1	Crop rotation and agri-environment schemes determine bumblebee communities via			
2	flower resources			
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# 26 Abstract

27	1. In many parts of the world, farmland pollinators decreased significantly during the last half
28	of the 20 <sup>th</sup> Century mainly due to land use changes and agricultural intensification.
29	2. We studied the effect of different typical crop rotations and agri-environment schemes
30	(AES) on bumblebee diversity in Estonia. We compared species abundances between four
31	crop rotation types [cereal rollover (no change from one year to the next), cereal to mass
32	flowering crops (hereafter MFC), MFC rollover, and MFC to cereal fields] where all counts
33	were conducted in the second year, and in three farming types (conventional farming, organic
34	farming and environmentally friendly management).
35	3. We surveyed bumblebees and flower cover along 401 field margins in five consecutive
36	years, and recorded twenty species and more than 6000 individuals. Abundances of long-
37	tongued and threatened bumblebee species were higher at the field margins of cereal rollover
38	fields than for the other three crop rotation types. In addition, cereal rollover field margins
39	had higher abundances of medium colony species, generalists, and forest scrub species than
40	MFC rollover and MFC to cereal or cereal to MFC field margins. Bumblebee species richness
41	was higher at the field margins of both AES types than those of conventional farming.
42	However, in general the strongest driver of bumblebee presence was flower cover.
43	4. Higher bumblebee abundances in cereal rollover field margins were probably owing to a
44	concentration effect there and/or a dilution effect into MFC fields. Both AES schemes
45	supported increasing flower cover in field margins and thereby diversity of bumblebees,
46	indicating positive AES impacts upon wild pollinators.
47	5. Synthesis and applications. Crop rotation and AES determine bumblebee richness and
48	abundance via the availability of flower resources, but crop rotation constrains bumblebees
49	differently based on their traits. Therefore, future agri-environmental policy should account

- 50 for these management options. Crop rotation could be a simple, but efficient solution to
- 51 increase the biodiversity of agricultural landscapes.
- 52
- 53 Keywords: agri-environment schemes, biodiversity, bumblebee, concentration effect, crop
- 54 rotation, dilution effect, functional traits, land use, organic management, pollinator

## 55 **1. Introduction**

56 Bumblebees, among other pollinating insects, contribute to wild plant and crop pollination, 57 and therefore to plant biodiversity and food production (Kremen *et al.*, 2007). Pollination by 58 bumblebees is known to increase the yields of almost 40 crops (Goulson, 2010). Thirty-five 59 percent of global crop production depends, to a degree, on pollinators (Klein et al., 2007), and 60 the global annual economic value of insect pollination is estimated to be between 215–529 61 billion dollars (IPBES, 2016). Therefore, conservation of farmland pollinators is one of the 62 key challenges of global crop production (Potts et al., 2016). 63 Industrial agriculture has caused remarkable declines in the diversity and abundance of 64 native flowers and semi-natural habitats, which in turn has caused decreases of wild 65 pollinators, particularly long-tongued bumblebees (Goulson, Lye & Darvill, 2008). Based on 66 a recent IUCN report, 46% of bumblebee species populations in Europe have declined (Nieto 67 et al., 2014). Drivers of the decline in pollinators include landscape homogenization, land-use 68 changes (e.g. the loss of semi-natural habitats and the increase in the area of cereal crops) and 69 the increasing use of synthetic pesticides and fertilizers (Winfree et al., 2009; Potts et al., 70 2010; Bommarco et al., 2012; Goulson et al., 2015). A reduction in the number of small-scale 71 farms has resulted in a decline in crop diversity and the loss of field margins (Sutcliffe *et al.*, 72 2015). Agri-environment schemes (AES), such as set-aside semi-natural habitat, organic 73 farming, and wildflower strips for pollinators, have been developed and introduced in the 74 European Union since the late 1980s as a tool to address the negative environmental impacts, 75 including declines in biodiversity, of large-scale agricultural intensification (Batáry et al., 76 2015). 77 Across the EU, the effectiveness of AES in terms of species conservation has been 78 questioned owing to goals remaining unachieved as a consequence of a lack of targeting

79 (Hole et al., 2005; Kleijn et al., 2011). Nonetheless, there is evidence of a positive effect of

80	many AES upon bumblebee abundances (recently e.g., Carvell et al., 2015; Wood et al.,
81	2015). However, AES availability and utilisation might not be enough to halt and reverse
82	declines in bumblebees and particularly threatened species. Therefore, agricultural intensity as
83	well as landscape structure are also important factors with regard to conservation efforts
84	(Tscharntke <i>et al.</i> , 2005, 2012).
85	Mass-flowering crops, such as clover species and oilseed rape, are significant food
86	resources for bumblebees and at the same time benefit from being pollinated. E.g. in Northern
87	Europe, sweet and red clover, which have deep corolla, benefit from being pollinated by long-
88	tongued bumblebee species (Westphal, Steffan-Dewenter & Tscharntke, 2003; Wood,
89	Holland & Goulson, 2015). In addition, resource continuity (Blüthgen & Klein, 2011) is
90	important, because mass-flowering crops are not always available to bumblebees during their
91	lifecycles. Therefore, the availability of wild flowers, especially those with deep corolla, is an
92	important driver of bumblebee diversity and population development (Williams & Osborne,
93	2009; Williams et al., 2015).
94	There is a knowledge gap regarding how temporal land-use change affects bumblebees.
95	To the best of our knowledge, this is the first multi-year study to evaluate the effect of crop
96	rotation on bumblebee communities. We investigated the impact of four different common
97	crop rotation types on bumblebee species richness and abundance, including comparisons
98	between species with different functional traits (tongue length, threat status, colony size,
99	habitat preference), during 2010–2014. In Estonia, crops are usually rotated every second
100	year, e.g. after being a cereal field for one or two years, there will be a rotation to mass
101	flowering crops or grasslands and vice versa. Hence, the overarching question is how does the
102	type of crop rotation determine the following year's bumblebee community (species richness,
103	total abundance, and tongue-length/threat status/colony size/habitat preference group
104	abundances)? We hypothesized that bumblebee species richness and abundance are higher in

