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Short-term effects of different genetically modified maize varieties on arthropod food web properties: an experimental field assessment

Ágnes Szénási[1], Zoltán Pálinkás[1], Mihály Zalai[1], Oswald J. Schmitz[2] & Adalbert Balog[2,3]

[1]Institute of Plant Protection, Faculty of Agriculture and Environmental Sciences, Szent István University, Gödöllő, Páter Károly str. 1. Hungary, [2]School of Forestry and Environmental Studies, Yale University, 370 Prospect Street, New Haven, Connecticut, USA, [3]Department of Horticulture, Faculty of Technical Science, Sapientia University. 1/C Sighisoara Street, Tg. Mures, Romania.

Correspondence and requests for materials should be addressed to A.B. (adalbert.balog@fulbrightmail.org; adalbert.balog@ms.sapientia.ro) or A.S. (Szenasi.Agnes@mkk.szie.hu)

There is concern that genetically modified (GM) plants may have adverse affects on the arthropod biodiversity comprising agricultural landscapes. The present study report on a two year field experimental test of whether four different genotypic lines, some are novel with no previous field tests, of GM maize hybrids alter the structure of arthropod food webs that they harbour, relative to non-GM maize (control) that is widely used in agriculture. The different GM genotypes produced either Bt toxins, conferred glyphosate tolerance or a combination of the two traits. Quantitative food web analysis, based on short-term assessment assigning a total of 243,896 arthropod individuals collected from the treatments to their positions in food webs, revealed that complex and stable food webs persisted in each maize treatment. Moreover, food web structure remained relatively unchanged by the GM-genotype. The results suggest that at least in short-term period these particular GM maize genotypes will not have adverse effects on arthropod biota of agricultural landscapes.

There is growing appreciation that the genotype of plants can be an important determinant of associated arthropod food webs[1–4]. These genetic effects on arthropod food webs may also have cascading effects on the functioning of ecosystems[3,5,6]. Given such relationships, it stands to reason that the release of genetically modified (GM) crops into the environment could disrupt arthropod food web structure and ecosystem functioning, especially through impacts on non-target arthropod species[3,7]. However, information on any potential link between GM genotypes and arthropod food web structure is scarce if nonexistent, thereby precluding the possibility to better inform policy about the broad impacts of GM crops are on the environment. The present study reports on the effects of four different genotypic lines of GM maize from which two with extra glyphosate treatment hybrids relative to non-GM maize on the structure of arthropod food webs that they harbour (Table 1). Through quantitative food web analysis the study offer insight into how food web properties such as bottom-up ($K_{bu.i}$) and top-down ($K_{td.i}$) indexes and connectance are affected by the GM maize genotype.

The approach is to examine the potential for tissue or genetic material from GM maize to enter no-target species via food chain effects or whether GM lines would disrupt food chain structure to the point where transmission is altered, in light of ecological observations that plant genotypes differentially affect arthropod species community structure. A potentially important route of exposure is through transgenic proteins that are bioaccumulated up trophic levels in a food chain after they are consumed by herbivores feeding on maize[8–10] that are then consumed by their predators (or consumption of pollen if prey are scarce[11]. Such bioaccumulation could have deleterious effects on predator survival, longevity and development, thereby shortening food chains on GM plants relative to non-GM plants, depending on genotype-specific toxicity. The presence of such affects have been examined in several laboratory studies with a host of arthropod species and Bt protein from crops[10,12–14]. These generally shown that effects of Bt protein may be negligible. But, these laboratory studies focus on single predator and prey species, and thus may fail to measure any potential sub-lethal effects that only become manifest when species are juxtaposed and interact with other species within their natural food webs, as is the case when examining effects of pesticides on predator-prey performance between lab and field settings[15,16]. Field studies are therefore needed to comprehensively assess the effects of Bt protein exposure to many insect species.

