

CONTRIBUTED PAPER

Negative effects of nitrogen deposition on Swiss butterflies

Tobias Roth^{1,2}  | Lukas Kohli² | Beat Rihm³ | Reto Meier⁴ | Valentin Amrhein¹

¹ Department of Environmental Sciences, Zoology, University of Basel, Basel, Switzerland

² Hintermann Weber AG, Reinach, Switzerland

³ Meteotest, Bern, Switzerland

⁴ Air Pollution Control and Chemicals Division, Federal Office for the Environment (FOEN), Bern, Switzerland

Correspondence

Tobias Roth, Department of Environmental Sciences, Zoology, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland.
Email: t.roth@unibas.ch

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Abstract

Nitrogen (N) deposition from agriculture and combustion of fossil fuels is a major threat to plant diversity, but its effects on organisms at higher trophic levels are unclear. We investigated how N deposition may affect species richness and abundance (number of individuals per species) in butterflies. We reviewed the peer-reviewed literature on variables used to explain spatial variation in butterfly species richness and found that vegetation variables appeared to be as important as climate and habitat variables in explaining butterfly species richness. It thus seemed likely that increased N deposition could indirectly affect butterfly communities via its influence on plant communities. To test this prediction, we analyzed data from the Swiss biodiversity monitoring program for vascular plants and butterflies in 383 study sites of 1 km² that are evenly distributed throughout Switzerland. The area has a modeled N deposition gradient of 2–44 kg N ha⁻¹ year⁻¹. We used traditional linear models and structural equation models to infer the drivers of the spatial variation in butterfly species richness across Switzerland. High N deposition was consistently linked to low butterfly diversity, suggesting a net loss of butterfly diversity through increased N deposition. We hypothesize that 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 may also be mediated by reduced plant species richness. In most butterfly species, abundance was negatively related to N deposition, but the strongest negative effects were found for species of conservation concern. We conclude that in addition to factors such as intensified agriculture, habitat fragmentation, and climate change, N deposition is likely to play a key role in negatively affecting butterfly diversity and abundance.

KEYWORDS

deposition model, elevational gradient, Lepidoptera, microclimate, microclimatic cooling, plant insect interactions, trophic interactions, vegetation

Efectos Negativos del Depósito de Nitrógeno sobre las Mariposas Suizas

Resumen: El depósito de nitrógeno (N) proveniente de la agricultura y la quema de combustibles fósiles es una gran amenaza para la diversidad botánica, pero sus efectos sobre organismos que se encuentran en niveles tróficos más altos no están claros. Investigamos cómo el depósito de N puede afectar a la riqueza y abundancia (número de individuos por especie) de especies de mariposas. Analizamos la literatura revisada por pares sobre las variables usadas para explicar la variación espacial en la riqueza de especies de mariposas y descubrimos que las variables de vegetación resultaron ser tan importantes como las variables climáticas y de hábitat para explicar la riqueza de especies de mariposas. Por lo tanto, parece probable que el incremento en el depósito de N podría afectar indirectamente a las comunidades de mariposas por medio de su influencia sobre las comunidades botánicas. Para probar esta predicción analizamos datos del programa de monitoreo de biodiversidad suiza de plantas vasculares y mariposas en 383 sitios de estudio de 1 km² que están distribuidos uniformemente por toda Suiza. El área tiene un gradiente modelado de depósito de N de 2–44 kg N ha⁻¹ año⁻¹. Usamos modelos lineales tradicionales y modelos de ecuación estructural para inferir los determinantes de la variación espacial en la riqueza de especies

de mariposas en Suiza. El nivel elevado de depósito de N estuvo vinculado consistentemente con la diversidad baja de mariposas, lo que sugiere una pérdida neta de diversidad de mariposas causada por el incremento en el depósito de N. Nuestra hipótesis establece que, a elevaciones bajas, el depósito de N puede contribuir a la reducción en la riqueza de especies de mariposas por medio del enfriamiento microclimático debido al incremento en la biomasa de las plantas. A elevaciones más altas, los efectos negativos del depósito de N sobre la riqueza de especies de mariposas también podrían ser mediados por la riqueza reducida de especies de plantas. En la mayoría de las especies de mariposas, la abundancia tuvo una relación negativa con el depósito de N, pero el efecto negativo más fuerte se halló para las especies de importancia para la conservación. Concluimos que además de los factores como la agricultura intensificada, la fragmentación del hábitat y el cambio climático, el depósito de N probablemente tenga un papel importante en los efectos negativos sobre la diversidad y abundancia de mariposas.

