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Urbanization drives cross-taxon declines in abundance and diversity at multiple spatial scales Peer-reviewed author version

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

2 spatial scales

3 Running title: urbanization impacts abundance and diversity

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43 Abstract

44 The increasing expansion of urban landscapes is hypothesized to drastically alter (semi-)natural 45 environments with a concomitant major decline in species abundance and diversity. Yet, studies on 46 this effect of urbanization, and the spatial scale at which it acts, are at present inconclusive due to 47 the large heterogeneity in taxonomic groups and spatial scales at which this relationship has been 48 investigated among studies. Comprehensive studies that analyse this relationship across multiple 49 animal groups and at multiple spatial scales are rare, hampering the assessment of how biodiversity 50 generally responds to urbanization. Here, we studied aquatic (cladocerans), limno-terrestrial 51 (bdelloid rotifers) and terrestrial (butterflies, ground beetles, ground- and web spiders, macro-52 moths, orthopterans and snails) invertebrate groups using a hierarchical spatial design wherein three 53 local-scale (200 m \times 200 m) urbanization levels were repeatedly sampled across three landscape-54 scale (3 km× 3 km) urbanization levels. We tested for local- and landscape urbanization effects on 55 abundance and species richness of each group, wherein species richness was partitioned into the 56 average richness of local communities and richness due to variation among local communities. 57 Abundances of the sampled terrestrial actively dispersing arthropods declined in response to local 58 urbanization, with reductions up to 85% for butterflies, while passive dispersers did not show any 59 clear trend. Species richness also declined with increasing levels of urbanization, but responses 60 were highly heterogeneous between the different groups with respect to the species richness 61 component and the spatial scale at which urbanization impacts species richness. Depending on the 62 group, species richness declined due to the homogenization of species assemblages and/or a 63 decrease in local species richness. This resulted in an overall decrease in total species richness 64 across groups in urban areas. Our results provide strong support to the general negative impact of 65 urbanization on abundance and species richness within habitat patches and highlight the importance 66 of considering multiple spatial scales and taxa to assess the impact of urbanization.

67

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

70 INTRODUCTION

71 The conversion of natural and rural land to urban environments increased drastically worldwide 72 over the last 30 years, with urban land cover expected to be tripled from 2000 to 2030 (Seto, 73 Güneralp & Hutyra 2012). Urbanization drives global environmental change and currently 74 represents one of the main anthropogenic impacts (Parris 2016) with expected drastic consequences 75 on biodiversity and ecosystem processes. Urbanization-associated changes in community structure 76 could result from several mechanisms (Rebele, 1994; Seto, Sánchez-Rodríguez & Fragkias, 2010), 77 which act at multiple spatial scales (Shochat, Warren, Faeth, McIntyre & Hope, 2006; Shochat et 78 al., 2010) and are strongly habitat-dependent (Hill et al., 2017). Ecological effects have been shown 79 to result from substantial changes to the local abiotic environmental conditions (e.g. high levels of 80 nutrients, pollution, and imperviousness) (Parris, 2016), and to landscape structure (e.g. reduced 81 size and connectivity and increased temporal turnover of habitat patches) (McDonnell, et al. 1997; 82 Parris, 2016).

83 Several studies investigated relationships between urbanization and two important determinants of 84 ecosystem functioning i.e. the abundance and/or diversity of species. Yet, their results are 85 surprisingly equivocal, as negative relationships (Chace & Walsh, 2006; Lagucki, Burdine & 86 McCluney, 2017; Niemelä & Kotze, 2009; Ramirez-Restrepo & Macgregor-Fors, 2017; Saari et al., 87 2016), no relationship (Christie & Hochuli, 2009) as well as positive relationships (Hill et al., 2017; 88 McKinney, 2008; Shochat et al., 2010), are reported. These heterogeneous results suggest that the 89 effect of increasing urbanization might strongly depend on the spatial scale and organism group at 90 which it is assessed (Concepción et al., 2015; Egerer et al., 2017; McKinney, 2008; Philpott et al., 91 2014).

92 First, the direction and magnitude of changes in species diversity in response to an environmental
93 driver may strongly depend on the spatial scale at which species diversity is measured (Chase &
94 Knight, 2013). For instance, urbanization may filter out species that are not pre-adapted to urban

95 conditions, with a consequent decrease in abundance or diversity at small (local) spatial scales 96 (Bates et al., 2011; Piano et al., 2017). Alternatively, the loss of species that are less adapted to 97 urban environments could be (over)compensated by an increase of species that are efficient in 98 exploiting urban resources, including exotic taxa (McKinney, 2006; Menke et al., 2011; Sattler, 99 Obrist, Duelli & Moretti, 2011). Both phenomena may cause biotic homogenization if local 100 communities are generally colonized by the same species, increasing in turn the compositional 101 similarity of urban species assemblages and, consequently, reducing species richness of urban areas 102 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 120 repeatedly sampled across the same three urbanization levels at the landscape scale (Merckx et al. 121 2018). The sampling design allows us to partition the total species richness (γ -diversity) into 122 richness within local communities (α -diversity) and richness due to variation in species composition 123 among local communities (β -diversity), and to relate these to both local and landscape-scale 124 urbanization levels. We tested (i) if, and in which direction, local and landscape-scale urbanization 125 affect total abundance; (ii) if local and landscape-scale urbanization affect species richness within 126 habitat patches, and if so at which spatial scale; and (iii) to what extent these responses are 127 consistent across animal groups.

