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The specific responses of Acari community to *Bt* cotton cultivation in agricultural soils in northern China

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ABSTRACT

Transgenic cotton expressing the *Bacillus thuringiensis* (*Bt*) insecticidal crystal (Cry1A) protein effectively controls the cotton bollworm and thus has been planted extensively in China. However, the large-scale release of *Bt* cotton may have undesirable effects on soil fauna due to *Bt* protein accumulation and the pleiotropic effects of genetic manipulation. A survey of soil mites was carried out monthly for two consecutive years (2009–2010) in *Bt* and non-*Bt* cotton fields. The soil mites were extracted using modified Tullgren funnels and were identified to the genus level, when possible. The results suggested that the effects of *Bt* cotton on soil mite community size were time dependent and taxonomic group specific. The cumulated abundance over a year was always higher in non-*Bt* fields for Oribatida; this effect was statistically significant in 2010 for Prostigmata and Astigmata. The changes in the community variables tested were similar between *Bt* and non-*Bt* cotton fields in 2009, whereas the taxonomic group richness, Shannon–Weaver index and evenness index were significantly different between *Bt* and non-*Bt* cotton fields in 2010. Additionally, sharp inter-annual fluctuations in the community composition of the soil mites were found, accompanied with the replacement of some taxonomic groups. Finally, the dominances of some taxonomic groups were significantly different between *Bt* and non-*Bt* cotton fields. *Bt* cotton cultivation fostered Laelapidae populations while inhibited *Tectocepheus* abundance in 2009. However, *Bt* cotton cultivation negatively impacted the abundances of *Scheloribates* and *Nothrus* in 2010. In conclusion, *Bt* cotton cultivation exerted specific impacts on soil mites.

1. Introduction

Transgenic cotton that has been genetically modified (GM) to express a gene derived from the bacterium *Bacillus thuringiensis* (*Bt*) effectively suppresses the cotton bollworm, *Helicoverpa armigera* (Hübner) and thus has been planted extensively throughout China. As *Bt* cotton is released in a large scale, the quantity of conventional pesticides applied and related expenditures have been reduced significantly (Huang et al., 2003; Wang et al., 2009). However, the introduction of *Bt* cotton has triggered public concerns about its potential ecological and environmental risks.

Soil organisms play an irreplaceable role in the decomposition of organic matter, and thus it is the soil fauna community that is most worthy of being monitored in ecological risk assessment of *Bt* plants. Soil invertebrates come into direct contact with the transgenic Cry endotoxin released from the root exudates, pollen and decomposing tissues of *Bt* crops during crop growth (Motavalli et al., 2004; Rui et al., 2005). Soil mites (Acari) are one of the most diverse and abundant components of the soil arthropod, and they regulate many key functional processes (Lavelle, 1996; Andrén and Balandreau, 1999; Fitter et al., 2005; Palacios-Vargas et al., 2007). Furthermore, prior studies have demonstrated that soil mites are sensitive to various physical and chemical perturbations. For example, soil mite community density, richness and structure are influenced by climate, vegetation type, soil porosity and pH, water content and altitude (Wauthy, 1981; Sinclair and Stevens, 2006; Bokhorst et al., 2008; Ducarme et al., 2004; Noti et al., 2003; Illig et al., 2010). To date, the soil mite community has been considered a useful bio-indicator for assessing ecosystem conditions (Parisi et al., 2005). In addition, it is customary to quantify the ecological stress or other environmental alterations in the soil ecosystem through some sensitive/warning indicators when performing risk assessment of transgenic plant cultivation.

Although most available studies support the view that *Bt* cotton has no hazardous effects on non-target organisms (NTO), the impacts of transgenic plants may change with gene transformation events or spatial and temporal environmental variables such as biotic activity, soil type, crop management practices, and other

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environmental conditions. Thus, the effects of transgenic plants may vary between sites and seasons. Furthermore, the rapid development of agricultural biotechnology and release of new GM plants (species and cultivars) in recent years has made the ecological risk assessment of GM plants even more important and urgent.

This study aimed to address the ecological safety of planting *Bt* cotton on the abundance, diversity and composition of soil mite community in small-scale cotton fields in northern China. We hypothesized that: (1) *Bt* cotton would exert no negative effect on the soil mite community as measured by abundance, species richness and composition due to the specificity of *Bt* proteins; (2) considering the temporal-spatial interaction between soil mites and plant, greater inter-annual discrepancies in community variables between *Bt* and non-*Bt* cotton fields were expected.

