

Contrasting impacts of urban and farmland cover on flying insect biomass

Authors

Cecilie S. Svenningsen ¹, Diana E. Bowler ^{2,3,4}, Susanne Hecker ^{4,2}, Jesper Bladt ⁵, Volker Grescho ^{2,4}, Nicole M. van Dam ^{2,3}, Jens Dauber ⁶, David Eichenberg ^{2,4}, Rasmus Ejrnæs ⁵, Camilla Fløjgaard ⁵, Mark Frenzel ⁷, Tobias Guldberg Frøslev ⁸, Anders Johannes Hansen ⁸, Jacob Heilmann-Clausen ⁹, Yuanyuan Huang ^{4,2}, Jonas Colling Larsen ¹, Juliana Menger ^{2,4,10}, Nur Liyana Binti Mat Nayan ^{4,2}, Lene Bruhn Pedersen ¹, Anett Richter ^{4,2,6}, Robert R. Dunn ^{1,11}, Anders P. Tøttrup ¹, Aletta Bonn ^{4,3,2*}

Institutional affiliation

¹ Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen, Denmark

² German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5a, 04103 Leipzig, Germany

³ Friedrich Schiller University Jena, Institute of Biodiversity, Dornburger Straße 159, 07743 Jena, Germany

⁴ Helmholtz Centre for Environmental Research - UFZ, Department of Ecosystem Services, Permoserstr. 15, 04318 Leipzig, Germany

⁵ Aarhus University, Department of Bioscience - Biodiversity and Conservation, Grenåvej 14, 8410 Rønde, Denmark

⁶ Thünen Institute of Biodiversity, Bundesallee 65, 38116 Braunschweig, Germany

⁷ Helmholtz Centre for Environmental Research - UFZ, Department of Community Ecology, Th.-Lieser-Str. 4, 06120 Halle, Germany

⁸ Centre for GeoGenetics, GLOBE Institute, University of Copenhagen, Denmark

⁹ Centre for Macroecology, Evolution and Climate, GLOBE Institute, University of Copenhagen, Denmark

¹⁰ Instituto Nacional de Pesquisas da Amazônia, Coordenação de Biodiversidade, Av. André Araújo 2936, CEP 69067-375, Manaus, Brazil

¹¹ Department of Applied Ecology, North Carolina State University, Campus Box 7617, NC State University Campus, Raleigh, NC 27695-7617, United States of America

*Aletta Bonn and Anders P. Tøttrup are joint senior author

Corresponding author

Cecilie Skræp Svenningsen, e-mail: cssvenningsen@snm.ku.dk, ORCID: 0000-0002-9216-2917, phone number: +45 35 33 63 92

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1 Abstract

2 Recent studies report declines in biomass, abundance and diversity of terrestrial insect
3 groups. While anthropogenic land use is one likely contributor to this decline, studies
4 assessing land cover as a driver of insect dynamics are rare and mostly restricted in spatial
5 scale and types of land cover. In this study, we used rooftop-mounted car nets in a citizen
6 science project ('InsectMobile') to allow for large-scale geographic sampling of flying insects
7 across Denmark and parts of Germany. Citizen scientists sampled insects along 278 10 km
8 routes in urban, farmland and semi-natural (grassland, wetland and forest) landscapes in the
9 summer of 2018. We assessed the importance of local to landscape-scale effects and land
10 use intensity by relating insect biomass to land cover in buffers of 50, 250, 500 and 1000 m
11 along the routes. We found a negative association of urban cover and a positive association
12 of farmland on insect biomass at a landscape-scale (1000 m buffer) in both countries. In
13 Denmark, we also found positive effects of all semi-natural land covers, i.e. grassland
14 (largest at the landscape-scale, 1000 m), forests (largest at intermediate scales, 250 m), and
15 wetlands (largest at the local-scale, 50 m). The negative association of insect biomass with
16 urban land cover and positive association with farmland were not clearly modified by any
17 variable associated with land use intensity. Our results show that land cover has an impact
18 on flying insect biomass with the magnitude of this effect varying across spatial scales. Since
19 we consistently found negative effects of urban land cover, our findings highlight the need for
20 the conservation of semi-natural areas, such as wetlands, grasslands and forests, in Europe.

21 Introduction

22 Insect decline related to changes in land cover and land use intensity
23 Agricultural production and urbanisation have increased over the centuries, with at least
24 three-quarters of the global land area currently affected by human activities (IPBES 2019).
25 The IPBES Global assessment draws a sobering picture of global biodiversity decline
26 associated with human activities; however, much of our understanding is based on

27 vertebrates and plants. Yet, the majority of terrestrial animal species are insects (Stork,
28 2018). Changes in their biomass, abundance, and community composition could have
29 diverse consequences, via alterations of food webs, nutrient recycling, pollination, and pest
30 control, among others. Recent studies have found declining populations for terrestrial
31 arthropod groups (e.g., Thomas *et al.* 2004; Hallmann *et al.* 2017, 2020; Valtonen *et al.*
32 2017; van Klink *et al.* 2020), with especially good evidence for some Lepidoptera, the order
33 that is most commonly subject to the most long-term monitoring (e.g. Conrad *et al.* 2006; van
34 Strien *et al.*, 2019; Bell, Blumgart, & Shortall, 2020). However, at the same time, some insect
35 taxa are expanding their distribution in Europe, including some dragonfly species, at least
36 over recent decades (Termaat *et al.* 2019). Other insect groups, such as ants, seem to be
37 able to persist even when exposed to extreme change (Guénard, Cardinal-De Casas &
38 Dunn, 2015). However, we still lack a comprehensive view of how land cover and land use
39 intensity affect insect populations (Seibold *et al.*, 2019). Few studies have simultaneously
40 compared insect biomass across multiple different habitat types and at different spatial
41 scales. Nonetheless, understanding relationships between insect biomass and land cover
42 and land use is essential for conservation strategies aiming to mitigate insect loss.

43 The effect of urbanisation on insect diversity and biomass

44 Arguably one of the most extreme land cover changes imposed by human activities is
45 urbanisation (Seto, Güneralp & Hutyrá, 2012). Across several insect taxa, Piano *et al.* (2020)
46 found that urbanisation was associated with a decline in insect diversity at multiple spatial
47 scales in Belgium. Similarly, a recent meta-analysis combining studies from across the world
48 found a mean negative effect of urbanisation on terrestrial arthropod diversity and
49 abundance, although the effect may differ among insect orders (Fenoglio, Rossetti & Videla,
50 2020). Long-term monitoring in Britain found lepidopteran biomass to be lower in urban sites
51 compared to woodland and grassland (Macgregor *et al.*, 2019). However, not all
52 urbanisation is created equally. Cities with greater amounts of green space harboured higher
53 insect pollinator abundances than cities with less green space (Turrini & Knop, 2015). Nor do

54 all taxa respond in the same ways to urbanisation. For example, in one study, Hymenoptera
55 showed higher species richness and flower visitation rates in urban areas compared to rural
56 areas, with the opposite pattern exhibited by Lepidoptera and Diptera (Theodorou *et al.*,
57 2020).

58 Agricultural land use effects on insect diversity and biomass

59 Similar to urbanisation, land conversion for crop production has substantial consequences
60 for biodiversity. In one study, Lepidopteran biomass was lower in arable areas compared to
61 woodland and grassland sites (Macgregor *et al.*, 2019). However, most studies on the
62 impact of agricultural habitats have focused on comparisons among farming systems, e.g.,
63 conventional vs organic, rather than comparisons to semi-natural habitats (e.g. Kleijn &
64 Sutherland, 2003; Bengtsson, Ahnström & Weibull, 2005; Bianchi, Booij & Tscharrntke, 2006;
65 Boutin, Martin & Baril, 2009). Overall, insect species richness has been reported as, on
66 average, 30% higher in areas with organic farming, although the positive effects vary over
67 spatial scales, and among taxa and functional groups (Bengtsson, Ahnström & Weibull,
68 2005). In Germany, Red List Lepidoptera biomass and species richness are twice as high in
69 organic farmland compared to conventional farmland (Hausmann *et al.*, 2020). Also, a high
70 proportion of semi-natural areas within farmland, in essence, a more extensively used
71 landscape, can have positive effects on insect taxa such as pollinators, that rely on the food
72 and nesting resources such extensive landscapes provide (Cole *et al.*, 2017). However, in
73 general, it is less clear how much insect biomass in farmland (whether organic or
74 conventional) differs from semi-natural areas, not to mention natural ecosystems, even
75 though effects of agriculture or agricultural intensification are often invoked to explain insect
76 declines (Goulson *et al.*, 2015; Goulson, Thompson & Croombs, 2018).

