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ORIGINAL ARTICLE

Importance of functional classification in the use of carabids for the environmental risk assessment of the GE crops and other agricultural practices

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Abstract Carabids (Coleoptera: Carabidae) seem to be suitable bioindicators of the environmental impacts of novel agrotechnologies, including deployment of the genetically engineered (GE) crops. In this article, we describe our effort to employ carabids in the environmental risk assessment (ERA). GE maize MON88017, its near-isogenic hybrid nontreated or treated with the soil insecticide chlorpyrifos, and two reference hybrids were used to compare three different ways how to utilize carabids in ERA. The analysis of abundance of all captured carabids or of the most abundant carabid species did not disclose any differences between the treatments. The analysis based on the categories of functional traits revealed distinct features of some treatments and proved suitable for ERA because it permitted field data transportability in spite of different species compositions. Our results indicate that GE maize has no detrimental environmental effect. On the other hand, we found significant trends toward lower abundance and lower species number (including analysis of all carabid species together) in plots treated with the insecticide, and some tendencies to higher abundance and higher species number in plots sown with the reference hybrid PR38N86. Using functional group indicators allows identification of unintended changes in ecological functions of agroecosystem and comparability across geographies. We recommend data evaluation at the level of the categories of functional traits in ERA of GE crops and other agricultural practices.

Key words carabid; Cry3Bb1; environmental risk assessment; functional diversity; GE maize; surrogate species

Introduction

Maize is a very important crop all over the world (222 Mha, FAO, 2017). In 2017, 32% of maize global hectarage was genetically engineered (GE, ISAAA, 2017) to be herbicide tolerant and/or insect resistant. Transgenic technology currently applied to insect pest management is based primarily on the insecticidal crystal (Cry) proteins produced by the soil-borne bacterium, *Bacillus thuringiensis* (in more detail in Zhang *et al*., 2017) but due to the diversification of pest management (Yang *et al*., 2018), various strategies are under development (Liu *et al*., 2016). Cry proteins and their derivatives are used to control effectively mainly the lepidopteran (Wang *et al*., 2016) and coleopteran pests including one of the most devastating maize pest, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae; Oyediran *et al*., 2016).

Although GE crops were planted on 185.1 million hectares in 24 countries worldwide in 2017 (ISAAA, 2017), their implementation is still the object of intense debate in European Union (EU). In 2017, the EU has issued 31 new approvals for the use of GE crops in

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food and feed, but just one for cultivation (European Commission, 2017). The deployment of GE crops is mostly rejected due to fear of possible negative impact on agricultural ecosystems. We worked with the glyphosate tolerant and *Diabrotica*-resistant GE maize MON88017 that expresses the Cry3Bb1 protein active mainly on the beetle family Chrysomelidae. MON88017 and its cross-breeds have been permitted in the EU for use in food and feed but not for cultivation (European Commission, 2017). We have assessed effects of MON88017 on epigeic spiders and staphylinids used as markers of environmental changes (Svobodová *et al*., 2013, 2016), and in this article we describe our study on carabids.

Since carabids represent the main component of the epigeic arthropod biomass in different geographic zones and occur in numbers allowing solid statistical analysis and data transportability (Romeis *et al*., 2014), they are often used as bioindicators of the impact of agricultural practices on the arthropod communities. Carabids appear to be suitable surrogates of beneficial arthropods (predators of pests) in agricultural landscapes (Wach *et al*., 2016). They are particularly fitting for investigations on MON88017 that was developed for maize protection against *Diabrotica*. Cross-reactivity with other beetles cannot be excluded. Carabids are exposed to the Cry proteins in maize directly and/or indirectly, depending mainly on their feeding behavior (EPA, 2002). High concentration of Cry3Bb1 was found in the phytophagous and polyphagous species feeding on the maize (pollen) but high concentrations were found also in the predators (four times lower than in the maize pollen; Priesnitz *et al*., 2013).

Field studies often compare impacts of the GE and non-GE crops and pay less attention to the standard insecticide treatments that should set the baseline of potential damage caused by existing pest management practices (Sanvido *et al*., 2011). We evaluated the effect of GE maize in comparison with the non-GE near-isogenic hybrid grown with or without insecticide treatment that is currently used in maize protection. We demonstrate that genetic engineering and insecticide application exert similar or even smaller impact on insect communities than some of the commonly used hybrids that are neither genetically engineered nor insecticide treated.

Carabids are often studied as a morphologically defined taxonomic group but this approach does not reveal their diverse environmental requirements nor their roles in the ecosystems (Romeis *et al*., 2014). Functional analyses were therefore recommended as a preferred approach (Brooks *et al*., 2003; Clough *et al*., 2007; Grabowski *et al*., 2010; Skoková Habuštová *et al*., 2017). Functional traits are well known marks of community responses to environmental changes that affect ecosystem properties and services (Nock *et al*., 2016). In search for a reliable and feasible ERA method, we performed a 3-year field study comparing the impact of five treatments (GE maize, near-isogenic non-GE maize with and without insecticide treatment, and two unrelated reference hybrids) on carabid communities. Data on the species number and activity abundance were evaluated in all treatments in respect to the entire populations of carabids, most dominant species, and species assigned to functional traits and categories.

Materials and methods

Site properties and study design

The study was conducted in the southwest of the Czech Republic (48°59′N, 14°20′E) in 2009–2011. Moderately fertile cambisol soil type with medium heavy clay-loam soil is typical for this area (FAO, 2015). The area in the size of 14 ha was divided into twenty-five 0.5 ha plots (63 × 81 m) separated by 3 m strips of bare land. The GE maize MON88017 (treatment G, YieldGard VT Rootworm/RR2^{TM}, MONSANTO Technology LLC, MO, USA), its near-isogenic hybrid DK 315 (MONSANTO Technology LLC) which was nontreated (N) or treated (I) with the wide-spectrum granular soil insecticide Dursban 10G, and the reference hybrids Kipous (A, SAAT AG, Germany) and PR38N86 (B, DuPont Pioneer, IA, USA) were each sown in five randomly distributed plots ($n = 5$ replications from which means in graphs and tables were calculated; Svobodová *et al*., 2013). The insecticide was applied (10% chlorpyrifos, 20 kg/ha, fumigation effect) simultaneously with sowing. All hybrids had similar maturity date (FAO 270–290 for seeds; Jugenheimer, 1958). Maize hybrid DKC 2870 (FAO 210, MONSANTO Technology LLC) was sown around the field margins.

Identical treatments G, N, I, A, and B, respectively, were repeated on the same plots every year in three successive years. The maize was always harvested at stage R5 (physiological maturity; Ritchie *et al*., 1992). In 2009, plants were shredded into small pieces and ploughed in the same plots where the respective maize hybrids had been grown. In 2010 and 2011, maize was used for biogas production and the remaining waste was ploughed into the soil (details in Svobodová *et al*., 2013, 2016).

Capture and identification of carabids

One pitfall trap was placed in the plot center and each of four other traps was set 16 m from the longer (81 m) and 20 m from the shorter (63 m) plot sides. The traps (plastic cups, Ø 9 cm and 0.5 L volume) were immersed in the

soil up to brim and supplied with 0.3 L of 10% NaCl with a few drops of detergent. Each trap was protected against rain by a metal cap set about 3 cm above the soil surface. Traps were installed for a fortnight prior to sowing and after the harvest, and in 7-d intervals at the maize stages VE (germination), V6 (six leaves unfolded, missing in 2009 due to rainy weather), VT (full flowering), and R5 (Ritchie *et al*., 1992, dates in Svobodová *et al*., 2013).

