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ORIGINAL PAPER

Impact of nitrogen deposition on larval habitats: the case of the Wall Brown butterfly *Lasiommata megera*

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Abstract Nitrogen deposition is considered as one of the main threats to biodiversity and ecosystem functioning. Three mechanisms have been proposed to explain the detrimental effect of excess nitrogen on butterflies: loss of host plants, deterioration of food plant quality and microclimatic cooling in spring. Here, we investigated whether these mechanisms might explain the dramatic recent decline of the Wall Brown butterfly *Lasiommata megera.* Monitoring data from the Netherlands indeed show a greater decline at higher critical load exceedance of nitrogen deposition. Loss of host plants is not a likely explanation of the decline for this grass-feeding species. In a greenhouse experiment, we only found beneficial effects of nitrogen fertilization on larval performance, which seems to rule out a nutritional cause; application of a drought treatment did not result in significant effects. Microclimatic conditions at overwintering larval sites of *L. megera* and the related but increasing *Pararge aegeria* provided a possible clue. In comparison with larval sites of *P. aegeria,* those of *L. megera* showed higher temperatures at the mesoscale and less plant cover and more dead plant material at the microscale. *L. megera* caterpillars were also found closer to the shelter of vertical structures. The greater dependence on warm microclimates suggests that microclimatic cooling through excess nitrogen contributes to the recent decline of *L. megera.*

Keywords Nitrogen deposition · Larval ecology · Microclimate · Habitat quality · Butterflies

Introduction

Despite growing public awareness, the global rate of biodiversity loss is not yet slowing down (Convention of Biological Diversity 2010). This is all the more alarming, because these estimates are largely based on vertebrate species, whereas invertebrates present the largest proportion of biodiversity and show signs of even higher rates of decline (Thomas et al. 2004). Changing land use, climate change and nitrogen deposition have been identified as the main drivers of anthropogenic biodiversity loss (Sala et al. 2000; Tylianakis et al. 2008). However, we still face a serious challenge to gain insight on how these drivers operate to explain current trends in insect biodiversity.

Based on an exceptional availability of distribution data, monitoring schemes and ecological knowledge for European butterflies, the most significant progress to explain the drivers of change has been made in this group of insects (Van Swaay et al. 2010). Knowledge on critical resources (Dennis et al. 2006) and dispersal capacity have increased our understanding of the impact of land use change and habitat fragmentation on butterflies (Dover and Settele 2009). Similarly, there is a growing insight in the effects of climate change on butterfly populations (e.g. WallisDeVries et al. 2011; Oliver et al. 2012a, b; Travis et al. 2013). However, these promising achievements have, as yet, not been seen with respect to the effects of nitrogen deposition, although its impact is expected to be especially important in the northern temperate zone (Sala et al. 2000). Experiments with nitrogen addition have shown strong impacts on insect community composition, with increases in the

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biomass but decreases in species richness of insects, both overall and for herbivores only (Haddad et al. 2000). Weiss (1999) already stressed the threat of excess nitrogen for *Euphydryas editha bayensis* in California. Recent trend analyses in the Netherlands have revealed greater declines of characteristic butterflies for low-productive habitat types in regions with higher nitrogen deposition (WallisDeVries and Van Swaay 2013). Conversely, in Sweden Betzholtz et al. (2013) found a greater range expansion in species with a nitrogen-favoured diet. These results underline the need for a greater focus on the influence of excess nitrogen on butterfly communities (see WallisDeVries 2014).

Three main mechanisms have been proposed to explain the detrimental effects of excess nitrogen on butterflies: loss of host plants with increasing dominance of competitive grasses (Weiss 1999; Haddad et al. 2000; WallisDeVries 2004), deterioration of food plant quality through nutritional imbalances resulting from excess nitrogen (Fischer and Fiedler 2000; Peñuelas et al. 2012; Turlure et al. 2013) and microclimatic cooling in spring within an increasingly productive and dense plant canopy that prevents caterpillars from absorbing solar radiation to attain optimal body temperatures (WallisDeVries and Van Swaay 2006). While analyses of butterfly declines often concern threatened species from rare habitats, a major challenge lies in identifying the causes of recent decline of common species (Van Dyck et al. 2009).

Here, we investigated whether these mechanisms operating under excessive nitrogen availability may explain the dramatic recent decline of a formerly common and spring-developing butterfly, the Wall Brown *Lasiommata megera.* This species has declined sharply over the last two decades, in the Netherlands as well as in other parts of northwestern Europe (Van Swaay et al. 2013). As a first step, we examined the existence of a relation between trends in abundance and nitrogen deposition. We then assessed the relative impact of two possible effects of nitrogen excess: on nutrition and on microclimatic conditions. The third mechanism, loss of host plants, is not a likely explanation of the decline for this polyphagous grass-feeding species. We expected that the loss of warm microclimatic conditions might be driving the decline, as the species has a relatively warm climatic niche among European butterflies (Schweiger et al. 2013). To exclude possible effects of nitrogen-driven nutritional imbalances, we tested the effects of nitrogen addition on larval performance in a greenhouse feeding experiment. We investigated larval habitat choice in relation to microclimatic conditions in its natural environment. In this field study, we compared the larval habitats of *L. megera* with a taxonomically related species, *P. aegeria,* which shares many life history characteristics with *L. megera* but is showing a contrasting trend of range expansion and increasing abundance (Warren et al. 2001; Van Dyck et al. 2009). We expected that the declining *L. megera* would be found in warmer microclimates than the increasing *P. aegeria.*