77 Grassland, wetland, and forest land cover effect on insect diversity and biomass

78 Grassland, wetland and forests are often considered semi-natural because they are to some
79 extent human-modified compared to natural ecosystems. In the cultural landscapes
80 of Europe, all these habitats have variable land use histories resulting in a continuum from

81 semi-natural to highly managed. Most forests in Western Europe are often managed for
82 timber production (McGrath *et al.*, 2015) and grasslands are often improved for extensive
83 agri-environmental practices, i.e. managed for high biomass yields of high energy content
84 fodder. These land covers might be expected to have greater abundance and biomass of
85 insects than urban and agricultural areas. Indeed, sites with more forest cover showed
86 weaker temporal declines of insect biomass in Germany (Hallman *et al.*, 2017); while
87 Lepidoptera biomass in woodland sites across Britain increased over a period of 10 years
88 (Macgregor *et al.*, 2019). Still, some studies show no significant differences in insect
89 abundances and diversity between managed and semi-natural forests (Young & Armstrong,
90 1994; Watt, Barbour & McBeath, 1997; Humphrey *et al.*, 1999), although natural habitat
91 structures related to deadwood, veteran trees and glades have been shown to be crucial for
92 threatened specialist species (Heilmann-Clausen & Christensen, 2004; Lassauce, *et al.*,
93 2011).

94 Study approach and expectations

95 Drivers of fluctuations in insect populations are challenging to assess since long-term spatio-
96 temporal population data are rare (De Palma *et al.*, 2018). However, analysis of spatial
97 patterns might indicate which land cover and land use changes are most harmful to insects.
98 In this study, we investigate drivers of insect biomass by examining spatial patterns across
99 two European countries, Denmark (northern Europe) and Germany (middle Europe).
100 Denmark is covered by 74% highly human-modified landscapes with 61% agricultural areas,
101 13% settlements and infrastructure, and the remaining landscape mainly covered by 13%
102 forests and 11% semi-natural areas (Statistics Denmark, 2019). Germany is similarly
103 covered by highly human-modified landscapes, with over 50% of the land area used for
104 farming, and the remaining area primarily used for forestry (31%) and human settlements
105 and infrastructure (13.7%) (German Federal Statistics Office, 2015). For our study, we
106 motivated citizen scientists to sample flying insects with car-nets as part of the InsectMobile
107 project. Car nets have been employed for biting flies, mosquito and beetle sampling by

108 professionals and amateurs for more than half a century (e.g. Bidlingmayer, 1966; Dyce,
109 Standfast and Kay, 1972; Roberts and Irving-Bell, 1985), but have not been used as a
110 standardised insect sampling method before. Our approach has the advantage of allowing
111 multiple land covers to be sampled nearly simultaneously across large scales in a uniform
112 and standardised way.

113 To our knowledge, this is the first study to simultaneously assess the effects of multiple land
114 covers and land use intensities on insect biomass. We compared insect biomass among
115 major land cover types: urban, farmland, grassland, wetland and forest across Denmark and
116 parts of Germany. We focused on insect biomass for several reasons: it aligns with reported
117 declines of insect biomass (Hallman *et al.*, 2017); it is a relevant measure for ecosystem
118 functioning (Barnes *et al.*, 2016), and it is a measure of resource availability for higher
119 trophic levels. Overall, we hypothesised that insect biomass would be lower in areas with
120 more human-modified land cover and more intense land use. Specifically, we assume (H1)
121 lower biomass in farmland areas compared to open semi-natural habitats (wetland and
122 grassland) due to agricultural practices such as pesticide use, homogenisation, and
123 ploughing and harvesting, either directly killing insects or removing habitats and resources
124 for insects. Further, we assume (H2) that urban cover would have the lowest insect biomass
125 among all land covers due to the high proportion of impervious surfaces and the low
126 proportion of blue and green space, meaning limited food, nesting and breeding resources.
127 Finally, we assume (H3) that insect biomass within highly-modified land cover types would
128 be negatively associated with increasing land use intensity, reflected by variables such as
129 intensity of farming and urban structural composition, i.e. larger cities and urban green
130 space.

131 **Materials & Methods**

132 **Citizen science sampling with car nets**

133 Flying insects were sampled by standardised nets attached to the rooftop of cars. The car
134 net is funnel-shaped with a detachable sampling bag at the far end for sample collection.
135 Metal guy line adjusters enable adjustment to car length and allow the net to be used on
136 most car types (Figure 1).

137

138 Citizen scientists were recruited by the Natural History Museum of Denmark in Denmark
139 (NHMD) and for a scoping study by the German Centre for Integrative Biodiversity Research
140 (iDiv) in Germany during spring 2018. The citizen scientists received a simple sampling
141 protocol as well as video tutorials and FAQ sheets along with the sampling equipment
142 (Supplementary Information (SI) V).

143 Sampling was carried out by 151 Danish and 29 German citizen scientists along 211 routes
144 from 1 - 30 June 2018 in Denmark, and along 67 routes between 25 June - 8 July 2018 in
145 Germany (Figure 2). Sampling of each route was carried out in two time intervals during the
146 day: between 12-15 h (midday) and between 17-20 h (evening) with a maximum speed of 50
147 km/h and weather conditions of at least 15°C, an average wind speed of maximum 6 m/s
148 and no rain. Samples were placed in 96% pure ethanol and sent back to NHMD and iDiv by
149 the citizen scientists.

150 Route design

151 Across both countries, routes were designed with a length of 5 km across five land cover
152 types: farmland, grassland, wetland, forest and urban areas. Each sampling event covered
153 10 km length in total - either driven in one direction or 5 km driven in both directions to cover
154 the total length. The routes were constructed in ArcGIS and QGIS using information from
155 Google Earth, Google Maps, OpenStreetMap (OSM), including data from Danish authorities
156 on land cover types in Denmark, and also using the German ATKIS data (Amtliches
157 Topographisch-Kartographisches Informationssystem) in Germany. The different land cover
158 data sources were used to assess the land cover along the routes to ensure as much

159 homogeneity in the chosen land cover type as possible. Routes were adjusted, if needed,
160 following incorporation of local area knowledge of the citizen scientists about land use,
161 accessibility, road condition and safety. In a few cases in Germany, routes were shorter due
162 to topographical limitations (e.g., extent of wetland and urban areas) and were therefore
163 driven several times back and forth to achieve a total length of 10 km.

164 Dry weight of bulk insect samples

165 In the laboratories of the NHMD and iDiv, insects were removed from the sampling bag with
166 a squeeze bottle containing 96% EtOH and forceps. Empty 15 or 50 ml centrifuge tubes
167 were weighed, and the insects were transferred to the tubes. The insects were dried
168 overnight at 50 °C in an oven (>18hrs), and the tubes containing the dry insects were
169 weighed to obtain the sample biomass (in mg).

170 Environmental data

171 According to Seibold *et al.* (2019), the effect of land cover levels off at a 1000 m buffer for
172 grassland and forest sites, we, therefore, extracted land use predictors for insect biomass
173 from four buffer zones for each route: 50 m, 250 m, 500 m, and 1000 m in five categories;
174 urban, farmland, grassland, wetland, and forest. Land use predictors were compiled into land
175 cover categories. A comprehensive overview of land cover categories and their definitions
176 are listed in Supplementary Information I. Land cover classifications were aligned across the
177 Danish and German data to the same categories.

178

179 Land use intensity data for Denmark were extracted for farmland and urban routes. The
180 farmland category consisted of crop types compiled into three overall categories: extensive,
181 semi-intensive, intensive, and agricultural areas with no associated crop type. Extensive
182 farmland is, e.g. fallow land etc., semi-intensive farmland is, e.g. orchards etc., and intensive
183 farmland is, e.g. wheat, rye, beans, etc. Grass leys (rotational grassland in an agricultural
184 area to ensure soil fertility) were included in all three intensity categories, whereas semi-

185 natural grassland only consisted of grasslands under the Danish Protection of Nature Act
186 Section 3. The three overall farmland categories in Denmark were further compiled into
187 organic and conventional farming practices. For the available German data, it was not
188 possible to make the distinction between grass leys and semi-natural grassland or farmland
189 practices. The urban category for Denmark consisted of various building type categories,
190 such as multistory buildings, residential areas, commercial areas and inner-city areas. Both
191 multistory buildings and inner-city cover are only found for larger cities. These data were not
192 available for Germany.

193

194 We extracted potential stop variables to account for sampling heterogeneity introduced by
195 the number of stops along each route. We obtained the number of traffic lights or stops of
196 any type (e.g. roundabouts, pedestrian crossings, stop signs, railroad crossings) within a 25-
197 30 m buffer using OSM. For Danish routes, we obtained the number of roundabouts using
198 data from the Danish Map Supply provided by SDFE (Agency for Data Supply and
199 Efficiency) (GeoDenmark-data), since data on roundabouts in Denmark was limited to three
200 records in OSM.