Carabids were stored in 70% ethanol, identified to the species level (Hůrka, 1996) and grouped into the following functional traits: body size, habitat and humidity affinity, breeding period (Hůrka, 1996), and food specialization (Larochelle, 1990; Table S1).

Statistical analysis

Rank activity abundance curves (Whittaker plots) were used to examine the distribution of species dominance within treatments (Navasero *et al*., 2016). Spearman's rank correlation coefficient (r) with Bonferroni correction of significance level (Dunn, 1961) was calculated to measure the strength of relationship of rank abundances between treatments (GraphPad Prism 5, GraphPad Software Inc., 2007).

Repeated measures analysis of variance (RM ANOVA, including interaction, Statistica 8, StatSoft Inc., 2015) was employed to evaluate the variations in activity abundance (Toschki *et al*., 2007) and in species number between treatments. The whole assemblage, the five most abundant species (*Pterostichus melanarius* Illiger, *Poecilus cupreus* L., *Calathus fuscipes* Goeze, *Carabus granulatus* L., *Trechus quadristriatus* Schrank, 95% of all captures), and the functional categories with sample dates as replicative units (within-subject factor) within analysis (data given per plot) were considered. The interaction analysis disclosed differences between treatments at sample dates. The Tukey's *post hoc* test followed significant results to specify between which treatments the difference was found. The homogeneity of variances was confirmed (the Cochran C, Hartley and Bartlett statistic), and normal approximation was applied.

The values of Chao 1 index (asymptote, estimated species number) were estimated in each treatment separately for the whole assemblage and for the functional categories (EstimateS 9.1.0, Colwell, 2013). Samples were randomly reordered 100 times. Data obtained in 2009–2011 were combined. The Boltzmann sigmoidal growth model was fitted to construct species accumulation curves (rarefaction) to determine whether enough collections had been made to get a reasonable estimate of the actual number of species. Curves, which were 10% lower than the Chao 1 (represents 100%), were assessed as indicators of inadequate species number (more samples would be needed to reach the asymptote). Since the confidence intervals of all rarefaction curves were overlapping, the chi-square test for trend with the Bonferroni correction was applied to see any differences within the trends of rarefaction in treatments (GraphPad Prism 5, GraphPad Software Inc., 2007).

The distribution of carabids in the field was examined using multivariate analysis. Data obtained in 2009–2011 were combined. The linear character of changes in abundance over the field was confirmed by detrended correspondence analysis (DCA: 0.001 attributed to each value, detrending by segments, log transformation: $x' = \log(x + 1)$, downweight rare species, length of gradient: 2.9 for species, 2.1 for functional categories) and permitted use of the redundancy analysis (RDA, 0.001 attributed to each value, log transformation, CANOCO for Windows 4.5; Lepš & Šmilauer, 2003). The treatment, sample date (time series used by Svobodová *et al*., 2016), and year were considered as environmental variables, while the traps and destroyed traps (dummy variable, 4.8% of the total trap number, missing values were substituted with arithmetical averages of catches in all undamaged traps at the respective sample dates in all types of treatment) were regarded as covariables (partial shape RDA). Environmental variables that could modify the effect of treatments included the spatial arrangement of plots (different distances from the adjacent fields and forest), slight field inclination and uneven moisture, and the presence of small grassy areas (ca 1.5 m^2) around 12 drainage wells in nine plots and a hunting hide in one plot. The Monte Carlo permutation test (MCPT, 999 permutations, forward selection) within RDA revealed effect of the spatial arrangement to rows (field inclination) that restricted MCPT to the split-plot design. The species and functional categories representing less than 10 individuals were not included in the analysis. The principal component analysis (PCA, 0.001 attributed to each value, log transformation) was used to give a comprehensive view of the variability in activity abundance of species and functional categories within the field. In other parameters of DCA, RDA and PCA, default settings were employed.

The assumption of the initial similarity of plots was verified by analyzing assemblages from one sample date prior to sowing in 2009. The activity abundance and the species number within each functional category were compared using one-way ANOVA (data given per plot) and MCPT (same data transformation as above). Analyses did not reveal any initial dissimilarity of plots intended for different treatments (Tables S2 and S3).

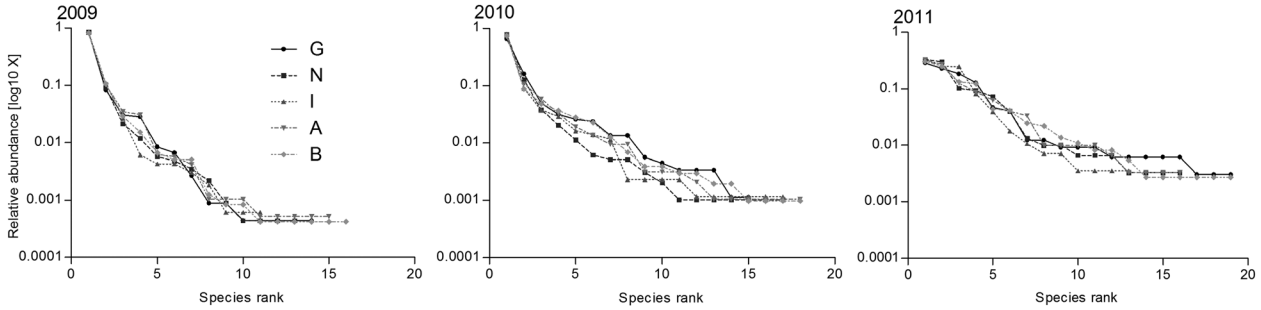

Fig. 1 Dominance distribution of the carabid communities (rank abundance plots) in plots with five different treatments (G, GE maize; N, near-isogenic; I, near-isogenic + insecticide; A, reference; B, reference). Each point represents one species.

All test statistics are provided in the Supporting Information.

Results

Carabid collection and dominance distribution

A total of 16 752 carabids comprising 38 species were captured (2009 : 10 456 individuals, 2010 : 4713 ind., 2011 : 1583 ind., Table S4). Dominance distributions were highly skewed (Fig. 1) due to high abundance of *P. melanarius* in 2009 (83% individuals) and 2010 (74%). A more balanced community was established in 2011, when *P. melanarius* was the second most abundant species (25%) after *P. cupreus* (29%) which had been the second most abundant species in 2009 (10%) and 2010 (11%). The order of dominance of other species varied between years. Spearman's rank correlation coefficients ($r =$ 0.965–0.999, Table S5) indicated very strong positive correlations between rank abundances in different treatments.

Effect of treatments on the overall activity abundance, species number, and dominance

The overall activity abundance was similar in different treatments but the species number in plots I was significantly lower than in G and B (Table S6). The abundances of the five most common species were not significantly different between treatments. Interactions were not significant (Table S6).

The trends in the species accumulation curves were significantly different between treatments (Table S7). The Chao 1 ranged from 24.94 ± 0.02, 26.61 ± 0.02, 30.12 ± 0.10, 41.70 ± 0.70 to 59.85 ± 5.77 for I, G, B, A, and N, respectively. Reasonable estimate of the species number was achieved in B (136%), I (99%), G (98%) and A (91%) but in N it was only 62%.

The MCPT did not reveal any significant effect of treatments. Plots N explained 0.1% and other treatments less than 0.1% variability. Time variables affected the incidence of species significantly except for 2010 with sample date as the most important environmental variable (explained highest portion of variability in comparison with other variables) followed by year 2009 and 2011 (Table S8). The model explained 22.5% of the total (model + error) variability. Cumulative percentage variance in PCA was 42.0%, that is, 1.9-fold higher than the variability explained by RDA.