128

129 MATERIALS AND METHODS

130 Sampling area and design

131 Sampling was conducted in Belgium, within a polygon of 8140 km², encompassing the cities of 132 Brussels, Antwerp and Ghent. It is a densely populated region (average human population density 133 of Belgium: 371 inhabitants/km², IBZ, 2018) that is composed of urban areas embedded within a 134 semi-natural and agricultural matrix. Because urbanization encompasses a range of factors that alter 135 the physical environment and landscape characteristics, we defined the percentage of built-up area 136 (%BU) as a proxy for urbanization and it was assessed with a GIS software using an object-oriented 137 reference map of Flanders as a vectoral layer (LRD, 2013). This layer included the precise contours 138 of all buildings, while roads and parking infrastructures were excluded. To test effects of 139 urbanization at the landscape scale, we selected 27 plots (i.e. squares of 3 km \times 3 km), among which nine located in low urbanized areas (low: 0%-3%BU), nine plots in areas with intermediate 140 141 urbanization (intermediate: 5%-10% BU) and nine in high urbanized areas (high: > 15% BU) (Figure 142 1). The latter encompassed city centres. Given that only buildings are considered for the calculation 143 of %BU, values of 15% can be considered highly urbanized. We first selected plots within this

144 highest %BU category that were approximately equidistant from each other within the study area. 145 Next, plots of the intermediate and lowest urbanization category were selected within 10 km - 25 146 km of the highest urbanized plots. This plot selection strategy resulted in an even spread of plots 147 within the same urbanization category across the study area and ensured a minimal spatial 148 autocorrelation of plot urbanization levels. Across plots, %BU was positively correlated with the 149 amount of other impervious substrates such as roads and artificial constructions (bridges, viaducts, 150 locks, ...) ($r_{\rm S} = 0.94$; P < 0.0001) and negatively correlated with the area of semi-natural habitat ($r_{\rm S}$ 151 = -0.85; P < 0.0001) (Figure S1), thus representing a reliable proxy of urbanization. To investigate 152 effects of local-scale urbanization, each plot was divided into local subplots of 200 m \times 200 m, 153 which were classified into urbanization categories using identical %BU thresholds as used at plot 154 level. Within each plot, we then selected one subplot of each urbanization category (i.e. low, 155 intermediate and high). This selection was random within the constraints imposed by the 156 availability of targeted habitats (e.g. pond, grassland, woodland), accessibility and the permission to 157 sample.

158 This sampling design resulted in a total of up to 81 sampling sites (i.e. 9 plots \times 3 landscape-scale 159 urbanization levels \times 3 local-scale urbanization levels) (Figure 1) and guaranteed that urbanization 160 at landscape and local scales are uncorrelated and, hence, that their effects, as well as their 161 interaction, could be tested simultaneously. The same sampling design was applied to all examined 162 groups, and all sampling was based on the identical set of plots (landscape-level of urbanisation). At 163 the local level too, the same sampling design was implemented across organism groups, but the 164 choice of specific subplots featuring a given level of local urbanisation within each plot could differ 165 between groups as sampling sites suitable for all groups were not always present within the same 166 200 m x 200 m subplot. With the exception of web spiders and macro-moths, most or all of the 81 167 subplots were sampled for each animal group (see Sampling methods).





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Figure 1 - Map of the study area, in the northern part of Belgium, showing the location of the 27 sampled landscapescale 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 \times 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.

177 Sampling methods

178 *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

182 lost during the last sampling campaign (end of June), data from the last sampling session were not 183 used for analysis. To reduce confounding effects of differences in habitat type between subplots 184 with varying levels of urbanization, pitfall traps were placed consistently in grassy-herbaceous 185 vegetation such as road verges, park grasslands and grasslands at the different subplot urbanization 186 levels. Samples were preserved in 4% formalin and sorted in the laboratory. Data from both pitfall 187 samples per site and the different sampling dates were pooled and treated as a single sampling unit. 188 All ground beetle and adult spider individuals were counted and identified to species level (Boeken, 189 2002; Duff, 2016; Roberts, 2009). Juvenile spiders were excluded from the final dataset since they 190 could only be identified to genus level.