PALABRAS CLAVE

enfriamiento microclimático, gradiente de elevación, interacciones planta-insecto, interacciones tróficas, Lepidoptera, microclima, modelo de depósito, vegetación

INTRODUCTION

Increased deposition of reactive nitrogen (N) to ecosystems is considered a major component of global change threatening biodiversity (Sala, 2000). Increased N availability usually results in increased biomass production, shifts in species composition, and often loss of plant species richness through competitive exclusion (Bobbink et al., 2010; Vellend et al., 2017). Although 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 have also been found in species groups higher in the food chain, for example, in insects (Haddad et al., 2001; WallisDeVries & van Swaay, 2017).

Because plant and insect communities are closely linked, N-induced changes in plant communities are likely to induce changes in insect communities (Sassi et al., 2012). For example, because insects are often specialized on one or a few plant species, the loss of plant diversity may negatively affect the diversity of insects (Haddad et al., 2001; Knops et al., 1999). 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 cool conditions, which will likely affect insects (WallisDeVries & van Swaay, 2017; WallisDeVries & van Swaay, 2006).

However, knowledge of how increased N availability affects consumer diversity is rather limited (Humbert et al., 2016; but see Haddad et al., 2001; WallisDeVries & van Swaay, 2017). For example, in a literature review from 2016, only 18 (10%) of the 187 effect sizes on species richness reported from N-addition experiments were about invertebrates (Murphy & Romanuk, 2016). The average effect size of those 18 studies suggests that the correlation between increased N availability and local-scale species richness of invertebrates is slightly positive (none of the 18 experimental studies from this literature review investigated butterflies). However, since the publication of this review in

2016, the number of studies on N deposition effects on consumer diversity increased (Schuldt et al., 2019; WallisDeVries & van Swaay, 2017).

We sought to complement the experimental studies on the effects of N deposition on organisms at higher trophic levels with an observational study of multiple field sites representing a large gradient of N deposition (i.e., a gradient study [Roth et al., 2017]). We used data from the Biodiversity Monitoring Switzerland program (BDM) that contains information on species richness of vascular plants and butterflies in 383 study sites of 1 km² (i.e., landscape scale) that cover an N deposition gradient of 2–44 kg N ha⁻¹ year⁻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 literature review. We searched for published studies investigating how butterfly species richness is related to environmental, land-use, and vegetation parameters. The aim of the literature review was to compile a comprehensive list of predictor variables that could be important for explaining the variation in butterfly richness among our 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 data from the BDM study sites and used traditional linear regression models to investigate how N deposition is correlated with butterfly species richness and how this correlation is affected by whether we accounted for all or only a selection of the other predictor variables. Because 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) to examine 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 via microclimatic cooling, for example, because the increasingly productive and dense plant canopy may prevent caterpillars from absorbing solar radiation (WallisDeVries & vanSwaay, 2006). Finally, we estimated the effect of N deposition on the abundance of the different butterfly species and examined how these effects differed between threatened species and species of less conservation concern.

METHODS

Literature review

On July 12, 2019, we searched Web of Science. We searched for original studies that applied multivariate regression models with several predictor variables and used variation in butterfly species richness among sites or grid cells as response variable. Because we aimed to quantify how often the different categories of predictor variables were used, we did not use specific search terms for N deposition or other predictor variables. Instead, we more generally searched for studies with titles that fulfilled the following search criteria: [(butterfly* 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]. Search settings are in Appendix S1. This search resulted in 95 studies. We excluded studies conducted in tropical rain forest and desert; thus, 32 studies met our criteria (Appendix S2).

