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Piano, E., C. Souffreau, T. Merckx, L.F. Baardsen, T. Backeljau, D. Bonte, K.I. Brans, M. Cours, M. Dahirel, N.Debortoli, E. Decaestecker, K. De Wolf, J.M.T. Engelen, D. Fontaneto, A.T. Gianuca, L. Govaert, F.T. Hanashiro, J. Higuti, L. Lens, K. Martens, H. Matheve, E. Matthysen, E. Pinseel, R. Sablon, I. Schön, R. Stoks, K. Van Doninck, H. Van Dyck, P. Vanormelingen, J. Van Wichelen, W. Vyverman, L. De Meester+ & F. Hendrickx+, 2019. Urbanization drives cross-taxon declines in abundance and diversity at multiple spatial scales. **Global Change Biology**.

Urbanization drives cross-taxon declines in abundance and diversity at multiple spatial scales

Running title: urbanization impacts abundance and diversity

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Abstract

The increasing expansion of urban landscapes is hypothesized to drastically alter (semi-)natural environments with a concomitant major decline in species abundance and diversity. Yet, studies on this effect of urbanization, and the spatial scale at which it acts, are at present inconclusive due to the large heterogeneity in taxonomic groups and spatial scales at which this relationship has been investigated among studies. Comprehensive studies that analyse this relationship across multiple animal groups and at multiple spatial scales are rare, hampering the assessment of how biodiversity generally responds to urbanization. Here, we studied aquatic (cladocerans), limno-terrestrial (bdelloid rotifers) and terrestrial (butterflies, ground beetles, ground- and web spiders, macro-moths, orthopterans and snails) invertebrate groups using a hierarchical spatial design wherein three local-scale (200 m × 200 m) urbanization levels were repeatedly sampled across three landscape-scale (3 km× 3 km) urbanization levels. We tested for local- and landscape urbanization effects on abundance and species richness of each group, wherein species richness was partitioned into the average richness of local communities and richness due to variation among local communities. Abundances of the sampled terrestrial arthropods declined – and all but one significantly so – in response to local urbanization, with reductions up to 85% for butterflies. Species richness also declined with increasing levels of urbanization, but responses were highly heterogeneous between the different groups with respect to the species richness component and the spatial scale at which urbanization impacts species richness. Depending on the group, species richness declined due to the homogenization of species assemblages and/or a decrease in local species richness. This resulted in an overall decrease in total species richness across groups in urban areas. Our results provide strong support to the general negative impact of urbanization on abundance and species richness within habitat patches and highlight the importance of considering multiple spatial scales and taxa to assess the impact of urbanization.

Keywords: biodiversity; biotic homogenization; diversity partitioning; insect decline; land use; spatial scale; urban ecology

INTRODUCTION

The conversion of natural and rural land to urban environments increased drastically worldwide over the last 30 years, with urban land cover expected to be tripled from 2000 to 2030 (Seto , Güneralp & Hutyra 2012). Urbanization drives global environmental change and currently represents one of the main anthropogenic impacts (Parris 2016) with expected drastic consequences on biodiversity and ecosystem processes. Urbanization-associated changes in community structure could result from several mechanisms (Rebele, 1994; Seto, Sánchez-Rodríguez & Fragkias, 2010), which act at multiple spatial scales (Shochat, Warren, Faeth, McIntyre & Hope, 2006; Shochat et al., 2010) and are strongly habitat-dependent (Hill et al., 2017). Ecological effects have been shown to result from substantial changes to the local abiotic environmental conditions (e.g. high levels of nutrients, pollution, and imperviousness) (Parris, 2016), and to landscape structure (e.g. reduced size and connectivity and increased temporal turnover of habitat patches) (McDonnell, et al. 1997; Parris, 2016).

Several studies investigated relationships between urbanization and two important determinants of ecosystem functioning i.e. the abundance and/or diversity of species. Yet, their results are surprisingly equivocal, as negative relationships (Chace & Walsh, 2006; Lagucki, Burdine & McCluney, 2017; Niemelä & Kotze, 2009; Ramirez-Restrepo & Macgregor-Fors, 2017; Saari et al., 2016), no relationship (Christie & Hochuli, 2009) as well as positive relationships (Hill et al., 2017; McKinney, 2008; Shochat et al., 2010), are reported. These heterogeneous results suggest that the effect of increasing urbanization might strongly depend on the spatial scale and organism group at which it is assessed (Concepción et al., 2015; Egerer et al., 2017; McKinney, 2008; Philpott et al., 2014).

First, the direction and magnitude of changes in species diversity in response to an environmental driver may strongly depend on the spatial scale at which species diversity is measured (Chase & Knight, 2013). For instance, urbanization may filter out species that are not pre-adapted to urban conditions, with a consequent decrease in abundance or diversity at small (local) spatial scales (Bates et al., 2011; Piano et al., 2017). Alternatively, the loss of species that are less adapted to urban environments could be (over)compensated by an increase of species that are efficient in exploiting urban resources, including exotic taxa (McKinney, 2006; Menke et al., 2011; Sattler, Obrist, Duelli & Moretti, 2011). Both phenomena may cause biotic homogenization if local communities are generally colonized by the same species, increasing in turn the compositional similarity of urban species assemblages and, consequently, reducing species richness of urban areas at large spatial scales (Knop, 2016; McKinney, 2006; Morelli et al., 2016).

Second, organisms may react to urbanization at different spatial scales (Concepción, Moretti, Altermatt, Nobis & Obrist, 2015; Fahrig, 2013; Merckx et al., 2018; Soininen, McDonald & Hillebrand, 2007; Wiens, 1989). Species traits, such as dispersal capacity, affect how organisms perceive and respond to their environment (Wiens, 1989), and hence, how species are spatially distributed (Finlay, Esteban, Brown, Fenchel & Hoef-Emden, 2006). Thus, urbanization effects may remain undetected if not assessed at relevant spatial scales (Jackson & Fahrig, 2015; Turrini & Knop, 2015).

A reliable assessment of the overall effects of urbanization on species communities is unlikely to be resolved by studies on single taxonomic groups and single spatial scales. Ideally, insights into general patterns of abundance and diversity change should be obtained by integrating data over multiple animal groups, while uncoupling the spatial scales at which urbanization and species richness are measured.

Here, we analysed data on abundance and species richness data of one limno-terrestrial (bdelloid rotifers), one aquatic (cladocerans) and seven terrestrial (butterflies, ground beetles, ground- and web spiders, macro-moths, orthopterans and snails) animal groups sampled along replicated urbanization gradients in Belgium. More specifically, we sampled communities according to a hierarchically nested sampling design, in which three local-scale urbanization levels were repeatedly sampled across the same three urbanization levels at the landscape scale (Merckx et al. 2018). The sampling design allows us to partition the total species richness (γ-diversity) into richness within local communities (α-diversity) and richness due to variation in species composition (β-diversity), and to relate these to both local and landscape-scale urbanization levels. We tested (i) if, and in which direction, local and landscape-scale urbanization affect total abundance; (ii) if local and landscape-scale urbanization affect species richness within habitat patches, and if so at which spatial scale; and (iii) to what extent these responses are consistent across animal groups.