105	the field margins of mass-flowering crops than in the field margins of cereal crops, regardless
106	of the previous year's crop in those fields (illustrative photos are shown in Fig. S1,
107	Supporting Information). In addition, we hypothesized a positive effect upon bumblebees of
108	organic and environmentally friendly management compared to conventional farming. We
109	collected data to test whether crop rotation and/or AES benefit bumblebees, and to identify
110	the possible drivers of bumblebee abundances (e.g., concentration or dilution effects
111	depending on the crop rotation type).
112	
113	2. Materials and methods
114	2.1. Monitoring areas
115	We sampled true bumblebees Bombus ssp. (hereafter bumblebees) as part of an ongoing
116	evaluation of AES under the framework of the Estonian Rural Development Plan 2007–2013
117	(Agricultural Research Centre, 2015). Two regions of Estonia were studied: Põlva, Võru and
118	Valga counties (hereafter referred to as Southern Estonia; centre coordinates 57°52'N,
119	26°57'E) and Lääne-Viru, Järva and Jõgeva counties (hereafter Northern Estonia; centre
120	coordinates 59°4'N, 26°12'E; a map of the study areas is available in Fig. S2, Supporting
121	Information). These regions were selected based on differences in agricultural yields, AES
122	uptake, and landscape structure. Southern Estonia has a more diverse landscape and lower
123	yields (average cereal yield over 2004–2013 was 2792 kg/ha). Northern Estonia is
124	characterized by larger fields, a more open landscape, and high yields by Estonian standards
125	(average cereal yield for 2004–2013 was 3011 kg/ha). Additional information about the
126	regions, and selection of study farms, is available in Marja et al. (2014).
127	In each region 11 organic, 11 environmentally friendly managed (both had five-year
128	AES obligations with the possibility to prolong the obligation to six years, started in 2009),
129	and 11 conventionally managed farms (non-AES) were surveyed, i.e. 66 in total. One of the

130 aims of environmentally friendly management scheme is to promote farmland biodiversity, 131 with the major requirements of farmers being to allocate a minimum of 15% of arable land 132 (including rotational grasslands) to legumes, use diversified crop rotation, take soil samples to 133 determine optimal fertilizer requirements and create a fertilization plan, maintain/create 134 permanent grassland field margins (2–5 m wide), not use black fallow (fallow land with bare 135 soil, where the height of weeds does not exceed 5 cm), protect landscape elements, and limit 136 glyphosate applications. Organic farmers followed the Organic Farming Act by not using any 137 synthetic pesticides or GMOs, and restricting their use of most mineral fertilizers. Detailed 138 information about AES requirements and conventional farming rules is provided in Table S1, 139 Supporting Information.

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### 141 2.2. Biodiversity survey and study design

142 Fieldwork for the evaluation of AES measures was carried out during the summers of 2010– 143 2014. Every year, each transect was surveyed three times (once in June, July, and August). The first visit was made during the  $23^{rd}$ - $30^{th}$  of June, the second visit from the  $15^{th}$ - $28^{th}$  of 144 July, and the third between the  $12^{th}$ – $23^{rd}$  of August. Bumblebees were surveyed by walking 145 146 slowly along a 2 m wide and 500 m long transect, of which 400 m was permanent between 147 vears and located in field margins (usually permanent grassland strips between the field and a 148 road/other field/ditch/forest etc., or if the margin was narrow, occasionally also on the edge of 149 a cropped field), with the remaining 100 m located in a field with an insect-pollinated crop 150 (e.g. clover) if present in the crop rotation, or if not, also in a field margin. Data from these 151 100 m section located in the field were not included in the analyses. Transects were divided 152 into shorter sections differentiated by crop types. The sections were marked on a map (scale 153 1:5000). During each fieldwork session, flower cover was estimated on a scale of 0-3 per 154 whole 2 m wide transect section where: 0 = no flowers suitable for bumblebees; 1 = >0 to 1/3

155	of the area with flowers suitable for bumblebees; $2 = 1/3$ to $2/3$ with suitable flowers, $3 = >2/3$
156	covered with suitable flowers (Marja et al., 2014). All flowering-plant species known to be
157	used by bumblebees for foraging were classified as suitable (Table S2, Supporting
158	Information).
159	The bumblebee counts were conducted between 11:00 and 16:00 under good weather
160	conditions (temperature always above 15°C, and no rain or strong wind). We mainly
161	identified bumblebees on flowers to species in the field. If identification on flowers was
162	impossible, individuals were caught, identified, and released in the field, or on very rare
163	occasions were retained to identify later in the laboratory. Each year the number of each
164	bumblebee species was summed per transect over the three counts.
165	To test our hypotheses we included only bumblebees, flower cover, and crop rotation
166	data of such transect sections which were located in the two most common types of field
167	margins, those alongside cereals and mass-flowering crops. Cereal fields included rye, oat,
168	barley, triticale, and wheat (hereafter cereals). The mass-flowering crop fields contained
169	legumes (pea, bean, clover, alfalfa, sweet clover spp.) and oilseed rape (hereafter MFC). Crop
170	harvest time depends on the crop and weather conditions and varies from June to September.
171	Legumes are typically harvested in June (first cut) and August (second cut), but sometimes
172	cut only once in July. Winter oilseed-rape is harvested at the end of July or in August, spring
173	oilseed-rape in September, cereals typically in August or at the beginning of September
174	(depending also if it is sown in autumn or in spring). The overall sample to test our
175	hypotheses comprised 401 transect sections, whose lengths varied between 40-500 m (mean
176	226 ±SEM 6 m). Sample size for each year (number of transect sections) were as follows:
177	2010: 80; 2011:78; 2012: 73, 2013: 84 and in 2014: 86 transect sections (401 in total). A cross
178	table of sample size by crop rotation and management type is given in Table 1. All other crop

179 rotation types, such as potato, short-term grassland, permanent grassland, and pasture were180 excluded from the analysis.

