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Effects of transgenic *cry1Ie* maize on non-lepidopteran pest abundance, diversity and community composition

Jingfei Guo · Kanglai He · Shuxiong Bai · Tiantao Zhang · Yunjun Liu · Fuxin Wang · Zhenying Wang

Abstract Non-lepidopteran pests are exposed to, and may be influenced by, Bt toxins when feeding on Bt maize that express insecticidal Cry proteins derived from *Bacillus thuringiensis* (Bt). In order to assess the potential effects of transgenic *cry1Ie* maize on non-lepidopteran pest species and ecological communities, a 2-year field study was conducted to compare the non-lepidopteran pest abundance, diversity and community composition between transgenic *cry1Ie* maize (Event IE09S034, Bt maize) and its near isoline (Zong 31, non-Bt maize) by whole plant inspections. Results showed that Bt maize had no effects on non-lepidopteran pest abundance and diversity (Shannon–Wiener diversity index, Simpson's diversity index, species richness, and Pielou's index). There was a significant effect of year and sampling time on those indices analyzed. Redundancy analysis indicated maize type, sampling time and year totally explained 20.43 % of the variance in the non-lepidopteran pest community composition, but no association was presented between maize type (Bt maize and non-Bt maize) and the variance. Nonmetric multidimensional scaling analysis showed that sampling time and year, rather than maize type had close relationship with the non-lepidopteran pest community composition. These results corroborated the hypothesis that, at least in the short-term, the transgenic *cry1Ie* maize had negligible effects on the non-lepidopteran pest abundance, diversity and community composition.

Keywords Transgenic *cry1Ie* maize · Non-lepidopteran pest · Abundance · Diversity · Community composition

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Introduction

The Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée), (Lepidoptera: Crambidae) is one of the most destructive insect pests of maize (*Zea mays* L.) in China, as well as in the Philippines, Korea, Malaysia, Indonesia, Thailand, Japan and several islands in the Pacific Ocean (Nafus and Schreiner 1991; Afidchao et al. 2013). Currently, various approaches are utilized for ACB control, including insecticide applications, agricultural control strategies, mating disruption techniques and *Trichogramma* releases, although each of these approaches have limitations (Guo et al. 2014;

Nafus and Schreiner 1991). Adoption of genetically modified (GM) maize that expresses *Bacillus thuringiensis* (Bt) Cry proteins can considerably reduce the population of ACB (Afidchao et al. 2013; He et al. 2003).

The cultivation of Bt crops has, in some cases, provided diverse economic and environmental benefits for the affected human population by reducing conventional insecticide applications, increasing crop yields, being less labor intensive and reducing ecological damage (Huang et al. 2005; Naranjo 2009; Wang et al. 2010). In addition, utilizing Bt crops is often compatible with other pest management methods (de la Poza et al. 2005). Given such positive economic and ecological returns, the total cultivation acreage of GM crops has increased an extraordinary 100-fold, since GM crops were first commercialized in 1996. This is especially true for Bt maize and Bt cotton (James 2014). However, there's still scientific uncertainty whether or not long-term and large-scale sustainability of Bt crops will disrupt non-target arthropod community structures (Romeis et al. 2008, 2011; Wolt et al. 2010). Before new Bt crops can be introduced commercially, it is essential to conduct environmental risk assessments (ERA) to determine the potential adverse effects on non-target species such as parasitoids, predators, decomposers, pollinators, and other non-target herbivores.

The non-target herbivore community is important to the maize ecosystem because the species involved can act as a food source for natural enemies and can potentially compete with the targeted insect pests for food and refuge. Non-target herbivores are exposed to Bt proteins directly by feeding on Bt-modified plant tissues (Groot and Dicke 2002), and mediate the potential effects of Bt crops to natural enemies through tritrophic interactions (Chen et al. 2009). It is essential, therefore, that prior to being approved for commercial cultivation, Bt crops, must be assessed to determine their potential impacts on non-target herbivores.

Currently, the vast majority of studies dealing with the impact of Bt crops on non-target herbivore community biodiversity have focused on limiting major non-target herbivores (Bernal et al. 2002; Burgio et al. 2007; Chen et al. 2007, 2012; Li et al. 2007; Liu et al. 2005; Rauschen et al. 2010; Wang et al. 2005). However, a study by Crawley (1999) suggests that field research assessing the impact of GM crops on the demography of non-target arthropods needs to encompass not only their entire life history but should also include several generations beyond the present. A focused analysis of a single non-target pest in laboratory studies may fail to uncover the potential lethal effects of Bt proteins on non-target herbivores when the effects are not immediately manifested (Szenasi et al. 2014). Because of this possibility, field assessments of the effects of new Bt crops on non-target herbivore insect species that occur in agricultural ecosystems are necessary prior to commercialization of new Bt crops. Although Guo et al. (2014) had reported that Bt maize expressing Cry1Ac protein had negligible effects on the density of non-target herbivores, new types of Bt maize, however, must be assessed for, possible unintended effects on non-target herbivores (Zurbrügg and Nentwig 2009).

