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

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(Article begins on next page)

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

3 **Running title:** Urbanization impacts abundance and diversity

4

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45 **Abstract**

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

68

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

71 INTRODUCTION

72 The conversion of natural and rural land to urban environments increased drastically worldwide
73 over the last 30 years, with urban land cover expected to be tripled from 2000 to 2030 (Seto,
74 Güneralp & Hutya 2012). Urbanization drives global environmental change and is currently one of
75 the main anthropogenic impacts (Parris 2016) with expected drastic consequences on biodiversity
76 and ecosystem processes. Urbanization-associated changes in community structure can result from
77 several mechanisms (Rebele, 1994; Seto, Sánchez-Rodríguez & Fragkias, 2010), which act at
78 multiple spatial scales (Shochat, Warren, Faeth, McIntyre & Hope, 2006; Shochat et al., 2010) and
79 are strongly habitat-dependent (Hill et al., 2017). Ecological effects are due to substantial changes
80 in local abiotic environmental conditions (e.g. high levels of nutrients, pollution, and
81 imperviousness) (Parris, 2016), and to landscape structure (e.g. reduced size and connectivity and
82 increased temporal turnover of habitat patches) (McDonnell, et al. 1997; 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.,
88 2017; McKinney, 2008; Shochat et al., 2010), are reported. These heterogeneous results suggest that
89 the effect of increasing urbanization might strongly depend on the spatial scale and taxon for which
90 it is assessed (Concepción et al., 2015; Egerer et al., 2017; McKinney, 2008; Philpott et al., 2014).

91 First, the direction and magnitude of changes in species diversity in response to an environmental
92 driver may strongly depend on the spatial scale at which species diversity is measured (Chase &
93 Knight, 2013). For instance, urbanization may filter out species that are not pre-adapted to urban
94 conditions, with a consequent decrease in abundance or diversity at small (local) spatial scales
95 (Bates et al., 2011; Piano et al., 2017). Alternatively, the loss of species that are less adapted to

96 urban environments could be (over)compensated by an increase of species that are efficient in
97 exploiting urban resources, including exotic taxa (McKinney, 2006; Menke et al., 2011; Sattler,
98 Obrist, Duelli & Moretti, 2011). Both phenomena may cause biotic homogenization if local
99 communities are colonized by the same species, increasing in turn the compositional similarity of
100 urban species assemblages and, consequently, reducing species richness of urban areas at large
101 spatial scales (Knop, 2016; McKinney, 2006; Morelli et al., 2016).

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

109 A comprehensive assessment of the overall effects of urbanization on species communities is
110 unlikely to be resolved by studying single taxa and single spatial scales. Instead, insights into
111 general patterns of abundance and diversity change should be obtained by integrating data over
112 multiple animal groups, while uncoupling the spatial scales at which urbanization and species
113 richness are measured.

114 Here, we analysed data on abundance and species richness data of one limno-terrestrial (bdelloid
115 rotifers), one aquatic (cladocerans) and seven terrestrial (butterflies, ground beetles, ground- and
116 web spiders, macro-moths, orthopterans and snails) animal groups sampled along replicated
117 urbanization gradients in Belgium. More specifically, we sampled communities according to a
118 hierarchically nested sampling design, in which three local-scale urbanization levels were
119 repeatedly sampled across the same three urbanization levels at the landscape scale (Merckx et al.
120 2018). This sampling design allowed us to partition the total species richness (γ -diversity) into

121 richness within local communities (α -diversity) and richness due to variation in species composition
122 among local communities (β -diversity), and to relate these to both local and landscape-scale
123 urbanization levels. We explored (i) if, and in which direction, local and landscape-scale
124 urbanization affect total abundance; (ii) if local and landscape-scale urbanization affect species
125 richness within habitat patches, and if so at which spatial scale; and (iii) to what extent these
126 responses are consistent across animal groups.

127

128 **MATERIALS AND METHODS**

129 **Sampling area and design**

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

145 within 10-25 km of the highly urbanized plots. This plot selection strategy guaranteed that plots
146 within the same urbanization category are evenly distributed across the study area and ensured a
147 minimal spatial autocorrelation of plot urbanization levels. Across plots, %BU was positively
148 correlated with the amount of other impervious substrates such as roads and artificial constructions
149 (for example bridges, viaducts, locks, ...) ($r_s = 0.94$; $P < 0.0001$) and negatively correlated with the
150 area of semi-natural habitat ($r_s = -0.85$; $P < 0.0001$) (Figure S1), thus representing a reliable proxy
151 of urbanization. To investigate effects of local-scale urbanization, each plot was divided into local
152 subplots of 200 m \times 200 m, which were classified into urbanization categories using identical %BU
153 thresholds as used at plot level. Within each plot, we then selected one subplot of each urbanization
154 category (i.e. low, intermediate and high) for a total of 81 sampling sites (i.e. 9 plots \times 3 landscape-
155 scale urbanization levels \times 3 local-scale urbanization levels) (Figure 1). This selection was random
156 within the constraints imposed by the availability of targeted habitats (e.g. pond, grassland,
157 woodland), accessibility and the permission to sample.

158 This setup guaranteed that urbanization at landscape and local scales are uncorrelated and, hence,
159 that urbanization effects at both scales, and their interaction, could be tested simultaneously. The
160 same sampling design was applied to all taxa, and all sampling was based on the same set of plots
161 (landscape-level of urbanization). At the local level too, the same sampling design was
162 implemented across organism groups, but the choice of specific subplots featuring a given level of
163 local urbanization within each plot could differ between groups as sampling sites suitable for all
164 groups were not always present within the same 200 m \times 200 m subplot. Except for web spiders and
165 macro-moths, all, or nearly all, of the 81 subplots were sampled for each animal group (see
166 *Sampling methods*).

167

168 **Sampling methods**

169 *Ground beetles and ground spiders*

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

181 *Web spiders*

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

193 *Macro-moths*

194 Sampling was restricted to a set of nine plots, three of each plot urbanization category, and
195 performed in woodland with Jalas type bait traps in three sampling sessions, which started on the
196 30th-31st of July 2014 (first session), 13th-14th of August 2014 (second session) and 30th-31st of
197 March and 1st of April 2015 (third session). Traps were emptied on 3rd-4th of August 2014 (first
198 session), 2nd-3rd of September 2014 (second session) and 24th-25th-26th of April 2015 (third session).
199 Traps were baited with sugar-saturated wine and sampled individuals were poisoned with
200 chloroform within the traps. Individuals were counted and identified to species level (Manley,
201 2010), except for two species pairs: *Mesapamea secalis/secalella* and *Hoplodrina*
202 *blanda/octogenaria*.

203 *Butterflies and orthopterans*

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

212 *Snails*

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

217 branches, piled wood, cardboard and construction/demolition materials, and (iii) along/on fences
218 and walls.