181	Part of the bumblebee dataset, the explanatory variables management type and flower
182	cover (years 2010–2012), is already published in Marja et al. (2014). However, in this study
183	we used a more comprehensive bumblebee dataset (2010-2014) that also included crop
184	rotation types. We added management type and flower cover into the analyses, as these are
185	important drivers of bumblebee abundances (Marja et al., 2014). Moreover, the present study
186	investigated different bumblebee variables: abundance of bumblebees sub-divided by
187	functional groups (tongue-length, colony size, and habitat preference), and threat status.
188	
189	2.3. Statistical analysis
190	We analysed flower cover and bumblebee variables using linear mixed-effects models in R (R
191	Development Core Team, 2016). The 'Ime4' (Bates et al., 2016) package for R was used to
192	conduct all analyses. Bumblebee response variables modelled were species richness,
193	abundance of all bumblebee species, abundance of long-tongued species (three species:
194	Bombus distinguendus, B. hortorum, and B. subterraneus), abundance of short- and medium-
195	tongued species (all other species, hereafter short-tongued species), abundance of threatened
196	species, and abundance of non-threatened species. We analysed long-tongued bumblebees
197	separately due to their specific ecological niche, i.e. only these species can pollinate flowers
198	with deep corollas, such as red clover and field bean. Species classified as vulnerable
199	(hereafter threatened) in Europe under the recent IUCN list (Nieto et al., 2014) were: Bombus
200	confusus, B. distinguendus, B. hypnorum, and B. muscorum. We also modelled pooled
201	bumblebee abundances based on species' colony size (large, medium, and small) and main
202	habitat (open-land specialists, forest specialists, and generalists). We used these life-history
203	traits, because a recent study indicated that bumblebees have trait-dependent vulnerability

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based on landscape heterogeneity (Persson *et al.*, 2015). We provide a list of the bumblebee
species with classification according to tongue length, colony size, preferred habitat, and
threat status in Table S3, Supporting Information.

207 Owing to the bumblebees being over-dispersed, we used mixed-effects models with a 208 negative binomial distribution. The explanatory variables of main interest were crop rotation 209 type [four factors: cereal rollover fields (rollover = no change from one year to the next); 210 cereal to MFC fields; MFC rollover fields; MFC to cereal fields], (e.g. in cereal to MFC 211 fields, surveying was done in MFC field margin), management type (three levels: 212 conventional; environmentally friendly management; organic farming), and flower cover 213 (average value over the three counts per transect). Note that bumblebee response variables 214 were always taken during the second year of crop rotation. First, we tested flower cover as a 215 dependent variable in relation to crop rotation and management. Second, we tested all 216 bumblebee variables against crop rotation, management, and flower cover. Since we had 217 multiple years and the study regions had different landscape structures (Northern Estonia has 218 a simpler landscape structure than Southern Estonia), we treated year and region as crossed 219 random factors in the model (R command: (1)year)+(1)region). As the length of transect 220 sections ranged from 40 to 500 m, they were treated as an offset function [R command: 221 offset=log(transect length)]. We also calculated the variance inflation factor between 222 explanatory variables (R package "car", Fox & Weisberg, 2011), and identified no values 223 exceeding 1.4 for any of the models, which suggests that no collinearity occurred. 224 225 3. Results

226 We observed a total of 6092 individuals of 20 bumblebee species during 2010–2014 (see

227 Table S3, Supporting Information). We provide mean values and standard errors of

228	investigated flower cover and bumblebee variables per transect sections length according to
229	crop rotation and management type in Tables S4 and S5, Supporting Information.
230	Flower cover was higher in organic and environmentally friendly managed field
231	margins, compared to the margins of conventional fields, but was not related with crop
232	rotation types (Fig. 1). As an explanatory variable, flower cover was positively associated
233	with all bumblebee groups (Fig. 2,3,4 and Fig. S3,S4).
234	Crop rotation type was not related to bumblebee species richness or abundance (Fig. S3,
235	Supporting Information). Bumblebee species richness in the field margins of both AES
236	management types were higher compared to the margins of conventional fields. Bumblebee
237	abundance was significantly higher in environmentally friendly managed field margins
238	compared to those of conventional fields; no significant difference in bumblebee abundance
239	occurred between the field margins of organic and conventionally managed fields.
240	Abundances of non-threatened species did not differ between crop rotation types, but
241	abundance of threatened species was highest in cereal rollover field margins, compared to the
242	other three rotation types (Fig. 2). Bumblebee abundance of non-threatened species was
243	significantly higher in environmentally friendly managed field margins compared to those of
244	conventional field margins. Abundances of threatened species were higher in both AES
245	management types field margins, compared to the margins of conventional fields.
246	Crop rotation type was associated with abundances of bumblebees of medium colony
247	sizes (Fig. 3). Abundance of medium colony sized species was higher in cereal rollover field
248	margins, compared to MFC rollover filed margins. Both AES management types had higher
249	abundances of small-sized colony species.
250	Abundance of open land bumblebee species did not differ between crop rotation types.
251	Abundance of generalist species was higher in cereal rollover field margins, compared to
252	cereal to MFC and MFC rollover field margins (Fig. 4). Abundance of forest-scrub species

253 was higher in cereal rollover field margins compared to MFC to cereal and MFC rollover 254 field margins. Abundances of open land species and generalists did not differ between field 255 margins under AES and conventional farming. Organic field margins hosted a higher 256 abundance of forest-scrub species compared to the margins of conventional fields. 257 Abundances of short-tongued species were similar in all investigated crop rotation types 258 (Fig. S4, Supporting Information). Abundance of long-tongued species was higher in cereal 259 rollover field margins compared to the other three crop rotation types. Bumblebee abundance 260 of short-tongued species was significantly higher in environmentally friendly managed field 261 margins compared to those of conventional field margins. Abundances of long-tongued 262 bumblebee species did not differ between management types. 263 264 4. Discussion 265 Our study shows that crop rotation has an important role in determining bumblebee 266 community. We found that some bumblebee abundances (e.g. of long-tongued and threatened 267 species) are higher at cereal rollover field margins than at the field margins of the other three 268 crop rotation types. Furthermore, we found higher abundances of medium sized colony 269 species, forest-scrub species, and habitat generalists in cereal rollover field margins than in 270 MFC rollover and MFC to cereal or cereal to MFC field margins. 271 272 4.1. Concentration and dilution effects of bumblebees at field margins 273 Our study suggests that crop rotation type is an important management driver of bumblebee 274 communities in field margins. Abundances of several bumblebee groups (e.g. long-tongued, 275 threatened, and forest-scrub species) were higher at the field margins of cereal rollover

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compared to MFC rollover. This may not indicate that the *status quo* of fields remaining as

cereals from one year to the next has a positive effect on bumblebee abundance, or that cerealmargins are more important to bumblebees than MFC margins.