Methods

Study species

Lasiommata megera (Family: Nymphalidae, subfamily: Satyrinae), the Wall Brown butterfly, has a distribution range from Ireland to West-Asia and Iran, and from Great Britain, South Scandinavia and Ireland to Northern Africa. *L. megera* is a mobile species which occurs in a variety of grassland types with patches of bare soil (Asher et al. 2001; Bos et al. 2006). The species usually flies in two generations; in the Netherlands the peak of the first generation is observed between 11 May and 15 July and the second, more abundant generation between 26 July and 25 August (Bos et al. 2006). In favourable years, a partial third generation is observed. The species hibernates as a half-grown caterpillar (Bink 1992). The caterpillars of *L. megera* feed on a range of perennial grasses including *Brachypodium pinnatum*, *Cynosurus cristatus*, *Elytrigia repens*, *Festuca rubra*, *Dactylis glomerata*, *Holcus lanatus* and *Arrhenatherum elatius* (Ebert & Rennwald 1991; Bink 1992; Asher et al. 2001; Bos et al. 2006). In the study area at least nine species of Poaceae have been recorded as host plants, with *F. rubra* and *D. glomerata* as the most important ones (B. Omon, unpubl. data). Over the period 1992–2013 *L. megera* has shown a decline in abundance of 17.0 % per year in the Netherlands (http://www.vlinderstichting.nl/actueel.php?id=301).

The Speckled Wood *Pararge aegeria* (Nymphalidae, subfamily: Satyrinae) is a close relative of *L. megera* and has a similar distribution, but a slightly cooler climatic niche (Schweiger et al. 2013). The species has an unusually long flight period between April and October, with two to three overlapping generations (Asher et al. 2001; Bos et al. 2006). The first generation emerges over a long period between early April to late June, because both caterpillars and pupae are hibernating (Ebert and Rennwald 1991), but with a predominance of overwintering pupae (Bink 1992). *P. aegeria* is more restricted to semi-open woodland habitats than the grassland species *L. megera* (Shreeve 1986; Ebert & Rennwald 1991). However, the species can also reproduce in more open habitats, and is increasingly found in agricultural landscapes (Merckx and Van Dyck 2006). The caterpillars feed on many different grass species and even *Carex* species (Ebert and Rennwald 1991; Shreeve 1986). In the study area at least 11 species of Poaceae serve as host plants of *P. aegeria* (B. Omon, unpubl. data). Since 1992 *P. aegeria* has increased in abundance by 8.6 % per year in the Netherlands (http://www.vlinderstichting.nl/actueel.php?id=301).

Trend analysis

Changes in annual abundance of *L. megera* were analysed in relation to nitrogen deposition using transect data from the Dutch Butterfly Monitoring Scheme. In this scheme, butterflies are counted weekly along fixed transects under suitable weather conditions, following the method described by Pollard and Yates (1993). Data are converted to annual indices as explained in Van Swaay et al. (2002). Only transects with a minimum of 8 years of monitoring data were included in the analysis, yielding a dataset of 202 transects for the period 1990–2010. Only data on the spring generation were analysed, because spring numbers are less variable and, therefore, more reliable in trend analysis (Van Strien et al. 1997).

Monitoring transects were characterized according to eight physico-geographical regions (Berendsen 2005): coastal dunes, calcareous hills, northern Pleistocene sandy soils, central Pleistocene sandy soils, southern Pleistocene sandy soils, lowland peats, riverine clay soils and marine clay soils.

Nitrogen deposition data were obtained as critical load exceedance (CLE) estimates for characteristic semi-natural grasslands, i.e. the positive values for the deposition of NO_x and NH_y minus the critical load for nitrogen tolerance of the characteristic semi-natural grassland types in a specific area (van Hinsberg et al. 2008; Velders et al. 2009). Data on average CLE during the period 1990–2010 were obtained for each transect at 1 × 1 km resolution from the National Institute for Public Health and the Environment (RIVM). Deposition values above the critical load induce changes in productivity and composition of plant communities. Critical load values differ between soil types (Bobbink and Hettelingh 2011). Hence, using CLE data provides a means to account for differences in abiotic conditions regarding the impact of nitrogen on plant communities. For the present study locations average critical load values varied from 1,258 to 1,355 mol N ha^{-1} $year^{-1}$ for the coastal dunes and Pleistocene sandy soils, through intermediate values of 1,516 N ha^{-1} $year^{-1}$ for the calcareous hills and 1,599 mol N ha^{-1} $year^{-1}$ for lowland peats to high values of 2,065 mol N ha^{-1} $year^{-1}$ on riverine clays and 2,384 mol N ha^{-1} $year^{-1}$ on marine clay soils. Average nitrogen deposition varied from a lower value of 1,501 mol N ha^{-1} $year^{-1}$ in the coastal dunes, through intermediate values of 2,006 mol N ha^{-1} $year^{-1}$ for lowland peats and 2,093 mol N ha^{-1} $year^{-1}$ for the northern Pleistocene sandy soils to higher values of 2,236–2,842 mol N ha^{-1} $year^{-1}$ in the other soil regions. CLE values are given in Fig. 1.