219 *Bdelloid rotifers*

220 Communities of bdelloid rotifers were sampled by collecting lichen patches of the genus *Xanthoria*,
221 for which bdelloid rotifer communities have been previously studied in Europe (Fontaneto,
222 Westberg & Hortal, 2011). Suitable *Xanthoria* patches could be found in all but one subplot.
223 Sampling was performed between June and July 2013. The selection of the lichen was haphazard:
224 the first lichen patch encountered in each subplot was collected. Dry lichen thalli between 3 and 10
225 cm² were cut from the substrate with a knife and kept in paper bags. For each lichen sample, an area
226 of 2.5 cm² was hydrated with distilled water in a plastic petri dish. All active bdelloid rotifers that
227 recovered from dormancy in the following four hours after hydration were sorted and identified to
228 species level (Donner, 1965). Previous studies on bdelloid rotifers in these lichens (Fontaneto et al.,
229 2011) revealed that animals start recovering between 10 and 40 minutes after hydration of the
230 sample and that no more bdelloid rotifers are recovered after four hours. The very few dormant
231 stages still found in the sample that did not recover after that time were considered dead and
232 excluded from the analyses.

233 *Cladocerans*

234 Water samples were collected from ponds using a tube sampler (length = 1.85 m; diameter = 75
235 mm; Gianuca et al. 2018). One pond was selected in each of the 81 selected subplots. Sampling was
236 performed once for each pond and all sampling was performed in the period from 29th of May to the
237 10th of July 2013. In each pond, eight sampling locations were selected using a predefined grid,
238 assuring that different microhabitats (shallow and deeper zone, different locations with respect to
239 wind direction) were represented to a similar extent. On each sample location, the exact place to be
240 sampled was chosen in a random way, regardless of the presence of macrophytes. At each of the

241 eight locations, 12 L of water was collected, resulting in a total of 96 L per pond. The tube sample
242 integrated the entire water column, but resuspension and subsequent sampling of bottom material
243 was avoided. For each pond, 40 L of water was filtered through a 64 μm conical net. The sample
244 was then collected in a 60 mL vial and fixed with formalin (4%). Additional sampling was
245 performed with a sweep-net (64 μm net) and preserved in the same way. These additional samples
246 served to guarantee sufficiently extensive sampling to reconstruct an as complete as possible
247 species list. Individuals in standardized subsamples were identified and counted; entire subsamples
248 were counted until at least 300 individuals were identified and no new species was found in the last
249 100 specimens. Samples containing less than 300 individuals were counted completely, and the
250 additional qualitative samples for those ponds were screened for additional species. Species
251 identification was based on Flößner (2000). *Daphnia longispina*, *Daphnia galeata* and *Daphnia*
252 *hyalina* were combined in the *Daphnia longispina* complex due to the morphological similarities
253 and possible hybridization between the species. Detailed information on the sampling and
254 identification of zooplankton are reported in Brans et al. (2017) and Gianuca et al. (2018). Densities
255 were calculated as number of individuals per L of the original sample.

256 **Abundance data and analysis**

257 The total number of sampled/observed individuals in each sample/transect was used as an estimate
258 for the abundance of each group in each subplot. For cladocerans, abundance data are based on the
259 total number of individuals in a standardized volume of 40 L. Differences in abundances in
260 response to local (subplot) and landscape (plot) scale urbanization levels were tested by means of a
261 Generalized Linear Mixed Model (GLMM) for each of the investigated groups. Local- (subplot)
262 and landscape-scale (plot) urbanization levels and their interaction were specified as fixed factors.
263 As each plot included three subplots, one for each urbanization category, a plot identifier (PlotID)
264 was incorporated as a random factor to account for the spatial dependency of subplots within the
265 same plot. Abundance data were assumed to be Poisson distributed and the sample variance instead

266 of the theoretical variance was used to account for potential overdispersion (Agresti et al. 1996).
267 Analyses were conducted with PROC GLIMMIX in SAS[®] 9.4 (SAS Institute Inc. 2013). We further
268 tested for a cross-group response in total abundance of individuals at both local- and landscape-
269 scale urbanization with the non-parametric Page test (Hollander & Wolfe, 1973). This test accounts
270 for the ordering of the urbanization levels (low – intermediate – high), with the nine groups
271 specified as blocks. *P*-values were based on permutations within blocks and obtained from StatXact
272 v5 (© Cytel Software, 2001).

273 **Species richness data and analysis**

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

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

288 For each animal group, we tested if total species richness declined significantly with increasing
289 local/landscape-scale urbanization level by means of the ordered heterogeneity test through the r_sP_c
290 statistic (Rice & Gaines, 1994), which combines the statistical evidence of differences between

291 sample means with their rank order. More precisely, we first tested for differences in species
292 richness among urbanization categories by comparing the observed average absolute differences in
293 total species richness for a total of nine samples (corresponding to the lowest sample size of the five
294 local/landscape-scale combinations) with those obtained by random shuffling samples across these
295 five combinations (*mobr* package 1.0; Xiao, McGlenn, May & Oliver, 2018 in R 3.4.2 (R
296 Development Core Team, 2017)). We then multiplied the complement of the obtained P -value (P_c)
297 with the Spearman Rank order correlation (r_s) between species richness and increasing urbanization
298 level to obtain the $r_s P_c$ statistic.

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

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

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

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

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

333 *Observed versus rarefied species richness*

334 Observed species richness is a composite measure and differences in this metric among samples
335 may result from variation in (i) the number of individuals present at a particular site, (ii) the spatial
336 aggregation of individuals of the same species, and (iii) the number and relative abundance of
337 species in the species pool (i.e. the species abundance distribution or SAD) (He & Legendre, 2002).
338 We therefore also calculated rarefied species richness as the expected number of species for each
339 diversity component for a standardized number of randomly selected individuals by means of
340 individual-based rarefaction curves. By removing the effect of individual densities, differences in

341 rarefied species richness provide more information on differences in the SAD between
342 communities. At the regional (γ) scale, we rarefied for each animal group to the number of
343 individuals in the urbanization category that yielded the smallest sample size.

344 *Overall pattern across groups*

345 While the above analyses were performed separately for each group, we further tested for a
346 significant change in the diversity components in response to the landscape- and local-scale
347 urbanization gradients across groups by means of the non-parametric Page test (Hollander & Wolfe,
348 1973) for both observed and rarefied richness values. The nine groups were specified as blocks and
349 P -values were obtained from StatXact v5 (© Cytel Software, 2001) based on permutations within
350 blocks.

351

352 **RESULTS**

353 *Abundance*

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

365 ***Total species richness***

366 Sample-based accumulation curves showed a trend towards a slower accumulation of species at
367 increasing local and/or regional urbanization levels for most of the investigated groups (Figure S2).
368 Rarefying richness to a size of nine samples for each combination revealed decreases in total
369 species richness for ground beetles, web spiders, macro-moths, butterflies and orthopterans ($r_s P_c <$
370 0.05 ; Figure 4a). A decline was also observed in total species richness across groups with increasing
371 urbanization levels (Page-test; $P < 0.001$). Samples originating from a mixture of high, intermediate
372 and low urbanized plots and subplots had a lower species richness compared to those based on
373 samples from subplots with low urbanization in plots with low urbanization only, indicating that
374 plots consisting of a mosaic of subplots with low and high urbanization harbour fewer species
375 across groups compared to plots with low urbanization (Page-test; $P = 0.007$). Other pairwise
376 comparisons between the urbanization categories were also significant (Page test; $P < 0.03$), except
377 for high local/landscape urbanization versus high landscape urbanization (Page test; $P = 0.15$) and
378 low local/landscape urbanization versus low landscape urbanization (Page test; $P = 0.45$).

