**Submission to Conservation Biology**

Effects of nitrogen deposition on butterfly species richness on the landscape scale

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Trophic interactions,

**Data accessibility and reproducibility of results**

Data and R-scripts to reproduce the results of this manuscript including figures and tables are provided at https://github.com/TobiasRoth/NDep\_butterflies. Raw data for analyses are provided in the folder "data" and the folder "R" contains the R-Script that was used to export the data from the BDM database and to produce the figures and tables of the manuscript.

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Effects of nitrogen deposition on butterfly species richness on the landscape scale

**Abstract**

Nitrogen (N) deposition caused by agriculture and combustion of fossil fuels is a major threat to plant diversity, but the effects on higher trophic levels are less clear. In this study we investigated how N deposition may affect butterfly species richness. We started with reviewing the literature and found that vegetation parameters might be as important as climate and habitat variables in explaining variation in butterfly species richness. It thus seems likely that increased N deposition indirectly affects butterfly communities via its influence on plant communities. We then analyzed data from the Biodiversity Monitoring Switzerland program surveying species diversity of vascular plants and butterflies in 383 study sites of 1 km2 that are regularly distributed over Switzerland, covering an N deposition gradient from 2 to 44 kg N ha-1 yr-1. Using traditional linear models and structural equation models we found that high N deposition was consistently linked with low butterfly diversity, suggesting a net loss of butterfly diversity through increased N deposition. At low elevations, N deposition may contribute to a reduction in butterfly species richness via microclimatic cooling due to increased plant biomass. At higher elevations, negative effects of N deposition on butterfly species richness seem also mediated by reduced plant species richness. We conclude that in addition to factors such as intensified agriculture, habitat fragmentation and climate change, nitrogen deposition is likely to play a key role in affecting butterfly diversity.

**Introduction**

The increased flux of reactive nitrogen (N) in the biosphere and its deposition to ecosystems is considered as a major component of global change threatening biodiversity (Weiss 1999; Sala 2000). Increased N availability usually results in increased biomass production, shifts in species composition (e.g., favoring eutrophic species), and often in a loss of plant species richness through competitive exclusion (Bobbink et al. 2010; Vellend et al. 2017). While the consequences of increased N availability are mainly documented for primary producers such as vascular plants (Bobbink et al. 2010), negative effects of increased N availability may also occur in species groups higher in the food chain, for example in insects.

Because plant and insect communities are closely linked (Zhu et al. 2015), N-induced changes on plants are likely to induce changes in insect communities (Sassi et al. 2012). For example, because insects are often specialized on one ore few plant species, the loss of plant diversity may decrease the diversity of insects (Siemann 1998; Knops et al. 1999; Haddad et al. 2001). Further, increased N availability favoring plant growth and biomass production is likely to alter the structure of the vegetation, thus leading to shifts in microclimatic conditions from open, dry and hot to more dense, humid and cooler conditions, which will likely affect insects (Mantilla-Contreras et al. 2011). Further, more direct effects of increased N availability on insects might occur through altered food quality of host plants (Awmack & Leather 2002).

However, our knowledge on how increased N availability affects consumer diversity is rather limited (Humbert et al. 2015). For example, in a recent literature review only 19 (10%) of the 187 reported effect sizes from N addition experiments on local-scale species richness were based on consumers (Murphy & Romanuk 2016). Interestingly, the data from those 19 studies suggested that the effect of increased N availability on local-scale species richness is slightly positive.

Here, we complement the experimental studies on the effects of N deposition at higher trophic levels with an observational study using multiple field sites representing a large gradient of N deposition (i.e., a gradient study Roth et al. 2017). The data are from the Biodiversity Monitoring Switzerland program (BDM) and contain information on species richness of vascular plants and butterflies in 383 study sites of 1 km2 covering an N deposition gradient from 2 to 44 kg N ha-1 yr-1 (Roth et al. 2015). In previous studies, we found that high N deposition in these landscapes was associated with low values of six measures of plant diversity, including species richness (Roth et al. 2015). The BDM data thus provide an opportunity to examine possible direct and indirect effects of N deposition on species diversity of butterflies.

We started with a systematic literature review, searching for published studies investigating how butterfly species richness is related to environmental, land-use and vegetation parameters. The aim of our literature review was to compile a comprehensive list of predictor variables that are probably important for explaining the variation in butterfly richness among study sites. A second aim was to quantify how often N deposition was used as a predictor variable in such studies.

We then compiled the available data from the BDM study sites for the predictor variables identified in the literature review. We used traditional linear regression models to investigate how N deposition is correlated with butterfly species richness and how this correlation is affected depending on whether we accounted for all or only a selection of the other predictor variables. Since we assumed that a possible negative effect of N deposition on butterflies would be mediated by plant communities, we predicted that a negative effect would be weaker in models accounting for variables describing plant communities. We then used structural equation models (SEM) for examining the different paths by which environmental variables could affect butterfly species richness (Grace et al. 2010). In particular, we investigated how N deposition may negatively affect butterfly diversity via a negative effect on plant diversity (Topp & Loos 2018) and, second, via microclimatic cooling, for example because the increasingly productive and dense plant canopy may prevent caterpillars from absorbing solar radiation (WallisDeVries & vanSwaay 2006; Klop et al. 2014).