Event IE09S034 transgenic maize produces the novel insecticidal protein Cry1Ie, which is targeted against ACB, soybean pod borers, *Leguminivora glycinivorella* (Matsumura) (Lepidoptera: Tortricidae), and cotton bollworms, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Song et al. 2003; Zhang et al. 2013). Cry1Ie protein was encoded by the *cry1Ie* gene, which was initially identified from *B. thuringiensis* isolate Btc007 by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (Song et al. 2003). A substantial body of literature has demonstrated that the Cry1Ie protein had no cross resistance with Cry1Ac, Cry1Ah, Cry1Ab, and Cry1F proteins (Han et al. 2009; He et al. 2013; Xu et al. 2013; Zhang et al. 2014, 2013), and could be integrated into the maize genome using other Bt genes such as the *cry1Ah* gene (Yang et al. 2012).

Cry1 class proteins are lepidopteran-active proteins (Duan et al. 2010), so, to distinguish the non-lepidopteran, non-target herbivores found on transgenic *cry1Ie* maize from the targeted lepidopteran species, were collectively referred to these as "non-lepidopteran pests". We surveyed the number of species (species richness) and the relative abundances of individuals of each species (species abundance) in Bt and non-Bt maize plots in 2012 and 2013. We then compared the non-lepidopteran pest abundance and diversity found in the Bt plots with the non-Bt maize plots. We were also interested in analyzing the non-target lepidopteran pest community composition and their responses to maize type (Bt and non-Bt maize), sampling time and year.

Materials and methods

Experimental design

The field trials were performed at the Langfang Experiment Station at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS), Hebei province (39°30′N, 116°36E) in 2012 and 2013. The seeds of Bt maize (Event IE09S034), and its corresponding non-transformed near isoline (Zong 31) used in this experiment were provided by the Institute of Crop Sciences, CAAS. Seeds were planted on 8 May 2012 and 29 May 2013. Bt and non-Bt maize were planted in a randomized block design involving three replications (Fig. 1). Each plot consisted of 25 rows spaced 60 cm apart, and plant spacing of 35 cm (length × width: 15 m × 15 m). Isolation belts between plots were established using 3 m wide bare aisles. Insecticides were not applied during the study. Other field management procedures were conducted according to local agricultural practices.

Sample collection

Species diversity and abundance of all non-lepidopteran pests occurring throughout the entire plant were recorded periodically at the 3rd (V3), 6th (V6), 9th (V9) 12th (V12) leaf stages, tasseling (VT), silking (R1), blister (R2), milk stage (R3), dent (R5) and physiological maturity (R6) in 2012 and 2013. In each plot, five points distributed at the two corner-to-corner diagonals (X shaped) (Fig. 1) were chosen and twenty plants in each point were randomly selected (100 plants in total per plot). The species and numbers of visible non-lepidopteran pests on leaves, stalks, tassels, sheaths, husks and ears were quickly counted. Easily recognized species were identified and recorded, while unknown species were stored in 5-ml plastic vials containing 75 % ethanol for later identification.

Statistical analysis

Measures of diversity were calculated using five diversity indices: (1) Shannon–Weaver diversity index (H'), $H' = -\sum_{i=1}^{s} P_i \ln(P_i)$, where P_i is the percentage of individuals belonging to the ith taxon of the total number of individuals surveyed (Shannon and Weaver 1949); (2) Simpson's diversity index (D), $D = 1 - \sum_{i=1}^{s} \frac{N_i(N_i-1)}{N(N-1)}$, where N_i is the number of individuals in the ith taxon; and N is the total number of individuals (Simpson 1949); (3) Number of species (S); (4) Pielou's evenness index (J), $J = H'/\ln S$, where S is the total number of species (Pielou 1966); (5) The total number of individuals (N). H', D and J were calculated using diversity function of the Vegan package in R (Oksanen et al. 2015). Abundance was measured by computing the total number of individuals captured per plot during each sampling time (Slade and Blair 2000).

A 3-way unequally spaced repeated-measures ANOVA ('proc mixed' procedure in SAS) was conducted to investigate the effects of maize type (Bt and non-Bt) on the diversity and abundance of non-lepidopteran pests. Maize type (Bt and non-Bt maize), year and sampling time were fixed factors, and the block was a random factor (Piepho et al. 2004). Because significant interaction of maize type × year × sampling time were observed for number of species (S), their interaction was further analyzed with Tukey HSD post hoc test.

