Nitrogen deposition and the reduction of butterfly biodiversity quality in the Netherlands

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A R T I C L E   I N F O

A B S T R A C T

Butterfly decline in Northern Europe is a cause of concern and it has been hypothesised that this is due to nitrogen deposition inducing excess early growth of plants. It has also been changing the quality of the food available to larvae. We tested these hypotheses by linking butterfly biodiversity quality indices (species richness, population, biomass, conservation value, evenness (Simpson's Index) and modelled species richness (Chao 1 and 2)) with nitrogen Critical Load Exceedence (nCLE) data. An index of butterfly sensitivity to nitrogen was also created (Species Nitrogen Value Index (SNVI)). Using PCA, datasets were tested for associations and relationships.

The results included multiple biodiversity quality indices based on 17 years of data (aggregated into three periods of six, six and five years to give 287 datasets) in four habitat types (grassland, heathland, woodland and farmland). With the exception of heathland the analysis showed that nitrogen deposition and all other indices (except SNVI) were in decline.

For heathland the last 11 years did not show any significant decline. Heathland also showed an anomalous biodiversity quality profile for these last 11 years, suggesting that the sensitivity of heathland to nitrogen deposition will require further considerable efforts to achieve a nitrogen deposition that is not in exceedence of the critical load.

Habitat restoration will take time due to the multiple hindrances to colonisation, which in the case of heathland might prevent successful butterfly colonisation for the foreseeable future. These results indicate the efficacy of butterfly biodiversity quality and nCLE as indicators for the SEBI 2020 process (Streamlining European Biodiversity Indicators) by showing the relationship between them.

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1. Introduction

Butterflies are one of the two indicator taxa (the other being birds) specifically identified by the European Environment Agency (EEA) among a total of 26 indicators of biodiversity in the Streamlining European Biodiversity Indicators 2010 (SEBI 2010) process. Butterflies are said to respond more quickly to environmental change than other taxonomic groups, such as birds or vascular plants (Erhardt and Thomas, 1991; Thomas et al., 2004). In particular Weiss (1999) showed a strong linkage between nitrogen deposition impact on plant growth and the population decline of the Bay Checkerspot) butterfly (*Euphydryas editha bayensis*)

alleviated by the removal of nitrogen by grazing cattle (nitrogen was exported in the form of cattle carcasses). Defined methods for butterfly population sampling exist, which in some cases have been conducted in a standardised way for a number of years (Pollard and Yates, 1993).

Butterfly populations are influenced by weather conditions and are expected to be affected by Global Climate Change (GCC), with the global expectation that many temperate region populations might extend their geographic range as temperatures increase (Settele et al., 2008; Devictor et al., 2012). However, studies by Settele et al. (2008) and Thomas et al. (2004) show many European butterflies appear to be in decline albeit obviously not the same species as are expanding.

Nitrogen pollution in the environment has several sources, all determined by human activity. The most obvious sources of these is the use of nitrates in agriculture. Many European habitats are heavily influenced by intensive farming practices and application of nitrogen. In particular, ammonia produced by cattle/pig slurry

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or from chicken deep litter/battery houses has a differential effect on the surrounding vegetation (Sutton et al., 2009). A more insidious nitrogen influence is general atmospheric deposition. Bobbink and Roelofs (1995) assessed atmospheric deposition to have a measureable effect on vegetation once it has reached a Critical Load. Below this load, the geology and vegetation are able to accommodate the nitrogen and it is incorporated into the ecosystem without too much change to the biotope. Above the Critical Load there is a possible effect on vegetation growth and species composition. This balance of deposition is modelled and expressed as the nitrogen Critical Load Exceedence (nCLE) (Bobbink and Roelofs, 1995; Hettelingh et al., 2009). These authors used remote sensing of atmospheric nitrogen combined with data derived from geological and vegetative sources to model the nitrogen effect. Nitrogen CLE is also a SEBI 2010 indicator and our working research hypothesis is that:

H nCLE above the modelled critical load has a deleterious effect on butterfly biodiversity quality

This hypothesis would therefore link to, and validate, two of the 26 indicators in the SEBI 2010 list. NCLE is here defined as nitrogen deposition per 1 km × 1 km modelled as deposition above background vegetation and geology accommodation levels and in this paper is measured as mol/ha/yr. Biodiversity quality of butterfly populations is used in the way described by Feest (2006), Feest et al. (2010, 2011) and Feest and Cardoso (2012) as the summation of the measured indices of characteristics of a population sampled in a standardised way. It is therefore expressed as a range of indices indicating the characteristics and thereby the quality of a butterfly population (see below).