**Methods**

*Literature study*

On 12 July 2019 we conducted a literature search in Web of Science to compile a list of relevant predictor variables for explaining butterfly species richness. We searched for original research that applied some sort of multi-variate regression models, with a measure of butterfly species richness as dependent variable and several predictor variables for explaining the variation in butterfly species richness among sites or grid cells. We searched for studies with titles that fulfilled the following search criteria: *[(butterfl\* OR lepidoptera) AND (diversity OR richness)]*; we excluded studies with *[island OR tropic\*]* in the title. Furthermore, the topic needed to contain *[“global change” OR driver\* OR predictor OR variable]*. See Appendix A1 for a screenshot of the search setting in web of science. This search resulted in 95 studies. We excluded off-topic studies and studies that were conducted in habitats distinct from the habitat types available in Switzerland (i.e. rain forests, deserts). We found 32 studies that complied with our criteria (Appendix A2).

From the 32 studies, we extracted the predictor variables that were used to model butterfly species richness and assigned them to one of the following categories: (1) environment, including climatic gradients (from cool and humid to hot and dry) and variability, and topographic variables (from low to high elevations; from northern to southern expositions; from low to high topographic variability), (2) habitat, including variables indicating the availability (from low to high amount of suitable habitat), configuration (from low to high suitability of habitat configuration) and diversity of habitat types (from low to high diversity) as well as land-use intensity (from low to high intensity), (3) vegetation, including variables indicating micro-climate (from dense vegetation with cool and humid microclimate to open vegetation with hot and dry microclimate) and resource diversity (from low to high plant or flower richness), and (4) other variables that did not fit (1) to (3).

For each of the first three categories (environmental, habitat and vegetation) we then grouped the predictor variables into sub-categories. The idea was that the predictor variables within a sub-category were used to measure the same underlying process that could affect butterfly diversity. Assigning predictor variables to categories and sub-categories was an iterative process and was to a certain extend arbitrary.

In Appendix A2 we present the entire list of predictor variables investigated in the 32 studies. For each study we extracted the investigated predictor variables with the reported effect on butterfly richness using the following coding: 1 = the effect of the category on butterfly diversity as measured by a predictor variable was positive; 0 = there was no obviously important effect; -1 = the effect of the category on butterfly diversity as measured by a predictor variable was negative; "interm" = the effect of the category on butterfly diversity peaked at intermediate level of the predictor variable.

To assign the effect size we first decided whether the reported effect should be considered important (that is 1, -1 or “interm”) or not important (that is 0). We considered an effect as important if the authors mentioned in the abstract or discussion that they consider the reported effect size as important or relevant. If the authors did not make a statement about the importance of the reported effect we judged the importance of the reported effect size ourselves based on the reported effect sizes and based on the accuracy of the results (confidence intervals or standard error). If the reported effect size was judged as important we then decided on the direction of the effect (1, -1 or “interm”) based on the reported effect size.

*Butterfly and plant data*

We analyzed the presence/absence of butterfly and plant species sampled between 2005 and 2009 in the Swiss Biodiversity Monitoring program (BDM, www.biodiversitymonitoring.ch) that was launched in 2001 to monitor Switzerland's biodiversity (Weber et al. 2004). To monitor species diversity at the landscape scale, a sample grid of 428 regularly spaced study sites, each of 1 km2 size, was selected using a randomly chosen starting cell from the national coordinate system of 41'285 1-km2 cells. From the 428 study sites, seven sites with 100% water surface and 25 sites that were too dangerous for fieldwork because of their exposed alpine terrain were excluded a priori, resulting in 396 study sites.

Within each study site, surveyors walked along a 2.5 km transect that followed existing trails preferably near the diagonal of the study site (Plattner et al. 2004); plants and butterflies were surveyed on the same transect. If no trails existed, surveyors marked the transect route in the field and plotted it on a map. The field protocol for butterflies was based on the British butterfly monitoring scheme (Pollard et al. 1995).

For the butterfly surveys, transects were walked seven times between 21 April and 21 September in the lowlands, and four times between July and August above approximately 2000 m. The difference in numbers of surveys corresponds to the shorter flying season of butterflies at higher elevations; sites at high and low elevations received approximately equal sampling effort per week of flight season. During each survey, surveyors walked the transects in both directions and recorded all day-flying butterfly species (presence/absence; including *Hesperiidae* and *Zygaenidae*) within 5 m to each side of the transects on the way forth and back, respectively. Detectability varied by butterfly species and averaged 88% per survey (Kéry et al. 2009). For more information on the butterfly surveys, see (Kéry & Plattner 2007; Roth et al. 2014).

For the plant surveys, transects were usually walked by qualified botanists once in spring and once in summer, assuring that data collection spanned a large variation in flowering phenologies (Pearman & Weber 2007). At study sites with short vegetation period above approximately 2000 m, only one survey per field season was conducted. During each survey, surveyors recorded all plant species (presence/absence) within 2.5 m to each side of the transects both on the way forth and back, respectively. The overall detection error was relatively small with an average of 6.6% undetected presences per plant species as estimated in an earlier study using site-occupancy models (Chen et al. 2012). For more information on the plant surveys, see (Plattner et al. 2004; Roth et al. 2014).