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: broad environment category at the landscape level, including climatic gradients (from cool and humid to hot and dry) and climatic variability and topographic variables (from low to high elevations, from northern to southern expositions, from low to high topographic variability); habitat category at the level of habitat patches, including variables indicating the availability (from low to high total area of habitat patches), configuration (from low to high suitability of habitat patch configuration), diversity of types of land cover (from low to high diversity of habitat patches), and land-use intensity (habitat patches with low to high land-use intensity); vegetation category describing the vegetation or the conditions within the vegetation, including resource diversity (from low to high plant or flower richness) and microclimate (from dense vegetation with cool and humid microclimate to open vegetation with hot and dry microclimate); and an others category that included variables that did not fit the other three categories, such as global vegetation index, area age, or soil parameters. All predictor variables are listed in Appendix S2.

For each study, we extracted the investigated predictor variables and assigned the reported effect on butterfly species richness: 1, effect of the category on butterfly diversity as measured by a predictor variable was positive; 0, no obviously important effect; -1, effect of the category on butterfly diversity as measured by a predictor variable was negative; intermediate

(intermediate), effect of the category on butterfly diversity peaked at intermediate levels of the predictor variable. We coded an effect as important (1, -1, or interm) if the authors of the study mentioned in the abstract or discussion that they considered the reported effect size important or relevant. If the authors did not make a statement about the importance of the reported effect, we judged the importance and direction of the effect ourselves, based on the reported point estimate and precision (compatibility interval or standard error).

Butterfly and plant data

We analyzed the presence and absence of butterfly and plant species sampled from 2005 to 2009 in the BDM (www.biodiversitymonitoring.ch). To monitor species diversity at the landscape scale, a sample grid of 428 evenly spaced study sites, each 1 km², was randomly selected. 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 grid cell (Plattner et al., 2004). The same transects were used to survey plants and butterflies. By using the existing trail network whenever possible, the location of the transects in the landscape was not random. As a consequence, the typical plant species of standing waters, marshes, and swamps were less fully represented than species of other major land-cover types (T.R., unpublished data).

For the butterfly surveys, transects were surveyed seven times from April 21 to September 21 in the lowlands and four times from July to August above approximately 2000 m. The number of surveys corresponded to the shorter flying season of butterflies at higher elevations. The number of sites were selected such that sites at high and low elevations received approximately equal sampling effort per week of the flight season. During each survey, surveyors walked the transects in both directions and recorded all day-flying butterfly species (including *Hesperiidae* and *Zygaenidae*) within 5 m of 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 the plant surveys, transects were surveyed 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 within 2.5 m to each side of the transects 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 based on site-occupancy models (Chen et al., 2012).

Plant and butterfly surveys were usually conducted in the same years; each year one fifth of the study sites were surveyed. Because we used the N deposition rates modeled for 2007 (see below), we selected the butterfly and plant data from the

TABLE 1 Predictor variables used to explain butterfly species richness

Category ^a	Abbreviation	Description	Unit	Relevance	Zero value ^b	Source
Climate gradient	amt	Annual mean temperature	°C	2	5	WorldClim
Climate gradient	mtcq	Mean temperature of coldest quarter	°C	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	°C (SD)	0.5	6	WorldClim
Climate variability	ps	Precipitation seasonality (coefficient of variation)	mm (CV)	5	20	WorldClim
Topography	ele	Elevation (meter above sea level)	m	200	500	GEOSTAT
Topography	ele_SD	Standard deviation of elevation within site	m (SD)	50	100	GEOSTAT
Topography	incli	Inclination	°	5	10	GEOSTAT
Topography	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)	%	80	10	GEOSTAT
Habitat availability	agri	Percent of agricultural land	%	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 ha ⁻¹ year ⁻¹	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 (square root)	1	–15	BDM plant surveys
Dependent variable	BSR	Butterfly species richness	Number (square root)	1	–5	BDM butterfly surveys

^aCategories obtained from a review of the literature on variables that were used to explain the spatial variation in butterfly species richness.

^bFor statistical analyses, we standardized predictor variables by subtracting the value in this column and dividing it by the value in the relevance column. See text for details.

survey year that was closest to 2007 for each study site; this was the reason survey data were from 2005 to 2009. In the analyses, we included only study sites for which both the plant and butterfly surveys met our standards of data collection or weather conditions were in accordance with the survey protocol. This resulted in omission of 13 study sites and use of data on plants and butterflies from 383 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 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 based on Landolt indicator values that were developed for the specific situation in Switzerland (Landolt et al., 2010). We used the mean Landolt indicator value of the recorded plant species for temperature and moisture as a measure of microclimatic conditions in vegetation. We used Landolt indicators for nutrients and mowing tolerance as a measure for land-use intensity, and Landolt indicators for light as a measure of vegetation density (Table 1). Additionally, we used the total number of recorded plants as a measure of resource diversity.

