Landscape filters of functional trait diversity and composition

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Abstract

Human activities are altering species' environments, consequently driving many to extinction and changing biotic communities worldwide. Functional traits are species' intrinsic characteristics that shape their roles in an environment. Loss of functional diversity compromises ecosystem processes and potentially the resilience of communities facing further change. Biological homogenization reflects the non-random loss of species and possibly also of trait distributions within community, leading to the increasing ubiquity of some traits and growing rarity of others. By changing the composition and configuration of species' local habitat and their regional surroundings, land use can alter community dynamics. The extent to which land uses within habitats and across the surrounding landscape matrix alter the distribution of functional traits in biotic communities remains highly uncertain but could determine how to design management strategies intended to aid conservation. Here, I investigate the relative contributions of compositional and configurational landscape characteristics at local and regional scales on the diversity and composition of functional traits within butterfly communities. I constructed models to identify landscape predictors of functional trait diversity but found no significant associations with individual traits. Managing habitat quality by fostering compositional and configurational heterogeneity in the local landscape can improve functional diversity. However, efforts to facilitate conservation of species with rare traits necessitates maintaining compositional and configurational variety within habitats and in the surrounding matrix.

Résumé

Les activités humaines modifient l'environnement des espèces, poussant de nombreuses à l'extinction et changeant les communautés biotiques dans le monde entier. Les traits fonctionnels sont les caractéristiques intrinsèques des espèces qui façonnent leurs rôles dans un environnement. La perte de diversité fonctionnelle compromet les processus écosystémiques et potentiellement la résilience des communautés confrontées à de nouveaux changements. L'homogénéisation biologique reflète la perte non aléatoire d'espèces et peut-être aussi de la distribution des traits au sein de la communauté, conduisant à la dominance croissante de certains caractères et à la rareté grandissante de d'autres. En modifiant la composition et la configuration de l'habitat local des espèces et de leur environnement régional, l'utilisation des terres peut modifier la dynamique des communautés. La mesure dans laquelle les utilisations des terres dans les habitats et à travers la matrice du paysage environnant modifient la distribution des traits fonctionnels dans les communautés biotiques reste très incertaine, mais pourrait déterminer comment concevoir des stratégies de gestion destinées à aider la conservation. Ici, j'étudie les contributions relatives des caractéristiques du paysage de composition et de configuration à l'échelle locale et régionale sur la diversité et la composition des traits fonctionnels au sein des communautés de papillons. J'ai construit des modèles pour identifier les prédicteurs du paysage de la diversité des traits fonctionnels, mais je n'ai trouvé aucune association significative avec les traits individuels. La gestion de la qualité de l'habitat en favorisant l'hétérogénéité de composition et de configuration dans le paysage local peut améliorer la diversité fonctionnelle. Cependant, les efforts pour faciliter la conservation des espèces aux traits rares nécessitent le maintien d'une variété de composition et de configuration dans les habitats et dans la matrice environnante.

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Introduction

Approximately one million of assessed plant and animal species are at risk of extinction due to human actions (*IPBES*, 2019). By changing environmental conditions, anthropogenic activities have become important catalysts in community dynamics (Williams and Jackson, 2007; Hobbs, Higgs and Harris, 2009), prompting biotic homogenization and compromising ecosystem functioning. With 75% of the terrestrial surface significantly altered (*IPBES*, 2019), land use alone is estimated to have already reduced communities' species richness by 13.6% globally (Newbold *et al.*, 2015).

Species ability to respond to changing conditions depends on both extrinsic environmental opportunities and intrinsic abilities (Robillard *et al*. 2015; Boulangeat, Gravel, and Thuiller 2012; Suding *et al*. 2008). Functional traits are organisms' inherent characteristics, including morphological, physiological, behavioral or phenological attributes, shaping how they function in their environment (Díaz *et al.*, 2013). Species' responses to environmental change and their contribution to ecosystem functioning are shaped by these attributes (Violle *et al.*, 2007; Wong, Guénard and Lewis, 2019; Woodcock *et al.*, 2019). A functionally diverse community is not only more likely to contribute more to ecosystem functioning (Roscher et al., 2018; Tilman *et al*, 1997), but also to be more resilient to change (Mori et al., 2013). Yet, anthropogenic activities are impoverishing the functional diversity of animal communities worldwide. Functional trait communities are homogenizing, and their composition is changing in a non-stochastic fashion, with some traits becoming conspicuous and others more and more rare (Eskildsen *et al.*, 2015; Schloss, Nuñez and Lawler, 2012). Ecological specialists are being replaced by generalists and species with high mobility and reproduction rates are becoming increasingly dominant in insect communities (Warren *et al.*, 2001; WallisDeVries, 2014; Frishkoff *et al.*, 2016; Habel *et al.*, 2016; Mangels *et al.*, 2017; Wagner, 2020).

Land use is an important driver of the ongoing biodiversity crisis (Haddad *et al.*, 2015). The conversion of a natural land cover type into a modified one alters the composition and configuration of species' habitat and the surrounding landscape. Compositional heterogeneity measures the diversity of vegetation resources and land cover classes, namely the availability of nectar sources and larval host plants. A compositionally heterogeneous land cover mosaic is more likely to host species with specialized needs as opposed to a simplified landscape (Gámez-Virués *et al.*, 2015; Aguirre-Gutiérrez *et al.*, 2017). In turn, configurational heterogeneity reflects the physical and structural complexity of vegetation and habitat patches. Vegetation structure may influence microclimate, movement ability, shelter and niche availability. The spatial arrangement of habitat patches within a landscape further impacts habitat accessibility (Duflot *et al.*, 2014; Carrié *et al.*, 2017). The presence of boundary area allows spillover between habitat patches and the use of resources in complementary land covers, which is generally favorable to species dispersal (van Halder *et al.*, 2015). Land use can thus destroy and degrade habitats through the depletion of resources and hinder connectivity by isolating patches.

The quality of a habitat patch affects the species that may occupy it, yet regional landscape constraints affect dispersal opportunities. A compilation of studies conducted on 10 European butterfly species (Thomas, Simcox and Hovestadt, 2011) revealed that between 10-40% of quality habitat patches remained unoccupied by well suited species, suggesting the importance of regional landscape scale variables. Local variables reflect habitat quality and the availability of resources required to sustain organisms. Regional variables reflect habitat connectivity and isolation. The landscape-moderated insurance hypothesis suggests that landscape scale variables can buffer the negative impacts of in-field management. Gaméz-Virtue *et al*. (2015) found that land cover type diversity (compositional heterogeneity) at the regional landscape mitigates the effect of intense local agricultural management on functional diversity. Similarly, semi-natural grassland patches and roadsides nested within agricultural landscapes provide highly important habitats and dispersal corridors for species with vulnerable trait values (van Halder *et al.*, 2015). Community composition is a result of landscape filters at the local and regional scales, yet the relative importance of local versus regional filters on taxonomic and functional community composition is unclear.

Novel environmental conditions can effectively exclude, or filter out, species on the basis of their traits, which drives functional homogenization, further compromising ecosystem functions and communities' resilience (Tilman *et al.*, 1997; Olden *et al.*, 2004; Hooper *et al.*, 2005). Functional communities are affected by the composition and configuration of the landscape within the habitat patch and in its surrounding extent (Duflot *et al.*, 2014; Carrié *et al.*, 2017). The relative contribution of landscape configuration versus composition at the local versus regional scale on both the diversity and composition of functional traits remains unclear. Understanding the importance of these landscape characteristics (configuration, composition, local scale, regional scale) on functional communities could help landscape management prioritize in conservation projects.

In this research, I investigate the contribution of compositional and configurational landscape characteristics at local and regional scales on functional trait diversity and on functional trait composition. Functional diversity (measured as functional dispersion) quantifies the spread of species in the multivariate trait space of a community. In turn, functional composition indicates which particular functional traits are present in the community (Tilman et al., 1997; Arruda Almeida et al., 2018). I ask the following questions: 1) How do landscape characteristics influence functional diversity? and 2) Are landscape characteristics predictable filters of functional traits?

The conservation actions and stakeholders involved differ greatly between landscape management at the regional and local scales, with the difficulty being generally greater for larger spatial extents (Kral *et al*., 2018; Pocewicz *et al*.,2009; Cozzi *et al*., 2008; Poiani *et al*., 2000). It is therefore important to understand how landscape characteristics influence functional diversity at different spatial extents. Doing so can allow for efficient resource allocation in landscape management plans. In addressing my first question, I will determine if local, regional or multi-scale factors most contribute to functional diversity. Further, I will identify whether the structural arrangement (*configuration),* the diversity (*composition)* of resources or a combination of both aspects are best predictors of a functionally diverse community. With my second question, I will determine which landscape characteristics act as environmental filters on which functional traits. The identification of significant filters could reveal concrete landscape characteristics that could be managed for the protection of vulnerable or desirable species' functions. Alternatively, the absence of detectable filters could suggest that multiple landscape characteristics may influence the presence of a singular trait, thus that protection of a trait value may require heterogeneous landscape characteristics on all fronts. It could also hint to the importance of other community assembly mechanisms.

Butterflies (order: *Lepidoptera,* Families: *Hesperiidae, Lyceanidae, Papillonidae, Nymphalidae, Pieridae*) served as model organisms for this study. Butterflies have been studied substantially in the context of global change due to their known sensitivity to environmental change, the contrasting responses observed across species, and the panoply of species-specific traits they harbor throughout their life cycle (Thomas, 2005; Wagner, 2020).

Methods

Data collection

Study sites

Field surveys were conducted in 24 sites around the Ottawa region between May 14th and September 4th, 2018 (123 days; Figure 1; Appendix, Table 1). Sites were visited between 9:30 and 16:30 on days with suitable conditions for butterfly activity, i.e. non-rainy days with minimal wind, air temperature above 13°C on clear days or above 17°C on cloudy days. All sites were surveyed between 5 and 6 times throughout the season. A minimum of two weeks' interval was maintained between each resampling at a location to account for different emergence times across species.

Study sites were selected based on known butterfly presence and to represent a gradient of landscape composition and configuration at local and regional extents. Butterfly occurrence points obtained from citizen science records (iNaturalist and eButterfly) or from anterior field surveys were overlapped on orthoimages and a national land cover map. Potential sites were selected to represent a gradient of local habitats (ranging from open grassland to wooded paths) located within a gradient of regional land cover composition (ranging from highly agricultural to forest dominated landscapes). Following site visits, 24 locations that were minimally 3 km apart were selected. To prevent spatial autocorrelation, effort was made to ensure that sites that were closer together to be dissimilar in terms of the local and regional landscape matrix. Five quadrats of 5 x 5m were set up at each site and sampled for vegetation structure and floral composition during each site visit. The quadrats were selected to be representative of the vegetation structure and floral composition in the habitat patch.

Butterfly surveys

Standard Pollard walk sampling techniques were used for butterfly surveys (Pollard, 1977). Transect length varied with the size of habitats, though most transects were 200 m in length. In 9 of the 24 field sites, transects were divided into two units of 100 m each to sample the habitat more representatively or, for the smallest habitats, made shorter so the total transect length was 100 m. The surveys were conducted by two observers who walked transects at a steady pace of 10 m/min for consistent sampling intensity, pausing to record, capture or identify specimens. Butterflies were identified in flight whenever possible or captured and released for identification when they could not be identified on the wing. A few specimens per species, with the exception of monarchs and swallowtail species, were collected and brought to the laboratory. Collected individuals were added as voucher specimens and their identifications confirmed. Morphological measurements were taken on all voucher specimens.

Vegetation structure survey

Vegetation structure surveys assessing the height category of the vegetation were conducted jointly by two examiners at every site visit. The percentage cover of the land cover within the following height classes was estimated in each quadrat: 0-meter, 0-0.5 meter, 0.5-2 meters, 2-5 meters, over 5 meters. The ground cover included in the 0-meter category comprised of bare ground, non-herbaceous layer or dead organic matter.

Floral composition survey

Floral composition surveys assessing the percent cover of flowering plants were conducted jointly by two examiners, in each quadrat and at every site visit. Only plants with blooming flowers (i.e., those providing a source of nectar to adult butterflies) at the time of survey were recorded. Species-level identifications were performed where possible and to the level of genus otherwise. Most specimens of the Asteraceae family (especially *Aster* and *Solidago*) were only identified to genus.

Trait data

I selected a set of butterfly traits known to relate to species function in the environment (Table 1). Only traits with available data for all observed species were chosen. The selected traits are voltinism, overwintering stage, larval host breadth, wingspan, wing elongation, wing brightness and start of the flight period.

Voltinism, overwintering stage, flight period and larval host breadth information were obtained from datasets compiling recognized sources of information and experts' knowledge. Flight period was assessed as the Julian day marking the beginning of a species' flight period.