MATERIALS AND METHODS

Sampling area and design

Sampling was conducted in Belgium, within a polygon of 8140 km^2, encompassing the cities of Brussels, Antwerp and Ghent. It is a densely populated region (average human population density of Belgium: 371 inhabitants/km^2, IBZ, 2018) that is composed of urban areas embedded within a semi-natural and agricultural matrix. Because urbanization encompasses a range of factors that alter the physical environment and landscape characteristics, we defined the percentage of built-up area (%BU) as a proxy for urbanization and it was assessed with a GIS software using an object-oriented reference map of Flanders as a vectoral layer (LRD, 2013). This layer included the precise contours of all buildings, while roads and parking infrastructures were excluded. To test effects of urbanization at the landscape scale, we selected 27 plots (i.e. squares of 3 km × 3 km), among which nine located in low urbanized areas (low: 0%-3%BU), nine plots in areas with intermediate urbanization (intermediate: 5%-10%BU) and nine in high urbanized areas (high: > 15%BU) (Figure 1). The latter encompassed city centres. Given that only buildings are considered for the calculation of %BU, values of 15% can be considered highly urbanized. We first selected plots within this highest %BU category that were approximately equidistant from each other within the study area. Next, plots of the intermediate and lowest urbanization category were selected within 10 km - 25 km of the highest urbanized plots. This plot selection strategy resulted in an even spread of plots within the same urbanization category across the study area and ensured a minimal spatial autocorrelation of plot urbanization levels. Across plots, %BU was positively correlated with the amount of other impervious substrates such as roads and artificial constructions (bridges, viaducts, locks, …) (r_S = 0.94; P < 0.0001) and negatively correlated with the area of semi-natural habitat (r_S = -0.85; P < 0.0001) (Figure S1), thus representing a reliable proxy of urbanization. To investigate effects of local-scale urbanization, each plot was divided into local subplots of 200 m × 200 m, which were classified into urbanization categories using identical %BU thresholds as used at plot level. Within each plot, we then randomly selected one subplot of each urbanization category (i.e. low, intermediate and high) as sampling sites, taking into account the suitability to sample within the subplot (e.g. availability of target habitat, sampling permission, accessibility). This sampling design resulted in a total of up to 81 sampling sites (i.e. 9 plots × 3 landscape-scale urbanization levels × 3 local-scale urbanization levels) (Figure 1) and guaranteed that urbanization at landscape and local scales are uncorrelated and, hence, that their effects, as well as their interaction, could be tested simultaneously. The same sampling design was applied to all examined groups, and all sampling was based on the identical set of plots (landscape-level of urbanisation). At the local level too, the same sampling design was implemented across organism groups, but the choice of specific subplots featuring a given level of local urbanisation within each plot could differ between groups as sampling sites suitable for all groups were not always present within the same 200 m x 200 m subplot. With the exception of web spiders and macro-moths, most or all of the 81 subplots were sampled for each animal group (see *Sampling methods*).

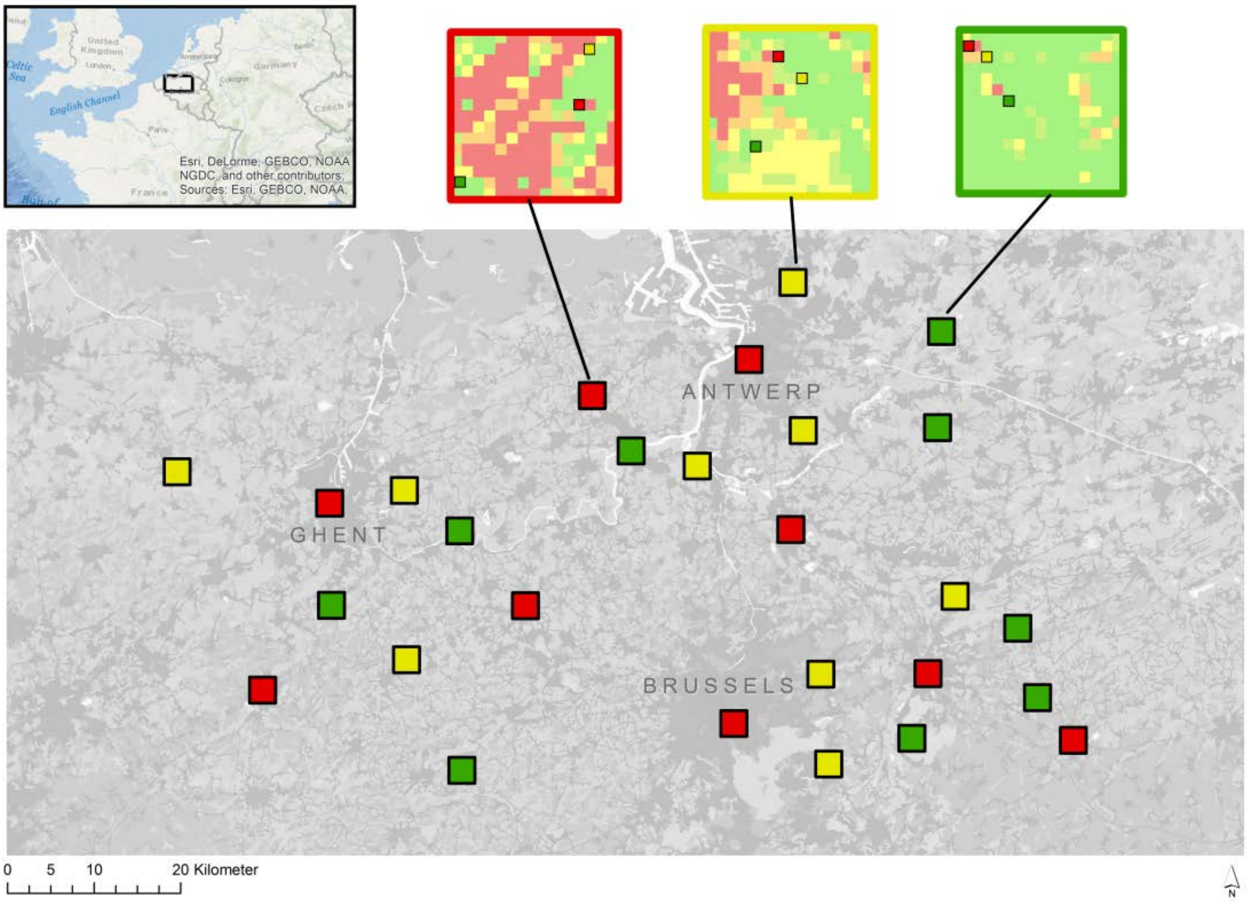


Figure 1 - Map of the study area, in the northern part of Belgium, showing the location of the 27 sampled landscape-scale plots. Colours refer to urbanization categories (green: low urbanization with < 3% of built-up area; yellow: intermediate urbanization with 5%-10% of built-up area; red: high urbanization with > 15% of built-up area). The plots are divided in 200 m × 200 m subplots, to which the same colour code used for the plots is assigned. Subplots characterized by urbanization values intermediate between these three classes are indicated in light green and orange. Within each plot, a subplot belonging to the low, intermediate and high urbanization category was selected as sampling sites.

Sampling methods

Ground beetles and ground spiders

Ground beetles and ground-dwelling spiders were sampled with pitfall traps from half of April till the end of June 2013. Within each subplot, two pitfall traps (diameter 8 cm) were installed (25-50 m apart) and emptied every two weeks for a total of six sampling sessions. Because four traps were lost during the last sampling campaign (end of June), data from the last sampling session were not used for analysis. To reduce confounding effects of differences in habitat type between subplots with varying levels of urbanization, pitfall traps were placed consistently in grassy-herbaceous vegetation such as road verges, park grasslands and grasslands at the different subplot urbanization levels. Samples were preserved in 4% formalin and sorted in the laboratory. Data from both pitfall samples per site and the different sampling dates were pooled and treated as a single sampling unit. All ground beetle and adult spider individuals were counted and identified to species level (Boeken, 2002; Duff, 2016; Roberts, 2009). Juvenile spiders were excluded from the final dataset since they could only be identified to genus level.

Web spiders

Web spiders were sampled by hand between the 27th of August and the 5th of October 2014 in 62 out of the 81 subplots. One landscape (3 subplots) was sampled per day. Each subplot was explored by the same two persons for about 4.5-person hours. Spiders were detected by looking for their webs and each subplot was completely explored searching for orb-weaving spiders until no new individual could be found after 15 min. Rainy days were avoided as spiders may be less likely to build webs and are thus less detectable. Every encountered spider was caught and stored in 70% ethanol. Identification was performed under a stereomicroscope to species level (Roberts, 2009). Juveniles were excluded from the final dataset since they could only be identified to genus level. Spiders captured according to this methodology are further referred to as ‘web spiders’ to distinguish them from the ’ground spiders’ that were captured by pitfall traps (see section *Ground beetles and ground spiders*).

Macro-moths

Sampling was restricted to a set of nine plots, three of each plot urbanization category, and performed with Jalas type bait traps in three sampling sessions, which started on the 30th-31th of July 2014 (first session), 13th-14th of August 2014 (second session) and 30th-31th of March and 1st of April 2015 (third session). Traps were emptied on 3rd-4th of August 2014 (first session), 2nd-3rd of September 2014 (second session) and 24th-25th-26th of April 2015 (third session). Traps were baited with sugar-saturated wine and sampled individuals were poisoned with chloroform within the traps. Individuals were counted and identified to species level (Manley, 2010), except for two species pairs: *Mesapamea secalis/secalella* and *Hoplodrina blanda/octogenaria*.