379 We further tested if the decrease in species richness is higher for those groups that show a strong
380 decrease in abundance, as this would indicate that the decrease in species richness is, at least partly,
381 due to a lower sampling effect in urbanized landscapes. More precisely, we correlated the relative
382 change in species richness in highly urbanized subplots in highly urbanized plots versus subplots
383 with low urbanization in plots with low urbanization with the relative change in abundance (Figure
384 4b). Groups showing the strongest decrease in abundance (macro-moths, butterflies, orthopterans,
385 ground beetles and ground spiders) showed a significant reduction in both local species richness
386 (i.e. average species richness within subplots) ($r_s = 0.95$, $P < 0.001$) and total species richness (i.e.
387 species richness across subplots) ($r_s = 0.69$, $P = 0.04$).

388 ***Species richness decomposition***

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

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

408 Results obtained from rarefied richness roughly corresponded with the results of observed richness,
409 but generally resulted in weaker urbanization effects at the α and γ levels (Table 2b). For example,
410 the effect of urbanization at local (α) scale was reduced for macro-moths, butterflies and
411 orthopterans when considering rarefied compared to observed richness. In contrast to observed
412 richness, there is no detectable across-group decline in rarefied total (γ) diversity due to either local

413 or landscape urbanization. Conversely, rarefying richness generally led to more negative effects of
414 local urbanization levels on additive species variation (β_A), with declines for six groups.
415 Across-group analysis revealed that increasing levels of landscape urbanization led to an average
416 decline in rarefied local (α) richness (Page test; $P = 0.023$) and an increase in proportional variation
417 in rarefied species richness (Page test; $P = 0.011$) within plots ($\beta_{P_{within}}$).

418

419 **DISCUSSION**

420 Urbanization is expected to inflict major impacts on biodiversity and ecosystem functioning,
421 together with other large-scale anthropogenic disturbances, such as agricultural intensification and
422 deforestation (Grimm et al., 2008; Shochat et al., 2010). Yet, studies show inconsistent responses
423 that are likely attributed to differences in the examined groups, the spatial extent at which
424 urbanization was assessed, the range of the urbanization gradient and the spatial scale at which the
425 responses to urbanization are measured (Aronson et al., 2014; Faeth, Bang & Saari, 2011; Marzluff,
426 2017; Saari et al., 2016). To account for variation in group- and scale-specific effects, we here
427 integrate data from multiple groups and multiple spatial scales in a study sampling identical
428 urbanization gradients and demonstrate that urbanization drives declines in the abundance for most
429 investigated groups and species richness across the examined groups. In line with the previously
430 reported heterogeneous patterns of biodiversity along urbanization gradients, we found that group-
431 specific responses strongly depended on the spatial scale at which urbanization and species richness
432 are assessed. Integrating data across multiple spatial scales and multiple taxa is therefore required to
433 provide an overall view of how biodiversity is affected by urbanization. There is currently little
434 consensus on the expected response of total abundance of organisms to urbanization, as both
435 increases and declines have been reported (Chace & Walsh, 2006; Grimm et al., 2008; Shochat et
436 al., 2010). Increases in abundance could be due to the dominance of a few synanthropic species
437 with superior competitive abilities, enhanced by increased human-mediated food resources and

438 reduced predation (Parris, 2016). Alternatively, the hostile environment imposed by urban
439 structures and the consequent decreased connectivity and size of suitable habitat patches may
440 deplete individuals and species from urban settlements (McKinney, 2008, Saari et al., 2016).
441 Although we could not demonstrate a decline in abundance across the entire set of examined groups
442 in response to local urbanization, significant declines were observed at the group-specific level for
443 ground beetles, ground and web spiders, butterflies and orthopterans, while macro-moths showed a
444 non-significant decreasing trend. Since ground beetles and ground spiders were sampled with pitfall
445 traps, their estimated abundances could potentially be biased by differences in species activity
446 between sites with high and low urbanization, due to variation in local physical parameters, such as
447 temperature. However, in a related study we demonstrated that temperatures are higher at the highly
448 urbanized sampling sites (i.e. UHI-effect, Merckx et al. 2018), thus higher arthropod numbers
449 would have been expected in the urbanized sites, which is opposite to what we observed. Our
450 measurements for these groups are hence highly conservative and thus further strengthen our
451 results.

452 The observed declines in diversity support the idea that poor environmental conditions in urban
453 environments decrease the average densities across major organism groups, notably actively
454 dispersing terrestrial arthropods. In contrast, we did not observe declines in abundance along the
455 urbanization gradient for snails, bdelloid rotifers and cladocerans. The latter two groups are small
456 (semi)aquatic passively dispersing organisms that have high dispersal capacities (Fontaneto et al.,
457 2019; Gianuca et al., 2018). As such, they do not need large habitat patches to thrive and, at the
458 same time, being passive dispersers, they cannot avoid cities during their dispersal process. Snails
459 host a number of species that prefer habitats that are abundant in cities, such as patches of soils that
460 are moist because they are covered with debris, stones and other building material.

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

463 al., 2012; Grubisic et al., 2018; Hallmann et al., 2017; Vogel, 2017). Identifying the main causes
464 driving this decline is, however, difficult given the multifaceted influence that urbanization exerts
465 on the environment (Parris, 2016). In particular, the urban-heat-island effect may be put forward as
466 a possible factor driving the observed decline in animal abundance. In fact, temperature increase has
467 recently been identified as one of the dominant factors affecting arthropod numbers, with bottom-up
468 effects towards higher trophic levels feeding on these organisms (Lister & Garcia, 2018). The
469 abundance response was only observed under local-scale urbanization levels, which is congruent
470 with the urban-heat-island effect that is indeed more pronounced at local spatial scales (Kaiser et al.
471 2016; Merckx et al., 2018; Brans et al., 2018).

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

481 By integrating species richness data from groups that widely differ in diversity, life-history traits
482 and ecological profiles, we showed an overall decrease in total species richness with increasing
483 levels of local and/or landscape-scale urbanization. We demonstrate that sites and landscapes with
484 low urbanization levels harbour a richer species pool compared to areas consisting of a mosaic of
485 urban and non-urban areas. This suggests that the faunal composition of urbanized regions is hardly
486 characterized by species that are absent in less urbanized regions. The significant decrease in
487 abundance for the insect groups also points in this direction, since synanthropic species are

488 expected to become dominant, and might thus increase total abundance in urban areas (Shochat et
489 al., 2010), opposite to what we observed.