Morphological traits (i.e. wing brightness, wingspan and wing elongation) were measured using standardized photographs of butterflies from each species (60) recorded during the field surveys. Data collection was done using the voucher specimens collected during the field surveys and supplemented with individuals from the National Collection of Insects. Sample sizes of 10 to 21 individuals per species, amounting to 655 individuals, were obtained. Butterflies were placed in a light box built to ensure standardized lighting conditions and both their outer- and upper wing sides were photographed with a Nikon D3100 mounted on a tripod. Morphological measurements were averaged to obtain mean trait values per species.

Coloration measurements were made in GIMP 2.0 using a brightness scale ranging from 0 (white) to 255 (black) (Zeuss, 2014; Stelbrink, 2019). The brightness value was extracted from the outer and upper wing with the color picker tool (radius 300) and the average brightness of both wing sides was computed.

Morphometric measurements were calculated using ImageJ 1.50e software (NIH, 2015). Forewing length was measured as the distance between the intersection of the R4 vein and wing margin and the end of the discal cell (Figure 2). Forewing width was measured as the longest straight-line parallel to the forewing length (DeVries, Penz and Hill, 2010). Wingspan and wing elongation were measured as follows:

$$\text{Wingspan (cm)} = (2 \times \text{Forewing Length}) + \text{Thorax Width}$$

$$\text{Wing Elongation (cm)} = \text{Forewing Length} / \text{Forewing Width}$$

Landscape data

Local landscape data were obtained *in situ* during field surveys, as detailed above. Regional landscape data were acquired from open source Geographic Information System (GIS). All GIS analyses were computed using ArcGIS 10.6 (ESRI, 2018).

Patch size was measured on orthoimages (i.e, satellite images corrected for distortion, also called orthophotos) sourced from a compilation of image collections to cover the extent of the region studied. All orthoimages had a 20 cm resolution. The 2017 Orthophotos from the City of Ottawa and the National Capital Commission were used when possible (9 sites) and 2014 Orthophotos from the City of Ottawa and the National Capital Commission (5 sites) or the 2014 Digital Raster Acquisition Project for the East (DRAPE) collection (10 sites) were used otherwise. The perimeter of the habitat patch was traced as a polygon feature enclosing the uninterrupted area of the same vegetation type and structure as the focal sampling point.

Land cover information was obtained from the 2010 Land Use map of Canada published by Agriculture and Agri-Food Canada (https://open.canada.ca/data/en/dataset/9e1efe92-e5a3-4f70-b313-68fb1283eadf). This classification was derived from Landsat TM5 mosaics at 30m resolution for all areas of Canada including or near agricultural land uses. The land use product identifies 13 land use/land cover classes, including Forest, Treed, Water, Wetland, Forest Wetland, Treed Wetland, Wetland Shrub, Wetland Herb, Cropland, Grassland unmanaged, Grassland managed, Roads, Settlement, Other land and Unclassified. Buffers at 500 m and 2000 m were traced around the perimeter of each habitat patch (Figure 3, Figure 4) and validated over repeated site visits to each habitat. The area of each land use/land cover class was extracted for both buffer distances with the Zonal Statistics as Table tool in ArcGIS.

Community diversity metrics

Diversity analyses were conducted using the Diverse (Miguel Guevara, 2017) and Vegan (Oksanen et al., 2019) packages in R (R-4.0.0; R Core Team, 2020).. Diversity indices were calculated for flowering plants and vegetation structure at the local scale, and for land cover Hill number for Shannon's diversity (entropy of Shannon's index, $q = 1$) served to calculate diversity indices for flowering plants and vegetation structure at the local landscape, and for land cover composition at the regional landscape(Chao et al., 2014). This metric provides an easily interpretable measure of diversity equivalent to the number of equally abundant "species" needed to obtain the same value.

Butterfly sampling intensity across sites was estimated with the iNEXT package in R and using individual-based abundance data (Hsieh et al., 2016) . Each site's total species richness served as the specified endpoint to rarefy and extrapolate both a coverage-based and a sample-size based sampling curve for each community. These sample estimates and their confidence intervals were used to draw a sample completeness curve per site (Appendix Figure A1).

Functional diversity was measured using the functional dispersion metric, FDis (Laliberté and Legendre, 2010). It is a robust metric that weighs trait diversity by species' relative abundance and is therefore less sensitive to the presence of rare species with extreme trait values (Aguirre-Gutiérrez et al., 2017; Pakeman, 2014). FDis creates a multidimensional trait space measuring the average distance of each species to the centroid of all species. Both the centroid and the average distance are weighted by species abundance to compute a functional dispersion value.

Statistical analyses

Response of functional diversity to multiple landscape variables

I assessed the response of functional diversity to predictor landscape variables using multiple linear regression with all measured quantitative variables. The assumptions of multiple linear regression are of linearity between the response variable and the predictor variables, no multicollinearity between the independent variables, and of homoscedastic and normal distribution of the residuals. To address the linearity assumption, the relationship between functional diversity and each potential predictor was first examined. Logarithmic transformations were applied to the independent variables *patch area*, percent cover of habitat within 500 m and percent cover of habitat within 2000 m to correct highly skewed relationships. Nonlinearity was also observed in the responses of functional diversity to the *diversity of vegetation structure* and to the *diversity of land cover classes within 500 m* variables. Visual inspection of the bivariate scatterplots suggested unimodal quadratic responses. ANOVA confirmed the better fit when the relationships were modelled as second order polynomial functions (vegetation structure diversity, p= 0.04873; land cover diversity within 500m, p= 0.0131). To avoid multicollinearity between the first and second orders, I fitted the quadratic relationships as orthogonal polynomials. Prior to model selection, all variables were standardized to make partial regression coefficients comparable.

I used an automated model selection approach to find the best model among a list of candidate models testing the main effect of predictor variables. The models were ranked using the Akaike Information Criterion corrected for small sample size (AICc). AICc estimates the quality of a model in comparison to other models by favoring high goodness-of-fit and penalizing model complexity.

Residuals of the best fit model were observed to confirm meeting the linearity and homoscedasticity assumptions of multiple linear regression (Appendix, Figure 2. Multicollinearity was tested with the Variance Inflation Factor (VIF).

Trait-landscape relationships

I assessed the relationship between landscape variables and specific functional traits using the combined RLQ and fourth-corner methods (Dray et al., 2014). The RLQ and fourth-corner analyses are two statistical approaches used to examine the relationship between trait composition and environmental variables in ecological communities. They both rely on the information contained in tables **R** (R-mode), **L** (Link), and **Q** (Q-mode), containing respectively environmental (i.e. landscape) variables per site, species abundance per site, and trait values per species. However, they differ in that the RLQ analysis is a multivariate method observing the joint structure among the tables, while the fourth-corner is a bivariate approach observing the associations between individual combinations of traits and environmental variables. Both methods are complementary to each other and, when combined, can provide an in-depth understanding of the trait-environment relationship (Dray et al., 2014; Dray & Legendre, 2008; Thioulouse et al., 2018).

RLQ analysis examines the covariance among its constituent three tables. Each of the **R**, **L**, and **Q** table independently undergoes ordination; correspondence analysis (CA) for the species abundance table (**L**) and principal component analysis (PCA) for the landscape variables and the trait tables (**R** and **Q**). Containing a combination of continuous and categorical variables, the PCA for **Q** was conducted with the Hill & Smith method designed for mixed variables (Dray et al., 2014b; Hill & Smith, 1976). The separate ordinations assign scores to each variable: traits, species, environmental variables and sites. RLQ analysis then computes linear combinations of traits (trait syndromes) and landscape variables (landscape gradients), weighted by sites and species scores, by maximizing their covariance. A global value measuring the total coinertia, i.e, the amount of multivariate variation shared by the tables, is computed. Observation of the RLQ biplot and outputs identifies main patterns of variation shared among groups of traits and environmental variables (Dolédec *et al.*, 1996; Dray *et al.*, 2014).

In turn, the fourth-corner analysis examines individual associations between each trait and environmental variable combination (Legendre, Galzin and Harmelin-Vivien, 1997). A fourth-corner statistic is computed as a measure of the bivariate trait-environment association. This approach uses double permutation as a randomization procedure (permutation on species and on sites), for which the level of type 1 error is corrected with the sequential approach (ter

Braak, Cormont and Dray, 2012). With the sequential approach, the largest of the two *P* values obtained from each permutation model is selected to assess the significance of the association. Dray & al. (2014) discuss the importance of correcting for multiple comparisons, an approach not initially included in earlier work using the fourth-corner method (Legendre, Galzin and Harmelin-Vivien, 1997; Dray and Legendre, 2008; Rueda, Godoy and Hawkins, 2018). With a total of 105 bivariate tests (15 trait categories/states, 7 landscape variables), I applied the False Discovery Rate (FDR) correction method along with a high number of permutations (n= 49999 repetitions) to detect significant associations (Dray *et al.*, 2014).

RLQ analysis summarizes the structure of **R**, **L**, and **Q** tables, without providing significance testing. In turn, the fourth-corner analysis identifies significant bivariate associations between traits and environmental variables, without examining the covariance within their datasets. Dray *et al*. (2014) suggested joining both analyses to address their respective limitations. Fourth-corner testing can be applied directly to the outputs of the RLQ analysis to test the significance of the global association of the three tables and to measure the relationship of individual traits and environmental gradients, or environmental variables and trait syndromes.

A joint RLQ-fourth-corner approach can test the significance of the global association representing the total covariance among the three tables. The global value (sum of eigenvalues) calculated in the RLQ analysis is compared to values from two null models obtained through double permutation. The null models are built by respectively permuting *sites (*Model 2) and *species (*Model 4) from the **R** table (n=49,999 repetitions). The global significance testing has a null hypothesis of no overall association between environmental and trait tables. Its alternative hypothesis is of greater covariance between the observed trait and environmental tables than produced by the null models. As such, the *p*-value is calculated as the number of null model values equal or greater than the observed. From the sequential approach, the highest of the two *p*-values obtained from model 1 and model 2 is selected for statistical testing with the observed value. A *p*-value less than the significance level of α=0.05 would inform the rejection of the null hypothesis.

Fourth-corner analysis can also be applied to RLQ outputs to test the association between an individual trait and environmental gradients (**A)**, or an environmental variable and a trait syndrome (**B)**. Environmental gradients (from the ordination of table R, **A**) and trait syndromes (from the ordination of table Q, **B**) computed in the RLQ analysis are inputted in the fourth-corner analysis *in lieu* of the raw data. Observed values of fourth-corner statistics are computed for each bivariate combination this way. As per the fourth-corner testing procedure, double permutation is then applied. Permutation of *species* in the **A** table generates a new environmental gradient (**A***) and permutation of *sites* in table **B** generates new trait syndromes (**B***). A first null model is built using the new environmental scores and the original trait data (**A*** and **Q**), and a second using original environmental data and the permuted trait data (**A and Q***). Another pair of null models is created using **B** and **R**, instead of **A** and **Q**. Significant associations between single traits and environmental gradients, and between single environmental variables and trait syndromes, along a number of selected axes can be identified using a sequential approach on *p*-values corrected for multiple comparisons.

Results

Data collection

I observed a total of 4798 individual butterflies and 60 different species across the 24 sites around the Ottawa region (Appendix , Table 2). European skippers accounted for 1204 sightings (25.09%) and Common ringlets for 971 (20.23%). Butterfly abundance per site ranges from 13 to 761 individuals encountered and species richness per site ranged from 7 to 29 species. Sample coverage estimates range from 0.705 to 0.993 across sites (Appendix, Figure 1).

One hundred and fifty-nine flowering plants were recorded, with species richness per site ranging from 21 to 55 species.

Response of functional diversity to multiple landscape variables

To explain the relationship of functional dispersion and landscape variables, I ranked an exhaustive list of candidate models using AICc as a selection criterion. The model with lowest AICc score (Model 1, AICc = 62.742, adj. $R^2 = 0.450$, $p = 0.001757$) contained two predictor variables relating to the local landscape: floral diversity (compositional) and vegetation structure diversity (configurational) (Table 2, Figure 5, Appendix Table 2). Three alternate models (Models 2-4) were within 2 AICc units of Model 1, thus could have similar predictive power (Burnham and Anderson, 2002). Four parameters are shared between the four models with lowest AICc value: vegetation structure diversity recurs in all four models, floral diversity in three models (Model 1, 3-4), percentage habitat within 500m appears in two models (Model 2-4) and the diversity of land cover classes within 500 m is found in one model (Model 3). Model 1 has a greater explanatory and marginally better predictive powers than Model 2 (Model 2, adj.r squared=0.424, *p*-value=0.002726, ΔAICc=1.098). Model 1 contains the two variables which were shared across all or almost all of the four models, thus I retained it as the best model. Functional dispersion increased with floral diversity (Coef. = 0.3798, *p*-value=0.03205). As for vegetation structural diversity, both the first (Coef. = 2.7097, *p*-value=0.00171) and second order (Coef. = 2.2901, *p*-value=0.00847) terms are positive (Figure 6).