Butterflies and orthopterans

Butterflies and orthopterans (grasshoppers and bush crickets) were sampled along standard transects in three sampling sessions performed in 2014, from July to early September. Walks of 20 minutes were performed in each of the 81 subplots in grasslands during the warmest hours of the day, i.e. between 10 a.m. and 4 p.m. avoiding cloudy and rainy days. Butterflies were sampled with visual counts along a transect (‘Pollard walk’, Pollard & Yates, 1993), with occasional netting of individuals when needed for species identification. All individuals were identified in the field to the species level following Bink (1992). Orthopterans were sampled through auditive counts with occasional visual inspection of individuals.

Snails

Snails were sampled by hand during visual search along transects. Each subplot was visited once from April to July 2014 and additional samplings were performed in 2015. Snails were searched along a ca. 150–200 m transect in an area of 50 m at both sides. Individuals were mainly searched in the most appropriate habitats, i.e. (i) at the bottom of/on herbs, shrubs and trees, (ii) under branches, piled wood, cardboard and construction/demolition materials, and (iii) along/on fences and walls.

Bdelloid rotifers

Communities of bdelloid rotifers were sampled by collecting lichen patches of the genus *Xanthoria*, for which bdelloid rotifer communities have been previously studied in Europe (Fontaneto, Westberg & Hortal, 2011). Suitable *Xanthoria* patches could be found in all but one subplot. Sampling was performed between June and July 2013. The selection of the lichen was haphazard: the first lichen patch encountered in each subplot was collected. Dry lichen thalli between 3 and 10 cm^2 were cut from the substrate with a knife and kept in paper bags. For each lichen sample, an area of 2.5 cm^2 was hydrated with distilled water in a plastic petri dish. All active bdelloid rotifers that recovered from dormancy in the following four hours after hydration were sorted and identified to species level (Donner, 1965). Previous studies on bdelloid rotifers in these lichens (Fontaneto et al., 2011) revealed that animals start recovering between 10 and 40 minutes after hydration of the sample and that no more bdelloid rotifers are recovered after four hours. The very few dormant stages still found in the sample that did not recover after that time were considered dead and excluded from the analyses.

Cladocerans

Water samples were collected from ponds using a tube sampler (length = 1.85 m; diameter = 75 mm; Gianuca et al. 2018). One pond was selected in each of the 81 selected subplots. Sampling was performed once for each pond and all sampling was performed in the period from 29th of May to the 10th of July 2013. In each pond, eight sampling locations were selected using a predefined grid, assuring that different microhabitats (shallow and deeper zone, different locations with respect to wind direction) were represented to a similar extent. On each sample location, the exact place to be sampled was chosen in a random way, regardless of the presence of macrophytes. At each of the eight locations, 12 L of water was collected, resulting in a total of 96 L per pond. The tube sample integrated the entire water column, but resuspension and subsequent sampling of bottom material was avoided. For each pond, 40 L of water was filtered through a 64 µm conical net. The sample was then collected in a 60 mL vial and fixed with formalin (4%). Additional sampling was performed with a sweep-net (64 µm net) and preserved in the same way. These additional samples served to guarantee sufficiently extensive sampling to reconstruct an as complete as possible species list. Individuals in standardized subsamples were identified and counted; entire subsamples were counted until at least 300 individuals were identified and no new species was found the last 100 specimens. Samples containing less than

300 individuals were counted completely, and the additional qualitative samples for those ponds were screened for additional species. Species identification was based on Flößner (2000). *Daphnia longispina*, *Daphnia galeata* and *Daphnia hyalina* were combined in the *Daphnia longispina* complex due to the morphological similarities and possible hybridization between the species. Detailed information on the sampling and identification of zooplankton are reported in Brans et al. (2017) and Gianuca et al. (2018). Densities were calculated as number of individuals per litre of the original sample.

Abundance data and analysis

The total number of sampled/observed individuals in each sample/transect was used as an estimate for the abundance of each group in each subplot. For cladocerans, our abundance data are based on the total number of individuals in a standardized volume of 40L. Differences in abundances in response to local (subplot) and landscape (plot) scale urbanization levels were tested by means of a Generalized Linear Mixed Model (GLMM) for each of the investigated groups. Local- (subplot) and landscape-scale (plot) urbanization levels and their interaction were specified as fixed factors. To account for the spatial dependency of subplots within the same plot, a plot identifier (PlotID) was incorporated as a random factor, nested within the landscape-scale urbanization levels. We assumed the abundance data to be Poisson distributed and used the sample variance instead of the theoretical variance to account for potential overdispersion (Agresti et al. 1996). Analyses were conducted with PROC GLIMMIX in SAS® 9.4 (SAS Institute Inc. 2013). We further tested for a cross-group response in total abundance of individuals at both local- and landscape-scale urbanization with the non-parametric Page test (Hollander & Wolfe, 1973). This test accounts for the ordering of the urbanization levels (low – intermediate – high), with the nine groups specified as blocks. *P*-values were based on permutations within blocks and obtained from StatXact v5 (© Cytel Software, 2001).

Species richness data and analysis

Effect of local- and landscape-scale urbanization on total species richness

We first assessed general responses in total species richness due to local- and landscape-scale urbanization by means of sample-based accumulation curves, which express the cumulative number of species when samples from a particular local- or landscape-scale urbanization category are added at random. Given that we aim at identifying responses in total (γ) species richness only, we restricted the analysis to five local/landscape-scale urbanization combinations. More specifically, we compared sample-based accumulation curves between: (i) low urbanized subplots in low urbanized plots (low end urbanization at both spatial scales); (ii) high urbanized subplots in high urbanized plots (high end urbanization at both spatial scales); (iii) low urbanized plots regardless of the degree of local urbanization; (iv) high urbanized plots regardless of the degree of local urbanization and (v) all samples regardless of the degree of local- and landscape-scale urbanization levels. This latter combination of samples thus represents a mixture of low and high urbanized plots and subplots. Settings (i) – (iii) – (v) – (iv) – (ii) represent a gradient of urbanization levels integrating both spatial scales.

For each animal group, we tested if total species richness declined significantly with increasing local/landscape-scale urbanization level by means of the ordered heterogeneity test through the $r_S P_c$ statistic (Rice & Gaines, 1994), which combines the statistical evidence of differences between sample means with their rank order. More precisely, we first tested for differences in species richness among urbanization categories by comparing the observed average absolute differences in total species richness for a total of nine samples (corresponding to the lowest sample size of the five local/landscape-scale combinations) with those obtained by random shuffling samples across these five combinations (*mobr* package 1.0; Xiao, McGlinn, May & Oliver, 2018 in R 3.4.2 (R Development Core Team, 2017)). We then multiplied the complement of the obtained P-value (P_c) with the Spearman Rank order correlation (r_S) between species richness and increasing urbanization level to obtain the $r_S P_c$ statistic.

Next, we tested for a cross-group response in total species richness among these five urbanization categories with the non-parametric Page test (Hollander & Wolfe, 1973), specifying the nine groups as blocks. *P*-values were based on permutations within blocks and obtained from StatXact v5 (© Cytel Software, 2001).

Effect of local- and landscape-scale urbanization on species richness components

To gain more insights into the spatial scale at which species richness of each group is most strongly affected by urbanization, we partitioned the total species richness observed at each local- or landscape-scale urbanization level into its underlying components. We used a diversity partitioning approach wherein the total diversity at larger spatial scales (γ) is decomposed into its average local species richness ($\bar{\alpha}$) and species richness due to variation between local communities (β). As a measure of variation in species composition between local communities, we calculated both the proportional differences in species composition of the local communities compared to the total species community ($\bar{\beta}_P = \gamma/\bar{\alpha}$) as well as additive variation ($\bar{\beta}_A = \gamma - \bar{\alpha}$) as these measures of β-diversity can be calculated and compared at multiple hierarchical spatial scales (Lande, 1996; Crist, Veech, Gering & Summerville, 2003; Anderson et al., 2011). While $\bar{\beta}_P$ expresses the number of times by which the richness at plot (or regional) level increases compared to the richness at subplot (or plot) level, $\bar{\beta}_A$ expresses the absolute increase in number of species between these two sampling levels.