490 When partitioning diversity into its components, the cross-group decline in species richness was
491 most clearly observed at the level of total (γ) diversity at both local and landscape scales. However,
492 we found strong differences among the animal groups with respect to the diversity component that
493 was most strongly affected, with significant trends either at α (e.g. web spiders, butterflies) or β
494 (e.g. ground beetles, orthopterans) level. Thus, although the overall declining trend of total diversity
495 summarizes the decline across all groups and all diversity components (Crist et al., 2003), the
496 differential response of each group points to the ecological and scale-dependent complexity of
497 metacommunity responses to urbanization (Chace & Walsh, 2006; Hill et al., 2017; Luck &
498 Smallbones, 2010; Leibold & Chase, 2017; McKinney, 2008).

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

502 For some groups, such as macro-moths, diversity components declined at multiple spatial scales.
503 For instance, local macro-moth communities are not only impoverished within sites located within
504 urban landscapes, but they are also highly homogeneous among sites within urban landscapes. We
505 further detected biotic homogenization at the largest spatial scale (i.e. across urban landscapes) for
506 ground beetles, ground spiders and orthopterans, and across groups. This suggests that more
507 homogeneous environmental conditions of urbanized areas may filter ecologically and
508 taxonomically similar species from the total species pool (Baldock et al., 2015; Ferenc et al., 2014;
509 La Sorte et al., 2014; McKinney, 2006; but see Brice et al., 2017 and Knop, 2016 for contrasting
510 results). The strong homogenizing effect of urban environments and landscapes has been most
511 clearly demonstrated by shifts in community life-history traits in response to urbanization
512 (Concepción et al., 2016; Croci et al. 2008; Knop, 2016; McCune & Vellend, 2013; Merckx et al.,

513 2018; Penone et al., 2013). For instance, elsewhere we demonstrated how urbanization causes a
514 clear depletion of ground beetle, butterfly and macro-moth species with poor dispersal capacity
515 (Piano et al., 2017; Merckx & Van Dyck, 2019). Although convergence of biotic communities in
516 urban environments has been shown to be more consistent at the level of community trait values
517 compared to at the taxonomic level (Brans et al., 2017; Gianuca et al., 2018), the results presented
518 here demonstrate that urbanization may not only decrease diversity in functional groups, but also at
519 the level of species richness itself.

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

535 Our sampling design did not allow to explicitly test whether urban plots have a different overall –
536 i.e. across habitats – species richness compared to less urbanized plots, as we sampled the same
537 habitat type within examined groups. It has been proposed that cities may sustain high levels of

538 biodiversity, playing an important role in the conservation of global biodiversity and threatened
539 species (Beninde, Veith & Hochkirch, 2015; Ives et al., 2016; Aronson et al., 2017) due to their
540 habitat heterogeneity that allows species with different habitat preferences to co-exist on small
541 spatial scales (Aronson et al., 2017). In other words, cities host several different habitat types (e.g.
542 ruderal habitats, grasslands, wooded areas, ...) within smaller areas compared to natural landscapes,
543 thus increasing the number of species per unit area. However, comparisons across habitats primarily
544 reflect the change in species number per unit area without providing clear information on loss of
545 species within each habitat. We could thus reveal that urbanization impoverishes the fauna within
546 habitat patches and, consequently, that future loss of species due to urbanization is to be expected.
547 This was further suggested by the higher number of species in more natural landscapes compared to
548 landscapes composed of a mosaic of subplots with high and low urbanization. It also indicates that
549 urban environments hardly contain species that are not found outside the urban areas.

550 Overall, by applying a multi-scale approach across multiple animal groups, we demonstrated a
551 negative overall effect of urbanization on insect abundance and diversity of a range of terrestrial
552 and (semi)aquatic taxa. In particular, we highlighted how passively dispersing taxa tend to be less
553 sensitive to urbanization than actively dispersing taxa. Further investigations should be performed
554 to better understand the mechanisms behind this pattern. Furthermore, our results suggest that
555 urbanization could exert a strong impact on ecosystem functioning and services, as it negatively
556 affects groups that play a central role in a variety of ecological processes, like nutrient cycling (e.g.
557 snails, butterflies, orthopterans and macro-moths), pollination (e.g. butterflies and macro-moths),
558 predation (ground beetles, ground and web spiders) and grazing (cladocerans). However, we also
559 highlight that the responses to urbanization strongly depend on the examined group, scale of
560 urbanization and scale at which diversity is assessed.

561 Results from our study stress the importance that the preservation of large and connected patches of
562 natural habitats is likely the most effective measure to halt further urbanization-driven biodiversity

563 loss. In fact, we demonstrate that patches embedded within urban areas hardly contribute in the
564 maintenance of species that do not occur outside urban areas, thus urban green spaces likely have
565 only a modest contribution in the maintenance of regional species richness. City planning should
566 therefore prioritize the preservation and enlargement of natural habitat relicts rather than focussing
567 on the design of new green infrastructures. In addition, as biodiversity decline in urban areas is
568 largely driven by the depletion of low dispersive and cold-dwelling species (e.g. Concepción et al.,
569 2015; Merckx & Van Dyck, 2019; Piano et al., 2017), fragmented and dynamic habitat patches
570 within cities will most likely be colonized by generalist species that would not contribute to
571 increase the size of the regional species pool. Avoiding the expansion of urban regions, as well as
572 preserving and expanding relict habitats within urban areas, combined with the development of
573 green infrastructures, is therefore the most optimal solution to preserve biodiversity within cities.

574

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585

586 **Data sharing and data accessibility**

587 The data that support the findings of this study are available from the corresponding author upon
588 reasonable request.