Plants and butterfly surveys were usually conducted in the same years; each year, one fifth of the study sites were surveyed. Since we used the N deposition rates modeled for 2007 (see below), for each study site we selected the butterfly and plant data from the survey year that was closest to 2007. This was the reason why survey data are from 2005 to 2009. In the analyses, we only included study sites for which both the plant and butterfly surveys met our standards of data collection or of weather conditions according to the protocol (Roth et al. 2014). This resulted in omission of 13 study sites and in a final dataset on plant and butterfly surveys in 383 study sites.

*Predictor variables*

For all categories that were assigned to the predictor variables found in the literature review we included at least one predictor variable that was available for the BDM study sites (Table 1). Climate variables were extracted from the WorldClim database (Fick & Hijmans 2017). The source for the topographic data package was the GEOSTAT data base of the Federal Statistical Office (FSO, version 2006). Habitat data were derived from aerial data and are available on a grid with a 100-m resolution in the land-cover data package also from the GEOSTAT data base of the FSO (version 2.0, 2013).

Predictor values for land-use intensity and microclimate were derived from the species lists of recorded plants using Landolt indicator values that were developed for the specific situation in Switzerland, published the first time in Landolt (1977) and recalibrated in Landolt (2010). Similar to the Ellenberg indicator values developed for the flora of Germany (Ellenberg 1974) Landolt values are ordinal numbers that express the realized ecological optima of plants species for different climate, soil or land-use variables. We used the mean Landolt indictor value of the recorded plant species for temperature, moisture, nutrients, mowing tolerance and light (Table 1).

Additionally, we used the total number of recorded plants as a measure of resource diversity and N deposition as a measure for atmospheric pollution. N deposition was estimated for the year 2007 in 100x100 m grid cells across Switzerland, using a pragmatic approach that combined monitoring data, spatial interpolation methods, emission inventories, statistical dispersion models, and inferential deposition models (Roth et al. 2015; Rihm & Achermann 2016). For each study site of 1 km2, we averaged N deposition values from the cells containing parts of the transect used for the BDM surveys.

To obtain meaningful parameter estimates from the statistical models (see below), we centered the predictor variables by subtracting the value of the column "Zero" in Table 1 and standardized the predictor values by dividing it by the value of the column "Relevance". Thus, the estimated intercept of the linear models is the predicted butterfly species richness for the values of the predictor variables as given in the column "Zero". We chose these values to lie within the range of available data. The estimated slopes of the predictor variables in the linear models are in the unit that is given in column "Relevance". The values that we chose for the respective unit reflects the minimum change of the predictor variable that we consider as a relevant change. Since the estimated effect sizes of all predictor values are in units of what we consider minimum relevant changes, they become directly comparable between each other, which puts the focus on parameter estimates rather than on significance thresholds (Schielzeth 2010; Amrhein et al. 2019). Note that centering and standardization of input variables does not affect the conclusions that may be drawn from the results. A matrix with the scatterplots between all centered and standardized predictor variables is given in Appendix A3.

*Statistical methods*

We used two different approaches for investigating the drivers of the spatial variation in butterfly species richness across Switzerland with a particular focus on N deposition effects. The first approach (i.e., the statistical approach) is based on linear models with the squared root of butterfly species richness as dependent variable and N deposition as focus variable included as predictor variables in all tested models. Additionally, some of the other variables in Table 1 were included as covariates (i.e., additional predictor variables). We applied the following models: (1) full model that included the linear terms of all predictor variables in Table 1, (2) full model without microclimate variables, because microclimate is rarely considered in other studies on butterfly species richness and climate and microclimate are usually correlated, (3) topo-climate model that included only the linear terms of the topography and climate-gradient variables, (4) climate model that included only the linear terms of the climate-gradient variables, (5) land-use model that included only the linear and quadratic term of elevation as a proxy for the climatic variation along the elevational gradient and the variables for habitat configuration, habitat diversity, habitat availability and land-use intensity, and (6) a minimalistic model that included only the linear and quadratic term of elevation as a proxy for climate and land-use intensity. All models assumed normal distribution of the residuals, and we examined this assumption for the full model using residual analyses. Model parameters were estimated in a Bayesian framework using the R-package arm (Gelman & Su 2018).

Our second approach was based on structural equation models (SEM, Hoyle 2012). For our purpose, SEMs have two main advantages over the more traditional linear models used in the first approach. First, SEM allow estimating direct versus indirect links, while linear models only allow separating the effects of two factors if they are mutually exclusive (Menéndez et al. 2007). Many of the variables in Table 1 may have a direct effect on butterfly species richness, but may also indirectly affect butterfly diversity through their effects on vegetation structure or plant diversity. Second, SEM can be used to model constructs that are measured by one or several predictor variables (Hoyle 2012). This is helpful, because we aim to compare the effects of climate, micro-climate and land-use intensity, each of them measured with several predictor variables.