279 Our results can be interpreted in two ways. First, this might have been caused by a 280 concentration effect in cereal field margins, similar to that found in Environmental 281 Stewardship AES in England (Carvell *et al.*, 2007). More flower resources are available in the 282 margins of cereal fields than inside the fields, owing to herbicide use controlling arable weeds 283 within crops, thus reducing nectar sources (Brittain et al., 2010). Second, a dilution effect in 284 MFC fields (Holzschuh et al., 2011) is likely as bumblebees may disperse into MFC fields, as 285 they have more nectar resources than cereal fields. June and July, when 2/3 of our data were 286 collected, is the main blooming time of legumes and oilseed rape in Estonia. Therefore, 287 dilution of bumblebee individuals from certain trait based groups onto MFC fields was 288 probably the main reason for the differences in bumblebee abundances between cereal and 289 MFC rollover field margins. One limitation of our investigation was that it only accounted for 290 bumblebees at field margins, not within fields. An important potential confounding factor that 291 needs to be mentioned vis-à-vie the concentration-dilution hypothesis of bumblebees (and 292 other pollinators) in cereal/MFC/other field margins, is the type of crop(s) being grown in 293 adjacent fields. For example, is there a stronger concentration effect if cereal fields are on 294 both sides of the field margin, than if the margin is between a cereal and MFC field? We 295 suggest that future studies test the concentration-dilution hypothesis by: i) also running 296 flower/pollinator transects from the edge to the centre of fields; *ii*) taking into account 297 adjacent fields.

Our results suggest a negative temporal effect of cereal fields upon the food resources of bumblebees. Abundances of threatened, long-tongued, and forest-scrub species were lower in the field margins of MFC to cereal than cereal rollover fields. We offer the following explanation: if cereals are grown for two consecutive years, this may already negatively

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302	influence the flowering plant community of the field, reducing food resources for bumblebees
303	within fields, thus making margins more attractive to bumblebees. In addition, as cereal
304	rollover fields were mainly on conventional farms (Table 1), such field margins are less likely
305	to: <i>i</i> ) have MFC dispersal into the margin from the previous year; <i>ii</i> ) be managed (including
306	the sowing of seed mixes) for wildflowers. From a recent study (Magrach et al., 2017) it is
307	known that honeybees spillover from mass-flowering orange groves to flower-rich woodlands
308	after orange bloom leading to a change in wild bee community composition and lower seed
309	set of the most common plant species. Nevertheless, for the honeybee itself this might be a
310	benefit. In a similar way, it is possible that for at least some bumblebee species, MFC can
311	provide a benefit the following year, as suggested by our results (MFC>cereal compared to
312	cereal rollover).
313	The importance of field margins is related to nectar and/or pollen continuity in agricultural
314	landscapes (Schellhorn, Gagic & Bommarco, 2015). Owing to the seasonality and duration of
315	nectar sources, legumes and oilseed rape fields are not fully available to bees throughout
316	spring and summer in Northern Europe, thus bumblebees likely also use semi-natural habitats,
317	such as field margins (Bäckman & Tiainen, 2002; Batáry et al., 2015). Therefore, flowering
318	field margins are of high importance during periods when legumes or oilseed rape resources
319	are not available, thus creating a resource bottleneck (Persson et al., 2015; Schellhorn, Gagic
320	& Bommarco, 2015). In our study areas, a resource bottleneck might occur if MFC are not
321	grown in certain years, do not flower until a certain date, or are harvested from a certain date
322	onwards. Thus, it is highly likely that a combination of all three presented reasons affects the
323	availability of food resources for bumblebees.
324	

325 4.2. AES has a role in determining the bumblebee communities of field margins

326	We found that both organic farming and environmentally friendly management promoted
327	bumblebee species richness in field margins. It might be possible that farming practice had a
328	confounding effect on the results, e.g. conventional farms had a higher percentage of cereal
329	rollover fields compared to organic and environmentally friendly management farms, but
330	owing to the lack of collinearity, a significant bias seems to be unlikely. Nonetheless, future
331	studies should aim to collect more balanced datasets. However, Marja et al. (2014), also
332	demonstrated that Estonian AES promoted bumblebees, both within the fields and at their
333	margins. Environmentally friendly management involves requirements to conserve or sow
334	field margins with a flower mix of at least three species (including graminaceous); organic
335	farming does not have such a requirement, but abundances of bumblebee threatened species,
336	small-sized colony species, and forest-scrub species were still higher than per conventional
337	farming. This was probably related to the strict management requirements (synthetic
338	pesticides and most mineral fertilizers are forbidden) of organic farming. Our results indicate
339	that threatened species are remarkably sensitive to agricultural management, and prefer more
340	AES, farms; non-threatened species seemed to be less sensitive to management.
341	We found that the abundances of species with small colonies were related to AES
342	management types, whereas abundances of species with medium and large colonies did not
343	differ between management types. These results can be related to the mobility potential.
344	Species with small colonies have more limited dispersal distances (Westphal, Steffan-
345	Dewenter & Tscharntke, 2006). This adaptation makes them more sensitive to local
346	environmental and agricultural conditions. It is also probable that there were more suitable
347	habitat conditions in organic and environmentally friendly management field margins for
348	bumblebee species with small colonies. Species with medium and large colonies are more
349	mobile and search for resources at larger scales, and are therefore less influenced by local
350	conditions.

## 352 4.3. Conservation of bumblebees

353 Both naturally-occurring plants and the sowing of seed mixes to provide nectar-rich plants 354 (e.g. clover) at field margins can benefit bumblebees and other pollinators in Estonia as well 355 as in Northern Europe in general (Scheper et al., 2013). It is important when sowing nectar-356 rich plants mixes, to use only local flora to avoid introducing alien species. The conservation 357 of non-cropped landscape elements, such as field margins and other flower resources, is 358 essential to support the diversity of wild pollinators and their food plants. For instance, the 359 latest results from Estonia showed that field margins need to be at least 3 m wide to support 360 'high nature value' plant species intolerant of modern farming practices (Aavik & Liira, 361 2010). For bumblebees, these plant species are potentially of higher value and provide more 362 temporally stable food resources than agro-tolerant plant species. Thus, non-cropped field 363 margins at least 3–5 m wide could be a key and simple solution to improve bumblebee 364 diversity in cereal-dominated agricultural landscapes. Furthermore, permanent field margins 365 are important for bumblebees in terms of the continuity of resources other than food, such as 366 nesting and wintering habitat (Bäckman & Tiainen, 2002; Batáry et al., 2015). 367 A recent study showed that almost 80% of crop pollination is performed by a limited number 368 of bee species, and threatened bee species contribute little (Kleijn *et al.*, 2015). However, 369 protecting the main, common pollinator species only is not a sustainable solution to the 370 conservation of pollinator biodiversity. Senapathi et al. (2015) highlighted that maintaining 371 whole pollinator species diversity, including widespread and rare species, is essential to 372 provide ecosystem resilience and functioning in the future. Therefore, the conservation of 373 different habitats and the whole pollinator species spectrum is crucial, because different 374 pollinator species visit different parts of crops, or crops at different times of the day or year, 375 and respond differently to environmental disturbances (Goulson et al., 2015).