589 **BIBLIOGRAPHY**

- 590 Agresti, A. (1996). *An introduction to categorical data analysis*. Wiley, New York, US.
- 591 Anderson, M. J., Crist, T. O., Chase, J. M., Vellend, M., Inouye, B. D., Freestone, A. L., Sanders,
592 N. J., Cornell, H. V., Comita, L. S., Davies, K. F., Harrison, S. P., Kraft, N. J. B., Stegen, J. C., &
593 Swenson, N. J. (2011). Navigating the multiple meanings of β diversity: a roadmap for the
594 practicing ecologist. *Ecology Letters*, **14**(1), 19-28. [https://doi.org/10.1111/j.1461-](https://doi.org/10.1111/j.1461-0248.2010.01552.x)
595 [0248.2010.01552.x](https://doi.org/10.1111/j.1461-0248.2010.01552.x)
- 596 Aronson, M. F., La Sorte, F. A., Nilon, C. H., Katti, M., Goddard, M. A., Lepczyk, C. A., Warren,
597 P. S., Williams, N. S. G., Cilliers, S., Clarckson, B., Dobbs, C., Dolan, R., Hedblom, M., Klotz, S.,
598 Kooijmans, J. L., Kühn, I., MacGregor-Fors, I., McDonnell, M., Mörtberg, U., Pyšek, P., Siebert,
599 S., Sushinsky, J., Werner, P., & Winter, M. (2014). A global analysis of the impacts of urbanization
600 on bird and plant diversity reveals key anthropogenic drivers. *Proceeding of the Royal Society B*,
601 **281**(1780), 20133330. <https://doi.org/10.1098/rspb.2013.3330>
- 602 Aronson, M.F.J, Lepczyk, C.A., Evans, K.L., Goddard, M.A., Lerman, S.B., MacIvor, J.S., Nilon,
603 C.H. & Vargo, T. (2017). Biodiversity in the city: key challenges for urban green space
604 management. *Frontiers in Ecology and the Environment*, **15**, 189-196.
605 <https://doi.org/10.1002/fee.1480>
- 606 Baldock, K. C., Goddard, M. A., Hicks, D. M., Kunin, W. E., Mitschunas, N., Osgathorpe, L. M.,
607 Potts, S. G., Robertson, K. M., Scott, A. V., Stone, G. N., Vaughan, I. P., & Memmott, J. (2015).
608 Where is the UK's pollinator biodiversity? The importance of urban areas for flower-visiting
609 insects. *Proceeding of the Royal Society B*, **282**(1803), 20142849.
610 <https://doi.org/10.1098/rspb.2014.2849>
- 611 Bates, A. J., Sadler, J. P., Fairbrass, A. J., Falk, S. J., Hale, J. D., & Matthews, T. J. (2011).
612 Changing bee and hoverfly pollinator assemblages along an urban-rural gradient. *PloS one*, **6**(8),
613 e23459. <https://doi.org/10.1371/journal.pone.0023459>
- 614 Beninde, J., Veith, M. & Hochkirch, A. (2015). Biodiversity in cities needs space: a meta-analysis
615 of factors determining intra-urban biodiversity variation. *Ecology Letters*, **18**, 581-592.
616 <https://doi.org/10.1111/ele.12427>
- 617 Bink, F. A. (1992). *Ecologische atlas van de Dagvlinders van Noordwest-Europa*. Schuyt & Co,
618 Haarlem, The Netherlands, 512 pp.

619 Boeken, M., Desender, K., Drost, B., van Gijzen, T., Koese, B., Muilwijk, J., Turin, H., &
620 Vermeulen, R. (2002). *De Loopkevers van Nederland en Vlaanderen (Coleoptera: Carabidae)*.
621 Stichting Jeugdbondsuitgeverij, Utrecht.

622 Brans, K. I., Govaert, L., Engelen, J. M., Gianuca, A. T., Souffreau, C., & De Meester, L. (2017).
623 Eco-evolutionary dynamics in urbanized landscapes: evolution, species sorting and the change in
624 zooplankton body size along urbanization gradients. *Philosophical Transactions of the Royal*
625 *Society B*, **372(1712)**, 20160030. <https://doi.org/10.1098/rstb.2016.0030>

626 Brans, K. I., Engelen, J. M., Souffreau, C., & De Meester, L. (2018). Urban hot-tubs: Local
627 urbanization has profound effects on average and extreme temperatures in ponds. *Landscape and*
628 *Urban Planning*, **176**, 22-29. <https://doi.org/10.1016/j.landurbplan.2018.03.013>

629 Brice, M. H., Pellerin, S., & Poulin, M. (2017). Does urbanization lead to taxonomic and functional
630 homogenization in riparian forests? *Diversity and Distributions*, **23(7)**, 828-840.
631 <https://doi.org/10.1111/ddi.12565>

632 Brooks, D. R., Bajer, J. E., Clark, S. J., Monteith, D. T., Andrews, C., Corbett, S. J., Beaumont, D.
633 A., & Chapman, J. W. (2012). Large carabid beetle declines in a United Kingdom monitoring
634 network increases evidence for a widespread loss in insect biodiversity. *Journal of Applied Ecology*,
635 **49(5)**, 1009–1019. <https://doi.org/10.1111/j.1365-2664.2012.02194.x>

636 Chace, J. F., & Walsh, J. J. (2006). Urban effects on native avifauna: a review. *Landscape and*
637 *Urban Planning*, **74(1)**, 46-69. <https://doi.org/10.1016/j.landurbplan.2004.08.007>

638 Chase, J. M., & Knight, T. M. (2013). Scale-dependent effect sizes of ecological drivers on
639 biodiversity: why standardised sampling is not enough. *Ecology Letters*, **16**, 17-26.
640 <https://doi.org/10.1111/ele.12112>

641 Christie, F. J., & Hochuli, D. F. (2009). Responses of wasp communities to urbanization: effects on
642 community resilience and species diversity. *Journal of Insect Conservation*, **13(2)**, 213-221.
643 <https://doi.org/10.1007/s10841-008-9146-5>

- 644 Concepción, E. D., Moretti, M., Altermatt, F., Nobis, M. P., & Obrist, M. K. (2015). Impacts of
645 urbanization on biodiversity: the role of species mobility, degree of specialisation and spatial scale.
646 *Oikos*, **124**, 1571-1582. <https://doi.org/10.1111/oik.02166>
- 647 Concepción, E. D., Götzenberger, L., Nobis, M. P., Bello, F., Obrist, M. K., & Moretti, M. (2016).
648 Contrasting trait assembly patterns in plant and bird communities along environmental and human-
649 induced land-use gradients. *Ecography*, **40(6)**, 753-763. <https://doi.org/10.1111/ecog.02121>
- 650 Crist, T. O., Veech, J. A., Gering, J. C., & Summerville, K. S. (2003). Partitioning species diversity
651 across landscapes and regions: a hierarchical analysis of α , β and γ diversity. *The American*
652 *Naturalist*, **162(6)**, 734-743.
- 653 Croci, S., Butet, A., & Clergeau, P. (2008). Does urbanization filter birds on the basis of their
654 biological traits? *The Condor*, **110(2)**, 223-240. <https://doi.org/10.1525/cond.2008.8409>
- 655 Donner, J. (1965). *Ordnung Bdelloidea. Bestimmungsbücher zur Bodenfauna Europas*. Akademie-
656 Verlag, Germany, 297 pp.
- 657 Duff, A. G. (2016). *Beetles of Britain and Ireland. Vol. 4*. A.G. Duff Publishing, West Runton, UK,
658 623 pp.
- 659 Egerer, M. H., Arel, C., Otoshi, M. D., Quistberg, R. D., Bichier, P., & Philpott, S. M. (2017).
660 Urban arthropods respond variably to changes in landscape context and spatial scale. *Journal of*
661 *Urban Ecology* **3**. <https://doi.org/10.1093/jue/jux001>
- 662 Faeth, S. H., Bang, C., & Saari, S. (2011). Urban biodiversity: patterns and mechanisms. *Annals of*
663 *the New York Academy of Sciences*, **1223(1)**, 69-81. [https://doi.org/10.1111/j.1749-](https://doi.org/10.1111/j.1749-6632.2010.05925.x)
664 [6632.2010.05925.x](https://doi.org/10.1111/j.1749-6632.2010.05925.x)
- 665 Fahrig, L. (2013). Rethinking patch size and isolation effects: the habitat amount hypothesis.
666 *Journal of Biogeography*, **40(9)**, 1649-1663. <https://doi.org/10.1111/jbi.12130>
- 667 Ferenc, M., Sedlacek, O., Fuchs, R., Dinetti, M., Fraissinet, M., & Storch, D. (2014). Are cities
668 different? Patterns of species richness and beta diversity of urban bird communities and regional
669 species assemblages in Europe. *Global Ecology and Biogeography*, **23**, 479-489.
670 <https://doi.org/10.1111/geb.12130>