2. Materials and methods

2.1. Site description and crop management

This study was conducted in the experimental station fields (39.538° N, 116.708° E) of the Chinese Academy of Agricultural Sciences, located in Anci County, Langfang city, Hebei province, China, from 2009 to 2010. The soil chemical properties of the fields were as follows: organic carbon, 10.07 g/kg; organic matter, 17.4 g/kg; total N, 0.44 g/kg; available N, 434.00 mg/kg; available P, 29.54 mg/kg; available K, 323 mg/kg; pH ($CaCl_2$), 8.13. This experiment was conducted in a large field with 90 m in length and 40 m wide. A randomized block design with four replications was used. The field was evenly divided into 4 blocks, and there were two plots per block, one plot was assigned for *Bt* cotton and another for non-*Bt* cotton. Each plot was 20 m in length and 15 m wide, the plots were divided by 2 m wide path. Plots were cultivated using standardized agricultural management practices. All plots were planted during the first or second week of May each year and received preventative in-furrow treatments but no foliar insecticide applications during the season.

The *Bt* cotton (cv. GK-12), expressing Cry1Ac and Cry1Ab fusion proteins, provides very good control of *H. armigera* and a number of other Lepidopteran insects. The parental line of the *Bt* cotton (cv. SiMian-3), which is susceptible to lepidopterous pests, was used as the non-*Bt* cotton. Each year, all plots were cultivated once during the growing season, and the plant stalks were tilled into the soil using a disk coulter immediately following the harvest of the cotton lint and seeds.

2.2. Sample collection and mite species identification

Soil samples were collected from a depth of 0 cm to 5 cm from July 13 to November 4 in 2009 and from June 29 to November 15 in 2010. During this period, 9 soil samples per plot were taken from 3 sites distributed as an "S" shape in fields of each cotton genotype each month. The soil cores (height: 52 mm, diameter: 70 mm) taken within 5 cm from the plant roots at each sampling site were combined to produce a mixed sample. The five sampling times included five cotton growth stages: seeding (from emergence to bud), budding (from bud to flower), flower and boll-1 (early flower stage to boll), flower and boll-2 (later flower stage to boll) and boll-opening (after boll opened). We also collected soil samples on May 15, 2010 to test whether the postharvest materials produced in 2009 had exerted an adverse impact on soil mites.

Soil mites were extracted using the modified Tullgren extraction method described by Crossley and Blair (1991). This method relies on a constant light source (40 W, 220 V lights) fitted inside beverage cans, which are placed above one end of the sample. These are fitted into baffles and suspended over collection funnels. A temperature gradient develops between the top and bottom of the sample, and the mites move down through the soil sample in response to the changes in the heat and humidity gradients (Merchant and Crossley, 1970). The extraction lasted 48 hours (no animals were captured after 2 days in our preliminary samples). The extracted organisms were preserved in 75% ethanol for subsequent identification. All specimens were identified according to the keys published by Krantz (1978).

2.3. Statistical analysis

The Shannon–Weaver Index (H') is commonly used to assess diversity, but as it may be dominated by abundant taxa or the overall number of taxa, the Pielou evenness index (J), Simpson index (D), and Margalef richness index (SR) are often calculated as well. The detailed calculation methods for these general biological indices are as follows: Diversity $H' = \sum_{i=1}^{s} P_i \ln P_i$, Simpson index: $D = \sum_{i=1}^{s} (P_i)^2$, Margalef richness index $SR = (S-1)/\ln N$, Evenness $J = H'/\ln S$. Where a given taxon is regarded as the ith taxon, P_i denotes the proportion of individuals in the ith taxon, S is the total number of taxa identified, and N is the number of individuals identified.

Prior to analysis, the abundance data from sampling sites within each replicated plot were standardized to the mean abundance of 3 mixed samples from each cotton plot per month. Thus, each treatment had four replicates (i.e., replicated plots) for each sampling event. Yearly accumulative abundances for *Bt* and non-*Bt* fields were generated from the sum of the monthly average numbers of sample events per year (5 months in 2009 and 6 months in 2010). Because the questions of interest were related to overall changes in the mite community size in *Bt* cotton relative to non-*Bt* cotton fields, taxon abundances were pooled to higher identified taxonomic groups (i.e., Prostigmata, Mesostigmata, Astigmata, and Oribatida). Simultaneously, this avoided the violation of ANOVA assumptions by ensuring that species occurring only at very low densities were considered. The Kolmogorov–Smirnov test was applied to assess the normality of the distribution of the datasets. Where normality was not satisfied, the data were transformed in $\log(x+1)$ for analysis, but untransformed averages are presented. The data were submitted to univariate repeated measures ANOVA, with cotton genotype and sampling date as fixed factors and replicated plots as random factors, to test the effects of cotton genotype, sampling date, and the interaction of cotton genotype and sampling date on the abundance and diversity indices of soil mites. These analyses were carried out using the Proc ANOVA function of SAS (SAS Institute, 1999–2001), with adaptation of the PROFILE statement as suggested by Cody and Smith (1997). In addition, monthly abundances, yearly accumulative abundances and dominance for the abundant taxa of *Bt* and non-*Bt* fields were compared with non-parameter Mann–Whitney U Test.