2. Materials and methods

The methodology for fieldwork was based on the 1993 (Pollard and Yates) survey methodology of the Dutch Butterfly Monitoring Scheme in the Netherlands for the years 1990–2006. Data was supplied by de Vlinderstichting and converted to biodiversity quality indices using the Fungib programme (free from ecosulis ltd.). Biodiversity quality for butterflies is defined in this research as the sum of characteristics presented by following indices (Feest, 2011) calculated for each survey site for each year: To alleviate the effects of the considerable annual variation in butterfly populations, that often incorporates at least a year of lag time when populations are recovering from depressed numbers, data was also aggregated into three sets of time periods data of six, six, and five years (a total of seventeen years).

The following biodiversity quality indices were calculated following Feest (2006) and Feest et al. (2010):

1. **Species Richness**: number of species in a unit sample, which in this case is the total of a year's observation of a defined site and survey route.
2. **Simpson's Index**: a measure of the evenness of the population numbers of a site in a year's sample. Simpson's Index was used (in preference to the Shannon–Wiener Index) since it is less influenced by sample size (Magurran, 2004) and has wider amplitude of scale, and thus greater sensitivity to change in species populations.
3. **Species Conservation Value Index (SCVI)**: a scale of the rarity of different species following the scale devised by Feest (2006) and based on the occurrence of different species in the de Vlinderstichting survey of the years 1990–2006. The scale ranged from two for abundant species to 100 for extremely rare species. The resultant list of species and their scores can be found in Appendix A.

4. **Population**: the total number of butterfly individuals recorded for a site in a year.
5. **Biomass**: the sum of the product of the number of individuals of each species multiplied by the wing width, which is an approximation for relative size. Since the size range of butterflies is small, this index was related to population, but in other organisms (e.g. macrofungi) these two indices can vary greatly (Feest, 2006).
6. **Species Nitrogen Value Index (SNVI)**: an index of the relative sensitivity of different butterfly species to nitrogen pollution. For the purposes of this research, it is the averaged transformed weighted $\log(n+1)$ data of an Ellenberg series of species allocations on a scale from 1 to 10. The method follows Oostermeyer and van Swaay (1998). In effect, the lower the value, the more a species prefers nitrogen-poor soils (nitrophobic). High values indicate nitrophilia. The resultant values for species can be found in Appendix A.
7. **Nitrogen Critical Load Exceedence (nCLE)**, which was calculated as the average modelled atmospheric nitrogen deposition at a 1 km × 1 km grid minus the average critical loads of semi-natural ecosystems present in the same 1 km × 1 km grid (as mol/ha/yr). The nitrogen deposition was derived from the OPS-model (van Jarsfeld et al., 1997), using information on the emission of nitrogen in the Netherlands and other EU countries. Modelled air concentrations were calibrated with air concentration measured in Dutch air-monitoring sites. Critical loads were derived from Bobbink and Lamers (2002). These empirical critical loads are based on observed changes in the structure and function of semi-natural ecosystems, reported in a number of publications (Achermann and Bobbink, 2003). Within the broad ranges of empirical critical loads, specific critical loads were assigned to different ecosystems using dynamic ecosystem models (Van Dobben et al., 2006). When modelled loads were outside the empirical ranges, the nearest limit was used to set the empirical load.

Habitats: butterfly populations were grouped into 4 broad habitat types found throughout the Netherlands (farmland, grassland, heathland and woodland) and assessed separately.

Statistical analysis: due to the annual variability and carry-over of year effects, the data was converted to mean values for the survey period 1990–2006 as periods of six (1990–1995), six (1996–2001) and five years (2002–2006) and relationships were examined by subjecting the data to Principal Component Analysis (PCA), conducted on both the aggregated data and the individual years as six-, six- and five-year assessments. PCA was used to isolate the relationships between factors with the indices not needing to be normally distributed and showing reduced interaction (Henderson and Seaby, 2008).

3. Results

A summary of the results are presented in Table 1 which shows a summary of the indicator values. Some patterns can be observed, such as that each habitat has:

a) an increased SNVI over the 17 years
b) Species Richness and Simpson's Index have no clear pattern and
c) Population declines sharply in three out of four habitats.

SCVI and nCLE declined (nCLE by over 1000 mol/ha/yr) over the 17 years (except for the notable third time period for data for heathland nCLE where a decline of only 11 mol/ha/yr is evident and the total decline is 566 mol/ha/yr for the 17 years. The final figure was still in excess of 1000 mol/ha/yr (Table 1).