Trait-landscape relationships

Following RLQ analysis, the first two principal components summarized the relationship between traits and landscape variables with a shared co-variance of 91.39% and were therefore kept for subsequent analysis (1st axis= 65.10%, 2nd axis=26.29%; Figure 7). Both the **R** and **Q** tables maintained a high proportion of their variability along the first (**R**=79.25%; **Q**=81.73%) and second (**R**=85.44%; **Q**=73.39%) axes. The sum of coinertia shared across the **R**, **L**, and **Q** tables was computed as 0.6545. Double permutation found this global value to be non-significant (Model 2 p = 0.3503, and Model 4 p = 0.2911). I did not reject the null hypothesis of no global association between butterfly traits and landscape characteristics (Table 5). Further, the fourth-corner analysis identified no significant bivariate associations between individual traits and landscape characteristics (Appendix, Table 4). Application of the fourth-corner analysis method on the RLQ outputs revealed the only significant associations between single variable and the first correspondence axis (projected inertia of 65.10%) to be land cover diversity at both 500m (adj. *p*-value= 0.0112) and 2000 m (adj. *p*-value= 0.01428) (Figure 8, Table 3; Appendix, Table 3). No trait contributed significantly to the inertia projected along the first axis. Accounting for 26.29% of the projected coinertia, the second axis displayed no significant association with any trait nor landscape variable.

Discussion

My results indicate that landscape characteristics are predictors of functional trait diversity of butterfly communities yet do not define trait composition. Local scale variables relating to both compositional and configurational heterogeneity promote functionally diverse communities, but neither local nor regional characteristics define the identity of those traits.

Landscape management for conservation purposes often aims to expand or improve the quality of habitat patches, counter habitat loss, enhance connectivity or restore landscape heterogeneity in the regional matrix (Fuentes-Montemayor *et al.*, 2017; Harlio *et al.*, 2019). The relevance of these strategies depends on the primary conservation objective. Decisions to manage the composition or configuration of the landscape at any spatial scale should therefore reflect the explicit conservation target (Harlio *et al.*, 2019).

In the context of biotic homogenization, a critical conservation priority is the protection of functional heterogeneity in biotic communities. Functional attributes contribute to biological entities' (e.g., species, communities, populations) ability to resist and recover from disturbances and maintain ecosystem functions (Tilman et al., 1997; Clavel et al., 2011; Oliver et al., 2015; Woodcock et al., 2019). My results suggest that conservation of functional diversity is contingent on the quality of habitat patches. At the local scale, variables measuring respectively the heterogeneity of landscape composition (floral diversity) and configuration (vegetation structure diversity) were found to predict functionally diverse butterfly communities. Also a local configurational variable, the size of the habitat patch did not predict functional diversity and neither did the composition and configuration of the landscape within the regional matrix. Floral diversity reflects the availability of resources for adult butterflies with varied feeding needs while vegetation structure diversity may reflect the provision of shelters, which influences butterfly species' distribution (Thomas, Simcox and Hovestadt, 2011). Hence, habitats with heterogeneous vegetation composition and configuration can host species with different sets of attributes, likely to differ in their environmental responses and contributions.

The prevalent importance of local over regional landscape variables in taxonomic and trait composition was previously observed in butterfly communities (Pöyry *et al.*, 2009; Krämer, Poniatowski and Fartmann, 2012; van Halder *et al.*, 2017). However, some studies have identified regional landscape structure has a more important filter of community composition than local variables (Kral *et al.*, 2018), namely in agricultural patches (Gámez-Virués *et al.*, 2015; Perović *et al.*, 2015). Compositionally heterogeneous regional matrices buffer the functional homogenization effect of highly managed sites (Gámez-Virués *et al.*, 2015). I suggest that the role of the regional landscape on community composition may be secondary; the importance of the regional landscape may increase with decreasing habitat patch quality. To host species with varied niches, a community must have access to a diversity of resources, which are provided for by compositional and configurational heterogeneity. When these resources can be obtained in the habitat patch, the regional landscape may be of lesser relative importance. In turn, when the habitat patch is of lower quality, heterogeneous land cover types in the regional matrix may serve to provide complementary resources. In fact, resources obtained from the regional landscape were found to buffer the negative impact of low local resources on butterfly species' composition. The contribution of regional landscape characteristics on the taxonomic and trait composition of butterfly communities showed to be stronger in low quality habitats identified as dispersal corridors than in grassland (van Halder *et al.*, 2017). More targeted studies may be needed to explore the interactive effect of resource availability in the local habitat and the regional landscape on functional diversity.

Species' extinction risks following environmental change are biased by their traits (Koh et al., 2004; Mattila et al., 2008). Conservation measures designed to protect species with vulnerable functions could curtail taxonomic loss. A significant association between traits and landscape characteristics would have revealed a filtering effect of landscape on functional composition. Yet, I recorded an absence of relationship between species' functions and compositional or configurational landscape characteristics at the local or regional scales. The lack of overall association between the structure of trait and landscape tables indicates that species distribution is not structured by traits (in *fixed environmental conditions*) nor by landscape composition or configuration (for *species with fixed traits*; Dray *et al.*, 2014). A significant landscape gradient was detected with land cover diversity at 500m and 2000m, yet it was not found to be an environmental filter of functional traits. The absence of links between individual traits and landscape variables or gradients observed in my research supports the alternative hypothesis that species' response to environmental variation is not determined by a single trait. Similarly, the lack of significant trait syndrome associated with an environmental characteristic suggests that multiple landscape features may influence trait presence. As such, seeking to ensure the presence of species with a particular trait may necessitate supporting compositional and configurational variety in the local and regional landscape.

Several functional traits have previously been associated with compositional, configurational, regional or local landscape characteristics, although the associations vary across studies. Barbaro & vanHalder (2009) linked butterfly traits in species with higher extinction risks (e.g. least developed overwintering stages, narrow feeding breadth and short flight period) with a more acute sensitivity to the proportional coverage of specific land cover (compositional), including shrub, croplands and pine forests, than to gradients of fragmentation (configurational).

Similarly, compositional simplification of the regional landscape was found to be a filter of feeding specialization in agricultural settings (Gámez-Virués *et al.*, 2015) and high coverage of forest (compositional) in a 50 m extent around the grassland positively influenced the presence of small-sized grassland species with low mobility (Toivonen *et al.*, 2017). Yet, configurational heterogeneity, locally and regionally, benefited larval specialists, sedentary and short-winged species in managed landscapes (Perović *et al.*, 2015). In turn, sedentary species surveyed in residential gardens were particularly influenced by local factors, as opposed to regional variations which more so affected mobile garden visitors (Olivier *et al.*, 2016). The absence of consensus in finding landscape filters of functional traits further suggests that maintaining landscape mosaics rather than enhancing single characteristics may better serve in fostering species with vulnerable functions (Villemey *et al.*, 2015). If the response of a species to a changing landscape characteristic is influenced by the interaction of multiple traits and that the interaction of landscape characteristics influences the presence of a single trait, maintaining habitats and regional landscapes with high heterogeneity of compositional and configurational structures matrix may be the best strategy to safeguard all traits. This result corroborates the conclusion of previous studies promoting the importance of multiscale conservation strategies preserving both high quality habitat patches and heterogeneous landscapes to support functionally and taxonomically rich Lepidoptera fauna (Samu *et al*., 2016; Liivamägi *et al*., 2014).

The absence of detectable landscape filters on functional traits could also suggest that other community assembly mechanisms are important drivers of functional trait composition (Adler et al., 2013; Pavoine & Bonsall, 2011; Wong et al., 2019). The presence of limiting factors may contribute to community assembly. The identity of floral resources or land cover type, more so than their diversity, may be a limiting factor for the presence of butterfly species with narrow feeding or habitat breadth (Curtis et al., 2015; Ekroos et al., 2010). Further, resource competition by dominant species may influence community composition (Kunte, 2008). The species with highest sightings in my field surveys, the European skipper *(Thymelicus lineola),* is non-native to North America. Only present in its adult form for approximately 2 weeks, it can dominate a community and potentially exclude species. Removing dominant or non-native species from further analyses might reveal greater taxonomic and trait diversity. The landscapes surveyed may possibly not be sufficiently degraded to filter functional traits. The absence of environmental filter on functional trait may therefore reflect landscapes without extreme disturbances or limiting conditions.

The heterogeneity of landscape matrices among my sites may have limited the ability to capture significant environmental gradients and detect associations with functional traits. Expanding my research to include more sites may increase the power to expose significant filters. This study could also easily be extended to test alternative sets of traits and landscape variables, including different metrics of landscape composition and configuration or additional traits known to relate to species function with their environment, such as oviposition (Valdés and Ehrlén, 2018) and longevity (Gibbs and van Dyck, 2010). Intraspecific trait variability was recognized to influence species' persistence in changing conditions (Lepš *et al.*, 2011). Moreso, it was found to contribute to changes in functional composition in lichen communities across a broad latitudinal gradient (Hurtado *et al.,* 2020). Integrating intraspecific trait variation into the present study would require extensive data collection yet might reveal new functional trait communities and reveal more precise relationships with compositional and configuration landscape components.

Conclusion

Functional traits are species' characteristics influencing how they function in their environment. Anthropogenic changes to the composition and configuration of species' local habitat and their regional surroundings contribute to the biotic homogenization which threatens ecosystem functioning and communities' resilience. Efficient science-informed landscape management may mitigate the loss of functional diversity and functional traits. Compositional and configurational heterogeneity in the habitat patch can improve functional diversity. However, landscape characteristics do not act as environmental filters of functional traits. This result suggests that maintaining heterogeneity at various landscape scales is required to facilitate the conservation of species with vulnerable traits and may also reflect the importance of alternative community assembly mechanisms.

