SAFEGUARD

Opportunities and risks of biodiversity offsetting for pollinator conservation

Deliverable D4.5

28 February 2024

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Safeguard
Safeguarding European wild pollinators

This project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101003476.

Prepared under contract from the European Commission

Grant agreement No. 101003476.
EU Horizon 2020 Research and Innovation action

Project acronym	**Safeguard**
Project full title	**Safeguarding European wild pollinators**
Start of the project	**September 2021**
Duration	**48 months**
Project coordinator	**Ingolf Steffan-Dewenter** **Julius-Maximilians-Universität Würzburg** **https://www.safeguard.biozentrum.uni-wuerzburg.de/**
Deliverable title	**Opportunities and risks of biodiversity offsetting for pollinator conservation**
Deliverable n°	**D4.5**
Nature of the deliverable	**Report**
Dissemination level	**Restricted**
WP responsible	**WP4**
Lead beneficiary	**WU**
Citation	**Leander Oh, K., Gazzea, E., Scheper, J., Marini, L., Kleijn, D. (2024).** ***Opportunities and risks of biodiversity offsetting for pollinator conservation.*** **Deliverable D4.5 EU Horizon 2020 Safeguard Project, Grant agreement No 101003476.**
Due date of deliverable	**Month 30°**
Actual submission date	**Month 30°**

Deliverable status:

Version	Status	Date	Author(s)	Reviewer(s)
1.0	Draft	28 February 2024	Leander Oh, Klara; Scheper, Jeroen; Kleijn, David (WU), Gazzea, Elena; Marini, Lorenzo (UNIPD)	WU, UNIPD

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Summary

Lead: WU

Contributors: UNIPD

Duration: 30 months

Task Description: Identifying opportunities and risks of biodiversity offsetting for pollinators, using a combined approach of a literature review and case studies (Netherlands, Italy).

Results: In both the Netherlands and Italy, we found no significant differences in hoverfly diversity metrics or hoverfly and wild bee community composition between the biodiversity offset sites and control sites. In the Netherlands, wild bee abundances were significantly higher, and evenness was lower in offset grassland sites. In Italy, lepidopteran abundances were significantly lower, and evenness was higher in offset forest sites. We also found significant differences between control and offset grassland vegetation and flower community composition in the Netherlands. In Italy, forest features had a stronger influence on pollinator responses than whether sites were offset or not. Biodiversity offsetting in the European Union shows promise for achieving No Net Loss objectives but there is uncertainty in its effectiveness due to limited empirical evidence. This can potentially result in offsets perpetuating biodiversity loss rather than restoring it. We emphasise the need for further research to better understand and quantify the success of biodiversity offsetting, particularly with regards to conserving pollinators within the European Union.

List of abbreviations

BBOP	**Business and Biodiversity Offsets Programme**
EU	**European Union**
NNL	**No Net Loss**

1. Introduction

Biodiversity offsetting can be defined as the creation, restoration, or protection of nature to compensate for the loss of other nature elsewhere (Business and Biodiversity Offsets Programme (BBOP), 2012). Offsetting is the last step in the mitigation hierarchy, once it has been determined that avoidance, minimisation, and remediation of development impacts are not sufficient (Bull et al., 2016; BBOP, 2012; Tucker et al., 2020). Biodiversity offsetting has long been proposed as a potential tool and novel conservation approach for achieving “No Net Loss” (NNL) objectives (Tucker et al., 2020) by conserving nature and its diversity while allowing for continued development. This is particularly relevant for the European Union, which has identified biodiversity offsetting as a key instrument of the No Net Loss initiative, with the initial goal of achieving NNL of biodiversity by 2020 (European Commission, 2011; Rayment et al., 2015) and now by 2030 (Tucker et al., 2020).

The European Union has a strict legal framework for offsetting within Natura 2000 network of protected areas. Under the Habitats Directive (Art. 6, 4) ‘compensation measures’, analogues to offsets (Wende et al., 2018), are required for unavoidable impacts on Natura 2000 sites (*Council Directive 92/43/EEC*, 1992). Furthermore, the potential of biodiversity offsetting to allow for infrastructure development while adhering to the conservation policy principle of NNL, has appealed the uptake of offsetting policies among several countries in Europe beyond Natura 2000 protected areas (Bull et al., 2018; Wende et al., 2018). Among these, for example, both the Netherlands and Italy have national or subnational legislation enabling offsetting (Bull et al., 2018; Rega, 2013). In the Netherlands, there should be no net loss of area, quality, or connectivity of protected nature due to development in accordance to several frameworks or acts, such as spatial planning decree ‘Barro’ (Ministerie van Infrastructuur en Milieu, 2012) and the Nature Conservation Act of 2017 (Ministerie van Economische Zaken, 2016). In Italy, physical or monetary compensation is only required in the context of woodland clearance (National Decree no. 227/2001) (Rega, 2013).

Guidance on the integration of ecosystems and their services into decision-making published by the European Union (Tucker et al., 2020) provides a comprehensive, general framework for biodiversity offsetting implementation, but it does not critically review the effectiveness of biodiversity offsets for mitigating biodiversity losses due to development. While some authors suggest that biodiversity offsets have the capacity for mitigating biodiversity loss (OECD, 2016; Tucker et al., 2018; Tucker et al., 2020), the success of these offsets can be highly variable (Quigley & Harper, 2006; zu Ermgassen et al., 2019). Recent global reviews highlighted the lack of evidence supporting the potential of offset sites to compensate for development-associated losses of biodiversity, thus questioning the effectiveness of biodiversity offsetting as a conservation strategy and advocating for long-term monitoring programmes (Josefsson et al., 2021; zu Ermgassen et al., 2019). Despite 67% of the world's biodiversity offsets being applied in forested ecosystems (Bull & Strange, 2018), only four offsetting projects were evaluated for their outcome, and none demonstrated successful target achievements for forested habitats or species (zu Ermgassen et al., 2019). Similarly, of the available published studies on the performance of biodiversity offsetting, most have mainly been conducted in North American freshwater biomes, with a particular focus on vegetation as the most common biodiversity indicator (Josefsson et al., 2021). In this context, the effectiveness of biodiversity offsetting on terrestrial habitats and specifically on invertebrates appear to be significantly understudied.

Given this background, the objective of Task 4.5 is to identify opportunities and risks of biodiversity offsetting as a conservation instrument targeting pollinators. To achieve this, we used a combined approach of a literature review and case studies. First, we conducted an updated literature review to identify published literature on the effects of biodiversity offsetting on pollinators. Second, we conducted experimental case studies in the Netherlands and in Italy, aiming at evaluating the effectiveness of existing biodiversity offsets, not specifically designed for pollinators, in hosting similar pollinator communities as in reference sites. Hence, within Task 4.5, we addressed the following specific questions:

1. What is the ecological effectiveness of biodiversity offsetting for the diversity of wild pollinators (wild bees, hoverflies, and lepidoptera) based on existing published and gray literature?
2. What is the ecological effectiveness of grassland restoration or creation—under the framework of biodiversity offsetting—for the diversity of wild bees and hoverflies based on a Dutch case study?
3. What is the ecological effectiveness of forest restoration or creation—under the framework of biodiversity offsetting—for the diversity of wild bees, hoverflies, and lepidopterans based on an Italian case study?

2. Methods

2.1. Literature Review

The literature review on biodiversity offsetting and impacts on pollinators was conducted following the PRISMA methodology (Page et al., 2021). The definition of biodiversity offsetting used was “measurable conservation outcomes resulting from actions designed to compensate for significant residual adverse biodiversity impacts arising from project development after appropriate prevention and mitigation measures have been taken” (BBOP, 2012). Papers were systematically searched for in the Web of Science Core Collection and Scopus by using search strings and methods adapted from Josefsson et al. (2021) and zu Ermgassen et al. (2019). At the time of this search (23/02/2022), these were the most recently published literature reviews of the impacts of biodiversity offsets. Keywords were adapted for wild pollinators by including “Pollinator*”, “Wild Pollinator*”, “Bee*”, “Wild bee*”, “Solitary bee*”, “Anthophila”, “Butterfl*”, “Lepidoptera”, “Rhopalocera”, “Hoverfl*”, “Syrphid*”, “NOT Apis mellifera”, “NOT Apis cerana”, “NOT Honeybee”, and “NOT Honey bee”. One nuisance term (“Banksia”) was identified after the first search and papers including it were removed prior to the full screening process. The search was not limited in geographic scope and date of publication but was limited to English-language publications.

The studies were screened according to several criteria:

1. English language
2. Refers to wild pollinator taxa (wild bees, hoverflies, lepidoptera)
3. Refers to biodiversity offsetting / compensation / banking, habitat offsetting / compensation / banking, mitigation hierarchy, or other synonyms listed in the search string
4. Is an empirical, field-based study of one or more offset sites that were restored, protected, or created under a biodiversity offsetting (or similar) scheme
5. Compared the offset site to a control site, either temporally (i.e., sampling the site pre-development) or spatially (i.e., using reference or degraded systems
6. Reports one or more response metrics (i.e., abundance, species diversity, density) of wild pollinator taxa (wild bees, hoverflies, butterflies)

2.2. Case Study: Netherlands

2.2.1. Study area

This study was conducted in 20 biodiversity offset grasslands and 20 control grasslands across the Netherlands (Figure 1). Of the biodiversity offset sites, 17 were classified as flora- and fauna-rich grassland, which are typically moderately nutrient-rich, can exist on dry to moist soil types, and contain common flower species; and three were classified as wet hay meadows, which are herb-rich grasslands that are typically low in nutrients and exist in wet conditions. 11 biodiversity offset sites were located on sandy soils, the most common soil type in the Netherlands, 7 on sandy loam soils, and 2 on clay soils. The offset sites ranged in area from 0.73 – 110 ha (SD ± 24.4 ha) and in age from 1–13 years (SD ± 3.7 years).

