






ORIGINAL ARTICLE

The Ecology and Conservation of Urban Insects

Disentangling how urbanisation influences moth diversity in grasslands

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Abstract

1. Urban areas have profound impacts on local species diversity and composition through a set of intertwined changes in the environment. As the world is rapidly urbanising while simultaneously facing a biodiversity crisis, a better understanding of how urbanisation influences biodiversity is necessary.
2. To test if and how urbanisation influences moth diversity and whether urbanisation is acting directly or indirectly via urbanisation-induced increased habitat isolation, smaller habitat area, higher light pollution and increased mowing intensity, we sampled moths with light trapping in 20 grasslands in the urban core of the city of Darmstadt (southwestern Germany) and 20 grasslands in the surrounding area.
3. Moth abundance and diversity decreased with increasing urbanisation. Smaller habitat area and high mowing intensity reduced moth abundance, while other environmental variables including isolation and light pollution had only indirect effects. High levels of urbanisation were associated with reduced moth abundance, which in turn drove declines in diversity. Urbanised sites favoured generalist species and differed in species composition compared to sites in the surrounding.
4. The results show that urbanisation is directly reducing moth abundance and diversity in cities. The negative effect of urbanisation is further attenuated by habitat fragmentation and high mowing intensity, which are both known drivers of biodiversity decline in urban areas and beyond.
5. While urbanisation itself is often irreversible, reducing mowing intensity and preserving larger grassland areas could facilitate moths and other taxa in and around cities.

KEYWORDS

artificial light at night, Lepidoptera, light trapping, moths, mowing, species communities, species diversity, urban ecology

INTRODUCTION

Urbanisation is a major threat to biodiversity (e.g., Grimm et al., 2008; Seto et al., 2012), not least because cities will inevitably expand as

urban populations are projected to further increase (United Nations, 2019). City expansion rates could even exceed human population growth rates (Angel et al., 2011) since there are multiple socio-economic factors that can drive expansion of cities into lower density

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areas (referred to as urban sprawl). Especially in Europe, urban sprawl is increasing despite slowing population growth (Haase et al., 2013; Rienow et al., 2014).

As urbanisation proceeds, more and more ecological communities are subjected to the associated alterations of habitats that include habitat loss, habitat fragmentation and habitat degradation (Elmqvist et al., 2013; Wagner, 2020). Urbanisation primarily causes habitat loss through construction and ground sealing. This form of habitat loss directly results in habitat fragmentation, which causes habitat isolation in addition to a reduction in habitat size (Liu et al., 2016). Often, the remaining habitats available in cities are further degraded and differ in structure, complexity and resource availability from natural habitats (Beninde et al., 2015), making them unsuitable for many species. For example, common urban habitat types include green spaces that are frequently mown (Hedblom et al., 2017; Robbins & Birkenholtz, 2003). Mortality due to mowing can be high and mowing reduces the availability of suitable sites for larval development in many insects. Thus, only a few species can reproduce in intensively mown urban green spaces (Wintergerst et al., 2021). Furthermore, urban areas tend to be more affected by air pollution and light pollution than surrounding habitats adjacent to cities (Knop et al., 2017; van Langevelde et al., 2018). Air pollution can lead to eutrophication or soil acidification (Koolen & Rothenberg, 2019; Lovett et al., 2009), while light pollution by artificial light at night (ALAN) has been shown to disrupt life cycles, especially of nocturnal animals (Boyes et al., 2021; Longcore & Rich, 2004; van Geffen et al., 2014). Cities are also known to accumulate heat, which is described as the urban heat island effect that can exacerbate background levels of climate warming (Deilami et al., 2018).

Urbanisation changes species communities and has been linked to biodiversity loss in many animal and plant taxa (Aronson et al., 2014; McKinney, 2002, 2008). For insects, increasing urbanisation usually reduces abundance and species richness, for example, in bees and hoverflies (Bates et al., 2011), coprophilous beetles (Foster et al., 2020) and some orthopteran groups (Penone et al., 2013). In this regard, urbanisation is likely contributing to the global insect decline (Wagner, 2020), as several drivers of decline interact in urban areas (Wagner et al., 2021). Multiple studies indicate that Lepidoptera are also negatively affected by urbanisation, especially in Europe (Fenoglio et al., 2020; Merckx & Van Dyck, 2019; Ramírez-Restrepo & MacGregor-Fors, 2017). Loss of Lepidoptera species with urbanisation could be related to habitat isolation, reduced habitat area, ground sealing, the urban heat island effect, light pollution, high nitrogen deposition, invasive plant species, a high proportion of non-native plants and ubiquitous pesticide use, among further drivers (e.g., Fenoglio et al., 2021; Wagner et al., 2021). However, how the management (such as mowing intensity) of the remaining habitats (usually green spaces) in urban areas influences Lepidoptera has not been studied in detail. Despite the predominantly negative impacts of urbanisation, suitable management of green spaces is one opportunity to enrich biodiversity in urban areas (Beninde et al., 2015; Elmqvist et al., 2013; Parris et al., 2018). Cities could, for example, create additional green spaces where currently sealed area dominates or improve

habitat quality of existing green spaces to attenuate the effects of urbanisation.

The effects that urban areas impose on species communities are intertwined and rarely independent. It is, therefore, important to disentangle whether the influences of urbanisation on species abundance and diversity are direct or mediated indirectly via changes in habitat properties, especially because multiple drivers may interact in complex ways (Fenoglio et al., 2021; Wagner et al., 2021), making it difficult to determine which effects of urbanisation contribute most to biodiversity decline. It is, for example, often unclear whether increased light pollution is the main driver of biodiversity loss in nocturnal animals or whether it is only correlated with other negative influences of urbanisation such as habitat loss or isolation. Moths (nocturnal Lepidoptera) are well suited to study the direct and indirect influences of urbanisation (Bachand et al., 2014; Rákósy & Schmitt, 2011) as they are a species-rich group with many ecological specialists that react quickly to changing environmental conditions. Despite this, relatively few studies have investigated the influence of urbanisation on moth communities in Europe (Franzén et al., 2020; Merckx et al., 2018; Merckx & van Dyck, 2019). Without a solid understanding of the outcomes of urbanisation, it will remain unclear how biodiversity of moths in cities can be best promoted and how the negative consequences of urbanisation can be mitigated in conservation schemes. Since moths are important nocturnal pollinators (Walton et al., 2020) and are a food source for many vertebrate species, this is also of importance for preserving biodiversity in general.

We investigated variation in moth abundance, species richness and diversity between an urban area and its surroundings to uncover the main drivers influencing moth diversity. We tested how urbanisation influences moths and whether moth communities are related to habitat area, habitat isolation, management intensity (i.e., mowing), light pollution or a combination of these variables. We hypothesized that small habitat area, high mowing intensity and low plant species richness would have the strongest negative influence on moth abundance, species richness and diversity. Light pollution and isolation were expected to also be negative for moth communities, but to a comparatively lower extent, because the mortality related to ALAN is in cities presumably less severe compared with the mortality inflicted by mowing and the constraints by low habitat availability.