Tables and Figures

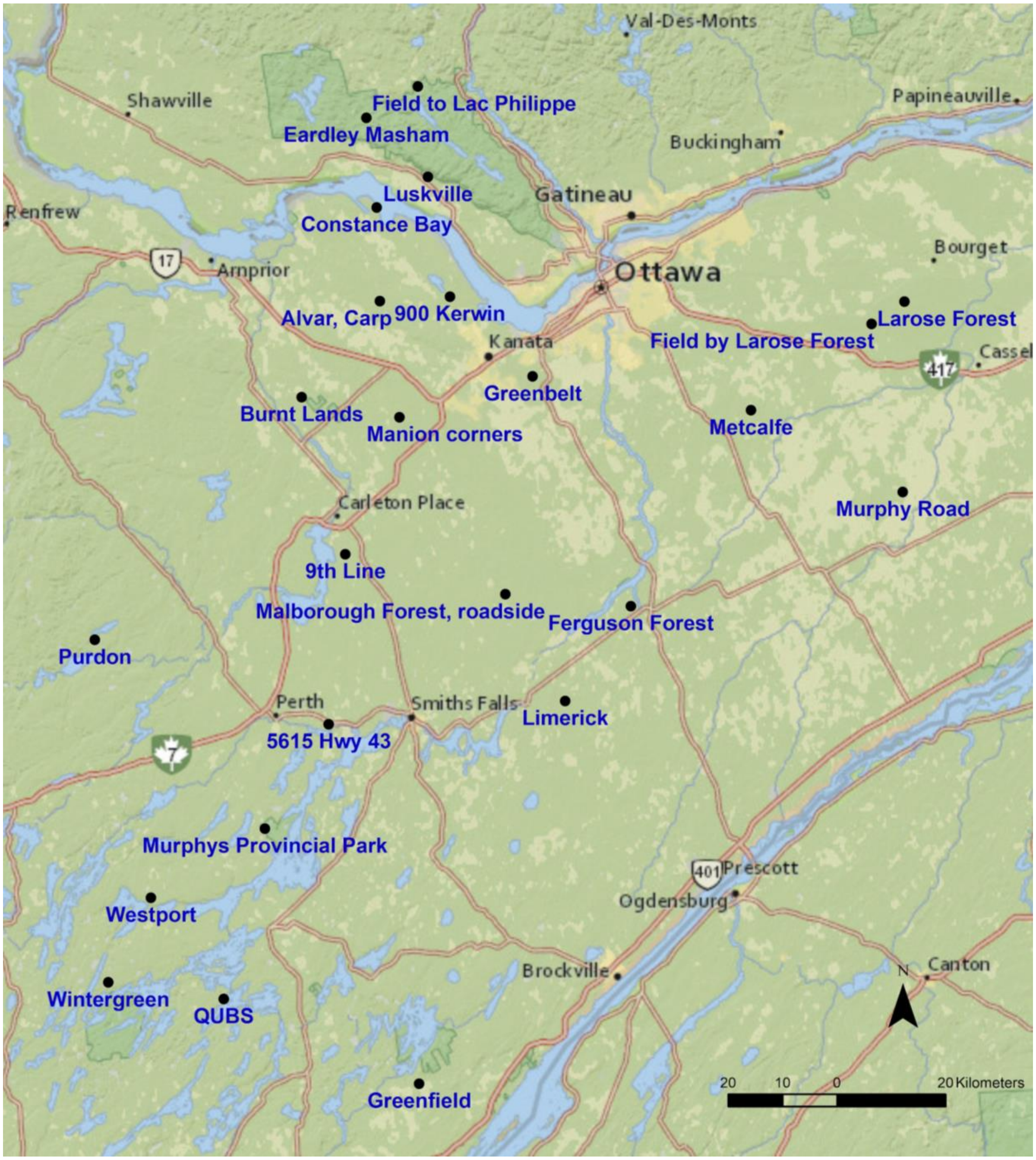
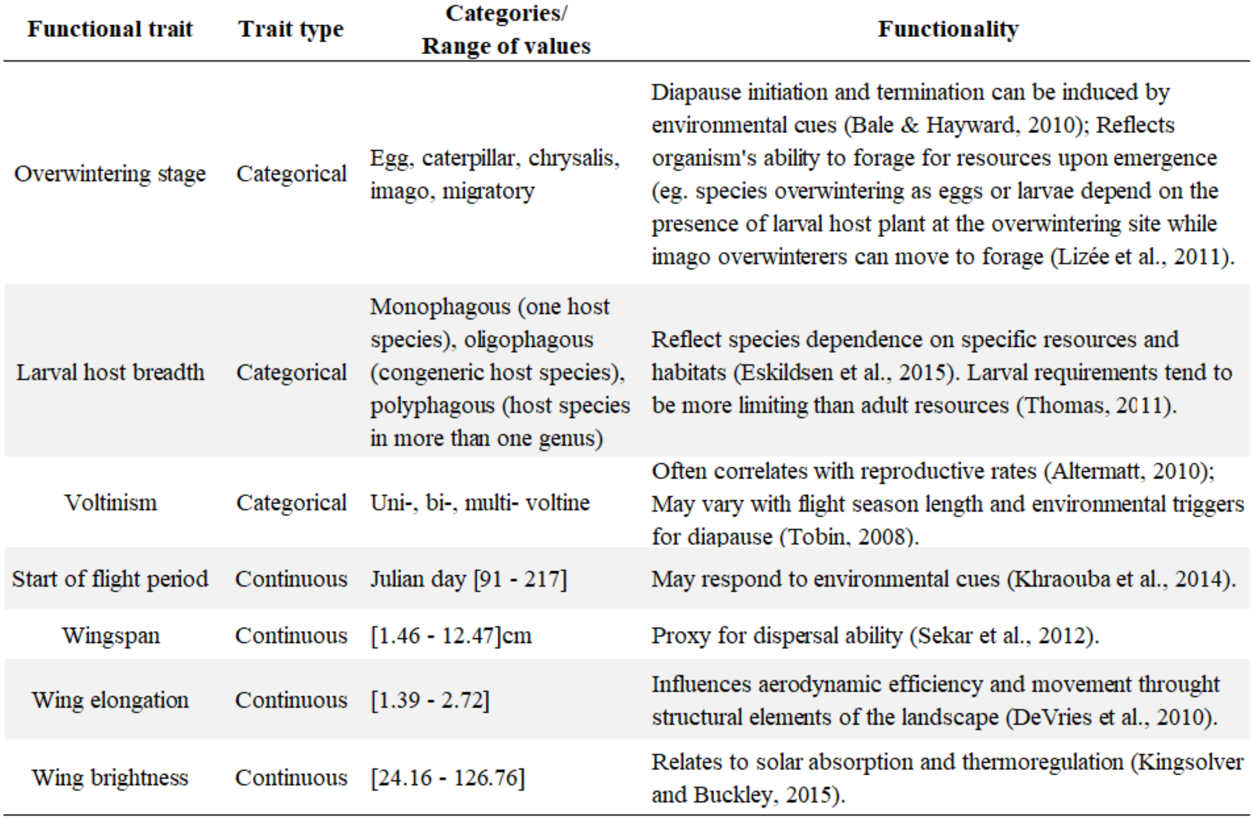


Figure 1. Distribution of 24 survey sites in the Ottawa-Gatineau region. The base map was sourced from ESRI

Table 1. Butterfly traits and examples of their functions in the environment.



Functional trait	Trait type	Categories/ Range of values	Functionality
Overwintering stage	Categorical	Egg, caterpillar, chrysalis, imago, migratory	Diapause initiation and termination can be induced by environmental cues (Bale & Hayward, 2010); Reflects organism's ability to forage for resources upon emergence (eg. species overwintering as eggs or larvae depend on the presence of larval host plant at the overwintering site while imago overwinterers can move to forage (Lizée et al., 2011).
Larval host breadth	Categorical	Monophagous (one host species), oligophagous (congeneric host species), polyphagous (host species in more than one genus)	Reflect species dependence on specific resources and habitats (Eskildsen et al., 2015). Larval requirements tend to be more limiting than adult resources (Thomas, 2011).
Voltinism	Categorical	Uni-, bi-, multi- voltine	Often correlates with reproductive rates (Altermatt, 2010); May vary with flight season length and environmental triggers for diapause (Tobin, 2008).
Start of flight period	Continuous	Julian day [91 - 217]	May respond to environmental cues (Khraouba et al., 2014).
Wingspan	Continuous	[1.46 - 12.47]cm	Proxy for dispersal ability (Sekar et al., 2012).
Wing elongation	Continuous	[1.39 - 2.72]	Influences aerodynamic efficiency and movement throught structural elements of the landscape (DeVries et al., 2010).
Wing brightness	Continuous	[24.16 - 126.76]	Relates to solar absorption and thermoregulation (Kingsolver and Buckley, 2015).

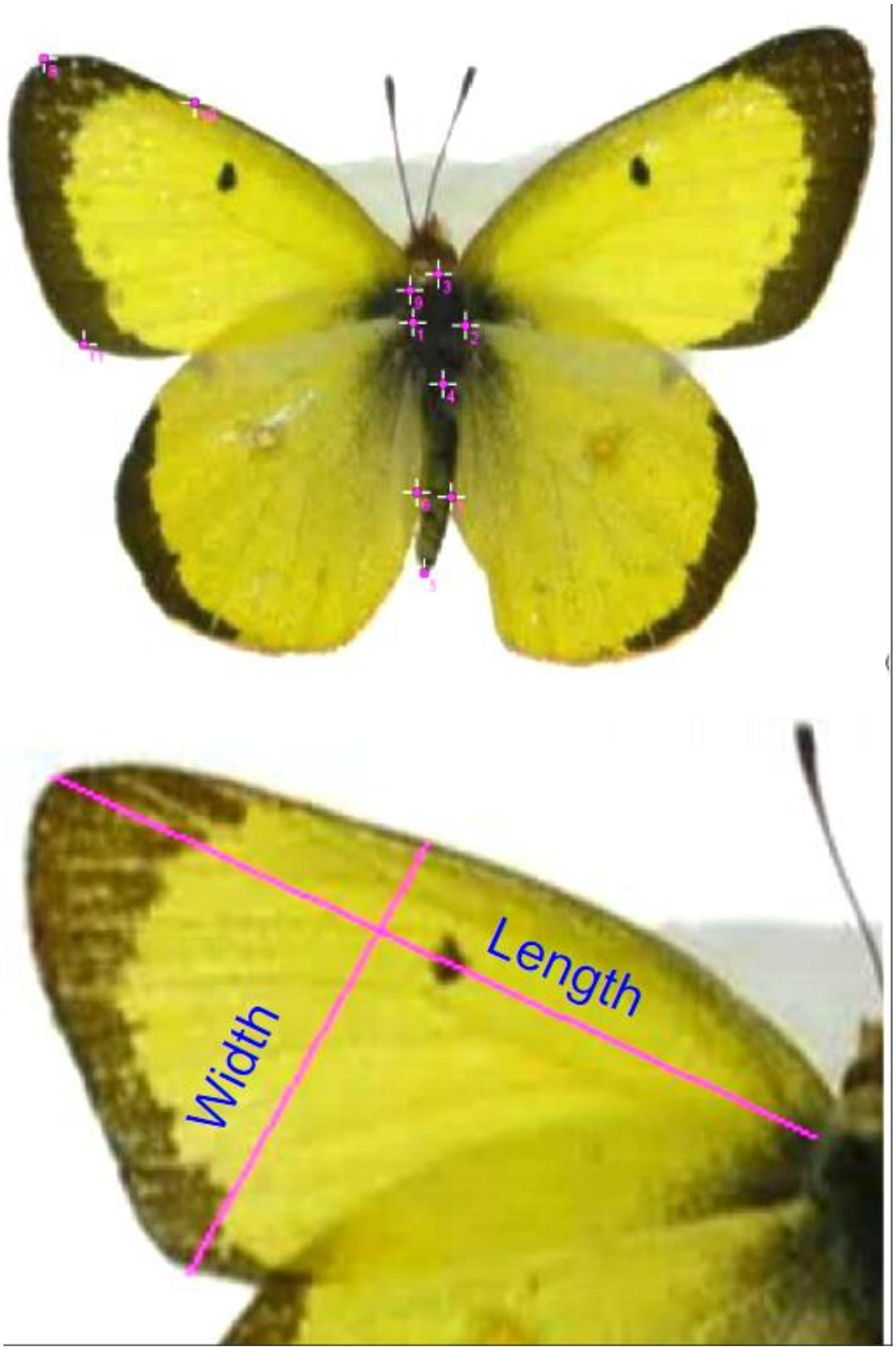


Figure 2. Example of morphometric measurements taken on a clouded sulphur (*Colias philodice*) using ImageJ software 1.50e Top: Eleven Landmarks identified butterfly for extraction of morphometric measurements. Bottom: Forewing width and length.

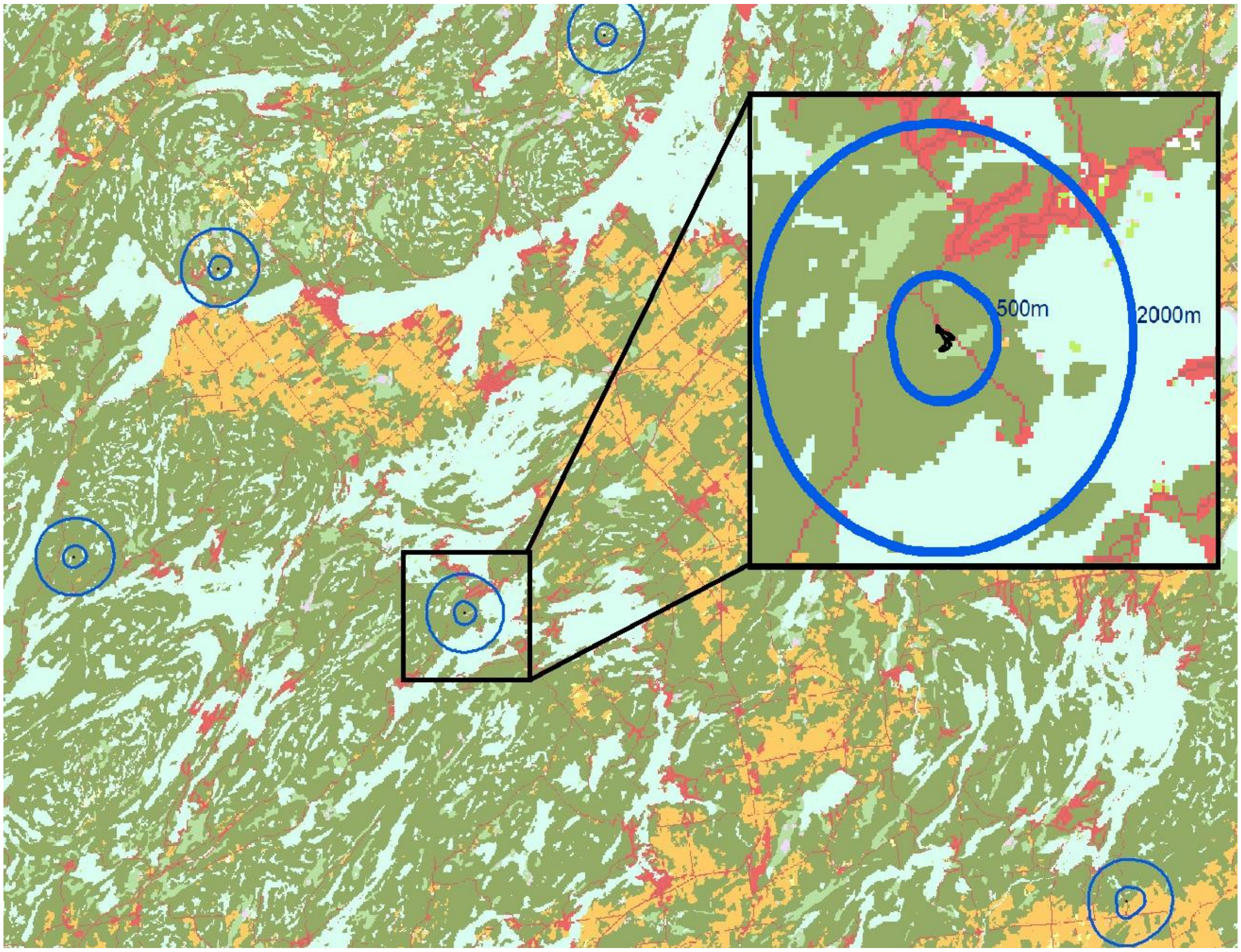


Figure 3. Survey sites and buffers (500m and 2000m) for extraction of land cover statistics using a 2010 Land Use map of Canada published by Agriculture and Agri-Food Canada at 30 m resolution.