191 Web spiders

Web spiders were sampled by hand between the 27th of August and the 5th of October 2014 in 62 192 193 out of the 81 subplots. One landscape (3 subplots) was sampled per day. Each subplot was explored 194 by the same two persons for about 4.5-person hours. Spiders were detected by looking for their 195 webs and each subplot was completely explored searching for orb-weaving spiders until no new 196 individual could be found after 15 min. Rainy days were avoided as spiders may be less likely to 197 build webs and are thus less detectable. Every encountered spider was caught and stored in 70% 198 ethanol. Identification was performed under a stereomicroscope to species level (Roberts, 2009). 199 Juveniles were excluded from the final dataset since they could only be identified to genus level. 200 Spiders captured according to this methodology are further referred to as 'web spiders' to 201 distinguish them from the 'ground spiders' that were captured by pitfall traps (see section Ground 202 beetles and ground spiders).

203 Macro-moths

204 Sampling was restricted to a set of nine plots, three of each plot urbanization category, and 205 performed in woodland 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 206 March and 1st of April 2015 (third session). Traps were emptied on 3rd-4th of August 2014 (first 207 session), 2nd-3rd of September 2014 (second session) and 24th-25th-26th of April 2015 (third session). 208 Traps were baited with sugar-saturated wine and sampled individuals were poisoned with 209 210 chloroform within the traps. Individuals were counted and identified to species level (Manley, 211 2010), except for two species pairs: Mesapamea secalis/secalella and Hoplodrina 212 blanda/octogenaria.

213 Butterflies and orthopterans

214 Butterflies and orthopterans (grasshoppers and bush crickets) were sampled along standard transects 215 in three sampling sessions performed in 2014, from July to early September. Walks of 20 minutes 216 were performed in each of the 81 subplots in grasslands during the warmest hours of the day, i.e. 217 between 10 a.m. and 4 p.m. avoiding cloudy and rainy days. Butterflies were sampled with visual 218 counts along a transect ('Pollard walk', Pollard & Yates, 1993), with occasional netting of 219 individuals when needed for species identification. All individuals were identified in the field to the 220 species level following Bink (1992). Orthopterans were sampled through auditive counts with 221 occasional visual inspection of individuals.

222 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.

229 Bdelloid rotifers

230 Communities of bdelloid rotifers were sampled by collecting lichen patches of the genus Xanthoria, 231 for which bdelloid rotifer communities have been previously studied in Europe (Fontaneto, 232 Westberg & Hortal, 2011). Suitable Xanthoria patches could be found in all but one subplot. 233 Sampling was performed between June and July 2013. The selection of the lichen was haphazard: 234 the first lichen patch encountered in each subplot was collected. Dry lichen thalli between 3 and 10 235 cm² were cut from the substrate with a knife and kept in paper bags. For each lichen sample, an area 236 of 2.5 cm² was hydrated with distilled water in a plastic petri dish. All active bdelloid rotifers that 237 recovered from dormancy in the following four hours after hydration were sorted and identified to 238 species level (Donner, 1965). Previous studies on bdelloid rotifers in these lichens (Fontaneto et al., 239 2011) revealed that animals start recovering between 10 and 40 minutes after hydration of the 240 sample and that no more bdelloid rotifers are recovered after four hours. The very few dormant 241 stages still found in the sample that did not recover after that time were considered dead and 242 excluded from the analyses.

243 Cladocerans

244 Water samples were collected from ponds using a tube sampler (length = 1.85 m; diameter = 75245 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 Mav to the 246 10th of July 2013. In each pond, eight sampling locations were selected using a predefined grid, 247 248 assuring that different microhabitats (shallow and deeper zone, different locations with respect to 249 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 250 251 eight locations, 12 L of water was collected, resulting in a total of 96 L per pond. The tube sample 252 integrated the entire water column, but resuspension and subsequent sampling of bottom material 253 was avoided. For each pond, 40 L of water was filtered through a 64 µm conical net. The sample 254 was then collected in a 60 mL vial and fixed with formalin (4%). Additional sampling was 255 performed with a sweep-net (64 µm net) and preserved in the same way. These additional samples 256 served to guarantee sufficiently extensive sampling to reconstruct an as complete as possible 257 species list. Individuals in standardized subsamples were identified and counted; entire subsamples 258 were counted until at least 300 individuals were identified and no new species was found the last 259 100 specimens. Samples containing less than 300 individuals were counted completely, and the 260 additional qualitative samples for those ponds were screened for additional species. Species 261 identification was based on Flößner (2000). Daphnia longispina, Daphnia galeata and Daphnia 262 hyalina were combined in the Daphnia longispina complex due to the morphological similarities 263 and possible hybridization between the species. Detailed information on the sampling and 264 identification of zooplankton are reported in Brans et al. (2017) and Gianuca et al. (2018). Densities 265 were calculated as number of individuals per litre of the original sample.