MATERIALS AND METHODS

Study sites

In and around the city of Darmstadt, situated in the southern part of the German federal state Hesse, 40 grassland sites were selected, 20 within the urban core (green spaces) of the city and 20 in the surrounding area (Figure 1). A site was considered within the city if it was surrounded on at least three sides by densely built-up, sealed areas and had a coverage of sealed area within a 500 m buffer zone of at least 25% (note that one nature reserve close to the boundary of the city had a coverage of over 25% but was not treated as a city site). All

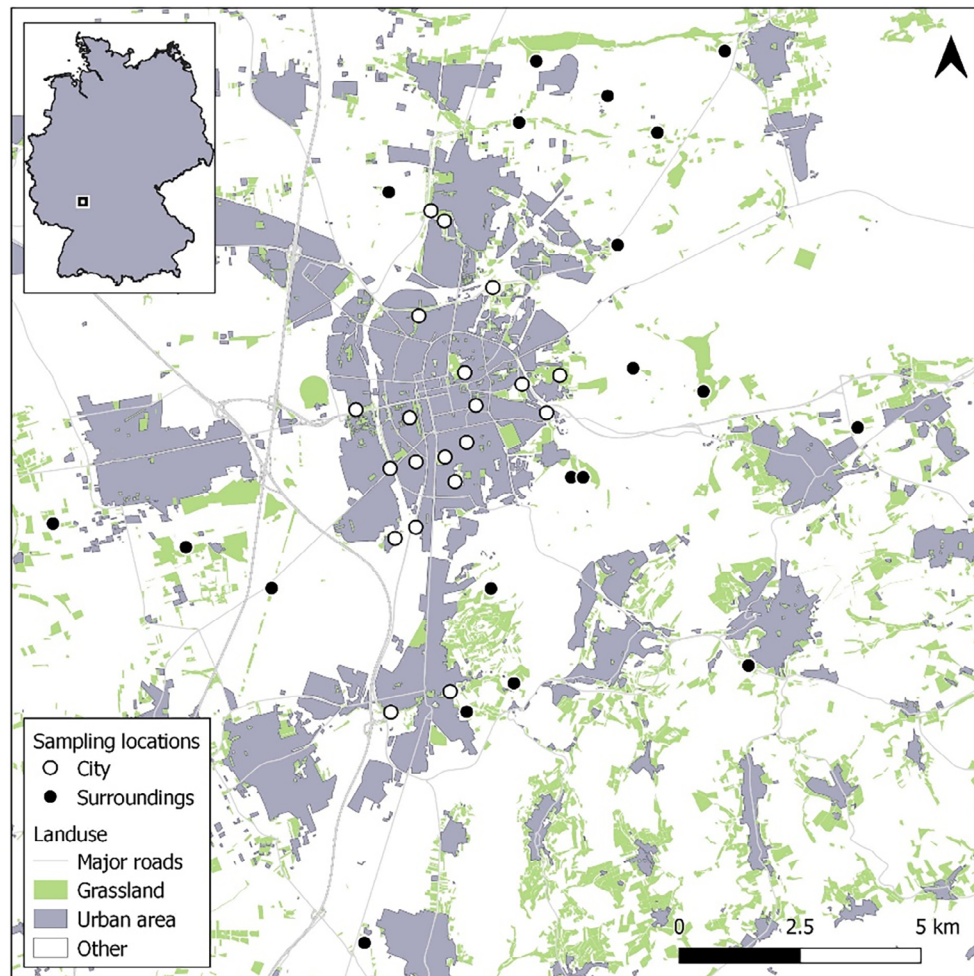


FIGURE 1 Map of the study sites showing sampling locations in the city and the surrounding areas of Darmstadt, Germany. Other land use includes arable land and forest.

grasslands had largely homogenous vegetation, dominated by grasses and herbaceous plants, and were managed in the same way over the entire area of each site. Sites were separated by at least 200 m. Climate in the area is maritime but with subcontinental influence. With a mean annual temperature of 10.6°C (mean annual precipitation 628 mm), Darmstadt is located in one of the warmest regions in Germany (Deutscher Wetterdienst, www.dwd.de).

Moth data

Moths were sampled for one night per study site between 20 June and 8 July 2022. This is within the flight period of a large proportion of the moth species in Germany, where species richness in light traps correlates with temperature and peaks in June and July (Jonason et al., 2014). In the study area, June and July are the warmest months and the sampling period was chosen around new moon (29 June 2022), limiting the influence of moonlight. At each site, one standardised light trap (bucket trap type with Phillips 18 W T8 black light bulb) was suspended from a tree at 0.5–1 m above ground. At two

sites lacking suitable trees, traps were placed directly onto the ground. For all traps, it was made sure that they were not obstructed by vegetation (traps were not directly adjacent to trunks or foliage). Using a programmed timer, traps were operating continuously from 22:30 in the evening until 4:30 in the morning, which roughly covered the time between civil twilight at the sampling dates. Sampling was restricted to nights with calm weather and temperatures above 10°C. As flight activity of moths is low in cool nights (Jonason et al., 2014), sampling was repeated in a subsequent night if the minimum temperature fell below 10°C (recorded on site with HOBO Pendant Temperature Data Loggers UA-001), with the exception of one site where the trap malfunctioned during the repeat. The number of sampled sites per night ranged from 1 to 8 and possible variation among nights (that may, e.g., be caused by differences in temperature, wind speed, humidity or cloud cover) was considered in the statistical analyses. Traps were emptied in the morning, and moths were directly identified by the first author and released on site if possible.

Only macro moths (traditionally comprised of the superfamilies Hepialoidea, Cossioidea, Lasiocampoidea, Bombycoidea, Drepanoidea, Geometroidea and Noctuoidea) were included for feasibility of

identification. In an effort to minimise the environmental impact of the study, only species that cannot be reliably identified in the field were collected. For all other species, only one or two voucher specimens were taken (deposited in the collection of the Ecological Networks Group at TU Darmstadt). We follow the taxonomy proposed by Steiner et al. (2014). As specimens in the species complexes *Diachrysis chrysitis/stenochrysis*, *Euxoa tritici/nigrofusca/eruta* and *Noctua janthina/janthe/tertia* cannot reliably be separated morphologically, we conservatively regard all individuals within each species complex as one species. Based on their larval food sources (Ebert, 1991–2003; Steiner et al., 2014), all species were considered as either specialists (monophagous or oligophagous; that is, host plants from three or fewer plant families) or generalists (polyphagous). Species whose larval host plants include trees and shrubs were considered woody feeders.

Even though only grassland type habitats were sampled, ‘ecotone’ or ‘forest’ moth species were not excluded, because while grassland species may be most affected by urbanisation induced habitat alterations (such as high mowing intensity), ecotones are often affected as well (e.g., Tölgyesi et al., 2018). Since the attraction radius of light traps was shown to be well below 100 m, often even just 10 m (Merckx & Slade, 2014; Truxa & Fiedler, 2012), species sampled by light trapping also had to be at least dwelling in the ecotone bordering the grassland or the grassland itself. Thus, it was assumed that the species found would be influenced in some way by grassland management, for example, through adults using nectar resources in grasslands.