Effects of local-scale urbanization on species richness were assessed by comparing decomposed species richness values along a gradient of local-scale urbanization. This is a two-step procedure. First, we decomposed the total species richness (γ) of all subplots belonging to the same urbanization level into the average species richness within subplots ($\bar{\alpha}$) and the average additive and proportional variation among subplots (β_{among}), and we did so for each of the three levels of local urbanization (Figure 2a). Second, differences in these species richness components across urbanization levels were tested with a randomization test, by permuting samples over the three local-scale urbanization levels (see McGlinn et al., 2019).

The effect of landscape-scale urbanization on species richness can be evaluated both within as well as between plots. For the former, we decomposed the total species richness within plots (γ_{within}) into the average local species richness of the three subplots within a plot (α) and the additive and proportional variation between these communities (β_{within}). For the latter, we decomposed the species richness across all plots (γ_{among}) into the average species richness within a plot (γ_{within}) and the additive and proportional variation in species richness among plots (β_{among}) (Figure 2b). Differences in species richness along the urbanization gradient at both scales were tested with a randomization test, by permuting samples over the three landscape-scale urbanization levels (McGlinn et al., 2019).

Observed versus rarefied species richness

Observed species richness is a composite measure and differences in this metric among samples may result from variation in (i) the number of individuals present at a particular site, (ii) the spatial aggregation of individuals of the same species, and (iii) the number and relative abundance of species in the species pool (i.e. the species abundance distribution or SAD) (He and Legendre 2002). We therefore also calculated rarefied species as the expected number of species for each diversity component for a standardized number of randomly selected individuals by means of individual-based rarefaction curves. By removing the effect of individual densities, differences in rarefied species richness provide more information on differences in the SAD between communities. At the regional (γ) scale, we rarefied for each animal group to the number of individuals in the urbanization category that yielded the smallest sample size.

Overall pattern across groups

While the above analyses were performed separately for each group, we further tested for a significant change in the diversity components in response to the landscape- and local-scale urbanization gradients across groups by means of the non-parametric Page test (Hollander & Wolfe, 1973) for both observed and rarefied richness values. The nine groups were specified as blocks and *P*-values were obtained from StatXact v5 (© Cytel Software, 2001) based on permutations within blocks.

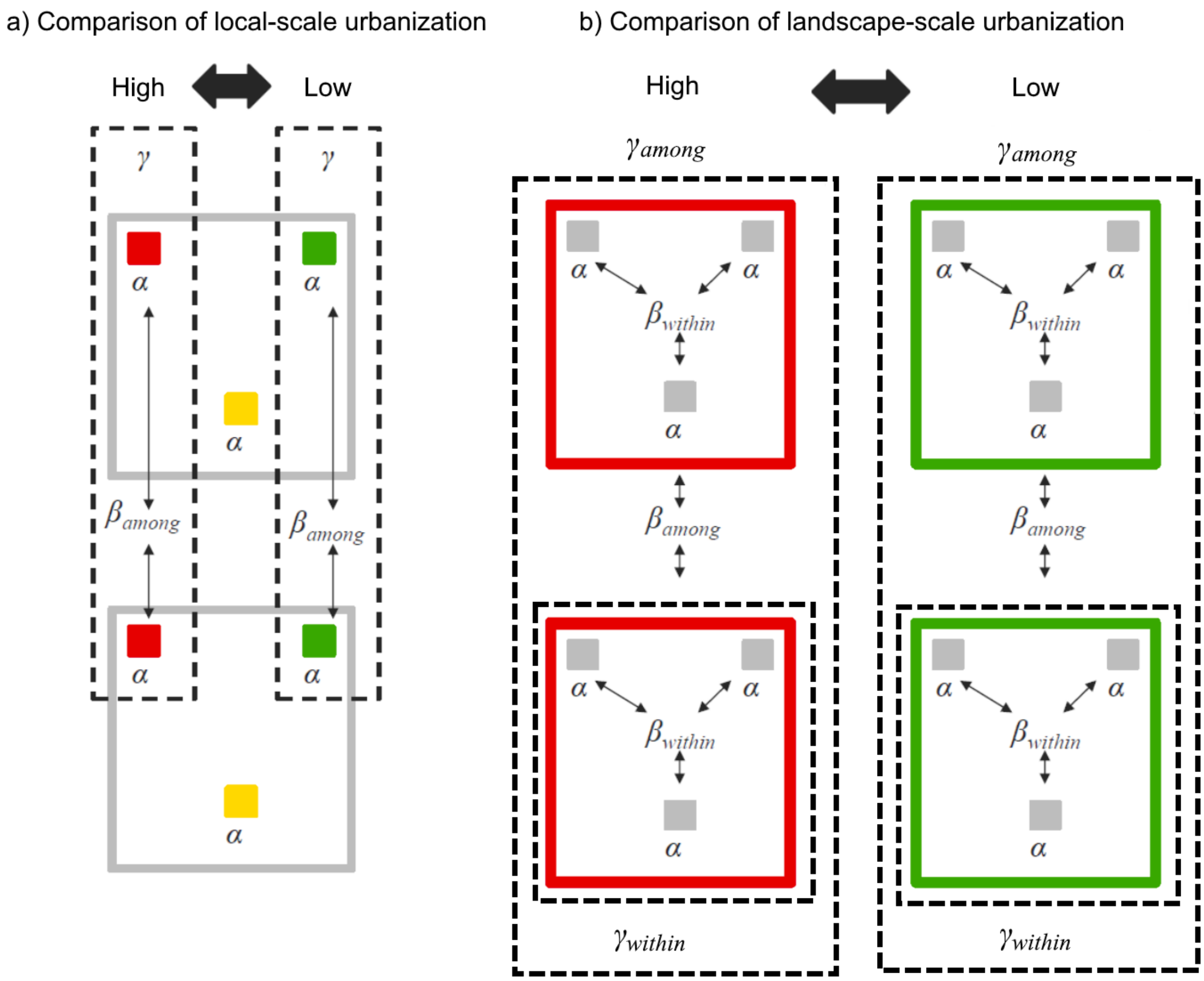


Figure 2 - Schematic overview of the calculated diversity components to test the effect of urbanization at local scale (a; 200 m x 200 m) and landscape scale (b; 3 km x 3 km) (low = green, intermediate = yellow, and high = red). Only the comparisons between low and high urbanization levels are shown for clarity.

RESULTS

Abundance

Although we could not detect an overall decrease in total abundance across the investigated groups along the urbanization gradient at both the local (Page test; $P > 0.05$) and landscape scale (Page test; $P > 0.05$), increasing the local-scale (subplot) urbanization level significantly decreased the abundance of all but one of the terrestrial arthropods: ground beetles, ground- and web spiders, butterflies and orthopterans (Table 1, Figure 3). This decline was most substantial for orthopterans and butterflies, with a reduction in abundance of 67.4% and 85.5% respectively, in the most urbanized compared to the least urbanized subplots. Local-scale urbanization had a much stronger effect on abundance than landscape-scale urbanization, which showed no effects in any of the investigated groups. An additional synergistic effect of local and landscape-scale urbanization was only observed for butterflies, with abundance decreasing stronger along the local urbanization gradient with increasing landscape-scale urbanization levels (Figure 3).