3. Results

3.1. The impact of Bt cotton cultivation on the seasonal dynamics and accumulative abundance of soil mites

When the total soil mite community was considered, the overall soil mite abundance varied widely among the sampling dates in 2009. However, no significant difference was observed between cotton genotypes, and there was no significant cotton genotype by sampling date interaction effect (Table 1; Fig. 1A). For separate groups, the abundances for all groups fluctuated dramatically across sampling dates (Table 1; Fig. 1C, E, G and I). The abundances of Mesostigmata between *Bt* and non-*Bt* cotton fields were significantly different in 2009 (Table 1), whereas the density of

Table 1
F-and *P*-statistics of repeated measures ANOVA of the effects of cotton genotype (*Bacillus thuringiensis* (*Bt*) vs. non-*Bt*), sampling date and their interaction on the abundance of total soil mites and mite taxonomic groups in a cotton field in northern China (statistically significant differences are shown in bold type).

Year	Factor	Df	Oribatida		Prostigmata		Mesostigmata		Astigmata		All	
			F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
2009	Genotype	1, 3	6.15	0.089	1.09	0.373	9.59	0.053	0.37	0. 585	0.57	0.504
	Date	4, 24	39.48	<0.001	36.27	<0.001	6.32	0.001	9.00	<0.001	31.23	<0.001
	Genotype* Date	4, 24	2.05	0.119	2.30	0.088	3.20	0.031	0.20	0.937	2.06	0.118
2010	Genotype	1, 3	6.49	0.084	1.23	0.348	19.44	0.022	4.54	0.123	8.11	0.065
	Date	5, 30	2.18	0.083	6.15	<0.001	9.10	<0.001	9.05	<0.001	3.60	0.012
	Genotype*Date	5, 30	2.18	0.083	0.40	0.847	2.16	0.086	0.94	0.469	2.31	0.069

Oribatida, Prostigmata and Astigmata varied but did so equally between *Bt* and non-*Bt* cotton fields (Table 1). There was a significant interaction effect of cotton genotype by sampling date for Mesostigmata in 2009 (Table 1). Great discrepancies in soil mite abundance across sampling dates were also found in 2010 for all groups, Oribatida, Prostigmata and Astigmata (Table 1). The abundances of Mesostigmata between *Bt* and non-*Bt* cotton fields were significantly different (Table 1), whereas the density of Oribatida,