Table 1
Biodiversity quality (Species Nitrogen Value Index (SNVI), Species Richness (SR), Simpson's Index, Population, Biomass, Nitrogen Critical Load Exceedence (nCLE) and sample size (*n*=)) data for four Dutch habitats over three time periods.

Habitat and time period	SNVI	SR	Simpson	SCVI	Population	Biomass	nCLE	*n*=
Farmland 1	5.65	15.35	4.72	2.67	714	10,147	1405	8
Farmland 2	5.67	16.17	5.19	2.69	871	17,338	1138	14
Farmland 3	6.08	14.56	4.83	2.61	539	11,548	145	21
Grassland 1	5.8	15.86	4.71	2.83	720	14,698	1765	23
Grassland 2	5.74	14.9	4.53	2.78	638	12,778	641	57
Grassland 3	6.22	16.48	6.33	2.72	527	11,352	572	54
Heathland 1	4.01	18.22	5.53	3.23	569	10,407	1626	12
Heathland 2	4.22	15.9	6.05	3.2	437	7423	1071	15
Heathland 3	4.42	17.42	6.06	3.05	385	7142	1060	20
Woodland 1	5.75	17.13	5.79	2.75	649	13,030	2133	14
Woodland 2	5.88	16.05	6.05	2.76	472	10,136	859	26
Woodland 3	6.22	16.47	6.33	2.72	527	11,351	572	23
								$\sum$287

Table 2
Principal Components Analysis of all butterfly and nitrogen deposition data for four habitats over three time periods.

	PC1	PC2
Species Richness	0.370	−0.331
Simpson's Index	0.340	0.231
Population	−0.395	−0.455
Biomass	−0.419	−0.326
SCVI	0.466	−0.212
SNVI	−0.434	0.258
nCLE	0.096	−0.642
% of variance	50.1	27.1

Principal Component Analysis of all of the data in Table 1 yields the data shown in Table 2, where the PC1 has a variance proportion of 50.1%, and yet the nCLE has almost no eigenvalue, so the relationships revealed do not have a bearing on nitrogen deposition. PC2 accounts for 27.1% of the variance and the major factor in the axis is nCLE (−0.642). As this is declining strongly, this appears to indicate that the other factors with the same negative sign (Species Richness, SCVI, Population and Biomass) are also in decline, whereas SNVI and Simpson's Index are increasing. Only PC1 and PC2 have eigenvalues greater than one, so only these were considered for analysis (Henderson and Seaby, 2008). This latter pairing (SNVI and Simpson's Index) indicates that the population was becoming dominated by nitrophilic species, whereas all other indices are in decline (they have the same negative sign as nCLE, which is declining strongly).

If the individual aggregations of years are subjected to PCA, a consistent pattern occurs (see Table 3).

3.1. PC1

The most consistent pattern found in all cases is the similarity of the nCLE and Simpson's Index eigenvalues. In all cases, they have the same +ve or −ve sign even if some of the values are small.

3.2. PC2

In PC2 (Table 4), Species Richness has many lower eigenvalues (only one >0.2), but nCLE has higher eigenvalues and therefore PC2 should show the relationships between the other indices and nCLE more clearly.

4. Discussion

Using PCA two EEA SEBI 2010 indicators examined in this paper show a relationship and nCLE has an impact on butterfly populations (as hypothesised). This could be explained (although not proven here but see paper by Weiss, 1999) on the grounds that nitrogen deposition affects plant food quality and vegetation structure. The hypothesis is also clearly supported by the different biodiversity relationship shown in the heathland data. It can be justified on the basis of the high susceptibility of this habitat to nitrogen deposition and nCLE remaining above 1000 mol/ha/yr. Indeed, it seems that the exceptional case of the heathland butterfly response to nitrogen (where nCLE does not decline between two of the time periods and remains above 1000 ml/ha/yr) validates the general theory for other habitat types where nCLE declines distinctly throughout the period (and is less than 600 mol/ha/yr). A

Table 3
Principal Components Analysis 1 values for five biodiversity quality characteristics and nitrogen deposition for four habitats over three time periods (grey cells show positive values and yellow negative values).

PC1 results.