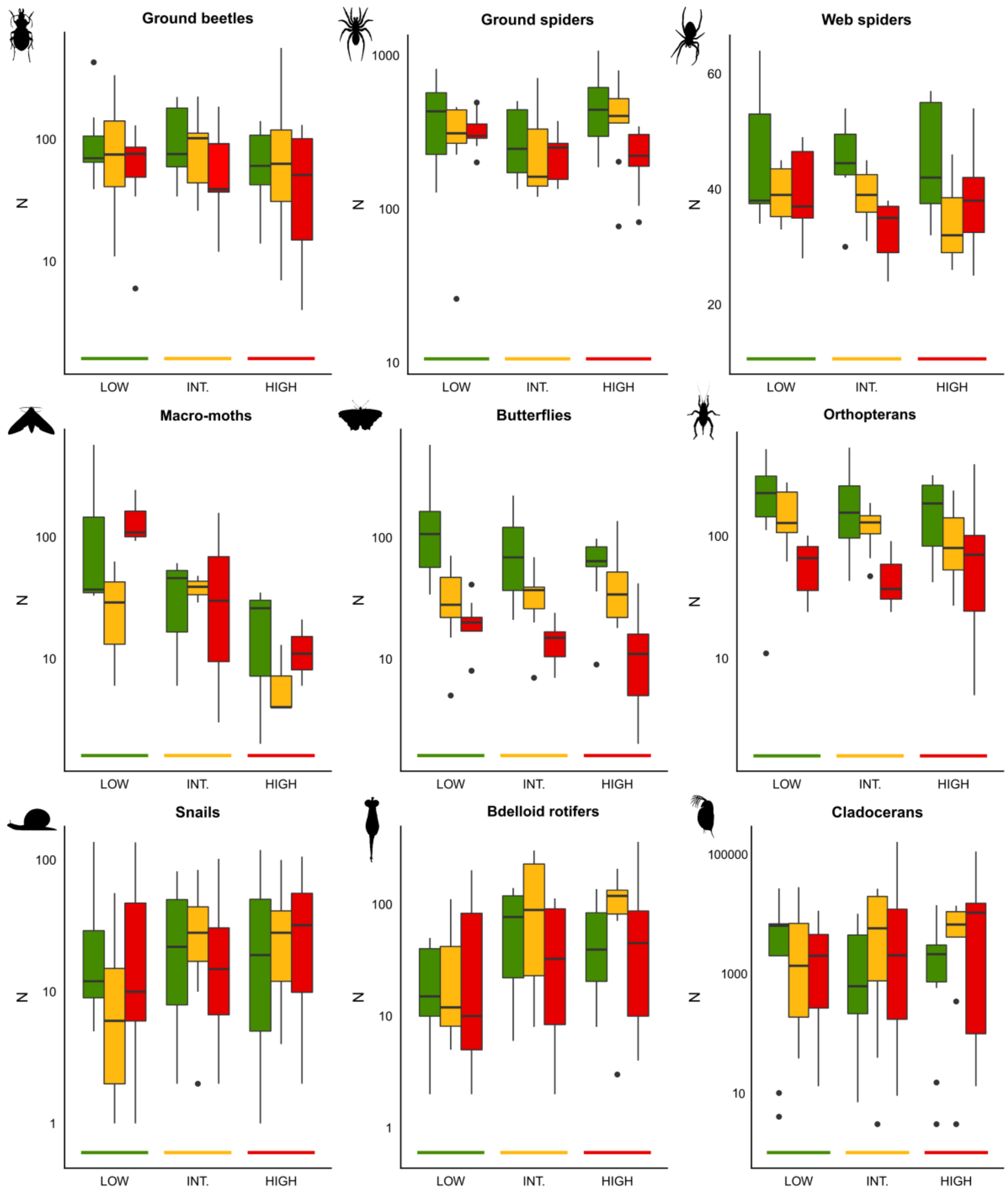


Figure 3 - Abundances (N) of the nine examined groups in response to local- (subplot) and landscape-scale (plot) urbanization levels. Labels at the x-axis represent the degree of urbanization at the landscape scale. Y-axis scale varies among groups and is $\log_{10}$-transformed, except for web spiders. Colours of the boxplots refer to urbanization levels at the local scale (green = low; yellow = intermediate; red = high). Boxplots display the median, 25% and 75% quartiles and 1.5 interquartile range. The nine animal silhouettes are from PhyloPic (http://www.phylopic.org) and fall under CC-BY 3.0 licences.

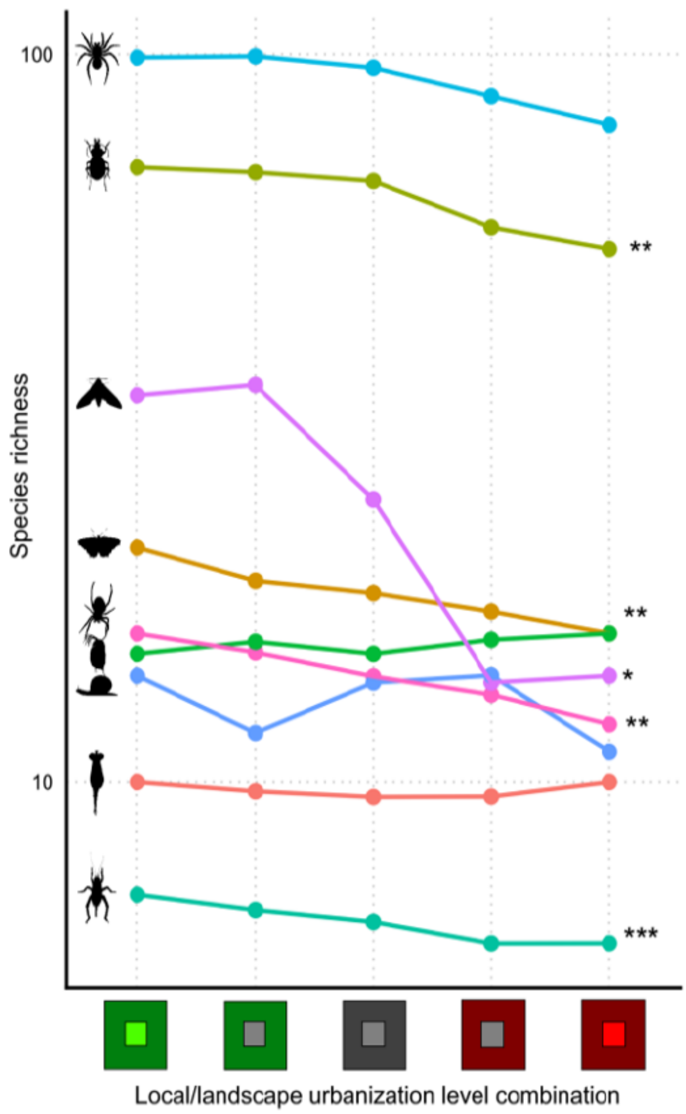
Table 1 - Test of the response in abundance towards urbanization at local (subplot) and landscape (plot) scale and their interaction. '% change' for the main effects is the percentage change in abundance in the highest compared to the lowest urbanization level. Significant effects are depicted in bold.

	Local (subplot) urbanization effect			**Landscape (plot) urbanization effect**			**Interaction**	
	F	*P*	*% change*	*F*	*P*	*% change*	*F*	*P*
Ground beetles	$F_{2,48}$ = 3.26	**0.047**	**-31.3**	$F_{2,48}$ = 0.430	0.654	-10.0	$F_{4,48}$ = 0.090	0.98
Ground spiders	$F_{2,48}$ = 5.16	**0.009**	**-36.5**	$F_{2,48}$ = 2.26	0.116	+8.1	$F_{4,48}$ = 1.11	0.36
Web spiders	$F_{2,35}$ = 8.15	**0.001**	**-19.2**	$F_{2,35}$ = 0.500	0.613	-5.1	$F_{4,35}$ = 1.19	0.33
Macro-moths	$F_{2,12}$ = 1.33	0.3	-17.5	$F_{2,12}$ = 2.62	0.114	-89.7	$F_{4,12}$ = 0.880	0.50
Butterflies	$F_{2,48}$ = 56.4	**0.001**	**-85.5**	$F_{2,48}$ = 0.340	0.71	-47.9	$F_{4,48}$ = 3.65	**0.01**
Orthopterans	$F_{2,48}$ = 18.4	**0.001**	**-67.4**	$F_{2,48}$ = 0.990	0.38	-23.0	$F_{4,48}$ = 1.94	0.11
Snails	$F_{2,48}$ = 0.220	0.8	-6.8	$F_{2,48}$ = 0.480	0.624	+33.3	$F_{4,48}$ = 0.670	0.61
Bdelloid rotifers	$F_{2,48}$ = 1.68	0.197	+29.3	$F_{2,48}$ = 2.90	0.065	+113.2	$F_{4,48}$ = 1.70	0.16
Cladocerans	$F_{2,48}$ = 0.61	0.547	+234.4	$F_{2,48}$ = 0.11	0.9	+54.0	$F_{4,48}$ = 0.36	0.83