We used the generic model of Fig. 1 as a starting point for the analyses using SEM. In this generic model we assume that butterfly species richness is mediated by vegetation structure and plant diversity (ovals and rectangulars with grey background in Fig. 1). Additionally, we assumed that different global change drivers such as climate, atmospheric nitrogen deposition or land-use intensity could each have independent effects on vegetation structure, plant diversity and butterfly diversity (white ovals). While butterfly and plant species richness are measured variables from the BDM data (depicted in rectangulars in Fig. 1), global change drivers and vegetation structure are latent variables that are measured by one or several of the predictor variables in Table 1. We will present the results of different implementations of this generic model that vary in the number of global change drivers considered and in the selection of predictor variables the are used to measure the latent variables. Parameters of the SEMs were estimated using the R-Package lavaan (Rosseel 2012).

**Results**

*Literature study*

From the 32 studies on butterfly species richness that we complied according to our inclusion criteria, we extracted the effect sizes of 252 predictor variables. Predictor environmental variables were included in 75% of studies, habitat variables were included in 84% of studies and vegetation variables were included in 47% of studies. Nitrogen deposition was considered in none of the compiled studies.

While predictor variables for the vegetation scale were less likely to be considered in these studies compared to environmental and habitat variables, the importance (as estimated by the proportion of times the variables were considered important) was similar to the importance of the predictor variables for the environmental and the habitat scale (Table 2). Furthermore, resource diversity of the vegetation was the variable with the most consistent effect (regarding the direction of the effects) across all variables considered in the reviewed studies (Table 2).

*Swiss butterfly study*

Based on the linear models that we applied to the BDM data, we found that butterfly diversity decreased with increasing N deposition. The amount of this negative effect of N deposition on butterfly species richness was similar for all considered models (Fig. 2). Except for the climatic variables (annual mean temperature, mean temperature of coldest quarter and temperature seasonality), N deposition was the variable with the highest absolute effect size in the full model (Table 3).

The results based on the structural equation models (SEM) that included climate, N deposition, land-use intensity and habitat availability as global change gradients potentially affecting vegetation structure, plant species richness and butterfly species richness are given in Fig. 3a. Based on the results of this model, butterfly species richness was affected (in descending order of the absolute value of the effect sizes) by climate (highest butterfly richness in warm and dry climate; estimate ± SE: 0.504 ± 0.054), plant species richness (butterfly richness increasing with plant richness; 0.379 ± 0.025), microclimate (higher butterfly richness in areas with warm, dry and open vegetation than in areas with closed and humid vegetation; 0.133 ± 0.041).

The effects of land-use intensity and habitat availability on butterfly richness seemed to be rather weak (land-use intensity: -0.035 ± 0.021; habitat availability: 0.026 ± 0.025). However, at sites below 1600 m where land-use is usually intense, the negative effect of land-use intensity on butterfly diversity was much stronger (-0.187 ± 0.034, Fig. 3b).

The results of applying the SEM to the data of all sites further suggest that higher deposition rates leaded to denser, more humid and cooler microclimates within the vegetation (-0.102 ± 0.042) and to lower plant species richness (-0.733 ± 0.154, Fig. 3a). While at elevations below 1600 m, the N deposition mainly affected vegetation structure (vegetation structure: -0.846 ± 0.097; plant species richness: -0.144 ± 0.177, Fig. 3b), the negative effect at higher elevations was strong both regarding vegetation structure and plant species richness (vegetation structure: -2.425 ± 1.011; plant species richness: -2.106 ± 1.09, Fig. 3c).

**Discussion**

*Drivers of butterfly species richness*

In this study, we systematically searched the peer-reviewed literature for studies that compared the importance of different drivers of butterfly species richness. In the reviewed studies vegetation variables were much less frequently included compared to environment or habitat variables. However, if the effect of vegetation variables on butterfly richness was studied, it usually had a relevant and consistent effect on butterfly richness. The results of our literature review thus suggest that vegetation variables representing microclimate or plant resource diversity are important but underrepresented in published studies on the spatial variation of butterfly species richness.

The results based on the structural equation models (SEM) applied to the BDM data confirm the importance of vegetation variables to explain butterfly species richness: butterfly species richness was similarly affected by plant species richness as it was affected by ambient temperature. In contrast, however, the effects of land-use intensity and habitat availability on butterfly richness we found were rather weak. A potential explanation might be that the available information about land-use intensity at the study plots of the BDM was limited. Our predictor variables are mainly derived from the plant surveys using mean indicator values hiding within-site variability. Further, land-use intensity is a rather multi-faced category that includes for example the amount of fertilization, insecticide use, cutting frequency of the meadows or the time of the first cut. The variables for land-use intensity used in this study might thus be too simple to realistically infer the effect of land-use intensity on butterfly and plant diversity and the small effect sizes of land-use intensity both on butterfly and plant diversity need to be interpreted with caution.

Both the literature review as well as the analyses of the BDM data suggest that drivers of butterfly species richness act at largely different scale including the large scale at which climatic effects are likely to act and the small scale of microclimatic conditions that are likely to change within the vegetation. As a consequence, the results of studies on butterfly species richness are likely to depend on the scale at which the studies were conducted. In our study, all predictor variables were measured at the scale of 1 km2. This seems to be an appropriate scale to investigate the effect of the environment on butterfly communities because large environmental variation occurs between landscapes. However, this scale may be considered too large to reliably infer vegetation structure effects on butterfly communities because variation in vegetation within one study plot of 1 km2 is large. It seems thus surprising that we were still able to obtain results that suggest negative effects on butterfly richness through microclimatic cooling caused by increased N deposition. This might suggest that the microclimatic cooling would be even more important for butterfly communities if measured at smaller scales.