Effect of treatments on the functional categories

The activity abundance of species included in the body size category 2, habitat category eurytopic, humidity category mesophilous, and categories autumn breeders and carnivores clearly dominated over the other functional categories of the respective traits (Table 1). Maximal activity abundances were recorded at the maize growth stage V6 and/or VT (third and fourth points in Fig. 2). Activity abundances were consistently lowest in the I treatment; the difference from the G treatment was significant in the category body size 3 and from the B treatment in the category open biotopes (Table 1). At the VE maize growth stage in 2009, interaction revealed significantly higher activity abundances in G and A than in N, I, and B (Table S9). Other comparisons were not significant (Table S9).

The numbers of species within functional categories were highest in the body size 3, open and hygrophilous biotopes, spring breeders and carnivores (Table 2). The seasonal dynamics of the species number (Fig. S1) were less dramatic than those of the activity abundance (Fig. 2). The numbers of species assigned to the categories body size 3, open biotopes and spring breeders were lowest in I but the difference was significant only between I and B. The number of hygrophilous and carnivorous carabids was lowest in I where it was significantly lower

Table 1 Mean ± SEM of the activity abundance of carabids assigned to the categories of functional traits. Data obtained in 2009–2011 were summed up for each treatment (G, GE maize; N, near-isogenic; I, near-isogenic + insecticide; A, reference; B, reference). The results of repeated measures (RM) ANOVA of treatments are indicated by small letters (test statistics in Table S9), the significance at the 5% level. Identical letters denote nonsignificant and diverse letters the significant differences between treatments. No letters are used when no treatments differed from one another. Body size categories (mid-range value) included 1 (> 22 mm), 2 (11–21.9 mm), 3 (6–10.9 mm) and 4 (< 5.9 mm).

Trait	Category	Treatment					Total[†]
		G	N	I	A	B	
Body size							
	1	0	0	0	0.03 ± 0.02	0	0.03 ± 0.03
	2	39.96 ± 6.45	42.28 ± 10.90	33.25 ± 1.73	36.83 ± 2.37	44.14 ± 7.69	196.45 ± 9.71
	3	1.73 ± 0.27***a***	0.84 ± 0.23***ab***	0.48 ± 0.14***b***	1.51 ± 0.39***ab***	1.61 ± 0.33***ab***	6.16 ± 1.22
	4	1.65 ± 0.26	1.40 ± 0.32	1.01 ± 0.13	1.33 ± 0.22	1.83 ± 0.32	7.21 ± 0.70
Habitat affinity							
	Eurytopic	37.20 ± 6.10	40.51 ± 10.80	31.03 ± 1.78	33.69 ± 2.88	41.23 ± 7.24	183.65 ± 9.78
	Open biotopes	4.94 ± 0.49***ab***	3.41 ± 0.33***ab***	2.56 ± 0.21***a***	4.60 ± 0.86***ab***	5.38 ± 0.73***b***	20.89 ± 2.59
	Silvicolous	1.20 ± 0.24	0.59 ± 0.07	1.15 ± 0.30	1.40 ± 0.44	0.98 ± 0.16	5.31 ± 0.68
Humidity affinity							
	Eurytopic	6.80 ± 0.46	6.33 ± 0.81	5.15 ± 0.28	6.23 ± 0.43	6.99 ± 0.61	31.49 ± 1.60
	Hygrophilous	2.34 ± 0.33	1.15 ± 0.24	1.38 ± 0.34	2.61 ± 0.63	1.98 ± 0.38	9.45 ± 1.39
	Mesophilous	32.96 ± 5.66	36.10 ± 9.86	27.36 ± 1.94	29.40 ± 2.59	36.91 ± 6.91	162.74 ± 9.26
	Xerophilous	1.24 ± 0.38	0.94 ± 0.13	0.85 ± 0.17	1.45 ± 0.58	1.70 ± 0.37	6.18 ± 0.79
Breeding period							
	Spring	8.66 ± 0.49	7.13 ± 0.65	6.20 ± 0.67	8.40 ± 0.89	8.59 ± 0.76	38.98 ± 2.43
	Summer	0.59 ± 0.14	0.28 ± 0.05	0.28 ± 0.09	0.33 ± 0.05	0.60 ± 0.13	2.06 ± 0.37
	Autumn	35.24 ± 6.04	37.61 ± 9.97	28.78 ± 2.09	31.58 ± 2.32	39.54 ± 7.41	172.74 ± 9.81
Food specialization							
	Carnivorous	37.04 ± 6.11	38.60 ± 9.75	29.84 ± 1.79	33.99 ± 1.94	41.09 ± 7.23	180.55 ± 9.73
	Granivorous	0.01 ± 0.01	0	0	0	0	0.01 ± 0.01
	Omnivorous	6.29 ± 0.57	5.91 ± 0.87	4.90 ± 0.25	5.70 ± 0.51	6.49 ± 0.65	29.29 ± 1.38

[†]Mean per treatment ($n = 5$) during field trial.

than in G and B (Table 2). At the V6 maize growth stage in 2011, the number of silvicolous species was highest in G and differences between G and I and A were significant (Table S10). Other tests were not significant (Table S10).

The trends in the increase of registered species in dependence on the total carabid abundance were significantly different between treatments in all functional categories except for the rarely trapped species in the categories 1 and 4, granivores, silvicolous, xerophilous, and summer breeders. Various differences between treatments were found (Fig. 3). The trend in B treatment was significantly different from A and G in 10 and 9 functional categories, respectively, and the trend in G was different from N and A 7 and 6 times, respectively. The trend in N differed from A and B in six functional categories and I was significantly different from N and B five times, from A four times, and from G twice (Table S11).

The range of Chao 1 depended on the functional category and treatment. Adequate species numbers were not reached in one functional category in treatment G, four in N, two in I, seven in A, and two in B. The lowest Chao 1 was found six times in I and the highest five times in N (reasonable value; Table 3).

The MCPT did not reveal any significant effect of treatments (Fig. 4). The I and B explained 0.1% and other treatments explained even less of the variability. The time variables were significantly correlated with community size with the exception of 2010 (Table S12) with sample date as the most important environmental variables followed by year 2009 and 2011. The model explained 34.8% of the total (model + error) variability (Fig. 4). Cumulative percentage variance in PCA was calculated to be 78.1%, that is, 2.2-fold more than variability explained by RDA.