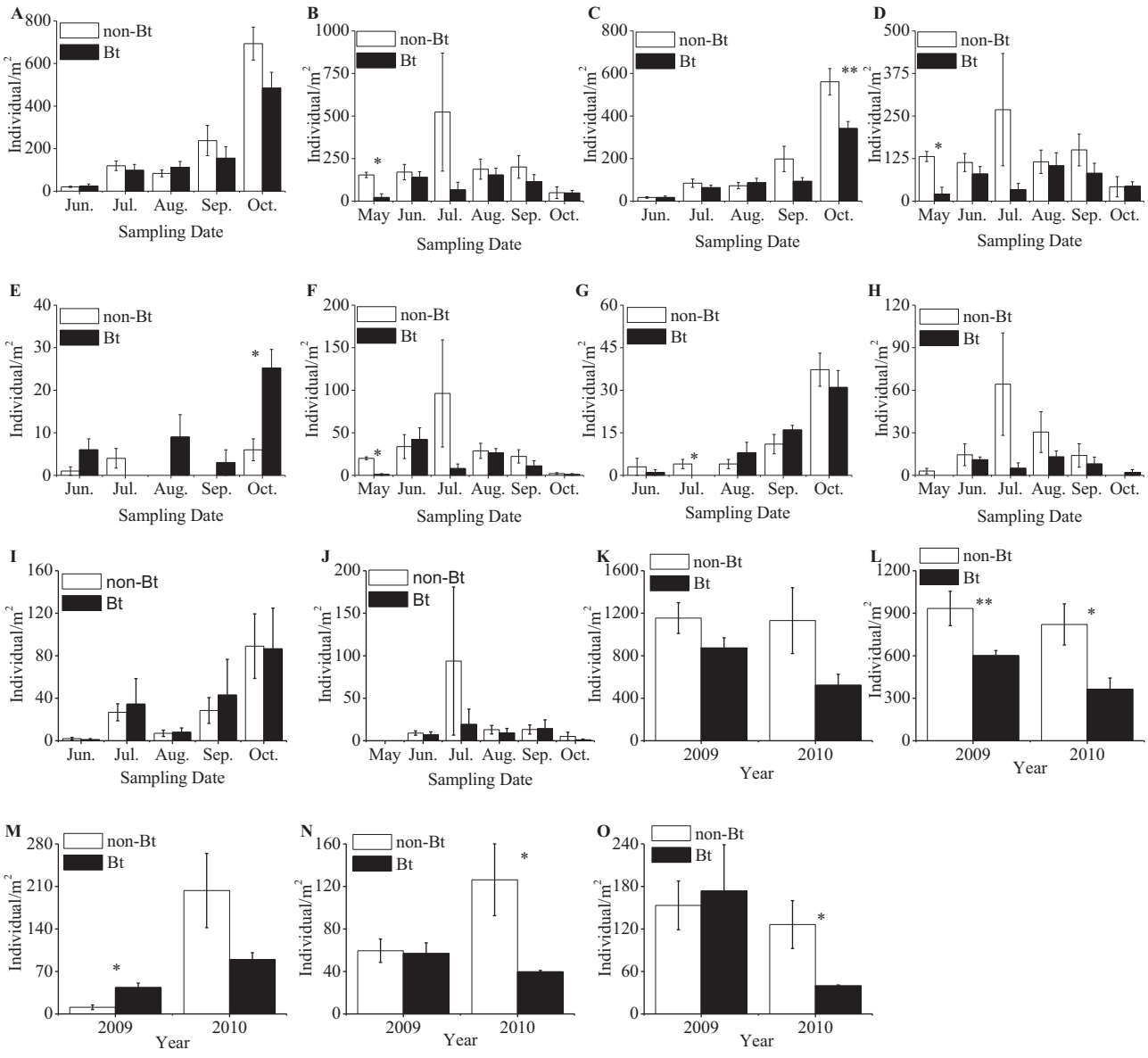

Fig. 1. Monthly and yearly abundances (Ind./m^2) of soil mite taxonomic group to *Bacillus thuringiensis* (*Bt*) cotton cultivation. Panels A–J represent monthly soil mite abundance of each taxonomic group in *Bt* and non-*Bt* cotton fields in 2009 (A, All; C, Oribatita; E, Mesostigmata; G, Prostigmata; I, Astigmata) and in 2010 (B, All; D, Oribatita; F, Mesostigmata; H, Prostigmata; J, Astigmata), and panels K-O represent yearly accumulative abundances of soil mites in *Bt* and non-*Bt* cotton fields during 2009–2010 (K, All; L, Oribatita; M, Mesostigmata; N, Prostigmata; O, Astigmata). All stands for all groups together. Means are averages of four replicate plots for each cotton genotype, and error bars represent ±1 SE. Differences between *Bt* and non-*Bt* cotton fields for each group are tested separately by Mann–Whitney U test, and statistical significances at $\alpha = 0.05$ between *Bt* and non-*Bt* cotton are denoted as follows: *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$.

Table 2
F-and *P*-statistics of repeated measures ANOVA of the effects of cotton genotype (*Bacillus thuringiensis* (*Bt*) vs. non-*Bt*) and date on the diversity indices of soil mite communities in a cotton field in northern China (*S* = Total number of taxa; *SR* = Margalef's richness; *D* = Simpson dominance index, *H′* = Shannon–Weaver diversity index, *J* = Shannon evenness index; significant *P*-values are indicated in bold type).

Year	Factor	DF	S		*SR*		*H′*		*D*		*J*	
			F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
2009	Genotype	1, 3	0.02	0.896	0.02	0.895	0.51	0.526	0.65	0.478	0.02	0.887
	Date	4, 24	57.24	<0.001	21.58	<0.001	34.20	<0.001	5.59	0.003	21.30	<0.001
	Genotype*Date	4, 24	1.54	0.222	1.74	0.174	4.46	0.008	2.05	0.119	1.86	0.150
2010	Genotype	1, 3	14.84	0.031	5.76	0.096	11.20	0.044	0.53	0.518	11.94	0.041
	Date	5, 30	4.25	0.005	2.33	0.071	8.16	<0.001	1.79	0.145	6.96	<0.001
	Genotype*Date	5, 30	2.58	0.047	6.62	<0.001	7.71	<0.001	0.47	0.796	5.30	0.001

Prostigmata and Astigmata varied but did so equally between *Bt* and non-*Bt* cotton fields (Table 1).