*Nitrogen deposition effect on butterfly species richness*

Given that negative effects of increased nitrogen (N) deposition on plant communities are well established (Bobbink et al. 2010; Vellend et al. 2017), indirect effects of increased N deposition on butterfly communities through its effects on plant communities are thus likely to occur. Indeed, several authors argued that increased N deposition has also consequences on species communities at higher trophic levels (Nijssen et al. 2017; Pöyry et al. 2017; Stevens et al. 2018). However, none of the studies that we compiled in our literature review included N deposition as predictor for butterfly species richness.

Based on the data from the Swiss biodiversity Monitoring (BDM) program, our results seem to confirm the importance of N deposition as a driver of butterfly species richness. The different variants of models (i.e., multivariate linear models or structural equation models, SEM) that we used to explain the spatial variation of butterfly species richness in Swiss landscapes consistently suggested that butterfly species richness is lower when N deposition is elevated. Previous studies have established that N deposition affects species differently depending on their preferred food plant or other species characteristics (WallisDeVries & vanSwaay 2006). For example, species that depend on nutrient-poor conditions tend to decrease while species that depend on nutrient-rich conditions or N-favored diet tend to increase (Öckinger et al. 2006; Betzholtz et al. 2013). Our study complements these studies by suggesting that species-dependent N-deposition effects sum up to a net loss of butterfly species richness due to increased N deposition.

At least three main mechanisms have been proposed to explain how butterfly communities react to increased N deposition: First, reduced plant diversity due to increased N deposition might result in reduced food diversity for butterflies (Zhu et al. 2015). Second, increased N deposition might result in microclimatic cooling that for example prevents caterpillars from absorbing solar radiation to attain optimal body temperatures (WallisDeVries & vanSwaay 2006; Klop et al. 2014). Third, the chemical composition of plants might change due to increased N deposition resulting in reduced food plant quality (Fischer & Fiedler 2000; Habel et al. 2016). The results of the SEM applied to the BDM data seem to support the first two pathways: Particularly at higher elevations where the negative effect of land-use intensity on plant species richness is reduced, increased N deposition results in reduced plant species richness, and plant species richness seems to be positively related to butterfly species richness (Fig. 3c). This suggests that at higher elevations, increased N deposition results in reduced butterfly species richness mediated by its negative effect on plant species richness. At low elevations, N deposition mainly resulted in denser, cooler and more humid vegetation, which is correlated with lower butterfly species richness. This suggest that at lower elevations, microclimatic cooling through increased N deposition contributes to a reduction in butterfly communities (Fig. 3b).

We do not have data to directly investigate the third pathway stating that reduced food plant quality results in reduced butterfly diversity. However, when we allowed for a direct effect of N deposition on butterfly species richness in the SEM (Appendix A4), the results from that model suggested a quite strong negative effect of N deposition on butterfly species richness. This effect is similar to the effect size found based on the traditional linear models (Fig. 2). The direct effect of N deposition on butterfly diversity, which is independent from vegetation structure and plant species richness, might be explained by N deposition resulting in reduced food plant quality. While we are not aware of other explanations that could convincingly explain a direct negative effect of N deposition on butterfly species richness, it seems nevertheless unlikely that high N deposition reduces food plant quality so much that it reduces the number of butterfly species in that community.

While in the published literature N deposition is usually not considered as a driver of butterfly species richness, our results on Swiss butterflies suggest that high N deposition is consistently linked with low butterfly diversity demonstrating a net loss of butterfly diversity through increased N deposition. In addition to agricultural intensity, habitat fragmentation and climate change, atmospheric nitrogen inputs might thus play an essential role in reducing butterfly diversity.

**Supporting Information**

**Figure A1:** Web of science search setting used for the systematic literature review. Webofscience\_Search\_Setting.png

**Table A2**: Excel file with one table listing the 32 studies used in the literature review and one table listing the predictor variables extracted from these studies.

**Figure A3**: Matrix of scatterplots between all predictor variables.

**Figure A4:** Results of structural equation model that allows for a direct effect of Nitrogen deposition on butterfly species richness. The size of the paths are relative to the absolute values of the effect sizes with paths in grey indicating positive effects and orange negative effects.

**Literature Cited**

Amrhein V, Greenland S, McShane B. 2019. Scientists rise up against statistical significance. Nature **567**:305–307. Nature Publishing Group.

Awmack CS, Leather SR. 2002. Host Plant Quality and Fecundity in Herbivorous Insects. Annual Review of Entomology **47**:817–844. Annual Reviews.

Betzholtz P-E, Pettersson LB, Ryrholm N, Franzén M. 2013. With that diet, you will go far: trait-based analysis reveals a link between rapid range expansion and a nitrogen-favoured diet. Proceedings of the Royal Society B: Biological Sciences **280**:20122305. The Royal Society.