Environmental data

The area of each site was calculated from polygons in QGIS (Version 3.26, QGIS Development Team, 2022). Around each polygon, a buffer of 500 m width was taken, which ensures that any analysis of the surrounding area is not biased by the size of the site as opposed to a simple radius based on the centre of each site. The degree of urbanisation for each site was measured as the proportion of sealed area using QGIS based on OpenStreetMap data (OpenStreetMap Contributors, 2022). In our study area, sealed area data in OpenStreetMap are accurate, as validated by haphazard ground-truthing for some of the sites. The 500 m buffer reflects a rough average dispersal distance for most non-migrating moth species (Merckx et al., 2009, 2010; Slade et al., 2013). To calculate the degree of isolation from surrounding grasslands, a 500 m buffer was likewise considered and one minus the proportion of area covered by grassland was calculated (excluding sports fields and lawns in private gardens as the former only provide suitable breeding habitat for a very small number of moth species and the latter contributed only little to grassland cover). The degree of light pollution by ALAN for each site was extracted from the world atlas of artificial night sky brightness (spatial resolution 15 arcsec, available from <https://www.lightpollutionmap.info>), using the zenith sky brightness in mcd/m^2 (Falchi et al., 2016).

For each site, information on mowing intensity, that is, the number of cuts per year, was obtained from local authorities and land owners or tenants. If a site was in the past mown with variable frequencies per year, the mean was taken. Six sites were only grazed but not mown (one in the city and five in the surrounding). As grazing was by sheep, light and not permanent, and as light grazing opposed to even a single mowing does not reduce moth diversity (Mangels et al., 2017), mowing intensity for grazed sites was set to 0. Park lawns were mown regularly but without a known fixed number of cuts. For sites that were likely mown at least 10 times per year, mowing intensity was set to 10. To assess plant species richness, one 20 m² area of continuous grassland vegetation (excluding ecotones, shrubs and trees) per site was surveyed for 20 min and all plant species present were identified to species level (with aggregates conservatively treated as a single species). Correlation coefficients among environmental variables are shown in Figure S1.

Data analyses

All analyses were conducted in R 4.1.2 (<https://www.R-project.org>) applying the packages ‘ape 5.7.1’ (Paradis & Schliep, 2019), ‘car 3.1.2’ (Fox & Weisberg, 2019), ‘indicpecies 1.7.14’ (De Caceres & Legendre, 2009), ‘lme4 1.1.34’ (Bates et al., 2015), ‘lmerTest 3.1.3’ (Kuznetsova et al., 2017), ‘MuMIn 1.43.17’ (Barton, 2020), ‘piecewiseSEM 2.3.0’ (Lefcheck, 2016), and ‘vegan 2.5.7’ (Oksanen et al., 2020). To assess sampling efficiency, sample-based species accumulation curves (10,000 permutations) and first-order jackknife species richness estimators were calculated for the full data set and separately for study sites in the city and in the surrounding (‘vegan’). Moth diversity was expressed as the effective number of species (Jost, 2006) by calculating e^H (‘vegan’). As moth species richness and moth diversity were highly correlated with moth abundance (Spearman’s $\rho = 0.947$ and 0.889 , Figure S2), we also rarefied species richness to a standardised number of five individuals (‘vegan’).

First, to test for differences in the environmental variables (urbanisation, sky brightness, habitat area, habitat isolation, plant species richness and mowing intensity) and Julian day (the continuous day of the year) between sites in the city and the surrounding, we used univariate (generalised) linear (Gaussian errors) mixed-effects models (‘lme4’) with Satterthwaite-approximated degrees of freedom (‘lmerTest’). Habitat area was log-transformed and mowing intensity was square-root-transformed to increase normality. For the count data on plant species richness, a Poisson model was used with overdispersion accounted for by including an observation-level random intercept following Harrison (2014). Sampling date was included as random intercept to account for variation in conditions among individual trapping nights. Using the same univariate approach, differences in the response variables moth abundance, moth species richness, moth diversity, rarefied moth species richness, specialist species richness and woody feeder species richness were assessed (‘lme4’, ‘lmerTest’). For the count data on moth abundance, moth species richness,

specialist species richness and woody feeder species richness, Poisson models (including an observation-level random intercept) were used.

Next, to disentangle the various and interrelated (Figure S1) influences of urbanisation on moth communities, we calculated multiple (generalised) mixed-effects models for the same response variables ('lme4', 'lmerTest'). Linear models were used for moth diversity and rarefied moth species richness, while Poisson models were used for all count data. The random effects structure was for each response variable identical to the univariate models above. As fixed effects, each model contained the environmental variables urbanisation, sky brightness, habitat area (log-transformed), habitat isolation, plant species richness, mowing intensity (square-root-transformed) and Julian day that were all scaled to mean = 0 and SD = 1. Because we had indication that for each response variable, there were several candidate models (based on combinations of fixed effects) with a similar fit to the data (Akaike information criterion, $\Delta\text{AICc} < 2$), we applied a model averaging approach ('MuMIn'). For this, one model for each possible combination of the fixed effects was calculated, and all candidate models within two AICc units of the model with the lowest AICc value were averaged by using the full average weighted by each candidate models' relative explanatory weight. Potential collinearity among fixed effects was checked with variance inflation factors ('car'). As variance inflation factors were consistently < 3 in all averaged models, collinearity was unlikely. Residuals were examined for assumptions of normality, which were always met. Spatial autocorrelation of model residuals was assessed with Moran's I coefficients ('ape') and never detected (maximum difference, $I_{\text{obs}} - I_{\text{exp}} = 0.019$).

We used a path model ('piecewiseSEM') to test if the influence of urbanisation on moth diversity is direct or if it is mediated via environmental changes associated with urbanisation and their influence on moth abundance. Thus, the a priori path model contained direct paths of urbanisation, mowing intensity, sky brightness and habitat area on moth diversity (e^H) and moth abundances as well as indirect paths of urbanisation via mowing intensity, sky brightness and habitat area. A path from moth abundance on moth diversity was included to test if urbanisation and related environmental changes are directly influencing moth diversity or if changes in diversity are driven by changes in individual numbers. To check whether the data from two sites where the light trap was placed on the ground are influencing the inference, this path model was recalculated after both sites were excluded. Furthermore, a path model with rarefied moth species richness (i.e., abundance already accounted for) and direct and indirect paths of urbanisation via mowing intensity, sky brightness and habitat area was calculated. Component models of the path models used the same standardised data, error families and random effects structure for the exogenous variables as the multiple (generalised) linear mixed-effects models above. Overall model fit was assessed with d -separation tests (Shipley, 2009).