We then used redundancy analysis (RDA) to explore the relationships between non-lepidopteran pest community composition and maize type (Bt and non-Bt maize), year (2012 and 2013) and sampling time (V3, V6, V9, V12, VT, R1, R2, R3, R5 and R6 stage). RDA is a constrained ordination tool used to map species occurrences along environmental/land-use gradients (Houlahan et al. 2006; Palmer 1993; Rao 1964; Ter Braak 1986). This method focused on inter-sample distances with rare species downweighted (Nielsen et al. 2010). In this analysis, maize type, sampling time and year were used as the environmental variables, and block as the covariable. Monte Carlo permutation tests (999 permutations) were performed to test the significance of the canonical axes of RDA, and the critical probability level for detecting significance was set at α = 0.01. The significance of the maize type, year and sampling time was defined by returning the environmental variables squared correlation coefficient, r^2, on all axes conjointly and by a permutations test (envfit function from the Vegan package in R with 999 Monte Carlo permutations) (Oksanen et al. 2015).

Non-lepidopteran pest assemblages among different samples were analyzed using nonmetric

Fig. 1 Distribution of plots for Bt and non-Bt maize in the fields in 2012 and 2013
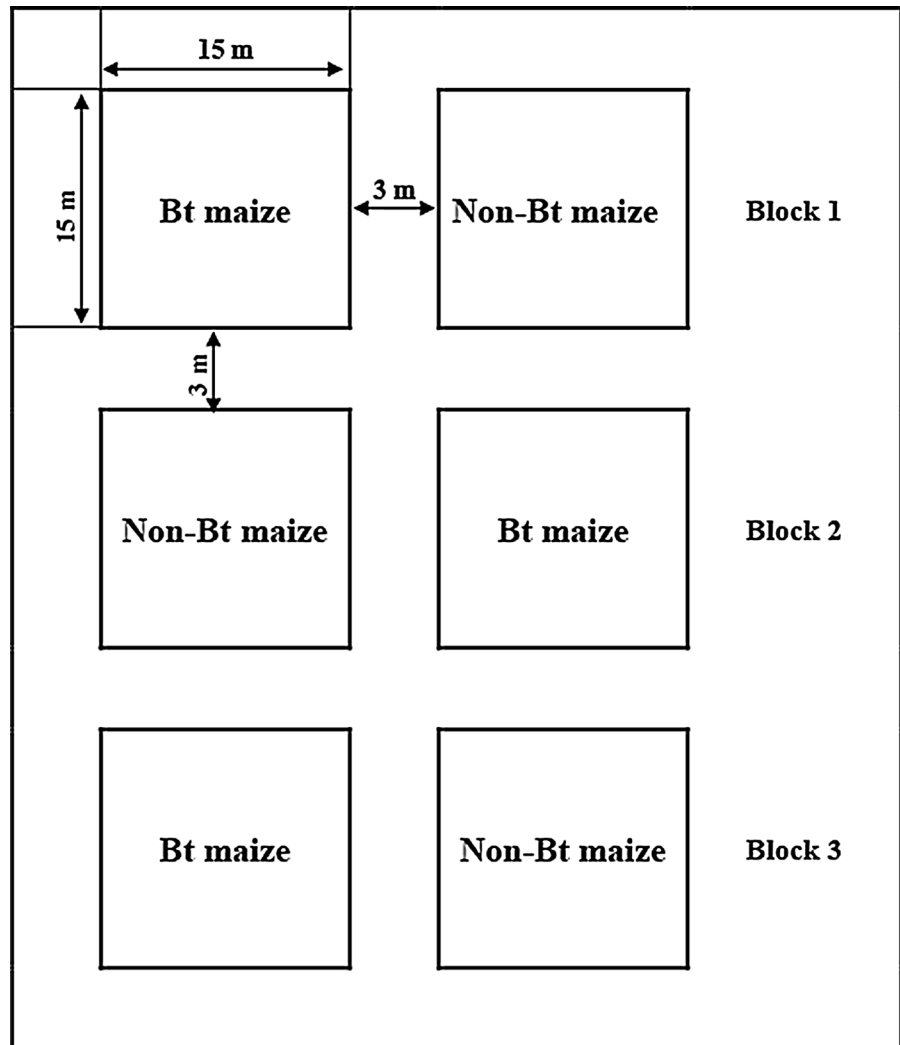
multidimensional scaling (nMDS) (Clarke 1993; Digby and Kempton 1987; Kruskal 1964; Kruskal and Whish 1978) with the packages Vegan and Mass of R version 3.0.3 (Oksanen et al. 2015). NMDS is a commonly used multivariate technique in arthropod community composition analysis (Wimp et al. 2005), which gives a comprehensive view of abundance, richness and family identity (Shepard 1962). Distances between communities per sample were computed by using the Bray–Curtis dissimilarity index (Bray and Curtis 1957). Here, non-lepidopteran pests surveyed in our study were distributed into 120 samples (10 sampling time × 2 maize types × 2 year × 3 replications). The result of goodness of fit (isoMDS function, Vegan package in R) and Shepard diagram (stress plot function, Vegan Package in R) of

non-metric fit between the ordination and the original data of non-lepidopteran pests illustrated that the ordination reflected the dissimilarity between samples well (Foulquier et al. 2013; Fig. 4). We also analyzed which factors (maize type, sampling time and year) were mainly responsible for the nMDS ordination of the non-lepidopteran pest community by envfit function of the Vegan package in R (Oksanen et al. 2015).