Table 1 | The characteristics of the investigated transgenic and isogenic maize hybrids

Treatm. ID	OECD Identifier	Toxin	Resistance/Tolerance	GM Yes or No	no. of blocks
1	DAS-59122-7	Cry34Ab1, Cry35Ab1	Coleoptera	YES	4
2	DAS-01507-1xDAS-59122-7	Cry34Ab1, Cry35Ab1xCry1F	Coleoptera and Lepidoptera	YES	4
5	DAS-01507-1xMON-00603-6	Cry1FxC4 EPSPS	Lepidoptera and glyphosate	YES	4
6	DAS-01507-1xMON-00603-6	Cry1FxC4 EPSPS	Lepidoptera and glyphosate + glyphosate treatment	YES	4
7	DAS-59122-7xDAS-01507-1xMON-00603-6	Cry34Ab1, Cry35Ab1xCry1FxC4 EPSPS	Coleoptera, Lepidoptera and glyphosate	YES	4
8	DAS-59122-7xDAS-01507-1xMON-00603-6	Cry34Ab1, Cry35Ab1xCry1FxC4 EPSPS	Coleoptera, Lepidoptera and glyphosate + glyphosate treatment	YES	4
901	PR-34A15	-	-	NO	4
903	PR-35A30	-	-	NO	4

More than 50 field studies have been conducted in commercial and experimental fields to evaluate the impact of GM crops that contain transgen from Bacillus thuringiensis (Bt crops) on beneficial non-target organisms[12]. The vast majority of studies have focused on Bt maize commercially grown since 1996[8,12]. These studies revealed minor, transient and inconsistent effects on non-target organisms when compared with non-Bt controls[9,12]. All important phytophagous and predators species were previously considered non-target organisms on which possible harmful effects of Bt toxins may have been directly (phytophagous) or indirectly (predators) detected[8–10,17]. Currently, there are no evidence to prove that Bt proteins accumulate in prey tissues[18]. Other studies individually analyzing these groups also revealed no deleterious effects of Bt maize on mortality (studies on lacewing larvae and lady beetles[12], adult and juvenile Theridion impressum spiders[10,19], field and laboratory studies on Orius spp., lacewings and Stethorus punctillum[9], staphylinidae[20] and Lepidoptera[21]. There were also no significant changes in heteropteran predator densities exposed with herbicide tolerant maize varieties[22]. Field studies can indeed assess the effects of exposure on many species[23,24], but quantitative food web analyses of arthropods on GM maize crops can provide a powerful tool to explore the structure of ecological communities and their responses to environmental factors that may not be revealed by conventional analyses of species presence or absence[25]. During this two-year field study detailed arthropod assessments were made on GM maize treatments and on non-GM controls (Table 1) to test the hypothesis that a similar and stable food-web exists on all maize crops. Although the total number of arthropods used for analyses exceeded 240,000 these can only be considered as a short-term (or acute) impact of transgenic crops on arthropod food webs. Therefore to increase the sensibility of the methods food-web analyses were mainly focus on a subset of organisms that are:

1. Most abundant during the maize vegetation period (but before pollen spreading to exclude errors by cross-pollination).
2. Their cumulative abundance as trophic groups (herbivores, predators) for each GM and non-GM crops exceeded 95%.
3. Were identified as test organisms in GM crops by EFSA[26].

The two target organisms western corn rootworm (*Diabrotica virgifera virgifera*) and the European corn borer (*Ostrinia nubilalis*) were also included in food-web analyses.

Study system. GM maize is grown annually on more than 35 million hectares globally[27]. Among EU countries, maize is cultivated on 13 million hectares, which represents 13% of the cultivated area in the EU or 8% of maize production area worldwide[28]. Currently, only one GM maize, MON810 that expresses active Bt toxin that protects it against insect pests, is legally grown in open field cultivation for commercial purposes in Europe.

The most important insect pests of non-GM field maize in Europe are the western corn rootworm (*Diabrotica virgifera virgifera*) and the European corn borer (*Ostrinia nubilalis*). Other insects, such as aphids and thrips, occur in all maize crops and may occasionally cause losses. The most important predators of these pests and other arthropod herbivores are lady beetles, predatory thrips, rove beetles (Staphylinidae), spiders, Syrphid larvae and minute pirate bugs (Orius spp)[28].

According to the ISSAA[27], the main applications for GM maize at present are: (1) pest resistance; (2) herbicide tolerance; and (3) a combination of the two. Pest resistant maize contains toxins against the western corn rootworm and the European corn borer. Herbicide tolerant maize, especially glyphosate-resistance genotypes are important for early growth stages of maize, because application of this broad spectrum herbicide reduces competition maize plants and weeds, and had no negative effects on maize plants.

The GM varieties studied in the present experiments contain toxins against insect pests, or proteins that confer tolerance to glyphosate, or in combination confer tolerance against both insects and glyphosate. Four GM varieties whose protein make-up conferred different combinations of resistance to pest species and tolerance to glyphosate were evaluated (Table 1). Resistance in different GM varieties was conferred through different *Bacillus thuringiensis* insecticidal crystal (*Cry*) proteins in different forms that can specifically target different insect pest species or the combinations of those pests. Some GM varieties also contain proteins that confer tolerance to glyphosate herbicides in addition to insecticidal proteins (Table 1).