Bobbink R et al. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications **20**:30–59. Wiley.

Chen G, Kéry M, Plattner M, Ma K, Gardner B. 2012. Imperfect detection is the rule rather than the exception in plant distribution studies. Journal of Ecology **101**:183–191. Wiley.

Ellenberg H. 1974. Zeigerwerte der Gefässpflanzen Mitteleuropas. Goltze, Göttingen.

Fick SE, Hijmans RJ. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology **37**:4302–4315. Wiley.

Fischer K, Fiedler K. 2000. Response of the copper butterfly Lycaena tityrus to increased leaf nitrogen in natural food plants: evidence against the nitrogen limitation hypothesis. Oecologia **124**:235–241. Springer.

Gelman A, Su Y-S. 2018. arm: Data Analysis Using Regression and Multilevel/Hierarchical Models. Available from https://CRAN.R-project.org/package=arm.

Grace JB, Anderson TM, Olff H, Scheiner SM. 2010. On the specification of structural equation models for ecological systems. Ecological Monographs **80**:67–87. Wiley.

Habel JC, Segerer A, Ulrich W, Torchyk O, Weisser WW, Schmitt T. 2016. Butterfly community shifts over two centuries. Conservation Biology **30**:754–762. Wiley Online Library.

Haddad NM, Tilman D, Haarstad J, Ritchie M, Knops JM. 2001. Contrasting effects of plant richness and composition on insect communities: a field experiment. The American Naturalist **158**:17–35. The University of Chicago Press.

Hoyle RH. 2012. Handbook of structural equation modeling. Guilford press.

Humbert J-Y, Dwyer JM, Andrey A, Arlettaz R. 2015. Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose application duration and climate: a systematic review. Global Change Biology **22**:110–120. Wiley.

Kéry M, Plattner M. 2007. Species richness estimation and determinants of species detectability in butterfly monitoring programmes. Ecological Entomology **32**:53–61. Wiley.

Kéry M, Royle JA, Plattner M, Dorazio RM. 2009. Species richness and occupancy estimation in communities subject to temporary emigration. Ecology **90**:1279–1290. Wiley.

Klop E, Omon B, WallisDeVries MF. 2014. Impact of nitrogen deposition on larval habitats: the case of the Wall Brown butterfly Lasiommata megera. Journal of Insect Conservation **19**:393–402. Springer Science and Business Media LLC.

Knops JM et al. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. Ecology Letters **2**:286–293. Wiley Online Library.

Landolt E. 1977. Ökologische zeigerwerte zur Schweizer Flora. Geobotanisches Institut der ETH Zürich.

Landolt E et al. 2010. Flora indicativa. Ecological inicator values and biological attributes of the flora of Switzerland and the Alps. Haupt Verlag.

Mantilla-Contreras J, Schirmel J, Zerbe S. 2011. Influence of soil and microclimate on species composition and grass encroachment in heath succession. Journal of Plant Ecology **5**:249–259. Oxford University Press (OUP).

Menéndez R, Gonzalez-Megias A, Collingham Y, Fox R, Roy DB, Ohlemüller R, Thomas CD. 2007. Direct and indirect effects of climate and habitat factors on butterfly diversity. Ecology **88**:605–611. Wiley Online Library.

Murphy GEP, Romanuk TN. 2016. Data gaps in anthropogenically driven local-scale species richness change studies across the Earth\textquotesingles terrestrial biomes. Ecology and Evolution **6**:2938–2947. Wiley.

Nijssen M, WallisDeVries M, Siepel H. 2017. Pathways for the effects of increased nitrogen deposition on fauna. Biological Conservation **212**:423–431. Elsevier.

Öckinger E, Hammarstedt O, Nilsson SG, Smith HG. 2006. The relationship between local extinctions of grassland butterflies and increased soil nitrogen levels. Biological Conservation **128**:564–573. Elsevier.

Pearman PB, Weber D. 2007. Common species determine richness patterns in biodiversity indicator taxa. Biological Conservation **138**:109–119. Elsevier BV.

Plattner M, Birrer S, Weber D. 2004. Data quality in monitoring plant species richness in Switzerland. Community Ecology **5**:135–143. Akademiai Kiado Zrt.

Pollard E, Moss D, Yates TJ. 1995. Population Trends of Common British Butterflies at Monitored Sites. The Journal of Applied Ecology **32**:9. JSTOR.

Pöyry J, Carvalheiro LG, Heikkinen RK, Kühn I, Kuussaari M, Schweiger O, Valtonen A, van Bodegom PM, Franzén M. 2017. The effects of soil eutrophication propagate to higher trophic levels. Global Ecology and Biogeography **26**:18–30. Wiley Online Library.

Rihm B, Achermann B. 2016. Critical Loads of Nitrogen and their Exceedances. Swiss contribution to the effects-oriented work under the Convention on Long-range Transboundary Air Pollution (UNECE). Page 78. 1642. Federal Office for the Environment, Bern. Environmental studies, Berne.

Rosseel Y. 2012. lavaan: An R Package for Structural Equation Modeling. Journal of Statistical Software **48**:1–36.