All statistical calculations were performed in SAS v. 9.2 statistics package (SAS Institute 2009) and R software environment (v. 3.2.3; R Development Core Team 2015). Non-lepidopteran pest abundance data were $\log(x + 1)$ prior to analysis to downweight the influence of dominant and rare species on the ordination (Luo et al. 2013; Meyer et al. 2012).

Results

Non-lepidopteran pest abundance and diversity in Bt and non-Bt maize

In 2012, there were 25,452 non-lepidopteran pests (22 species/families) documented in Bt maize plots and 23,105 (25 species/families) documented in non-Bt maize plots. In 2013, 14,815 individuals (25 species/families) and 18,258 (25 species/families) were documented in Bt and non-Bt maize plots, respectively—fewer than the amounts recorded in 2012 (Table S1). During both years, the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), heavily dominated the non-lepidopteran pest communities in the Bt and non-Bt maize plots. In 2012, *R. maidis* represented more than half of the total non-lepidopteran pest community found in the Bt and non-Bt maize plots (57.12 % for Bt maize and 56.09 % for non-Bt maize). Other relatively abundant species found in 2012 were *Frankliniella tenuicornis* (Uzel) (14.96 % for Bt maize and 14.64 % for non-Bt maize), *R. padi* L. (13.04 % for Bt maize and 8.51 % for non-Bt maize), *Tetranychus cinnabarinus* (Boisduval) (6.02 % for Bt maize and 7.87 % for non-Bt maize), *Sitobion avenae* (F.) (3.24 % for Bt and 4.99 % for non-Bt maize), *Haplothrips aculeatus* (F.) (1.69 % for Bt and 2.32 % for non-Bt maize), and *Laodelphax striatellus* (Fallen) (1.59 % for Bt and 1.98 % for non-Bt maize) (Table S1; Fig. 2). In 2013, *R. maidis* was even more dominant, accounting for 91.83 % of the non-lepidopteran pest community composition in Bt maize plots and 93.73 % in non-Bt maize plots, followed by *Erthesina fullo* (Thunberg) and *L. striatellu*. The percentages of all other species were below 1 % (Table S1; Fig. 2).

Impacts of maize type, year and sampling time on the non-lepidopteran pest abundance and diversity

Unequally spaced repeated-measures ANOVA results showed that maize type had no significant effect on the Shannon–Wiener diversity index (H'), Simpson's diversity index (D), number of species (S), Pielou's evenness index (J) and the total number of individuals (N) (Table 1). In contrast, the year, the sampling time and their interaction all had highly significant impacts on all of the abundance and diversity indices

(Table 1). No interaction of year—maize type, and maize type—sampling time was detected in any of the diversity and abundance indices (Table 1). The significant interaction of year × maize type × sampling time was only observed in number of species (S) (Table 1, Table S2). There were no significant differences between number of species (S) in Bt and non-Bt maize plots for all the sampling time in the 2 years except for R1 stage in 2013 (t = −2.55, $P = 0.013$) (Table S2).

Effects of maize type, year and sampling time on non-lepidopteran pest community composition

The effects of maize type, year and sampling time on the non-lepidopteran pest community compositions were examined using redundancy analysis. Maize type, year and sampling time totally explained 20.43 % of the total variability in the community compositions as explanatory variables ($P = 0.001$, 999 Monte Carlo permutation test) (Table S3). Axis 1 and 2 explained 10.88 and 8.94 % of variations in community composition, respectively ($P = 0.001$ for axis 1 and 2, 999 Monte Carlo permutation test) (Fig. 3; Table S3). Figure 3 also clearly illustrated that axis 1 was correlated with year, and axis 2 was correlated with sampling time.

RDA also showed which factors (the maize types, sampling times and year) were most important to the non-lepidopteran pest community composition. Both sampling time ($r^2 = 0.68$, $P = 0.001$, 999 Monte Carlo permutations) and year ($r^2 = 0.82$, $P = 0.001$, 999 Monte Carlo permutations) had a significant correlation with the non-lepidopteran pests community composition, except maize type ($r^2 = 0.00$, $P = 0.996$, 999 Monte Carlo permutations) (Table S4).

The similarity of non-lepidopteran pest assemblages between Bt and non-Bt maize plots