Results

The total number of individuals used for food-web construction varied between treatments, ranging from the highest with 32,237 in Lepidoptera resistant and glyphosate tolerant + glyphosate treated blocks and the lowest with 24,972 in Coleoptera and Lepidoptera resistant blocks (Table 2). Different treatments had very similar food web structure with relative constant numbers of trophic species (S) and trophic links (L) between species (Figures 1 A–H). Predatory groups of all treatments were dominated by ladybird beetles, Lacewing larvae, predatory thrips, rove beetles (*Staphylinidae*), minute pirate bugs (*Orius*), dipteral syrphidae, and spiders. Phytophagous groups were dominated by aphids, Cicadellidae, thrips, flea beetles, and *Diabrotica virgifera virgifera* was also present. The most frequent weed species were *Echinochloa crus-galli*,

Table 2 | The number of total individuals used for the quantitative food-web constructions

Trophic species	Treatm. 1	Treatm. 2	Treatm. 5	Treatm. 6	Treatm. 7	Treatm. 8	Treatm. 901	Treatm. 903	Total
Predatory guild									
Coccinellidae	703	498	635	698	659	703	944	946	**5,786**
Neuroptera larvae	852	896	909	853	833	852	941	985	**7,121**
Aeolothripidae	33	30	26	29	23	33	45	61	**280**
Staphylinidae	380	333	341	378	367	380	500	386	**3,065**
Orius spp.	1,317	1,259	1,360	1,354	1,149	1,317	1,454	1,150	**10,360**
Syrphidae larvae	237	158	239	259	247	237	246	288	**1,911**
Araneae	861	846	789	593	983	861	722	463	**6,118**
TOTAL	**4,383**	**4,019**	**4,297**	**4,164**	**4,262**	**4,383**	**4,852**	**4,279**	**34,639**
Phytophagous guild									
Aphididae	2,511	1,782	2,109	3,456	2,081	2,511	3,562	3,786	**21,798**
Cicadellidae	3,783	3,810	3,903	3,940	3,471	3,783	4,385	2,751	**29,826**
Thysanoptera	17,291	14,461	17,399	17,866	18,635	17,291	17,907	17,081	**137,931**
Alticinae	6,571	3,316	4,481	4,879	3,331	6,571	5,907	3,950	**39,006**
Diabrotica virgifera virgifera	1,771	1,603	2,019	2,097	1,591	1,771	2,352	1,930	**15,134**
Helicoverpa armigera	71	47	24	28	18	12	1	0	**201**
TOTAL	**31,926**	**24,972**	**29,912**	**32,237**	**29,109**	**31,926**	**34,113**	**29,498**	**243,896**

Cynodon dactylon, *Sorghum halapense*, *Convolvulus arvensis*, *Amaranthus retroflexus*, and *Rubus caesius*. (Figures 1 A–H). All these non-targett organisms were defined as test organisms in GM crops. Specialist insects that depend more on target pests may be the exception that Bt maize does not impact non-target organisms. This is particularly true for parasitoids, which may become less abundant along with their herbivorous hosts. The present study revealed however very low parasitisms rate in both GM and non-GM maize, therefore they importance needs to be further evaluated. Target insects as the western corn rootworm and the European corn borer were also present. Diabrotica adults were able to persist in Coleoptera-resistant maize (treatments 1, 2, 7 and 8) leaves where its intensive predation from the ladybirds beetles were detected. Very low density of European corn borer was detected in all plots; its abundance reached a maximum of 0.2% between herbivores.

The structure of interactions between trophic groups shows small if any differences between GM and non-GM maize. The complexity parameters together (S and L) indicate that neither Bt toxins, glyphosate tolerance or the extra glyphosate treatment had any effect on trophic groups relative to non-GM maize hybrids. The persistence of a food web increases with connectance and values between 0.2 and 0.5 suggest the existence of stable food webs[29,30]. Connectance in all GM and non-GM maize treatments was within this range of values with no difference between treatments (Table 3). No significant effects of bottom-up ($F = 0.034$, $p = 0.96$) and top-down ($F = 0.021$, $p = 0.98$) indexes were detected between treatments. Similar and relatively low top-down indexes from predators to phytophagous species and from phytophagous species to plants indicate strong interferences. Bottom-up indexes also reveal that a strong interference exists between trophic groups (Table 3).