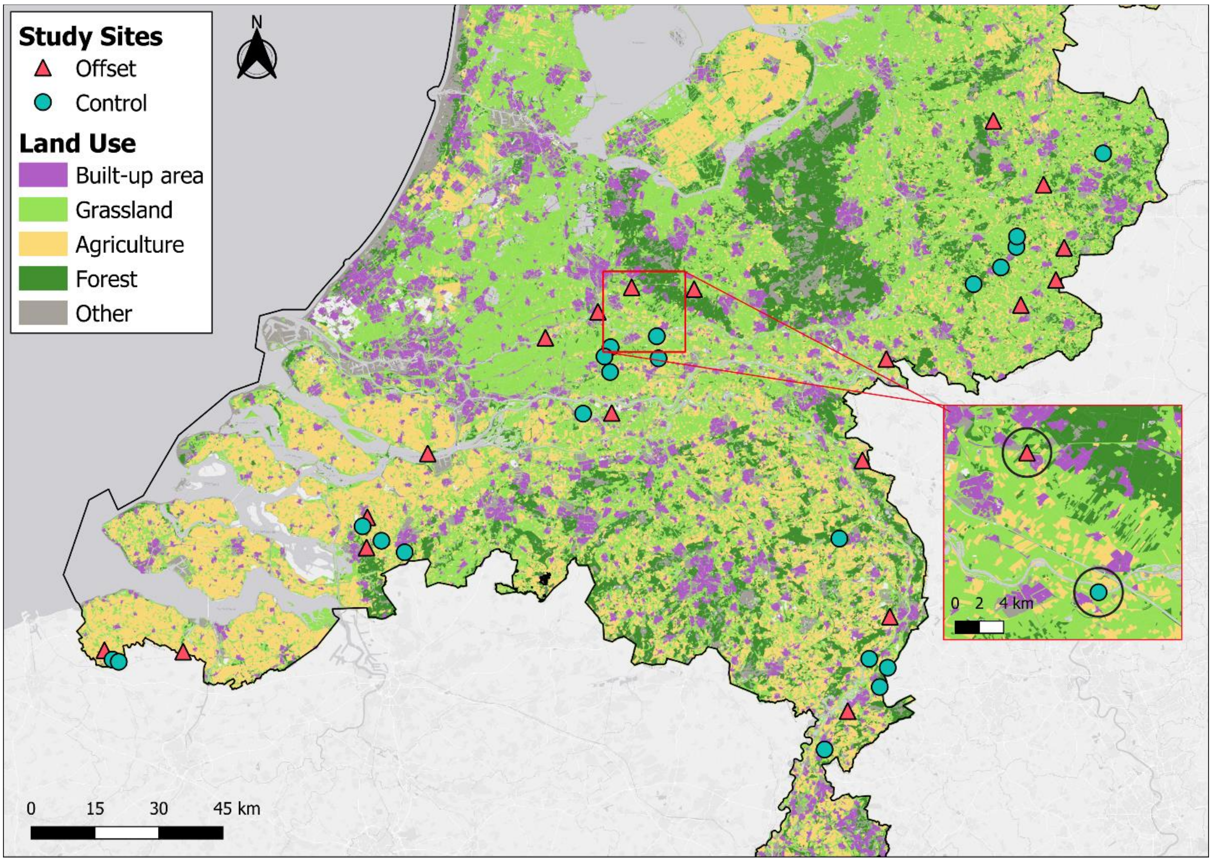

Figure 1. Map of the study areas and study sites in The Netherlands. The map inset shows two paired study sites (#9 – offset Rijswaardseveld and #29 – control Beusichem Waarden) and their landscape context within a 2 km buffer. Land use data for this map was acquired from TOP100NL (CC BY 4.0) and further summarised into four broad land use classes: built-up (urban) area, grassland, agriculture, and forest. Any land use type that did not belong to these categories was classified as “other”.

2.2.2. Site selection

20 biodiversity offset sites were selected based on the following criteria:

1. The habitat type is grassland. According to the Dutch Nature and Landscape Index (BIJ12), grasslands suitable for pollinators fall under the habitat types N10 (wet rough grasslands), N11 (dry rough grasslands), and N12 (rich grasslands and fields).

2. The compensation occurred under the frameworks of *Besluit algemene regels ruimtelijke ordening* 2012 (Barro; Decree on general rules on spatial planning of 2012), or Natuurbeschermingswet 1998/2017 (Nature Conservation Act of 1998/2017) and Wet natuurbescherming 2017 (Nature Conservation Act of 2017). Barro protects nature areas that that are part of the Natuurnetwerk Nederland (NNN; Dutch national nature network). The Nature Conservation Acts of 1998/2017 and 2017 implement the Birds and Habitats Directive (*Council Directive 92/43/EEC*, 1992), which protects EU-wide Natura 2000 sites, and additionally cover Dutch “natuurmonumenten” (natural monuments).

3. The site is designated as a compensation site for a specific development project. Thus, the site cannot be part of a compensation pool or bank.

4. It is possible to determine a) what habitat type was lost due to development and b) if the offset site is explicitly intended to offset that loss.

These offset sites were then paired to control sites across the Netherlands. Control sites were determined by matching habitat type, soil type, and landscape composition (2 km buffer) of offset sites with nature areas managed by the Staatsbosbeheer. This was done by using national GIS data from BIJ12 (habitat types) (BIJ12, 2022), top10NL (landscape composition) (Beheer PDOK, 2020), and Grondsoortenkaart 2006 (simplified soil types) (Wageningen UR - Alterra, 2006) to establish control site characteristics in ArcGIS Pro (v2.6.3). A map of potential control sites, limited to a 20 km radius around each offset site (allowing for same-day sampling of pairs) was then compared to each biodiversity offset site using the Similarity Search tool. The 10 most similar potential control sites were manually assessed, where records with non-matching habitat and soil types were removed and the remainder sorted by similarity. The control sites were then selected from these lists, going by most to least similar, depending on whether the location was available for fieldwork according to the Staatsbosbeheer.

2.2.3. Data collection

Pollinator sampling

Including and between the months of May and August in 2022, two transects on each site were sampled in three rounds. Bees and hoverflies were sampled for 30 minutes of pure sampling time in two 150x1 m transects (Scheper et al., 2015). Where necessary, the transect dimensions (but not area) were adjusted to follow the layout of the site. The transects were divided into 3 sub-transects of 50 m^2, each sampled for 5 minutes.

Floral resource surveys

In the same visit as the pollinator sampling, total forb flower diversity and cover for each transect was estimated by counting the abundance of each species present. Only currently flowering individuals were included in the count. Transect flower cover (%) was calculated by multiplying the average diameter per number of flowers per species, then summing all flower areas and dividing by transect size (150 m^2). A database of average flower diameters per species collected by the Plant Ecology and Nature Conservation chair group was used for this purpose.

Vegetation surveys

Vegetation was surveyed once in each site during the field season using ten 0.5x0.5 m quadrats (Bretagnolle et al., 2022). These quadrats were placed randomly in the site within 100 m of, but not overlapping, the transect. Each quadrat was surveyed for a total species list of vascular plants. The percent cover of each species, as well as bare ground, was assessed using a birds-eye view, adding up to 100% (no overlap included).

2.2.4. Statistical analysis

All analyses were done in R version 4.2.3 (R Core Team, 2023). Wild bees (including bumblebees but excluding honeybees) and hoverflies were analysed separately.

Pollinator diversity and floral resources

We used (generalised) linear models to analyse the impact of treatment (control or offset) on pollinator diversity (abundance, species richness, and community evenness), and on floral resources (percent cover and species richness). Flower cover per species was calculated by multiplying the total number of flower units by mean surface area per unit, then dividing by transect size (150 m^2). Flower cover per site was then calculated by summing flower cover from both transects, averaging across rounds, and multiplying by 100. Pollinator abundances, and species richness were also pooled across transect (sum) and round (mean) before analysis. To meet the assumption of normally distributed and homoscedastic residuals, pollinator abundances were $\ln(x + 1)$ transformed. Pair ID was initially included as a random effect in all models to account for variation between paired sets of control-offset sites. As there was not a significant amount of variance between pairs, this random effect was removed from all models except for hoverfly species richness and flower species richness. One site (#4) had no wild bee observations and was thus excluded from the analyses of wild bee evenness and community composition, as these cannot be calculated when there are no observations. All residual diagnostics were checked using the package *DHARMa* (Hartig, 2022).

Community analyses

Vegetation, flower, and pollinator communities were first converted to Bray-Curtis distance matrices, scaled to relative species abundances. Site 21 was identified as an outlier as it contained only 1 observation of a species (*Andrena labiata*) that was not present in any other site (i.e., site 21 was completely dissimilar from all other locations). It was therefore removed from the wild bee community dataset before analysis. Treatment differences per community were then compared using a PERMANOVA test (Anderson, 2001) with 999 permutations (package *vegan*) (Oksanen et al., 2022) and treatment (control or offset) as a fixed factor. A permutation test of multivariate group variances per community showed that all communities, excluding hoverflies, were homogenous (Table S1). Heterogeneous group variances can affect the reliability of such resemblance-based permutation methods, however PERMANOVA has been found to be robust to heterogeneity so long as the study design is balanced (Anderson & Walsh, 2013), as it is here. Thus, using a PERMANOVA was still appropriate. Community compositions were then visualised using nonmetric Multi-dimensional Scaling (NMDS).