671 Finlay, B. J., Esteban, G. F., Brown, S., Fenchel, T., & Hoef-Emden, K. (2006). Multiple
672 cosmopolitan ecotypes within a microbial eukaryote morphospecies. *Protist*, **157**(4), 377-390.
673 <https://doi.org/10.1016/j.protis.2006.05.012>

674 Flößner, D. (2000). *Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas*. Backhuys,
675 Netherlands: 140-143.

676 Fontaneto, D., Westberg, M., & Hortal, J. (2011). Evidence of weak habitat specialisation in
677 microscopic animals. *PLoSone*, **6**(8), e23969. <https://doi.org/10.1371/journal.pone.0023969>

678 Fontaneto D. (2019). Long-distance passive dispersal in microscopic aquatic animals. *Movement*
679 *Ecology*, **7**(1), 10. <https://doi.org/10.1186/s40462-019-0155-7>

680 Gianuca, A. T., Engelen, J., Brans, K. I., Hanashiro, F. T., Vanhamel, M., van den Berg, E. M.,
681 Souffreau, C., & De Meester, L. (2018). Taxonomic, functional and phylogenetic metacommunity
682 ecology of cladoceran zooplankton along urbanization gradients. *Ecography*, **41**(1), 183-194.
683 <https://doi.org/10.1111/ecog.02926>

684 Grimm, N. B., Foster, D., Groffman, P., Grove, J. M., Hopkinson, C. S., Nadelhoffer, K. J., Pataki
685 DE, & Peters, D. P. (2008). The changing landscape: ecosystem responses to urbanization and
686 pollution across climatic and societal gradients. *Frontiers in Ecology and the Environment*, **6**(5),
687 264-272. <https://doi.org/10.1890/070147>

688 Grubisic, M., van Grunsven, R. H. A., Kyba, C. C. M., Manfrin, A., & Hölker, F. (2018). Insect
689 declines and agroecosystems: does light pollution matter? *Annals of Applied Biology*, **173**(2), 180-
690 189. <https://doi.org/10.1111/aab.12440>

691 Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W.,
692 Müller, A., Sumser, H., Hörren, T., Goulson, D., & de Kroon, H. (2017). More than 75 percent
693 decline over 27 years in total flying insect biomass in protected areas. *PLoS one*, **12** (10), e0185809.
694 <https://doi.org/10.1371/journal.pone.0185809>

695 He, F.L. & Legendre, P. (2002). Species diversity patterns derived from species-area models.
696 *Ecology* **83**, 1185-1198. [https://doi.org/10.1890/0012-9658\(2002\)083](https://doi.org/10.1890/0012-9658(2002)083)

697 Hill, M. J., Biggs, J., Thornhill, I., Briers, R. A., Gledhill, D. G., White, J. C., Wood PJ, & Hassall,
698 C. (2017). Urban ponds as an aquatic biodiversity resource in modified landscapes. *Global Change*
699 *Biology*, **23**(3), 986-999. <https://doi.org/10.1111/gcb.13401>

- 700 Hollander, M., & Wolfe, D. A. (1973). *Nonparametric statistical methods*. New York: John Wiley
701 and Sons, 503 pp.
- 702 IBZ (2018). *FOD Binnenlandse Zaken, Algemene Directie Instellingen en Bevolking, Rijksregister:*
703 [http://www.ibz.rrn.fgov.be/fileadmin/user_upload/fr/pop/statistiques/population-bevolking-](http://www.ibz.rrn.fgov.be/fileadmin/user_upload/fr/pop/statistiques/population-bevolking-20170101.pdf)
704 [20170101.pdf](http://www.ibz.rrn.fgov.be/fileadmin/user_upload/fr/pop/statistiques/population-bevolking-20170101.pdf).
- 705 Ives, C.D., Lentin, P.E., Threfall, C.G., Ikin, K., Shanahan, D.F., Garrard, G.E., Bekessy, S.A.,
706 Fuller, R.A., Mumaw, L., Rayner, L., Rowe, R., Valentine, L.E. & Kendal, D. (2016). Cities are
707 hotspots for threatened species. *Global Ecology and Biogeography*, **25**, 117-126.
708 <https://doi.org/10.1111/geb.12404>
- 709 Jackson, H. B., & Fahrig, L. (2015). Are ecologists conducting research at the optimal scale?
710 *Global Ecology and Biogeography*, **24(1)**, 52-63. <https://doi.org/10.1111/geb.12233>
- 711 Kaiser, A., Merckx, T., & Van Dyck, H. (2016). The Urban Heat Island and its spatial scale
712 dependent impact on survival and development in butterflies of different thermal sensitivity.
713 *Ecology and Evolution*, **6(12)**, 4129-4140. <https://doi.org/10.1002/ece3.2166>
- 714 Knop, E. (2016). Biotic homogenization of three insect groups due to urbanization. *Global Change*
715 *Biology*, **22**, 228-236. <https://doi.org/10.1111/gcb.13091>
- 716 Lagucki, E., Burdine, J.D., & McCluney, K.E. (2017). Urbanization alters communities of flying
717 arthropods in parks and gardens of a medium-sized city. *PeerJ*, **5**, e3620. [https://doi.org/](https://doi.org/10.7717/peerj.3620)
718 [10.7717/peerj.3620](https://doi.org/10.7717/peerj.3620).
- 719 Lande, R. (1966). Statistics and partitioning of species diversity and similarity among multiple
720 communities. *Oikos*, **76**, 5-13.
- 721 La Sorte, F. A., Aronson, M. F., Williams, N. S., Celesti-Grapow, L., Cilliers, S., Clarkson, B. D.,
722 Dolan, R. W., Hipp, A., Klotz, S., Kühn, I., Pyšek, P., Siebert, S., & Winter, M. (2014). Beta
723 diversity of urban floras among European and non-European cities. *Global Ecology and*
724 *Biogeography*, **23(7)**, 769-779. <https://doi.org/10.1111/geb.12159>
- 725 Leibold, M. A., & Chase, J. M. (2017). *Metacommunity Ecology (Vol. 59)*. Princeton University
726 Press.