Linear contrast tested between bottom-up and top-down effects of the two control maize and GM crops indicate a well fit with our initial prediction that similar food web exits in both non-GM and GM maize. A strong positive correlation between predators' top-down effects, herbivores top-down effects and plants bottom-upp effects in controls and GM maize were observed (Table 4). Similar trend were observed for herbivores bottom-up effects but only for treatments 7 and 8 (Coleoptera, Lepidoptera and glyphosate resistance and Coleoptera, Lepidoptera and glyphosate resistance + glyphosate treatment) (Table 4).

Discussion

Several conventional studies have been previously conducted in commercial and experimental fields to evaluate the impact of GM maize crops that contain transgen from *Bacillus thuringiensis* (Bt crops) on beneficial non-target organisms[12]. Field studies are useful tools to assess the effects of exposure on many species[23,24], but quantitative food web analyses can provide a much comprehensive information to understand the effect of Bt toxins to the structure of ecological communities. Concerns still exist that Coleopteran and Lepidopteran resistant GM maize may have cascading effects on the functioning of ecosystems and could disrupt community structure and ecosystem functioning[28]. Phytophagous and predator trophic groups in current study were previously considered as non-target organisms on which effects of Bt proteins may have possible direct (phytophagous species) or indirect (predators) harmful effects[9,10,12,20,31,32]. These studies revealed no significant effects on non target organisms via direct (phytophagous) or indirect (predator) pathway. Target pests as the western corn rootworm and the European corn borer were also present, nevertheless in low density. The extra glyphosate treatment reduced the overall weed density; broadleaf weeds were still able to persist in low mass because of existing seeds in soil. This reduced weed density was an important source for phytophagous groups that further explains the food-web complexity in these GM plots. Quantitative analyses of food web properties that includes all of the above species and more, revealed no negative effects of different of Bt toxins singly or in combination with glyphosate tolerance traits on arthropod food web structure, relative to non-GM maize. Evaluating effects of GM crops can be usefully examined using quantitative food web analyses as it helps to conduct field evaluations of effects of Bt toxins on target and non-target organisms as well as evaluate effects on the functional diversity and relations between trophic groups existing on different GM maize stands. However a long-term ramifications of the transgenic crops with multiple Bt genes and other traits are still to be examined in the coming decades and centuries, the present short-term analyses and method makes possible simple comparison between GM and non-GM maize stands in terms of potential shifts in vertical interactions of trophic groups. Both bottom-up and top-down values indicate a strong interference between trophic groups (Table 3). Other studies revealed that human modification of ecosystems can influence the degree of which bottom-up control is replaced by top-down control[33]. In cases when Bt crops or herbicide-resistant crops are compared with control different impact would be expected. To test this multiple regressions were performed between bottom-up and top-down values. Almost in all cases a strong positive relationship existed between control and GM K_{bu} and K_{td} variables. Exceptions were