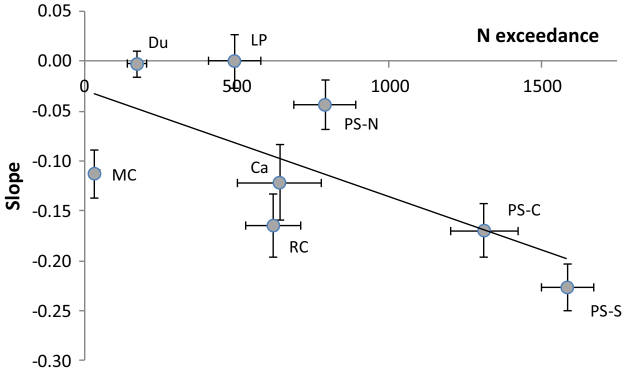

Fig. 1 Trend slopes for the period 1990–2010 in the annual presence of the spring generation of *Lasiommata megera* butterflies on monitoring transects in eight physico-geographical regions of the Netherlands, in relation to the critical load exceedance for nitrogen deposition (in mol N ha^{-1} $year^{-1}$; *error bars* denote standard errors; the *line* gives the linear trend $r = -0.68$). Abbreviations of physico-geographical regions: *Ca* calcareous soils, *Du* dunes, *LP* lowland peat, *PS-N* northern Pleistocene sandy soils, *PS-C* central Pleistocene sandy soils, *PS-S* southern Pleistocene sandy soils, *MC* marine clay soils, *RC* riverine clay soils

Larval feeding experiment

For the larval experiment, we subjected *L. megera* caterpillars to three different nitrogen (N) fertilization levels and two water treatments in a replicated block design, resulting in six treatments with five block replicates. A drought treatment was added to a normal water regime in order to evaluate possible effects of water shortage, which could be a limiting factor on sandy soils.

The grass species *D. glomerata* was used as host plant. Plants had been sown in the previous year and tillers were ca 0.15 m high at the start of the experiment. Ten tillers were planted in pots of 27 cm diameter filled with nutrient-poor sandy substrate on May 1st 2012. All plants were initially fertilized with NH_4NO_3 and KCl dissolved in water (60 and 60 mg K dm^{-2}). In the first weeks after planting, all plants received sufficient water. Generative sprouts were removed from the plants during the whole experiment to increase vegetative growth of the plants. After 3 weeks, plants were subjected to three different N fertilization treatments (control: no N added, medium: 40 mg N dm^{-2}, high: 80 mg N dm^{-2}), applied weekly; additional KCl was added occasionally to all treatments. Water regimes simulated a normal precipitation regime for the Netherlands and a drought regime where plants received half the normal amount of water.

Eggs were obtained in the last week of May and early June from four wild-caught females from a lowland peat population near Zegveld (52°08′N, 4°39′E). Newly hatched larvae were directly placed on the experimental plants, with each pot receiving between one and six caterpillars.

Caterpillar placement was carried out simultaneously on all treatments within each block and different blocks were supplied on subsequent days. Plants were covered with nets to prevent larvae from escaping. Development times were recorded until pupation and adult eclosion. Pupae were weighed and adult weight and forewing length were measured after eclosion. Butterflies were subsequently released.

Leaf samples were collected from each pot during larval development (June 19th) and at the end of the experiment (July 9th). Samples were weighed before and after drying at 70 °C and processed for chemical analysis of N, P, K, Ca, Mg and Na concentrations. Results for the two sampling dates were pooled. For comparison, samples of *D. glomerata* were also taken from 12 monitoring sites where *L. megera* was or had been recorded.

Field study

The field study was carried out in the Krimpenerwaard, a lowland peat area near Gouda (51°58′N, 4°40′E). In this area both study species, *L. megera* and *P. aegeria,* are common. Ten study plots of 2,500 m^2 were selected in 2013 on locations where either or both species had been frequently recorded during the last 5 years. Distances between plots were >700 m. In March and April, all plots were searched under sunny weather and temperatures >10 °C for the presence of caterpillars of the study species. Searching time was fixed at 6 h per plot, with half the time spent searching randomly and half the time searching on potentially suitable locations at warm or sheltered places, such as the vicinity of vertical structures such as walls, stones, tree trunks and posts that give shelter or accumulate solar heat (Stoutjesdijk and Barkman 1992; Dennis 2004).

