Applied Soil Ecology 73 (2014) 156–164

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Bt cotton planting does not affect the community characteristics of rhizosphere soil nematodes

Bing Yang[a,b], Hui Chen[c], Xianghui Liu[a], Feng Ge[a,*], Qunying Chen[a]

[a] State Key Laboratory of Integrated Management of Pest and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[b] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China
[c] Sichuan Remote Sensing Geomatics Institute, Chengdu 610100, China

ARTICLE INFO

Article history:
Received 20 April 2013
Received in revised form 1 September 2013
Accepted 2 September 2013

Keywords:
Nematode
Abundance
Taxonomic indices
Functional indices
Community composition

ABSTRACT

Transgenic cotton plants expressing Cry1Ac proteins from the soil bacterium *Bacillus thuringiensis* (Bt) provide effective control of Lepidopteran pests and thus reduce pesticide application. However, whether Bt cotton exerts undesirable effects on soil nematodes is largely unknown. Here we report the seasonal variations of soil nematode populations and the associated community indices in the rhizosphere soil of Bt and non-Bt cotton fields in northern China. Soil samples were collected at the main growing stages of the cotton during 2009–2010. Nematodes were extracted and recovered from soil samples using a modified cotton–wool filter method and identified under a light microscope according to their morphological characteristics. In addition, the nematodes were also classified to trophic group according to their feeding habits. Two years of cultivating transgenic Bt cotton failed to affect the total abundance, community diversity index or functional index of soil nematodes in 0–15 cm layer of the rhizosphere soil. Bt cotton consistently exerted detectable effects on nematode community composition when measured as direct effects on the densities of nematode genera, while slight effects were found using the principal response curve (PRC) analysis of repeated sampling events. These results suggest that Bt cotton has no significant adverse impact on soil nematodes community.

1. Introduction

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae), one of the most destructive lepidopteran pests on cotton, was drastically reduced in northern China by the introduction of transgenic cotton with the *Cry1Ac* gene from *Bacillus thuringiensis* (*Bt*) bacteria (Wu et al., 2008). Due to its promising effect on pest suppression, the total cultivation acreage of *Bt* cotton has expanded dramatically in northern China since 1997 (Huang and Pray, 2002). However, *Bt* proteins such as *Cry1Ac* and *Cry1Ab* produced in leaves, pollen and roots of *Bt* cotton can be introduced to soil through biomass incorporation and root exudates (Gupta and Watson, 2004; Knox et al., 2007; Li et al., 2012; Mina et al., 2008). Once released in soil, *Bt* toxin get adsorbed or bound on clay particles, humic components, or organic mineral complexes and in this way is protected against degradation by soil microorganisms (Tapp et al., 1995). Therefore, repeated cultivation of *Bt* cotton on same location might affect the composition and activity of soil microbial communities (Donegan and Seidler, 1999; Rui et al., 2005) and the soil biochemical properties (Rui et al., 2005; Sarkar et al., 2009; Sun et al., 2007) due to the accumulation of *Bt* toxin.

Soil-dwelling invertebrates, such as earthworms, nematodes, collembolans and mites, are an essential link in food webs as decomposers. They play key roles in soil nutrient cycling and organic matter degradation, and their abundance and diversity are directly related to the health and quality of soil (Blair et al., 1996; Icoz and Stotzky, 2008). Hence, the potential adverse effects of *Bt* cotton on soil organisms continued as a major subject of public concern and also an crucial aspect needed cautious consideration for government when they approve the application for releasing of new *Bt* cultivars.

Soil nematodes, one of the most abundant groups of soil invertebrates (Fu et al., 2000), live in the water films of soil and feed on a range of resources, including bacteria, fungi, plant roots and other nematodes (Yeates et al., 1993a,b). Furthermore, they reflect changes in ecological structure and function of soils in ways more predictable and efficient than other soil flora or fauna (Fiscus and Neher, 2002). The abundance, community diversity indices and functional indices of soil nematodes have been evaluated for their ability to detect changes in response to environmental characteristics in many types of ecosystems (Bongers and Ferris, 1999; de Geode and Dekker, 1993; Freckman and Ettema, 1993; Ettema et al.,

* Corresponding author. Tel.: +86 010 6480 7123; fax: +86 010 6480 7099.
E-mail address: gef@ioz.ac.cn (F. Ge).