- 727 Lister, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a
728 rainforest food web. *Proceedings of the National Academy of Sciences of the USA*, **115(44)**, 10397-
729 10406. <https://doi.org/10.1073/pnas.1722477115>
- 730 LRD (2013). *Large-scale Reference Database, an object-oriented reference map of Flanders:*
731 <https://www.agiv.be/international/en/products/grb-en>.
- 732 Luck, G. W., & Smallbone, L. T. (2010). Species diversity and urbanization: patterns, drivers and
733 implications. *Urban Ecology*, 88-119.
- 734 Manley, C. (2010). *British moths and butterflies: a photographic guide*. Bloomsbury Publishing.
- 735 Marzluff, J. M. (2017). A decadal review of urban ornithology and a prospectus for the future. *Ibis*,
736 **159(1)**, 1-13. <https://doi.org/10.1111/ibi.12430>
- 737 McCune, J. L., & Vellend, M. (2013). Gains in native species promote biotic homogenization over
738 four decades in a human-dominated landscape. *Journal of Ecology*, **101(6)**, 1542-1551.
739 <https://doi.org/10.1111/1365-2745.12156>
- 740 McDonnell, M. J., Pickett, S. T., Groffman, P., Bohlen, P., Pouyat, R. V., Zipperer, W. C.,
741 Parmelee R. W., Carreiro M. M., & Medley, K. (1997). Ecosystem processes along an urban-to-
742 rural gradient. *Urban Ecosystems*, **1(1)**, 21-36. <https://doi.org/10.1023/A:1014359024275>
- 743 McGlenn, D. J., Xiao, X., May, F., Gotelli, N. J., Engel, T., Blowes, S. A., Knight, T. M., Purschke,
744 O., Chase, J. M., & McGill, B. J. (2019). Measurement of Biodiversity (MoB): A method to
745 separate the scale-dependent effects of species abundance distribution, density, and aggregation on
746 diversity change. *Methods in Ecology and Evolution*, **10(2)**, 258-269. [https://doi.org/10.1111/2041-](https://doi.org/10.1111/2041-210X.13102)
747 [210X.13102](https://doi.org/10.1111/2041-210X.13102)
- 748 McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological*
749 *Conservation*, **127**, 247-260. <https://doi.org/10.1016/j.biocon.2005.09.005>
- 750 McKinney, M. L. (2008). Effects of urbanization of species richness: a review of plants and
751 animals. *Urban Ecosystems*, **11**, 161-176. <https://doi.org/10.1007/s11252-007-0045-4>
- 752 Menke, S. B., Guénard, B., Sexton, J. O., Weiser, M. D., Dunn, R. R., & Silverman, J. (2011).
753 Urban areas may serve as habitat and corridors for dry-adapted, heat tolerant species; an example
754 from ants. *Urban Ecosystems*, **14(2)**, 135-163. <https://doi.org/10.1007/s11252-010-0150-7>

755 Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L. F., Backeljau, T., Bonte, D., Brans, K. I., Cours,
756 M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J. M. T., Fontaneto, D., Gianuca, A. T.,
757 Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., Matheve, H., Matthysen, E., Piano, E.,
758 Sablon, R., Schön, I., Van Donink, K., De Meester, L., & Van Dyck, H. (2018a). Body-size shifts in
759 aquatic and terrestrial urban communities. *Nature*, **558**, 113–116. [https://doi.org/10.1038/s41586-](https://doi.org/10.1038/s41586-018-0140-0)
760 018-0140-0

761 Merckx, T., & Van Dyck, H. (2019). Urbanization-driven homogenization is more pronounced and
762 happens at wider spatial scales in nocturnal and mobile flying insects. *Global Ecology and*
763 *Biogeography*. <https://doi.org/10.1111/geb.12969>

764 Morelli, F., Benedetti, Y., Ibáñez-Álamo, J. D., Jokimäki, J., Mänd, R., Tryjanowski, P., & Møller,
765 A. P. (2016). Evidence of evolutionary homogenization of bird communities in urban environments
766 across Europe. *Global Ecology and Biogeography*, **25(11)**, 1284-1293.
767 <https://doi.org/10.1111/geb.12486>

768 Niemelä, J., & Kotze, D. J. (2009). Carabid beetle assemblages along urban to rural gradients: a
769 review. *Landscape and Urban Planning*, **92(2)**, 65-71.
770 <https://doi.org/10.1016/j.landurbplan.2009.05.016>

771 Parris, K.M. (2016). *Ecology of Urban Environments*. Wiley-Blackwell, Chichester, West Sussex,
772 UK.

773 Penone, C., Kerbiriou, C., Julien, J. F., Julliard R., Machon, N., & Viol, I. (2013). Urbanization
774 effect on Orthoptera: which scale matters? *Insect Conservation and Diversity*, **6(3)**, 319-327.
775 <https://doi.org/10.1111/j.1752-4598.2012.00217.x>

776 Philpott, S. M., Cotton, J., Bichier, P., Friedrich, R. L., Moorhead, L. C., Uno, S., & Valdez, M.
777 (2014). Local and landscape drivers of arthropod abundance, richness, and trophic composition in
778 urban habitats. *Urban Ecosystems*, **17**, 513–532. <https://doi.org/10.1007/s11252-013-0333-0>

779 Piano, E., De Wolf, K., Bona, F., Bonte, D., Bowler, D. E., Isaia, M., Lens L, Merckx T, Mertens
780 D, van Kerckvoorde M, De Meester, L., & Hendrickx, F. (2017). Urbanization drives community
781 shifts towards thermophilic and dispersive species at local and landscape scales. *Global Change*
782 *Biology*, **23(7)**, 2554-2564. <https://doi.org/10.1111/gcb.13606>

783 Pollard, E., & Yates, T. (1993). *Monitoring butterflies for ecology and conservation: the British*
784 *butterfly monitoring scheme*. Chapman & Hall, London, UK.

785 R Development Core Team (2017). *R: A language and environment for statistical computing*. R
786 Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>

787 Ramírez-Restrepo, L., & MacGregor-Fors, I. (2017). Butterflies in the city: a review of urban
788 diurnal Lepidoptera. *Urban Ecosystems*, **20(1)**, 171-182. [https://doi.org/10.1007/s11252-016-0579-](https://doi.org/10.1007/s11252-016-0579-4)
789 4

790 Rebele, F. (1994). Ecology and special features of urban ecosystems. *Global Ecology and*
791 *Biogeography Letters*, **4**, 173–187. <https://www.jstor.org/stable/2997649>

792 Rice, W. R., & Gaines, S. D. (1994). Extending nondirectional heterogeneity tests to evaluate
793 simply ordered alternative hypotheses. *Proceedings of the National Academy of Sciences of the*
794 *USA*, **91(1)**, 225-226. <https://doi.org/10.1073/pnas.91.1.225>

795 Roberts, M. J. (2009). *The Spiders of Great Britain and Ireland: Compact Edition*. Apollo Books,
796 Denmark, 714 pp.