266 Abundance data and analysis

267 The total number of sampled/observed individuals in each sample/transect was used as an estimate 268 for the abundance of each group in each subplot. For cladocerans, our abundance data are based on 269 the total number of individuals in a standardized volume of 40L. Differences in abundances in 270 response to local (subplot) and landscape (plot) scale urbanization levels were tested by means of a 271 Generalized Linear Mixed Model (GLMM) for each of the investigated groups. Local- (subplot) 272 and landscape-scale (plot) urbanization levels and their interaction were specified as fixed factors. 273 To account for the spatial dependency of subplots within the same plot, a plot identifier (PlotID) 274 was incorporated as a random factor, nested within the landscape-scale urbanization levels. We 275 assumed the abundance data to be Poisson distributed and used the sample variance instead of the 276 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-277 278 group response in total abundance of individuals at both local- and landscape-scale urbanization 279 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).

283 Species richness data and analysis

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

285 We first assessed general responses in total species richness due to local- and landscape-scale 286 urbanization by means of sample-based accumulation curves, which express the cumulative number 287 of species when samples from a particular local- or landscape-scale urbanization category are added 288 at random. Given that we aim at identifying responses in total (γ) species richness only, we 289 restricted the analysis to five local/landscape-scale urbanization combinations. More specifically, 290 we compared sample-based accumulation curves between: (i) low urbanized subplots in low 291 urbanized plots (low end urbanization at both spatial scales); (ii) high urbanized subplots in high 292 urbanized plots (high end urbanization at both spatial scales); (iii) low urbanized plots regardless of 293 the degree of local urbanization; (iv) high urbanized plots regardless of the degree of local 294 urbanization and (v) all samples regardless of the degree of local- and landscape-scale urbanization 295 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 296 297 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 $r_{\rm S}P_{\rm c}$ 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 305 five combinations (*mobr* package 1.0; Xiao, McGlinn, May & Oliver, 2018 in R 3.4.2 (R 306 Development Core Team, 2017)). We then multiplied the complement of the obtained *P*-value (P_c) 307 with the Spearman Rank order correlation (r_s) between species richness and increasing urbanization 308 level to obtain the r_sP_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).

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

314 To gain more insights into the spatial scale at which species richness of each group is most strongly 315 affected by urbanization, we partitioned the total species richness observed at each local- or 316 landscape-scale urbanization level into its underlying components. We used a diversity partitioning 317 approach wherein the total diversity at larger spatial scales (γ) is decomposed into its average local 318 species richness ($\overline{\alpha}$) and species richness due to variation between local communities (β). As a 319 measure of variation in species composition between local communities, we calculated both the 320 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 -321 322 diversity can be calculated and compared at multiple hierarchical spatial scales (Lande, 1996; Crist, 323 Veech, Gering & Summerville, 2003; Anderson et al., 2011). While $\bar{\beta}_P$ expresses how much the richness at plot (or regional) level increases compared to the richness at subplot (or plot) level, $\bar{\beta}_A$ 324 325 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 330 proportional variation among subplots (β_{among}), and we did so for each of the three levels of local 331 urbanization (Figure 2a). Second, differences in these species richness components across 332 urbanization levels were tested with a randomization test, by permuting samples over the three 333 local-scale urbanization levels (see McGlinn et al., 2019).

334 The effect of landscape-scale urbanization on species richness can be evaluated both within as well 335 as between plots. For the former, we decomposed the total species richness within plots (γ_{within}) into 336 the average local species richness of the three subplots within a plot (α) and the additive and 337 proportional variation between these communities (β_{within}). For the latter, we decomposed the 338 species richness across all plots (γ_{among}) into the average species richness within a plot (γ_{within}) and 339 the additive and proportional variation in species richness among plots (β_{among}) (Figure 2b). 340 Differences in species richness along the urbanization gradient at both scales were tested with a 341 randomization test, by permuting samples over the three landscape-scale urbanization levels 342 (McGlinn et al., 2019).

343 *Observed versus rarefied species richness*

344 Observed species richness is a composite measure and differences in this metric among samples 345 may result from variation in (i) the number of individuals present at a particular site, (ii) the spatial 346 aggregation of individuals of the same species, and (iii) the number and relative abundance of 347 species in the species pool (i.e. the species abundance distribution or SAD) (He and Legendre 348 2002). We therefore also calculated rarefied species as the expected number of species for each 349 diversity component for a standardized number of randomly selected individuals by means of 350 individual-based rarefaction curves. By removing the effect of individual densities, differences in 351 rarefied species richness provide more information on differences in the SAD between 352 communities. At the regional (γ) scale, we rarefied for each animal group to the number of 353 individuals in the urbanization category that yielded the smallest sample size.