201

202 Mean hourly temperature and wind was extracted for each route including date and time
203 band from the nearest weather station using the rdwd R package for German routes. For
204 Danish routes, temperature, average wind speed, and sampling time were registered by the
205 citizen scientists.

206 **Statistical analyses**

207 The German and the Danish datasets were analysed separately while applying the same
208 modelling approaches and methods to enable comparison.

209 **Correlation and PCA**

210 We first investigated the correlations among the land cover variables to explore land use
211 gradients and assess whether multicollinearity would be an issue in multiple regression
212 models. We investigated this by calculating pairwise Pearson correlation coefficient as well
213 as principal components analysis (PCA). Correlations among land cover types were
214 strongest between urban and farmland, with increasing farmland associated with decreasing
215 urban cover (Denmark, $r = -0.6$; Germany, $r = -0.46$, both calculated for the 1000 m buffer).
216 However, since the correlations among the land cover types were not strong (see SI IV:
217 Figure 4.1), and hence none were redundant, we analysed the land cover variables as
218 separate variables, but later considered the patterns with the land covers simplified to the
219 first two PCA axes (see SI II). We used a varimax-rotated PCA to maximise the variation
220 explained by each axis, using the psych R package (Revelle, 2020). Using the same model
221 structure as below (equation 1), we used the first two PCA axes, as described above, as
222 land cover explanatory variables in an additional set of models (SI II). Results from
223 correlation tests and PCA can be found in the supplementary information.

224 General model

225 To test the impact of land cover on insect biomass, we analysed log biomass (+1, since
226 there were a few zeros) as the response in mixed-effects models assuming a normal
227 distribution, with land cover or land use variables as our main explanatory variables. To
228 control for other factors causing variation in insect biomass, we included the day of the year,
229 time band (midday vs evening), time of day (centred around each time band, and then
230 nested within time-band as a predictor), weather variables (temperature and wind) and other
231 measures of possible sampling variation (log-transformed number of traffic lights, or other
232 stops) (hereafter, called controlling variables). Additionally, to account for potential non-
233 independence of data points, we included random effects for route and citizen scientists (i.e.,
234 driver and car). The mixed-effects models were fit using lmer in the lme4 R package (Bates
235 *et al.*, 2015).

236 Hence, the general form of the mixed-effects model was:

$$\begin{aligned} 237 \quad \log(\text{Biomass} + 1) &\sim \text{Land.cover} + \text{Time.band} + \text{Time.band:Time} + \text{Day} + \log(\text{StopNumber} + 1) \\ 238 \quad &\quad \text{DriverID} + \text{RouteID} \text{ (equation 1)} \end{aligned}$$

239 We consistently found no effect of weather variables (probably because of little variation, as
240 the samples were taken under similar weather conditions), and therefore they were not
241 included in the final models.

242 Spatial autocorrelation

243 Since the sampling points were spatially-structured, we investigated whether the models of
244 insect biomass should account for spatial autocorrelation. We plotted correlograms and
245 tested for spatial autocorrelation with Moran's I (simulated residuals from the lmer model,
246 DHARMA R package (Hartig, 2020)) but did not find evidence for spatial autocorrelation in
247 the residuals of the fitted model of equation 1 ($p = 0.3$). Moreover, we also used a
248 generalised least squares model (GLS, in R package nlme (Pinheiro *et al.*, 2020)), with the
249 same response and explanatory variables described above and the geographic coordinates
250 of each route as an exponential spatial correlation structure (nugget = TRUE). These models
251 produced very similar results and models without the spatial term had a lower AIC. Based on
252 these findings, we analysed the findings of the model without the explicit spatial structure.

253 Land cover as ecological predictors

254 Using models of the general form of equation 1, we tested the effect of each land cover
255 variable. We used % coverage of each land use type (see documentation in SI I) within the
256 four different buffer zones (50 m, 250 m, 500 m, and 1000 m) around each route,
257 representing local to landscape-scale effects. To facilitate comparison of the effects of each
258 land cover within and across countries, covariates were kept in their original units; hence,
259 effect sizes of the land cover relate to change in biomass per 1% land cover change.
260 Because some of the variables were skewed, we also checked the effects of applying
261 square-root transformations to the land cover data.

262 Simple regression models

263 We first tested the effect of each land cover and buffer combination (5 land covers x 4 buffer
264 widths) on insect biomass in simple regression models (i.e., one land cover variable per
265 model, but including controlling variables of time, day and stops as well) (SI II: Figure 2.1).
266 We used these simple models to identify the best buffer width (i.e., one with the largest
267 effect size) for each land cover.

268

269 For the Danish data, we found a grassland outlier route containing around 40% grassland
270 cover, where all other routes with grassland contained less than half of that cover (<20%).
271 We excluded this route from the analysis, as it could introduce bias in our models (see SI II
272 for model outputs and visualisation with the outlier).

273 Multiple regression models

274 Full model

275 We then built a linear mixed-effects model that included all five of the land cover variables
276 (at the best buffer width for each one) and the controlling variables day of the year, time
277 band, time of day, and log-transformed number of traffic lights or stops. We examined
278 variation inflation factors to check whether collinearity among explanatory variables (i.e.,
279 variable redundancy) was an issue.

280 Best fit model

281 We identified the best fit model using AIC, i.e. the model with the lowest AIC, and ran the
282 analysis with the modified models for each country (see included variables in both the full
283 and the best fit model in Table 1). To examine the partial effects of each land cover variable,
284 we used the effects R package (Fox & Weisberg, 2018) to predict the change in biomass
285 with increased land cover for each land cover type, controlling for effects of other land
286 covers as well as controlling variables, at their mean values (Figure 4), based on the model
287 output from the full model.

288

289 We additionally tested whether the effect of land use depends on time band (i.e., a time
290 band:land use interaction) as different insect communities are expected to be sampled at
291 different times of the day, which may respond differently to land cover.

292 Land use intensity as ecological predictors

293 For routes dominated by urban or farmland, we further investigated whether variables
294 associated with the intensity of land use within the 1000 m buffer explained variation in
295 insect biomass. We restricted this analysis to the Danish routes because of the larger
296 sample size. We first selected the routes where the dominant land cover (i.e., the highest
297 proportion among land covers) was farmland within the 1000 m buffer for farmland intensity
298 analysis, or urban within the 1000 m buffer, for urban intensity analysis. In this subset, 34
299 routes were dominated by urban areas, and 255 routes were dominated by farmland. We
300 tested for correlation between land use variables by calculating pairwise Pearson correlation
301 coefficient and PCA (see SI III).

302

303 To account for the association between general land cover and the land use intensity
304 variables, we calculated the proportional cover of the land use intensity variable within the
305 land cover variable (i.e., the proportion of green space within the urban land cover.) We then
306 constructed models similar to equation 1, but included an interaction term between the
307 overall land cover and the proportional land use intensity variables (equation 2). These
308 interactions tested whether the effect of urban cover depended on the land use intensity
309 properties of the urban cover, and similarly whether the effect of farmland cover depends on
310 the land use intensity properties of the farmland cover:

311

$$312 \quad \log(\text{Biomass} + 1) \sim \text{Land.cover} * \text{Intensity} + \text{Time.band} + \text{Time.band:Time} + \text{Day} + \\ 313 \quad \log(\text{StopNumber} + 1) \text{ DriverID} + \text{RouteID} \text{ (equation 2)}$$

314 We examined whether there was a strong correlation between land covers and land use
315 intensity variables after calculating the proportional cover of the intensity variables (SI III:

316 Figure 3.4 & 3.8) and removed highly correlated variables in urban land use intensity
317 analysis from the model.

318

319 All analyses were carried out in R (version 3.6.3).

320 Results

321 Land cover

322 We found a negative effect of urban land cover on insect biomass and higher biomass in all
323 rural land covers, including farmland cover. Especially at the broader landscape scale, we
324 found significant associations.

325

326 In Denmark, largest effect sizes for urban, farmland and grassland were associated with
327 buffers of 1000 m as well as for 250 m for forest and 50 m for wetland (SI II: Figure 2.1A). In
328 Germany, all land covers except forest had largest effect sizes associated with 1000 m
329 buffers; forest cover had similar effect sizes with buffers between 250, 500 and 1000 m (SI
330 II: Figure 2.1B). The dominant land cover types within the routes were farmland (mean
331 coverage of 54% in Denmark, and 37% in Germany), urban (mean coverage of 12% in
332 Denmark and 21% in Germany) and forest (mean coverage of 16% in Denmark and 26% in
333 Germany), which reflect the coverage of these cover types in the two countries.