Total species richness

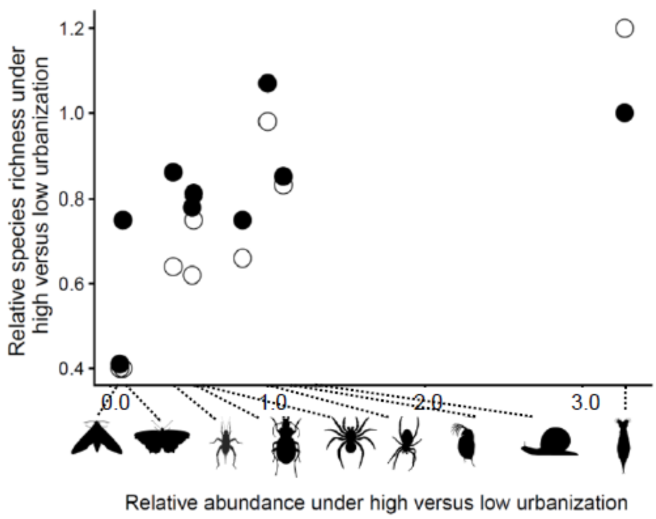
Sample-based accumulation curves showed a trend towards a slower accumulation of species at increasing local and/or regional urbanization levels for the majority of investigated groups (Figure S1). Rarefying richness to a size of nine samples for each combination revealed decreases in total species richness for five groups (i.e. ground beetles, web spiders, macro-moths, butterflies and orthopterans; $r_S P_c$ < 0.05; Figure 4a). A decline was also observed in total species richness across groups with increasing urbanization levels (Page-test; *P* < 0.001). Samples originating from a mixture of high, intermediate and low urbanized plots and subplots had a lower species richness compared to those based on samples from low urbanized subplots in low urbanized plots only, indicating that plots consisting of a mosaic of high and low urbanized subplots harbour less species across groups compared to low urbanized plots (Page-test; *P* = 0.007). Other pairwise comparisons between the urbanization categories were also significant (Page test; *P* < 0.03), except for high local/landscape urbanization versus high landscape urbanization (Page test; *P* = 0.15) and low local/landscape urbanization versus low landscape urbanization (Page test; *P* = 0.45).

a)



100
10
Species richness
**
**
*
**
***
Local/landscape urbanization level combination

b)



1.2
1.0
0.8
0.6
0.4
Relative species richness under high versus low urbanization
0.0
1.0
2.0
3.0
Relative abundance under high versus low urbanization

403

Figure 4 – (a) Estimated total number of species for each examined group in nine random samples from five different local/landscape urbanization level combinations using raw data. Y-axis scale is $\log_{10}$-transformed to improve visualization. Pictograms on the x-axis depict (from left to right): (i) low urbanized subplots in low urbanized landscapes (light green square in dark green square); (ii) low urbanized landscapes regardless of the degree of local urbanization (light grey square in dark green square); (iii) samples regardless of the degree of local and landscape urbanization level (light grey square in dark grey square); (iv) high urbanized landscapes regardless of the degree of local urbanization (light grey square in dark red square) and (v) high urbanized subplots in high urbanized landscapes (light red square in dark red square). Asterisks (* = $0.01 < P < 0.05$, ** = $0.01 < P < 0.001$, *** = $P < 0.001$) depict results of the directional ordered heterogeneity test $r_S P_c$.. (b) Correlation between urbanization-related change in abundance versus change in local (open circles) and total (closed circles) observed species richness across taxonomic groups. Values on both axes represent the relative abundance (x-axis) and species richness (y-axis) in high urbanized subplots in high urbanized landscapes versus those in low urbanized subplots in low urbanized landscapes. Animal silhouettes are from PhyloPic (http://www.phylopic.org) and fall under CC-BY 3.0 licences.

We further tested if the decrease in species richness is higher for taxonomic groups that show a strong decrease in abundance, as this would indicate that the decrease in species richness is, at least partly, due to a lower sampling effect in urbanized landscapes. More precisely, we correlated the relative change in species richness in high urbanized subplots in high urbanized landscapes versus low urbanized subplots in low urbanized landscapes with the relative change in abundance (Figure 4b). Groups showing the strongest decrease in abundance (moths, butterflies, grasshoppers, ground beetles and ground spiders) showed a significant reduction in local species richness (i.e. average species richness within subplots) ($r = 0.88$, $P = 0.001$), but not for total species richness (i.e. species richness across subplots) ($r = 0.59$, $P = 0.1$).

Species richness decomposition

High local- and landscape-level urbanization reduced total (γ) species richness across the investigated groups by 7% and 14%, respectively (Page test; P = 0.026 and P = 0.003, respectively; Figure 5; Table 2). Increased landscape-level urbanization also decreased average local (α) species richness by 14% (Page test; P = 0.047) but did not result in a consistent change in species variation (β) across the investigated groups (Figure 5; Table 2).

Group specific responses were highly heterogeneous, but, except for bdelloid rotifers and cladocerans, all groups showed a significantly negative response towards increasing local- and/or landscape-scale urbanization for at least one of the calculated diversity components (Table 2). Increased local urbanization primarily decreased local (α) diversity of butterflies and orthopterans and decreased (additive) variation in species composition (β_A) of ground beetles, snails and orthopterans. The effects of landscape-scale urbanization resulted in decreases in local diversity of web spiders and macro-moths, a decrease in variation among local communities within urbanized landscapes ($\beta_{A,within}$) in macro-moths and a decrease in variation among urbanized landscapes ($\beta_{A,among}$) in ground beetles, ground spiders and orthopterans. Positive relationships with increasing urbanization were observed in butterflies, showing positive responses in both proportional and additive variation in species composition among locally urbanized sites. A positive relationship with increasing urbanization was also observed for web spiders, with an increase in variation among urbanized landscapes ($\beta_{A,among}$). Similar results were observed for cladocerans, which showed increasing local diversity within urbanized landscapes along the urbanization gradient.