Because urbanisation is also expected to influence species composition, we used a permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarity to test for differences between study sites in the city and the surroundings ('vegan'). Variation in composition was visualised with two-dimensional non-metric multidimensional scaling (NMDS) using Bray–Curtis

dissimilarity of normalised species abundances ('vegan'). To test how urbanisation, sky brightness, habitat area (log-transformed), habitat isolation, plant species richness, mowing intensity (square-root-transformed) and Julian day relate to species composition, the scores of the first two NMDS-axes were correlated in a post-hoc permutation test with these environmental variables. All multivariate procedures used 10,000 permutations and were calculated in 'vegan'. Finally, to assess if particular moth species are distinctly associated with sites in the city or the surroundings, we applied an indicator species analysis ('indicspecies') based on group-equalised ϕ -coefficients (10,000 permutations).

RESULTS

In total, 518 macro moth individuals belonging to 127 species were collected, of which 58 species occurred in the city and 106 species in the surroundings (Table S1). The most abundant species were *Eilema lurideola* (Zincken, 1817) (81 individuals), *Chiasmia clathrata* (Linnaeus, 1758) (23 individuals), *Agrotis exclamatoris* (Linnaeus, 1758) (18 individuals) and *Hoplodrina octogenaria* (Goeze, 1781) (18 individuals). These species were also most frequent, with *E. lurideola* occurring at 20 sites, *H. octogenaria* at 13 sites, and *C. clathrata* and *A. exclamatoris* at 12 sites. Total abundance per site ranged from 3 to 65 individuals (city: 3–21 and surroundings: 4–65) with a mean of 13.0 ± 12.5 SD individuals (city: 7.2 ± 4.7 and surroundings: 18.7 ± 15.2). Between 3 and 31 species were collected per site (city: 3–13, surroundings: 4–31) with an average of 8.8 ± 6.1 species (city: 5.5 ± 2.8 and surroundings: 12.0 ± 6.9). Total sampling efficiency was 68%, with 187 ± 16 (first-order jackknife \pm SE) species expected. Sampling efficiency for city (64%, 91 ± 11 expected species) and surroundings (66%, 160 ± 18 expected species) was similar (Figure S3).

The environmental variables urbanisation, sky brightness and mowing intensity were on average significantly higher in the city than the surrounding (Table 1; Figure 2). Habitat area was significantly lower in the city while habitat isolation, plant species richness and the Julian day of the sampling did not differ. Moth abundance, species richness, diversity and rarefied species richness, but not specialist and woody feeder species richness, were significantly lower in the city (Table 1; Figure 2).

Moth abundance increased with larger habitat area ($z = 2.840$, $p = 0.005$; Figure 3a) and decreased with mowing intensity ($z = -2.522$, $p = 0.012$; Figure 3b) (Table 2). Moth species richness, in turn, decreased towards more urbanised sites ($z = -2.902$, $p = 0.004$; Figure 3c) and more moth species were found when plant species richness was higher ($z = 2.592$, $p = 0.010$; Figure 3d). Like moth species richness, both moth diversity (Figure 3e) and rarefied moth species richness (Figure 3f) were negatively related to urbanisation (respectively, $t = -2.362$, $p = 0.018$; $t = -3.390$, $p = 0.001$). Specialist species richness decreased with urbanisation ($z = -2.300$, $p = 0.021$) while woody feeder species richness increased with habitat area ($z = 2.581$, $p = 0.010$) (Table 2; Figure S4). Julian day was not retained as a significant fixed effect in any averaged model.

TABLE 1 Summary information of study sites and sampled moth communities, separated for the 20 sites in the city and the 20 sites in the surrounding.

Variable	City	Surrounding	t/z-value	p-value
Urbanisation (%)	0.23–0.93	0.00–0.40	8.473	<0.001
	0.64 ± 0.23	0.07 ± 0.09		
Sky brightness (mcd/m ²)	0.82–2.25	0.60–1.35	6.901	<0.001
	1.58 ± 0.39	0.82 ± 0.16		
Habitat area ^a (ha)	0.21–10.2	1.24–55.05	–4.144	<0.001
	2.04 ± 2.34	8.31 ± 12.66		
Habitat isolation	0.69–0.97	0.73–0.99	0.067	0.947
	0.91 ± 0.07	0.90 ± 0.08		
Plant species richness	4–26	4–36	–0.788	0.430
	15.9 ± 6.5	18.0 ± 8.3		
Mowing intensity ^b	0–10	0–10	2.357	0.024
	3.9 ± 4.2	1.6 ± 2.2		
Julian day	171–186	171–189	0.404	0.686
	178.1 ± 3.6	176.4 ± 4.9		
Moth abundance	3–21	4–65	–3.158	0.002
	7.2 ± 4.7	18.7 ± 15.2		
Moth species richness	3–13	4–31	–3.042	0.002
	5.5 ± 2.8	12.0 ± 6.9		
Moth diversity e ^H	2.8–10.7	4.0–19.7	–2.657	0.012
	5.1 ± 2.3	9.9 ± 4.4		
Rarefied moth species richness	2.9–5.0	3.6–5.0	–3.034	0.004
	3.8 ± 0.8	4.4 ± 0.4		
Specialist species richness	0–6	1–18	–1.423	0.155
	2.1 ± 1.7	4.5 ± 4.4		
Woody feeder species richness	0–5	0–14	–1.331	0.183
	1.3 ± 1.4	3.2 ± 3.5		

Note: Range (min–max) and mean (±SD) are given each. Test statistics refer to univariate (generalised) linear mixed-effects models (with t-values for linear models and z-values for Poisson models on species richness and abundance) testing for differences between city and surrounding (with differences significant at $p < 0.050$ highlighted in bold).

^aLog-transformed before analyses.

^bSquare-root-transformed before analyses.

The hypothesized a priori path model received high statistical support (d -separation test, Fisher's $C = 2.057$, $p = 0.914$; note that p -values > 0.05 indicate significant support) and revealed that influences of urbanisation on moth diversity were indirect and mediated by moth abundance (Table S2; Figure 4). With increasing urbanisation, mowing intensity ($p = 0.005$) and sky brightness ($p < 0.001$) increased while habitat area decreased ($p = 0.023$). However, urbanisation was not directly associated with moth abundance and diversity. Rather, moth abundance decreased with mowing intensity ($p = 0.005$) and increased with habitat area ($p = 0.003$) (but not sky brightness, $p = 0.764$), which led to higher moth diversity at sites with more individuals ($p < 0.001$). These results did qualitatively and quantitatively not change if the two sites at which traps were placed on the ground were excluded (Table S3). The alternative path model with rarefied moth species richness as the endogenous variable (Table S4 and

Figure S5) had similar model fit (d -separation test, Fisher's $C = 2.057$, $p = 0.914$) but indicated no direct or indirect influence of urbanisation on rarefied moth species richness. Differences between marginal (fixed effects) and conditional (fixed and random effects) variances in the path models were small.