2.3. Case Study: Italy

2.3.1. Study area and sampling design

This study was conducted in the Po Valley (NE Italy), the largest floodplain in Southern Europe. The study area is dominated by intensive agriculture, interspersed with urban settlements and semi-natural features, such as hedges and short-rotation poplar plantations. Within this landscape, forest patches are mainly oak-hornbeam forests typical of temperate lowlands that have been subjected to severe habitat fragmentation and loss. Such habitat type (Sub-Atlantic and medio-European oak or oak-hornbeam forests of the *Carpinion betuli*, Natura 2000 code 9160) is considered in an unfavorable conservation status throughout Europe (European Environmental Agency, 2009).

In the Italian context, forest clearance is legally required by National Decree no. 227/2001 to be offset by i) the creation of new forested areas, ii) the ecological enhancement of existing forests or iii) monetary compensation. Following devolution of forest management, observance of national matters on forests is provided at the regional level (Rega, 2013). In this context, while most of the forest clearance and conversion to other land uses occurs in highly forested landscapes within the region (Basso, 2019), the land compensation efforts are concentrated by the regional administrations on the ecological restoration of lowland landscapes, where forests are extremely scarce.

Within the study area, included in Veneto and Friuli Venezia Giulia regions, we selected a total of 34 forest sites: 17 forest restoration patches and 17 primary forest remnants (Figure 2). Due to the spatial mismatch between originally cleared forests and offsetting sites at the regional level, we selected primary forest remnants as controls. As forest patches are extremely rare in the study area, their spatial arrangement did not allow for a paired design. The characteristics of each forest type and the results of two-sample t tests used to test their differences are summarized in Table 1. We selected reforested sites with a gradient in years since forest establishment (min = 22 years, max = 37 years). Stand age was obtained from records of various forest administrations. Sampled forest patches were either managed as conservation sites or unmanaged.

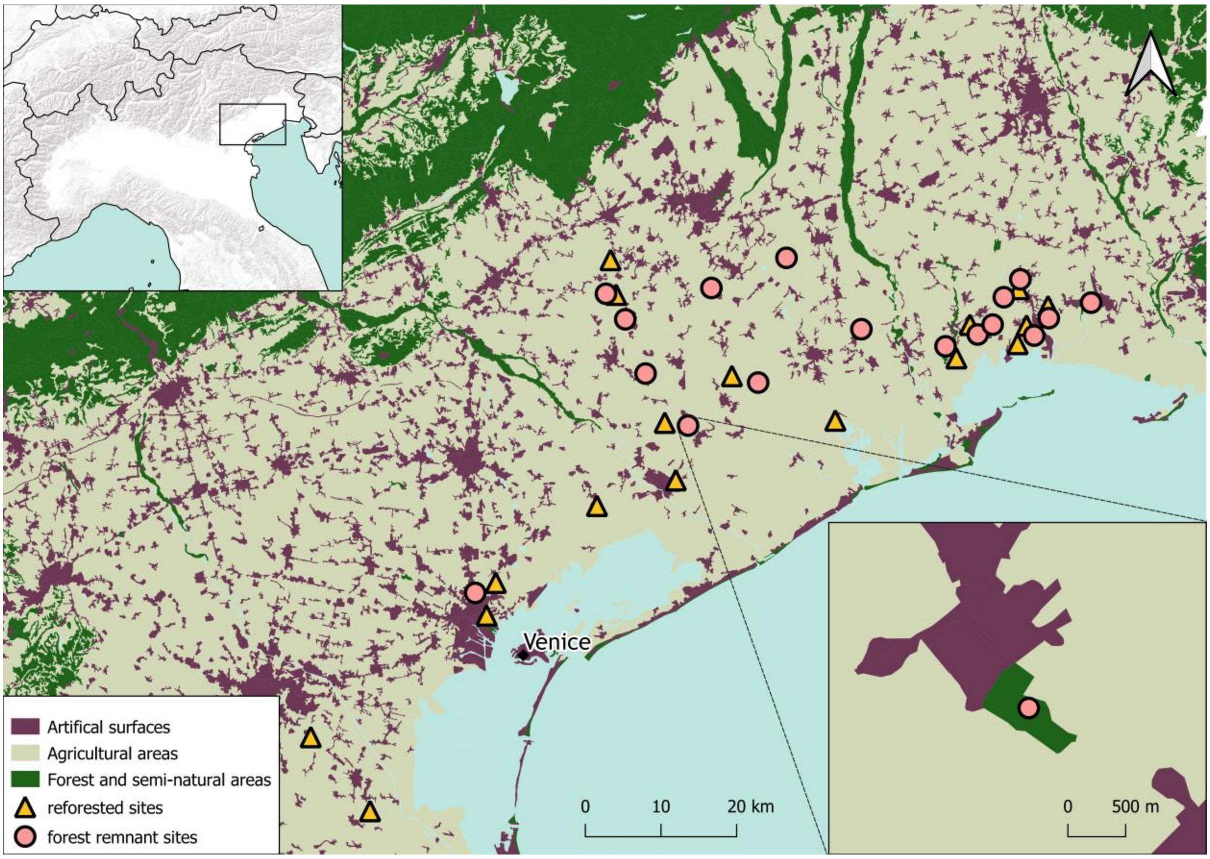

Figure 2. Map of the study area and the sampling sites in NE Italy. The inset map at the bottom shows an example of sampling site with the surrounding land use.

2.3.2. Data collection

Pollinator sampling

Sites were surveyed three times from the end of April to the end of June 2022. Within each forest site, we conducted a 40-minute transect to sample pollinators, including bees, hoverflies, and lepidopterans. Sampled lepidopterans included butterflies and burnet moths. Transects were corridors ca. 5 m wide and were conducted by two surveyors simultaneously, walking ca. 2 m apart. Along the transects, pollinator individuals were caught either while flying, or resting on the vegetation, or while visiting flowers. In the latter case, we stored insects in separate vials for each flowering species. Pollinators that could be identified to species level in the field were not collected and were directly recorded. All other specimens were identified to species level later.

Environmental variables

We estimated several local environmental characteristics. For each sub-transect, we estimated cover of bare ground, grass, herb, and shrub/tree cover, the latter being included when lower than 2 m in height. We further estimated the total flower percentage cover, and the cover of each flowering plant species. Moreover, we estimated the canopy cover three times in each sub-transect using the GLAMA app (Tichý, 2016), resulting in 24 estimates per site and per sampling round. Next, we recorded the tree composition by identifying and visually estimating the basal area percentage of each tree species higher than 2 m in each sub-transect. We computed tree species Shannon diversity (H) using *vegan* package (Oksanen et al., 2022). Finally, we visually estimated the mean height and diameter of the dominant tree layer for each site.

At the landscape scale, we considered variables related to forest fragmentation, including habitat area and connectivity using the Hanski's connectivity index (Si). Si was calculated by measuring edge-to-edge distances between each study site and all other forest patches in a 10 km radius, using the equation (1):

$$Si_i = \sum_{i \neq j} \exp(-\alpha\, d_{ij})\, A_j^b \tag{1}$$

where Si_i is connectivity index of patch *i*, α is a coefficient of the negative exponential function that determines how the weight given to the surrounding patches decreases with distance and it is related to the dispersal ability (1/average migration distance in km), d_{ij} is distance between patches *i* and *j*, A_j is area of patch *j*, related to emigration by factor *b*, which scales movement to the size of the surrounding habitat patches (Moilanen & Hanski, 2006). We chose α=1, as we expect an average migration distance of 1 km for the studied pollinator groups (Kleijn & van Langevelde, 2006; Krauss et al., 2003; Zurbuchen et al., 2010). For the scaling parameter *b,* we chose *b* = 0.3. The connectivity gradient of our landscapes ranged from 0 to 54 (Table 1).

Table 1. Summary statistics of environmental variables (mean ± SD) estimated in the restored and the remnants forest patches sampled in this study. Highlighted p values indicate significant differences in means detected by two sample t-tests.

Variable	Unit	Restored	Remnant	P value
Canopy cover	%	80.06 ± 6.64	81.97 ± 5.13	0.3551
Flower cover	%	2.84 ± 2.42	4.38 ± 4.59	0.2307
Flower richness	N	9.71 ±6.16	10.24 ± 4.47	0.7763
Tree species	N	10.76 ± 4.04	7.76 ± 2.36	**0.0138**
Diameter	cm	20.76 ± 6.91	35.35 ± 5.33	**<0.001**
Area	ha	21.77 ± 33.16	29.07 ± 48.40	0.6121
Connectivity (Si)	-	9.83 ± 13.68	10.24 ± 14.14	0.9309
Grass cover	%	5.04 ± 3.89	2.92 ± 5.08	0.1837
Herb cover	%	24.17 ± 24.83	36.38 ± 12.92	0.0846
Shrub and tree cover	%	43.90 ± 19.81	31.54 ± 8.83	**0.0282**
Age	years	25.65 ± 4.14	-	-

2.3.3. Statistical analyses

All statistical analyses were conducted with the Software R version 4.3.1 (R Core Team, 2023). Due to the large number of potential predictors, we first tested only the effect of forest type (i.e., forest remnant versus reforested) on pollinator species richness, abundance, and community evenness using general linear models. We tested each sampled pollinator group (i.e., bees, hoverflies, and lepidopterans) separately, pooling their number of species and abundance observed at site level. To meet the assumption of normally distributed residuals, we used a natural logarithmic transformation of pollinators abundance.