376	
377	5. Conclusions
378	Our results indicate that cereal field margins can act as refugia to forest-scrub, long-tongued,
379	and threatened bumblebee species, such as B. hypnorum, B. distinguendus, and B. muscorum,
380	which are vulnerable in Europe (Nieto et al., 2014). Semi-natural field margins, especially in
381	intensively managed cropland, may be a viable option to support these species in Europe,
382	because they represent permanent valuable landscape elements, offering places to nest and
383	overwinter, as well as providing food resources. It is possible that the field margin
384	requirement of Estonian AES is one of the reasons why Estonian bumblebee abundances were
385	stable over a recent five year period (Agriculture Research Centre, 2015). Our study indicated
386	a concentration-dilution effect of field margins upon bumblebee abundances, dependant on
387	the type of crop being grown in the field (cereal = concentration at the margin; MFC =
388	dilution into the field). To test the concentration-dilution hypothesis of field margins upon
389	pollinators, future studies should account for within-field pollinator/flower abundances, and
390	the influence of adjacent fields (or even landscape composition). Nonetheless, our results
391	show that management of flower rich field margins, especially in cereal rollover fields, where
392	few alternative nectar sources exist, is important and should form part of all AES targeting
393	pollinators.
• • •	

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- 407

## 408 Authors' Contributions

- 409 RM and PB conceived the study and designed the methodology; EV and MM coordinated
- 410 data collection; RM analysed the data; RM led the writing of the manuscript. All authors
- 411 (RM, EV, MM, JP, AMK, and PB) contributed critically to the manuscript and approved the

412 submission.

413

### 414 Data accessibility

- 415 Data availability. The biodiversity and environmental data used in the analyses are archived at
- 416 the research data repository Zenodo (https://zenodo.org/record/1161431).

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# **Table captions**

- **Table 1** Cross-table of sample sizes by crop rotation and management types. Cereal (all rye,
- 538 oat, barley, triticale, and wheat fields), MFC = mass-flowering crops (pea, bean, clover,
- 539 alfalfa, sweet clover species, and oilseed rape).

Management type/ Crop rotation	Conventional farming	Environmentally friendly management	Organic farming	Crop rotation total
Cereal→cereal	86	22	9	117
Cereal→MFC	17	46	24	87
MFC→cereal	28	36	19	83
MFC→MFC	17	31	66	114
Management type total	148	135	118	401

543	Figure captions
544	Fig. 1. Comparison of flower cover in field margins between different crop rotation and
545	management types. The figure shows results from linear mixed-effects models (p-value, lower
546	and upper boundary of 95% CI). Indicated are effect sizes (y-axis) compared to the crop
547	rotation type control group (cereal rollover field margins) and management type control group
548	(conventional farming). The effect size is significantly different if the CIs do not overlap with
549	zero. Asterisk symbols represent statistically significant p-values below 0.05, 0.01, and 0.001
550	(*, **, and ***, respectively). Cer = cereals (all rye, oat, barley, triticale, and wheat fields),
551	MFC = mass flowering crops (pea, bean, clover, alfalfa, sweet clover species, and oilseed
552	rape), Environmental = environmentally friendly management, Organic = organic farming.
553	

555	Fig. 2. Comparison of bumblebee abundances in field margins between different crop rotation
556	types, management types, and effect of flower cover for (a) non-threatened and (b) threatened
557	bumblebee species. The figure shows results from linear mixed-effects models (p-value,
558	lower and upper boundary of 95% CI). Indicated are effect sizes (y-axis) compared to the crop
559	rotation type control group (cereal rollover field margins) and management type control group
560	(conventional farming). The effect size is significantly different if the CIs do not overlap with
561	zero. Asterisk symbols represent statistically significant p-values below 0.05, 0.01, and 0.001
562	(*, **, and ***, respectively). Cer = cereals (all rye, oat, barley, triticale, and wheat fields),
563	MFC = mass flowering crops (pea, bean, clover, alfalfa, sweet clover species, and oilseed
564	rape), Environmental = environmentally friendly management, Organic = organic farming,
565	Flowers = flower cover.

568	Fig. 3. Comparison of bumblebee abundances in field margins between different crop rotation
569	types, management types, and effect of flower cover for species based on their colony size,
570	i.e. (a) large, (b) medium and (c) small colonies. The figure shows results from linear mixed-
571	effects models (p-value, lower and upper boundary of 95% CI). Indicated are effect sizes (y-
572	axis) compared to the crop rotation type control group (cereal rollover field margins) and
573	management type control group (conventional farming). The effect size is significantly
574	different if the CIs do not overlap with zero. Asterisk symbols represent statistically
575	significant p-values below 0.05, 0.01, and 0.001 (*, ** and, ***, respectively). Cer = cereals
576	(all rye, oat, barley, triticale, and wheat fields), MFC = mass flowering crops (pea, bean,
577	clover, alfalfa, sweet clover species, and oilseed rape), Environmental = environmentally
578	friendly management, Organic = organic farming, Flowers = flower cover.

580	Fig. 4. Comparison of bumblebee abundances in field margins between different crop rotation
581	types, management types, and effect of flower cover for species based on their habitat
582	preference, i.e. (a) open land, (b) generalists, and (c) forest-scrub. The figure shows results
583	from linear mixed-effects models (p-value, lower and upper boundary of 95% CI). Indicated
584	are effect sizes (y-axis) compared to the crop rotation type control group (cereal rollover field
585	margins) and management type control group (conventional farming). The effect size is
586	significantly different if the CIs do not overlap with zero. Asterisk symbols represent
587	statistically significant p-values below 0.05, 0.01, and 0.001 (*, ** and, *** respectively). Cer
588	= cereals (all rye, oat, barley, triticale, and wheat fields), MFC = mass flowering crops (pea,
589	bean, clover, alfalfa, sweet clover species, and oilseed rape), Environmental =

590 environmentally friendly management, Organic = organic farming, Flowers = flower cover.