Nitrogen deposition was estimated for the year 2007 in 100×100 m grid cells across Switzerland based on a pragmatic approach that combined monitoring data, spatial interpolation methods, emission inventories, statistical dispersion models, and inferential deposition models (Rihm & Achermann, 2016; Roth et al., 2015). For each study site of 1 km^2 , we averaged N deposition values from the cells containing parts of the transect used for the BDM surveys.

Often predictor variables are standardized by subtracting the mean and dividing by the standard deviation, that is, the z -transformation (Schielzeth, 2010). To obtain parameter estimates that are easier to interpret than estimates from z -transformed variables, we subtracted the value of the zero column in Table 1 and standardized the predictor values by dividing them by the value in the relevance column. Thus, the estimated intercept of the linear models was the predicted butterfly species richness for the values of the predictor variables as shown in column zero. We chose these values to lie within the range of available data. The estimated slopes of the predictor variables indicated how much the butterfly species richness changed when the predictor variable was increasing by the number given in the relevance column in Table 1. To determine the number in the relevance column, we asked ourselves what the minimum difference in the predictor value between two study plots would be that would result in detectable differences in, for example, vegetation. Although the choice of relevance value was arbitrary to a certain degree, it made it easier to make comparisons among parameter estimates, which put the focus on parameter estimates rather than on significance thresholds (Amrhein et al., 2019; Schielzeth, 2010). A matrix with the scatterplots between all centered and standardized predictor variables is in Appendix S3.

Statistical analyses

We used two different approaches for investigating the drivers of the spatial variation in butterfly species richness across Switzerland. The first approach was based on linear models, with the square root of butterfly species richness as response variable, and N deposition was the focus variable included among the 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: full model that included the linear terms of all predictor variables in Table 1; full model without microclimate variables, because microclimate is rarely considered in other studies on butterfly species richness, and climate and microclimate are usually correlated; topoclimate model that included only the linear terms of the topography and climate-gradient variables; climate model that included only the linear terms of the climate-gradient variables; 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 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 with residual analyses. Model parameters were estimated in a Bayesian framework in R-package arm (Gelman & Su, 2018).

Our second approach was based on SEM (Hoyle, 2012). We used the generic model in Appendix S4 as a starting point for the analyses with SEM. In this generic model, we assumed that butterfly species richness is mediated by vegetation structure and plant diversity (ovals and rectangles with gray background in Appendix S4). We further assumed that vegetation structure has an effect on plant diversity and, therefore, vegetation structure may affect butterfly diversity indirectly through its effect on plant diversity. Plant diversity is also likely to influence vegetation structure, and a bidirectional arrow between the two may have been more appropriate. Bidirectional arrows, however, are not possible to implement in SEMs.

Additionally, we assumed that different global-change drivers, such as climate, N deposition, and land-use intensity (white ovals), could each have independent effects on vegetation structure, plant diversity, and butterfly diversity. Although butterfly and plant species richness are measured variables in the BDM program (rectangles in Appendix S4), global-change drivers and vegetation structure were latent variables that were measured by one or several of the predictor variables in Table 1. We present the results of different implementations of this generic model that varied in the number of global-change drivers considered and in the selection of predictor variables used to measure the latent variables. Parameters of the SEMs were estimated with the R-package lavaan (Rosseel, 2012).

Finally, we tested for all butterfly species recorded in at least 20 study sites how the abundance of the species was related to N deposition. We used a generalized linear model with Poisson distribution; the number of recorded individuals of a species was the dependent variable and the linear terms of all variables in Table 1 were predictor variables. We then compared the estimated effect size of N deposition between the red list of threatened butterfly species in Switzerland and the number of target species for which Swiss agriculture has particular conservation responsibility (Bafu, 2008; Wermeille et al., 2014).

RESULTS

Literature review

From the 32 studies on butterfly species richness, we extracted the effect sizes of 252 predictor variables. Environmental predictor 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.