City and surroundings differed in moth species composition (PERMANOVA, $F = 2.557$, $p < 0.001$) and sites separated in the NMDS ordination largely according to location (Figure 5). Variation in the NMDS scores was associated with urbanisation (post-hoc permutation test, $p = 0.003$; Table S5) and sky brightness ($p = 0.004$), which aligned approximately to the first NMDS axis, but not with any other variable including Julian day. *Eilema caniola* (Hübner, 1808) was the only species significantly associated with the city (indicator species analysis, $\phi = 0.500$, $p = 0.049$). In contrast, the occurrence of the six species *E. lurideola* ($\phi = 0.816$, $p = 0.001$), *H. octogenaria* ($\phi = 0.624$,

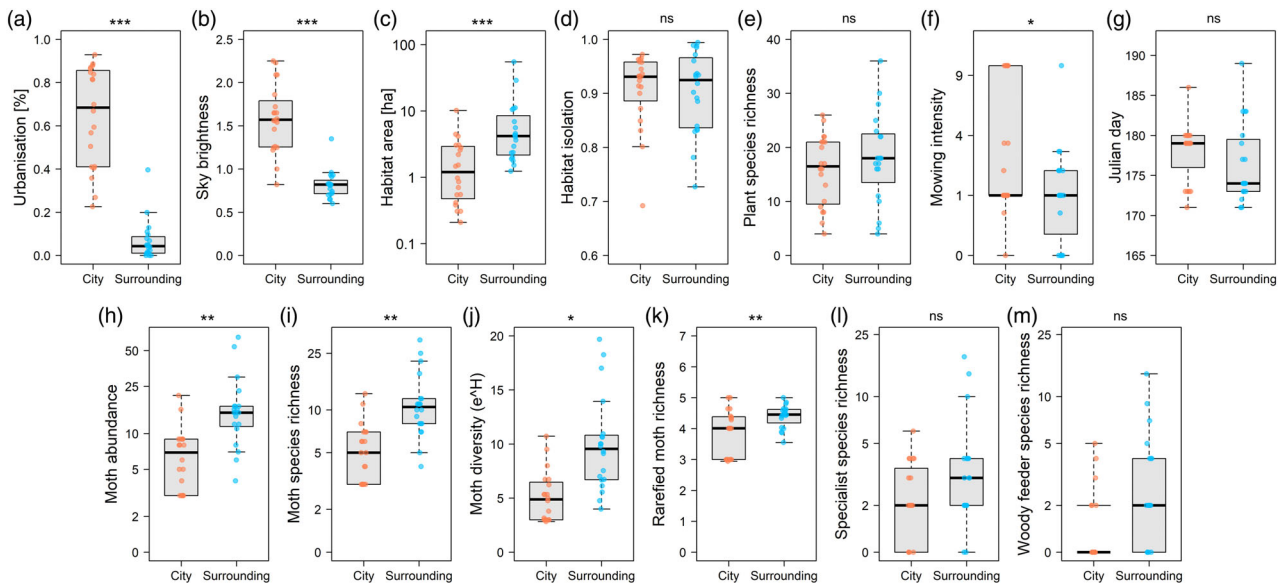


FIGURE 2 Comparison of (a)–(g) environmental variables and (h)–(m) response variables between study sites in the city and surroundings. See Table 1 for statistical parameters (indicated significances are *** $p < 0.001$, ** $p < 0.010$ and * $p < 0.050$).

$p = 0.029$), *Mythimna impura* (Hübner, 1808) ($\varphi = 0.500$, $p = 0.046$), *Mythimna pudorina* (Denis & Schiffermüller, 1775) ($\varphi = 0.500$, $p = 0.046$), *Phalera bucephala* (Linnaeus, 1758) ($\varphi = 0.564$, $p = 0.035$) and *Phragmatobia fuliginosa* (Linnaeus, 1758) ($\varphi = 0.606$, $p = 0.014$) was associated to the surroundings.

DISCUSSION

Influence of urbanisation on moth diversity

Cities with their high proportion of sealed surfaces (i.e., urbanisation) provide only limited habitat for the majority of organisms. In our study, moth abundance, species richness and diversity (e^H) were substantially lower within the city compared with its surrounding areas. This trend agrees with the few other studies on moths focusing on urbanisation in Europe that consistently reported fewer individuals and species within cities in Belgium, Sweden and Germany (Franzén et al., 2020; Merckx & Van Dyck, 2019). Although less pronounced, urbanisation also reduces the populations of butterflies (Bergerot et al., 2011; Kuussaari et al., 2021; Merckx & Van Dyck, 2019), indicating a generally negative influence of urbanisation on Lepidoptera across life histories. The path model revealed that this reduction in diversity was mainly mediated via abundance and by urbanisation-dependent relationships between abundance, habitat area and mowing intensity but not ALAN. This suggests that urban habitats per se support fewer individuals across species by reducing habitat availability and quality ('more individuals' mechanism, Srivastava & Lawton, 1998), which partly explains the lower species richness and diversity of moths in cities. The negative influence of urbanisation on species richness prevailed even after abundance was accounted for

by rarefaction, indicating that, in addition to mechanisms mediated by abundance, urbanisation can also have direct negative relationships with diversity that warrant further study. Even though our sampling was restricted to a single night per site, the recorded number of 518 macromoth individuals is low. Likely, moths have in the study area already substantially declined in the past. In Germany, 'insect decline' is particularly severe for Lepidoptera (Habel et al., 2019; Roth et al., 2021), which is probably one reason why only few individuals occurred.

Influence of urbanisation related to changes in the environment

Urbanisation leads to manifold changes in the environment, which may further accentuate the negative relationship between urban areas and species abundance and diversity. For example, grasslands, such as green spaces in cities, are subjected to high mowing intensity, which reduced moth abundance in our study, likely due to the high mortality particularly of larvae during the mowing process (see Humbert et al., 2010). Furthermore, mowing reduces shelter from predators and induces a sudden change in microclimate that many species may not be able to cope with. Beyond these direct effects, mowing also reduces the availability and diversity of plant species, so that feeding specialists can no longer find suitable host plants for reproduction and adults are subjected to reduced nectar availability (Halbritter et al., 2015). While some mowing or other management is necessary for grassland insects to prevent succession into woodland, high mowing intensity consistently reduces the abundance and species richness of moths and other insects and leads to changes in community composition such as reduction of habitat specialists (Chisté et al., 2016;