Indices/habitat	Fa1	Fa2	Fa3	Gr1	Gr2	Gr3	Wo1	Wo2	Wo3	He1	He2	He3
Species Richness	0.523	0.478	-0.451	0.487	0.473	-0.554	-0.451	-0.490	-0.484	-0.435	0.508	-0.276
Simpson's Index	-0.058	0.316	-0.102	0.295	0.148	-0.354	-0.294	-0.406	-0.352	-0.320	0.206	0.118
Population	0.290	0.407	-0.427	0.479	0.465	-0.334	-0.381	-0.341	-0.433	-0.407	0.430	-0.481
SCVI	0.466	0.439	-0.403	0.346	0.381	-0.411	-0.417	-0.433	-0.324	0.367	-0.419	-0.458
SNVI	-0.507	-0.367	0.362	-0.311	-0.345	0.318	0.395	0.281	0.332	-0.385	0.402	0.447
nCLE	0.350	0.001	-0.361	0.003	0.230	-0.256	-0.299	-0.327	-0.234	-0.303	0.021	0.279
% of variance	45.0	45.0	56.5	55.2	48.6	40.7	66.6	53.2	53.5	64.8	43.2	45.1

Table 4
Principal Components Analysis 2 values for five biodiversity quality characteristics and nitrogen deposition for four habitats over three time periods (grey cells show positive values and yellow negative values).

PC2 results.

Indices/habitat	Fa1	Fa2	Fa3	Gr1	Gr2	Gr3	Wo1	Wo2	Wo3	He1	He2	He3
Species Richness	-0.003	0.015	-0.150	-0.122	0.154	0.048	0.013	0.069	0.040	0.183	0.125	-0.533
Simpson's Index	-0.460	-0.415	-0.796	-0.373	0.550	-0.333	-0.430	-0.399	-0.109	-0.619	-0.492	-0.432
Population	0.547	0.472	0.378	0.178	-0.342	0.481	0.486	0.057	0.549	0.295	0.439	-0.298
SCVI	-0.223	-0.326	-0.102	0.232	-0.101	0.027	0.017	0.565	-0.298	0.031	0.333	0.325
SNVI	0.133	0.292	0.134	0.326	-0.168	0.452	0.154	-0.392	0.415	0.215	-0.480	-0.288
nCLE	-0.358	-0.477	-0.208	-0.777	0.528	-0.430	-0.505	-0.592	-0.313	-0.628	-0.123	-0.378
% of variance	31.2	25.3	15.6	19.7	18.9	23.5	16.5	16.1	24.7	15.0	26.4	31.1

relationship can be established between the different biodiversity characteristics and qualities.

There are at least three possible hypotheses for implicating nitrogen deposition in this decline:

1) As nitrogen deposition and warmer temperatures increase, spring plant growth increases shading at soil level, so that increased temperatures due to GCC are not sensed at the soil surface by poikilothermic invertebrates (Wallis de Vries and van Swaay, 2006). The importance of nitrogen as a possible influence on biodiversity, therefore, could be a direct one of a relative cooling of the ground, and those stages of the life cycle located at ground level will be slower to respond to spring warming.

2) Nitrogen is a limiting nutrient in natural habitats and the dramatic changes wrought by nitrogen can be exampled by the impact of deposition on mycorrhizal sporocarp production where an input of 100 kg/ha/yr almost completely eliminated the production of sporocarps (Hasselquist et al., 2012). The added uptake of nitrogen by plants has a profound influence on plant biochemistry, with mineral nitrates being present in the cells and a disturbance of the amino acid content (Fischer and Fiedler, 2000; Mevi-Schutz and Erhardt, 2005). The net result is that invertebrates feeding on nitrogen-affected plants are feeding on a less than optimum resource (Fischer and Fiedler, 2000). Invertebrate larvae require amino acids and minerals for their skeletons and cannot use nitrates for this purpose (van Duinen et al., 2010), but total nitrogen content determines feeding rate. The net effect is a disturbance of the nutrition of the feeding larvae.

3) Nitrogen deposition will change plant species directly as nitrophobic species are replaced by nitrophilic species and therefore the food plants of larvae will also change distribution.

The complexity of nitrogen dynamics is shown by three habitats having similar patterns of nCLE relationships (everything, including nCLE, is declining, except for SNVI), with the notable exception being heathland, where it appears that the sensitivity of the habitat to nitrogen pollution results in nCLE not declining (almost any deposition is excessive and the nCLE remains above 1000 mol/ha/yr) and thus resulting patterns of relationships are different. One index that is particularly uninformative is Species Richness. In PC1, it has the same sign as nCLE when nCLE has any eigenvalue of note (e.g. >0.2), so the trend shown is a decline matching the nCLE decline.