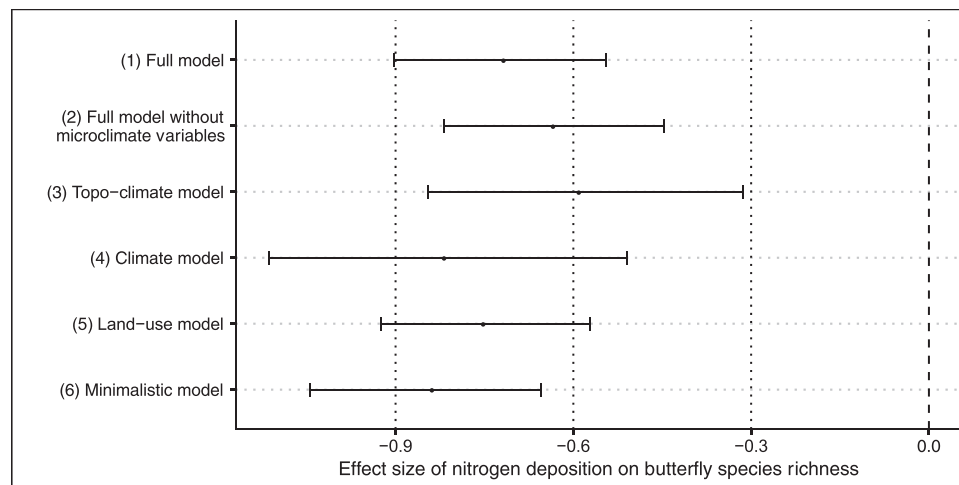
Although predictor variables for the vegetation category were less likely to be considered in these studies relative to environment and habitat variables, their importance (as estimated by the proportion of times the variables were considered important) was similar to the importance of the predictor variables

TABLE 2 Summary of the review of the literature on variables that were used to explain the spatial variation in butterfly species richness

Category	Subcategory	Number of different predictor variables	Number of studies	Importance ^a	Direction ^b
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

^aProportion of times an important effect was identified.

^bDirection of the important effect size (mean direction of effects not coded as zero [i.e., no effect], excluding effects that peaked at intermediate levels).

**FIGURE 1** Results (mean effect sizes and 95% compatibility intervals) of the linear models to explain the spatial variation of butterfly species richness with nitrogen deposition and other variables listed in Table 1

for the environment and the habitat category (Table 2). Furthermore, resource diversity of 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).

Field study

Based on the linear models that we applied to the BDM data, we found that butterfly diversity decreased as N deposition increased. The amount of this negative effect of N deposition on butterfly species richness was similar for all considered models (Figure 1). Except for the climatic variables (annual mean temperature, mean temperature of coldest quarter of the year, and temperature seasonality), N deposition was the variable with the highest absolute effect size in the full model (Table 3).

The results of the generic SEM (Appendix S4) showed that 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; effect size of climate: 0.50 [SE 0.054]), plant species richness (butterfly richness increased as plant richness increased; 0.38 [0.025]), and microclimate (butterfly richness was higher in areas with warm, dry, and open vegetation than in areas with closed and humid vegetation; 0.13 [0.041]) (Figure 2a).

The effects of land-use intensity and habitat availability on butterfly species richness seemed weak (effect size of land-use intensity: -0.035 [SE 0.021]; habitat availability: 0.026 [0.025]). However, at sites below 1600 m, where land use was usually intense, the negative effect of land-use intensity on butterfly diversity was much stronger (-0.19 [0.034]) (Figure 2b).

The results of applying the SEM to the data of all sites further suggested that higher N deposition rates led to denser, more

TABLE 3 Parameter estimates of the full model that explains butterfly species richness with the linear terms of all the variables listed in Table 1^a

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

^aOrder of variables is according to the absolute value of the estimate.

humid, and cooler microclimates in the vegetation (effect size of N deposition: -0.10 [SE 0.042]) and to lower plant species richness (-0.73 [0.15]) (Figure 2a). At elevations below 1600 m, N deposition mainly affected vegetation structure (vegetation structure: -0.85 [0.097]; plant species richness: -0.14 [0.17]) (Figure 2b), and the negative effect of N deposition at higher elevations was strong regarding vegetation structure and plant species richness (vegetation structure: -2.43 [1.01]; plant species richness: -2.11 [1.09]) (Figure 2c). However, the latent variable vegetation structure had a different meaning below and above 1600 m because the mean Landolt indicator value for light was positively correlated below 1600 m (Figure 2b), whereas it was negatively correlated above 1600 m (Figure 2c). This makes intuitive sense because below 1600 m the coldest areas are the shaded ones in the forest, whereas above 1600 m the coldest areas are open areas with hardly any vegetation that would protect from freezing temperatures.