591	Supporting Information
592	
593	Table S1. Requirements of conventional farming and the two agri-environment schemes.
594	
595	<b>Table S2.</b> Flowering plant species known to be used by bumblebees for foraging.
596	
597	Table S3. Bumblebee species' traits based on tongue length, threat status, colony size and
598	main habitat type.
599	
600	Table S4. Investigated plant and bumblebee variables depending on crop rotation type (mean
601	values and standard error of mean).
602	
603	Table S5. Investigated plant and bumblebee variables depending on management type (mean
604	values and standard error of mean).
605	
606	Figure S1. Illustrative photos of field margins.
607	
608	Figure S2. Study areas in the two regions of Northern and Southern Estonia.
609	
610	Figure S3. Comparisons of bumblebee species richness and abundance in field margins
611	between different crop rotation types, management types, and effect of flower cover.
612	
613	Figure S4. Comparisons of bumblebee abundance of short- and long-tongued bumblebee
614	species in field margins between different crop rotation types, management types, and effect
615	of flower cover



Fig. 1. Comparison of flower cover in field margins between different crop rotation and management types. The figure shows results from linear mixed-effects models (p-value, lower and upper boundary of 95% CI). Indicated are effect sizes (y-axis) compared to the crop rotation type control group (cereal rollover field margins) and management type control group (conventional farming). The effect size is significantly different if the CIs do not overlap with zero. Asterisk symbols represent statistically significant p-values below 0.05, 0.01, and 0.001 (\*, \*\*, and \*\*\*, respectively). Cer = cereals (all rye, oat, barley, triticale, and wheat fields), MFC = mass flowering crops (pea, bean, clover, alfalfa, sweet clover species, and oilseed rape), Environmental = environmentally friendly management, Organic = organic farming.



Fig. 2. Comparison of bumblebee abundances in field margins between different crop rotation types, management types, and effect of flower cover for (a) non-threatened and (b) threatened bumblebee species. The figure shows results from linear mixed-effects models (p-value, lower and upper boundary of 95% CI). Indicated are effect sizes (y-axis) compared to the crop rotation type control group (cereal rollover field margins) and management type control group (conventional farming). The effect size is significantly different if the CIs do not overlap with zero. Asterisk symbols represent statistically significant p-values below 0.05, 0.01, and 0.001 (\*, \*\*, and \*\*\*, respectively). Cer = cereals (all rye, oat, barley, triticale, and wheat fields), MFC = mass flowering crops (pea, bean, clover, alfalfa, sweet clover species, and oilseed rape), Environmental = environmentally friendly management, Organic = organic farming, Flowers = flower cover.



Figure 2 B

253x279mm (90 x 90 DPI)



Fig. 3. Comparison of bumblebee abundances in field margins between different crop rotation types, management types, and effect of flower cover for species based on their colony size, i.e. (a) large, (b) medium and (c) small colonies. The figure shows results from linear mixed-effects models (p-value, lower and upper boundary of 95% CI). Indicated are effect sizes (y-axis) compared to the crop rotation type control group (cereal rollover field margins) and management type control group (conventional farming). The effect size is significantly different if the CIs do not overlap with zero. Asterisk symbols represent statistically significant p-values below 0.05, 0.01, and 0.001 (\*, \*\* and, \*\*\*, respectively). Cer = cereals (all rye, oat, barley, triticale, and wheat fields), MFC = mass flowering crops (pea, bean, clover, alfalfa, sweet clover species, and oilseed rape), Environmental = environmentally friendly management, Organic = organic farming, Flowers = flower cover.



Figure 3 b

253x279mm (90 x 90 DPI)



Figure 3 c 253x279mm (90 x 90 DPI)



Fig. 4. Comparison of bumblebee abundances in field margins between different crop rotation types, management types, and effect of flower cover for species based on their habitat preference, i.e. (a) open land, (b) generalists, and (c) forest-scrub. The figure shows results from linear mixed-effects models (p-value, lower and upper boundary of 95% CI). Indicated are effect sizes (y-axis) compared to the crop rotation type control group (cereal rollover field margins) and management type control group (conventional farming). The effect size is significantly different if the CIs do not overlap with zero. Asterisk symbols represent statistically significant p-values below 0.05, 0.01, and 0.001 (\*, \*\* and, \*\*\* respectively). Cer = cereals (all rye, oat, barley, triticale, and wheat fields), MFC = mass flowering crops (pea, bean, clover, alfalfa, sweet clover species, and oilseed rape), Environmental = environmentally friendly management, Organic = organic farming, Flowers = flower cover.



Figure 4 b

253x279mm (90 x 90 DPI)



Figure 4 c

253x279mm (90 x 90 DPI)

Crop rotation and agri-environment schemes determine bumblebee communities via flower resources

Riho Marja, Eneli Viik, Marika Mänd, James Phillips, Alexandra-Maria Klein, Péter Batáry

## SUPPORTING INFORMATION

**Table S1.** Requirements of conventional farming (single area payment scheme) and two agri-environment schemes (environmentally friendly management, and organic farming), of the Estonian Rural Development Plan 2007–2013 (Estonian Rural Development Plan 2007–2013, 2010).

Management type	Pre-requisites of applying for support	Baseline requirements for obtaining agri- environment support	Additional requirements for obtaining agri-environment support, specific to each scheme
Conventional farming	Cross-Compliance requirements. Minimum 1 ha of agricultural land entered into the register of agricultural support and agricultural parcels.		
Environmentally friendly management	Cross-Compliance requirements. Minimum requirements for the application of fertilizers and plant protection products. Self-employed person engaged in agriculture or a legal person. Minimum 1 ha of arable land entered into the register of agricultural support and agricultural parcels (permanent grassland is not eligible). 5-year obligation.	Keeping a field book. Compiling a cropping or crop rotation plan. Plant protection equipment have to pass a technical inspection after every three years. Agricultural crops are sown or planted by the 15 <sup>th</sup> of June (spread of weeds avoided) or the agricultural land is kept as black fallow. In certain parishes, at least 30% of the agricultural land must remain under winter cover. Restrictions on using nitrogen. In certain cases, there have to be a grassland strip of at least 0.5 meters or another kind of landscape border element between the road and	Basic scheme requirements: Compiling a standard fertilization plan. Requirement of a cropping or crop rotation plan (e.g. 1 <sup>st</sup> November to 31 <sup>st</sup> March at least 30% under winter vegetation). At least 15% of agricultural crops sown with certified seed. Collection of soil samples once during the obligation period, and in the case of manure storage facilities, manure samples. To leave or establish a 2-5 m wide grassland strip with perennial vegetation or other kind of landscape element between the field and public road if the arable land area is larger than 20 ha (also some more detailed requirements). Cultural heritage sites and other valuable landscape elements