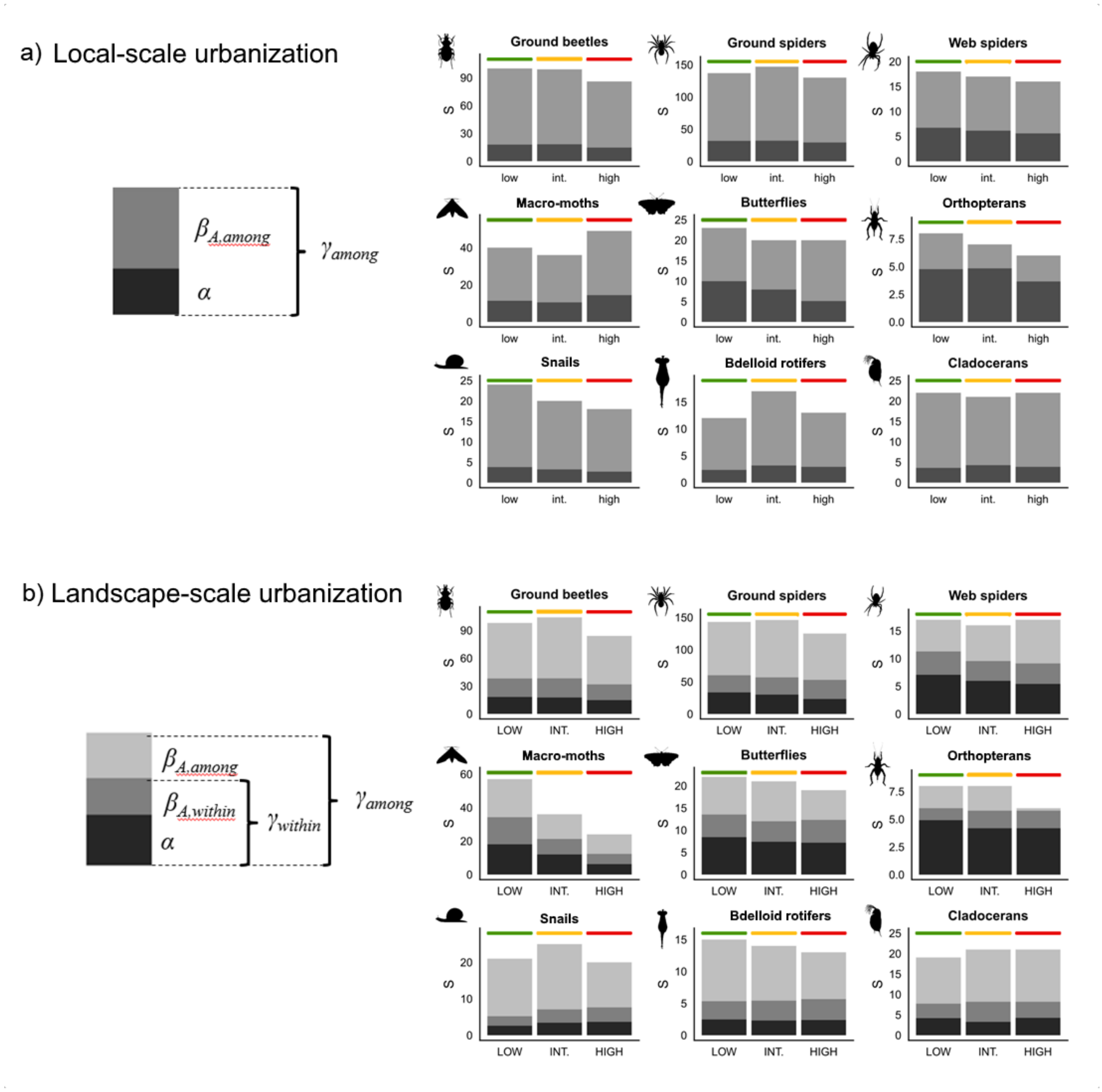


Figure 5 - Total observed diversity (S) partitioning for each examined group and for each of three (a) local- and (b) landscape-scale urbanization levels (green = low; yellow = intermediate; red = high). See Figure 2 for an explanation of the different diversity components. The nine animal silhouettes are from PhyloPic (http://www.phylopic.org) and fall under CC-BY 3.0 licences.

Table 2 – Differences in observed (a) and rarefied (b) species richness components across the three urbanization categories. Plus and minus signs indicate an increase and decrease in species richness from the lowest towards the highest urbanization category respectively, while NT indicates that no difference was detected. Asterisks refer to comparisons wherein the intermediate urbanization level showed higher or lower values compared to the low and high urbanized categories. Colour codes refer to significance values (light red/light green/light yellow -/+: 0.05 > P > 0.01,

red/green/yellow --/++: 0.01 > P > 0.001 and dark red/dark green/dark yellow ---/+++: P < 0.001). $\bar{\beta}_P$ and $\bar{\beta}_A$ refer to proportional ($\bar{\beta}_P = \gamma/\bar{\alpha}$) and additive ($\bar{\beta}_A = \gamma - \bar{\alpha}$) beta diversity, respectively, wherein $\bar{\beta}_P$ expresses the number of times by which the richness at plot (or regional) level increases compared to the richness at subplot (or plot) level, while $\bar{\beta}_A$ expresses the absolute increase in number of species between these two sampling levels.

a	**Local urbanization**				**Landscape urbanization**						
	α	β_P	β_A	γ	α	$\beta_{P,within}$	$\beta_{A,within}$	γ_{within}	$\beta_{P,among}$	$\beta_{A,among}$	γ
Ground beetles	-	-	---	-	-	+	+	-	+	---	-
Ground spiders	-	+	-	-	-	-	-	-	-	-	-
Web spiders	-	+	-	-	-	+	-	-	+	+	NT
Macro-moths	-	+	+	+	--	-	-	-	+	-	-
Butterflies	--	++	+	-	-	+	-	-	-	-	-
Orthopterans	-	-	-	-	-	+	+	NT	--	---	-
Snails	-	+	---	-	+	+	+	+	-	-	-
Bdelloid rotifers	+	+	+	+	-	+	+	+	-	-	-
Cladocerans	+	+	---*	-	+	-	-	NT	+	+	+
Across groups	-	+	-	-	-	+	-	-	-	-	--
b	**Local urbanization**				**Landscape urbanization**						
	α	β_P	β_A	γ	α	$\beta_{P,within}$	$\beta_{A,within}$	γ_{within}	$\beta_{P,among}$	$\beta_{A,among}$	γ
Ground beetles	-	-	--	-	-	+	+	--	+	---*	-*
Ground spiders	NT	NT	-	NT	-	+	-	-	-	---*	-
Web spiders	-	NT	-	-	--	NT	-	-	+	+	NT
Macro-moths	+	+	+++*	+	-	-	-	-	+	-	NT
Butterflies	NT	+	+++	+	-	NT*	+	-	-	NT	-
Orthopterans	-	-	---	-	-	+	+	NT	-	-	-
Snails	-	NT	---	-	+	NT	+	+	-	-	-
Bdelloid rotifers	-	+	---	+	NT	NT	NT	NT	-	-	-
Cladocerans	+	-	--*	-	+*	+	-	+	+	+	+
Across groups	-	+	-	-	-	+	-	-	+	-	-

Results obtained from rarefied richness roughly corresponded with the results of observed richness, but generally resulted in weaker urbanization effects at the α and γ levels (Table 2b). For example, the effect of urbanization at local (α) scale was reduced for some groups (e.g. macro-moths, butterflies and orthopterans) when considering rarefied compared to observed richness. In contrast to observed richness, there is no detectable across-group decline in rarefied total (γ) diversity due to either local or landscape urbanization. Conversely, rarefying richness generally led to more negative effects of local urbanization levels on additive species variation (β_A), with declines for six groups.

Across-group analysis revealed that increasing levels of landscape urbanization led to an average decline in rarefied local (α) richness (Page test; P = 0.023) and an increase in proportional variation in rarefied species richness (Page test; P = 0.011) within plots ($\beta_{Pwithin}$).

DISCUSSION

Urbanization is expected to inflict major impacts on biodiversity and ecosystem functioning, together with other large-scale anthropogenic disturbances, such as agricultural intensification and deforestation (Grimm et al., 2008; Shochat et al., 2010). Yet, studies show inconsistent responses that are likely attributed to differences in the examined groups, spatial extent at which urbanization was assessed, the range of the urbanization gradient and the spatial scale at which the responses to urbanization are measured (Aronson et al., 2014; Faeth, Bang & Saari, 2011; Marzluff, 2017; Saari et al., 2016). To account for variation in group- and scale-specific effects, we here integrate data from multiple groups and multiple spatial scales in a study sampling identical urbanization gradients and demonstrate that urbanization drives declines in the abundance for most investigated groups and species richness across the examined groups. In line with the previously reported heterogeneous patterns of biodiversity along urbanization gradients, we found that group-specific responses strongly depended on the spatial scale at which urbanization and species richness are assessed. Integrating data across multiple spatial scales and multiple taxa is therefore required to provide an overall view of these general relationships. There is currently little consensus on the expected response of total abundance of organisms to urbanization, as both increases and declines have been reported (Chace & Walsh, 2006; Grimm et al., 2008; Shochat et al., 2010). Increases in abundance could be due to the dominance of a few synanthropic species with superior competitive abilities, enhanced by increased human-mediated food resources and reduced predation (Parris, 2016). Alternatively, the hostile environment imposed by urban structures and the consequent decreased connectivity and size of suitable habitat patches may deplete individuals and species from urban settlements (McKinney, 2008, Saari et al., 2016). Although we could not demonstrate a decline in abundance across the entire set of examined groups in response to local urbanization, significant declines were observed at the group-specific level for ground beetles, ground- and web spiders, butterflies and orthopterans. Since ground beetles and ground spiders were sampled with pitfall traps, their estimated abundances could potentially be biased by differences in species activity between high and low urbanized sites, due to variation in local physical parameters, such as temperature. However, in a related study we demonstrated that temperatures are higher at the highly urbanized sampling sites (i.e. UHI-effect, Merckx et al. 2018), thus higher arthropod numbers would have been expected in the urbanized sites, which is opposite to what we observed.

The observed declines support the idea that poor environmental conditions in urban environments decrease the average densities across major organism groups, notably terrestrial arthropods in our study. There were three organism groups for which we did not observe declines in abundance along the urbanization gradient: snails, bdelloids and cladocerans. The latter two groups are small (semi)aquatic organisms that have high dispersal capacities (Fontaneto et al., 2011; Gianuca et al., 2018) and do not need large habitat patches to thrive. Snails host a number of species that prefer habitats that are abundant in cities, such as patches of soils that are moist because they are covered with debris, stones and other building material.