Caterpillar locations were paired with a control location without caterpillars at 10 m distance in a random direction. At both locations we established a plot of 1×1 m^2. In each plot we estimated the horizontal cover of the total herb layer, grasses and bare ground. A central subplot of 20×20 cm^2 was used to record maximum vegetation height (cm). The percentage of green material in the vegetation was assessed from a grid of 10×10 points plotted on a picture of the subplot. In order to assess the possible role of shelter, the distance to the nearest vertical structure was measured in both caterpillar and control locations.

Thermal measurements were taken between 11 and 15 h on a sunny day, with each pair of caterpillar location and random control being surveyed within a 5 min interval. We recorded air temperature and relative humidity at plant canopy level and, as a reference point at breast height in the shade, using a SPER 800014 thermometer. In the central subplot, we recorded air temperature and relative humidity close to the caterpillar and at the same height in the control plot. Further measurements were obtained on the effect of solar radiation on surface temperatures, using a FLIR B2 infrared thermacam image of the central subplot. Images were analysed to record the surface temperature at the caterpillar (or corresponding control location) as well as the average, minimum and maximum temperatures in the entire subplot and again in a smaller area of 10×10 cm^2.

Statistical analysis

As the distribution of abundance indices showed strong overdispersion, abundance indices were transformed into binary presence-absence data. The dependence of occupancy trend slopes for individual transects on nitrogen CLE was analysed by logistic regression.

For the larval feeding experiment, treatment effects on plant nutrient concentrations, larval development time and pupal weight were pooled for each pot and analysed by standard least square analysis in Generalised Linear Models (GLM) with block as a random factor, N and water treatments and their interaction as fixed effects; variation in egg production and timing between females did not allow a proper analysis of possible influences of maternal origin. For the butterfly data, we used the fraction of females in each pot as a covariate to account for the longer development time and larger size of females. Data were checked for normality of residuals and homogeneity of variances. In addition, we calculated N:P ratios as an indication of possible imbalance in stoichiometry (Peñuelas et al. 2012; Tao and Hunter 2012) and carried out linear regression analysis to explain development time and pupal weight as a function of plant N:P ratio, again with the fraction of females in each pot as a covariate.

Data from the field study were analysed by Principal Component Analysis (PCA) to obtain the main independent components of associated variables. Temperature and humidity data were analysed as differences of plot measurements with the reference values at breast height to control for time-related variation in these variables. Differences of main variables and average principal component values between the four plot categories (*L.megera*-caterpillar, *L.megera*-control, *P. aegeria*-caterpillar, *P. aegeria*-control) were analysed in a oneway ANOVA or a Kruskal–Wallis test in the case of non-normal distributions. All statistical analyses were carried out in JMP 5.0.1 (Sall et al. 2005).

Results

Trend analysis

The trend slope in occupancy of *L. megera* on monitoring transects over the period 1990–2010 was significantly lower with increasing levels of nitrogen CLE (coefficient—0.00027 ± 0.00005, $F_{1,171} = 26.7$, $p < 0.0001$). This was also apparent when comparing the trend slopes in relation to nitrogen CLE for the different physic-geographical regions (Fig. 1): the central and southern Pleistocene sandy soil regions showed the greatest decline at the highest nitrogen exceedance levels, whereas nitrogen exceedance was much lower in the dunes and lowland peat region, where *L. megera* shows a stable occupancy.

Larval feeding experiment

Nitrogen addition significantly increased N content and slightly lowered P concentrations, resulting in significantly higher N:P ratios in N-fertilized *D. glomerata* host plants (Table 1). Concentrations of K and Mg were not affected by N addition, but were augmented under water shortage. Water content was a little lower in drought-affected plants. Plant concentrations of Na (0.38 ± 0.02 %) and Ca (0.90 ± 0.03 %) were not significantly affected by the treatments. Leaf samples collected on monitoring transects showed a wide range of concentrations for N (2.28 ± 0.24 %; range 1.45–4.30 %) and P (0.30 ± 0.03 %; range 0.12–0.56 %), which encompassed the range in the experimental treatments. However, the N:P ratio of the high N treatment (15.0 and 15.9; Table 1) substantially exceeded the values found in the field (8.2 ± 0.7; range 3.3–11.8).

Larval survival did not differ between treatments ($F_{6,23} = 0.82$; $p = 0.56$), with 96 out of 138 first instar larvae successfully completing their development until adult eclosion. Mortality occurred mainly during the larval stage: pupal mortality was limited to six individuals. The sex ratio of eclosed butterflies was 0.78:1 (male:female), which did not deviate significantly from the expected 1:1 ratio.

Larval development time was significantly shortened by N addition ($F_{2,18} = 8.83$, $p = 0.0021$), but not by water regime or the interaction between N and water treatments ($p > 0.25$) (Fig. 2a). The difference between treatments could be largely attributed to the caterpillar stage, as the pupal development time (mean 11.6 ± 1.2 days) was unaffected by treatments ($F_{6,23} = 0.96$; $p = 0.51$). Total development time decreased significantly with increasing plant N:P ratio ($F_{1,26} = 12.55$; $p = 0.0015$).