Temporal variability within the overall non-lepidopteran pest community structure of Bt and non-Bt maize with 3 replications in 20 sampling times during the 2 years samples was further explored by nonmetric multidimensional scaling (nMDS) (Fig. 4). The distance between two points was estimated using the pairwise Bray–Curtis similarity index between sampling events (Wells et al. 2011). Differences in the
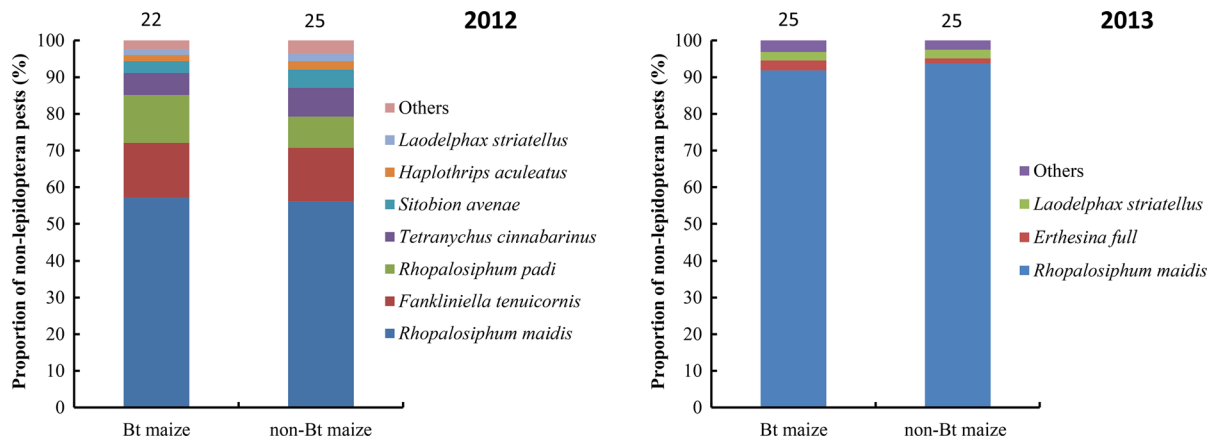
Fig. 2 Proportional representation of non-lepidopteran pests found in Bt and non-Bt maize plots in a total of 2 years (2012, 2013). The Y axis shows the percentage of taxa in all collected non-lepidopteran pests and the X axis shows the maize type. This diagram illustrates dominant non-lepidopteran pests (proportion >1 %) and the total proportion of taxa with percentages below 1 % (others). *Numbers* above the *columns* show the total numbers of taxa collected in Bt and non-Bt maize in each year

Table 1 Multi-factor effects of year (2012 and 2013), maize type (Bt maize and non-Bt maize) and sampling time on non-lepidopteran pest diversity and abundance

Diversity and abundance parameters	Shannon–Weaver's index (H')		Simpson's diversity index (D)		Number of species (S)		Pielou's evenness index (J)		Abundance (N)	
	F	P	F	P	F	P	F	P	F	P
Year	40.47	**0.000*****	43.82	**0.000*****	33.03	**0.000*****	34.36	**0.000*****	14.25	**0.005****
Maize type	0.20	0.669	0.14	0.716	0.17	0.690	0.08	0.7842	0.06	0.806
Sampling time	4.93	**<0.0001*****	4.51	**0.000*****	3.52	**0.010****	9.41	**<0.0001*****	18.62	**<0.0001*****
Year × maize type	0.01	0.941	0.14	0.716	0.26	0.627	0.03	0.870	1.94	0.201
Year × sampling time	4.58	**<0.0001*****	2.69	**0.010****	5.28	**<0.0001*****	3.66	**0.001*****	15.19	**<0.0001*****
Maize × sampling time	1.17	0.326	1.21	0.303	0.92	0.517	1.57	0.140	0.97	0.470
Year × maize type × sampling time	1.63	0.124	1.14	0.348	2.14	**0.037***	1.81	0.080	1.61	0.130
Mean ± SD (Bt maize)	1.898 ± 0.039		0.816 ± 0.006		9.000 ± 0.351		0.897 ± 0.003		6711 ± 253	
Mean ± SD (non-Bt maize)	1.522 ± 0.026		0.697 ± 0.001		7.000 ± 0.130		0.827 ± 0.005		6885 ± 209	

All data was analyzed using a 3-way unequally spaced repeated-measure ANOVA. The values highlighted in bold are statistically significant (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

non-lepidopteran pest community composition between all samples were visualized in an nMDS plot (Fig. 4), where an intuitive separation of samples attributed to sampling time and year was evidenced. In contrast, a clear separation of the samples according to maize type was not seen in nMDS plots (Fig. 4). This was substantiated by a more detailed analysis of similarity (ANOSIM). Significant correlations between year, sampling time and community composition were found, however, no correlation was detected between maize type and community composition (Table 2).