Second, we analyzed changes in the community composition between forest remnants and reforested sites. To visualize the spatial community dissimilarity of species composition, we performed two-dimensional nonmetric multidimensional scaling ordination (NMDS) on presence-absence data. To test for differences in community composition between forest types, we performed permutational multivariate analyses of variance (PERMANOVA) using the function *adonis2*. All analyses were performed separately for bees, hoverflies, and lepidopterans. We repeated the same analysis including only rare pollinator species, which were defined as those species of bees, hoverflies, and lepidopterans with an incidence of 5 or less occurrences across all sampling sites.

Third, we investigated the effects of forest features on pollinator diversity in lowland forests using multiple linear regression models. To avoid overfitting of the models due to the large number of potential interactions, we first tested the effect of forest characteristics in interaction with forest type alone. Forest type and tree diameter were correlated, so we excluded the latter in the analyses. Since no significant interactions were detected, in the following models we did not include forest type as explanatory variable, i.e., we used both restored and remnant sites together. We examined the effects of both local variables related to the composition, structure and heterogeneity of forest stands, and landscape variables related to the dispersal ability of studied insect groups. We used multi-model inference within an information theoretic framework to compare the fit of a set of models rather than selecting one single best model based on *p* values. Global models included pollinator species richness as response variables and mean flower cover, flowering plant species richness, tree species Shannon diversity (H), mean canopy cover, mean tree diameter, forest patch area, and Hanski connectivity index (Si) as explanatory variables. Furthermore, we included interaction terms between connectivity and forest patch area, and between canopy cover and forest patch area. Vegetation cover estimates were averaged across sub-transects and sampling rounds, and all analyses were conducted per site. All explanatory variables were scaled to mean 0 and standard deviation 1 to make slopes comparable. To assess possible collinearity between predictors, we estimated variance inflation factors (VIFs). As no VIF>2 were detected, we retained all candidate predictors.

Model selection was performed using a multi-model inference approach based on AICc. Nested models within each global model were ordered based on their second-order AICc, with the best fitting model having lowest AICc, indicating the best trade-off between number of parameters and explanatory power. For each nested model, we calculated the difference between the model AICc and the lowest AICc detected (ΔAIC). We set a cutoff value of ΔAIC = 7 to define a top model set, whereas lower-ranked models were treated as less meaningful (Burnham et al., 2011). Finally, we computed the model-averaged partial coefficient for each explanatory variable using all models within the top model set and estimated the 95% confidence intervals around each model-averaged partial coefficient. Multi-model inference analyses were performed with the *MuMIn* package (Bartoń, 2023) and all residual diagnostic was checked using *DHARMa* package (Hartig, 2022). Finally, we explored the effect of restoration age on the diversity of the individual pollinator groups on the subset of the dataset related to offset sites.

3. Results:

3.1. Literature review

In total, the literature search resulted in 33 hits, of which two were duplicates. After screening the remaining 31 records, we found that none of the studies contained relevant information on the effects of biodiversity offsetting on pollinator abundance, species richness or composition.

3.2. Case Study: Netherlands

3.2.1. Pollinator observations and site characteristics

We sampled a total of 2128 hoverflies (86 species), 799 bumblebees (9 species), and 192 other wild bees (42 species). The most common bee species was *Bombus pascuorum* (410 individuals) and the most common hoverfly species was *Eristalis tenax* (703 individuals). We counted 215 species of flowering plants.

3.2.2. Pollinator diversity analyses

We found significant differences in wild bee abundance (Figure 3; $R^2 = 0.11$, $F(1, 38) = 4.66$, $p = 0.037$), representing a mean increase in log-transformed wild bee abundance of 0.591 (95% CI [0.037, 1.15]) in offset sites compared to control sites. Wild bee evenness was lower average by -0.113 (95% CI [-0.21, -0.016]) in offset sites relative to control sites (Figure 4; $R^2 = 0.13$, $F(1, 37) = 5.62$, $p = 0.023$). There was no significant difference between treatments of the species richness of wild bees, nor of any hoverfly diversity measures (Figure S1, Table 2).

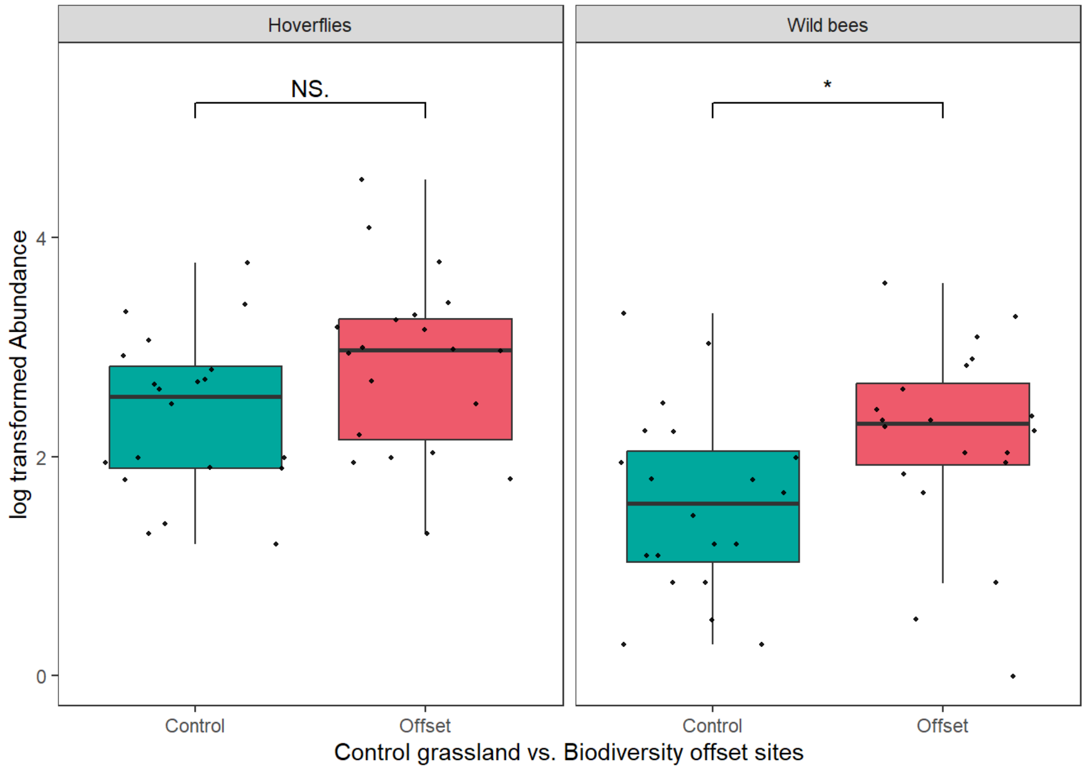

Figure 3. Boxplots comparing the log-transformed abundances of pollinator groups hoverflies (left) and wild bees (right) between control and offset grasslands in the Netherlands. * denotes a p-value < 0.05.

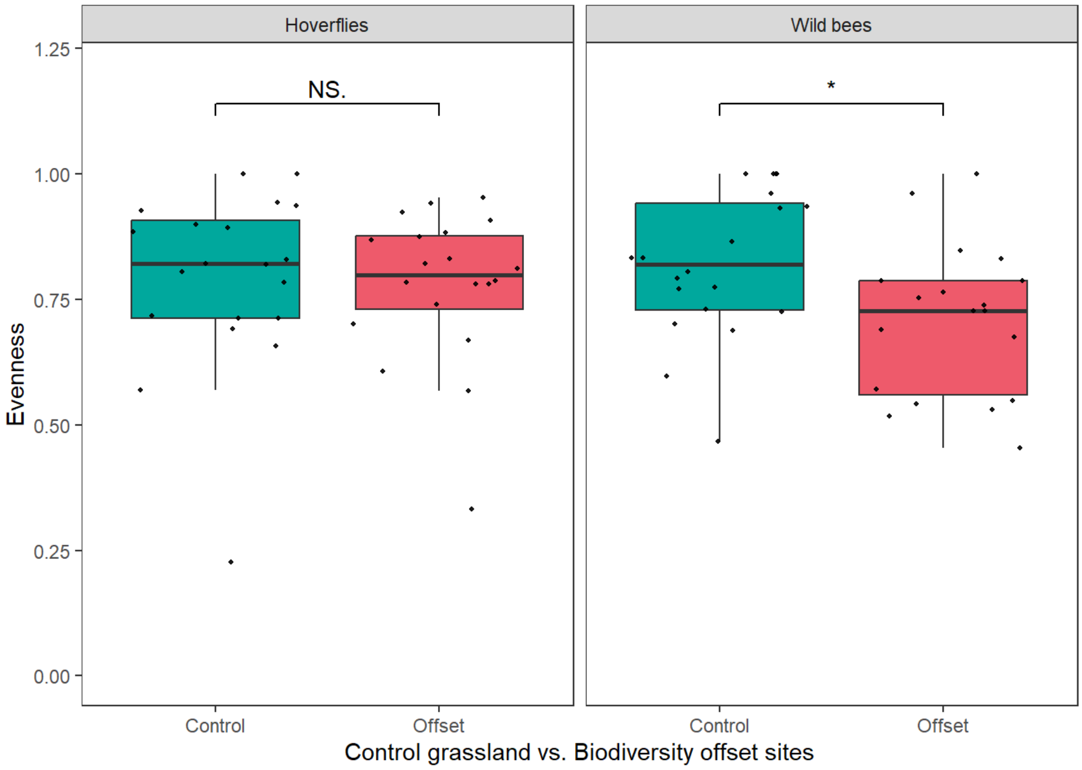

Figure 4. Boxplots comparing Pielou evenness of pollinator groups hoverflies (left) and wild bees (right)between control and offset grasslands in the Netherlands. * denotes a p-value < 0.05.**Table 2.** (General) linear model results on the effect of treatment (control versus offset

grasslands) on wild pollinator abundances, species richness, and evenness. Results are reported as model estimates with p-values in parentheses. The row SD refers to the standard deviation of the random effect term. Bolded estimates indicate significant differences. Asterisks indicate the degree of significance (* p < 0.05, ** p < 0.01, *** p < 0.001).