Pupal weights were raised by N addition ($F_{2,19} = 5.16$, $p = 0.0162$) and tended to be higher under the drought treatment ($F_{1,19} = 3.43$, $p = 0.08$), but were not affected by the interaction between treatments ($p = 0.90$) (Fig. 2b). Adult weight and forewing length were significantly correlated with pupal weight ($r = 0.76$ and $r = 0.70$, respectively, $p < 0.001$ for both). Pupal weights grew significantly heavier with increasing plant N:P ratio ($F_{1,27} = 9.98$; $p = 0.0039$).

Larval microhabitat

Three principal components explained 71.3 % of the variation in the 16 variables used to quantify larval microhabitat conditions for *L. megera* (n = 61) and *P. aegeria* (n = 19) in the field (Table 2). The first component explained 31.4 % of the variation and was mainly positively correlated with temperature range, average temperature and maximum temperatures at both the 10 × 10 and 20 × 20 cm^2 scales. The second component explained 17.0 % of the variation and mainly reflected vegetation and grass cover, vegetation height and relative humidity; higher values of this component indicate low cover, short vegetation and low humidity. The third component was determined by the minimum temperatures at both scales and by the distance to the nearest vertical structure. It explained 22.9 % and shows high values at low minimum temperatures and short distances to vertical structures.

All three components were significantly affected by the variation between plots depending on study species and caterpillar presence, but all components showed a different

Table 1 Average chemical composition and water content (% dry matter ± SE) of *Dactylis glomerata* host plants for the three nitrogen levels and the two water regimes applied in the larval feeding experiment, with the significance of treatment effects

Water	Normal			Drought			Treatment effect		
Nitrogen	Low	Medium	High	Low	Medium	High	Nitrogen	Water	N × W
N (%)	1.79 ± 0.11	2.49 ± 0.10	3.17 ± 0.14	1.85 ± 0.04	2.52 ± 0.06	3.11 ± 0.10	****	ns	ns
P (%)	0.27 ± 0.01	0.26 ± 0.00	0.21 ± 0.01	0.27 ± 0.00	0.22 ± 0.01	0.22 ± 0.00	****	ns	ns
N:P ratio	6.82 ± 0.62	9.80 ± 0.53	14.96 ± 0.39	6.76 ± 0.19	11.41 ± 0.75	15.90 ± 0.65	****	ns	ns
K (%)	2.06 ± 0.11	2.11 ± 0.07	2.05 ± 0.08	2.43 ± 0.12	2.38 ± 0.17	2.30 ± 0.07	ns	*	ns
Mg (%)	0.28 ± 0.01	0.31 ± 0.01	0.38 ± 0.02	0.37 ± 0.03	0.33 ± 0.01	0.39 ± 0.02	ns	**	ns
Water (%)	63.7 ± 0.7	65.5 ± 0.4	66.4 ± 0.4	63.2 ± 1.2	61.4 ± 0.8	63.3 ± 0.7	ns	***	ns

ns not significant

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$)

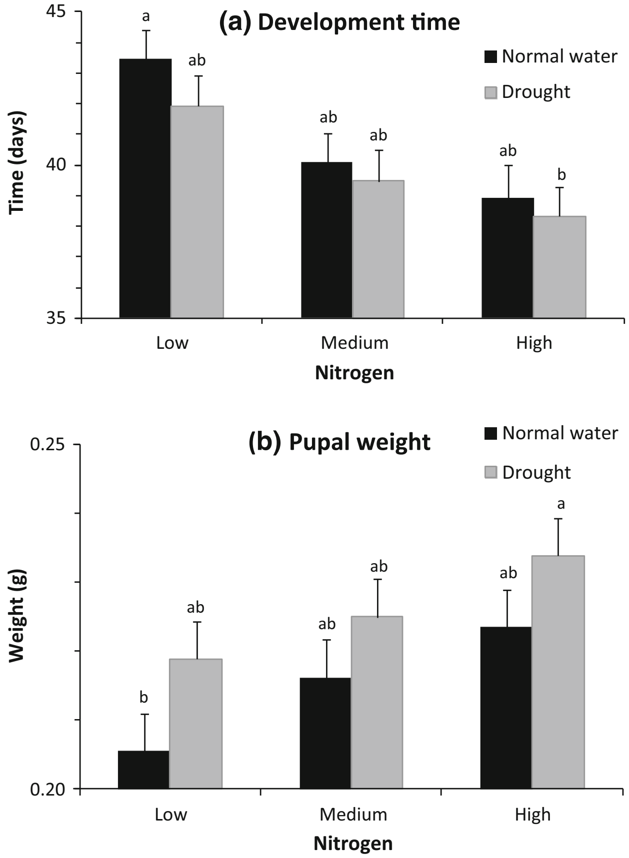

Fig. 2 Effects of nitrogen addition and water regime on larvae of *Lasiommata megera* in the feeding experiment for **a** mean development time from egg to adult and **b** pupal weight (+SE). *Different letters* indicate significant differences between treatment combinations (Tukey HSD, $p < 0.05$)