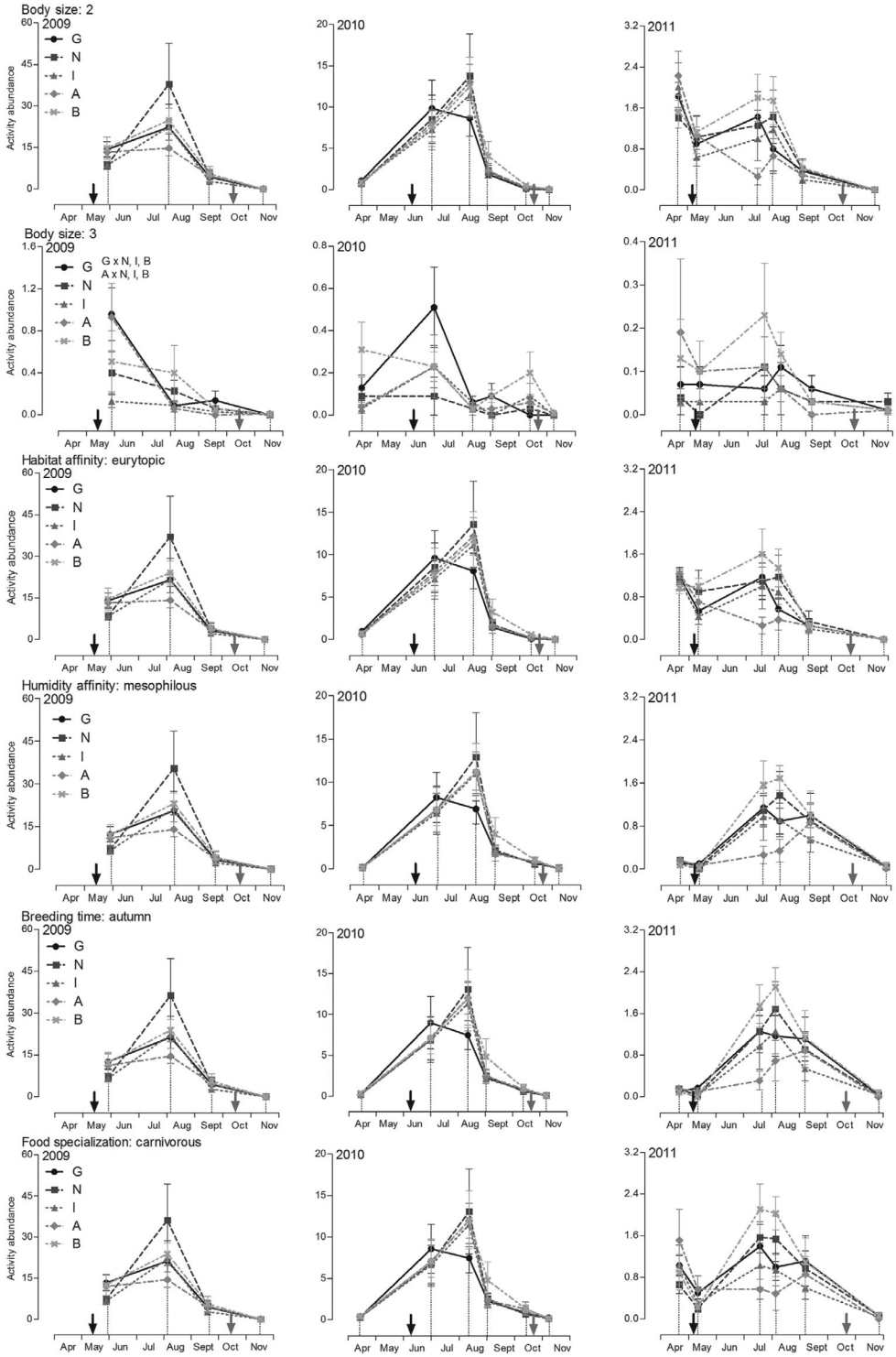

Fig. 2 Seasonal dynamics of the activity abundance of the most abundant categories of functional traits and carabids of body size 3 in plots with five different treatments (G, GE maize; N, near-isogenic; I, near-isogenic + insecticide; A, reference; B, reference). Means ± SEM per 1 plot and 1 d are displayed. The black and grey arrows indicate maize sowing and harvest, respectively. The points in graphs represents sample dates (chronological order): prior to sowing, at maize stages of VE, V6 (missing in 2009), VT and R5, and after the harvest. The dashed lines indicate sample dates on *x*-axis.

Table 2 Mean ± SEM of the numbers of carabid species assigned to the categories of functional traits. Data obtained in 2009–2011 were summed up for each treatment (G, GE maize; N, near-isogenic; I, near-isogenic + insecticide; A, reference; B, reference). The results of repeated measures (RM) ANOVA of treatments are indicated by small letters (test statistics in Table S10), the significance at the 5% level. Identical letters denote nonsignificant and diverse letters significant differences between treatments. No letters are used when no treatments differed from one another. Body size categories (mid-range value) included 1 (> 22 mm), 2 (11–21.9 mm), 3 (6–10.9 mm) and 4 (< 5.9 mm).

Trait	Category	Treatment					Total[†]
		G	N	I	A	B	
Body size							
	1	0	0	0	0.03 ± 0.02	0	0.03 ± 0.03
	2	2.13 ± 0.07	1.93 ± 0.11	1.93 ± 0.06	2.06 ± 0.13	2.08 ± 0.13	10.11 ± 0.21
	3	0.58 ± 0.05***ab***	0.46 ± 0.04***ab***	0.33 ± 0.08***a***	0.49 ± 0.09***ab***	0.71 ± 0.11***b***	2.56 ± 0.32
	4	0.51 ± 0.06	0.54 ± 0.05	0.45 ± 0.07	0.40 ± 0.06	0.54 ± 0.06	2.44 ± 0.14
Habitat affinity							
	Eurytopic	1.25 ± 0.05	1.21 ± 0.03	1.26 ± 0.03	1.28 ± 0.04	1.30 ± 0.08	6.30 ± 0.07
	Open biotopes	1.54 ± 0.05***ab***	1.44 ± 0.07***ab***	1.15 ± 0.12***a***	1.34 ± 0.14***ab***	1.71 ± 0.12***b***	7.18 ± 0.47
	Silvicolous	0.43 ± 0.04	0.28 ± 0.04	0.29 ± 0.03	0.36 ± 0.04	0.31 ± 0.05	1.66 ± 0.14
Humidity affinity							
	Eurytopic	1.01 ± 0.04	1.06 ± 0.08	0.99 ± 0.08	1.05 ± 0.06	1.23 ± 0.05	5.34 ± 0.21
	Hygrophilous	0.78 ± 0.04***a***	0.55 ± 0.09***ab***	0.46 ± 0.03***bc***	0.68 ± 0.06***ac***	0.74 ± 0.05***a***	3.20 ± 0.29
	Mesophilous	1.09 ± 0.04	0.99 ± 0.02	0.98 ± 0.09	0.93 ± 0.04	0.96 ± 0.05	4.94 ± 0.14
	Xerophilous	0.34 ± 0.04	0.33 ± 0.04	0.28 ± 0.03	0.33 ± 0.03	0.40 ± 0.03	1.66 ± 0.10
Breeding period							
	Spring	1.54 ± 0.03***ab***	1.40 ± 0.06***ab***	1.25 ± 0.09***a***	1.50 ± 0.11***ab***	1.76 ± 0.13***b***	7.45 ± 0.42
	Summer	0.20 ± 0.02	0.19 ± 0.03	0.18 ± 0.05	0.20 ± 0.01	0.26 ± 0.03	1.03 ± 0.08
	Autumn	1.85 ± 0.10	1.66 ± 0.06	1.59 ± 0.07	1.64 ± 0.10	1.78 ± 0.16	8.51 ± 0.24
Food specialization							
	Carnivorous	2.38 ± 0.04***a***	2.06 ± 0.09***ab***	1.88 ± 0.08***bc***	2.08 ± 0.11***ac***	2.33 ± 0.10***a***	10.71 ± 0.46
	Granivorous	0.01 ± 0.01	0	0	0	0	0.01 ± 0.01
	Omnivorous	0.83 ± 0.04	0.86 ± 0.07	0.83 ± 0.04	0.90 ± 0.04	1.00 ± 0	4.41 ± 0.16

[†]Mean per treatment ($n = 5$) during field trial.