334 Denmark

335 In the best fit model, we found a positive effect of wetland, grassland, forest and farmland on
336 insect biomass, and a negative effect of urban land cover on insect biomass. Furthermore,
337 we found a positive effect of increasing biomass throughout the month of June and higher
338 biomass in the evening compared to midday. Fixed effects of land cover type and control
339 variables explained 33% of the variation in biomass. Results were similar when land cover
340 types with skewed distribution were transformed by a square-root transformation. The mean
341 landscape composition for samples with high biomass (within top 20% of biomass samples,

342 >262 mg) was dominated by farmland cover. In comparison, the mean landscape
343 composition for samples with low biomass (within the bottom 20% of biomass samples,
344 <48.8 mg) was dominated by urban areas as well as farmland (see Figure 3C).

345

346 In the full model, we found positive effects of farmland, forest and grassland cover on insect
347 biomass, and a trend towards a positive effect of wetland on insect biomass (Table 1 &
348 Figure 4A). The negative effect of urbanisation was, however, not significant. The fixed
349 effects explained 37% of the variation in the model. In addition, we found a positive effect of
350 sampling day with an increase in biomass throughout June, higher biomass in the evening
351 compared to midday and an increase in biomass within the three-hour evening sampling
352 (Table 1). The urban cover had a high correlation with potential stops along the routes (SI IV:
353 Figure 4.1).

354

355 In the composite land cover analysis, the two axes of the varimax rotated PCA were driven
356 by an urbanisation gradient (axis 1) and a forest gradient (axis 2) (SI II: Figure 2.2). We
357 found a significant negative effect of the urbanisation gradient on insect biomass ($p = 0.002$),
358 but no effect of the forest gradient ($p = 0.31$) (SI II: Table 2.1). The fixed effects in this
359 model explained a third (34%) of the variation in insect biomass among routes.

360

361 Since we found an effect of timeband (more insects in the evening; Table 1, Figure 5A), we
362 explored whether the effect of land cover differed with sampling time, but we did not find any
363 evidence of an interaction between land cover and timeband.

364 Germany

365 In the best fit model, lower insect biomass was associated with higher urban cover, and
366 higher biomass was found in the evening (Table 1 & Figure 4B). Urban cover and time of
367 day were the only variables retained in the model. The fixed effects of this model explained
368 30% of the variation in insect biomass. Consistent with these patterns, routes with low

369 biomass samples (within the bottom 20%, <46 mg), were dominated by the urban cover. By
370 contrast, in the routes with high biomass yields (within the top 20%, >502 mg), the mean
371 landscape composition was dominated by farmland cover (see Figure 3C). Similar results
372 were found when land cover variables were square-root transformed, and this reduced
373 multicollinearity problems highlighted by the variance inflation factors found in the full model.
374 Just as for Denmark, there was no evidence of interactions between land cover and time of
375 day, but overall, biomass was higher in the evening compared to midday (Figure 5).

376

377 In the full model, including each land cover variable, none of the land cover variables were
378 significant (Table 1). Insect biomass was generally higher in the evening than at midday and
379 further increased with a later start time of sampling during the evening time band (Table 1).

380

381 The two main axes identified by the PCA of the land covers were an urbanisation gradient
382 (from urban to farmland) and a forest gradient (from forest to grassland/wetland) (SI II:
383 Figure 2.2B), just as for Denmark. In a model including all control variables, only the
384 urbanisation effect was significant ($p=0.036$) and not the forest gradient ($p=0.20$) on insect
385 biomass, again, just as for Denmark. The fixed effects of this model explained 32% of the
386 variation in the data (SI II: Table 2.1).

387 Land use intensity in Denmark

388

389 The most pronounced effects on insect biomass in both Denmark and Germany were due to
390 urbanisation. To better understand these effects, we considered, within land cover types, a
391 set of sub-types, focused on the intensity of urbanisation. We did the same for sub-types of
392 agricultural land use types. Here, we considered only Denmark for which our sample size
393 was sufficient to allow within land cover type analyses.

394

395 When we considered the different subtypes of urban land cover separately, we found a
396 negative effect of urban cover with a high proportional hedge cover and a positive effect of
397 urban areas that had a high cover of commercial areas (Figure 6A & SI III: Table 3.2).
398 However, multicollinearity was an issue for the hedge/urban interaction; hence the result was
399 highly uncertain. For agricultural land use intensity, we found that farmland with a high
400 proportional cover of intensive conventional agriculture had a negative effect on insect
401 biomass; however, multicollinearity was again an issue, and the result was thus highly
402 uncertain. Furthermore, we detected a trend of increased biomass in semi-intensive
403 managed farmland (Figure 6B & SI III: Table 3.4). The partial effect analysis revealed lower
404 insect biomass in intensive conventional agriculture land use compared to the other
405 agricultural land uses.

406

407 Throughout all models for land cover and land use, the random effects explained between
408 28-37% of the variation in Denmark (mean site ID variance = 0.07, mean driver ID variance
409 = 0.35) and 47-52% of the variation in Germany (mean site ID variance = 0.44, mean driver
410 ID variance = 0.84).

411 Discussion

412 Using an innovative citizen science method with car nets, we could simultaneously sample
413 over a large geographic area with a total of 278 transects/routes. In doing so, we sampled
414 the flying insects associated adjacent to both public and private lands, including highly
415 populated cities, relatively remote forests and wetlands, and intensive agricultural fields. This
416 sampling approach revealed a consistent spatial pattern in insect biomass across the two
417 countries, namely lower biomass associated with urbanisation.

418 Urban land use has a strong negative effect on insect biomass

419 In both countries, we found the lowest biomass in urban routes compared with all other land
420 covers, confirming our assumption (H2). However, our results did not confirm our

421 assumption of lower biomass associated with increasing land use intensity (H3), since we
422 found a slightly positive effect of commercial areas on insect biomass. The estimated effect
423 of urban areas was still negative after accounting for potential stops during sampling (Figure
424 4). Our results are consistent with a recent meta-analysis combining data from multiple
425 studies to show an overall negative effect of urbanisation for arthropod diversity and
426 abundance (Fenoglio, Rossetti & Videla, 2020) and the decline of insect diversity with
427 urbanisation at multiple spatial scales (Piano *et al.*, 2020). In large part, this may be due to
428 the reduced biomass and productivity per unit area in urban habitats where much of the
429 landscape is impervious surface, such as cement or rooftops, on which vegetation does not
430 grow. While studies that focus on local, green habitats in cities often find those habitats to be
431 biological diverse (Guénard, Cardinal-De Casas & Dunn, 2015; Turrini & Knop, 2015;
432 Brunbjerg *et al.*, 2018; Mody *et al.*, 2020; Theodorou *et al.*, 2020), such studies may risk
433 missing the broader picture, that the unsampled grey spaces of cities are likely to have low
434 biomass, a reality reflected in our results from both Denmark and Germany. Our approach of
435 sampling across a transect of several km, while having limitations, integrates the effects of
436 green and grey spaces on biomass and provides a more complete picture of the mean
437 biomass of insects in a volume of air space over the city. In doing so, it reveals that there is
438 much lower insect biomass in the urban realm than in all other habitats.

439 **Insect biomass is positively associated with agricultural land** 440 **cover, but the positive association may be due to specific land** 441 **use intensities**

442 We found a positive effect of farmland cover on insect biomass in Denmark and a similar
443 tendency was found in Germany, thus not confirming our assumption of lower biomass in
444 agricultural areas (H1). We found this effect, despite a lack of different land use intensity
445 measures available to test, e.g. data on the amount of fertiliser, pesticide application, and
446 pastoral land cover and land use. Although there was some indication that insect biomass
447 generally was lower in intensive conventional agriculture, thus confirming our assumption

448 (H3), the effect was uncertain and perhaps affected by the fact that most of the farmland
449 cover in Denmark is intensive conventional agriculture. Hence, more sampling in agricultural
450 areas might be helpful to test the effect of agricultural management schemes better. Since
451 random effects explained a large part of the variation, e.g. site and sampling variability, more
452 replicates and detailed explanatory variables would benefit future analysis.

453 Some studies have previously found a positive association between insect biomass and
454 agricultural land use. Hallmann *et al.* (2017) reported substantial declines in insect biomass
455 in protected areas, many of which are cultural habitats in Europe, having been shaped by
456 human activities (Hurford & Schneider, 2007). However, they found weaker declines in areas
457 with a higher proportion of arable land than natural habitats (measured at a 200 m
458 resolution). In a recent global study, van Klink *et al.* (2020) also found weaker declines in
459 terrestrial insect biomass in areas with high crop cover compared to areas with low crop
460 cover at a local scale, but not at a landscape scale.