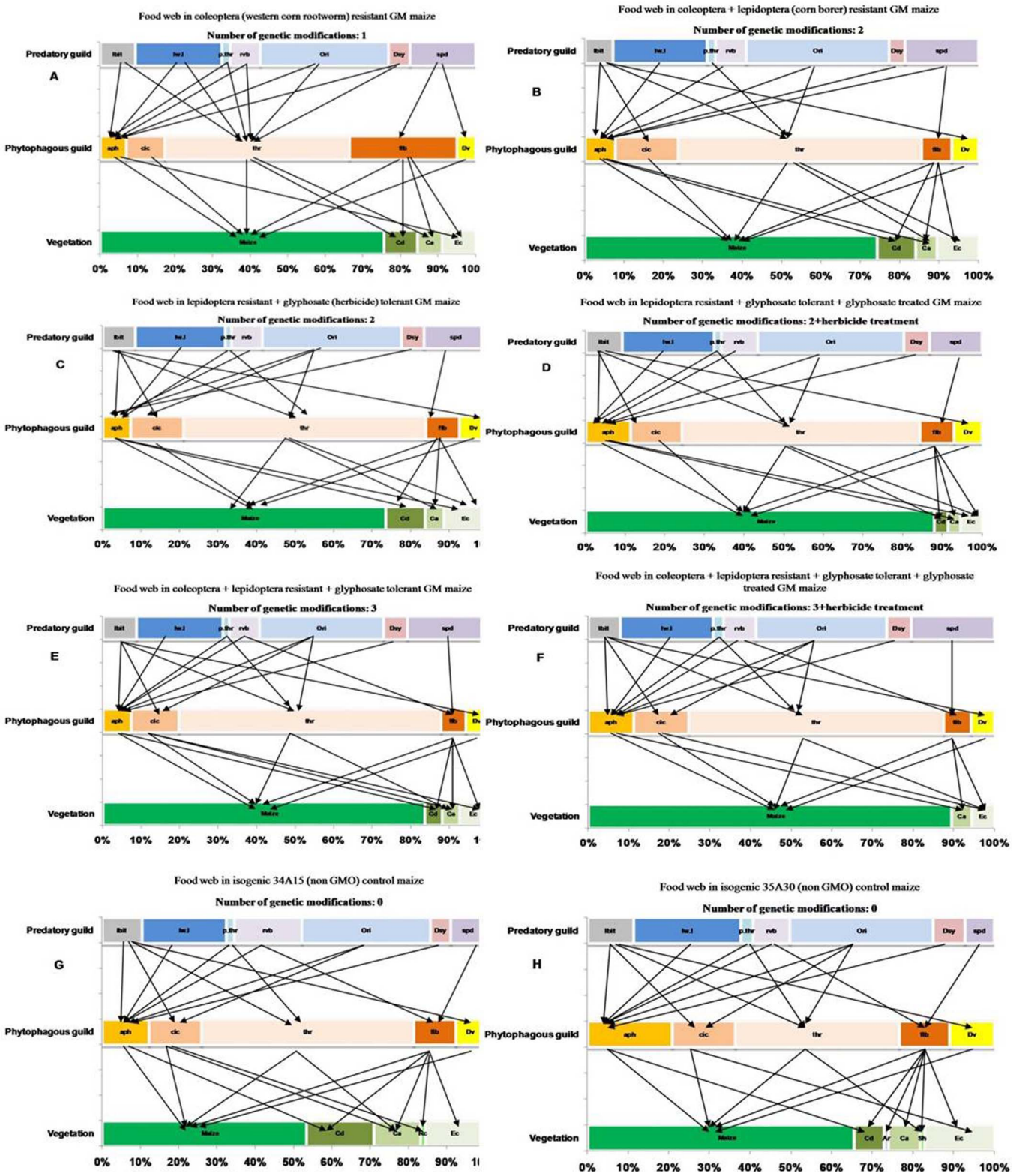

Figure 1 | (A–H) Food-webs of predatory and phytophagous guilds in different GM maize stands and their isogenic control. The size of each band represents the proportion of the trophic group. The food web for each GM and non-GM maize were made by considering the mean number of throphic groups/replicates/maize growing stage. Analyses were made on a subset of organisms that are the most abundant during the maize vegetation period (but before pollen spreading to exclude errors by cross-pollination), their cumulative abundance as trophic groups (herbivores, predators) for each GM and non-GM crops exceeded 95% and they were identified as test organisms in GM crops by EFSA. All food webs of GM and non-GM treatment presents the interactions in the same time periods.

observed when herbivores bottom-up effects were compared between controls and Coleopta resistant, Coleoptera and Lepidoptera resistant, Lepidoptera and glyphosate resistant and Lepidoptera and glyphosate resistanat + glyphosate treatment data (Table 4). This however can be explained by the lover K_{bu} indexes (0.57) of a single group, Cicadellidae in these treatments (K_{bu} = 1.07 in the other

Table 3 | Food web parameter on GM and isogenic maize stands (CoSBiLab). ANOVA was used to test the effect variation on top down and bottom-up effects. Comparisons were made by sampling events/treatments and their interactions for the following responses: weed coverage of four maize growth stages and abundance of phytophagous and predators