Discussion

The community structure of carabids in our field was similar to other European regions (Brooks *et al*., 2003; Kalushkov *et al*., 2009; Grabowski *et al*., 2010; Skoková Habuštová *et al*., 2015; Vician *et al*., 2015; Lee & Albajes, 2016; Wach *et al*., 2016) and certain similarities could be found also with other parts of the world (Hatten *et al*., 2007; Leslie *et al*., 2010). Strong dominance of one or a few ubiquitous species and low abundance of other species is typical for all agrocoenosis but the composition of dominating species differs between biotopes and years (Irmler, 2003; Lee & Albajes, 2016; Skoková Habuštová *et al*., 2017).

In our study, the dominance distributions on different plots were similar in spite of different treatments (Leslie *et al*., 2010). There were no differences in abundance when all species were analyzed together or when five most abundant species were analyzed. However, several significant differences were found when data were evaluated at the level of functional categories. Differences in the species number were more pronounced than differences in the activity abundance. Most of the significant differences followed the same pattern characterized by lowest abundance and/or species number in plots treated with the insecticide (I).

Significant differences in the species accumulation curves were common in the functional categories with highest activity abundance followed by functional categories with highest species number. More significant differences were found between hybrids (different genetic background) than between near-isogenic maize (G, N, I). Consistently with previous reports (Rauschen *et al*., 2009; Twardowski *et al*., 2012; Priesnitz *et al*., 2013;

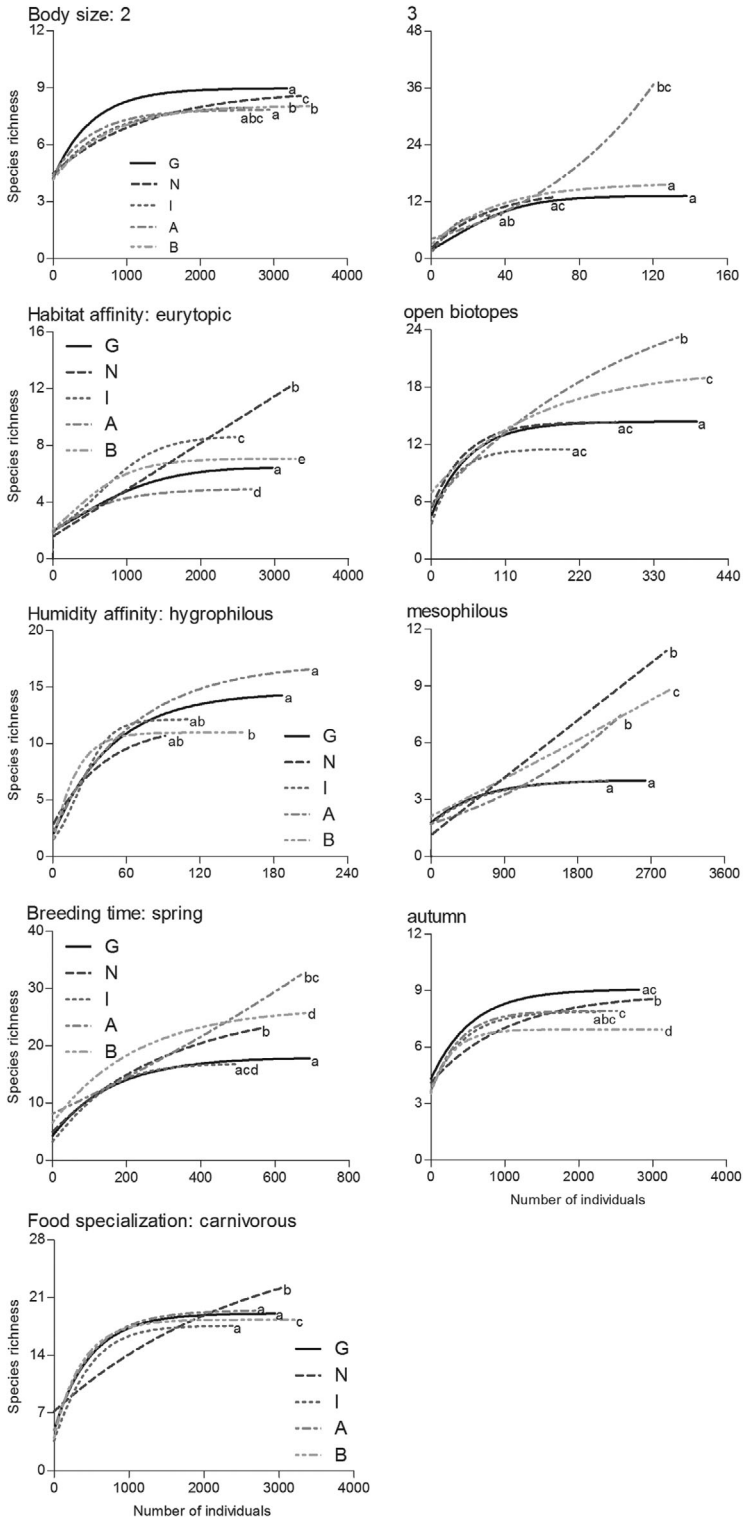

Fig. 3 Species accumulation curves (rarefaction) in the categories of functional traits with the highest activity abundance and/or species number in plots with five different treatments (G, GE maize; N, near-isogenic; I, near-isogenic + insecticide; A, reference; B, reference). Data obtained in 2009–2011 were combined. Small letters indicate differences in the trends of curves in different treatments (test statistics in Table S11).

Table 3 Mean ± SEM of the Chao 1 for the categories of functional traits and comparison with values that emerged from the estimation analysis (% of Chao 1). Values of the estimation analysis lower than 10% of Chao 1 (in bold) indicate that the adequate species number was not collected. Data obtained in 2009–2011 were summed up for each treatment (G, GE maize; N, near-isogenic; I, near-isogenic + insecticide; A, reference; B, reference).

Trait	Category[†]	G		N		I		A		B	
		Chao 1	% of Chao 1	Chao 1	% of Chao 1	Chao 1	% of Chao 1	Chao 1	% of Chao 1	Chao 1	% of Chao 1
Body size											
	1[‡]										
	2	8.98 ± 0.01	100	8.87 ± 0.05	101	8.05 ± 0.02	99	7.85 ± 0.01	102	8.06 ± 0.01	99
	3	13.23 ± 0.05	94	14.54 ± 1.01	**89**	9.20 ± 0.10	98	42373 ± 1.74E+07	**0**	15.91 ± 0.42	119
	4	3.98 ± 0.01	101	6.69 ± 0.83	90	6.84 ± 0.72	**88**	3.96 ± 0.03	101	5.01 ± 0.03	100
Habitat affinity											
	Eurytopic	6.52 ± 0.01	100	23.05 ± 0.71	**56**	8.68 ± 0.01	92	4.95 ± <0.01	101	7.07 ± <0.01	99
	Open biotopes	14.39 ± 0.03	100	14.35 ± 0.12	101	11.51 ± 0.05	100	30.70 ± 2.35	**76**	19.89 ± 0.48	111
	Silvicolous	4.36 ± 0.08	92	3.01 ± 0.11	100	3.39 ± 0.10	**88**	7.11 ± 0.99	**77**	3.17 ± 0.06	174
Humidity affinity											
	Eurytopic	5.89 ± 0.01	102	5.82 ± 0.02	103	5.76 ± 0.02	104	5.85 ± 0.01	103	8.35 ± 0.36	96
	Hygrophilous	14.52 ± 0.07	96	11.76 ± 0.99	102	12.15 ± 0.05	95	17.14 ± 0.28	105	10.98 ± 0.02	100
	Mesophilous	4.03 ± <0.01	99	12.00 ± n.a.[§]	100	4.10 ± 0.01	98	1712 ± 49440	**0**	430.4 ± 15510	**2**
	Xerophilous	25.65 ± 3404	**8**	2.10 ± 0.02	95	n.a.[§]	104	3.90 ± 4.34	**51**	2.04 ± 0.01	98
Breeding period											
	Spring	18.01 ± 0.05	97	27.29 ± 0.99	**86**	16.88 ± 0.05	98	4692 ± 841972	**1**	26.72 ± 0.65	131
	Summer	2.10 ± 0.05	95	2.01 ± 0.02	100	3.21 ± 0.11	93	2.05 ± 0.04	98	2.01 ± 0.01	100
	Autumn	9.01 ± 0.01	100	8.82 ± 0.05	102	7.91 ± 0.01	101	7.94 ± 0.01	101	6.95 ± <0.01	101
Food specialization											
	Carnivorous	19.10 ± 0.02	98	28.96 ± 2.43	90	17.61 ± 0.01	99	19.45 ± 0.03	103	18.33 ± 0.01	104
	Granivorous[‡]										
	Omnivorous	5.29 ± 0.05	95	9.92 ± 0.21	**86**	5.89 ± 0.37	102	5819 ± 195098	**0**	4063 ± 576075	**0**