354 Overall pattern across groups

355 While the above analyses were performed separately for each group, we further tested for a 356 significant change in the diversity components in response to the landscape- and local-scale 357 urbanization gradients across groups by means of the non-parametric Page test (Hollander & Wolfe, 358 1973) for both observed and rarefied richness values. The nine groups were specified as blocks and 359 P-values were obtained from StatXact v5 (© Cytel Software, 2001) based on permutations within 360 blocks.





b) Comparison of landscape-scale urbanization

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.

366

367 **RESULTS**

368 Abundance

369 Although we could not detect an overall decrease in total abundance across the investigated groups 370 along the urbanization gradient at both the local (Page test; P > 0.05) and landscape scale (Page 371 test; P > 0.05), increasing the local-scale (subplot) urbanization level significantly decreased the 372 abundance of all but one of the terrestrial arthropods: ground beetles, ground- and web spiders, 373 butterflies and orthopterans (Table 1, Figure 3). This decline was most substantial for orthopterans 374 and butterflies, with a reduction in abundance of 67.4% and 85.5% respectively, in the most 375 urbanized compared to the least urbanized subplots. Local-scale urbanization had a much stronger 376 effect on abundance than landscape-scale urbanization, which showed no effects in any of the 377 investigated groups. An additional synergistic effect of local and landscape-scale urbanization was 378 only observed for butterflies, with abundance decreasing stronger along the local urbanization 379 gradient with increasing landscape-scale urbanization levels (Figure 3).



380

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 log₁₀-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.

387

388

389 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

	Local (subplot) urbanization effect			Lan urba	ndscape (plot nization effe	Interaction		
	F	Р	% change	F	Р	% change	F	Р
Ground beetles	$F_{2,48} = 3.26$	0.047	-31.3	$F_{2,48} = 0.430$	0.654	-10.0	$F_{4,48} = 0.090$	0.984
Ground spiders	$F_{2,48} = 5.16$	0.009	-36.5	$F_{2,48} = 2.26$	0.116	+8.1	$F_{4,48} = 1.11$	0.363
Web spiders	$F_{2,35} = 8.15$	0.001	-19.2	$F_{2,35} = 0.500$	0.613	-5.1	$F_{4,35} = 1.19$	0.332
Macro-moths	$F_{2,12} = 1.33$	0.3	-17.5	$F_{2,12} = 2.62$	0.114	-89.7	$F_{4,12} = 0.880$	0.506
Butterflies	$F_{2,48} = 56.4$	0.001	-85.5	$F_{2,48} = 0.340$	0.71	-47.9	$F_{4,48} = 3.65$	0.011
Orthopterans	$F_{2,48} = 18.4$	0.001	-67.4	$F_{2,48} = 0.990$	0.38	-23.0	$F_{4,48} = 1.94$	0.119
Snails	$F_{2,48} = 0.220$	0.8	-6.8	$F_{2,48} = 0.480$	0.624	+33.3	$F_{4,48} = 0.670$	0.617
Bdelloid rotifers	$F_{2,48} = 1.68$	0.197	+29.3	$F_{2,48} = 2.90$	0.065	+113.2	$F_{4,48} = 1.70$	0.166
Cladocerans	$F_{2,48} = 0.61$	0.547	+234.4	$F_{2,48} = 0.11$	0.9	+54.0	$F_{4,48} = 0.36$	0.834

391 lowest urbanization level. Significant effects are depicted in bold.

392

393 *Total species richness*

394 Sample-based accumulation curves showed a trend towards a slower accumulation of species at 395 increasing local and/or regional urbanization levels for the majority of investigated groups (Figure 396 S1). Rarefying richness to a size of nine samples for each combination revealed decreases in total 397 species richness for five groups (i.e. ground beetles, web spiders, macro-moths, butterflies and 398 orthopterans; $r_{\rm S}P_{\rm c} < 0.05$; Figure 4a). A decline was also observed in total species richness across 399 groups with increasing urbanization levels (Page-test; P < 0.001). Samples originating from a 400 mixture of high, intermediate and low urbanized plots and subplots had a lower species richness 401 compared to those based on samples from low urbanized subplots in low urbanized plots only, 402 indicating that plots consisting of a mosaic of high and low urbanized subplots harbour less species 403 across groups compared to low urbanized plots (Page-test; P = 0.007). Other pairwise comparisons 404 between the urbanization categories were also significant (Page test; P < 0.03), except for high 405 local/landscape urbanization versus high landscape urbanization (Page test; P = 0.15) and low 406 local/landscape urbanization versus low landscape urbanization (Page test; P = 0.45).