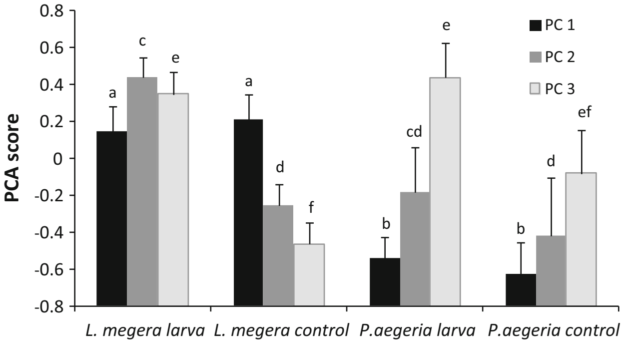

Fig. 3 Average values of the three main principal components (+SE) describing microhabitat conditions at larval and control sites of *Lasiommata megera* and *Pararge aegeria* in the field (see Table 2 for the variables correlated with the principal components). *Different letters* indicate significant differences between groups for each principal component (Tukey HSD $p < 0.05$)

Table 2 Principal components for the characteristics of larval plots for *L. megera* and *P. aegeria* and corresponding control plots in the field, with correlation coefficients of plot variables with the rotated principal component axes

	PCA1	PCA2	PCA3
Eigenvalue	6.62	2.87	1.92
Variance	5.02	2.72	3.66
% explained variance	31.4	17.0	22.9
Cumulative % explained	31.4	48.4	71.3
Air temperature (plot centre)	**0.47**	–0.06	–0.08
Surface temperature (caterpillar)	**0.65**	0.11	–0.63
Surface temperature average 10 × 10 cm	**0.71**	0.12	–0.65
Surface temperature range 10 × 10 cm	**0.93**	0.04	0.13
Surface temperature maximum 10 × 10 cm	**0.92**	0.06	–0.30
Surface temperature average 20 × 20 cm	**0.71**	0.14	–0.66
Surface temperature range 20 × 20 cm	**0.89**	0.12	0.06
Surface temperature maximum 20 × 20 cm	**0.86**	0.10	–0.35
% Herb layer	0.05	**–0.90**	–0.23
% Grass cover	0.02	**–0.79**	–0.24
% Green leaves	–0.12	**–0.84**	–0.03
Maximum vegetation height	–0.25	**–0.45**	0.26
% Relative humidity (plot centre)	–0.01	**–0.51**	0.20
Surface temperature minimum 10 × 10 cm	0.10	0.05	**–0.93**
Surface temperature minimum 20 × 20 cm	0.16	–0.01	**–0.89**
Distance to vertical structure (cm)	0.01	–0.23	**–0.55**

The explained variation of the components concerns the values after rotation of the three main components. The strongest correlations for each variable to the components have been marked in bold (correlations with $r > 0.30$ are significant at $p < 0.0001$)

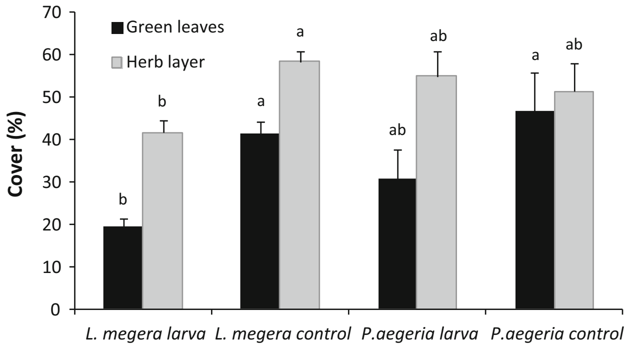

Fig. 4 Average percentage horizontal cover (+SE) of green leaves and herb layer at larval and control sites of *Lasiommata megera* and *Pararge aegeria* in the field. *Different letters* indicate significant differences between groups for each variable (Tukey HSD, $p < 0.05$)

response (Fig. 3). PC1 reflected the differences of species-specific locations, with high values, indicating warmer and more heterogeneous microhabitats, at both larval and control plots of *L. megera* in comparison to those of *P. aegeria* ($F_{3,156} = 6.24$; $p = 0.005$); average and maximum surface temperatures in the 20x20 cm^2 *L. megera* subplots were, respectively, 3.1 and 6.4 °C higher than in *P. aegeria* plots. PC2 was higher at the caterpillar locations of *L. megera* than in all other locations ($F_{3,156} = 7.37$; $p = 0.0001$), reflecting a less green, shorter and more open vegetation structure. Finally, PC3 differed between the larval locations of both species and the control locations ($F_{3,156} = 9.39$; $p < 0.0001$), associated with a short distance to vertical structures and low minimum temperatures.

Analysis of individual variables confirmed the selective occurrence of *L. megera* caterpillars at locations with a lesser proportion of green plant material (Kruskal–Wallis test; $chi^2 = 28.4$, $p < 0.0001$) and a lower vegetation cover (Kruskal–Wallis test; $chi^2 = 19.5$, $p = 0.0004$) (Fig. 4). All caterpillars of both species were found within 50 cm of vertical structures such as walls, stones, tree trunks and posts, whereas in the random control plots the median distance to the nearest vertical structure was much longer: 5 m in *P. aegeria* and even >10 m for *L. megera.* Caterpillars of *L. megera* were found significantly closer to vertical structures (median 2 cm) than those of *P. aegeria* (median 10 cm; Wilcoxon rank sums test, $S = 1{,}162$, $Z = 4.46$, $p < 0.0001$).