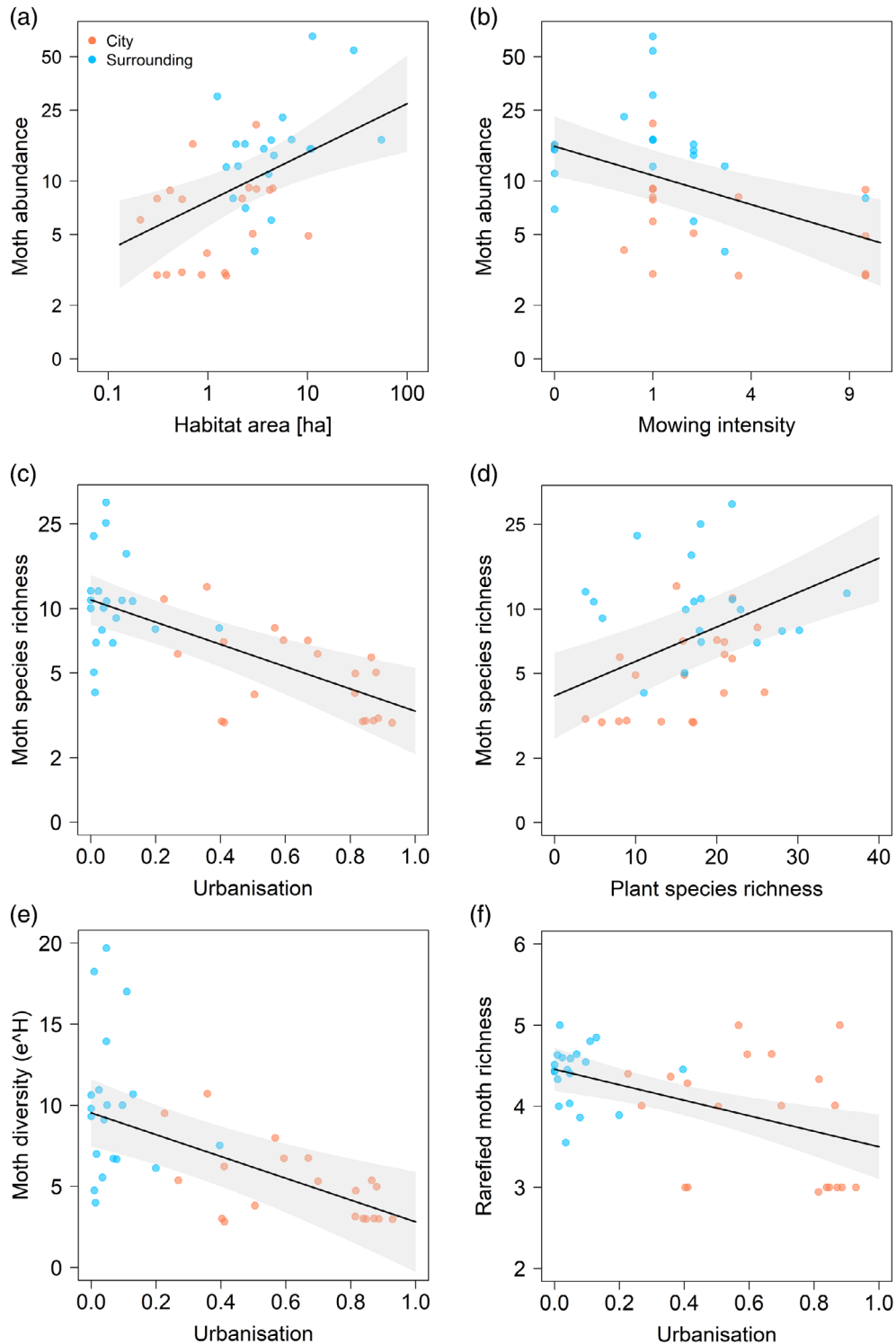


FIGURE 3 Moth abundance (a) increased with larger habitat area but (b) decreased with higher mowing intensity. In turn, moth species richness decreased with (c) urbanisation while it (d) increased with higher plant species richness. (e) Moth diversity and (f) rarefied moth species richness both decreased with urbanisation. Regression lines (95% CI as shaded polygons) indicate the predictions of averaged (generalised) linear mixed-effects models. Full statistical parameters are available in Table 2. Note that the x-axis in (a) is on a log-scale, the x-axis in (b) is on a square-root scale, and the y-axes in (a)–(d) are on a log-scale.

Mangels et al., 2017; Wintergerst et al., 2021). However, once grasslands and green spaces are mown less often, plant and insect diversity increases (Chollet et al., 2018; Sehart et al., 2020; Wastian et al., 2016),

which is why reducing mowing intensity is a promising strategy for promoting urban insect diversity, including Lepidoptera (Proske et al., 2022).

TABLE 2 Results of averaged (within 2 AICc units of the model with lowest AICc) multiple (generalised) linear mixed-effects models (with *t*-values for linear models and *z*-values for Poisson models on species richness and abundance) testing for relationships between response variables and environmental variables.

Variable	Estimate ± SE	t/z-value	p-value
Moth abundance			
Habitat area ^a	0.262 ± 0.092	2.840	0.005
Mowing intensity ^b	−0.280 ± 0.111	2.522	0.012
Urbanisation	−0.138 ± 0.151	0.915	0.360
Plant species richness	0.060 ± 0.095	0.637	0.524
Sky brightness	−0.026 ± 0.076	0.348	0.728
Moth species richness			
Urbanisation	−0.298 ± 0.103	2.902	0.004
Plant species richness	0.182 ± 0.070	2.593	0.010
Habitat area ^a	0.014 ± 0.044	0.321	0.748
Mowing intensity ^b	−0.015 ± 0.050	0.297	0.767
Habitat isolation	0.010 ± 0.034	0.286	0.775
Moth diversity e ^H			
Urbanisation	−1.717 ± 0.727	2.363	0.018
Plant species richness	0.886 ± 0.573	1.547	0.122
Habitat isolation	0.074 ± 0.242	0.303	0.762
Mowing intensity ^b	−0.032 ± 0.210	0.155	0.877
Julian day	−0.036 ± 0.380	0.094	0.925
Sky brightness	0.002 ± 0.318	0.007	0.994
Rarefied moth species richness			
Urbanisation	−0.360 ± 0.106	3.390	<0.001
Julian day	0.087 ± 0.123	0.710	0.478
Specialist species richness			
Urbanisation	−0.474 ± 0.206	2.300	0.021
Habitat area ^a	0.044 ± 0.104	0.426	0.670
Plant species richness	0.029 ± 0.084	0.339	0.735
Sky brightness	0.033 ± 0.126	0.260	0.795
Woody feeder species richness			
Habitat area ^a	0.413 ± 0.160	2.581	0.010
Plant species richness	0.222 ± 0.179	1.242	0.214
Mowing intensity ^b	−0.185 ± 0.230	0.802	0.423
Urbanisation	−0.028 ± 0.104	0.265	0.791

Note: Significant relationships (at $p < 0.050$) are in bold.

^aLog-transformed before analyses.

^bSquare-root-transformed before analyses.

In our study, habitat and host plant availability were major constraints for moths, since moth abundance was also higher when habitat area was larger. A positive relationship between individual and species numbers of insects and area of green spaces has often but not always (Fattorini et al., 2018; Lizée et al., 2012; Öckinger et al., 2009) been found for butterflies (Knapp et al., 2008; Saarinen et al., 2005). Larger areas have usually a higher carrying capacity and provide more habitat and plant diversity. However, species richness and diversity

were not directly related to area in our data, although species-area-relationships for moths are known for true islands (Franzén et al., 2012). Indeed, urban green spaces are literally habitat islands within a matrix of uninhabitable space and many predictions of equilibrium theory of island biogeography hold for urban areas (Fattorini et al., 2018). This would predict that larger habitat area and lower isolation would influence species richness positively.