	Wild bee abundance (LM)	Hoverfly abundance (LM)	Wild bee richness (GLM)	Hoverfly richness (GLM)	Wild bee evenness (LM)	Hoverfly evenness (LM)	Flower cover (LM)	Flower species richness (GLMM)
(Intercept)	1.567	2.391	1.423	2.115	0.820	0.791	0.689	2.964
	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)
Treatment (Offset)	**0.591***	0.459	0.196	0.193	**−0.113***	−0.013	0.373	**0.271*****
	(0.037)	(0.067)	(0.185)	(0.063)	(0.023)	(0.796)	-0.146	(<0.001)
SD (Intercept: Pair)				0.193				0.376
N Obs.	40	40	40	40	39	40	40	40
R2	0.109	0.086			0.132	0.002	0.055	
R2 Adj.	0.086	0.062			0.108	−0.024	0.03	
R2 Marg.				0.064				0.093
R2 Cond.				0.311				0.785
F	4.655	3.566	1.755		5.623	0.068	2.208	
RMSE	0.84	0.75	2.17	3.08	0.14	0.16	0.77	7.21

3.2.3. Floral resources

We found that there was no difference in flower cover between treatments (Figure S2; R^2 = 0.055, F(1, 38) = 2.21, p = 0.15). However, flower species richness was significantly higher in offset sites than treatment sites (Figure 5; β = 0.27, SE = 0.065, z(40) = 4.16, p = 3.22e^{-05}).

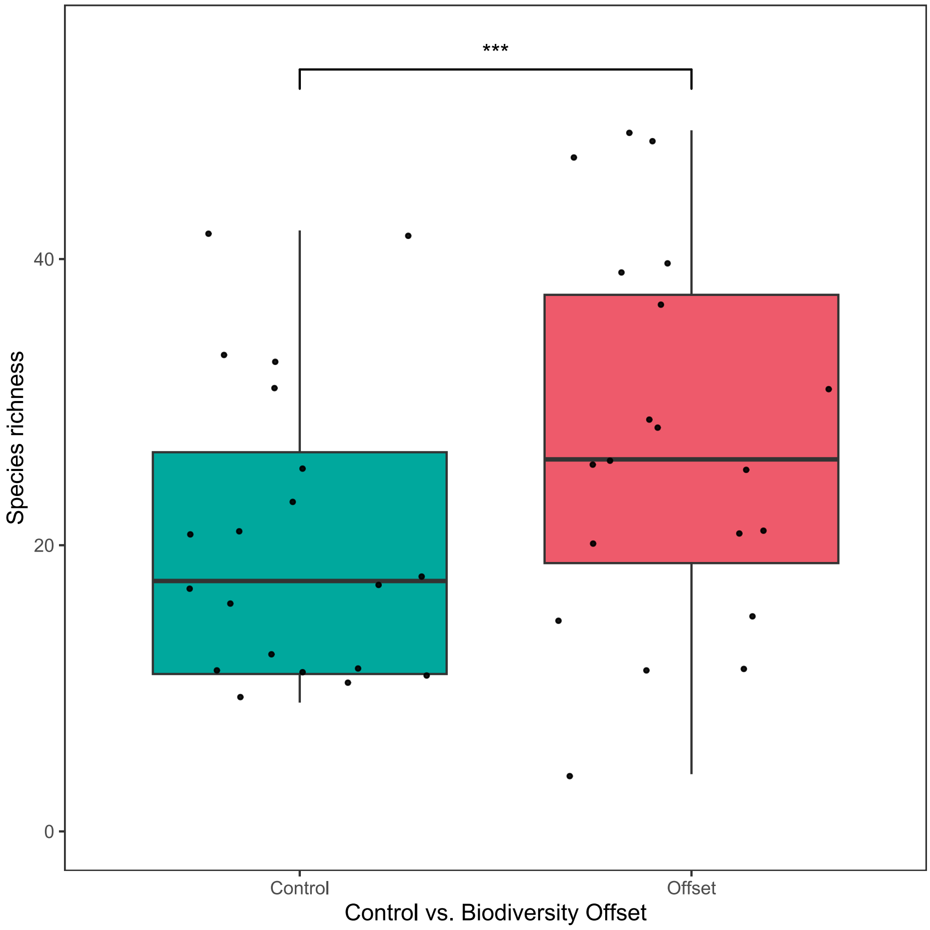

Figure 5. Boxplot of flower species richness compared between control and offset grasslands in the Netherlands. *** denotes a p-value < 0.001.

3.2.4. Community composition analyses

Significant differences in vegetation and flower species communities were found between offset and control sites, but the amount of variation in community composition explained by treatment was consistently low (< 6%; Figure 6c, Figure 6d, Table S1). We did not find a significant difference in hoverfly or wild bee species communities between control and offset sites (Figure 6a, Figure 6b, Table S1).

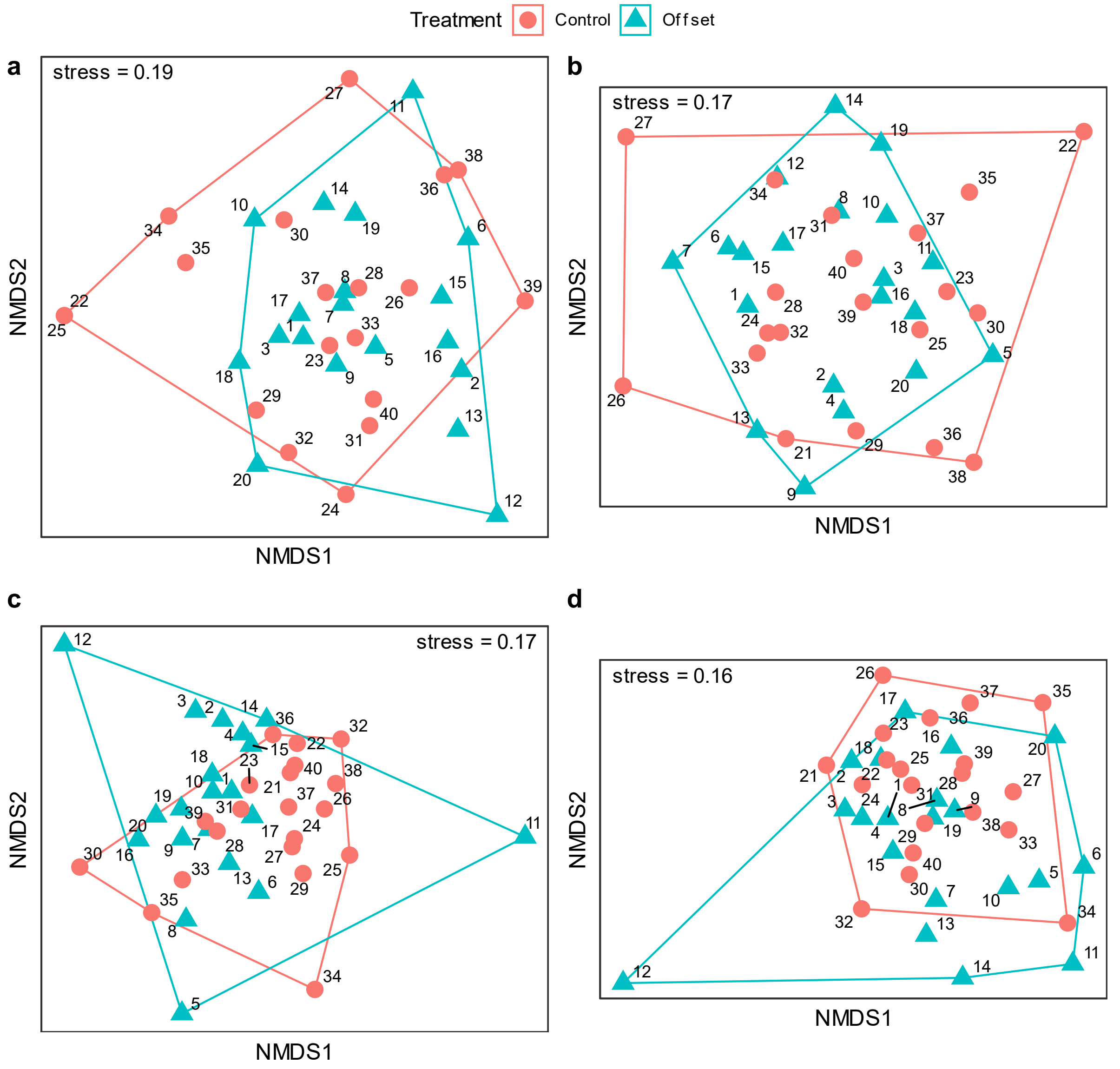

Figure 6. 2-Dimensional NMDS (Bray-Curtis) plots representing a) wild bee, b) hoverfly, c) vegetation, and d) flower communities in control and offset grasslands in the Netherlands.