The results of each sampling date showed that the effects of *Bt* cotton on soil mites were time dependent and taxon specific. In 2009, there was a significant inhibitory effect of *Bt* cultivation on Prostigmata in July (Fig. 1G). Meanwhile, there were contrasting effects of *Bt* cotton cultivation on Oribatida and Mesostigmata in October. The abundance of Oribatida was significantly higher in *Bt* cotton fields than in non-*Bt* cotton fields (Fig. 1C), whereas the abundance of Mesostigmata in *Bt* cotton fields was lower than that in non-*Bt* fields (Fig. 1E). In May 2010, the abundances of all soil mites (Fig. 1B), Oribatida (Fig. 1D) and Mesostigmata (Fig. 1F) were significantly lower in *Bt* cotton fields than in non-*Bt* cotton fields. However, there was no significant effect of cotton genotype on the soil mite community size on any other sampling dates.

With respect to the yearly accumulative abundance of soil mites, the effect of *Bt* cotton cultivation varied with taxonomic group and year. Oribatida was less abundant in *Bt* cotton fields than in non-*Bt* fields in 2009, and a similar phenomenon was observed in 2010 (Fig. 1L). Mesostigmata was significantly more abundant in *Bt* cotton fields than in non-*Bt* cotton fields in 2009, whereas its abundance was not significantly different between field types in 2010 (Fig. 1M). Neither Prostigmata nor Astigmata differed in abundance in fields with different cotton genotypes in 2009, whereas *Bt* cotton cultivation suppressed the accumulation of these groups in 2010 (Fig. 1N and 1O).

3.2. The impact of Bt cotton on soil mite community diversity indices

In 2009, all community variables fluctuated significantly across sampling dates but did so equally between *Bt* and non-*Bt* cotton fields (Table 2). The genotype by sampling date interactions did not have significant effects on most of the diversity variables (Table 2), while exerted a significant effect on Shannon–Weaver index (*H′*). Further analysis suggested that only the Shannon–Weaver index (*H′*) between *Bt* and non-*Bt* cotton fields was significantly different in July.

In 2010, all diversity indices varied greatly across sampling times for the *Bt* as well as non-*Bt* cotton (Table 2). Simultaneously, there were statistically significant differences in the taxonomic group richness (*S*), Shannon–Weaver index (*H′*) and evenness index (*J*) between *Bt* and non-*Bt* cotton fields (Table 2). In addition, the interaction effects between genotype and sampling date on all the diversity indices, except for the Simpson dominance index (*D*), were observed. This suggested that these variables differed between *Bt* and non-*Bt* cotton, but the interaction strength changed with sampling date, and no uniform pattern was found. Finally, analyses based on monthly investigations showed differences in the taxonomic group richness (*S*), Shannon–Weaver index (*H′*) and evenness index (*J*) between *Bt* and non-*Bt* cotton fields occurring in May.

3.3. The impact of Bt cotton on soil mites community composition

In 2009, a total of 1103 individuals were collected from cotton fields during our study period (from June to October), 42% from *Bt* cotton fields and 58% from non-*Bt* cotton fields. Thirty-two groups of soil mites were identified in both the *Bt* and non-*Bt* cotton fields. The eu-dominant taxonomic groups (dominance above 10%) in the *Bt* cotton fields were *Epilohmannia*, *Zygoribatula*, nymph Oribatida and Acaridae, whereas the eu-dominant taxonomic groups in non-*Bt* cotton fields were *Epilohmannia* and *Zygoribatula* (Table 3). However, the abundances of these groups were not statistically significant between *Bt* and non-*Bt* fields (Mann–Whitney U Test, $P > 0.05$). *Bt* cotton cultivation had different effects on Laelapidae and *Tectocepheus* (Mann–Whitney U Test, $P < 0.05$). Specifically, *Bt* cotton cultivation increased the abundance of Laelapidae and suppressed the abundance of *Tectocepheus*.

In 2010, a total of 1791 individuals were collected from cotton fields during our study period (from May to October), 32% from *Bt* cotton fields and 68% from non-*Bt* cotton fields. Thirty-four and thirty-one groups of soil mites were identified in the *Bt* and non-*Bt* cotton fields, respectively. The eu-dominant taxonomic group in the *Bt* cotton fields was *Arcoppia*, whereas the eu-dominant taxonomic groups in the non-*Bt* cotton fields included *Arcoppia* and *Rhodacarus* (Table 3). However, the differences in their abundances between *Bt* and non-*Bt* fields were not statistically significant (Mann–Whitney U Test, $P > 0.05$). In addition, the abundances of *Scheloribates* and *Nothrus* were significantly reduced in the *Bt* cotton fields compared with the non-*Bt* cotton fields (Mann–Whitney-U Test, $P < 0.05$).

In general, the community composition of soil mites is time dependent, accompanied with the replacement of some taxonomic groups throughout the growing season (Table 3). However, these changes were limited to some sub-dominant or rare groups, the abundances of which were below 10% of the total community composition.