From the 183 butterfly species that were recorded, 113 (62%) species were recorded in at least 20 study sites. The abundance (number of recorded individuals) of most of these 113 species decreased as N deposition increased, as revealed by generalized linear models applied to each species separately. The negative effect was strongest for near-threatened and vulnerable species (near threatened: 24 species; vulnerable: three species); intermediate for the target species for which Swiss agriculture has

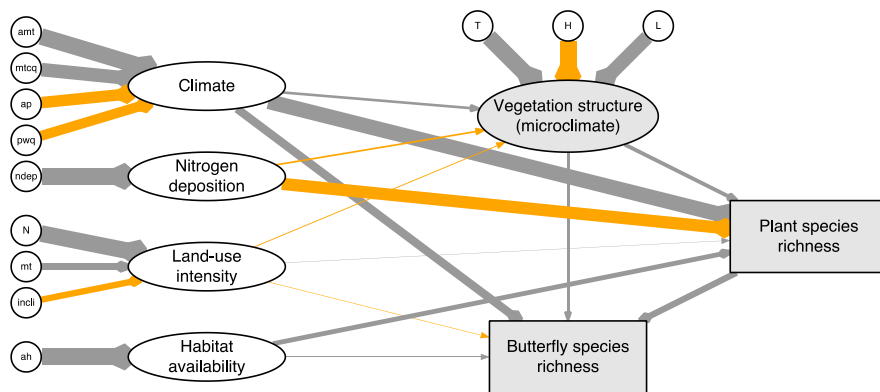
particular responsibility of conservation (58 species, including 19 of the near-threatened and two of the vulnerable species); and weakest for the remaining species (Figure 3). No critically endangered or endangered species were among the 113 analyzed species. The estimated effect size for N deposition for each species is in Appendix S5.

DISCUSSION

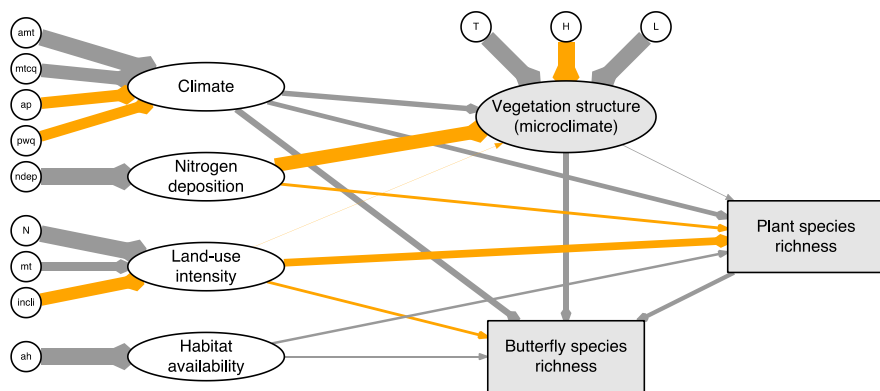
Nitrogen deposition effect on butterflies and its consequences for conservation

Our results confirm the importance of N deposition as a largely negative driver of butterfly species richness and abundance (number of recorded individuals) in Switzerland. Previous studies show that N deposition affects butterfly species differently, depending on their preferred food plant or other factors (WallisDeVries & vanSwaay, 2006). For example, population sizes of butterfly species that depend on nutrient-poor conditions tend to decrease as N deposition increases, whereas population sizes of species that depend on nutrient-rich conditions, or N-favored plant species, tend to increase (Betzholtz et al., 2013; Öckinger et al., 2006). Our study complements these results by suggesting that species of conservation concern are particularly affected by