Table 3. One-way PERMANOVA results (N permutations = 999) on the effect of treatment (control versus offset grasslands) on species communities. Bolded headers indicate the type of community. Bolded p-values indicate significant differences. Asterisks indicate the degree of significance (* p < 0.05, ** p < 0.01, *** p < 0.001).

	Df	SumOfSqs	R2	F	Pr(>F)
Vegetation					
Treatment	1	0.542	0.045	1.784	**0.007*** **
Residual	38	11.555	0.955		
Total	39	12.097	1.000		
Flowers					
Treatment	1	0.733	0.054	2.178	**0.004** **
Residual	38	12.796	0.946		
Total	39	13.529	1.000		
Wild bees					
Treatment	1	0.323	0.045	1.683	0.115
Residual	36	6.907	0.955		
Total	37	7.230	1.000		
Hoverflies					
Treatment	1	0.106	0.013	0.509	0.931
Residual	38	7.941	0.987		
Total	39	8.048	1.000		

3.3. Case Study: Italy

3.3.1. Pollinator observations and vegetation characteristics

We sampled a total of 893 bees (63 species), 1360 hoverflies (65 species), and 457 lepidopterans (16 species). The honeybee (*Apis mellifera* L.) was the most abundant bee species (434 individuals), followed by *Bombus pascuorum* (61 individuals). The most common hoverfly and lepidopteran species were, respectively, *Episyrphus balteatus* (860 individuals) and *Amata phegea* (252 individuals). In total, we identified 92 flowering plant species. The average flower cover was rather low in the sampled transects of both remnants and reforested sites (Table 1). On the contrary, the average canopy cover was high in both forest types (Table 1). Most (70%) of the sampled insects were recorded while flying or resting on the vegetation, whereas only 30% when visiting flowers.

3.3.2. Pollinator diversity analyses

We did not detect significant differences in bee and in hoverfly species richness, abundance, and community evenness between restored and remnant forest sites. Similarly, lepidopteran species richness did not differ between the two habitat types. We only detected significant differences in lepidopteran abundance, which was higher in remnant sites, and in lepidopteran evenness, which was higher in restored sites (Table 4; Figure 7).

Table 4. Results of general linear models testing for differences in species richness, abundance, and evenness of the sampled pollinator groups between restored and remnants forest habitats. Highlighted p values indicate significant differences.

Response variable	df	F value	P value
Bee richness	1,32	0.6519	0.4254
Bee abundance	1,32	0.6187	0.4373
Bee evenness	1,32	0.4163	0.5234
Hoverfly richness	1,32	0.7903	0.3806
Hoverfly abundance	1,32	1.7921	0.1901
Hoverfly evenness	1,32	0.8602	0.3606
Lepidopteran richness	1,32	0.2911	0.5932
Lepidopteran abundance	1,32	4.1667	**0.0496**
Lepidopteran evenness	1,32	8.4922	**0.0065**

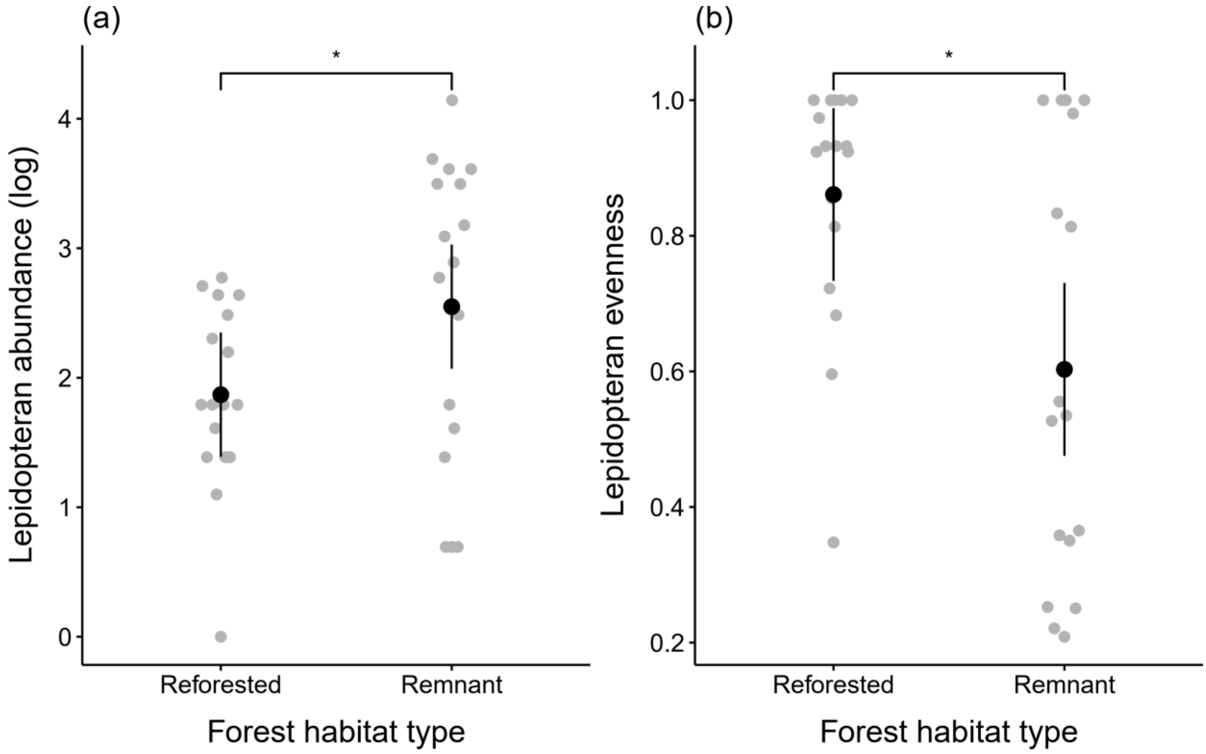

Figure 7. Lepidopteran abundance and evenness between restored and remnant sites. Dots show mean values and whiskers show 95% confidence intervals.

PERMANOVA tests based on presence-absence data of pollinator community composition revealed no significant differences between forest remnants and reforested areas for both bees ($R^2 = 0.031$, $p = 0.445$), and for hoverflies ($R^2 = 0.040$, $p = 0.101$). Moreover, no significant differences in community composition were found when considering only the presence of rare pollinator species (i.e., those occurring 5 or less times across all sampled sites; 108 pollinator species in 32 sites) ($R^2 = 0.028$, $p = 0.807$). We only detected significant but weak differences in the composition of lepidopterans between the two forest types ($R^2 = 0.070$, $p = 0.019$; Figure S3).

3.3.3. Effects of forest features on pollinator diversity

Models testing the effect of forest local and landscape characteristics in interaction with forest type did not reveal significant interactions for any groups (Table S1. Results of per-community permutation tests of multivariate group variances. A significant p-value (bolded) indicates that group variances were not homogenous.

	Df	Sum Sq	Mean Sq	F	Number of Permutations	Pr(>F)
Vegetation						
Groups	1	0.014	0.014	1.548	999	0.229
Residuals	38	0.346	0.009			
Flowers						
Groups	1	0.008	0.008	1.194	999	0.277
Residuals	38	0.268	0.007			
Wild bees						
Groups	1	0.023	0.023	1.227	999	0.263

	Df	Sum Sq	Mean Sq	F	Number of Permutations	Pr(>F)
Residuals	36	0.68	0.019			
Hoverflies						
Groups	1	0.038	0.038	6.189	999	**0.015**
Residuals	38	0.235	0.006			

Table S2). To avoid overfitting, we therefore present here only models with no interactions with forest type.

For bee species richness as response variable, multi-model inference considering both remnants and restored sites together identified 96 models constituting the top model set with a ΔAIC < 7. Our model showed that bee species richness was mainly driven by increasing flower cover and decreasing forest canopy cover (Figure 8).

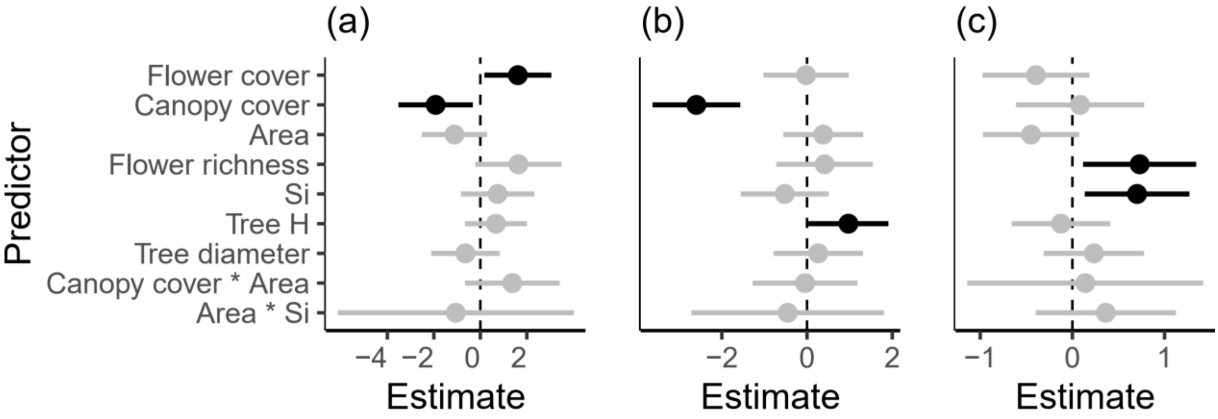

Figure 8. Model-averaged estimates of bee (a), hoverfly (b), and Lepidoptera (c) species richness varying with tested predictors. Estimates means and confidence intervals not overlapping zero are considered significant. Si = Hanski's connectivity index; Tree H = Tree Shannon diversity.