Fig. 3 Redundancy analysis (RDA) for non-lepidopteran pest community composition and their relationship with maize type, sampling time and year
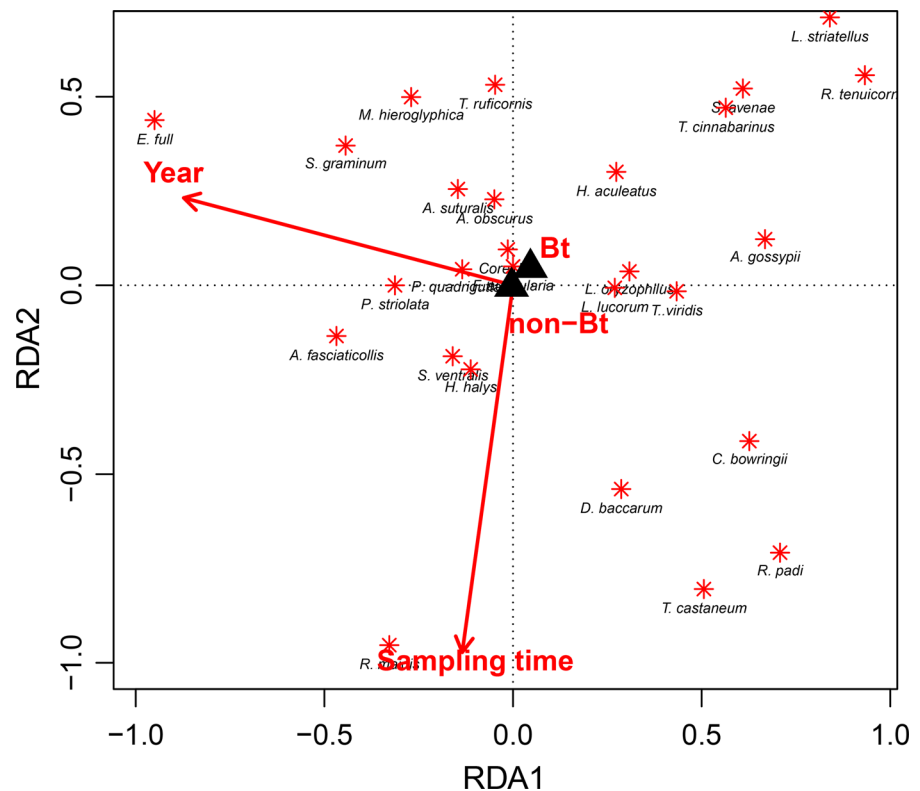
Discussion

Cry insecticidal proteins are produced in Bt crops throughout the entire growing season, non-target herbivores, therefore, have direct access to Bt proteins while feeding on Bt crops (Groot and Dicke 2002). As the area under cultivation with Bt crops has steadily increased worldwide, environmental risk assessments (ERA) of Bt crops on non-target arthropods have attracted increasing attention from a number of researchers (Devos et al. 2012; Guo et al. 2014; Habuštová et al. 2014; Romeis et al. 2008). Laboratory and field studies have evaluated the effects of Bt maize on non-target arthropods. For example, lab experiments have demonstrated that Cry1Ab maize had no negative effects on the intrinsic rate of *R. padi* and the spider mite, *Tetranychus urticae* (Dutton et al. 2002), as well as the population parameters of *R. maidis* (Li et al. 2007). Likewise, Cry3Bb1 maize did not harm the life history parameters of *T. urticae* (Li and Romeis 2010) and Cry1F maize did not negatively affected egg production and hatching rate of the corn leafhopper *Dalbulus maidis* (Virla et al. 2010). Field