Food web metrics	Treatm. 1	Treatm. 2	Treatm. 5	Treatm. 6	Treatm. 7	Treatm. 8	Treatm. 901	Treatm. 903
Predators								
S	7	7	7	7	7	7	7	7
L	15	12	13	12	14	14	13	14
C	0.30	0.24	0.26	0.24	0.28	0.28	0.26	0.28
K_{td}	1.28(0.12)^{ns}	1.28(0.11)^{ns}	1.28(0.04)^{ns}	1.28(0.08)^{ns}	1.28(0.05)^{ns}	1.14(0.04)^{ns}	1.42(0.22)^{ns}	1.57(0.34)^{ns}
Phytophagous								
S	5	5	5	5	5	5	5	5
L	11	12	12	12	12	10	13	13
C	0.44	0.48	0.48	0.48	0.48	0.4	0.52	0.52
K_{bu}	1.39(0.36)^{ns}	1.40(0.36)^{ns}	1.39(0.36)^{ns}	1.40(0.37)^{ns}	1.39(0.21)^{ns}	1.39(0.21)^{ns}	1.40(0.31)^{ns}	1.39(0.41)^{ns}
K_{td}	0.80(0.19)^{ns}	0.79(0.19)^{ns}	0.79(0.13)^{ns}	0.79(0.19)^{ns}	0.79(0.08)^{ns}	0.59(0.09)^{ns}	0.99(0.12)^{ns}	1.20(0.11)^{ns}
Plants								
S	4	4	4	4	4	3	5	6
K_{bu}	1.71(0.05)^{ns}	1.71(0.04)^{ns}	1.71(0.03)^{ns}	1.71(0.03)^{ns}	1.71(0.03)^{ns}	1.71(0.03)^{ns}	1.71(0.08)^{ns}	1.71(0.38)^{ns}

S – nr. of trophic species, L – nr. of links between trophic groups, C – connectance, K_{bu} – arithmetic means of bottom-up indexes, K_{td} – arithmetic means of top-down indexes. ns – not significant. Standard Errors are given in brackets

Table 4 | Linear contrasts of bottom-up and top-down effects between the controls and GM maize crops. Bottom-up and top-down indexes were considered as separate variables for each trophic group

Resistance	Control 901 PR-34A15					Control 903 PR-35A30				
	Predators K_{td}									
	Coeff.	S.E.	t	p	R^2	Coeff.	S.E.	t	p	R^2
Col.	0.91	0.10	9.53	**0.00**	0.93	0.99	0.14	7.32	**0.00**	0.89
Col. and Lep.	0.93	0.09	10.64	**0.00**	0.94	1.02	0.13	7.85	**0.00**	0.91
Lep. and glyph.	0.84	0.03	31.46	**0.00**	0.99	0.93	0.05	18.57	**0.00**	0.98
Lep. and glyph.+ glyph. tr.	0.87	0.07	13.22	**0.00**	0.96	0.96	0.08	11.36	**0.00**	0.95
Col., Lep. and glyph.	0.79	0.04	22.69	**0.00**	0.98	0.87	0.06	14.05	**0.00**	0.97
Col., Lep. and glyph. + glyph. tr.	0.76	0.03	23.41	**0.00**	0.98	0.84	0.05	15.30	**0.00**	0.97
	Herbivores K_{bu}									
	Coeff.	S.E.	t	p	R^2	Coeff.	S.E.	t	p	R^2
Col.	0.58	0.31	1.86	0.14	0.46	0.63	0.41	1.54	0.20	0.37
Col. and Lep.	0.58	0.31	1.86	0.14	0.46	0.63	0.41	1.54	0.20	0.37
Lep. and glyph.	0.83	0.32	2.60	0.06	0.62	0.99	0.41	2.43	0.07	0.59
Lep. and glyph.+ glyph. tr.	0.85	0.33	2.60	0.06	0.62	1.01	0.42	2.42	0.07	0.59
Col., Lep. and glyph.	0.97	0.18	5.40	**0.01**	0.87	1.17	0.23	5.00	**0.01**	0.86
Col., Lep. and glyph. + glyph. tr.	0.97	0.18	5.40	**0.01**	0.87	1.17	0.23	5.00	**0.01**	0.86
	Herbivores K_{td}									
	Coeff.	S.E.	t	p	R^2	Coeff.	S.E.	t	p	R^2
Col.	1.02	0.22	4.64	**0.01**	0.84	1.33	0.16	8.11	**0.00**	0.94
Col. and Lep.	1.02	0.22	4.64	**0.01**	0.84	1.33	0.16	8.11	**0.00**	0.94
Lep. and glyph.	0.88	0.15	5.91	**0.00**	0.89	1.12	0.10	10.91	**0.00**	0.96
Lep. and glyph.+ glyph. tr.	1.02	0.22	4.64	**0.01**	0.84	1.33	0.16	8.11	**0.00**	0.94
Col., Lep. and glyph.	1.04	0.03	36.43	**0.00**	0.99	1.25	0.13	9.47	**0.00**	0.95
Col., Lep. and glyph. + glyph. tr.	0.89	0.08	10.49	**0.00**	0.96	1.09	0.09	12.67	**0.00**	0.97
	Plants K_{bu}									
	Coeff.	S.E.	t	p	R^2	Coeff.	S.E.	t	p	R^2
Col.	0.72	0.02	33.06	**0.00**	0.99	0.65	0.07	9.34	**0.00**	0.96
Col. and Lep.	0.73	0.02	47.45	**0.00**	0.99	0.67	0.07	10.14	**0.00**	0.97
Lep. and glyph.	0.73	0.01	68.17	**0.00**	0.99	0.67	0.05	12.82	**0.00**	0.98
Lep. and glyph.+ glyph. tr.	0.70	0.01	51.00	**0.00**	0.99	0.65	0.05	13.52	**0.00**	0.98
Col., Lep. and glyph.	0.68	0.01	104.85	**0.00**	0.99	0.63	0.05	12.34	**0.00**	0.98
Col., Lep. and glyph. + glyph. tr.	0.77	0.01	87.34	**0.00**	0.99	0.71	0.06	12.63	**0.00**	0.98