[†]Categories with highest activity abundance: underlined. Categories with highest species number: italic.

[‡]Not enough number of individuals and species for statistical comparison.

[§]n.a., not available.

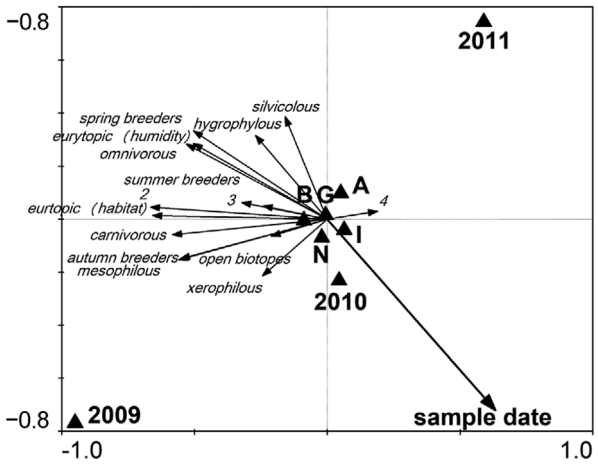

Fig. 4 Redundancy analysis (RDA) of the correlation of functional traits of carabids with treatments (G, GE maize; N, near-isogenic; I, near-isogenic + insecticide; A, reference; B, reference). Data obtained in 2009–2011 were combined.

Garcia-Alonso *et al*., 2014), genetic engineering (treatment G) had smaller effect on carabid communities in comparison with unknown differences between the genotypes.

Chao 1 for all data showed lowest asymptote in I and highest in the N treatments. When analyzed in respect to the functional categories, assemblages in B were nearest to Chao 1 while those in A reached very high Chao 1 values. This extreme in A was a consequence of reduced activity abundance while the species number remained similar. The lowest species number was most often found in the I treatment.

RDA did not show any significant association of treatments with the species distribution or with the functional traits. The centroids for all five treatments were around the center of the ordination diagram, reflecting the low role of treatments in explaining the distribution of functional categories. Relatively long arrows of functional categories positioned close to the first axis indicate strong relationships with the axis–time variables. Negative correlation of the sample date with most functional categories reflected lower abundance of such categories later in the season. The direction of most arrows evidenced very high carabid abundance in 2009 when maize was grown after wheat that provided better living conditions than maize (Duflot *et al*., 2013). The decrease of carabids in the next 2 years was mainly caused by the sharp decline of *P. melanarius* and partly also of *P. cupreus* and *P. versicolor*. The short arrows for the categories of body size 3 and 4, affinity to open biotopes, and summer breeders around the center of diagram indicate their stability over time.

Consistently with other studies, we did not find any negative effect of the GE maize expressing the Cry3Bb1 insecticidal protein (Al-Deeb & Wilde, 2003; Ahmad *et al*., 2005; Leslie *et al*., 2010; Priesnitz *et al*., 2013). We found clear trends toward reduced abundance and species numbers in the I treatment. This can be explained by increased mortality. Some previous results also indicated that insecticide application into the soil or on the seeds affected carabids negatively (Bhatti *et al*., 2005; Leslie *et al*., 2010) but other authors did not find any effect (Al-Deeb & Wilde, 2003; Ahmad *et al*., 2005). The tendency of better carabid performance in B can be explained by massive *Ostrinia nubilalis* Hübner infestation that lured carabids (Toschki *et al*., 2007; Svobodová *et al*., 2015).

Cumulative percentage variance in PCA was twice higher than variability explained by RDA, confirming that different treatments are not good predictors of invertebrate abundance in the field as shown by Skoková Habuštová *et al*. (2017). Generally, year-to-year changes in environmental conditions and in the use of agrotechniques have much stronger effects on the arthropod community than the use of GE maize (Guo *et al*., 2016). It is difficult to detect small effects of maize varieties on other organisms (Harrigan *et al*., 2010; Arias-Martín *et al*., 2018). Meissle and Lang (2005) showed that enormous numbers of plots would be necessary to determine unequivocal effects. The situation is even more complicated with the highly mobile flying carabids (*Harpalus affinis* Schrank, *Pseudoophonus rufipes* De Geer), for which plots should be sufficiently large to reduce population exchanges. Our 0.5-ha plots were probably large enough (Priesnitz *et al*., 2013) and in any case unusually large in comparison with other studies (Al-Deeb & Wilde, 2003; Ahmad *et al*., 2005).

Insect grouping according to taxonomic relatedness is not suitable for assessing the effect of environmental factors because it conceals differences in particular functional categories. The analysis of most abundant species is also not appropriate because it limits data transportability due to big differences in the species abundance in various localities. By contrast, the representation of functional categories is similar in different regions and years and is independent of species composition (Grabowski *et al*., 2010; Skoková Habuštová *et al*., 2017). The analysis based on functional traits permits inclusion of the less abundant species, thereby increasing the number of species and individuals and enhancing the power of statistical analysis. Due to the possibility to compare species that perform identical functions in different geographic regions, functional approach enables transportability, and/or interpolation of diverse data (Ahmad *et al*., 2016).

In summary, no adverse effect of GE maize MON88017 on carabids was observed in our 3-year field trial and no environmental risk of growing MON88017 was detected. Our findings on the carabid community composition and fluctuation are consistent with the published reports. Observed patterns of differences between five treatments suggest that the comparisons of species numbers and abundances in all collected carabids or in the most common carabid species is unsuitable for ERA because of very limited comparability between data collected in different sites or in different years. This shortcoming is eliminated by the comparisons of carabid communities in respect to the functional traits. The use of functional group indicators allows recognition of changes in the carabid community despite differences in the identity of species. We recommend data evaluation at the level of functional categories because the treatment effects (relatively small at any rate) are easier to detect when carabids are organized in functional group. Transportability of functional groups allows better comparison of treatments and effects in diverse regions. Ubiquitous occurrence and functional diversification of carabids render them particularly suitable for this approach.

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Disclosure

The authors have no conflict of interest to declare.

References

Ahmad, A., Negri, I., Oliveira, W., Brown, C., Asiimwe, P., Sammons, B. *et al*. (2016) Transportable data from nontarget arthropod field studies for the environmental risk assessment of genetically modified maize expressing an insecticidal double-stranded RNA. *Transgenic Research*, 25, 1–17.