Relative abundance under high versus low urbanization

408 Figure 4 - (a) Estimated total number of species for each examined group in nine random samples from five different 409 local/landscape urbanization level combinations using raw data. Y-axis scale is log₁₀-transformed to improve 410 visualization. Pictograms on the x-axis depict (from left to right): (i) low urbanized subplots in low urbanized 411 landscapes (light green square in dark green square); (ii) low urbanized landscapes regardless of the degree of local 412 urbanization (light grey square in dark green square); (iii) samples regardless of the degree of local and landscape 413 urbanization level (light grey square in dark grey square); (iv) high urbanized landscapes regardless of the degree of 414 local urbanization (light grey square in dark red square) and (v) high urbanized subplots in high urbanized landscapes 415 (light red square in dark red square). Asterisks (* = 0.01 < P < 0.05, ** = 0.01 < P < 0.001, *** = P < 0.001) depict 416 results of the directional ordered heterogeneity test $r_s P_c$.. (b) Correlation between urbanization-related change in 417 abundance versus change in local (open circles) and total (closed circles) observed species richness across taxonomic 418 groups. Values on both axes represent the relative abundance (x-axis) and species richness (y-axis) in high urbanized 419 subplots in high urbanized landscapes versus those in low urbanized subplots in low urbanized landscapes. Animal 420 silhouettes are from PhyloPic (http://www.phylopic.org) and fall under CC-BY 3.0 licences.

421

422 We further tested if the decrease in species richness is higher for taxonomic groups that show a 423 strong decrease in abundance, as this would indicate that the decrease in species richness is, at least 424 partly, due to a lower sampling effect in urbanized landscapes. More precisely, we correlated the 425 relative change in species richness in high urbanized subplots in high urbanized landscapes versus 426 low urbanized subplots in low urbanized landscapes with the relative change in abundance (Figure 427 4b). Groups showing the strongest decrease in abundance (moths, butterflies, grasshoppers, ground 428 beetles and ground spiders) showed a significant reduction in local species richness (i.e. average 429 species richness within subplots) (r = 0.88, P = 0.001), but not for total species richness (i.e. species 430 richness across subplots) (r = 0.59, P = 0.1).

431 Species richness decomposition

432 High local- and landscape-level urbanization reduced total (γ) species richness across the 433 investigated groups by 7% and 14%, respectively (Page test; P = 0.026 and P = 0.003, respectively; 434 Figure 5; Table 2). Increased landscape-level urbanization also decreased average local (α) species 435 richness by 14% (Page test; P = 0.047) but did not result in a consistent change in species variation 436 (β) across the investigated groups (Figure 5; Table 2).

437 Group specific responses were highly heterogeneous, but, except for bdelloid rotifers and 438 cladocerans, all groups showed a significantly negative response towards increasing local- and/or 439 landscape-scale urbanization for at least one of the calculated diversity components (Table 2). 440 Increased local urbanization primarily decreased local (α) diversity of butterflies and orthopterans 441 and decreased (additive) variation in species composition (β_A) of ground beetles, snails and 442 orthopterans. The effects of landscape-scale urbanization resulted in decreases in local diversity of 443 web spiders and macro-moths, a decrease in variation among local communities within urbanized 444 landscapes ($\beta_{A,within}$) in macro-moths and a decrease in variation among urbanized landscapes 445 $(\beta_{A,among})$ in ground beetles, ground spiders and orthopterans. Positive relationships with increasing 446 urbanization were observed in butterflies, showing positive responses in both proportional and additive variation in species composition among locally urbanized sites. A positive relationship 447 448 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 449 450 increasing local diversity within urbanized landscapes along the urbanization gradient.



451

452 Figure 5 - Total observed diversity (S) partitioning for each examined group and for each of three (a) local- and (b)
453 landscape-scale urbanization levels (green = low; yellow = intermediate; red = high). See Figure 2 for an explanation of
454 the different diversity components. The nine animal silhouettes are from PhyloPic (http://www.phylopic.org) and fall
455 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, 461 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 462 proportional ($\bar{\beta}_P = \gamma/\bar{\alpha}$) and additive ($\bar{\beta}_A = \gamma - \bar{\alpha}$) beta diversity, respectively, wherein $\bar{\beta}_P$ expresses how much the 463 richness at plot (or regional) level increases compared to the richness at subplot (or plot) level, while $\bar{\beta}_A$ expresses the 464 absolute increase in number of species between these two sampling levels.

465

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

466

467 Results obtained from rarefied richness roughly corresponded with the results of observed richness, 468 but generally resulted in weaker urbanization effects at the α and γ levels (Table 2b). For example, 469 the effect of urbanization at local (α) scale was reduced for some groups (e.g. macro-moths, 470 butterflies and orthopterans) when considering rarefied compared to observed richness. In contrast 471 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.

474 Across-group analysis revealed that increasing levels of landscape urbanization led to an average

475 decline in rarefied local (α) richness (Page test; P = 0.023) and an increase in proportional variation

476 in rarefied species richness (Page test; P = 0.011) within plots ($\beta_{Pwithin}$).