Discussion

The mechanisms causing loss of insect diversity under excessive nitrogen input still remain largely unresolved, despite growing insights on the impact of nitrogen on broader functional groups (e.g. Haddad et al. 2000; Tylianakis et al. 2008). In this study we used the case of a formerly common, declining and spring-developing (hence thermophilous) species, *L. megera,* to examine the possible role of nitrogen excess in explaining population declines of similar thermophilous species, which represent the majority of the regional butterfly fauna (WallisDeVries and Van Swaay 2006). We focused on three aspects: population trends in relation to nitrogen CLE, larval performance under nitrogen addition to host plants and larval microhabitat characteristics. In the feeding experiment, larval performance was taken as a measure of individual fitness, which should be expected to translate into population dynamics. Larval microhabitats were studied for the declining *L. megera* in comparison to the related but increasing species *P. aegeria* to test whether the former shows a stronger selectivity for warmer microclimates as we expected.

Population trends under nitrogen deposition

We found stronger declines of *L. megera* at higher levels of exceedance above critical loads for nitrogen deposition in predominant semi-natural plant communities. The greatest declines were indeed observed in regions with highest nitrogen deposition. This confirms the effects of nitrogen deposition reported for characteristic butterflies in N-sensitive habitat types by WallisDeVries and Van Swaay (2013). Despite the recognition that nitrogen excess is one of the major drivers of biodiversity loss, good data on the effect of nitrogen deposition on the insect fauna are still extremely scarce (Bobbink and Hettelingh 2011). Our results stress the need to extend such analyses to other regions at a European scale.

Nitrogen impact on plant quality and larval performance

In our feeding experiment with *L. megera,* we investigated the effects of nitrogen addition on larval performance and found no evidence for detrimental effects of nutritional imbalances resulting from nitrogen excess. At high nitrogen levels and increased N:P ratios, larval developmental times were shortened, pupal weight increased and was significantly correlated with adult weight and forewing length, whereas survival remained unaffected. In females, pupal and adult weights can be taken as a proxy for potential fecundity (Mevi-Schütz and Erhardt 2003) and this is also true for wing length (Karlsson and Van Dyck 2009). Thus, in this case the overall effect of nitrogen addition on larval nutrition appeared rather more beneficial than detrimental. This was true despite the fact that our high N treatment caused even higher N:P ratios than found in the field, at levels of >14, which indicate P limitation in plants (Güsewell et al. 2003).

Similar beneficial effects of N addition have been reported in a feeding experiment of *L. megera* on *Molinia caerulea* at different nitrogen levels by Bink and Siepel (1996). In a feeding experiment using caterpillars of the Monarch butterfly *Danaus plexippus*, Tao & Hunter (2012) also observed positive effects of increased N:P ratios on larval performance. These were attributed to a lowered plant defence by latex production due to P limitation. Grasses mainly rely on their coarse cell structure for defence against herbivores and generally do not produce significant amounts of nitrogen-based secondary plant compounds (Munguira et al. 2009). Fischer and Fiedler (2000) did find detrimental effects of nitrogen fertilization on pupal weight and pupal survival for the Sooty Copper butterfly *Lycaena tityrus* feeding on *Rumex acetosa.* In this herb, excess nitrogen might indeed be causing higher levels of anti-herbivorous secondary plant compounds. Thus, the detrimental effects of excess nitrogen on nutritional quality through increased plant defence might be limited to species with dicotyledonous host plants. An alternative explanation was proposed by Nijssen and Siepel (2010), who suggested that especially species from oligotrophic environments, such as heathlands, should be expected to be vulnerable for nutritional imbalances. Our grass-feeding study species *L. megera* is mainly found in rather more productive environments. Hence, nutritional imbalances resulting from nitrogen deposition may be considered less likely for this species.

Drought effects

The greater decline of *L. megera* on the generally dry Pleistocene sandy soils might also be seen as a response to summer drought. Our experimental drought treatment showed no detrimental effects on larval performance, however. It should be noted that an interaction between water and nitrogen treatments occurred, because the less productive plants at the low N treatments used up less water than the fertilized treatments. As a consequence, water availability within the same water regime was greater in the unfertilised than in the fertilised treatments. This may have masked a possible drought effect. However, the results rather show a tendency towards a beneficial than a detrimental effect of drought on pupal weight. Indeed, trend analyses for *L. megera* in relation to weather conditions have not revealed that drought effects are of great importance to explain year-to-year variation in abundance or occupancy (Morecroft et al. 2002; WallisDeVries et al. 2011; E. Klop and B. Omon, unpublished data). Although detrimental interactions of drought stress and excessive nitrogen availability could still play a role (Mattson 1980; Huberty and Denno 2004), we have found no clear indications that these are of great importance in explaining the decline of *L. megera.*