Roth T, Kohli L, Rihm B, Amrhein V, Achermann B. 2015. Nitrogen deposition and multi-dimensional plant diversity at the landscape scale. Royal Society Open Science **2**:150017. The Royal Society.

Roth T, Kohli L, Rihm B, Meier R, Achermann B. 2017. Using change-point models to estimate empirical critical loads for nitrogen in mountain ecosystems. Environmental Pollution **220**:1480–1487.

Roth T, Plattner M, Amrhein V. 2014. Plants Birds and Butterflies: Short-Term Responses of Species Communities to Climate Warming Vary by Taxon and with Altitude. PLoS ONE **9**:e82490. Public Library of Science (PLoS).

Sala OE. 2000. Global Biodiversity Scenarios for the Year 2100. Science **287**:1770–1774. American Association for the Advancement of Science (AAAS).

Sassi C de, Lewis OT, Tylianakis JM. 2012. Plant-mediated and nonadditive effects of two global change drivers on an insect herbivore community. Ecology **93**:1892–1901. Wiley.

Schielzeth H. 2010. Simple means to improve the interpretability of regression coefficients. Methods in Ecology and Evolution **1**:103–113. Wiley.

Siemann E. 1998. Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. Ecology **79**:2057–2070. Wiley Online Library.

Stevens CJ, David TI, Storkey J. 2018. Atmospheric nitrogen deposition in terrestrial ecosystems: Its impact on plant communities and consequences across trophic levels. Functional ecology **32**:1757–1769. Wiley Online Library.

Topp EN, Loos J. 2018. Local and landscape level variables influence butterfly diversity in critically endangered South African renosterveld. Journal of Insect Conservation **23**:225–237. Springer Science and Business Media LLC.

Vellend M, Baeten L, Becker-Scarpitta A, Boucher-Lalonde V, McCune JL, Messier J, Myers-Smith IH, Sax DF. 2017. Plant Biodiversity Change Across Scales During the Anthropocene. Annual Review of Plant Biology **68**:563–586. Annual Reviews.

WallisDeVries MF, vanSwaay CAM. 2006. Global warming and excess nitrogen may induce butterfly decline by microclimatic cooling. Global Change Biology **12**:1620–1626. Wiley.

Weber D, Hintermann U, Zangger A. 2004. Scale and trends in species richness: considerations for monitoring biological diversity for political purposes. Global Ecology and Biogeography **13**:97–104. Wiley.

Weiss SB. 1999. Cars Cows, and Checkerspot Butterflies: Nitrogen Deposition and Management of Nutrient-Poor Grasslands for a Threatened Species. Conservation Biology **13**:1476–1486. Wiley.

Zhu H, Zou X, Wang D, Wan S, Wang L, Guo J. 2015. Responses of community-level plant-insect interactions to climate warming in a meadow steppe. Scientific Reports **5**. Springer Science and Business Media LLC. Available from https://doi.org/10.1038%2Fsrep18654.

**Tables**

**Table 1:** List of the predictor variables used in the Swiss Butterfly study. The categories are the ones obtained from the literature review and reflect different processes that may affect butterfly diversity. For the statistical analyses we standardized the predictor variables with subtracting “Zero” and dividing it by “Relevance”. See main text for more details.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Category** | **Acronym** | **Description** | **Unit** | **Relevance** | **Zero** | **Source** |
| Climate-Gradient | amt | Annual mean temperature | degree Celsius | 2 | 5 | WorldClim |
| Climate-Gradient | mtcq | Mean temperature of coldest quarter | degree Celsius | 2 | 0 | WorldClim |
| Climate-Gradient | ap | Annual precipitation | mm | 200 | 1000 | WorldClim |
| Climate-Gradient | pwq | Precipitation of warmest quarter | mm | 50 | 400 | WorldClim |
| Climate-Variability | ts | Temperature Seasonality | degree Celsius (SD) | 0.5 | 6 | WorldClim |
| Climate-Variability | ps | Precipitation Seasonality (Coefficient of Variation) | mm (CV) | 5 | 20 | WorldClim |
| Topgraphy | ele | Elevation (meter above sea level) | m | 200 | 500 | GEOSTAT |
| Topgraphy | ele\_SD | Standard deviation of elevation within site | m (SD) | 50 | 100 | GEOSTAT |
| Topgraphy | incli | Inclination | degrees | 5 | 10 | GEOSTAT |
| Topgraphy | cd | Number of the eight cardinal directions | number | 2 | 4 | GEOSTAT |
| Habitat configuration | fe | Forest edges | m | 1000 | 5000 | GEOSTAT |
| Habitat diversity | nlut | Number of land-use types | number | 3 | 10 | GEOSTAT |
| Habitat availability | ah | Available habitat (total area minus sealed areas and open water) | percentage | 80 | 10 | GEOSTAT |
| Habitat availability | agri | Percent of agricultural land | percentage | 10 | 50 | GEOSTAT |
| Land-use intensity | N | Mean Landolt indicator value for nutrients | [1-5] | 0.1 | 3 | BDM plant surveys |
| Land-use intensity | mt | Mean Landolt indicator value for mowing tolerance | [1-5] | 0.1 | 2.5 | BDM plant surveys |
| Atmospheric pollution | ndep | Nitrogen deposition | kg per ha and yr | 10 | 10 | Roth et al 2015 |
| Microclimate | T | Mean Landolt indicator value for temperature | [1-5] | 0.1 | 3.5 | BDM plant surveys |
| Microclimate | H | Mean Landolt indicator value for humidity | [1-5] | 0.1 | 3 | BDM plant surveys |
| Microclimate | L | Mean Landolt indicator value for light | [1-5] | 0.1 | 3.5 | BDM plant surveys |
| Resource diversity | PSR | Plant species richness | number (sqrt) | 1 | -15 | BDM plant surveys |
| Dependend variable | BSR | Butterfly species richness | number (sqrt) | 1 | -5 | BDM butterfly surveys |