477 **DISCUSSION**

478 Urbanization is expected to inflict major impacts on biodiversity and ecosystem functioning, 479 together with other large-scale anthropogenic disturbances, such as agricultural intensification and 480 deforestation (Grimm et al., 2008; Shochat et al., 2010). Yet, studies show inconsistent responses 481 that are likely attributed to differences in the examined groups, spatial extent at which urbanization 482 was assessed, the range of the urbanization gradient and the spatial scale at which the responses to 483 urbanization are measured (Aronson et al., 2014; Faeth, Bang & Saari, 2011; Marzluff, 2017; Saari 484 et al., 2016). To account for variation in group- and scale-specific effects, we here integrate data 485 from multiple groups and multiple spatial scales in a study sampling identical urbanization 486 gradients and demonstrate that urbanization drives declines in the abundance for most investigated 487 groups and species richness across the examined groups. In line with the previously reported 488 heterogeneous patterns of biodiversity along urbanization gradients, we found that group-specific 489 responses strongly depended on the spatial scale at which urbanization and species richness are 490 assessed. Integrating data across multiple spatial scales and multiple taxa is therefore required to 491 provide an overall view of how biodiversity is affected by urbanization. There is currently little 492 consensus on the expected response of total abundance of organisms to urbanization, as both 493 increases and declines have been reported (Chace & Walsh, 2006; Grimm et al., 2008; Shochat et 494 al., 2010). Increases in abundance could be due to the dominance of a few synanthropic species 495 with superior competitive abilities, enhanced by increased human-mediated food resources and 496 reduced predation (Parris, 2016). Alternatively, the hostile environment imposed by urban 497 structures and the consequent decreased connectivity and size of suitable habitat patches may 498 deplete individuals and species from urban settlements (McKinney, 2008, Saari et al., 2016). 499 Although we could not demonstrate a decline in abundance across the entire set of examined groups 500 in response to local urbanization, significant declines were observed at the group-specific level for 501 ground beetles, ground- and web spiders, butterflies and orthopterans, while macro-moths showed a 502 non significant decreasing trend. Since ground beetles and ground spiders were sampled with pitfall 503 traps, their estimated abundances could potentially be biased by differences in species activity 504 between high and low urbanized sites, due to variation in local physical parameters, such as 505 temperature. However, in a related study we demonstrated that temperatures are higher at the highly 506 urbanized sampling sites (i.e. UHI-effect, Merckx et al. 2018), thus higher arthropod numbers 507 would have been expected in the urbanized sites, which is opposite to what we observed. Our 508 measurements for these groups are consequently highly conservative, furtherly strengthening our 509 results.

510 The observed declines support the idea that poor environmental conditions in urban environments 511 decrease the average densities across major organism groups, notably terrestrial active dispersive 512 arthropods in our study. There were three organism groups for which we did not observe declines in 513 abundance along the urbanization gradient: snails, bdelloids and cladocerans. The latter two groups 514 are small (semi)aquatic passive dispersive organisms that have high dispersal capacities (Fontaneto 515 et al., 2011; Gianuca et al., 2018). As a consequence, they do not need large habitat patches to 516 thrive and, at the same time, being passive dispersers, they cannot avoid cities during their dispersal 517 process. Snails host a number of species that prefer habitats that are abundant in cities, such as 518 patches of soils that are moist because they are covered with debris, stones and other building 519 material.

520 The obvious decline we observed for terrestrial arthropods parallels the recent reports on global521 declines of insects, even in areas safeguarded from obvious anthropogenic disturbances (Brooks et

522 al., 2012; Grubisic et al., 2018; Hallmann et al., 2017; Vogel, 2017). Identifying the main causes 523 driving this decline is, however, difficult given the multifaceted influence that urbanization exerts 524 on the environment (Parris, 2016). In particular, the urban-heat-island effect may be put forward as 525 a possible factor driving the observed decline in animal abundance. In fact, temperature increase has 526 recently been identified as one of the dominant factors affecting arthropod numbers, with bottom-up 527 effects towards higher trophic levels feeding on these organisms (Lister & Garcia, 2018). The 528 abundance response was only observed under local-scale urbanization levels, which is congruent 529 with the urban-heat-island effect being indeed more pronounced at local spatial scales (Kaiser et al. 530 2016; Merckx et al., 2018; Brans et al., 2018).

531 The observed declines in abundance likely represent a rather conservative view on the actual 532 abundance patterns in urban landscapes. To allow comparison between high and low urbanized 533 landscapes, sampling was restricted to green infrastructures (e.g. grassy/herbaceous vegetation, 534 ponds). In the most urbanized landscapes, such as cities, these sampled green infrastructures might 535 be less common than in rural areas, as they are embedded within built-up areas that likely harbor 536 even lower abundances of the investigated groups. It can thus be expected that the observed 537 declines in abundances, and their consequences for ecosystem functioning, are even more 538 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 546 expected to become dominant, and might thus increase total abundance in urban areas (Shochat et547 al., 2010), opposite to what we observed.