Nevertheless, we also could not find effects of isolation and neither could Franzén et al. (2012) for moths on oceanic islands. As evidence for isolation effects in Lepidoptera is mixed (Bräuniger et al., 2010; Lizée et al., 2012; Öckinger et al., 2009), responses to isolation might be context-dependent on the isolating matrix or geographically restricted. Here, we note that every land use type that was not equated to a grassland type habitat was considered as an isolating element, which might be an oversimplification (Prugh et al., 2008). At least on community level, it seems that most moth species are little affected by isolation, which is corroborated by the dispersal distances of non-migrating moths (Merckx et al., 2009, 2010; Slade et al., 2013), which would enable most moths to overcome isolating elements in a typical urban environment.

Surprisingly, light pollution quantified as ALAN was not related to any moth variable. Even though many parts of moths' life history including dispersal and reproduction might be hampered by ALAN (Degen et al., 2016) and background skyglow and point source lighting can detract moths from light traps, it was consistently urbanisation per se rather than ALAN alone that influenced moth diversity. While negative effects of light pollution and ALAN on moths' larval development (van Geffen et al., 2014), abundance and species richness (Boyes et al., 2021; Macgregor et al., 2017; van Langevelde et al., 2018), as well as nocturnal flower visits (Knop et al., 2017) are firmly established, impacts of urbanisation other than ALAN on moth diversity were stronger in our study area. Even though in most cities there has recently been a switch to white LED lighting, which is even more problematic for nocturnal moths than previous light types, the general negative impacts of ALAN are probably long-term effects (Kalinkat et al., 2021) that have been introduced decades ago. Thus, the consequences also might have already plateaued. As our densely populated study area has high levels of light pollution in general, and as urban sites had been illuminated for many decades, sensitive species may have already disappeared or local populations increasingly adapted to ALAN, for example, by reduced phototactic behaviour (Altermatt & Ebert, 2016). Furthermore, sky brightness is intertwined with urbanisation in a way that is difficult to disentangle without experiments (Knop et al., 2017). As some sites fell within the same pixel in the ALAN data, masking potential variation, it is possible that the spatial resolution of the ALAN data was not fine enough to unambiguously detect differences among sites.

The low sample size per site is a limitation of this study. Every site was for logistical reasons sampled only once. Thus, sampling efficiency (64%–68%) was rather moderate. Since moth communities have seasonal turnover, operating light traps for multiple nights would have resulted in more complete species inventories across sites. However, Julian day, that is, the day of the year at which a site was sampled,

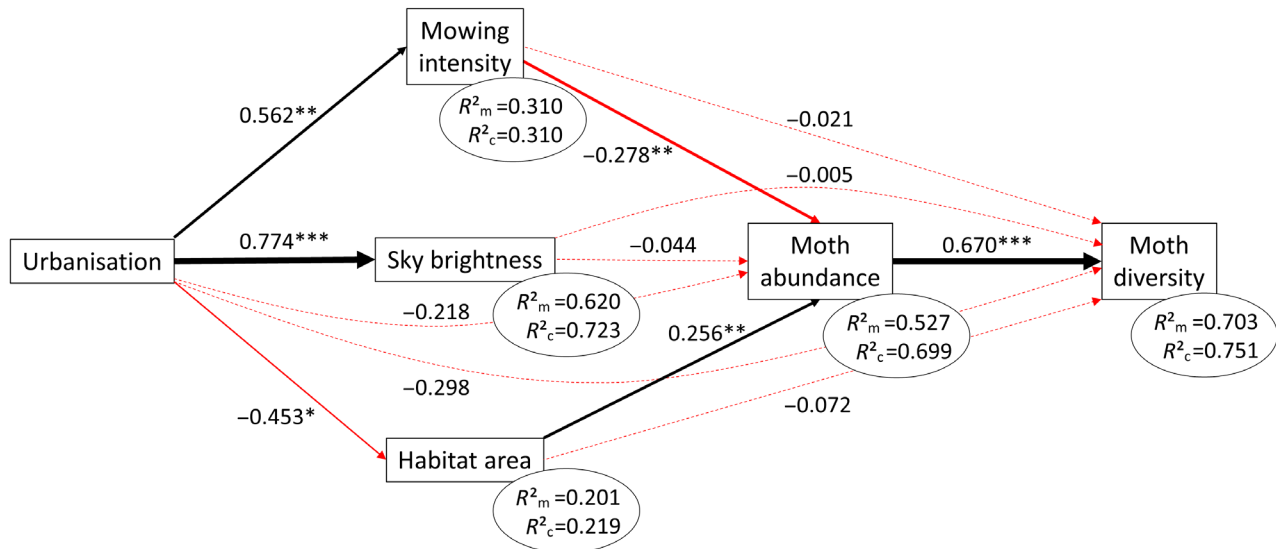


FIGURE 4 Path model (Fisher's $C = 2.057$, $p = 0.914$) illustrating that influences of urbanisation on moth diversity were mediated by moth abundance, and only indirect via mowing intensity and habitat area. Numbers next to arrows give the standardised path coefficients (** $p < 0.01$, *** $p < 0.001$ and * $p < 0.050$). Arrow width scales with the strength of association, black arrows indicate positive relationships, red arrows negative relationships, with non-significant relationships indicated by dashed arrows. Values below endogenous variables are the explained marginal (R^2_m , fixed effects) and conditional (R^2_c , fixed and random effects) variances. Full statistical parameters are given in Table S2.

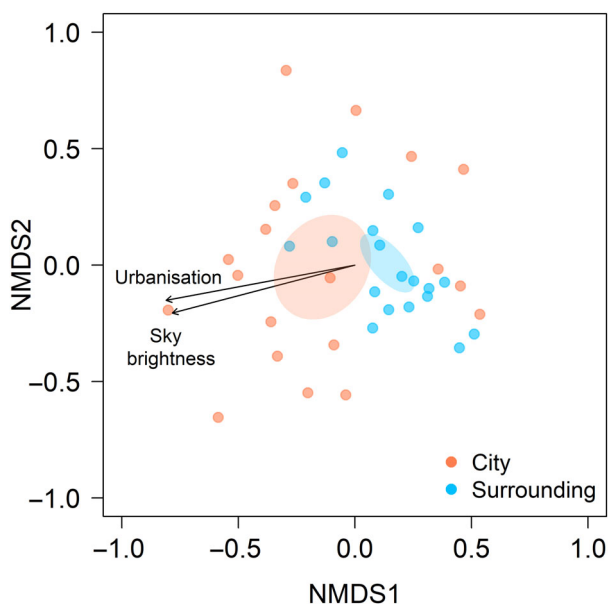


FIGURE 5 City and surrounding had largely separated moth species composition (2-dimensional NMDS ordination, stress = 0.169), with turnover among sites being primarily associated to urbanisation and sky brightness (post-hoc permutation test, lengths of arrows proportional to strength of correlation; details in Table S5). Shaded polygons indicate the 95% CI of ordination centroids for city and surrounding.

was not significant in any analyses and possible variation among sampling nights (e.g., caused by differences in temperature, wind speed, humidity or cloud cover) were considered in the hierarchical model structure. Variance explained by the random intercept 'sampling date'

was much smaller than the variance explained by the fixed effects, indicating that there was no systematic influence of the single sampling on the results. Thus, the limited sampling effort likely only resulted in error rather than bias and is not weakening the inference.