(a) all data



(b) data from sites <1600 m



(c) data from sites >1600 m

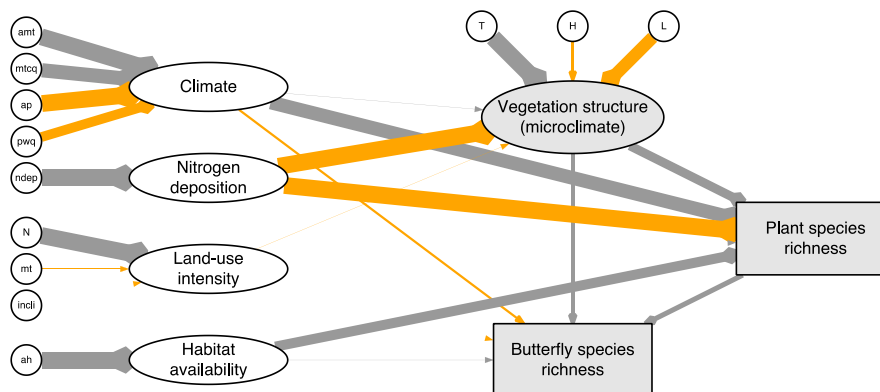


FIGURE 2 Results of the application of the structural equation model to infer how climate, nitrogen deposition, land-use intensity, and habitat availability affect butterfly species richness through their effects on vegetation: (a) all data, (b) data from sites <1600 m, and (c) data from sites >1600 m. Variable abbreviations defined in Table 3

N deposition (Figure 3) and that at the landscape scale, species-dependent N-deposition effects sum up to a net loss of butterfly species richness due to increased N deposition.

None of the studies included N deposition as a predictor variable for butterfly species richness. Although our literature review was not exhaustive, the absence of N deposition as a

variable for butterfly species richness in the 32 reviewed studies suggests that the negative effect of N deposition on butterfly communities has probably been underestimated. Given the global insect decline in terrestrial ecosystems (van Klink et al., 2020), the lack of awareness of N deposition as a negative driver of insect populations seems particularly relevant because

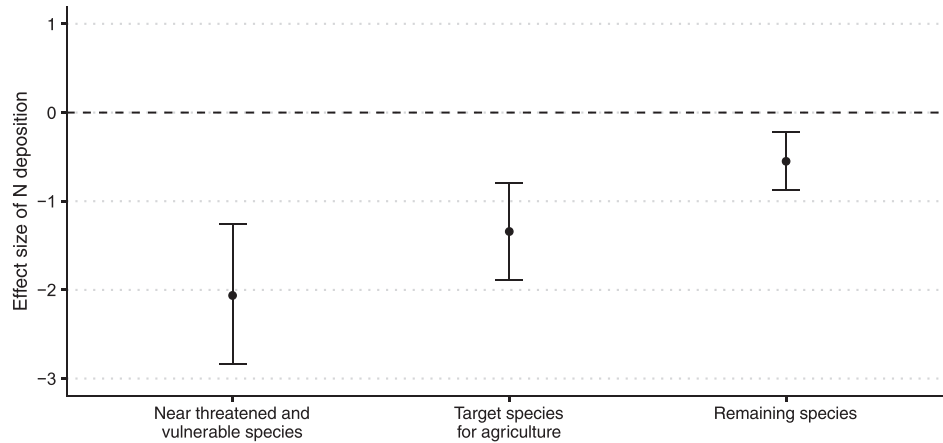


FIGURE 3 Mean (95% compatibility intervals) effects of N deposition on abundance (number of recorded individuals of near threatened or vulnerable butterfly species, target species for which Swiss agriculture has particular responsibility for conservation that are not near threatened or vulnerable, and all remaining species)

promising strategies to mitigate or even reverse the negative trends in insect populations might be overlooked. For example, mowing with biomass removal or grazing, which both remove large amounts of N, may have a positive effects on butterfly diversity, at least if the intensity of mowing or the density of grazers is not too high (Jones et al., 2017). Indeed, in a recent review of the factors believed to be responsible for the observed collapses of insect populations, Wagner (2020) concludes that “the potential consequences of atmospheric nitrogen deposition are grave and worthy of greater attention.”