548 When partitioning diversity into its components, the cross-group decline in species richness was 549 most clearly observed at the level of total (γ) diversity at both local and landscape scales. However, 550 we found strong differences among the animal groups with respect to the diversity component that 551 was most strongly affected, with significant trends either at α (e.g. web spiders, butterflies) or β 552 (e.g. ground beetles, orthopterans) level. Thus, although the overall declining trend of total diversity 553 summarizes the decline across all groups and all diversity components (Crist et al., 2003), the 554 differential response of each group points to the ecological and scale-dependent complexity of 555 metacommunity responses to urbanization (Chace & Walsh, 2006; Hill et al., 2017; Luck & 556 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.

560 For some groups, such as macro-moths, diversity components declined at multiple spatial scales. 561 Local macro-moth communities are thus not only impoverished within sites located within urban 562 landscapes, but they are also highly homogeneous among sites within urban landscapes. We further 563 detected biotic homogenization at the largest spatial scale (i.e. across urban landscapes) for ground 564 beetles, ground spiders and orthopterans, and across groups. This suggests that more homogeneous 565 environmental conditions of urbanized areas may filter ecologically and taxonomically similar 566 species from the total species pool (Baldock et al., 2015; Ferenc et al., 2014; La Sorte et al., 2014; 567 McKinney, 2006; but see Brice et al., 2017 and Knop, 2016 for contrasting results). The strong 568 homogenizing effect of urban environments and landscapes has been most clearly demonstrated by 569 shifts in community life-history traits in response to urbanization (Concepción et al., 2016; Croci et 570 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.

577 Rarefying species richness generally resulted in less strong urbanization effects, in particular at the 578 local scale. We showed that groups with a strong decline in abundance, like orthopterans and 579 butterflies, showed a concomitant decline in local species richness. This suggests that the decrease 580 in local species richness with increasing urbanization might, at least partly, be driven by a sampling 581 effect due the decrease in individual abundances and less so by changes in the local species pool 582 and/or evenness of local communities (Chase & Knight, 2013). However, although we rarefied 583 richness to the lowest number of individuals within each group, this procedure could potentially 584 lead to the comparison of different points in the rarefaction curves among urbanization categories, 585 e.g. the end of the curve (total richness in the regional pool) in high urbanized sites against the base 586 of the curve (evenness) in low urbanized sites (McGlinn et al., 2019). Therefore, one must be 587 prudent in interpreting the decrease in local (α) species richness as a mere sampling effect. 588 Alternatively, rarefying species richness resulted in a stronger effect of local urbanization on 589 variation in species composition among plots, with ground beetles, ground spiders, orthopterans, 590 snails and bdelloid rotifers all showing significant decreases in beta diversity. Only for butterflies 591 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 596 biodiversity and threatened species (Beninde, Veith & Hochkirch 2015, Ives et al. 2016, Aronson et 597 al. 2017).due to their habitat heterogeneity that allow species with different habitat preferences to 598 co-exist on small spatial scales (Aronson et al. 2017). In other words, cities host several different 599 habitat types (e.g. ruderal habitats, grasslands, wooded areas,...) within smaller areas compared to 600 natural landscapes, thus increasing the number of species per unit area. However, comparisons 601 across habitats primarily reflect the change in species number per unit area without providing clear 602 information on loss of species within each habitat. Our sampling design allowed us to investigate 603 diversity patterns without confounding factors related to habitat type. We could thus reveal that 604 urbanization impoverishes the fauna within habitat patches and, consequently, that future loss of 605 species due to urbanization is to be expected. This was further suggested by the higher number of 606 species in more natural landscapes compared to landscapes composed of a mosaic of high and low 607 urbanized subplots and indicates that urban environments hardly contain species that are not found 608 outside the urban areas.

609 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 610 611 and (semi)aquatic taxa. In particular, we highlighted how passively dispersing taxa tend to be less 612 sensitive to urbanization than actively dispersing taxa. Further investigations should be performed 613 to better understand the mechanisms behind this pattern. Furthermore, our results suggest that 614 urbanization could exert a strong impact on ecosystem functioning and services, as it negatively 615 affects groups that play a central role in a variety of ecological processes, like nutrient cycling (e.g. 616 snails, butterflies, orthopterans and macro-moths), pollination (e.g. butterflies and macro-moths), 617 predation (ground beetles, ground- and web spiders) and grazing (cladocerans). However, we also 618 highlight that the responses to urbanization strongly depend on the examined group, scale of 619 urbanization and scale at which diversity is assessed. This might indicate that city planning should 620 include measures at both local and more regional spatial scales as well as green infrastructure to 621 make urban areas more attractive to active highly dispersive species.

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