4. Discussion

The impact of a commercialized transgenic cotton line compared to its non-transgenic iso-line on the community parameters of soil mites was determined in fields that received no insecticide treatments. We hypothesized that *Bt* cotton cultivation would exert no undesirable effect on soil mites, whereas the effect would vary with planting time. In the current study, the effects of *Bt* cotton on soil mites were time dependent and taxonomic group specific. This finding supported some evidences for our null hypothesis although it is contrasted with the findings of most studies, which suggest that *Bt* crops have no adverse effects on non-target soil organisms (e.g., Donegan et al., 1995; Saxena and Stotzky, 2001; Rui et al., 2005; Sarkar et al., 2009).

Furthermore, this phenomenon is in disagreement with the results of the limited studies addressing the potential impact of

Table 3
Mean accumulative abundance of soil mites (Mean ± SE, $n = 4$) in *Bacillus thuringiensis* (*Bt*) and non-*Bt* commercial cotton plots in northern China during 2009 and 2010 (Mann–Whitney U Test, statistically significant differences between the *Bt* and non-*Bt* cotton plots are shown in bold type). Abundances are averages of four replicate plots sampled.

Order	Family	Taxa	2009		2010	
			non-*Bt* (Ind./m^2)	*Bt* (Ind./m^2)	non-*Bt* (Ind./m^2)	*Bt* (Ind./m^2)
Prostigmata	Stigmaedae		29.3 ± 6.9	16.0 ± 6.3	0	0
Prostigmata	Erythraeidae		0	2.0 ± 2.0	5.0 ± 3.0	4.0 ± 2.3
Prostigmata	Cryptognathidae		4.0 ± 1.6	0	0	0
Prostigmata	Eupodidae		4.0 ± 2.3	8.0 ± 2.8	4.0 ± 1.6	1.0 ± 1.0
Prostigmata	Raphignathidae		3.0 ± 1.0	6.0 ± 2.0	0	2.0 ± 1.2
Prostigmata	Microdispidae	*Allopygmephorus*	9.0 ± 6.6	12.3 ± 8.7	91.0 ± 45.0	31.8 ± 8.4
Prostigmata	Cunaxidae		1.0 ± 1.0	8.3 ± 3.5	1.0 ± 1.0	0
Prostigmata	Bdellidae		0	0	2.0 ± 2.0	1.0 ± 1.0
Prostigmata	Scutacaridae		1.0 ± 1.0	0	10.3 ± 4.9	4.0 ± 2.3
Prostigmata	Trombidiidae		2.0 ± 2.0	0	0	0
Prostigmata	Tarsonmidae		4.0 ± 2.3	7.0 ± 4.7	1.0 ± 1.0	0
Prostigmata		Larvae	2.0 ± 2.0	0	11.0 ± 6.2	10.0 ± 3.5
Astigmata	Anoetidae		6.0 ± 2.6	8.3 ± 4.6	107.3 ± 73.3	47.8 ± 18.4
Astigmata	Acaridae		80.0 ± 24.9	85.8 ± 69.6	27.3 ± 18.4	9.0 ± 4.4
Astigmata	Acaridae	Hypopodes	68.3 ± 22.3	76.5 ± 29.0	0	0
Mesostigmata	Pachylaelapidae		0	1.0 ± 1.0	0	0
Mesostigmata	Uropodidae		0	0	54.3 ± 41.2	11.3 ± 7.8
Mesostigmata	Parholaspididae	*Parholaspis*	0	2.0 ± 2.0	0	0
Mesostigmata	Parasitidae		0	2.0 ± 1.2	1.0 ± 1.0	1.0 ± 1.0
Mesostigmata	Rhodacaridae	*Rhodacarellus*	2.0 ± 2.0	6.0 ± 2.0	7.0 ± 3.4	4.0 ± 1.6
Mesostigmata	Laelapidae		2.0 ± 2.0	14.0 ± 2.0	16.3 ± 8.4	2.0 ± 1.2
Mesostigmata	Ascidae		6.0 ± 2.6	5.0 ± 5.0	27.5 ± 8.1	11.0 ± 2.5
Mesostigmata	Phytoseiidae		0	2.0 ± 2.0	0	0
Mesostigmata	Rhodacaridae	*Rhodacarellus*	3.0 ± 1.9	8.0 ± 3.7	95.3 ± 20.1	68.5 ± 11.7
Mesostigmata		Nymphs	0	1.0 ± 1.0	0	0
Oribatida	Scheloribatidae	*Scheloribates*	0	0	47.0 ± 8.7	7.0 ± 2.5
Oribatida	Suctobelbidae	*Flagrosuctobelba*	2.0 ± 2.0	4.0 ± 1.6	3.0 ± 1.9	0
Oribatida	Suctobelbidae	*Suctobelbella*	0	0	2.0 ± 2.0	1.0 ± 1.0
Oribatida	Oppiidae	*Arcoppia*	77.5 ± 16.7	50.5 ± 17.7	270.8 ± 97.9	138.0 ± 47.4
Oribatida	Epilohmanniidae	*Epilohmannia*	132.5 ± 22.3	148.8 ± 15.1	41.3 ± 9.9	34.5 ± 9.0
Oribatida	Tectocepheidae	*Tectocepheus*	70.5 ± 8.7	25.3 ± 8.1	40.8 ± 6.7	27.8 ± 4.5
Oribatida	Oribatulidae	*Zygoribatula*	318.0 ± 129.7	102.5 ± 8.5	66.3 ± 9.5	28.5 ± 15.5
Oribatida	Ceratozetes	*Ceratozetes*	67.3 ± 15.5	37.8 ± 15.3	54.3 ± 11.0	24.5 ± 7.4
Oribatida	Nothridae	*Nothrus*	21.5 ± 6.0	30.5 ± 14.8	48.0 ± 26.6	4.0 ± 1.6
Oribatida	Euphthiracaridae	*Rhysotritia*	41.8 ± 12.3	33.5 ± 6.0	17.3 ± 6.8	9.3 ± 6.0
Oribatida	Lohmanniidae	*Vepracarus*	35.5 ± 21.5	0	49.0 ± 25.1	10.0 ± 3.5
Oribatida	Xylobatidae	*Xylobates*	4.0 ± 2.8	7.0 ± 3.4	55.0 ± 25.9	50.3 ± 8.3
Oribatida	Lohmanniidae	*Papillacarus*	26.8 ± 20.4	2.0 ± 1.2	3.0 ± 3.0	0
Oribatida	Oppiidae	*Graptoppia*	2.0 ± 1.2	0	0	0
Oribatida	Oppiidae	*Oppia*	0	0	14.3 ± 9.1	6.0 ± 3.5
Oribatida	Hypochthoniidae	*Hypochthonius*	0	0	0	1.0 ± 1.0
Oribatida	Otocepheidae	*Fissicepheus*	0	0	1.0 ± 1.0	0
Oribatida	Galumnidae	*Galumna*	7.3 ± 7.3	2.0 ± 2.0	6.0 ± 3.8	3.0 ± 1.9
Oribatida		Nymphs	59.5 ± 12.6	88.5 ± 13.0	81.0 ± 30.7	43.8 ± 7.4
Oribatida		Larvae	17.3 ± 8.1	11.0 ± 1.0	28.5 ± 19.3	3.0 ± 1.0