surveys showed that Bt-maize had no significant impact on the field densities of the plant bug *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera, Miridae) (Rauschen et al. 2009) and the leafhopper *Zyginidia scutellaris* (Herrich-Schäffer) (Hemiptera: Cicadellidae) (Rauschen et al. 2010), as well as on the abundance and diversity of non-target arthropods (Guo et al. 2014; Habuštová et al. 2014; Svobodová et al. 2015). The abundance and species of non-target herbivores may not only reflect the potential effects that Bt crops have on non-target arthropods, but may also indirectly affect the abundance of parasitoids or predators through disruption of food webs (Balog et al. 2010; Lundgren et al. 2009; Svobodová et al. 2016). Therefore, evaluating the effects of Bt crops on non-target herbivore biodiversity should be an important step in environmental safety assessment of Bt crops. This will also aid in identifying species which are most likely to be at risk and selecting appropriate species for laboratory bioassay, higher-tier studies, and post-market environmental monitoring (Romeis et al. 2013, 2014). In addition, comparing the differences between Bt and non-Bt maize on non-target herbivore
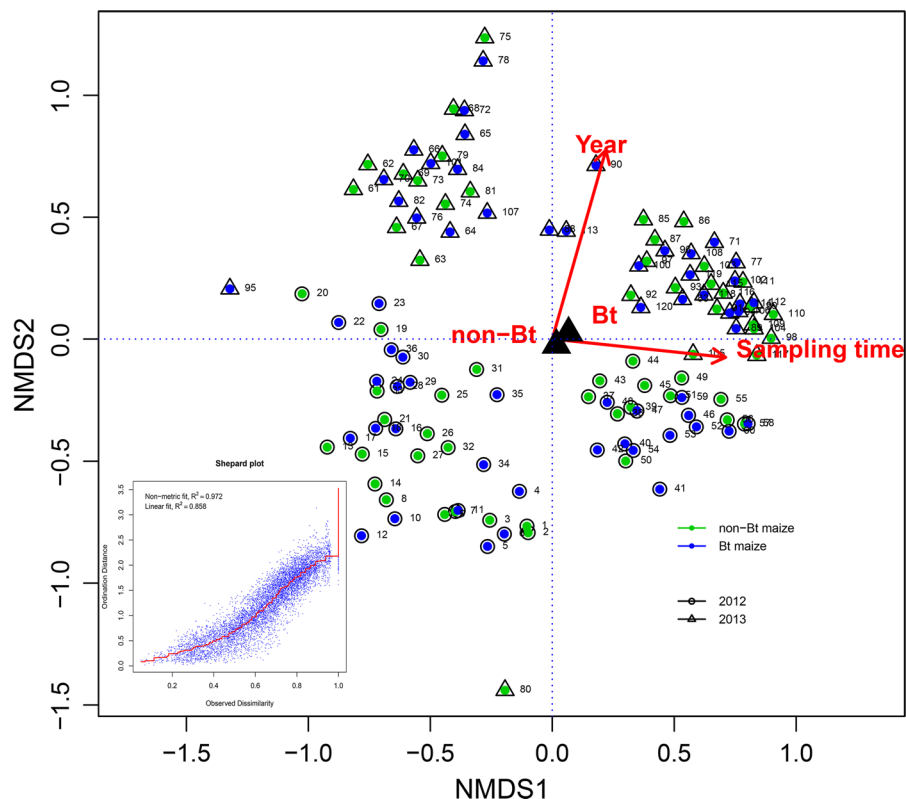
Fig. 4 Non-metric multidimensional scaling (nMDS) plot of non-lepidopteran pest community structure from Bt and non-Bt maize in each sampling time in 2012 and 2013. *Green* indicates Bt maize, and *red* indicates non-Bt maize. *Circles* with associated *numbers* from 1 to 60 indicate sampling points analyzed in temporal order in 2012 (1–3: non-Bt maize at V3 stage, 4–6: Bt maize at V3 stage, 7–9: non-Bt maize at V6 stage, 10–12: Bt maize at V6 stage, 13–15: non-Bt maize at V9 stage in 2012, 16–18: Bt maize at V9 stage, 19–21: non-Bt maize at V12 stage, 22–24: Bt maize at V12 stage, 25–27: non-Bt maize at VT stage, 28–30: Bt maize at VT stage, 31–33: non-Bt maize at R1 stage, 34–36: Bt maize at R1 stage, 37–39: non-Bt maize at R2 stage, 40–42: Bt maize at R2 stage, 43–45: non-Bt maize at R3 stage, 46–48: Bt maize at R3 stage, 49–51: non-Bt maize at R5 stage, 52–54: Bt maize at R5 stage, 55–57: non-Bt maize at R6 stage, 58–60: Bt maize at R6 stage). *Triangles* with associated *numbers* from 61 to 120 indicate sampling points analyzed in temporal order in 2013 (61–63: non-Bt maize at V3 stage, 64–66: Bt maize at V3 stage, 67–69: non-Bt maize at V6 stage, 70–72: Bt maize at V6 stage, 73–75: non-Bt maize at V9 stage in 2012, 76–78: Bt maize at V9 stage, 79–81: non-Bt maize at V12 stage, 82–84: Bt maize at V12 stage, 85–87: non-Bt maize at VT stage, 88–90: Bt maize at VT stage, 91–93: non-Bt maize at R1 stage, 94–96: Bt maize at R1 stage, 97–99: non-Bt maize at R2 stage, 100–102: Bt maize at R2 stage, 103–105: non-Bt maize at R3 stage, 106–108: Bt maize at R3 stage, 109–111: non-Bt maize at R5 stage, 112–114: Bt maize at R5 stage, 115–117: non-Bt maize at R6 stage, 118–120: Bt maize at R6 stage). Smaller diagram represents Shepard's stress plot. (Color figure online)

abundance and diversity may offer insight into the reasons why some species of secondary insect pests benefit from planting Bt crops. These include: (1) reducing insecticide application (Lu et al. 2010), (2) crops being more susceptible to non-target herbivores (Faria et al. 2007), (3) reducing competition from target pests (Pons et al. 2005; Zeilinger et al. 2015).

Methods for analyzing the effects of environmental variables on species and ecological communities are generally focused on the distributions of individual species, and differences in the community

composition. Diversity indices (e.g., Shannon–Weiner's, Simpson's, Pielou's diversity index), and relative abundance of the species are commonly used as the simplest expression of measuring the disturbance of community structure in agricultural areas (Clergue et al. 2005). The drawback to using these indices, however, is that they are often weak in identifying complex diversity patterns because they pool multi-species information into a single value for each sample (Ramette 2007). Multivariate analyses (e.g., redundancy analysis, RDA; nonmetric multidimensional

Table 2 Effects of maize type (Bt maize and non-Bt maize), sampling time and year on non-lepidopteran pest community structure (nMDS structure) in 2012 and 2013

Correlation with nMDS structure	R^2	P
Maize type	0.00	0.904
Sampling time	0.51	**0.001*****
Year	0.65	**0.001*****