treatments). Lower bottom-up effects of Cicadellidae here indicates stronger interference and weak host dependence[33,35] but these cannot be attributed directly to genetic modifications. Factors such as competitions between herbivores, abiotic factors (possible higher temperature, humidity, etc) may also influence herbivores bottom-up effects. The connectance offers complementary insight into food web persistence within an area, because connectance values for a particular trophic species reflect its foraging behaviour. As long as the most connected species are unlikely to go extinct, stability increases with connectance[29,34], with connectance values between 0.2 and 0.3 are indicative of the most stable food web configurations across many terrestrial ecosystems[29,30]. Connectance values among our GM treatments and non-GM controls fell within this range (Table 3), suggesting the existence of stable food web on both GM and non-GM maize.

Conclusions

Analyses focused on those non-target organisms that are defined as test organisms in GM crops. All these organisms were in the same times the most abundant in both GM and non-GM maize. Target insects as the western corn rootworm and the European corn borer were also present in lower percentages. Diabrotica adults were able to persist in Coleoptera-resistant maize and very low density of European corn borer was detected, its abundance reached a maximum of 0.2%. However arthropod movements between plots cannot be excluded, our data assessment and the realistically parameterized food web analyses based on a total of 243,896 arthropod individuals can be considered as new and useful method to assess the deleterious effects of Bt toxins and glyphosate on non-target arthropods. Despite the presence of different maize genotypes and different Bt toxins, glyphosate tolerance and extra glyphosate treatment, a complex and stable food web exist in maize ecosystems.

Methods

Ethics statement. For arthropod collection in maize field, no permits were required. Ethics approval for arthropods used for survey was not required by any specific committee. All animal work was conducted according to relevant national and international guidelines.

Site characterization and sampling procedure. Thirty-two plots were established in Central Europe, near Budapest on chernozem soil in a randomized complete block design with each of the four GM varieties and 2 non-GM controls (Table 1) represented in each block. In addition, the two glyphosate tolerant varieties were replicated again in each block and subject to a glyphosate treatment in order to distinguish effects of GM genotype vs. effects of glyphosate chemical application on food web composition. Glyphosate in these treatments was applied in a total amount of 1060 g/ha in each year at the four and eight leaf stages of the maize plants.

Each maize treatment was planted in monoculture in 625 m^2 (25 m × 25 m) plots spaced 3 m apart within each block. Maize plants of similar maturity were planted in the retention zone surrounding the experimental fields to capture pollen surrounding the entire experimental field site to prevent pollen contamination of non-GM fields of the wider area around the experimental fields. Maize was planted in between late April and early May; and harvested between late October and early November.

For two years, arthropod and weed were sampled weekly from April until the end of harvest. Because many arthropods could have moved between plots, thereby reducing impacts of the different treatments, samples were taken only from a 10 × 10 m area within the centre of each 625 m^2 plot. In this way there was a distance of approximately 18–20 meter between traps of different blocks. Three standard methods were used to sample arthropods. Ground-dwelling arthropods were sampled using pitfall traps. Three traps were placed in each plot (a total of 96 traps) and were operated weekly from May to October. All individuals collected were counted and assigned to a trophic group (herbivores and predators). Plant canopy dwelling insects were collected weekly with 30 × 20 cm Pherocon (trade mark name) yellow sticky traps. Again, three/plot (a total of 96), were used to collect insects from plants. Traps were operated for seven days in each year from May to October. In each seven-day interval, traps were changed and arthropods were identified in laboratory. Surveys for other arthropods that are not sensitive to yellow colour were assessed by visual observations during each sampling period. Fifteen plants per plot were randomly selected weekly (480 plants per assessment) and all arthropods were counted and identified to trophic group. Weed plant species and their area coverage were estimated in all blocks by visual survey on 3 × 1 m^2 area of each plot as 0%, 10%, 20%, 30% … and 100% at four different growth stages of maize (eight leaves stage (V8), twelve leaves stage (V12), vegetative stage, tasseling (VT) and reproductive stage, milk (R3)).