461 The relatively high insect biomass found in farmland might be explained by the high
462 availability of food sources for some insects. Indeed, the density of herbivorous insects have
463 been positively correlated with nitrogen loading in the landscape (Haddad *et al.*, 2000;
464 Ritchie, 2000), and nitrogen loading is expected to be highest in areas with high farmland
465 cover. Hence, higher plant biomass, more nutrient input and higher leaf N content may
466 explain the positive correlation of insect biomass with intensive agriculture. Since we
467 focused on biomass, greater biomass might be primarily caused by a few common and
468 highly abundant species, i.e. agricultural pests and their predators. Further work is needed to
469 assess variation in species diversity and composition, which may show contrasting patterns
470 to biomass.

471 Considering that our car-based sweeping of insects, like most other forms of insect
472 sampling, records activity rather than directly the local abundance of flying insects, an
473 alternative explanation may be that flying insects more easily traverse farmland, while not

474 necessarily breeding or feeding there. To disentangle activity and habitat association, it
475 would be optimal to have additional biomass data from vegetation sweeping along a subset
476 of routes. If our results contrast with patterns derived from other sampling methods, it may
477 suggest that the higher abundance in farmland is rather due to changes in movement
478 behaviour in hostile landscapes.

479 **Grassland is sparse and an essential habitat for insects**

480 We found higher biomass of insects in forest, wetland and grassland sites in Denmark
481 compared to agricultural sites, similar to a study on Lepidoptera in Britain (Macgregor *et al.*,
482 2019). The grassland land cover category in Denmark consisted of meadows, salt meadows
483 and grassland under the Danish Protection of Nature Act Section 3. Grassland is an
484 important habitat in a European context, with one-third of all grassland used in an
485 agricultural context with management schemes ranging from extensive to intensive land use
486 (Smit, Metzger & Ewert, 2008). Management schemes can have a large impact on insect
487 populations (Plantureux, Peeters & McCracken, 2005). For instance, nutrient loading, i.e.
488 manure or inorganic fertilisers, in managed grasslands, can decrease insect diversity but
489 tends to increase insect biomass and abundance (Haddad *et al.* 2000), so the biomass
490 found in this study could be associated with specific insect groups, e.g. herbivorous and
491 detritivorous species that may thrive under such conditions (Haddad *et al.* 2000).
492 Management of grasslands in Denmark has changed within the last couple of decades with
493 less grazing by large herbivores leading to lower rates of deposition of organic nutrients, i.e.
494 dung. For example, the dairy cow population grazing outside has decreased by more than a
495 third since the middle of the 1980s (Statistics Denmark, 2019). These management changes
496 have resulted in shifts in nutrient loading amount and frequency. Loss of outdoor grazing
497 dairy cows is associated with a 60% decrease in starling populations (Heldbjerg *et al.*, 2016),
498 most likely due to a loss of insects as a food source due to shifts in nutrient loading with
499 consequences for insect diversity and abundance (Plantureux, Peeters & McCracken, 2005).
500 Grasslands in Germany did not differ from other habitats in terms of insect biomass, which

501 may be explained by the difference in the grassland data in Germany compared to Denmark
502 (in Germany, the semi-natural grassland cover could not be distinguished from agricultural
503 grassland, e.g. grass leys). However, it is also possible that this lack of effect was simply an
504 issue of sample size.

505 **Even a little wetland cover goes a long way**

506 Recent studies suggest that freshwater insects have increased in abundance and biomass
507 over the last decades, possibly due to improved wastewater regulation, such as through the
508 Water Framework Directive (van Klink *et al.*, 2020; Termaat *et al.*, 2019). However, wetland
509 land cover has decreased by two thirds over the last century in Europe (European
510 Commission, 1995). In Germany, relatively small areas of wetland were sampled by our
511 study, while in contrast, in Denmark, more samples were obtained in proximity to wetlands.
512 In Denmark, despite the low proportional area, wetland had a significant positive effect on
513 flying insect biomass at the local scale, indicating that even small areas of wetland can be
514 important for flying insects, most likely as breeding habitats. In our study, Danish wetland
515 areas had the highest estimated effect on insect biomass compared to the other land covers
516 in the country (Figure 4A).

517 **A positive effect of forests on insect biomass**

518 We found a positive effect of forest cover on insect biomass in Denmark at an intermediate
519 spatial scale (250 m). In a study of 30 forest sites in Germany, Seibold *et al.* (2019) found
520 complex patterns of insect changes over the last decade. While they found significant overall
521 declines in biomass and species numbers, forest plots exhibited increases in species
522 numbers and abundance of herbivorous species, especially for invasive and potential pest
523 species, as well as for short-range dispersers. In our study, there were no available data on
524 measures of land use intensity in forests; however, especially deadwood volume is expected
525 to have a significant impact on insect biomass, by providing a rich carbon source that is

526 utilised by saproxylic species (Stokland et al. 2012). However, this should be tested by more
527 focused studies incorporating direct measures on the abundance of these habitat types.

528 Limitations and opportunities

529 We found strong trends and effects of land cover types on insect biomass, especially in
530 Denmark. Interestingly, the summer of our surveys was hot and dry. As such, the differences
531 in biomass among the land cover types might have been increased or reduced due to the
532 drought. We found some unexplained site-specific variability (variation between sites and
533 drivers) that may be explained by including temporal effects. As more samples were
534 obtained from Denmark, it was clear, from comparing sample sizes in Denmark and
535 Germany, that increased sample size could also alleviate some of the variations between
536 sites and citizen scientists. Moreover, there inherently are some issues with the
537 independence of hypothesis tests in this study, since the proportional land cover of each
538 land cover was a part of a 100% cover for each route. Thus, an increase in one type of land
539 cover inevitably leads to a loss in others. Hence, both the loss and gain of land cover have to
540 be considered to understand the impact of land use change on insects. This shift is apparent
541 in both countries where increasing farmland cover is associated with decreasing urban cover
542 (SI IV).

543 Biomass as a measure of insect community change

544 We focused our analysis on insect biomass for a number of reasons. Biomass is readily
545 measurable, relates to some ecosystem services (Barnes *et al.*, 2016) and has been
546 reported to be declining in several studies (Hallman *et al.*, 2017, van Klink *et al.*, 2020).
547 Indeed, our findings of reduced biomass in urban areas are consistent with a recent food
548 supplementation experiment suggesting that urban bird populations are more limited by
549 insect food availability than forest bird populations (Seress *et al.*, 2020). Moreover, since we
550 found similar effects of land cover for insects flying during midday and evening, there is
551 some evidence that taxa active during different parts of the day are similarly impacted.

552 However, biomass is only one measure of an insect community and other measures, such
553 as richness and composition, may show contrasting patterns. For instance, biomass may
554 increase, but species richness may decrease if the increase in biomass is driven by common
555 large-bodied or multiple small generalist species. Relationships between body size, rarity
556 and sensitivity to land use will play roles in determining the relationship between biomass
557 and other metrics.

558 Car net sample at landscape scales

559 The car net sampling approach allowed us to sample across a large geographical extent with
560 several citizen scientists sampling under similar conditions in multiple habitats. In addition,
561 car nets provide an alternative to traditional stationary traps, such as Malaise traps or
562 window traps, since they sample at the landscape scale and integrate over local spatial
563 variation. However, the car net shares some of the same sampling bias as other sampling
564 methods, i.e. they sample insect activity, especially taxa that disperse well, rather than the
565 entire insect fauna of the habitat. Moreover, compared to stationary traps, our car net
566 covered quite a short sampling period and specific taxonomic groups like, e.g. butterflies are
567 underrepresented. This is reflected by the biomass of insects which is mostly <5 gram per
568 sample, whereas, e.g. Malaise trap samples may yield up to several hundred grams within
569 the sampling period (Hausmann *et al.*, 2020). For this study, the sampling period was usually
570 10-20 minutes per route; however, the sampling protocol can be designed to have more
571 extended sampling periods with increased frequency, if the purpose is to monitor biomass,
572 abundance and diversity over time. Since we relied on citizen scientists to collect our
573 samples, we designed a sampling protocol that made it possible for as many people as
574 possible to contribute, without specific insect knowledge or expertise. The simple sampling
575 protocol proved to be quite useful, with a response rate, i.e. samples returned to the
576 research institutions, of 86% in Denmark and a response rate of 96% in Germany. The
577 numbers suggest that standardised citizen science schemes can be a powerful approach to
578 monitor insect diversity simultaneously.