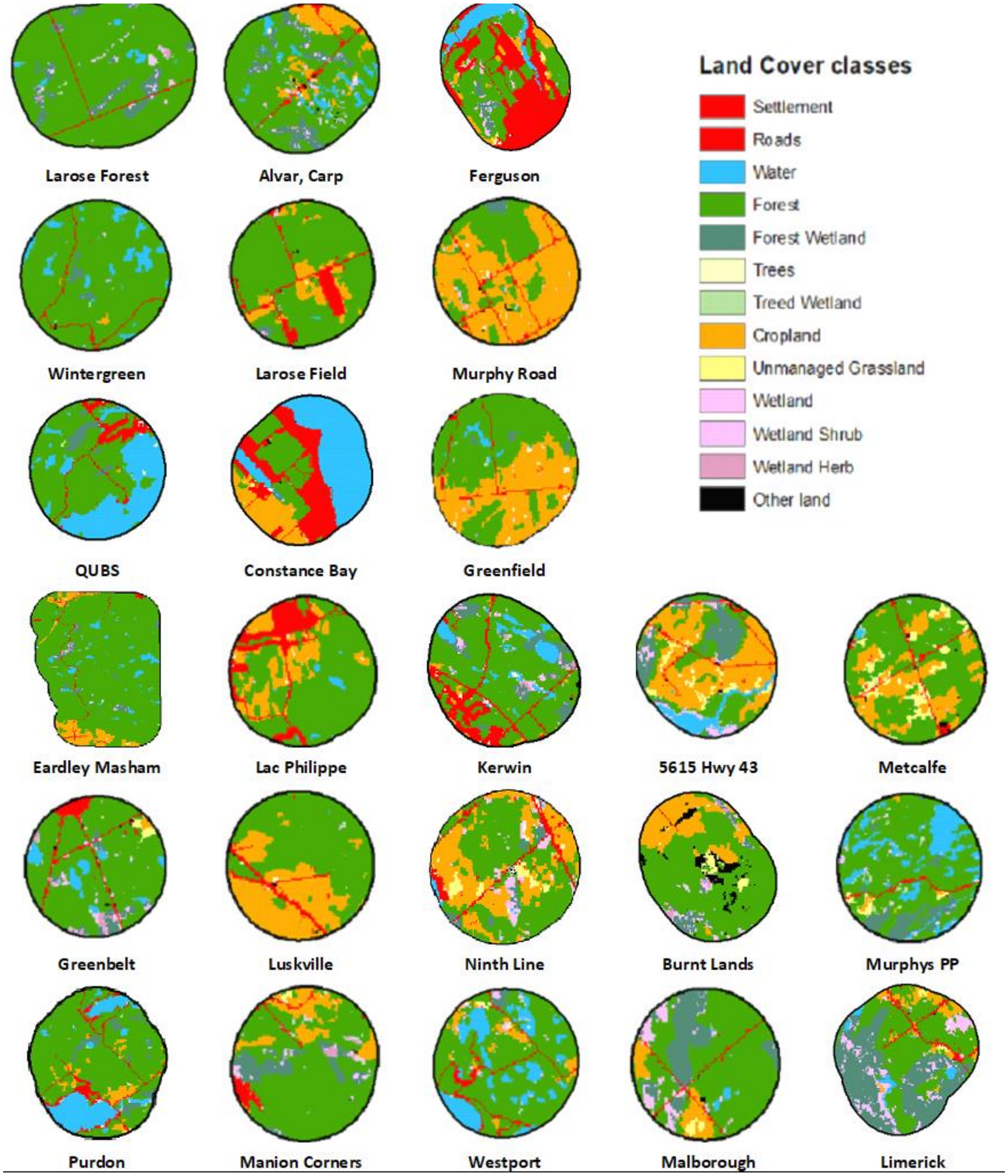
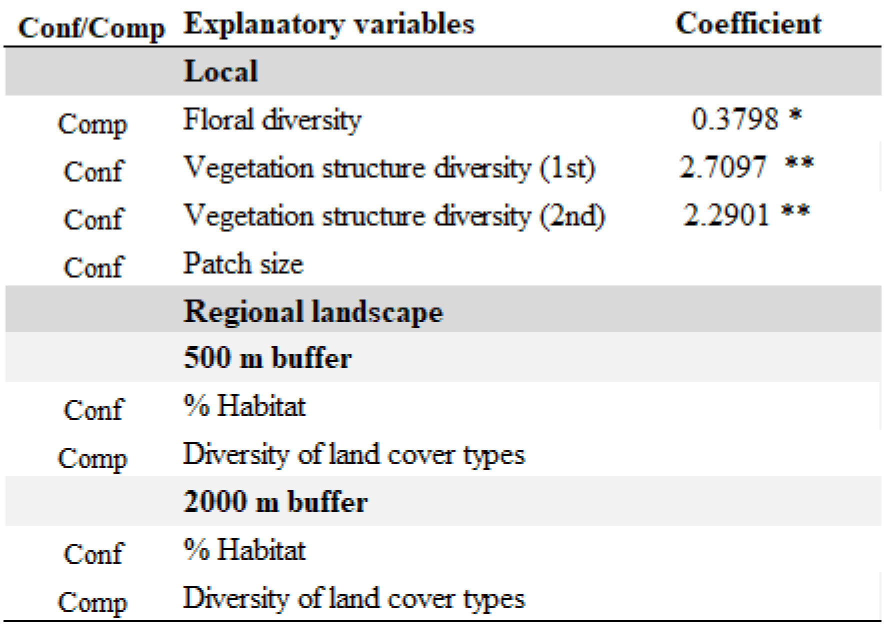


Figure 4. Land cover composition of the regional landscape of 24 survey sites in the Ottawa-Gatineau region obtained from a 2010 Land Use map of Canada published by Agriculture and Agri-Food Canada at 30 m resolution. The regional landscape includes a 2,000m buffer around the perimeter of the site.

Table 2. Effect of compositional (comp) and configurational (conf) landscape variables at the local and regional landscape on functional diversity in the best multiple linear regression model following automated model selection. Predictor variables included in the best model are presented along with their significance (p<0.001= ‘***’, p<0.01= ‘**’, p<0.05= ‘*’, p<0.1=‘ . ’, p>0.1 =‘ ’). The presence of a first (1st) and second (2nd) order for the vegetation structure diversity parameter reflects a quadratic relationship with the response variable.



Conf/Comp	Explanatory variables	Coefficient
	Local	
Comp	Floral diversity	0.3798 *
Conf	Vegetation structure diversity (1st)	2.7097 **
Conf	Vegetation structure diversity (2nd)	2.2901 **
Conf	Patch size	
	Regional landscape	
	500 m buffer	
Conf	% Habitat	
Comp	Diversity of land cover types	
	2000 m buffer	
Conf	% Habitat	
Comp	Diversity of land cover types	

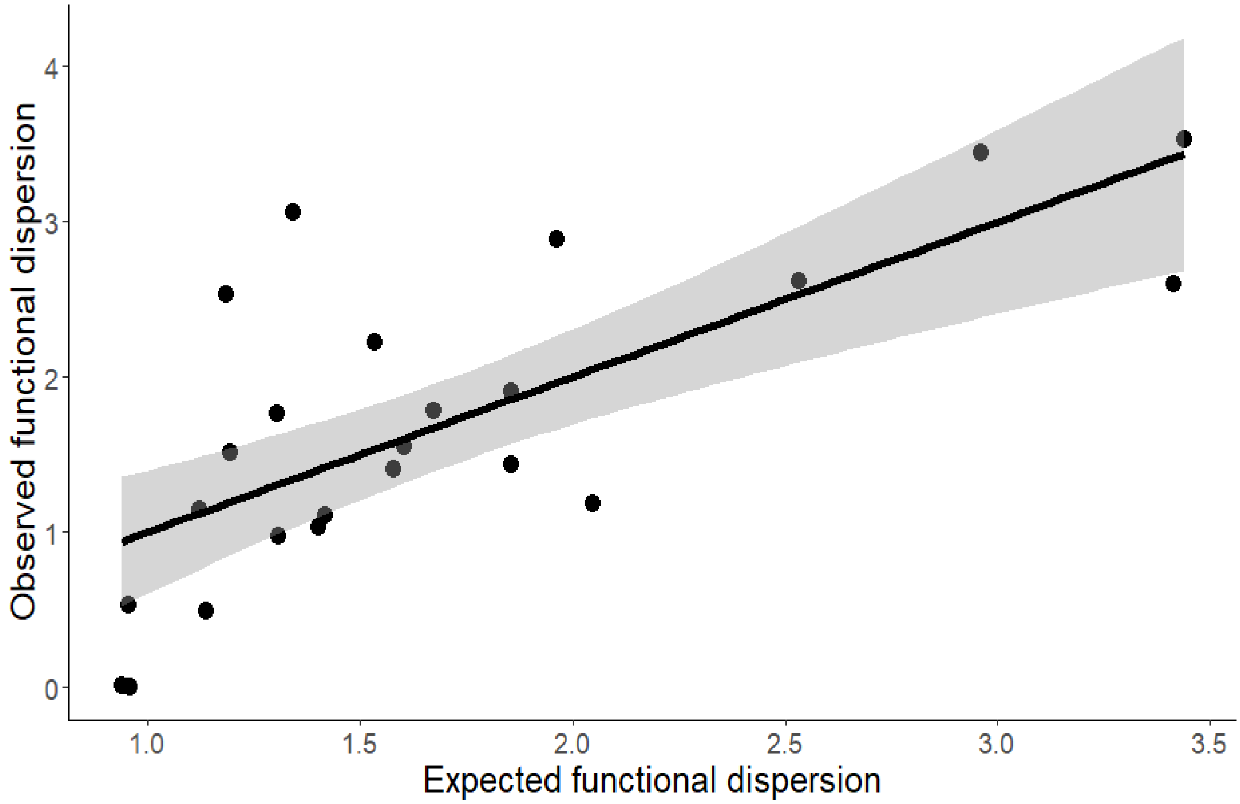


Figure 5. Observed vs expected plot derived from the multiple regression of Model 1. Observed functional dispersion values for each of the 24 sites are represented as black dots scattered along the best fit regression line predicted from the equation of Model 1 (adjusted $R^2 = 0.417$, $p = 0.000852$.

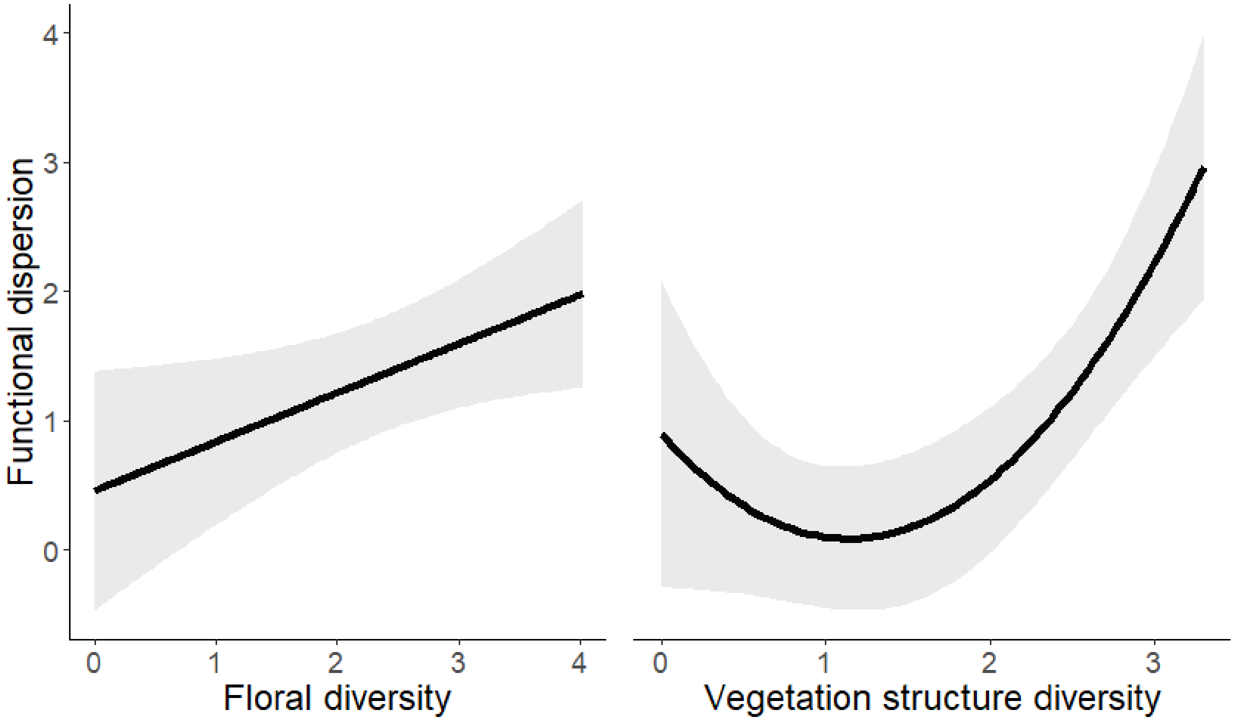


Figure 6. Plots of each predictor variables included in Model 1 on the response variable, functional dispersion. The partial slope for a predictor variable is represented when the other predictor is held constant. The 95% confidence interval based on the standard errors measured at each point is represented as a grey band. .