Data analyses. Arthropods of similar prey preferences were assigned to the same trophic groups, where trophic group contains taxa that share the same set of predators and/or prey within a food web. This was done by direct observations of species interactions during the weekly plant surveys on the 480 plants (i.e. lady beetle predation on aphids and/or lady beetle predation on thrips etc.) and all interaction events noted. Designating species into trophic groups this way is a widely accepted convention in structural food-web studies because it reduces methodological biases related to uneven resolution of taxa within and among food webs[33].

Designation of trophic links in the food web followed methods presented by[25]. All observed interactions among the food web components were grouped according to the numbers of interactions (e.g., the number of times that lady beetle predation on aphids were observed) per sample period. To increase the sensibility of the methods two important factors were considered and data used for food web construction adjusted according to these factors:

i. Because maize pollen moves long distances, this may resulted in the non-Bt plots producing some or many kernels with Bt toxins (production of Bt toxins is a dominant trait), the quantitative food web associated with each GM and non-GM maize was constructed using information on the trophic groups until vegetative stage, tasseling (VT).
ii. Because presence and abundance of some or all of the organisms (insects and weeds) investigated varying importantly during the vegetation period. For example, corn rootworms adults are absent in April, May, June, present in July and August, and absent again in September, October and November. Therefore food-webs for each GM and non-GM crops were constructed from data when the abundance of the most frequent species are the highest, but before pollen spreading (end of June, mead July).

This resulted that the food webs were constructed by using 24,972 to 32,237 arthropod individuals per treatment. All food webs of GM and non-GM treatment presents the interactions in the same time periods.

Quantitative, weighted measures of bottom-up ($K_{bu.i}$), top-down ($K_{td.i}$) indexes and connectance (C) were computed for all trophic groups in each sampling period[33]. Bottom-up and top-down indices emphasize vertical interactions and are used for analyzing food webs in which top-down and/or bottom-up processes are of particular interest[33]. These indices emphasize vertical interactions and indicate a strong interference between trophic groups. Large top-down effects require weak interference, while large bottom-up effects require both weak interference and strong prey dependence[33,35]:

$$K_{bu.i} = \sum_{c=1}^{n} \frac{1}{d_c(1+K_{bc})} \text{ and } K_{td.i} = \sum_{e=1}^{m} \frac{1}{f_e(1+K_{te})}$$

For species "i", the first formula quantifies the bottom-up effect ($K_{bu,i}$) while the second formula quantifies the top-down effect ($K_{td,i}$), where n in the first formula is the number of predators preying species "i", d_c is the number of prey of its c^{th} predator and K_{bc} is the bottom-up keystone index of the c^{th} predator. Symmetrically, m in the second formula is the number of prey eaten by species "i", f_e is the number of predators of its e^{th} prey and K_{te} is the top-down keystone index of the eth prey[35].

A common Connectance (C) index was calculated which quantifies the linkage probability between any pair of trophic groups within food-web (L/S^2) where L is the number of links between trophic groups and S the number of trophic groups[33]. S and L can be derived by counting the number of trophic groups that interact and number of links (interactions) between them. If for example lady beetle predation on aphids were observed several times in a sampling period, this was considered as one interaction.

ANOVA was used to test the effect variation on top down and bottom-up effects[25,36]. Bottom-up and top-down indexes were considered as separate variables for each trophic group.

Linear contrasts were used to test bottom-up and top-down effects between the controls and GM crops. Because genetic modifications between maize plots varied and extra glyphosat treatments were used, impacts between bottom-up and top-down effects would be expected when Bt crops or herbicide-resistant crops are compared with control plots. Again bottom-up and top-down indexes were considered as separate variables for each trophic group. In this way the contrast analysis became a particular case of multiple regression analysis where R-square are determined as correlation coefficient between control and GM bottom-up and top-down values[37].

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Author contributions

Á.Sz., Z.P. and M.Z. performed the experiment, O.J.S. and A.B. wrote the main manuscript text and A.B. prepared figures and tables. All authors reviewed the manuscript and were agree with submission.

Additional information

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