The complications of different patterns of nCLE between habitats due to the sensitivity of heathland required that the heathland data be analysed separately. Heathland does not show a decline

between periods 2 and 3 and nCLE remains above 1000 mol/ha/yr whilst for other sites nCLE is declining and SNVI and Simpson's Index increase. This latter could be interpreted as populations becoming dominated by larger nitrophilic species. For Heathland PC2 where nCLE is not apparently decreasing, only He1 and He3 have nCLE values greater than 0.2, so only these two can be expected to show the relationship between nCLE and other factors. Species Richness has only low values, so the number of species is not changing, although nCLE and Simpson's Index are declining and SNVI is increasing. This could be interpreted as a turnover of species through the invasion of the heathland by nitrophilic species and an expansion of these populations. He1 generally follows the trends of the other habitats (and shows declining nCLE), whereas He2 and He3 do not. He3 is particularly interesting in that it has a high percentage of the variance (31.1%) and all indices have larger eigenvalues. Since there appears to be no overall decline in nCLE, the analysis will present differences between sites, rather than trends. SCVI is shown to be strongly in decline on these sites. The other indices are relating positively (Species Richness, Population, SNVI and nCLE) which again could be interpreted as nitrophilic species becoming dominant in sites with higher nCLE.

A complicating process is the lag-time of restoration of butterfly populations following recovery from an insult (Feest, 2011) and this will be prolonged: (a) compared to the immediate effects due to pollution as a result of colonisation effects; (b) due to the process of elimination of the influence (N in this case) from the habitat; and (c) due to stochastic influences.

The problem of nitrogen deposition is common throughout Europe, and very high levels are also found in countries such as China and India (Erisman, 2011). It would be a valuable exercise to determine if the results established here were found in other ecosystems; in particular, tropical rainforest biodiversity hotspots. Given the results we have shown extra attention should be given to heathlands. This habitat is found on poor sandy soils, which makes it very vulnerable to nitrogen deposition similar to the effect on Serpentinic soils used by Weiss (1999). Given the levelling off of the nCLE values at these Dutch sites, a further reduction of the nitrogen deposition below 1000 mg/ha/yr seems necessary for successful restoration of the former high biodiversity quality values of this habitat for butterflies.

We have shown that a strong negative relationship between nCLE and butterfly biodiversity quality can be demonstrated in an analysis of national populations in the Netherlands (satisfying the hypothesis) and in Fig. 1 we give an example of a specific heathland site where these trends can be detected (a wet heathland site in the province of Overijssel). Nitrogen does not act on the invertebrates per se: it is mediated through the effects on food
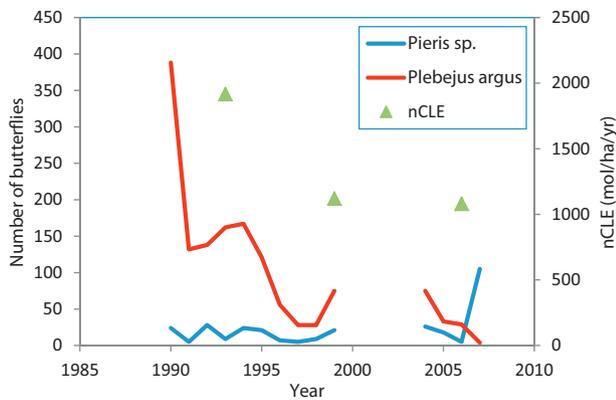
Fig. 1. The decline of a nitrophobic species (*Plebejus argus*) contrasted with the increase of a nitrophilic taxon (*Pieris* sp.) despite the apparent decline in nitrogen deposition between 1990 and 2007 on a heathland site in the province of Overijssel (Netherlands).

plants, and we have postulated mechanisms for this. It is clear that in the conservation of butterflies the impact and role of nitrogen pollution must be of great importance and that despite the influence of Global Climate Change, without deposition levels reverting to less than the modelled exceedence, butterfly conservation will be problematic. Butterflies, therefore, are confirmed to be good indicators of the pressures and status given in the SEBI 2010 list.

5. Conclusion

The approach used here utilises a multiple index/characteristic definition of biodiversity quality, derived from a metadata assessment of survey data (Feest et al., 2011). It has been justified since on occasions however the prevailing interpretation of biodiversity, that it is equal to Species Richness alone, is likely to be misleading. The differing nitrogen sensitivity and population characteristics (SCVI, Biomass, Simpson) of the butterfly species results in a species turnover situation induced by nitrogen deposition. As advocated by Feest (2006), Feest et al. (2010, 2011) and Feest and Cardoso (2012), this approach allows much greater understanding of the dynamics of biodiversity quality, in which nitrogen deposition is only one factor.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2013.12.008.

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