Community characteristics and composition

The most common species found in the single collection in high summer were all polyphagous generalists that inhabit a variety of habitats. With increasing urbanisation, specialist species decreased significantly, which indicates that urbanised environments favour generalists. Feeding specialists are often more sensitive to altered environmental conditions (Bergerot et al., 2011; Merckx & Van Dyck, 2019) as they are more likely to be affected by habitat loss and face higher risk of local extinction (Nieminen, 1996; Wagner et al., 2021). Subsequently, we found that urban and surrounding areas had a different community composition. Apart from the decrease in specialists, community composition was related to urbanisation and ALAN (which itself was highly correlated to urbanisation). Likely, this observed turnover in species between urban and surrounding areas is mediated via species-specific life history characteristics. For example, characteristics associated with the city may include thermophily, high mobility and polyphagy (Franzén et al., 2020; Merckx et al., 2018).

Indeed, the only indicator species for the city was the thermophilic *Eilema caniola*. Originally, this species inhabited xerothermic, rocky slopes south of the Alps, but recently expanded northwards by accessing moss and lichen covered roofs within cities, likely profiting from the urban heat island effect. In the surrounding areas, *E. caniola*

was replaced by *E. lurideola*, which was one of the several indicator species for the surroundings. Larvae of all *Eilema* feed mainly on lichen and algae on rocks, branches, tree trunks and similar substrates (Ebert, 1991–2003). These resources are abundant also in urbanised areas and almost unaffected by management, which may contribute to their wide occurrence. The other indicator species of the surroundings differ in their ecology. The oligophagous *M. impura* and *M. pudorina* develop on Poaceae and prefer more continuous and wet grasslands. *Mythimna pudorina* dwells particularly in habitats that are sensitive to land use such as reeds and sedge reeds (Ebert, 1991–2003). *H. octogenaria* and *P. fuliginosa*, in contrast, are polyphagous on various herbs and are, thus, adversely affected when grasslands become dominated by grasses. The indicator species of the city surroundings have in common that they are more sensitive to intensive management (including mowing and fertilisation) and require structurally diverse habitats with open areas as well as fringe vegetation in conjunction with scrub or woodlands, which is rare in cities.

CONCLUSIONS

Impacts of urban areas on moths are direct and indirect, negative and favour a limited set of generalist species. Among other relationships, high mowing intensity, by reducing moth abundance, was a major driver of declining moth diversity, which could easily be mitigated by mowing less. However, measures to improve biodiversity in cities will usually be limited, as green spaces cover only a comparatively small proportion of the total land. The results, albeit based on the limited data from a single sampling night per site in and around a single city only, also highlight the importance of habitats in the surroundings of cities, as many species simply cannot survive in urban areas. As larger habitat area additionally supported higher moth abundance, further fragmentation, especially urban sprawl, should be avoided to protect diverse communities still surviving on the urban fringes but also to safeguard diversity within the city boundaries. In order to increase the evidence base and geographic coverage, future studies could sample urban and surrounding habitats in multiple cities. As many consequences of urbanisation are inevitably not independent from each other, dedicated experiments (Weisser et al., 2023) isolating the effects of single environmental variables such as mowing would be desirable.

AUTHOR CONTRIBUTIONS

Dennis Sanetra: Conceptualization; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. **Johanna Berger:** Investigation; writing – review and editing. **Margarita Hartlieb:** Investigation; writing – review and editing. **Nadja K. Simons:** Funding acquisition; investigation; writing – review and editing. **Genevieve Walther:** Investigation; project administration; writing – review and editing. **Nico Blüthgen:** Conceptualization; formal analysis; funding acquisition; investigation; supervision; writing – original draft; writing – review and editing. **Michael Staab:** Formal analysis;

investigation; methodology; supervision; visualization; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All raw data are archived in Figshare (<https://doi.org/10.6084/m9.figshare.24920226.v1>).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.

Table S1. Number of individuals of each moth species collected. Given are the number of individuals for sites in the city and surroundings

(with the number of sites a species was found in parentheses). Nomenclature of species follows Steiner et al. (2014).

Table S2. Full results of the path model (Fisher's $C=2.057$, $p=0.914$) with moth diversity as terminal endogenous variable (Figure 3). Estimates give standardized path coefficients \pm SE. Significant paths are shown in bold. Marginal (R^2_m , fixed effects) and conditional (R^2_c , fixed and random effects) variances are also given.

Table S3. Full results of the path model (Fisher's $C=2.551$, $p=0.863$) with moth diversity as terminal endogenous variable in which the two sites where the trap was placed on the ground have been excluded. Estimates give standardized path coefficients \pm SE. Significant paths are shown in bold. Marginal (R^2_m , fixed effects) and conditional (R^2_c , fixed and random effects) variances are also given. Results are qualitatively and quantitatively very similar to the model (Table S2; Figure 3) with the full data, indicating that a bias by those two sites is unlikely.

Table S4. Full results of the path model (Fisher's $C=2.057$, $p=0.914$) with rarefied moth species richness as terminal endogenous variable (Figure S5). Estimates give standardized path coefficients \pm SE. Significant paths are shown in bold. Marginal (R^2_m , fixed effects) and conditional (R^2_c , fixed and random effects) variances are also given.

Table S5. Results of the post-hoc permutation test ($n=10,000$ permutations) relating the scores of the first two NMDS axes to environmental variables (ordered by decreasing R^2). Significant (at $p<0.05$) variables are in bold.

Figure S1. Correlations (Spearman's ρ) among all environmental and response variables.

Figure S2. The a) species richness and b) diversity (e^H) of moths was strongly related to their abundance (Spearman's $\rho=0.947$ and 0.889 , respectively). Note that all axes are on a log-scale.

Figure S3. Sample-based species accumulation curves (based on $n=10,000$ permutations, shaded polygons indicate 95% CI) for all sites and separated for city and surrounding. The expected number of species (jackknife 1 estimator, all sites: 187, city: 91, surrounding: 160) is each indicated by horizontal lines. Total sampling efficiency was comparable each (all sites: 68%, city: 64%, surrounding: 66%).

Figure S4. Specialist species richness decreased a) with higher urbanisation while b) woody feeder species richness increased with larger habitat area. Regression lines (95% CI as shaded polygons) indicate the predictions of averaged (generalized) linear mixed-effects models. Full statistical parameters are available in Table 2. Note that the x-axis in b) and all y-axes are on a log-scale.

Figure S5. Path model (Fisher's $C=2.057$, $p=0.914$) illustrating that urbanisation did not directly influence rarefied moth species richness once the simultaneous relationships with environmental variables related to urbanisation was considered. Numbers next to arrows give the standardized path coefficients (** $p<0.001$, * $p<0.010$, $p<0.050$). Arrow width scales with the strength of association, black arrows indicate positive relationships, red arrows negative relationships, with non-significant relationships indicated by dashed arrows. Values below endogenous variables are the explained marginal (R^2_m , fixed effects) and conditional (R^2_c , fixed and random effects) variances. Full statistical parameters are given in Table S3.

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