		field.	cannot be damaged or destroyed.
		Valuable landscape elements cannot be damaged or destroyed.	Basic + additional scheme requirements:
		Compulsory training (6+6 hours).	Basic scheme requirements.
			At least 15% of the eligible land is under leguminous crops.
			The application of glyphosates is prohibited from the time of the emergence of cultivated plants until harvesting. It is also prohibited on grasslands used as green manures.
			Plant growth regulators can only be used in case of growing winter cereals.
			Black fallow is prohibited.
			The amount of nitrogen fertilization is restricted.
Organic farming	Cross compliance requirements.	Keeping a field book.	Requirements for organic plant production and for organic
	Minimum requirements for the usage of fertilizers and plant protection products.	Agricultural crops are sown or planted by 15 <sup>th</sup> of June (spread of weeds avoided) or the	animal husbandry.
	Self-employed person engaged in agriculture or a legal person.	agricultural land is kept as black fallow. Grasslands and orchards must be mowed once or	
	Minimum 1 ha of agricultural land entered into the register of agricultural support and	grazed before 31 <sup>st</sup> July and mowed grass removed or chopped.	
	agricultural parcels.	Destruction or spoiling of natural protected	
	The enterprise must be approved according to	objects is prohibited.	
	the Organic Farming Act.	Damaging of semi-natural habitats is prohibited.	
	To follow the Organic Farming Act.	Compulsory training (12+12 hours).	
	5-year obligation.		

Estonian Rural Development Plan 2007–2013 (2010. URL: http://www.agri.ee/mak).

•	*	2
Plant species	Plant species	Plant species
Aegopodium podagraria	Galopsis tetrahit	Symphytum officinale
Anchusa arvensis	Geranium pratense	Trifolium hybridum
Anchusa officinalis	Hieracium spp	Trifolium medium
Arctium lappa	Hypericum maculatum	Trifolium pratense
Arctium minus	Hypericum perforatum	Trifolium repens
Arctium tomentosum	Knautia arvensis	Veronica longifolia
Bunias orientalis	Lamium album	Vicia cracca
Campanula cervicaria	Lamium hybridum	Vicia sepium
Campanula glomerata	Lamium purpureum	Vicia villosa
Campanula latifolia	Lathyrus pratensis	
Campanula medium	Linaria vulgaris	
Campanula persicifolia	Lonicera xylosteum	
Campanula rapunculoides	Lotus corniculatus	
Capsella bursa bastoris	Lupinus polyphyllus	
Carduus crispus	Lythrum salicaria	
Centaurea cyanus	Medicago lupulina	
Centaurea jacea	Medicago sativa	
Centaurea phrygia	Medicago varia	
Centaurea scabiosa	Melampyrum nemorosum	
Cirsium arvense	Melilotus albus	
Cirsium heterophyllum	Mentha arvensis	
Cirsium palustre	Odontites serotina	
Consolida regalis	Odontites verna	
Echium vulgare	Origanum vulgare	
Epilobium angustifolium	Phacelia tanacetifolia	
Fragaria vesca	Rubus idaeus	
Galega orientalis	Silene alba	
Galeopsis bifida	Silene vulgaris	
Galeopsis speciosa	Sonchus oleraceus	
Galeopsis tetrahit	Stachys palustris	
Galium album	Symphytum asperum	

**Table S2.** Flowering plant species known to be used by bumblebees for foraging in Estonian agricultural landscapes based on our 2014 unpublished survey.

**Table S3.** Bumblebee species' traits based on tongue length, threat status, colony size, and main habitat type, and their abundance in our sample. Colony size information is based on Benton (2006), Pawlikowski (2008), von Hagen & Aichhorn (2014), del Castillo *et al.* (2015), Weronika Banaszak-Cibicka (pers. comm.), and our unpublished data. Main habitat classification is based on Bäckman & Tiainen (2002), Diaz-Forero *et al.* (2011), and our own unpublished data. Threatened species at a European scale were classified as vulnerable under the recent IUCN list (Nieto *et al.*, 2014).

Bumblebee species	Tongue length	Threat status	Colony size	Main habitat	Total number of individuals
Bombus confusus	short- or medium-tongued	threatened	small	generalist	2
B. cryptarum	short- or medium-tongued	non-threatened	medium	generalist	11
B. distinguendus	long-tongued	threatened	small	forest-scrub	160
B. hortorum	long-tongued	non-threatened	medium	open	526
B. humilis	short- or medium-tongued	non-threatened	small	open	32
B. hypnorum	short- or medium-tongued	threatened	large	generalist	240
B. jonellus	short- or medium-tongued	non-threatened	small	forest-scrub	24
B. lapidarius	short- or medium-tongued	non-threatened	large	open	1006
B. lucorum	short- or medium-tongued	non-threatened	large	open	1150
B. muscorum	short- or medium-tongued	threatened	small	forest-scrub	61
B. pascuorum	short- or medium-tongued	non-threatened	medium	forest-scrub	785
B. pratorum	short- or medium-tongued	non-threatened	small	forest-scrub	165
B. ruderarius	short- or medium-tongued	non-threatened	small	open	486
B. schrencki	short- or medium-tongued	non-threatened	small	forest-scrub	50
B. semenoviellus	short- or medium-tongued	non-threatened	small	open	4
B. soroeensis	short- or medium-tongued	non-threatened	medium	generalist	405
B. subterraneus	long-tongued	non-threatened	small	open	46
B. sylvarum	short- or medium-tongued	non-threatened	small	open	419
B. terrestris	short- or medium-tongued	non-threatened	large	open	213
B. veteranus	short- or medium-tongued	non-threatened	small	open	307

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**Table S4.** Investigated plant and bumblebee variables depending on crop rotation type (mean values and standard error of mean per transect section) and transect sections length mean values and standard error of mean. Cereal (all rye, oat, barley, triticale, and wheat fields), MFC = mass-flowering crops (pea, bean, clover, alfalfa, sweet clover species, and oilseed rape). Scale of flower cover 0-3: 0 = no flowers suitable for bumblebees; 1 = >0 to 1/3 of the area with flowers suitable for bumblebees; 2 = 1/3 to 2/3 with suitable flowers, 3 = >2/3 covered with suitable flowers.

