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

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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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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

INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

Sampling area and design

130 Sampling was conducted in Belgium, within a polygon of 8140 km^2, 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 x 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 cm^2 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 $r_S P_c$

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, McGlinn, 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 (McGlinn 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   (McGlinn 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 ($\beta_{Pwithin}$).

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 (McGlinn 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

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.

BIBLIOGRAPHY

Agresti, A. (1996). *An introduction to categorical data analysis*. Wiley, New York, US.

Anderson, M. J., Crist, T. O., Chase, J. M., Vellend, M., Inouye, B. D., Freestone, A. L., Sanders, N. J., Cornell, H. V., Comita, L. S., Davies, K. F., Harrison, S. P., Kraft, N. J. B., Stegen, J. C., & Swenson, N. J. (2011). Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. *Ecology Letters*, **14(1)**, 19-28. https://doi.org/10.1111/j.1461-0248.2010.01552.x

Aronson, M. F., La Sorte, F. A., Nilon, C. H., Katti, M., Goddard, M. A., Lepczyk, C. A., Warren, P. S., Williams, N. S. G., Cilliers, S., Clarckson, B., Dobbs, C., Dolan, R., Hedblom, M., Klotz, S., Kooijmans, J. L., Kühn, I., MacGregor-Fors, I., McDonnell, M., Mörtberg, U., Pyšek, P., Siebert, S., Sushinsky, J., Werner, P., & Winter, M. (2014). A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. *Proceeding of the Royal Society B,* **281(1780)**, 20133330. https://doi.org/10.1098/rspb.2013.3330

Aronson, M.F.J, Lepczyk, C.A., Evans, K.L., Goddard, M.A., Lerman, S.B., MacIvor, J.S., Nilon, C.H. & Vargo, T. (2017). Biodiversity in the city: key challenges for urban green space management. *Frontiers in Ecology and the Environment*, **15**, 189-196. https://doi.org/10.1002/fee.1480

Baldock, K. C., Goddard, M. A., Hicks, D. M., Kunin, W. E., Mitschunas, N., Osgathorpe, L. M., Potts, S. G., Robertson, K. M., Scott, A. V., Stone, G. N., Vaughan, I. P., & Memmott, J. (2015). Where is the UK's pollinator biodiversity? The importance of urban areas for flower-visiting insects. *Proceeding of the Royal Society B*, **282(1803)**, 20142849. https://doi.org/10.1098/rspb.2014.2849

Bates, A. J., Sadler, J. P., Fairbrass, A. J., Falk, S. J., Hale, J. D., & Matthews, T. J. (2011). Changing bee and hoverfly pollinator assemblages along an urban-rural gradient. *PloS one*, **6(8),** e23459. https://doi.org/10.1371/journal.pone.0023459

Beninde, J., Veith, M. & Hochkirch, A. (2015). Biodiversity in cities needs space: a meta-analysis of factors determining intra-urban biodiversity variation. *Ecology Letters*, **18**, 581-592. https://doi.org/10.1111/ele.12427

Bink, F. A. (1992). *Ecologische atlas van de Dagvlinders van Noordwest-Europa*. Schuyt & Co, Haarlem, The Netherlands, 512 pp.

619   Boeken, M., Desender, K., Drost, B., van Gijzen, T., Koese, B., Muilvijk, 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., Bater, 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

Concepción, E. D., Moretti, M., Altermatt, F., Nobis, M. P., & Obrist, M. K. (2015). Impacts of urbanization on biodiversity: the role of species mobility, degree of specialisation and spatial scale. *Oikos*, **124**, 1571-1582. https://doi.org/10.1111/oik.02166

Concepción, E. D., Götzenberger, L., Nobis, M. P., Bello, F., Obrist, M. K., & Moretti, M. (2016). Contrasting trait assembly patterns in plant and bird communities along environmental and human-induced land-use gradients. *Ecography*, **40(6)**, 753-763. https://doi.org/10.1111/ecog.02121

Crist, T. O., Veech, J. A., Gering, J. C., & Summerville, K. S. (2003). Partitioning species diversity across landscapes and regions: a hierarchical analysis of α, β and γ diversity. *The American Naturalist*, **162(6)**, 734-743.

Croci, S., Butet, A., & Clergeau, P. (2008). Does urbanization filter birds on the basis of their biological traits? *The Condor*, **110(2)**, 223-240. https://doi.org/10.1525/cond.2008.8409

Donner, J. (1965). *Ordnung Bdelloidea. Bestimmungsbücher zur Bodenfauna Europas*. Akademie-Verlag, Germany, 297 pp.