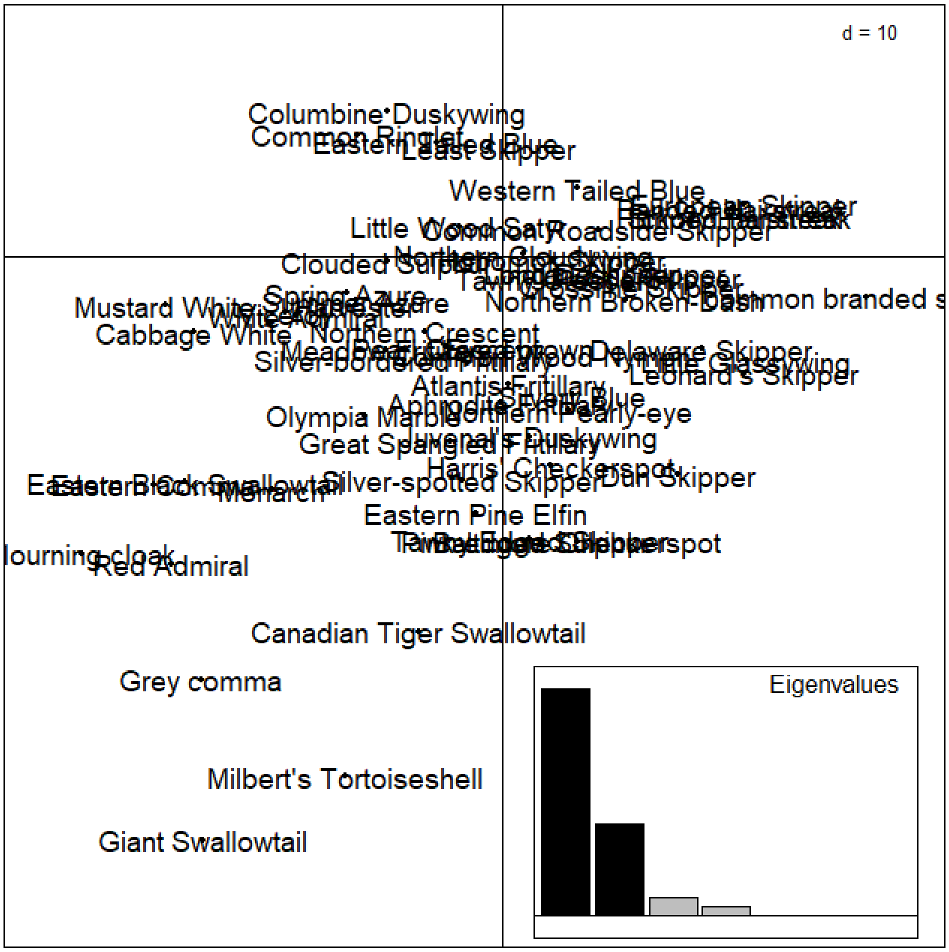


Figure 7. RLQ biplot of species scores along the first and second axis with insert of eigenvalues in the bottom-right corner.

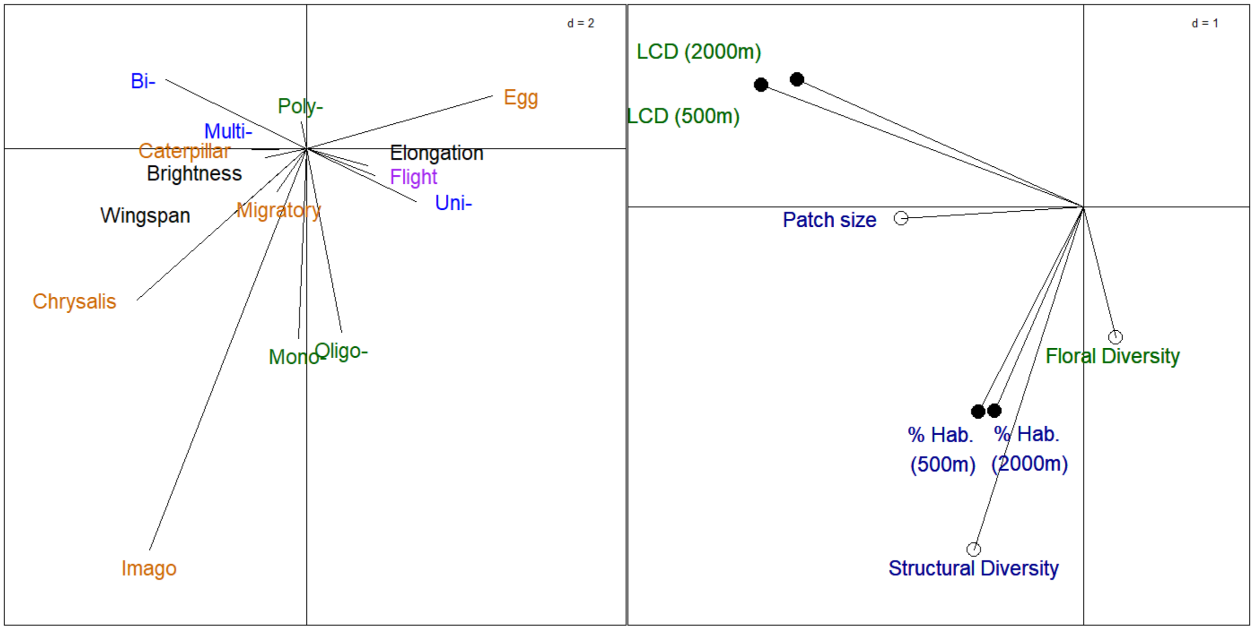


Figure 8. Biplots of RLQ scores along the first and second axis across all sites. Left: Scores of 14 traits following Hill and Smith analysis ordination for mixed variables. Color indicates trait categories: Orange=Overwintering stage, Green=Larval host breadth, Blue=Voltinism (uni-, bi-, or multi-voltine), Black=Wing morphometrics, Purple= Flight period. Right: Scores of 7 environmental variables following Principal component analysis (PCA). Color distinguishes compositional (Green) and configurational (Blue) variables. LCD = Land cover diversity, % Hab. = Percentage habitat. Symbol distinguishes local (empty) and regional (filled) landscape variables.

Table 3. Principal component loadings for environmental variables along the first and second axis of RLQ ordination.

Trait category	Trait value	CS1	CS2
Overwintering stage	Egg	0.924719	0.411123
	Caterpillar	-0.27043	-0.01147
	Chrysalis	-0.84025	-1.18918
	Imago	-0.77766	-3.14688
	Migratory	-0.14656	-0.33586
Larval host breadth	Monophagous	-0.04081	-1.48872
	Oligophagous	0.17338	-1.438
	Polyphagous	-0.02515	0.210812
Wing morphometrics	Wingspan	-0.36448	-0.51434
	Elongation	0.304491	-0.13734
	Brightness	-0.20315	-0.06828
Voltinism	Univoltine	0.544512	-0.41619
	Bivoltine	-0.7011	0.53741
	Multivoltine	-0.19106	0.143987
Flight period	Start	0.340657	-0.21514

Table 4. Principal component loadings for trait variables along the first and second axis of RLQ ordination. Significant relationships between the variable and RLQ axis are indicated with an asterisk (*).

Conf/Comp	**Landscape variable**	**RS1**	**RS2**
	Local		
Conf	Structural diversity	-0.21933	-0.68705
Comp	Floral diversity	0.065025	-0.26213
Conf	Patch size	-0.36416	-0.02461
	Regional		
Conf	% Habitat (*500m*)	-0.20899	-0.41096
Comp	Land cover diversity (*500m*)	-0.6426 *	0.242557
Conf	% Habitat (*2000m*)	-0.17671	-0.40847
Comp	Land cover diversity (*2000m*)	-0.57203 *	0.253142

Table 5. Significance testing of the global coinertia between the R, L and Q table. Sum of eigenvalues (Obs) calculated in the RLQ analysis tested against two null models built by permuting sites (model 1) and species (model 2) from the R table (n=49999 repetitions).

Test	**Obs**	**Std. Obs**	***p*-value**
Model 2	0.6544	0.1931	0.3503
Model 4	0.6544	0.5327	0.2911

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Appendix: Supplemental results

Table 1. Coordinates and identification number of 24 sampling sites visited 5 or 6 times throughout the summer, 2018

ID	Site	Latitude	Longitude
1	5615 Hwy 43	44.894205	-76.158496
2	900 Kerwin	45.393065	-75.95759
3	9th Line	45.09311	-76.130678
4	Alvar, Carp	45.387735	-76.073677
5	Burnt Lands	45.276185	-76.202928
6	Constance Bay	45.496475	-76.079134
7	Eardley Masham	45.600351	-76.096163
8	Ferguson Forest	45.03242	-75.65858
9	Field by Larose Forest	45.361313	-75.260826
10	Field to Lac Philippe	45.636594	-76.010886
11	Greenbelt	45.300332	-75.821057
12	Greenfield	44.471425	-76.009061
13	Larose Forest	45.387221	-75.206345
14	Limerick	44.921439	-75.767273
15	Luskville	45.531957	-75.994346
16	Malborough Forest, roadside	45.04635	-75.866016
17	Manion corners	45.252826	-76.041108
18	Metcalfe	45.260999	-75.460234
19	Murphy Road	45.16568	-75.20903
20	Murphy’s Provincial Park	44.771702	-76.263632
21	Purdon	44.992957	-76.54506
22	QUBS	44.571342	-76.331811
23	Westport	44.6906	-76.452281
24	Wintergreen	44.590976	-76.522784

Table 2. Butterfly species abundance at 24 sampling sites around the Ottawa region throughout spring and summer 2018.

		Sites																							
Species	**Common name**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Speyeria aphrodite	Aphrodite Fritillary	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Speyeria atlantis	Atlantis Fritillary	0	0	1	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euphydryas phaeton	Baltimore Checkerspot	0	0	0	0	0	0	0	0	0	3	3	0	0	0	2	0	0	0	0	0	0	0	0	0
Satyrium calanus	Banded Hairstreak	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0
Pieris rapae	Cabbage White	5	2	11	1	1	0	0	0	4	2	3	8	0	0	5	6	4	34	8	2	1	6	12	3
Papilio canadensis	Canadian Tiger Swallowtail	1	0	0	0	0	2	0	1	1	0	0	9	2	0	1	1	0	0	0	3	0	3	0	2
Colias philodice	Clouded Sulphur	74	0	53	36	14	0	0	0	66	32	24	44	19	0	37	52	10	35	39	6	0	70	69	43
Erynnis lucilius	Columbine Duskywing	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	41	0	0	0	0	0	0	10
Hesperia Comma	common branded skipper	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coenonympha tullia	Common Ringlet	406	0	30	25	28	0	0	0	70	13	28	36	0	0	36	69	13	32	2	0	1	92	28	62
Amblyscirtes vialis	Common Roadside Skipper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0
Cercyonis pegala	Common Wood-Nymph	2	0	0	0	0	0	0	0	0	18	0	1	0	0	7	0	0	2	0	8	7	3	1	28
Polites origenes	Crossline Skipper	0	0	0	1	0	0	0	0	1	1	0	4	0	0	0	5	1	0	1	0	0	5	0	3
Anatrytone logan	Delaware Skipper	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	1	0
Euphyes vestris	Dun Skipper	0	0	0	0	2	0	0	0	2	0	1	11	3	3	2	10	21	2	0	5	17	83	16	41
Papilio polyxenes	Eastern Black Swallowtail	0	0	4	0	0	0	0	0	1	0	0	0	3	0	2	0	0	1	2	0	0	0	0	0
Polygonia comma	Eastern Comma	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	2	0	3	1	0	1
Callophrys niphon	Eastern Pine Elfin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
cupido comyntas	Eastern Tailed Blue	48	0	51	1	0	0	0	0	0	1	2	11	0	0	2	6	0	5	1	0	0	3	1	2
Thymelicus lineola	European Skipper	186	1	1	5	5	0	0	0	26	60	17	19	0	0	401	5	8	19	12	2	19	202	200	16