The values highlighted in bold are statistically significant (*** $P < 0.001$)

scaling, nMDS; canonical correspondence analysis, CCA) of community diversity patterns do explain community composition very well and are also better tools for analyzing the response of community composition to environmental variables (Ramette 2007). Therefore, diversity indices and multivariate analyses are often combined together to assess the effects of Bt crops on non-target arthropods in the field, which help to obtain a more comprehensive view of species abundance, diversity and community composition of arthropods in the field. An increasing number of studies have adopted multivariate analyses, such as PRC, CCA and RDA to compare the non-target arthropod communities of Bt crops with those of non-Bt crops (Guo et al. 2014; Habuštová et al. 2014; Li and Liu 2013; Lu et al. 2014; Svobodová et al. 2013, 2016). All of these studies have demonstrated that non-target arthropod community in Bt crops are similar to those found in corresponding non-Bt crops, which has been further substantiated by our multivariate analyses results.

In the current study, Shannon–Weiner's, Simpson's, Pielou's index, and species richness and abundance values were calculated to compare the differences of non-lepidopteran pest diversity and abundance between Bt and non-Bt maize plots. Results showed that maize type (Bt and non-Bt maize) had no significant effects on any of the indices analyzed (Table 1). In addition, the abundance of major insect pests were similar in the Bt and non-Bt maize plots, which was consistent with earlier research demonstrating that the densities of non-target herbivores were not affected significantly by Bt maize expressing Cry1Ac proteins (Guo et al. 2014). There was, however, a significant year and sampling time effect on those indices (Table 1). Significant year and sampling time effects indicated that environmental factors (e.g., weather, rainfall) had a greater impact on

the non-lepidopteran pest diversity and abundance than the maize type itself (Bt or non-Bt). The significant effects of sampling year or time on arthropod communities and biodiversity has been observed in a number of other studies regarding the effects of Bt crops on non-target arthropods (Guo et al. 2014; Habuštová et al. 2014; Priestley and Brownbridge 2009; Svobodová et al. 2013).

RDA analysis embodied an accurate relation of maize type, sampling time, year and other undefined factors with variations in non-lepidopteran pest community composition. Maize type, sampling time and year totally explained 20.43 % of the variance in the non-lepidopteran pest community, while no association was also found between maize type and the variance. Therefore, most of the variance must have been attributable to other factors, including: (1) plot size and isolation between plots (Prasifka et al. 2005); (2) complex interactions among natural enemies, target pests and non-target herbivores.

The occurrence of changes in community structure in non-target arthropods was often the basis used by scientists to evaluate the potential effects of Bt maize on non-target arthropods (Li et al. 2008; Pons et al. 2005; Zhang et al. 2011). However, the quantitative measures of compositional dissimilarities between sites, i.e., the Bray–Curtis dissimilarity (Faith et al. 1987) of non-target arthropods communities between Bt and non-Bt crop plots were neglected (Guo et al. 2014). When analyzing the abundance and diversity indices of Bt versus non-Bt crops, the rarely occurring species may influence the results (Faith et al. 1987). While the Bray–Curtis dissimilarity index may analyze the community compositions between Bt and non-Bt crops more effectively (Faith et al. 1987). In our study, nMDS, an ordination method, was initially used to measure the non-lepidopteran pest community dissimilarities between Bt and non-Bt maize from various sampling times during the 2 years. NMDS intuitively demonstrated the similarity of non-lepidopteran pest communities between the Bt and non-Bt maize plots by showing the Bray–Curtis distance between samples (Fig. 4). The multivariate permutation test of effects of maize type, sampling time and year on Bray–Curtis distance illustrated that sampling time and year had close relationships with the Bray–Curtis distances, but maize type did not. These results were in keeping with the previous conclusion mentioned above where environmental factors were found

to greatly influence the non-lepidopteran pest community composition, whereas the Bt maize itself did not.

In summary, we found that Bt maize itself neither negatively nor positively affected the non-lepidopteran pest community biodiversity. This is contradictory to those previous studies that indicated the wide-scale adoption of Bt crops would lead to outbreaks of secondary pests (Faria et al. 2007; Lu et al. 2010). However, secondary pest outbreaks in Bt crop cultivation areas were driven by the reduction of broad-spectrum insecticide applications (Wu et al. 2002). For example, the population increases of mirid bugs in Bt cotton fields in China have been shown by over 10 years of field investigation to be directly related to reductions in insecticide use in this crop (Lu et al. 2010). In addition, our results also provide evidence indicating that sampling time and year to year variations can affect the abundance, diversity and community composition of non-lepidopteran pests in Bt and non-Bt maize plots. It is also necessary to mention that since our investigation was conducted during the same season in the 2 years, some cumulative effects of the Bt crop on non-lepidopteran species may have been overlooked (Bourguet et al. 2002). It is, therefore, imperative that the impacts of Bt maize on non-lepidopteran pest community characteristics need to be documented over an extended period of time on a broad range of Asian environments. Meanwhile, the complex interactions among Bt maize, target pests, non-target herbivores and herbivore enemies of Bt maize require additional study.

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