Bt cotton on soil mites to date. Yu et al. (1997) tested the toxicological effects of the Cry1Ab and Cry1Ac toxins expressed by *Bt* cotton leaves on the population growth rates of *Oppia nitens* Koch in the laboratory, and their findings suggested that the total production of *O. nitens* adults and nymphs feeding on transgenic cotton leaves was unaffected by these toxins. Furthermore, Oliveira et al. (2007) demonstrated that the intensive ingestion of the *B. thuringiensis* toxins expressed by the Bollgard and Dipel strains did not affect the survival or development of *Scheloribates praeincisus* in the laboratory. In addition, some adverse affects of *B. thuringiensis* products on Astigmata, Mesostigmata and Prostigmata have been reported (Saleh et al., 1991; Payne et al., 1993, 1994; van der Geest et al., 2000). Here, the adverse effect of *Bt* cotton on soil mites was taxon specific and was limited to certain dates.

As the expression and release of *Bt* toxins from GM plants are the foremost and original changes compared to their parental lines, the root expression levels of modified genes, root exudation, soil persistence of transgenic proteins and unintentional changes in the chemical compositions of the transgenic plants are the main factors determining the interaction strength of *Bt* crops and soil fauna. Previous studies demonstrated no adverse effects of *Bt* crops on non-target soil organisms due to the specificity of the *Bt* proteins (Hönemann et al., 2008). Simultaneously, other reports suggested that most of the *Bt* toxins remained active in the soil for weeks or several months before degradation or complete adsorption by the soil particles (Palm et al., 1996; Sims and Ream, 1997; Saxena et al., 1999; Stotzky, 2000; Saxena et al., 2002; Zwahlen et al., 2003), and the persistence of *Bt* toxin depended on the interactions among many variables, such as biotic activity, soil type, crop management practices, and environmental conditions. One study demonstrated that the *B. thuringiensis* toxins from root exudates, leaves or other parts of *Bt* plants incorporated into the soil are progressively degraded by decomposition (Head et al., 2002). Taking these factors into consideration, it is reasonable to speculate that the adsorption and decomposition of *Bt* toxins may vary between sites and seasons. Finally, different soil organisms are likely to have different interactive strengths and exposure pathways to *Bt* crops due to their specific food resource and habitat requirements. Therefore, it is not surprising that *Bt* cotton exerts contrasting effects on soil mites and other soil fauna.