Duff, A. G. (2016). *Beetles of Britain and Ireland. Vol. 4*. A.G. Duff Publishing, West Runton, UK, 623 pp.

Egerer, M. H., Arel, C., Otoshi, M. D., Quistberg, R. D., Bichier, P., & Philpott, S. M. (2017). Urban arthropods respond variably to changes in landscape context and spatial scale. *Journal of Urban Ecology* **3**. https://doi.org/10.1093/jue/jux001

Faeth, S. H., Bang, C., & Saari, S. (2011). Urban biodiversity: patterns and mechanisms. *Annals of the New York Academy of Sciences*, **1223(1)**, 69-81. https://doi.org/10.1111/j.1749-6632.2010.05925.x

Fahrig, L. (2013). Rethinking patch size and isolation effects: the habitat amount hypothesis. *Journal of Biogeography*, **40(9),** 1649-1663. https://doi.org/10.1111/jbi.12130

Ferenc, M., Sedlacek, O., Fuchs, R., Dinetti, M., Fraissinet, M., & Storch, D. (2014). Are cities different? Patterns of species richness and beta diversity of urban bird communities and regional species assemblages in Europe. *Global Ecology and Biogeography,* **23**, 479–489. https://doi.org/10.1111/geb.12130

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

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

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

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

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

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

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

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

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

Hill, M. J., Biggs, J., Thornhill, I., Briers, R. A., Gledhill, D. G., White, J. C., Wood PJ, & Hassall, C. (2017). Urban ponds as an aquatic biodiversity resource in modified landscapes. *Global Change 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-
704 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/
718 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.

Lister, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web. *Proceedings of the National Academy of Sciences of the USA*, **115(44)**, 10397-10406. https://doi.org/10.1073/pnas.1722477115

LRD (2013). *Large-scale Reference Database, an object-oriented reference map of Flanders:* https://www.agiv.be/international/en/products/grb-en.

Luck, G. W., & Smallbone, L. T. (2010). Species diversity and urbanization: patterns, drivers and implications. *Urban Ecology*, 88-119.

Manley, C. (2010). *British moths and butterflies: a photographic guide.* Bloomsbury Publishing.

Marzluff, J. M. (2017). A decadal review of urban ornithology and a prospectus for the future. *Ibis*, **159(1)**, 1-13. https://doi.org/10.1111/ibi.12430

McCune, J. L., & Vellend, M. (2013). Gains in native species promote biotic homogenization over four decades in a human-dominated landscape. *Journal of Ecology*, **101(6)**, 1542-1551. https://doi.org/10.1111/1365-2745.12156

McDonnell, M. J., Pickett, S. T., Groffman, P., Bohlen, P., Pouyat, R. V., Zipperer, W. C., Parmelee R. W., Carreiro M. M., & Medley, K. (1997). Ecosystem processes along an urban-to-rural gradient. *Urban Ecosystems*, **1(1)**, 21-36. https://doi.org/10.1023/A:1014359024275

McGlinn, D. J., Xiao, X., May, F., Gotelli, N. J., Engel, T., Blowes, S. A., Knight, T. M., Purschke, O., Chase, J. M., & McGill, B. J. (2019). Measurement of Biodiversity (MoB): A method to separate the scale-dependent effects of species abundance distribution, density, and aggregation on diversity change. *Methods in Ecology and Evolution*, **10(2),** 258-269. https://doi.org/10.1111/2041-210X.13102

McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological Conservation*, **127**, 247-260. https://doi.org/10.1016/j.biocon.2005.09.005

McKinney, M. L. (2008). Effects of urbanization of species richness: a review of plants and animals. *Urban Ecosystems*, **11**, 161-176. https://doi.org/10.1007/s11252-007-0045-4

Menke, S. B., Guénard, B., Sexton, J. O., Weiser, M. D., Dunn, R. R., & Silverman, J. (2011). Urban areas may serve as habitat and corridors for dry-adapted, heat tolerant species; an example 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-

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-

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., McGlinn, 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	
	F	*P*	*% change*	*F*	*P*	*% change*	*F*	*P*
Ground beetles	$F_{2,48} = 3.26$	**0.047**	**-31.3**	$F_{2,48} = 0.430$	0.654	-10.0	$F_{4,48} = 0.090$	0.984
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

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

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

Bdelloid rotifers	-	+	---	+	NT	NT	NT	NT	-	-	-
Cladocerans	+	-	--*	-	+*	+	-	+	+	+	+
Across groups	-	+	-	-	-	+	-	-	+	-	-

845

846

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