Ahmad, A., Wilde, G.E. and Zhu, K.Y. (2005) Detectability of coleopteran-specific Cry3Bb1 protein in soil and its effect on nontarget surface and below-ground arthropods. *Environmental Entomology*, 34, 385–394.

Al-Deeb, M.A. and Wilde, G.E. (2003) Effect of *Bt* corn expressing the Cry3Bb1 toxin for corn rootworm control on aboveground nontarget arthropods. *Environmental Entomology*, 32, 1164–1170.

Arias-Martín, M., García, M., Castañera, P., Ortego, F. and Farinós, G.P. (2018) Farm-scale evaluation of the impact of Cry1Ab Bt maize on canopy nontarget arthropods: a 3-year study. *Insect Science*, 25, 87–98.

Bhatti, M.A., Duan, J., Head, G., Jiang, C.J., Mckee, M.J., Nickson, T.E. *et al*. (2005) Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected Bt corn on ground-dwelling invertebrates. *Environmental Entomology*, 34, 1325–1335.

Brooks, D.R., Bohan, D.A., Champion, G.T., Haughton, A.J., Hawes, C., Heard, M.S. *et al*. (2003) Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates. *Philosophical Transactions of the Royal Society of London B*, 358, 1847–1862.

Clough, Y., Kruess, A. and Tscharntke, T. (2007) Organic versus conventional arable farming systems: functional grouping helps understand staphylinid response. *Agriculture, Ecosystems and Environment*, 118, 285–290.

Colwell, R.K. (2013) EstimateS: statistical estimation of species richness and shared species from samples. Version 9. User's Guide and application. http://purl.oclc.org/estimates. Accessed 12 March 2017.

Dunn, O.J. (1961) Multiple comparisons among means. *Journal of the American Statistical Association*, 56, 52–64.

Duflot, R., Aviron, S. and Burel, F. (2013) Farmland heterogeneity & complementation a case study on carabid beetles. ESA Annual Meeting 2013, Minneapolis, USA. https://www.researchgate.net/profile/Remi_Duflot/publication/264047099_Effects_of_farmland_landscape_heterogeneity_on_biodiversity_is_there_a_spatio-temporal_complementation_between_crop_fields/links/0a85e53cbe6364033e000000/Effects-of-farmland-landscape-heterogeneity-on-biodiversity-is-there-a-spatio-temporal-complementation-between-crop-fields.pdf. Accessed 10 April 2018.

Environmental Protection Agency [EPA]. Scientific Advisory Panel. (2002) Corn rootworm plant-incorporated protectant insect resistance management and non-target insect issues. Transmittal of meeting minutes of the FIFRA Scientific Advisory Panel Meeting August 27–29, 2002 Arlington, VA.

https://www.regulations.gov/docketBrowser?rpp=50&po=0&D=EPA-HQ-OPP-2002-0157. Accessed 14 October 2017.

European Commission (2017) EU Register of authorised GMOs. http://ec.europa.eu/food/dyna/gm_register/index_en.cfm. Accessed 17 October 2017.

Food and Agriculture Organization of the United Nations [FAO] (2017) FAOSTAT. http://www.fao.org/faostat/en/#data/QC. Accessed 13 October 2017.

Food and Agriculture Organization of the United Nations [FAO] (2015) *World Reference Base for Soil Resources 2014: International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*, pp. 1–192. *Update 2015. World soil resources reports*. FAO, Rome.

Garcia-Alonso, M., Hendley, P., Bigler, F., Mayeregger, E., Parker, R., Rubinstein, C. *et al*. (2014) Transportability of confined field trial data for environmental risk assessment of genetically engineered plants: a conceptual framework. *Transgenic Research*, 23, 1025–1041.

Grabowski, M., Bereś, P.K. and Dąbrowski, Z.T. (2010) Charakterystyka wybranych gatunków biegaczowatych (Coleoptera: Carabidae) pod kątem ich przydatności dla oceny ryzyka i monitoringu uwalniania GMO do środowiska. *Progress in Plant Protection*, 50, 1602–1606.

GraphPad Software Inc. (2007) *GraphPad Prism 5.0 User´s Guide*. GraphPad Software Inc., San Diego, CA. https://www.graphpad.com/guides/prism/7/user-guide/index.htm?citing_graphpad_prism.htm. Accessed 19 May 2018.

Guo, J.F., He, K.L., Bai, S.X., Zhang, T.T., Liu, Y.T., Wang, F.X. *et al*. (2016) Effects of transgenic *cry1Ie* maize on non-lepidopteran pest abundance, diversity and community composition. *Transgenic Research*, 25, 761–772.

Harrigan, G.G., Lundry, D., Drury, S., Berman, K., Riordan, S.G., Nemeth, M.A. *et al*. (2010) Natural variation in crop composition and the impact of transgenesis. *Nature Biotechnology*, 28, 402–404.

Hatten, T.D., Bosque-Pérez, N.A., Labonte, J.R., Guy, S.O. and Eigenbrode, S.D. (2007) Effects of tillage on the activity density and biological diversity of carabid beetles in spring and winter crops. *Environmental Entomology*, 36, 356–368.

Hůrka, K. (1996) *Carabidae of the Czech and Slovak Republics. Illustrated Key 1. 16 col. Plates*, pp. 1–565. Kabourek Publishing, Zlín.

Irmler, U. (2003) The spatial and temporal pattern of carabid beetles on arable fields in northern Germany (Schleswig-Holstein) and their value as ecological indicators. *Agriculture, Ecosystems and Environment*, 98, 141–151.

International Service for the Acquisition of Agri-biotech Applications [ISAAA]. (2017) Global Status of Commercialized Biotech/GM Crops: 2017. ISAAA Brief No. 53. Ithaca: ISAAA.

Jugenheimer, R.W. (1958) Hybrid maize breeding and seed production. *FAO Agricultural Development Paper*, 62, 99–103.

Kalushkov, P., Gueorguiev, B., Spitzer, L. and Nedved, O. (2009) Biodiversity of ground beetles (Coleoptera: Carabidae) in genetically modified (Bt) and conventional (non-Bt) potato fields in Bulgaria. *Biotechnology and Biotechnological Equipment*, 23, 1346–1350.

Larochelle, A. (1990) The food of carabid beetles (Coleoptera: Carabidae, including Cicindelidae). *Fabreries Supplement*, 5, 1–132.

Lee, M.S. and Albajes, R. (2016) Monitoring carabid indicators could reveal environmental impacts of genetically modified maize. *Agricultural and Forest Entomology*, 18, 238–249.

Lepš, J. and Šmilauer, P. (2003) *Multivariate Analysis of Ecological Data Using CANOCO*. Cambridge University Press, Cambridge. pp. 1–269.

Leslie, T.W., Biddinger, D.J., Rohr, J.R. and Fleischer, S.J. (2010) Conventional and seed-based insect management strategies similarly influence nontarget coleopteran communities in maize. *Environmental Entomology*, 39, 2045–2055.

Liu, S.M., Li, J., Zhu, J.Q., Wang, X.W., Wang, C.S., Liu, S.S. *et al*. (2016) Transgenic plants expressing the AaIT/GNA fusion protein show increased resistance and toxicity to both chewing and sucking pests. *Insect Science*, 23, 265–276.

Meissle, M. and Lang, A. (2005) Comparing methods to evaluate the effects of *Bt* maize and insecticide on spider assemblages. *Agriculture, Ecosystems and Environment*, 107, 359–370.