Satyrodes eurydice	Eyed brown	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Papilio cresphontes	Giant Swallowtail	1	3	3	0	0	0	0	0	0	0	0	0	0	4	0	2	0	4	1	5	0	0	0	5
Speyeria cybele	Great Spangled Fritillary	0	0	1	0	1	0	1	0	0	0	0	4	6	0	0	16	6	0	0	0	0	0	0	0
Polygonia progne	Grey comma	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	1
Chlosyne harrisii	Harris' Checkerspot	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Feniseca tarquinius	Harvester	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Satyrium caryaevorus	Hickory Hairstreak	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	2
Poanes hobomok	Hobomok Skipper	0	1	0	0	0	2	3	0	2	0	0	1	0	3	3	0	3	0	0	10	0	19	14	26
Hesperia sassacus	Indian Skipper	0	0	0	1	5	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	1	0
Erynnis juvenalis	Juvenal's Duskywing	0	0	0	0	0	3	0	0	0	0	0	0	1	0	3	3	0	0	0	12	0	6	1	6
Ancyloxypha numitor	Least Skipper	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	9	0
Hesperia leonardus	Leonard's Skipper	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pompeius verna	Little Glassywing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0
Megisto cymela	Little Wood Satyr	0	8	0	0	0	0	0	4	0	0	0	7	0	19	0	0	0	0	0	33	0	23	4	5
Polites mystic	Long Dash Skipper	1	0	5	1	0	0	0	0	9	2	2	4	0	0	0	3	0	0	0	0	0	4	0	10
Boloria bellona	Meadow Fritillary	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Nymphalis milberti	Milbert's Tortoiseshell	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	1	0	0
Danaus plexippus	Monarch	3	0	8	1	3	0	0	0	7	4	2	21	2	2	5	3	2	6	2	2	0	6	0	4
Nymphalis antiopa	Mourning cloak	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Pieris oleracea	Mustard White	1	0	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	3	0	3	0	0	0
Wallengrenia egeremet	Northern Broken-Dash	1	0	0	0	0	0	0	0	1	0	0	2	0	1	0	8	0	0	0	0	1	40	0	23
Thorybes pylades	Northern Cloudywing	0	1	0	0	3	0	0	0	1	0	0	0	0	0	0	0	5	0	3	4	0	3	1	4
Phyciodes cocyta	Northern Crescent	6	3	0	7	6	0	1	0	4	9	4	4	2	7	17	12	9	5	3	1	2	8	0	5
Enodia anthedon	Northern Pearly-eye	0	1	0	0	0	2	2	1	0	0	0	0	0	0	0	0	0	0	0	7	12	1	0	0
Euchloe olympia	Olympia Marble	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Phyciodes tharos	Pearl Crescent	0	4	8	2	0	0	0	0	7	1	0	3	0	1	16	20	6	6	3	0	0	22	3	6
Polites peckius	Peck's Skipper	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0

Colias interior	Pink-edged Sulphur	0	0	2	0	0	0	0	0	0	3	0	4	0	0	9	3	0	16	10	0	0	0	8	4
Vanessa atalanta	Red Admiral	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	2	0	0
Boloria selene	Silver-bordered Fritillary	0	0	0	0	0	0	2	0	3	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Epargyreus clarus	Silver-spotted Skipper	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Glaucopsyche lygdamus	Silvery Blue	13	0	2	0	8	0	0	0	0	0	1	5	1	0	21	0	0	6	0	0	0	1	1	0
Celastrina ladon	Spring Azure	0	0	0	1	8	5	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Satyrium liparops	Striped Hairstreak	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Celastrina neglecta	Summer Azure	0	0	1	0	1	0	0	0	1	0	5	3	2	1	2	1	2	1	5	0	0	0	0	0
Phyciodes batesii	Tawny Crescent	9	0	0	0	0	0	0	0	6	10	0	0	0	0	3	3	0	0	0	0	0	0	14	4
Polites themistocles	Tawny Edged Skipper	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Limenitis archippus	Viceroy	2	0	0	0	2	0	0	0	4	1	8	9	2	1	2	4	5	1	1	0	0	0	0	0
Cupido amyntula	Western Tailed Blue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Limenitis arthemis	White Admiral	0	1	0	0	2	0	6	3	0	4	1	2	1	2	1	0	0	0	0	1	1	8	1	18

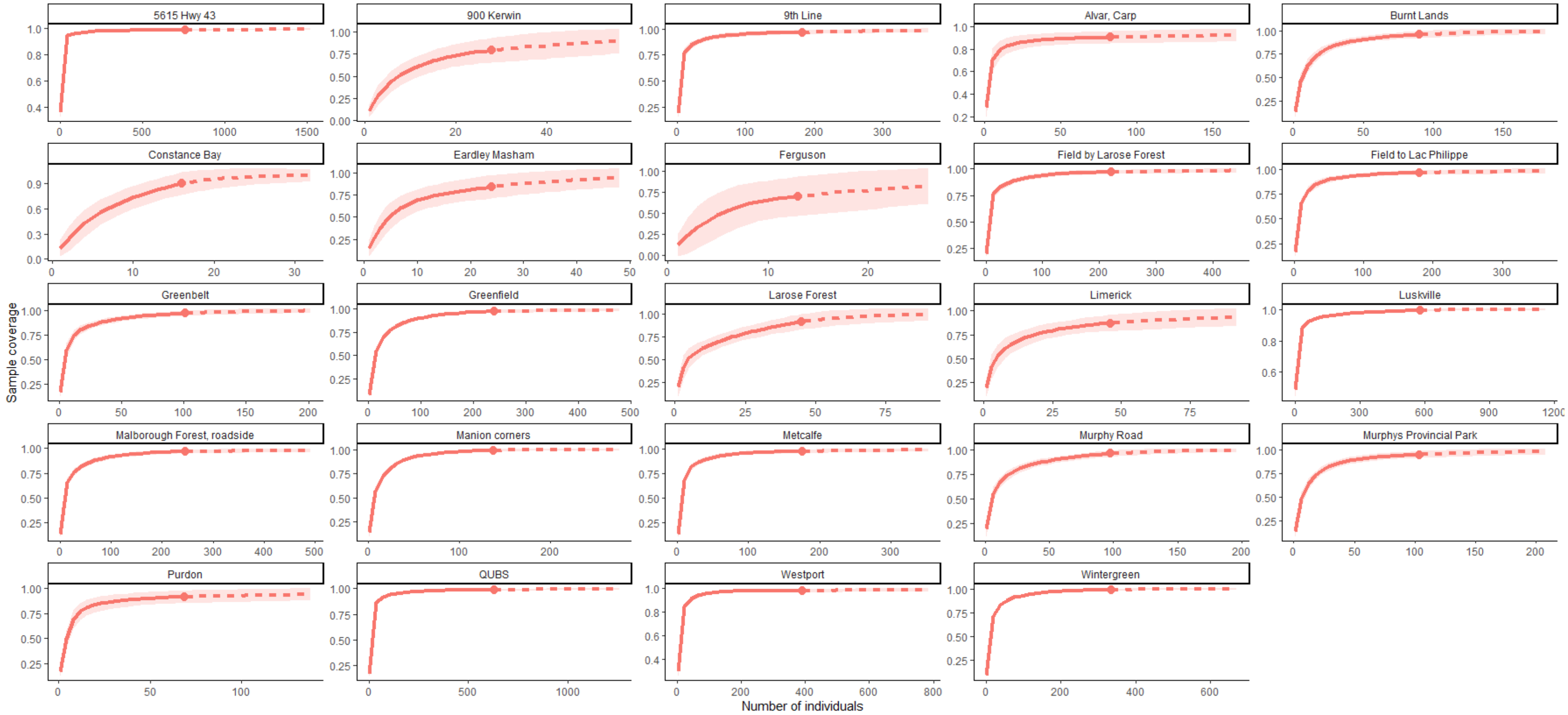

Figure 1. Sample completeness curves depicting the relationship between sample coverage of butterfly communities and sample size for 24 sites surveyed repeatedly (5 or 6 times) throughout spring and summer 2018. Each curve shows the observed sample (solid dot), rarefied sample (solid line), extrapolated sample (dashed line) up to an endpoint double the reference sample size.

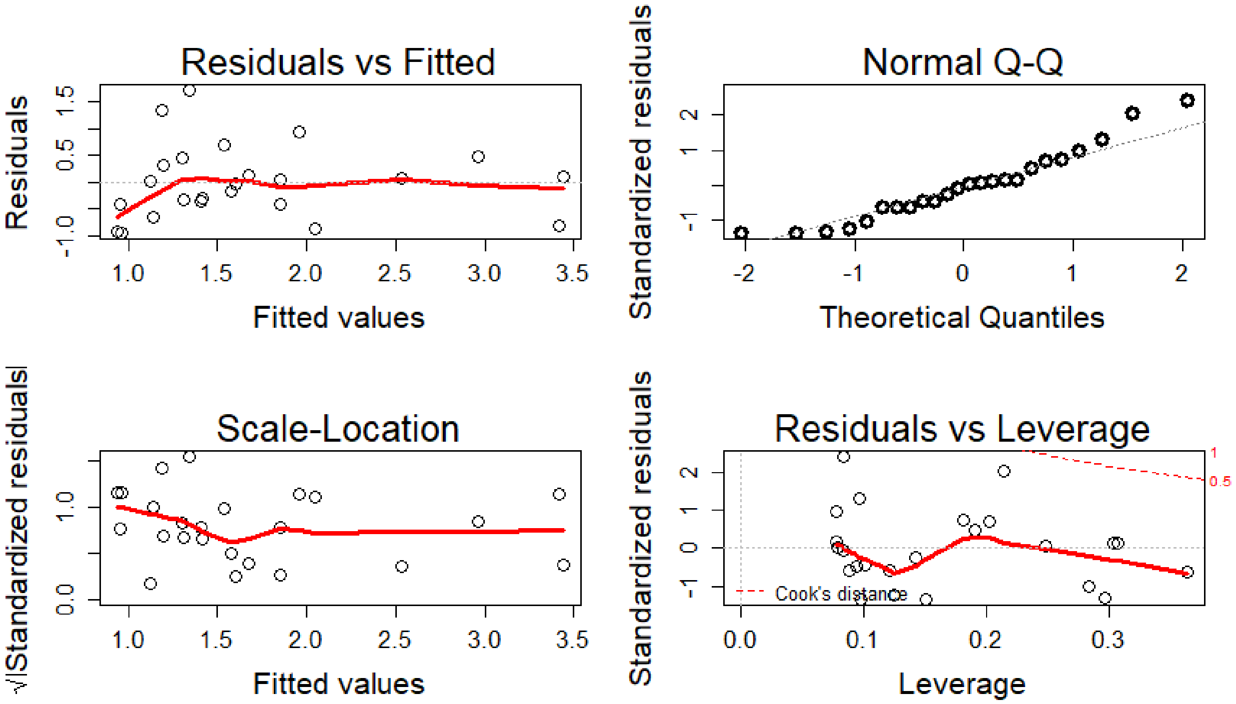

Figure 2. Diagnostic plots of the multiple linear regression of Model 1.

Table 3. Fourth-corner analysis applied to RLQ output. Fourth-corner association between single traits and the first two RLQ axes for environmental variables (AxcR1/AxcR2) and between single environmental variables and the first two RLQ axes for trait variables (AxcQ1/AxcQ2). Correction for multiple comparisons applied with the false discovery rate (FDR). Significant *p*-values are in bold.