Mechanistic links between nitrogen deposition and butterfly communities

Given that negative effects of increased N deposition on plant communities are well established (e.g., reduced plant diversity or increased vegetation density [Bobbink et al., 2010; Vellend et al., 2017]), negative effects of increased N deposition on butterfly diversity through its effects on plant communities are likely to occur (Schuldt et al., 2019). At least three main mechanisms have been proposed. First, reduced plant diversity due to increased N deposition could result in reduced food diversity for butterflies (Zhu et al., 2016). Second, increased N deposition resulting in higher plant biomass and denser vegetation could lead to microclimatic cooling that, for example, may prevent caterpillars from absorbing solar radiation to attain optimal body temperatures (WallisDeVries & vanSwaay, 2006). Third, the chemical composition of plants could change due to increased N deposition, resulting in reduced quality of food plants (Habel et al., 2016; but see Pullin, 1987).

The results of the SEM seem to support the first two pathways. Particularly at higher elevations, where the negative effect of land-use intensity on plant species richness was reduced, increased N deposition was correlated with reduced plant species richness, and plant species richness was positively related to butterfly species richness (Figure 2c). This suggests

that at higher elevations, the negative effect of N deposition on butterfly species richness is mediated by its negative effect on plant species richness. At lower elevations, N deposition was mainly correlated with denser vegetation (i.e., with plant indicator values associated with less light) and with cooler and more humid vegetation, which was correlated with lower butterfly species richness. This suggests that at lower elevations, microclimatic cooling through increased N deposition contributes to a reduction in butterfly species richness (Figure 2b).

We did not have data to directly investigate the third explanation: decreased butterfly diversity due to N deposition could be caused by reduced food plant quality. However, when we allowed for a direct effect of N deposition on butterfly species richness in the SEM (Appendix S6), the results suggested a relatively strong negative effect of N deposition on butterfly species richness. This effect was similar to the effect size found based on the traditional linear models (Figure 1). The direct effect of N deposition on butterfly species richness, which is independent from vegetation structure and plant species richness, might be caused by N deposition resulting in reduced food plant quality. Although 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 this reduces considerably the number of butterfly species.

The mechanistic links between N deposition and butterfly communities discussed above are mostly based on the assumption that links exist between plant communities and caterpillars. However, the counts of butterfly populations and species richness we analyzed were based on surveys of adult butterflies. Thus, our results rely on the assumption that the number of adult butterflies counted reflected caterpillar populations at the same site. The clear effects between N deposition and butterfly populations we found for many species (Appendix S5) suggest that this assumption was fulfilled for many species. However, particularly for the more mobile species (e.g., *Pieris* spp., *Vanessa atalanta*, *Aglais urticae*, or *Papilio machaon*), we could not rule

out the possibility that the comparatively low effect sizes of N deposition for these species were due to adults recorded away from their larval sites.

Importance of the vegetation

In the reviewed studies that compared different drivers of butterfly species richness, vegetation variables were much less frequently investigated than environmental or habitat variables. However, when the effects of vegetation variables on butterfly species richness were studied, they were usually described as relevant and consistent. The results of our literature review thus suggest that vegetation variables representing microclimate or plant resource diversity are important but underrepresented in published research on the spatial variation of butterfly species richness.

Our SEM results confirmed the importance of vegetation variables: butterfly species richness was correlated with plant species richness to a similar degree as was ambient temperature. In contrast, the observed effects of land-use intensity and habitat availability were rather weak. An explanation might be that the available information about land-use intensity at the study plots of the BDM was limited. Our predictor variables were derived mainly by the plant surveys and contained average indicator values per 1-km² study plot; therefore, within-site variability was hidden.

Although in the published literature, N deposition was rarely investigated as a driver of butterfly species richness, we found that in Swiss landscapes, high N deposition was consistently linked with low butterfly diversity and low butterfly abundance, suggesting a net loss of butterfly diversity caused by increased N deposition. Conservation strategies, such as biomass removal or grazing to remove the added N, are thus essential to mitigate these negative effects. In addition to agricultural intensity, habitat fragmentation, and climate change, N deposition is likely to play an essential, yet apparently underestimated, role in threatening butterfly diversity and abundance.

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ORCID

Tobias Roth  <https://orcid.org/0000-0001-9742-8297>

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SUPPORTING INFORMATION

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