579 Conclusions

580 Overall, we found that urbanisation is associated with decreases in insect biomass. Given
581 the rapid growth of cities around the world, this decrease has the potential for widespread
582 consequences and cascading effects on other species. By sampling both grey and green
583 urban areas, we show clear effects of reduced biomass that were not evidenced before. In
584 addition, we show the relative importance of other land covers, particularly in Denmark,
585 where we had more samples. Conventional intensive agriculture tended to be associated
586 with reduced biomass, even when agriculture overall showed relatively high biomass.
587 Because of the difficulty of sampling conventional intensive agricultural fields, we think our
588 results may be the first evidence of such an effect. In Denmark, semi-natural areas tended to
589 have more insect biomass than either urban areas or farmland. Given the geographic extent
590 of urban areas and farmland in Europe, these findings suggests that massive declines in
591 total insect biomass could have already occurred.

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Conflicts of interest

The authors state no conflicts of interest.

Author's contributions

CSS, JHC, RE, JB, CF, APT, AB, RRD conceptualised the project. JCL, CSS, APT, AR, AB, DE VG and SH organised and coordinated the citizen science sampling. JB and VG extracted environmental data for Denmark and Germany, respectively. CSS, LBP and JM carried out the lab work with support from AJH, NMD and TGF. DEB, AB, RRD, APT and CSS developed analysis models and DEB and CSS wrote scripts for statistical analysis and analysed the data. All authors contributed to the development of the manuscript.

Data accessibility

Files documenting the analyses and all files necessary to reproduce the analyses, including links to raw data and metadata, are available on GitHub (https://github.com/CecSve/InsectMobile_Biomass).

Appendix A. Supplementary Information

Supplementary data to this article can be found online at:

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Tables

Table 1: Regression coefficients of the linear mixed-effects model on insect log(biomass+1) (significant variables in bold). The full model includes all land cover and controlling variables. The best fit model was identified by the lowest AIC. All land cover variables were kept in their original units to facilitate interpretation. Shown is the mean (standard error) of each regression coefficient. Explained variation by the fixed effects in each model indicated in percent.

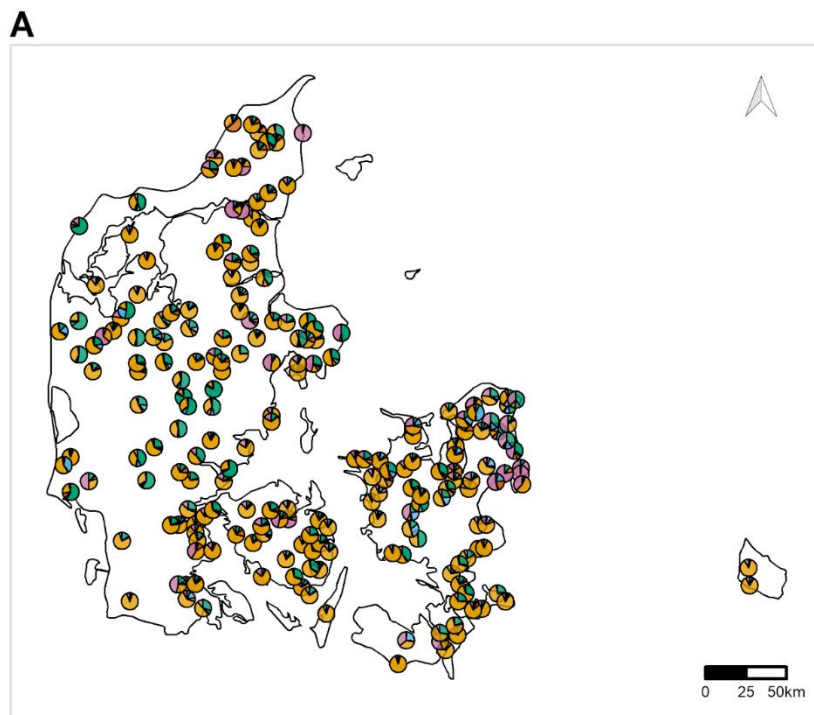
Variable	Denmark (full model: 37%) [∇]	Denmark (best fit model: 33%)	Germany (full model: 36%) [∇]	Germany (best fit model: 30%)
Urban - 1000 m	-0.013 (0.009)	-0.017 (0.007) *	-0.028 (0.024)	-0.040 (0.008) *
Farmland - 1000 m	0.012 (0.005) *	0.014 (0.005) *	0.024 (0.016)	-
Grassland - 1000 m	0.054 (0.019) *	0.058 (0.018) *	0.018 (0.021)	-
Wetland; DE: 1000 m, DK: 50 m	0.051 (0.029) ^Δ	0.057 (0.028) *	0.005 (0.044)	-
Forest: DE: 250 m, DK: 250 m	0.013 (0.085) *	0.016 (0.005) *	0.014 (0.013)	-
Day of year	0.028 (0.006) *	0.027 (0.006) *	-0.046 (0.042)	-
Time band: midday vs evening	0.33 (0.09) *	0.32 (0.09) *	0.383 (0.115) *	0.416 (0.116) *
Time within band (change in biomass per minute within time band)	Midday: -0.0005 (0.002) Evening: 0.006 (0.001) *	-	Midday: 0.0002 (0.002) Evening: 0.005 (0.002) *	-
Number of Stops/Traffic lights	-0.26 (0.19)	-	0.4013(0.3217)	-

* $p < 0.05$, ^Δ $p < 0.1$, [∇] Generalised variance inflation factor for the full models; DE: 5 for grassland and stops, 10 for farmland, urban and forest, DK: 2.87 for stops and forest, 5.4 for urban cover, 4.8 for farmland, 1 for wetland, 1.2 for grassland.

Figures



Figure 1: Car net used to sample flying insects. Picture from The Natural History Museum of Denmark's promotional video. Photo: Anders Drud | Natural History Museum of Denmark.



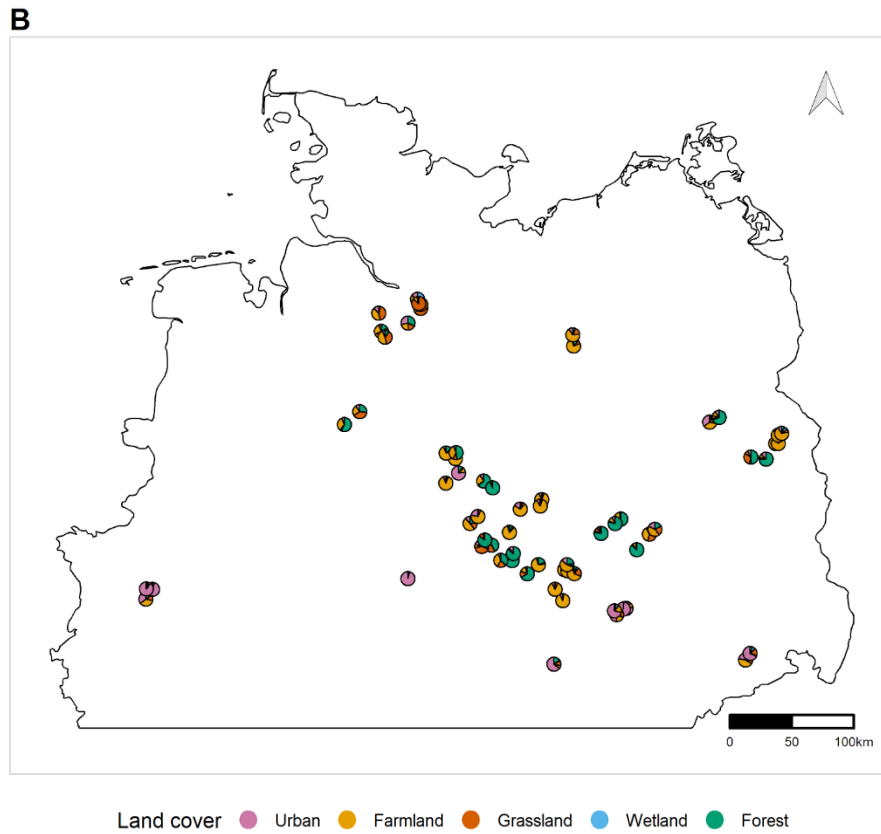


Figure 2: Location of car net sampling routes in two European countries A) Denmark (211 routes) and B) Germany (67 routes). Pie chart points illustrate the proportional land cover at the 1000 m buffer for each sampling location.