Oribatida live in the uppermost soil layers in agricultural systems, where they mainly feed on decomposing plant debris and

fungi and play a crucial role in the process of recycling organic matter (Luxton, 1972; Travé et al., 1996). Some studies have demonstrated that Oribatida have little capacity to respond numerically to short-term environmental perturbations, but Oribatida density declines rapidly when their habitat is damaged, making them an excellent indicator of environmental degradation (Lebrun and Straalen, 1995; Siepel, 1996). More importantly, oribatid mites are particularly susceptible to the effects of toxic compounds of GM crops or pesticides accumulated in the soil, and should be considered as potential indicators in risk assessment studies (Oliveira et al., 2007). Among the groups investigated in the current study, the Oribatida are the most diverse and abundant, and their abundance fluctuated throughout the season. Meanwhile, the abundance of Oribatida in *Bt* cotton fields was consistently lower than that in non-*Bt* fields in October 2009 (Fig. 1C) and May 2010 (Fig. 1D). This finding contradicts that of other field studies, which demonstrated that neither *Bt* corn Cry toxins nor *B. thuringiensis* var. *kurstaki* spores had significant effects on oribatid mites (Al-Deeb et al., 2003; Beck et al., 2004; Addison et al., 2006). However, the adverse effects of *Bt* cotton on the Oribatida coincided with crop senescence and tillage practices. Most senescent tissues and crop residues entered the soil in October and were distributed due to tillage in April of the following year. To some extent, these results imply that the adverse impact of *Bt* cotton residues on soil mites is more important than the effects of root exudations and pollen. Unfortunately, we failed to identify a specific mechanism corresponding to the observed phenomena. Namely, we cannot clarify whether this inhibition effect is a direct or indirect effect of the *Bt* cotton.

Taxon diversity indices have been used to detect the responses of ecological communities to stressors (Carcamo and Parkinson, 2001). In our study, the changes in the community variables were similar between *Bt* and non-*Bt* cotton fields in 2009, but were significantly different in 2010 (i.e., the taxonomic group richness (*S*), Shannon–Weaver index (*H′*) and evenness index (*J*)). These results indicated that *Bt* cotton was deleterious to soil mites. Of course, the use of diversity indices to detect changes in community structure as a result of land management practices has not always proven useful (Siepel and Van de Bund, 1988). For example, diversity indices do not identify which species are contributing to the diversity (Magurran, 1988). However, analyses of the community compositions can compensate for this limitation.

Mina et al. (2007) postulated that any change in the structure or function of biological soil representatives, i.e., either a single species or whole community of species in a transgenic agro-ecosystem, could represent the impact of a transgenic crop. In our study, the community composition of soil mites was time dependent and accompanied by the replacement of some taxonomic groups. Meanwhile, the frequencies of dominant taxonomic groups differed between *Bt* and non-*Bt* cotton fields. This result also suggested that *Bt* cotton exerted a detectable impact on soil mites.

In conclusion, three lines of evidence supported the hypothesis that *Bt* cotton planting exerted a specific impact on ground-dwelling mites. However, this report only represents a case study of cotton in the GK series, which are widely planted *Bt*-cotton cultivars developed by the Institute of Biotechnology Research, Chinese Academy of Agricultural Sciences, in northern China over a relatively short time period. Given the complex interactions among ecological region, crop variety and *Bt* gene type transferred, it remains challenging to understand the overall response of soil mites to *Bt* cotton cultivation. Therefore, more multi-site experiments covering all of the *Bt* cotton genotypes available should be conducted to further explore the effects of *Bt* cotton on soil mites and their interaction mechanisms. Long-term monitoring experiments to detect slowly changing variables, such as biodiversity or the accumulation of transgenic crop products in the soil, are also seriously lacking. Furthermore, the soil biota is inextricably linked to aboveground communities, including plants, herbivores, pathogens and parasites; therefore, the linkage between aboveground and belowground communities requires explicit consideration. In spite of the limitations mentioned above, this baseline study filled an information gap in the risk assessment of *Bt* cotton on soil mites and provided information that will be useful for related studies in the future. Finally, these results may partially validate the increasing public concern about the potential effects of planting large areas of *Bt* crops on non-target soil organisms.

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