Association tests	Obs	Std.Obs	*p*-value	Adj. *p*-value
Environmental gradient - trait variable				
AxR 1 / Overwinter - Egg	0.216862421	1.45132650	0.15456	0.42152727
AxR 2 / Overwinter - Egg	0.075432328	0.47333891	0.67462	0.87327692
AxR 1 / Overwinter - Caterpillar	-0.149316829	-1.17130843	0.27154	0.62663077
AxR 2 / Overwinter - Caterpillar	-0.004955641	-0.01908217	0.98664	0.98664
AxR 1 / Overwinter - Chrysalis	-0.087032477	-1.01880237	0.31522	0.639075
AxR 2 / Overwinter - Chrysalis	-0.096367468	-1.03553482	0.34084	0.639075
AxR 1 / Overwinter - Imago	-0.022938206	-0.08427252	0.90556	0.97024286
AxR 2 / Overwinter - Imago	-0.072620605	-0.75832140	0.39622	0.64458947
AxR 1 / Overwinter - Migratory	-0.007770691	0.12581587	0.9511	0.98389655
AxR 2 / Overwinter - Migratory	-0.013931817	0.05554591	1	1
AxR 1 / Monophagous	-0.000526691	-0.02207883	0.98466	0.98664
AxR 2 / Monophagous	-0.015032604	0.10807812	0.90426	0.97024286
AxR 1 / Oligophagous	0.026349375	0.38147752	0.73914	0.87327692
AxR 2 / Oligophagous	-0.170979089	-2.00380326	**0.0357**	0.15771429
AxR 1 / Polyphagous	-0.026206082	-0.37995209	0.74172	0.87327692
AxR 2 / Polyphagous	0.171835500	2.00258719	**0.0358**	0.15771429
AxR 1 / Wingspan	-0.145371703	-1.50090497	0.13964	0.41892
AxR 2 / Wingspan	-0.160498950	-1.60880763	0.10844	0.417975
AxR 1 / Wing elongation	0.121446949	1.32419554	0.20658	0.51645

AxR 2 / Wing elongation	-0.042856624	-0.45580298	0.68586	0.87327692
AxR 1 / Flight period	0.135871730	1.77481276	0.07174	0.417975
AxR 2 / Flight period	-0.067133825	-0.90899940	0.39062	0.68932941
AxR 1 / Univoltine	0.204551702	1.60154971	0.11146	0.417975
AxR 2 / Univoltine	-0.122320728	-0.97643176	0.36248	0.68932941
AxR 1 / Bivoltine	-0.184525727	-1.63911969	0.09602	0.417975
AxR 2 / Bivoltine	0.110659956	0.98055473	0.36736	0.68932941
AxR 1 / Multivoltine	-0.041244488	-0.52387411	0.61446	0.87327692
AxR 2 / Multivoltine	0.024318432	0.35069707	0.73432	0.87327692
AxR 1 / Wing brightness	-0.081027831	-0.93075328	0.37096	0.68932941
AxR 2 / Wing brightness	-0.021305218	-0.13349647	0.90488	0.97024286
Environmental variable- trait gradient				
Land cover diversity (500m) / AxQ 1	-0.271522329	-2.28844390	**8e-04**	**0.0112**
Land cover diversity (2000m) / AxQ 1	-0.241703509	-2.24686099	**0.00204**	**0.01428**
Vegetation structure diversity / AxQ 1	-0.092674464	-0.71450444	0.5214	0.6083
Floral cover / AxQ 1	0.027475456	0.28435824	0.791	0.85184615
Patch size / AxQ 1	-0.153869140	-1.62973657	0.10546	0.24588
% Habitat (2000m) / AxQ 1	-0.074665453	-0.72298911	0.50476	0.58888666
% Habitat (500m) / AxQ 1	-0.088305830	-0.88063656	0.40734	0.51843272
Land cover diversity (500m) / AxQ 2	0.095428003	0.82773019	0.47486	0.60436727
Land cover diversity (2000m) / AxQ 2	0.099592246	0.95631790	0.4009	0.56126
Vegetation structure diversity / AxQ 2	-0.270302232	-2.09305775	**0.02252**	0.05632666
Floral cover / AxQ 2	-0.103128039	-1.38583391	0.1752	0.3504
Patch size / AxQ 2	-0.009682697	-0.08538937	0.94102	0.94102
% Habitat (2000m) / AxQ 2	-0.160700649	-2.30709958	**0.01136**	0.05301333
% Habitat (500m) / AxQ 2	-0.161680712	-2.11516903	**0.02414**	0.05632666

Table 4. Output of the fourth-corner analysis following double permutation (model 6) which combines the output from the permutation of the rows (value= sites; model 2) and columns (value=species, model 4) in table L . The observed fourth-corner statistic (Obs) quantifies the association between each of the 14 traits and each of the 7 environmental variables, for a total of 98 bivariate tests. Correction for multiple comparisons applied with the false discovery rate (FDR). Significant *p*-value are in bold.

Environment - trait association	**Obs**	**Std.Obs**	***p*-value**	**Adj. *p*-value**
Land cover diversity (500m) / Overwinter - Egg	-0.20569	-1.37623	0.18302	0.835526
Land cover diversity (2000m) / Overwinter - Egg	-0.19385	-1.32874	0.19768	0.835716
Vegetation structure diversity / Overwinter - Egg	-0.13531	-0.89868	0.41666	0.903624
Floral cover / Overwinter - Egg	-0.06169	-0.42535	0.68366	0.935884
Patch size / Overwinter - Egg	-0.12354	-0.80137	0.47302	0.903624
% Habitat (2000m) / Overwinter - Egg	-0.09905	-0.60075	0.59406	0.903624
% Habitat (500m) / Overwinter - Egg	-0.11338	-0.70123	0.54884	0.903624
Land cover diversity (500m) / Overwinter - Caterpillar	0.149944	1.170202	0.27866	0.886645
Land cover diversity (2000m) / Overwinter - Caterpillar	0.173045	1.376012	0.17962	0.835526
Vegetation structure diversity / Overwinter - Caterpillar	0.037219	0.359382	0.76576	0.971592
Floral cover / Overwinter - Caterpillar	0.074041	0.58882	0.57634	0.903624
Patch size / Overwinter - Caterpillar	0.073537	0.558653	0.63138	0.903624
% Habitat (2000m) / Overwinter - Caterpillar	0.04596	0.317996	0.77298	0.943755
% Habitat (500m) / Overwinter - Caterpillar	0.051587	0.366672	0.75762	0.935884
Land cover diversity (500m) / Overwinter - Chrysalis	0.073592	0.650765	0.44176	0.878821
Land cover diversity (2000m) / Overwinter - Chrysalis	0.027387	0.249707	0.76802	0.971592
Vegetation structure diversity / Overwinter - Chrysalis	0.142481	0.98203	0.35092	0.878821
Floral cover / Overwinter - Chrysalis	-0.0329	-0.56982	0.58888	0.903624

Patch size / Overwinter - Chrysalis	0.045309	0.760627	0.46356	0.903624
% Habitat (2000m) / Overwinter - Chrysalis	0.070646	1.133626	0.25872	0.879697
% Habitat (500m) / Overwinter - Chrysalis	0.081858	0.912706	0.39672	0.878821
Land cover diversity (500m) / Overwinter - Imago	-0.00101	-0.02715	0.97988	0.9819
Land cover diversity (2000m) / Overwinter - Imago	-0.02699	-0.93643	0.36412	0.903624
Vegetation structure diversity / Overwinter - Imago	0.054516	0.229874	0.75056	0.971592
Floral cover / Overwinter - Imago	0.003835	0.152261	0.8848	0.9819
Patch size / Overwinter - Imago	0.050539	0.669297	0.49854	0.878821
% Habitat (2000m) / Overwinter - Imago	0.057469	0.628777	0.4787	0.878821
% Habitat (500m) / Overwinter - Imago	0.063938	0.656473	0.45298	0.878821
Land cover diversity (500m) / Overwinter - Migratory	0.006627	0.171379	0.87512	0.9819
Land cover diversity (2000m) / Overwinter - Migratory	-0.01623	-0.40954	0.69986	0.935884
Vegetation structure diversity / Overwinter - Migratory	0.02128	0.026547	0.99222	0.99222
Floral cover / Overwinter - Migratory	-0.0036	-0.08143	0.9392	0.9819
Patch size / Overwinter - Migratory	0.033165	0.82427	0.43686	0.903624
% Habitat (2000m) / Overwinter - Migratory	0.000378	-0.05331	0.95752	0.9819
% Habitat (500m) / Overwinter - Migratory	0.003334	0.033977	0.97432	0.9819
Land cover diversity (500m) / Monophagous	-0.00857	-0.16519	0.7439	0.971592
Land cover diversity (2000m) / Monophagous	-0.02459	-1.11469	0.27	0.885938
Vegetation structure diversity / Monophagous	-0.00765	-0.34679	0.7573	0.935884
Floral cover / Monophagous	-0.02354	-1.05602	0.29958	0.887195
Patch size / Monophagous	0.027737	0.315152	0.74294	0.971592
% Habitat (2000m) / Monophagous	0.029715	0.205831	0.83448	0.976724
% Habitat (500m) / Monophagous	0.025032	0.08872	0.93402	0.976724
Land cover diversity (500m) / Oligophagous	-0.07508	-1.01944	0.35544	0.903624
Land cover diversity (2000m) / Oligophagous	-0.05487	-0.74639	0.49938	0.903624
Vegetation structure diversity / Oligophagous	0.160964	1.122582	0.2666	0.878821
Floral cover / Oligophagous	0.126813	1.684911	0.08938	0.6714

Patch size / Oligophagous	-0.0418	-0.60525	0.59522	0.903624
% Habitat (2000m) / Oligophagous	0.063117	0.829645	0.43452	0.903624
% Habitat (500m) / Oligophagous	0.064702	0.846915	0.4398	0.903624
Land cover diversity (500m) / Polyphagous	0.075647	1.028257	0.35102	0.903624
Land cover diversity (2000m) / Polyphagous	0.057062	0.778023	0.48096	0.903624
Vegetation structure diversity / Polyphagous	-0.15966	-1.1114	0.26934	0.878821
Floral cover / Polyphagous	-0.12409	-1.65142	0.09824	0.68768
Patch size / Polyphagous	0.038966	0.566908	0.62024	0.903624
% Habitat (2000m) / Polyphagous	-0.06577	-0.86815	0.4126	0.903624
% Habitat (500m) / Polyphagous	-0.0669	-0.87824	0.42322	0.903624
Land cover diversity (500m) / Wingspan	0.082718	0.867552	0.4088	0.903624
Land cover diversity (2000m) / Wingspan	0.076432	0.829957	0.43484	0.903624
Vegetation structure diversity / Wingspan	0.18536	1.414424	0.16076	0.84399
Floral cover / Wingspan	0.001136	0.02569	0.9819	0.9819
Patch size / Wingspan	0.117852	1.184671	0.25338	0.879697
% Habitat (2000m) / Wingspan	0.148538	1.444268	0.14922	0.809445
% Habitat (500m) / Wingspan	0.149734	1.457768	0.15418	0.809445
Land cover diversity (500m) / Wing elongation	-0.14222	-1.23545	0.2598	0.878821
Land cover diversity (2000m) / Wing elongation	-0.10215	-1.1018	0.30908	0.887195
Vegetation structure diversity / Wing elongation	0.040388	0.266335	0.80814	0.976724
Floral cover / Wing elongation	0.033253	0.435721	0.6824	0.942789
Patch size / Wing elongation	-0.10403	-1.11618	0.3064	0.887195
% Habitat (2000m) / Wing elongation	-0.03928	-0.45036	0.60952	0.903624
% Habitat (500m) / Wing elongation	-0.0517	-0.57158	0.5622	0.903624
Land cover diversity (500m) / Flight period	-0.17015	-1.40137	0.1779	0.878821
Land cover diversity (2000m) / Flight period	-0.16252	-1.47631	0.1449	0.84399
Vegetation structure diversity / Flight period	-0.04221	-0.34694	0.75838	0.971592
Floral cover / Flight period	0.056691	0.739645	0.4915	0.903624

Patch size / Flight period	-0.04739	-0.59524	0.58086	0.903624
% Habitat (2000m) / Flight period	0.03584	0.53096	0.63684	0.903624
% Habitat (500m) / Flight period	0.018238	0.256974	0.81394	0.976724
Land cover diversity (500m) / Univoltine	-0.26109	-2.01717	**0.03262**	0.421454
Land cover diversity (2000m) / Univoltine	-0.24217	-1.90613	**0.04574**	0.421454
Vegetation structure diversity / Univoltine	0.027948	0.212875	0.84786	0.9819
Floral cover / Univoltine	0.047203	0.368675	0.74114	0.935884
Patch size / Univoltine	-0.09779	-0.75047	0.49176	0.903624
% Habitat (2000m) / Univoltine	0.012392	0.132496	0.90952	0.9819
% Habitat (500m) / Univoltine	0.009084	0.096334	0.93336	0.9819
Land cover diversity (500m) / Bivoltine	0.211512	1.76269	0.06196	0.54215
Land cover diversity (2000m) / Bivoltine	0.207248	1.827322	0.05218	0.421454
Vegetation structure diversity / Bivoltine	-0.06709	-0.64395	0.65186	0.912604
Floral cover / Bivoltine	-0.04791	-0.42302	0.71314	0.935884
Patch size / Bivoltine	0.146992	1.284367	0.22594	0.847275
% Habitat (2000m) / Bivoltine	0.011527	0.035802	0.9778	0.987202
% Habitat (500m) / Bivoltine	0.017029	0.153543	0.8891	0.9819
Land cover diversity (500m) / Multivoltine	0.079022	1.029253	0.32108	0.887195
Land cover diversity (2000m) / Multivoltine	0.061144	0.800483	0.44128	0.903624
Vegetation structure diversity / Multivoltine	0.040355	0.103261	0.8809	0.976724
Floral cover / Multivoltine	-0.00367	-0.04329	0.96784	0.9819
Patch size / Multivoltine	-0.04483	-0.60475	0.56318	0.903624
% Habitat (2000m) / Multivoltine	-0.02743	-0.42084	0.68828	0.935884
% Habitat (500m) / Multivoltine	-0.02953	-0.43446	0.67864	0.935884
Land cover diversity (500m) / Wing brightness	0.099335	1.157671	0.25972	0.879697
Land cover diversity (2000m) / Wing brightness	0.067334	0.800887	0.4457	0.903624
Vegetation structure diversity / Wing brightness	0.088881	1.031404	0.3174	0.887195
Floral cover / Wing brightness	-0.0213	-0.39517	0.71476	0.971592

Patch size / Wing brightness	0.008115	0.062247	0.95358	0.9819
% Habitat (2000m) / Wing brightness	0.018707	0.129231	0.9035	0.9819
% Habitat (500m) / Wing brightness	0.01493	0.102531	0.92108	0.9819