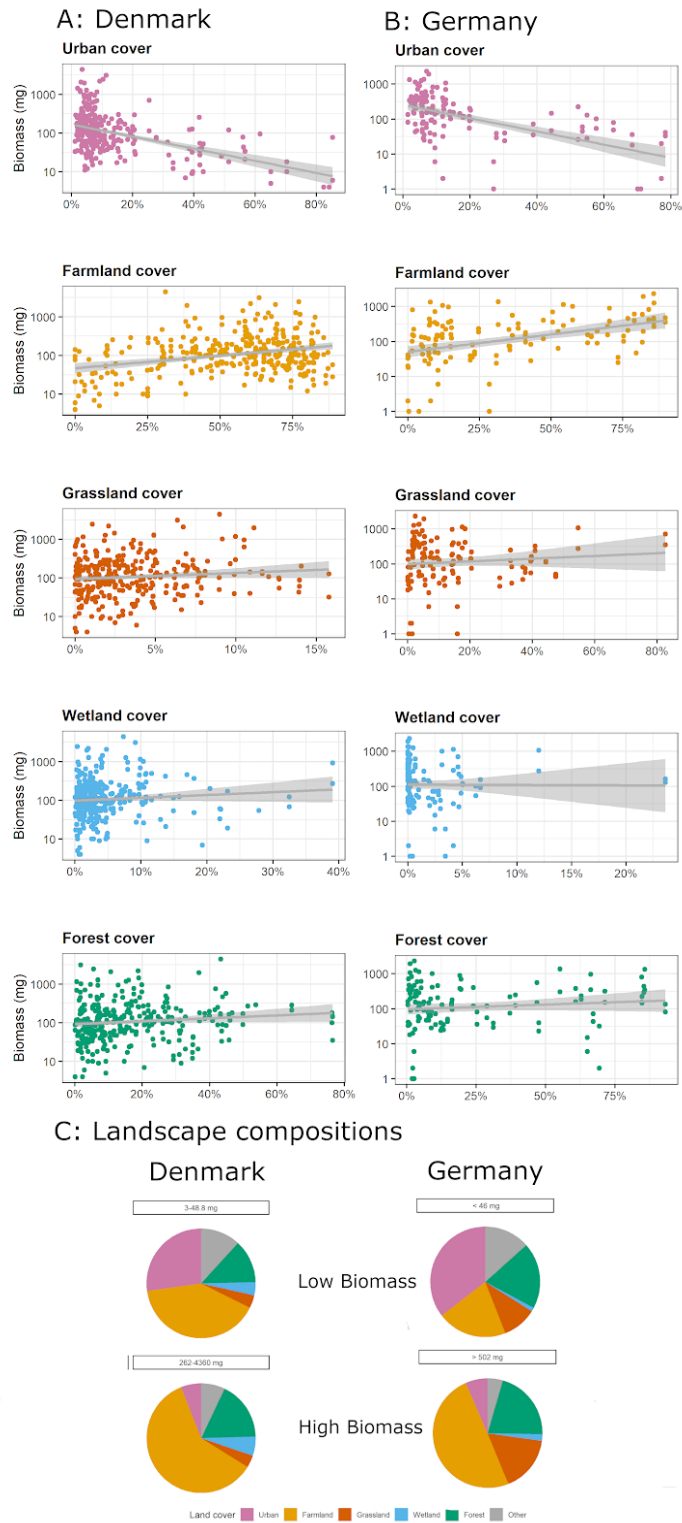


Figure 3: Scatterplots show the simple relationships between percent of each land cover and insect biomass. A) Denmark, B) Germany. C) Pie Charts show the mean land cover composition of routes with the lowest 20% quantile and upper 20% quantile of biomass samples.

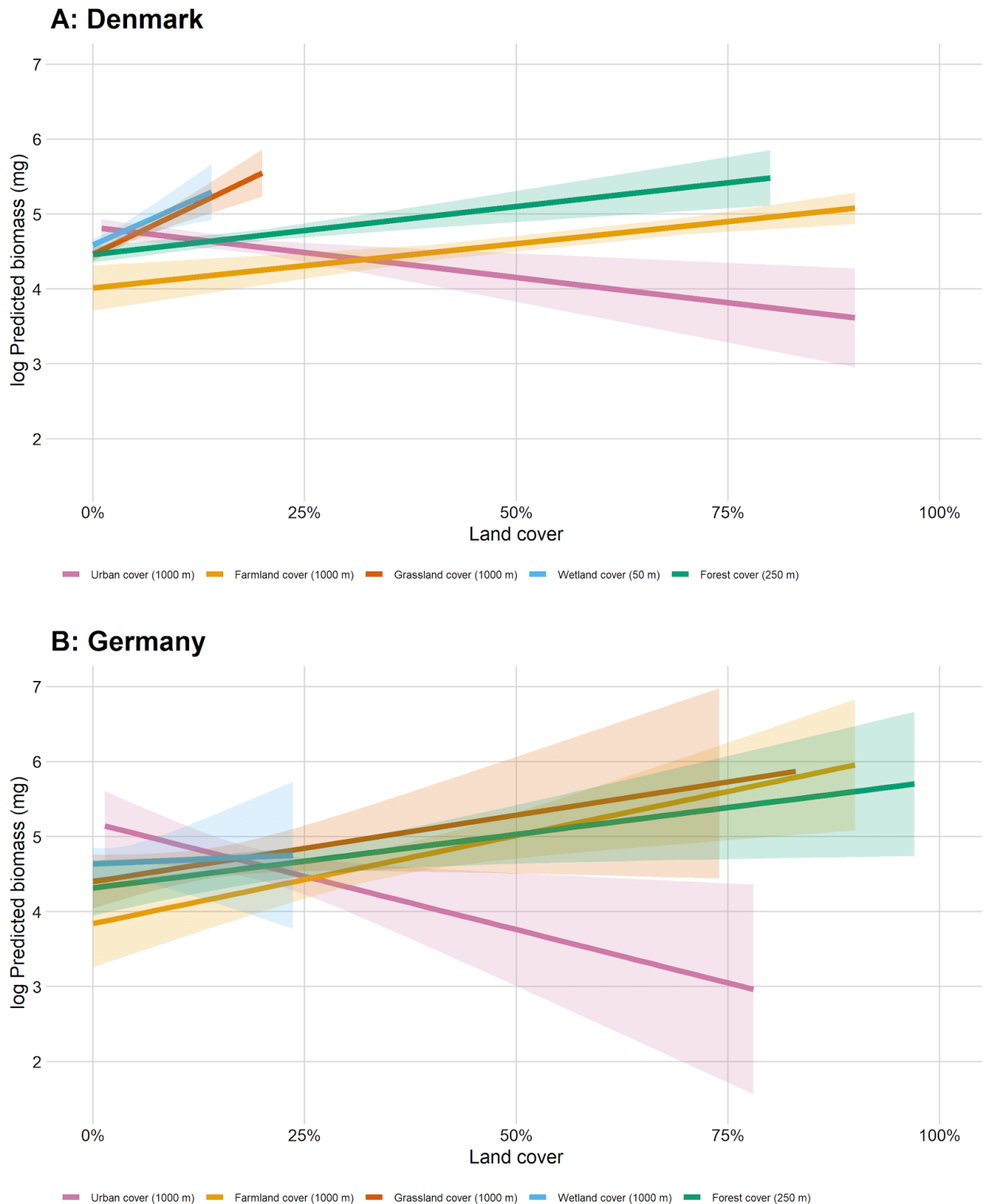
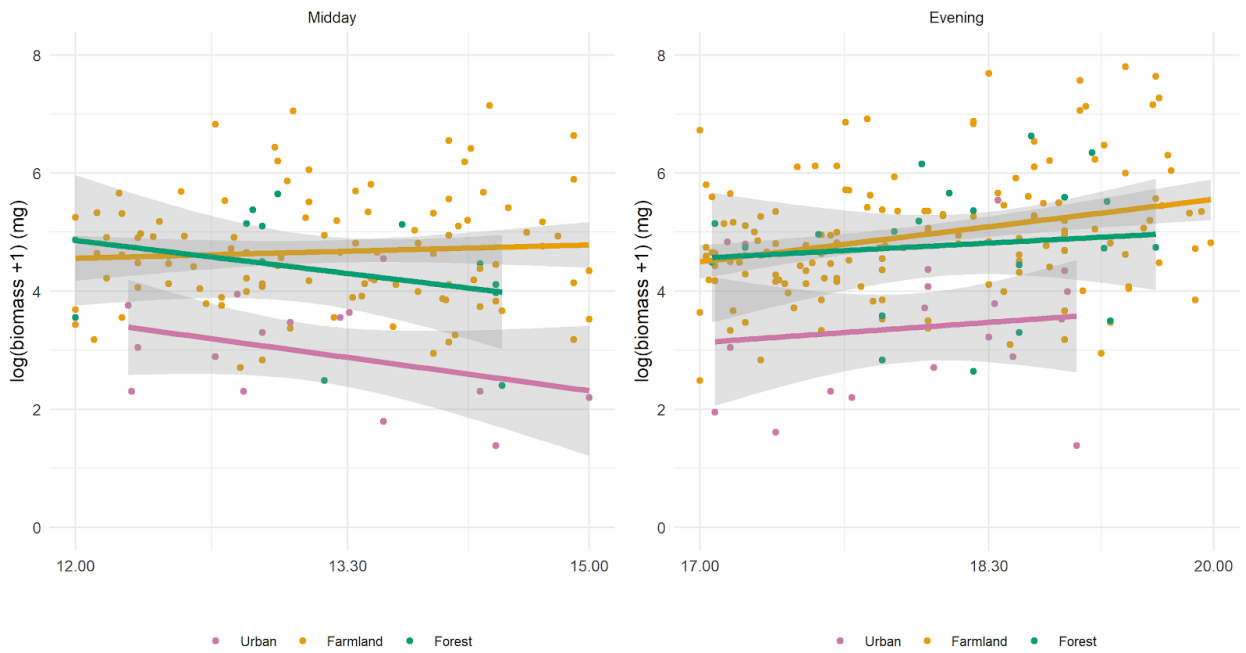