	Cereal→cereal	Cereal→MFC	MFC→cereal	MFC→MFC
Plants				
Flower cover	$0.85\pm0.05$	$1.32 \pm 0.06$	$1.03\pm0.06$	$1.36 \pm 0.06$
Bumblebees				
Species richness	$3.92 \pm 0.25$	$5.18\pm0.33$	$4.31 \pm 0.29$	$5.19\pm0.32$
Abundance	$12.19\pm1.16$	$18.64\pm2.42$	$12.10 \pm 1.36$	$17.89 \pm 1.67$
Short-tongued abundance	$10.53\pm1.03$	$16.69\pm2.11$	$10.88 \pm 1.22$	$15.55 \pm 1.47$
Long-tongued abundance	$1.66 \pm 0.24$	$1.95\pm0.40$	$1.22\pm0.22$	$2.34\pm0.38$
Non-threatened abundance	$10.91 \pm 1.04$	$17.53 \pm 2.22$	$11.47 \pm 1.31$	$16.46 \pm 1.53$
Threatened abundance	$1.28 \pm 0.21$	$1.11\pm0.29$	$0.63 \pm 0.14$	$1.44\pm0.28$
Large colony abundance	$5.46 \pm 0.64$	8.45 ± 1.23	$4.61\pm0.60$	$7.47\pm0.92$
Medium colony abundance	$3.68\pm0.41$	$5.47\pm0.96$	$3.61\pm0.48$	$4.57\pm0.53$
Small colony abundance	$3.05\pm0.40$	$4.72\pm0.78$	$3.87\pm0.54$	$5.85\pm0.67$
Open land abundance	$7.93\pm0.82$	$12.91 \pm 1.66$	$8.10\pm0.97$	$12.86 \pm 1.30$
Generalists abundance	$1.63 \pm 0.23$	$1.87\pm0.34$	$1.36 \pm 0.26$	$1.68\pm0.26$
Forest-scrub abundance	$2.62\pm0.36$	$3.86\pm0.92$	$2.64\pm0.43$	$3.36\pm0.46$
Transect sections length	227.5 ± 11.7	$208.7 \pm 13.5$	$223.6 \pm 13.7$	$224.0 \pm 11.2$

**Table S5.** Investigated plant and bumblebee variables depending on management type (mean values and standard error of mean per transect section) and transect sections length mean values and standard error of mean. Scale of flower cover 0-3: 0 = no flowers suitable for bumblebees; 1 = >0 to 1/3 of the area with flowers suitable for bumblebees; 2 = 1/3 to 2/3 with suitable flowers, 3 = >2/3 covered with suitable flowers.

	Conventional farming	Environmentally friendly management	Organic farming
Plants			
Flower cover	$0.84\pm0.04$	$1.23 \pm 0.05$	$1.39\pm0.06$
Bumblebees			
Species richness	$3.61\pm0.21$	$5.19\pm0.26$	$5.31\pm0.30$
Abundance	$10.26\pm0.88$	$18.88 \pm 1.70$	$17.16\pm1.69$
Short-tongued abundance	$9.01\pm0.79$	$16.61 \pm 1.48$	$15.13 \pm 1.49$
Long-tongued abundance	$1.25\pm0.18$	$2.27\pm0.34$	$2.03\pm0.31$
Non-threatened abundance	$9.47\pm0.81$	$17.70 \pm 1.59$	$15.58 \pm 1.51$
Threatened abundance	$0.78\pm0.15$	$1.19\pm0.20$	$1.58\pm0.29$
Large colony abundance	$4.69\pm0.46$	$8.67\pm0.97$	$6.31\pm0.77$
Medium colony abundance	$3.02\pm0.35$	$5.13\pm0.53$	$4.98\pm0.68$
Small colony abundance	$2.55\pm0.34$	$5.09\pm0.53$	$5.86\pm0.69$
Open land abundance	$7.14\pm0.66$	$13.34 \pm 1.28$	$11.28 \pm 1.15$
Generalists abundance	$1.26\pm0.20$	$2.11\pm0.27$	$1.58\pm0.23$
Forest-scrub abundance	$1.86\pm0.24$	$3.43 \pm 0.43$	$4.30\pm0.73$
Transect sections length	$236.0\pm10.9$	$223.2 \pm 11.0$	$201.8\pm9.8$



Fig. S1. Illustrative photos of studied field margins.





Fig. S2. Study sites (black dotes) in the two regions of Northern and Southern Estonia.

**Fig. S3.** Comparisons of bumblebee (a) species richness and (b) abundance in field margins between different crop rotation types, management types, and effect of flower cover. The figure shows results from linear mixed-effects models (p-value, lower and upper boundary of 95% CI). Indicated are effect sizes (y-axis) compared to the crop rotation type control group (cereal rollover field margins) and management type control group (conventional farming). The effect size is significantly different if the CIs do not overlap with zero. Asterisk symbols represent statistically significant p-values below 0.05, 0.01, and 0.001 (\*, \*\*, and \*\*\*, respectively). Cer = cereals (all rye, oat, barley, triticale, and wheat fields), MFC = mass-flowering crops (pea, bean, clover, alfalfa, sweet clover species, and oilseed rape). Environmental = environmentally friendly management, Organic = organic farming, Flowers = flower cover.





**Fig. S4.** Comparisons of bumblebee abundance in field margins between different crop rotation types, management types, and effect of flower cover for (a) short- and (b) long-tongued bumblebee species. The figure shows results from linear mixed-effects models (p-value, lower and upper boundary of 95% CI). Indicated are effect sizes (y-axis) compared to the crop rotation type control group (cereal rollover field margins) and management type control group (conventional farming). The effect size is significantly different if the CIs do not overlap with zero. Asterisk symbols represent statistically significant p-values below 0.05, 0.01, and 0.001 (\*, \*\*, and \*\*\*, respectively). Cer = cereals (all rye, oat, barley, triticale, and wheat fields), MFC = mass-flowering crops (pea, bean, clover, alfalfa, sweet clover species, and oilseed rape). Environmental = environmentally friendly management, Organic = organic farming, Flowers = flower cover.