797 Saari, S., Richter, S., Higgins, M., Oberhofer, M., Jennings, A., & Faeth, S. H. (2016). Urbanization
798 is not associated with increased abundance or decreased richness of terrestrial animals - dissecting
799 the literature through meta-analysis. *Urban Ecosystems*, **19**, 1251–1264.
800 <https://doi.org/10.1007/s11252-016-0549-x>

801 Sánchez-Bayo, F., & Wyckhuys, K. A. (2019). Worldwide decline of the entomofauna: A review of
802 its drivers. *Biological Conservation*, **232**, 8-27. <https://doi.org/10.1016/j.biocon.2019.01.020>

803 Sattler, T., Obrist, M. K., Duelli, P., & Moretti, M. (2011). Urban arthropod communities: Added
804 value or just a blend of surrounding biodiversity? *Landscape and Urban Planning*, **103(3-4)**, 347-
805 361. <https://doi.org/10.1016/j.landurbplan.2011.08.008>

806 Seto, K. C., Sánchez-Rodríguez, R., & Fragkias, M. (2010). The new geography of contemporary
807 urbanization and the environment. *Annual Review of Environment and Resources*, **35**, 167-194.
808 <https://doi.org/10.1146/annurev-environ-100809-125336>

809 Seto, K. C., Güneralp, B., & Hutyra, L. R. (2012). Global forecasts of urban expansion to 2030 and
810 direct impacts on biodiversity and carbon pools. *Proceedings of the National Academy of Sciences*
811 *of the USA*, **109(40)**, 16083-16088. <https://doi.org/10.1073/pnas.1211658109>

812 Shochat, E., Warren, P. S., Faeth, S. H., McIntyre, N. E., & Hope, D. (2006). From patterns to
813 emerging processes in mechanistic urban ecology. *Trends in Ecology & Evolution*, **21(4)**, 186-191.
814 <https://doi.org/10.1016/j.tree.2005.11.019>

815 Shochat, E., Lerman, S. B., Anderies, J. M., Warren, P. S., Faeth, S. H., & Nilon, C. H. (2010).
816 Invasion, competition, and biodiversity loss in urban ecosystems. *BioScience*, **60(3)**, 199-208.
817 <https://doi.org/10.1525/bio.2010.60.3.6>

818 Soininen, J., McDonald, R., & Hillebrand, H. (2007). The distance decay of similarity in ecological
819 communities. *Ecography*, **30(1)**, 3-12. <https://doi.org/10.1111/j.0906-7590.2007.04817.x>

820 Turrini, T., & Knop, E. (2015). A landscape ecology approach identifies important drivers of urban
821 biodiversity. *Global Change Biology*, **21(4)**, 1652-1667. <https://doi.org/10.1111/gcb.12825>

822 Vogel, G. (2017). Where have all the insects gone? *Science*, **356(6338)**, 576-579.

823 Wiens, J. A. (1989). Spatial scaling in ecology. *Functional Ecology*, **3(4)**, 385-397.
824 <https://www.jstor.org/stable/2389612>

825 Xiao, X., McGlenn, D., May F., & Oliver, C. (2018). mobr: Measurement of Biodiversity in R. R
826 package version 1.0.

827

828 **Tables**

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

	Local (subplot) urbanization effect			Landscape (plot) urbanization effect			Interaction	
	<i>F</i>	<i>P</i>	% change	<i>F</i>	<i>P</i>	% change	<i>F</i>	<i>P</i>
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

832

833 **Table 2** – Differences in observed (a) and rarefied (b) species richness components across the three
834 urbanization categories. Plus and minus signs indicate an increase and decrease in species richness
835 from the lowest towards the highest urbanization category respectively, while NT indicates that no
836 difference was detected. Asterisks refer to comparisons wherein the intermediate urbanization level
837 showed higher or lower values compared to the low and high urbanized categories. Colour codes
838 refer to significance values (light red/light green/light yellow (light grey in printed version) -/+:
839 $0.05 > P > 0.01$, red/green/yellow (medium grey in printed version) --/++: $0.01 > P > 0.001$ and
840 dark red/dark green/dark yellow (dark grey in printed version) ---/+++ : $P < 0.001$). $\bar{\beta}_P$ and $\bar{\beta}_A$ refer
841 to proportional ($\bar{\beta}_P = \gamma/\bar{\alpha}$) and additive ($\bar{\beta}_A = \gamma - \bar{\alpha}$) beta diversity, respectively, wherein $\bar{\beta}_P$
842 expresses how much the richness at plot (or regional) level increases compared to the richness at
843 subplot (or plot) level, while $\bar{\beta}_A$ expresses the absolute increase in number of species between these
844 two sampling levels.

<i>a</i>	Local urbanization				Landscape urbanization						
	<i>A</i>	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	-	+	-	-	-	+	-	-	-	-	--
<i>b</i>	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	-	+	-	-	-	+	-	-	+	-	-

845

846

847 **Figure captions**

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

857 **Figure 2** – Schematic overview of the calculated diversity components to test the effect of
858 urbanization at local scale (a; 200 m x 200 m) and landscape scale (b; 3 km x 3 km) (low = green
859 (medium grey in printed version), intermediate = yellow (light grey in printed version), and high =
860 red (dark grey in printed version)). Only the comparisons between low and high urbanization levels
861 are shown.

862 **Figure 3** – Abundances (N) of the nine examined groups in response to local- (subplot) and
863 landscape-scale (plot) urbanization levels. Labels at the X-axis represent the degree of urbanization
864 at the landscape scale. Y-axis scale varies among groups and is log₁₀-transformed, except for web
865 spiders. Colours of the boxplots refer to urbanization levels at the local scale (green (medium grey
866 in printed version) = low; yellow (light grey in printed version) = intermediate; red (dark grey in
867 printed version) = high). Boxplots display the median, 25% and 75% quartiles and 1.5 interquartile
868 range. The nine animal silhouettes are from PhyloPic (<http://www.phylopic.org>) and fall under CC-
869 BY 3.0 licences.

870 **Figure 4** – (a) Estimated total number of species for each examined group in nine random samples
871 from five different local/landscape urbanization level combinations using raw data. Y-axis scale is

872 log₁₀-transformed to improve visualization. Pictograms on the x-axis depict (from left to right): (i)
873 subplots with low urbanization in plots with low urbanization (light green square in dark green
874 square); (ii) plots with low urbanization regardless of the degree of local urbanization (light grey
875 square in dark green square); (iii) samples regardless of the degree of local and landscape
876 urbanization level (light grey square in dark grey square); (iv) highly urbanized plots regardless of
877 the degree of local urbanization (light grey square in dark red square) and (v) highly urbanized
878 subplots in highly urbanized plots (light red square in dark red square). Asterisks (* = 0.01 < P <
879 0.05, ** = 0.01 < P < 0.001, *** = P < 0.001) depict results of the directional ordered heterogeneity
880 test rSPc. (b) Correlation between urbanization-related change in abundance versus change in local
881 (open circles) and total (closed circles) observed species richness across examined groups. Values
882 on both axes represent the relative abundance (X-axis) and species richness (Y-axis) in highly
883 urbanized subplots in highly urbanized plots versus those in subplots with low urbanization in plots
884 with low urbanization. Animal silhouettes are from PhyloPic (<http://www.phylopic.org>) and fall
885 under CC-BY 3.0 licences.

886 **Figure 5** – Total observed diversity (S; Y-axis) partitioning for each examined group and for each
887 of three (a) local- and (b) landscape-scale urbanization levels (green (medium grey in printed
888 version) = low; yellow (light grey in printed version) = intermediate; red (dark grey in printed
889 version) = high). See Figure 2 for an explanation of the different diversity components. The animal
890 silhouettes are from PhyloPic (<http://www.phylopic.org>) and fall under CC-BY 3.0 licences.

891