Nitrogen impact on microclimatic conditions

With an abundance of host plants and a lack of support for a nutritional cause, the impact of nitrogen deposition on microclimate remains the main candidate to explain the observed relation between nitrogen exceedance levels and the decline of *L. megera.* The raised levels of green plant biomass under excessive nitrogen availability leads to an increase of both shading and green:dead ratios in the vegetation, which should be expected to result in a cooling of microclimatic conditions (Stoutjesdijk and Barkman 1992; WallisDeVries and Van Swaay 2006). For species such as *L. megera,* that hibernate as caterpillars and have high thermal requirements for larval development in spring, microclimatic cooling may well lead to increased mortality and, ultimately, population decline and biodiversity loss of thermophilous species. Such population declines were indeed observed by WallisDeVries and Van Swaay (2006) and could be explained by a combination of excess nitrogen and climatic warming, with both factors enhancing plant growth in early spring and reducing the availability of warm microclimates.

Our study provides badly needed field evidence to support the explanation of microclimatic cooling as a result of nitrogen deposition. *L. megera* showed stronger declines at higher levels of nitrogen deposition exceeding the critical loads for the occurring semi-natural plant communities. Caterpillars of *L. megera* were found in microhabitats reflecting warm spring conditions that, as argued above, grow rarer under excessive nitrogen deposition. At the mesoscale of 100 m^2, i.e. the scale of each pair of caterpillar plot and its control, higher average and maximum temperatures were found at *L. megera* locations than at *P. aegeria* locations. At the microscale of 1 m^2, lower vegetation cover and a higher proportion of dead plant material of *L. megera* caterpillar microhabitats in comparison with random control plots also indicated warmer conditions, as found by WallisDeVries (2006). Finally, *L. megera* caterpillars were found closer to the shelter of vertical structures than the caterpillars of *P. aegeria,* although these too were always found within 50 cm of vertical structures.

In general, the locations where *P. aegeria* caterpillars were found did not differ much from random control locations: this was true both for vegetation structure and for thermal characteristics. This tolerance of a wide range of circumstances in its larval habitat may be part of the explanation of the recent expansion of *P. aegeria* in northwestern and northern Europe. Another, perhaps even more important, advantage is that *P. aegeria* is a highly flexible species that may hibernate either as a caterpillar or (even more frequently) as a pupa. This further increases its advantage over *L. megera* (see WallisDeVries 2014).

When considering microclimatic conditions we focused on the daylight period. Satyrine species have a reputation of being predominantly nocturnal during the larval stage (Ebert and Rennwald 1991). However, to our knowledge good field data are lacking from spring conditions, when night temperatures typically fall below the thresholds for foraging activity. Caterpillars from both study species have been frequently observed foraging during spring days: at cool temperatures even in sunny weather and at warmer temperatures under more cloudy conditions (B. Omon, pers. obs.). Moreover, warm microclimatic conditions during the day will even favour food digestion when caterpillars appear inactive (Kingsolver and Woods 1997).

The seeming puzzle that the temperatures were not significantly higher at the caterpillar locations of *L. megera* than at the control locations is probably due to the shading effect of vertical structures (walls, stones, tree trunks and posts). This is shown by the association of their presence with lower minimum temperatures, as seen in PC 3 (Table 2). Thermal measurements were only carried out at a single moment between 11 and 15 h. It may be that a continuous temperature recording during the entire daylight period would show the overall warmer character of the caterpillar locations compared to the controls. However, it should be noted that the shading from a vertical structure is quite different from that of a moist green plant canopy and typically covers only a small area. Thus, the caterpillar would need to move only a short distance to stay in the sun. There might even be advantageous effects of the shade in providing a refuge from desiccation. Moreover, vertical structures may offer additional thermal benefits in reducing wind chill and in supplying radiation heat at the end of the day. Indeed, our results point out the need for a closer study of the benefits of vertical structures on the microclimatic conditions in larval habitats (see Dennis 2004).

Implications for conservation

In a recent analysis of butterfly traits in relation to environmental change, WallisDeVries (2014) stressed the urgency for a better understanding of the impact of nitrogen deposition on habitat conditions. Our study provides field evidence suggesting that the cooling of microclimates by nitrogen deposition may be an important driver of the decline of *L. megera*. This supports the microclimatic cooling hypothesis advanced by WallisDeVries and Van Swaay (2006) on the basis of an analysis of butterfly population trends from northwestern Europe. This hypothesis also may explain the failure of many species to expand into new climatically suitable niches under climatic warming (Oliver et al. 2012b).

The vulnerability of butterflies for microclimatic cooling is thought to be especially strong for slow-developing species that hibernate as eggs or caterpillars (WallisDeVries 2014). However, the present study indicates that even a fast-developing species such as *L. megera* may suffer from microclimatic cooling during its larval development in spring. This suggests that the problem may affect a larger number of species than one might expect. Further work is necessary to underpin the mechanism of microclimatic cooling under nitrogen deposition and its impacts on larval performance. Our findings also highlight the possible function of vertical structures and small-scale heterogeneity in enhancing suitable microclimates for insect larvae. Ultimately, increasing our understanding of microhabitat conditions for larval development will provide valuable input for effective conservation management.

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