Figure 4: Partial effects of each land cover when all other predictors are held fixed at their means for (A) Denmark and (B) Germany. Predicted $\log(\text{biomass}+1)$ (mg) on the y-axis and proportional land cover on x-axis. Based on the full model for each country to illustrate the relative effect of each land cover. Shaded areas around each line is the standard error for the fit.

A: Denmark



B: Germany

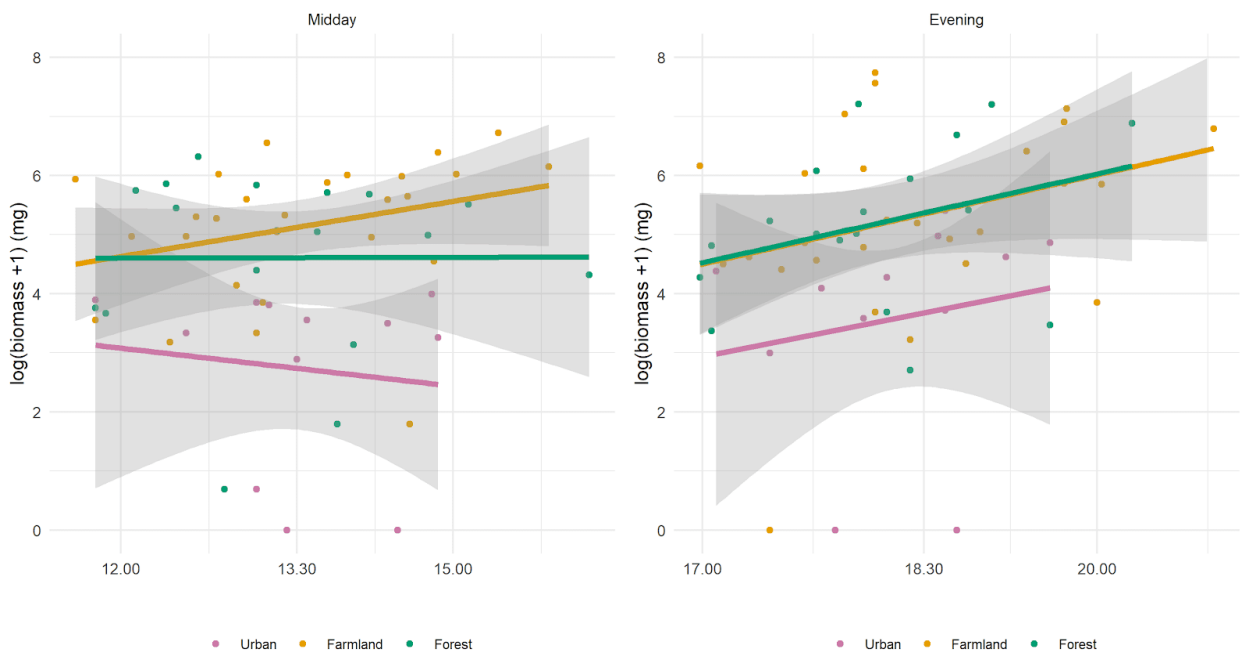


Figure 5: Sampling time effects on insect biomass. A) Denmark and B) Germany: overall effect of sampling time on insect biomass on land covers where the maximum proportional cover could be assigned to a specific land cover category at the 1000 m buffer. Coloured by land covers and shaded areas correspond to the standard error of the fit. We do not show wetland and grassland since these were rarely the dominant land cover along a route.

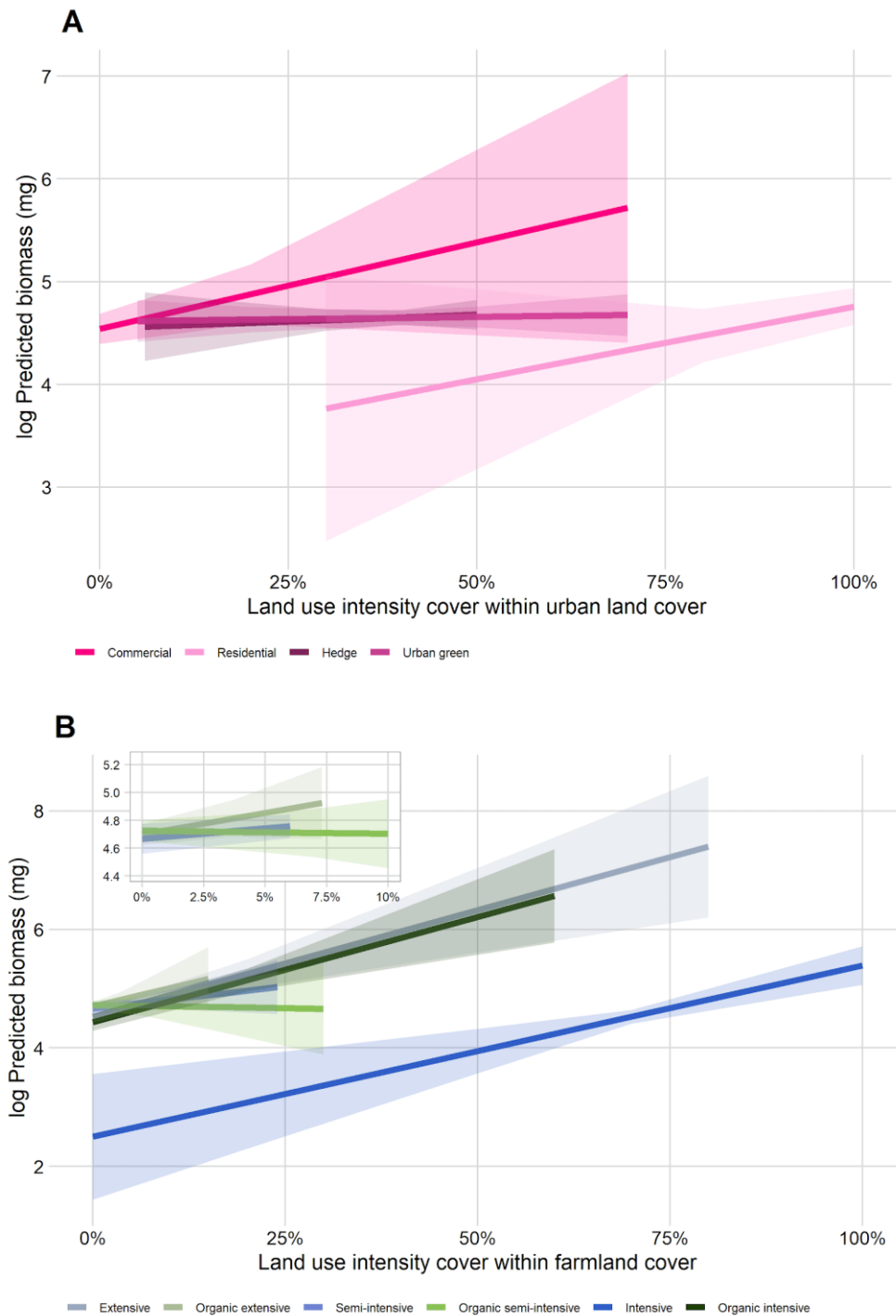


Figure 6: Partial effects of each proportional land use cover in Denmark when all other predictors are held fixed at their means for (A) urban land use within the cover of the 34 most urban routes and (B) farmland land use within the cover of the 255 most agricultural routes, green hues = organic farming, blue hues = conventional farming and general farmland cover. Figure zoomed in for the 10% cover to show the partial effects of the land uses with low coverage on the routes. Only observations in the 1000 m buffer. Predicted $\log(\text{biomass}+1)$ (mg) on the y-axis and proportional land use cover on x-axis. Shaded areas around each line is the standard error for the fit.