For hoverfly species richness as response variable, 40 models showed ΔAIC < 7. Top model averaging indicated that canopy cover and tree diversity significantly affected hoverfly species richness in forests (Figure 8). In particular, hoverfly species richness increased with increasing diversity of tree species in the forest, while it decreased with increasing canopy cover. For lepidopteran species richness as response variable, we identified 55 models with ΔAIC < 7. Top model averaging indicated that increasing flower richness and connectivity positively affect butterfly species richness in forest habitats (Figure 8). The hypothesized interactions between site area and connectivity with other forest patches and between site area and canopy cover did not affect the richness of neither bee, nor hoverfly, nor lepidopteran species. We also tested the effect of forest age for restored sites. This was not significant for any of the studied pollinator groups.

4. Discussion:

The use of biodiversity offsetting as a tool for halting biodiversity decline and achieving No Net Loss by 2030 (Tucker et al., 2020), both at a regional (Wende et al., 2018) and European (*Council Directive 92/43/EEC*, 1992) scale, highlights the importance of assessing its ecological effectiveness for pollinator conservation amidst significant declines (Potts et al., 2016).

To our knowledge there is no existing research in the current literature on whether compensatory nature supports similar pollinator diversity or communities to reference habitats. Given this, our case studies provide the first empirical evidence of whether biodiversity offsetting is an effective strategy for mitigating pollinator biodiversity loss. In both the Netherlands and Italy, we find variable responses of pollinator biodiversity, which we have defined in terms of diversity metrics (abundance, species richness, and evenness) and community composition. While pollinator species richness may be similar in compensation sites compared to reference sites, other diversity metrics still varied. In the Netherlands, wild bee abundances were higher in offset sites, yet community evenness declined, indicating that while offset sites may have on average larger populations of wild bees compared to controls (despite having similar floral cover), this increase in abundance might be driven by a few common species becoming even more prevalent (Hillebrand, 2003) or vice versa (Kremen et al., 2018), though further study into the exact changes in the species assemblage would be needed to determine this. In Italy, the opposite pattern was found for remnant forests: lepidopteran abundances were significantly higher in remnants, but their community evenness was significantly lower. Moreover, pollinator communities were not always equivalent in compensation sites compared to their references. In the Netherlands, we found that wild bee communities differed significantly between treatments, and in Italy we found the same for lepidopteran communities. Hoverfly communities were similar between compensation and control sites in both countries. This suggests that of all the pollinator groups evaluated, biodiversity offsetting could be ecologically effective specifically for hoverflies, perhaps given their broader feeding niche compared to wild bees (Biesmeijer et al., 2006; Lucas et al., 2018).

However, we caution interpreting these results as such that biodiversity offsets are necessarily ecologically effective, equivalent to or possibly even “better” than their reference sites. In the Netherlands, while wild bee abundances were higher, the lower evenness in offset sites suggests declines in the populations of rare wild bee species, putting them more at risk of local extinction. In Italy, the opposite relationship for lepidopterans is possibly driven by a decline in a common, though characteristic, woodland species (*Amata phegea*) within restored forests (personal observation, Elena Gazzea). The resulting lepidopteran communities are not necessarily less species rich, and are more even, but their species assemblages are no longer similar, and as such it is possible that the restored forests are not replacing (i.e., compensating for) the remnant forest pollinator communities, but are perhaps instead developing assemblages unique to restored forest. Further, in the Netherlands we found significant differences in vegetation and flower communities between controls and offsets. While this did not translate to differences in pollinator communities, it could potentially affect plant-pollinator networks as these same pollinator species might be utilising different vegetation and floral resources in the environment. Shifts in the flower community may result in lower quality forage available to pollinators (with a subsequent influence on long-term pollinator health) (Jones &

Rader, 2022; Parreño et al., 2022) or shift the relative proportions of generalist and specialist pollinator species (e.g., declining wild bee evenness versus higher flower species richness in Dutch offsets) (Hillebrand, 2003; Kremen et al., 2018). However, further research into the effectiveness of biodiversity offsets for maintaining pollinator-plant networks would be needed to determine the importance of alternative vegetation and floral species assemblages when pollinator community composition does not differ.

Our case studies utilised “target” habitat as controls to which our compensation sites were compared. In the Netherlands these controls matched the habitat type index of destroyed nature sites (protected grassland), while in Italy they matched the locally relevant goal habitat type (remnant forest). An obvious and critical issue with our space-for-time study design is the lack of ecological information on the sites that were destroyed, and the subsequent assumption that our controls were equivalent to them (Kujala et al., 2022). In the Netherlands, semi-natural grasslands like our controls have historically been heavily managed by humans and it is estimated that around two-thirds are of poor quality (Luske et al., 2023). In Italy, forest remnants in the Po Valley are highly fragmented habitats within an intense agricultural matrix (Bertolasi et al., 2015; Marchetti, 2002). Moreover, our case studies represent only two of the 27 countries in the European Union, and while for hoverflies the effectiveness of biodiversity offsetting was consistent across both the Netherlands and Italy, this was not the case for wild bees.

While biodiversity offsetting holds promise for achieving No Net Loss in the European Union, its effectiveness remains uncertain due to factors such as limited empirical evidence and reliance on space-for-time substitutions (Kujala et al., 2022). National laws governing biodiversity offsetting vary widely in scope, implementation, and success, impacting conservation outcomes for non-Natura 2000 habitats across the EU (Bull & Strange, 2018). It is possible that the offsets in our case studies are replacing destroyed (relatively) poor quality habitat with new, equivalently poor, quality habitat. While this might fulfil the requirements of “No Net Loss”, continued habitat degradation in Europe may result in biodiversity offsets simply following or reinforcing the trend of biodiversity loss rather than improving or restoring European nature. Thus, we advocate for increased empirical research to better understand and quantify the effectiveness of biodiversity offsetting, particularly with regards to conserving pollinators within the EU.

5. Acknowledgements

We would like to thank the site managers of our study sites whose cooperation made our research possible. We would also like to thank Francesca Sodano, Bram de Vries, and Liyenne Hagenberg (Wageningen University and Research) for executing the data collection in the Netherlands; and Davide Gobbo (University of Padova) for helping with data collection, Maurizio Mei (Sapienza University of Rome), Dino Paniccia, Giacomo Trotta (University of Trieste, University of Udine), and Francesco Boscutti (University of Udine) for identifying the sampled plants and pollinators in Italy.

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7. Supplementary information

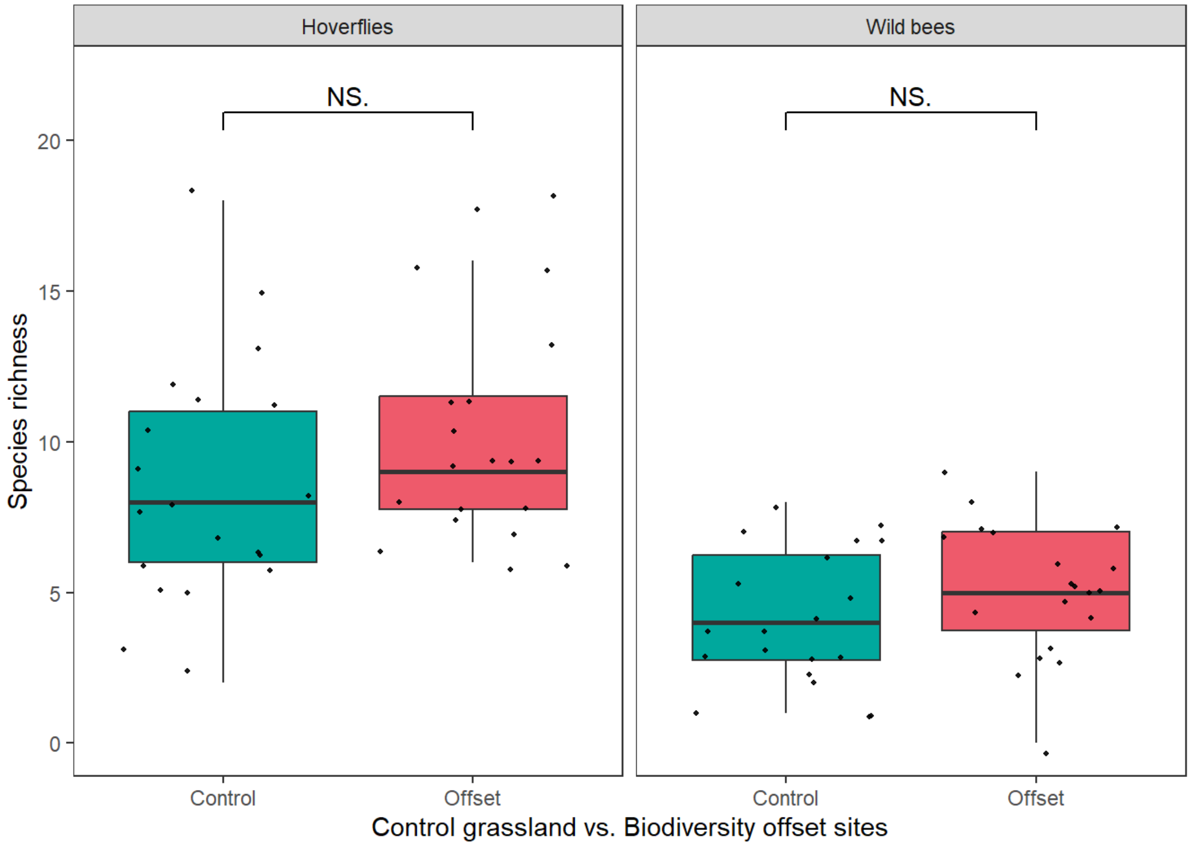

Figure S1. Boxplots comparing pollinator species richness of hoverflies (left) and wild bees (right) between control and offset grasslands in the Netherlands.