Navasero, M.V., Candano, R.N., Hautea, D.M., Hautea, R.A., Shotkoski, F.A. and Shelton, A.M. (2016) Assessing potential impact of Bt egg plants on non-target arthropods in the Philippines. *PLoS ONE*, 11, e0165190.

Nock, C.A., Vogt, R.J. and Beisner, B.E. (2016) Functional Traits. *eLS*, pp. 1–8. John Wiley & Sons, Ltd, Chichester.

Oyediran, I.O., Matthews, P., Palekar, N., French, W., Conville, J. and Burd, T. (2016) Susceptibility of northern corn rootworm *Diabrotica barberi* (Coleoptera: Chrysomelidae) to mCry3A and eCry3.1Ab *Bacillus thuringiensis* proteins. *Insect Science*, 23, 913–917.

Priesnitz, K.U., Benker, U. and Schaarschmidt, F. (2013) Assessment of the potential impact of a *Bt* maize hybrid expressing Cry3Bb1 on ground beetles (Carabidae). *Journal of Plant Diseases and Protection*, 120, 131–140.

Rauschen, S., Schultheis, E., Pagel-Wieder, S., Schuphan, I. and Eber, S. (2009) Impact of Bt-corn MON88017 in comparison to three conventional lines on *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae) field densities. *Transgenic Research*, 18, 203–214.

Ritchie, S.W., Hanway, J.J. and Benson, G.O. (1992) How a corn plant develops. Special Report No. 48. Iowa State

University of Science and Technology, Ames. https://s10.lite.msu.edu/res/msu/botonl/b_online/library/maize/www.ag.iastate.edu/departments/agronomy/corngrows.html#vegetative. Accessed 31 July 2017.

Romeis, J., Meissle, M., Alvarez-Alfageme, F., Bigler, F., Bohan, D.A., Devos, Y. *et al*. (2014) Potential use of an arthropod database to support the nontarget risk assessment and monitoring of transgenic plants. *Transgenic Research*, 23, 995–1013.

Sanvido, O., De Schrijver, A., Devos, Y. and Bartsch, D. (2011) Post market environmental monitoring of genetically modified herbicide tolerant crops (Working group report from the 4th International Workshop on PMEM of Genetically Modified Plants, Quedlinburg, Germany 2010. *Journal für Kulturpflanzen*, 63, 211–216.

Skoková Habuštová, O., Svobodová, Z., Cagáň, Ľ. and Sehnal, F. (2017) Use of carabids for the post-market environmental monitoring of genetically modified crops. *Toxins*, 9, 121.

Skoková Habuštová, O., Svobodová, Z., Spitzer, L., Doležal, P., Hussein, H.M. and Sehnal, F. (2015) Communities of ground-dwelling arthropods in conventional and transgenic maize: background data for the post-market environmental monitoring. *Journal of Applied Entomology*, 139, 31–45.

StatSoft Inc. (2015) *Statistica Electronic Manual*. StatSoft Inc., Tulsa, OK. http://documentation.statsoft.com/STATISTICAHelp.aspx?path=common/AboutSTATISTICA/ElectronicManualIndex. Accessed 12 March 2017.

Svobodová, Z., Habuštová, O., Sehnal, F., Holec, M. and Hussein, H.M. (2013) Epigeic spiders are not affected by the genetically modified maize MON 88017. *Journal of Applied Entomology*, 137, 56–67.

Svobodová, Z., Skoková Habuštová, O., Boháč, J. and Sehnal, F. (2016) Functional diversity of staphylinid beetles (Coleoptera: Staphylinidae) in maize fields: testing the possible effect of genetically modified, insect resistant maize. *Bulletin of Entomological Research*, 106, 432–445.

Svobodová, Z., Skoková Habuštová, O., Hutchison, W.D., Hussein, H.M. and Sehnal, F. (2015) Risk assessment of genetically engineered maize resistant to *Diabrotica* spp.: influence on above-ground arthropods in the Czech Republic. *PLoS ONE*, 1, e0130656.

Toschki, A., Hothorn, L.A. and Roß-Nickoll, M. (2007) Effects of cultivation of genetically modified *Bt* maize on epigeic arthropods (Araneae; Carabidae). *Environmental Entomology*, 36, 967–981.

Twardowski, J.P., Bereś, P.K., Hurej, M., Klukowski, Z., Dąbrowski, Z.T., Sowa, S. *et al*. (2012) The quantitative changes of ground beetles (Col., Carabidae) in Bt and conventional maize crop in southern Poland. *Journal of Plant Protection Research*, 52, 404–409.

Vician, V., Svitok, M., Kočík, K. and Stašiov, S. (2015) The influence of agricultural management on the structure of ground beetle (Coleoptera: Carabidae) assemblages. *Biologia*, 70, 240–251.

Wach, M., Hellmich, R.L., Layton, R., Romeis, J. and Gadaleta, P.G. (2016) Dynamic role and importance of surrogate species for assessing potential adverse environmental impacts of genetically engineered insect-resistant plants on non-target organisms. *Transgenic Research*, 25, 499–505.

Wang, Y.N., Ke, K.Q., Li, Y.H., Han, L.Z., Liu, Y.M., Hua, H.X. *et al*. (2016) Comparison of three transgenic *Bt* rice lines for insecticidal protein expression and resistance against a target pest, *Chilo suppressalis* (Lepidoptera: Crambidae). *Insect Science*, 23, 78–87.

Yang, F., Chen, M., Gowda, A., Kerns, D.L. and Huang, F.N. (2018) Possibly similar genetic basis of resistance to *Bacillus thuringiensis* Cry1Ab protein in 3 resistant colonies of the sugarcane borer collected from Louisiana, USA. *Insect Science*, 25, 241–250.

Zhang, Q., Hua, G. and Adang, M.J. (2017) Effects and mechanisms of *Bacillus thuringiensis* crystal toxins for mosquito larvae. *Insect Science*, 24, 714–729.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Taxonomic affiliation and functional traits of captured carabids.

Table S2. Testing the assumption of the initial similarity of experimental plots prior to maize sowing in 2009. Results of one-way ANOVA of differences in the activity abundance and species number of carabids assigned to the functional categories.

Table S3. Testing the assumption of the initial similarity of experimental plots. Results of Monte Carlo permutation tests of correlation between functional categories and plot positions in carabids captured prior to maize sowing in 2009.

Table S4. The activity abundance and number of species of carabids.

Table S5. Spearman's rank correlation coefficients with Bonferroni correction of significance level for rank activity abundance curves (Whittaker plots) of carabid species.

Table S6. The results of repeated measures analysis of variance (RM ANOVA). Effects of treatments on the carabid number of species, overall activity abundance, and activity abundances of five dominant species.

Table S7. The results of chi-square test for trend with Bonferroni correction of significance level for Boltzmann sigmoidal growth model of species accumulations curves (rarefaction) for overall carabid abundance.

Table S8. The results of Monte Carlo permutation tests showing correlation of all carabid species activity abundance with experimental treatments and time variables.

Table S9. The results of repeated measures (RM) ANOVA. Effects of treatments on the activity abundances of carabids belonging to different functional categories.

Table S10. The results of repeated measures (RM) ANOVA. Effects of treatments on the number of carabid species in functional categories.

Table S11. Significant results of the chi-square test for trend with Bonferroni correction of significance level for Boltzmann sigmoidal growth model of species accumulations curves (rarefaction) for the carabid functional categories.

Table S12. The results of Monte Carlo permutation tests showing correlation of the carabid functional categories with experimental treatments and time variables.

Fig. S1. Seasonal dynamics of the number of carabid species in the most species-rich categories of functional traits and carabids with affinity to silvicolous habitats.