**Table 2:** Summary of the literature review. Given are for each category the number of different indicator variables (Indicators), the number of studies (Studies), the proportion of times an effect was identified (Importance) and the direction of the effect size (i.e. the mean of effects excluding effects that peaked at intermediate level).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Category** | **Subcategory** | **Number of variables** | **Studies** | **Importance** | **Direction** |
| Environment | Climate-Gradient | 10 | 18 | 0.63 | 0.26 |
| Environment | Climate-Variability | 4 | 8 | 0.56 | 0.00 |
| Environment | Topography | 3 | 13 | 0.45 | 0.19 |
| Habitat | Habitat configuration | 6 | 7 | 0.58 | -0.10 |
| Habitat | Habitat diversity | 4 | 11 | 0.64 | 0.27 |
| Habitat | Habitat-availability | 11 | 20 | 0.64 | 0.15 |
| Habitat | Land-use intensity | 25 | 19 | 0.57 | -0.42 |
| Vegetation | Microclimate | 3 | 7 | 0.64 | 0.11 |
| Vegetation | Resource diversity | 8 | 13 | 0.59 | 0.57 |
| Others | – | 4 | 6 | 0.29 | 0.00 |

**Table 3:** Parameter estimates of the full model that explains the butterfly species richness with the linear terms of all the variables listed in Table 1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Predictor variable** | **Description** | **Estimate** | **Std-Error** | **P-value** |
| amt | Annual mean temperature | -2.179 | 1.072 | 0.043 |
| mtcq | Mean temperature of coldest quarter | 2.405 | 1.032 | 0.020 |
| ap | Annual precipitation | -0.113 | 0.124 | 0.364 |
| pwq | Precipitation of warmest quarter | 0.236 | 0.105 | 0.026 |
| ts | Temperature Seasonality | 1.341 | 0.475 | 0.005 |
| ps | Precipitation Seasonality (Coefficient of Variation) | -0.081 | 0.061 | 0.188 |
| ele | Elevation (meter above sea level) | 0.429 | 0.090 | 0.000 |
| ele\_SD | Standard deviation of elevation within site | -0.193 | 0.102 | 0.059 |
| incli | Inclination | 0.165 | 0.064 | 0.010 |
| cd | Number of the eight cardinal directions | -0.017 | 0.043 | 0.690 |
| fe | Forest edges | 0.030 | 0.012 | 0.018 |
| nlut | Number of land-use types | 0.028 | 0.051 | 0.580 |
| ah | Available habitat (total area minus sealed areas and open water) | 0.058 | 0.033 | 0.082 |
| agri | Percent of agricultural land | 0.091 | 0.021 | 0.000 |
| N | Mean Landolt indicator value for nutrients | -0.198 | 0.060 | 0.001 |
| mt | Mean Landolt indicator value for mowing tolerance | 0.018 | 0.054 | 0.738 |
| ndep | Nitrogen deposition | -0.719 | 0.094 | <0.001 |
| T | Mean Landolt indicator value for temperature | 0.200 | 0.045 | <0.001 |
| H | Mean Landolt indicator value for humidity | -0.016 | 0.043 | 0.712 |
| L | Mean Landolt indicator value for light | -0.167 | 0.031 | <0.001 |
| PSR | Plant species richness | 0.248 | 0.031 | <0.001 |

**Figure-legends**

**Figure 1:** Path diagram of the generic model that we used as a starting point for the analyses using structural equation models. Observed variables are depicted in rectangles; latent variables that were measured using several of the predictor variables are depicted in ovals. Arrows indicate assumed causal relationships between variables.

**Figure 2:** Effect sizes (with 95%-compatibility intervals) of nitrogen deposition on the squared root of butterfly species richness based on linear models that different in predictor variables. See statistical methods for a description of the different models.

**Figure 3:** Results obtained from applying the structural equation model (a) to all data of the Swiss biodiversity monitoring, (b) to the data of the sites below 1600 m and (c) to the data of sites above 1600 m. Depicted are presumed effects of different global change drivers and vegetation variables on butterfly species richness at 1km^2 resolution in Switzerland: The size of the paths are relative to the absolute values of the effect sizes with paths in grey indicating positive effects and orange negative effects.

**Figures**

**Figure 1:** Generic model.



**Figure 2:** Linear model results



**Figure 3:** Results obtained from applying the structural equation model.

(a) all data



(b) data from sites <1600 m



(c) data from sites >1600 m