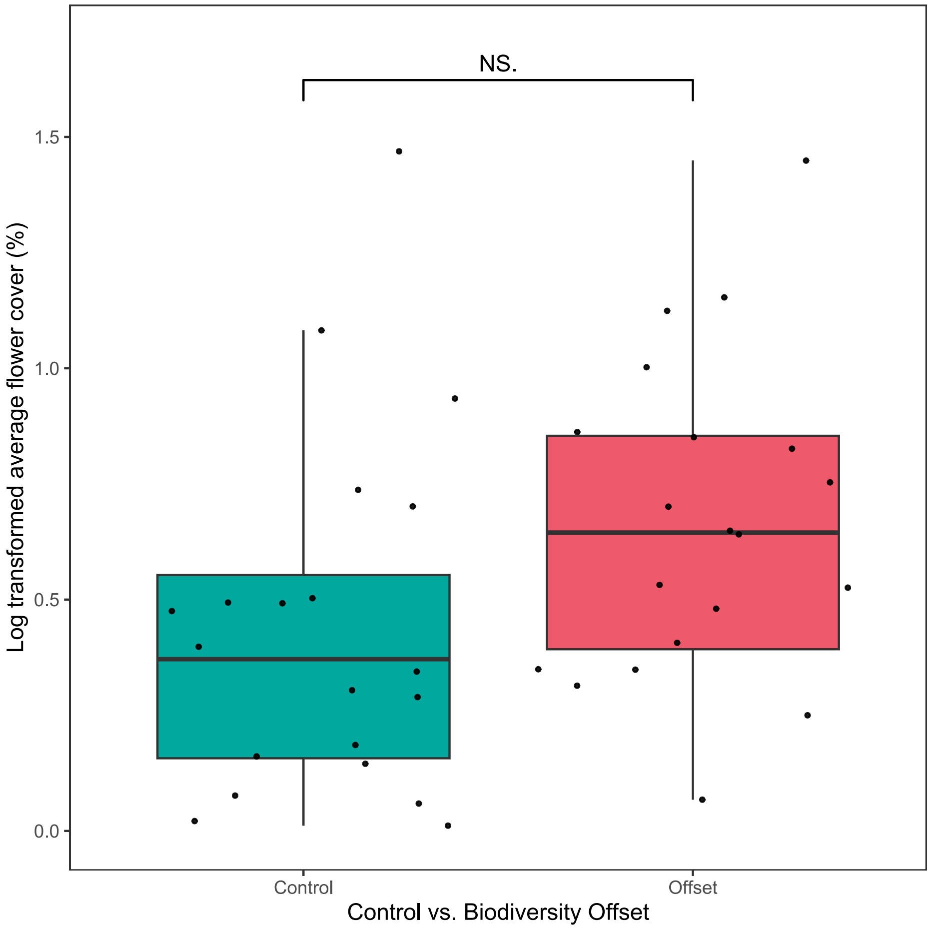

Figure S2. Boxplot of log-transformed flower cover (%) compared between control and offset grasslands in the Netherlands.

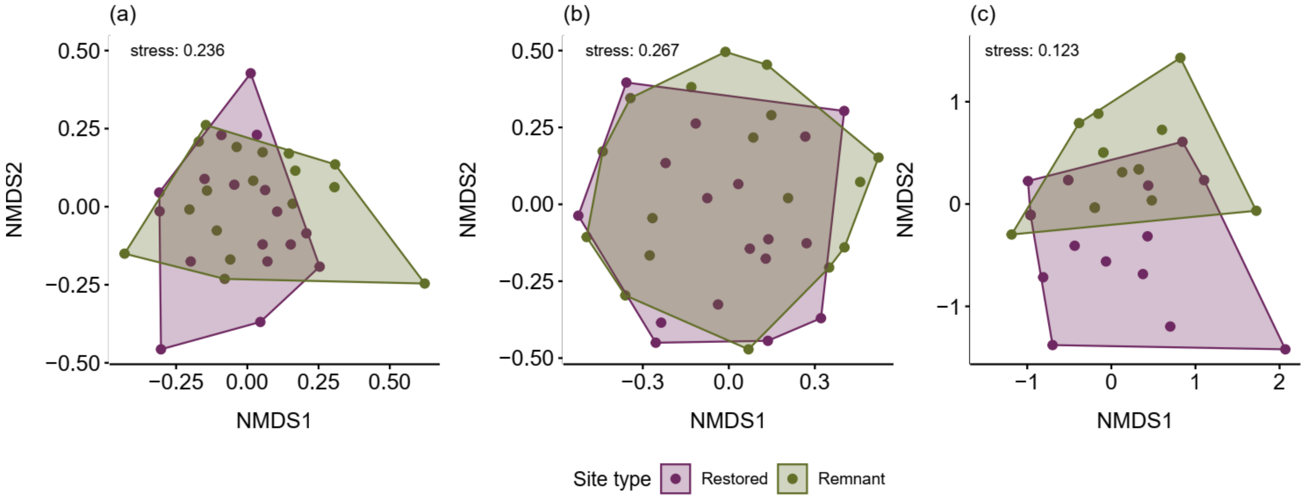

Figure S3. 2-Dimensional NMDS representing bee (a), hoverfly (b), and lepidopteran (c) communities in reforested and remnant forests.

Table S1. Results of per-community permutation tests of multivariate group variances. A significant p-value (bolded) indicates that group variances were not homogenous.

	Df	Sum Sq	Mean Sq	F	Number of Permutations	Pr(>F)
Vegetation						
Groups	1	0.014	0.014	1.548	999	0.229
Residuals	38	0.346	0.009			
Flowers						
Groups	1	0.008	0.008	1.194	999	0.277
Residuals	38	0.268	0.007			
Wild bees						
Groups	1	0.023	0.023	1.227	999	0.263
Residuals	36	0.68	0.019			
Hoverflies						
Groups	1	0.038	0.038	6.189	999	**0.015**
Residuals	38	0.235	0.006			

Table S2. Model-averaged conditional results obtained after multi-model inference analysis on the effects of interactions between pollinator diversity and forest features. Si = Hanski’s connectivity index; Tree H = Tree Shannon diversity.

Bees:

	Estimate	SE	Adj. SE	z	P
Intercept	8.31469	0.61278	0.63884	13.015	<2e-16
Flower cover	1.50492	0.82834	0.85768	1.755	0.0793
Canopy cover	-1.67657	0.84014	0.86825	1.931	0.0535
Area	-1.03667	0.6806	0.70746	1.465	0.1428
Flower richness	1.72348	0.87355	0.904	1.907	0.0566
Forest type	0.7749	0.66341	0.69162	1.12	0.2625
Canopy cover * Forest type	1.3443	0.82676	0.85669	1.569	0.1166
Flower richness * Forest type	1.48868	0.82784	0.8584	1.734	0.0829
Si	0.82664	0.69614	0.72569	1.139	0.2547
Tree H	0.56873	0.70291	0.73154	0.777	0.4369
Flower cover * Forest type	-1.25383	1.03276	1.07213	1.169	0.2422
Area * Forest type	0.11977	0.75366	0.78364	0.153	0.8785
Si * Forest type	0.02779	0.64808	0.6784	0.041	0.9673
Tree H * Forest type	0.17879	0.81165	0.84827	0.211	0.8331

Hoverflies:

	Estimate	SE	Adj. SE	z	P
Intercept	8.76525	0.43904	0.45747	19.16	<2e-16
Canopy cover	-2.59842	0.50698	0.52655	4.935	8.00E-07
Tree H	1.00605	0.48094	0.50054	2.01	0.0444
Si	-0.52774	0.495	0.51615	1.022	0.3066
Area	0.3988	0.46073	0.47994	0.831	0.406
Forest type	-0.31816	0.54722	0.56788	0.56	0.5753
Flower richness	0.40936	0.55885	0.58156	0.704	0.4815
Flower cover	-0.02699	0.49056	0.51102	0.053	0.9579
Area * Forest type	0.76485	0.49305	0.51549	1.484	0.1379
Canopy cover * Forest type	0.25006	0.49821	0.51983	0.481	0.6305
Tree H * Forest type	0.19679	0.57081	0.59592	0.33	0.7412
Si * Forest type	-0.2711	0.45106	0.47142	0.575	0.5652

Lepidopterans:

	Estimate	SE	Adj. SE	z	P
Intercept	2.7923	0.24114	0.25144	11.105	<2e-16
Area	-0.45946	0.25659	0.26737	1.718	0.0857
Flower richness	0.73174	0.30344	0.31466	2.326	0.02
Si	0.68104	0.26705	0.27802	2.45	0.0143
Flower cover	-0.40707	0.29323	0.30518	1.334	0.1822
Forest type	-0.17393	0.25629	0.26717	0.651	0.515
Canopy cover	0.09311	0.34253	0.35467	0.263	0.7929
Tree H	-0.12759	0.26319	0.27438	0.465	0.6419
Area * Forest type	-0.30586	0.27457	0.28656	1.067	0.2858
Si * Forest type	0.28113	0.24979	0.26081	1.078	0.2811
Flower richness * Forest type	-0.22352	0.27544	0.2871	0.779	0.4363
Flower cover * Forest type	-0.28964	0.34308	0.35847	0.808	0.4191