The obvious decline we observed for terrestrial arthropods parallels the recent reports on global declines of insects, even in areas safeguarded from obvious anthropogenic disturbances (Hallmann et al., 2017; Vogel, 2017; Sánchez-Bayo & Wyckhuys, 2019). Identifying the main causes driving this decline is, however, difficult given the multifaceted influence that urbanization exerts on the environment (Parris, 2016). In particular, the urban-heat-island effect may be put forward as a possible factor driving the observed decline in animal abundance. In fact, temperature increase has recently been identified as one of the dominant factors affecting arthropod numbers, with bottom-up effects towards higher trophic levels feeding on these organisms (Lister & Garcia, 2018). The abundance response was only observed under local-scale urbanization levels, which is congruent with the urban-heat-island effect being indeed more pronounced at local spatial scales (Kaiser et al. 2016; Merckx et al., 2018; Brans et al., 2018).

The observed declines in abundance likely represent a rather conservative view on the actual abundance patterns in urban landscapes. To allow comparison between high and low urbanized landscapes, sampling was restricted to green infrastructures (e.g. grassy/herbaceous vegetation, ponds). In the most urbanized landscapes, such as cities, these sampled green infrastructures might be less common than in rural areas, as they are embedded within built-up areas that likely harbor even lower abundances of the investigated groups. It can thus be expected that the observed declines in abundances, and their consequences for ecosystem functioning, are even more pronounced in the most urbanized areas than suggested by our analyses.

By integrating species richness data from groups that widely differ in diversity, life-history traits and ecological profiles, we showed an overall decrease in total species richness with increasing levels of local and/or landscape-scale urbanization. We demonstrate that sites and landscapes of low urbanization level harbour a richer species pool compared to areas consisting of a mosaic of urban and non-urban areas. This suggests that the faunal composition of urbanized regions is hardly characterized by species that are absent in less urbanized regions. The significant decrease in abundance for the insect groups also points in this direction, since synanthropic species are expected to become dominant, and might thus increase total abundance in urban areas (Shochat et al., 2010), opposite to what we observed.

When partitioning diversity into its components, the cross-group decline in species richness was most clearly observed at the level of total (γ) diversity at both local and landscape scales. However, we found strong differences among the animal groups with respect to the diversity component that was most strongly affected, with significant trends either at α (e.g. web spiders, butterflies) or β (e.g.

ground beetles, orthopterans) level. Thus, although the overall declining trend of total diversity summarizes the decline across all groups and all diversity components (Crist et al., 2003), the differential response of each group points to the ecological and scale-dependent complexity of metacommunity responses to urbanization (Chace & Walsh, 2006; Hill et al., 2017; Luck & Smallbones, 2010; Leibold & Chase, 2017; McKinney, 2008).

For all diversity components we observed a significant decrease for at least one of the examined groups, thus demonstrating that both local species loss (α-diversity) and biotic homogenization (β-diversity) at all spatial levels may potentially contribute to a decrease in total species richness.

For some groups, such as macro-moths, diversity components declined at multiple spatial scales. Local macro-moth communities are thus not only impoverished within sites located within urban landscapes, but they are also highly homogeneous among sites within urban landscapes. We further detected biotic homogenization at the largest spatial scale (i.e. across urban landscapes) for ground beetles, ground spiders and orthopterans, and across groups. This suggests that more homogeneous environmental conditions of urbanized areas may filter ecologically and taxonomically similar species from the total species pool (Baldock et al., 2015; Ferenc et al., 2014; La Sorte et al., 2014; McKinney, 2006; but see Brice et al., 2017 and Knop, 2016 for contrasting results). The strong homogenizing effect of urban environments and landscapes has been most clearly demonstrated by shifts in community life-history traits in response to urbanization (Concepción et al., 2016; Croci et al. 2008; Knop, 2016; McCune & Vellend, 2013; Merckx et al., 2018; Penone et al., 2013). For instance, elsewhere we demonstrated how urbanization causes a clear depletion of ground beetle, butterfly and macro-moth species with poor dispersal capacity (Piano et al., 2017; Merckx & Van Dyck, 2019). Although convergence of biotic communities in urban environments has been shown to be more consistent at the level of community trait values compared to at the taxonomic level (Brans et al., 2017; Gianuca et al., 2018), the results presented here demonstrate that urbanization may not only decrease diversity in functional groups, but also at the level of species richness itself.

Rarefying species richness generally resulted in less strong urbanization effects, in particular at the local scale. We showed that groups with a strong decline in abundance, like orthopterans and butterflies, showed a concomitant decline in local species richness. This suggests that the decrease in local species richness with increasing urbanization might, at least partly, be driven by a sampling effect due the decrease in individual abundances and less so by changes in the local species pool and/or evenness of local communities (Chase & Knight, 2013). However, although we rarefied richness to the lowest number of individuals within each group, this procedure could potentially lead to the comparison of different points in the rarefaction curves among urbanization categories, e.g. the end of the curve (total richness in the regional pool) in high urbanized sites against the base of the curve (evenness) in low urbanized sites (McGlinn et al., 2019). Therefore, one must be prudent in interpreting the decrease in local (α) species richness as a mere sampling effect. Alternatively, rarefying species richness resulted in a stronger effect of local urbanization on variation in species composition among plots, with ground beetles, ground spiders, orthopterans, snails and bdelloid rotifers all showing significant decreases in beta diversity. Only for butterflies we observed positive effects of local urbanization on beta diversity.

It should be pointed out that our sampling design did not allow to explicitly test whether urban plots have a different overall – i.e. across habitats – species richness compared to less urbanized plots, as we sampled the same habitat type within taxonomic groups. It has been proposed that cities may sustain high levels of biodiversity, playing an important role in the conservation of global biodiversity and threatened species (Beninde, Veith & Hochkirch 2015, Ives et al. 2016, Aronson et al. 2017).due to their habitat heterogeneity that allow species with different habitat preferences to co-exist on small spatial scales (Aronson et al. 2017). In other words, cities host several different habitat types (e.g. ruderal habitats, grasslands, wooded areas,…) within smaller areas compared to natural landscapes, thus increasing the number of species per unit area. However, comparisons across habitats primarily reflect the change in species number per unit area without providing clear information on loss of species within each habitat. Our sampling design allowed us to investigate diversity patterns without confounding factors related to habitat type. We could thus reveal that urbanization impoverishes the fauna within habitat patches and, consequently, that future loss of species due to urbanization is to be expected. This was further suggested by the higher number of species in more natural landscapes compared to landscapes composed of a mosaic of high and low urbanized subplots and indicates that urban environments hardly contain species that are not found outside the urban areas.

Overall, by applying a multi-scale approach across multiple animal groups, we demonstrated a negative overall effect of urbanization on insect abundance and diversity of a range of terrestrial and (semi)aquatic taxa. Our results suggest that urbanization could exert a strong impact on ecosystem functioning and services, as it negatively affects groups that play a central role in a variety of ecological processes, like nutrient cycling (e.g. snails, butterflies, orthopterans and macro-moths), pollination (e.g. butterflies and macro-moths), predation (ground beetles, ground- and web spiders) and grazing (cladocerans). However, we also highlight that the responses to urbanization strongly depend on the examined group, scale of urbanization and scale at which diversity is assessed. These complex interactions might strongly impact the way urbanization affects ecological interactions.

Acknowledgements

This research has been funded by the Interuniversity Attraction Poles Programme Phase VII (P07/4) initiated by the Belgian Science Policy Office. Chantal Van Nieuwenhove and Pieter Vantieghem are gratefully acknowledged for sorting out the large amount of pitfall samples. We thank Aurélien Kaiser for his contribution to the sampling of butterflies and orthopterans, Edwin van den Berg for counting and identifying species in the zooplankton samples, Jasper Dierick for the sampling and identification of web spiders, and Marc Van Kerckvoorde and Marc Hanssen for the identification of ground beetles and snails respectively. FTTH was supported by the Science without Borders program [process number: 45968/2012-1] of Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brazil. The authors declare no conflicts of interest.

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