outcome of increasing yields and biodiversity (Wurz et al. 2022). Our results found that agroforestry preserves rare genetic groups and greater numbers of individuals compared to conventional plantations that have a negative impact on butterfly biodiversity at both levels. The absence of some genetic groups in conventional plantations may be due to pesticide use, acting as a strong filter. We propose that further studies are urgently needed to investigate this hypothesis across a wider range of species and agricultural systems. We find that the conventional plantations have the greatest impact on population genetic composition, while at the community level, the greatest difference is between the native forest and either

type of plantation. Our study strongly supports the importance of native forest habitats, even in small patches, to preserve the distinct genetic groups and species in these areas. Finally, these complementary approaches have therefore allowed us to assess distinct aspects of biodiversity on an eco-evolutionary level, that would not have been detected using just traditional methods focussing purely on species differences and diversity metrics (Logue et al. 2011).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10531-025-03075-0>.

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Author contributions D.O.R, N.N., L.D. and C.A.I. conceived the ideas and designed the methodology; D.O.R. collected and analysed the genetic data; D.O.R. and G.N. analysed the community data; D.O.R. and N.N. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability The DNA sequence data used in this work are deposited in the European Nucleotide Archive under study ID PRJEB45832 (ERP129991).

Declarations

Competing interests The authors declare no competing interests.

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