0929-1393/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsoil.2013.09.001

1999; Ekschimitt et al., 2001; Verschoor et al., 2001), and have been regarded as sensitive indicators of the stress caused by ecological disturbance and soil pollutants in natural ecosystems (Sochová et al., 2006). Simultaneously, soil nematodes are susceptible to the toxin from *B. thuringiensis* (Bottjer et al., 1985; Meadows et al., 1990). Thus, information from soil nematodes community analysis can be used to indicate the impacts of *Bt* cotton on soil processes and soil fertility.

This study aimed to distinguish whether the planting of *Bt* cotton would exert considerable adverse effects on soil nematodes, such as reduction in abundance, lower diversity and simpler community structure. We were also interested in whether the effect of *Bt* cotton on soil nematodes would show an apparent temporary variation, because the impact of the genetic modification may be transient and minor compared to the variation caused by soil condition and growth stage of the crop. Here we report the seasonal dynamics, diversity, function and community structure of soil nematodes in *Bt* and non-*Bt* cotton fields during 2009–2010. In addition, we attempted to identify the sensitive taxa which could serve as bio-indicators in the risk assessment of *Bt* crops. Through this research, we hope to clear the impacts of *Bt* cotton cultivation on soil nematodes, and address the compatibility of transgenic *Bt* cotton with the sustainable agricultural development comprehensively and efficiently.

2. Materials and methods

2.1. Experimental design

The present study was conducted in the field of the Langfang Experiment Station (39.538°N, 116.708°E), Hebei Province, China, in 2009 and 2010. The soil chemical properties of the fields were as follows: organic carbon, 10.1 g/kg; organic matter, 17.4 g/kg; total N, 0.44 g/kg; available N, 434 mg/kg; available P, 29.5 mg/kg; available K, 323 mg/kg; pH ($CaCl_2$), 8.1. 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 8 plots. 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 (Yang et al., 2013).

The *Bt* cotton (GK-12), a transgenic cotton expressing fusion protein of *Cry1Ac* and *Cry1Ab*, and its parental line non-*Bt* cotton (Simian-3) were used. Both seeds were provided by the Biotechnology Research Institute of Chinese Academy of Agricultural Sciences (Beijing, China). The plants were sown at a density of approximately 40,000 plants/ha, and the standard agronomic practices common to northern China were followed in all the tested plots. However, no insecticides or fungicides were applied. Lepidopteran pests were killed by a black light trap with *UV* lamp near the fields. To avoid any effect of weeds on the soil nematodes, weeds were manually removed at irregular intervals. In addition, the plants were watered as needed and fertilized with a controlled release fertilizer.

2.2. Sample collection and species identification

Soil samples were collected from the 0–15 cm layer rhizosphere of the *Bt* and non-*Bt* plants at 30, 60, 90, 120 and 150 days after planting, which corresponded to the seedling, squaring, flowering, bolling and harvesting stages of cotton plants. Soil was sampled from the rhizosphere of both *Bt* and non-*Bt* plants using the same procedure. During this period, five soil samples per plot were taken from five sites distributed at the diagonal for the two linear transects of each plot per month. For each site, an area of 30 cm around 5 randomly selected plants was marked, and totally 25 plants each plot were selected for every sampling date. Sampling was limited to the rhizosphere soil due to the greater abundance of microbes in the rhizosphere compared to the bulk soil (Alphei et al., 1996). The core samples were 15 cm deep with a 7.5 cm wide bore. At each site, 3 core samples were taken from the marked area, the core samples were mixed thoroughly and 0.5 kg of each soil composite samples was drawn as a representative sample. Then, the two composite samples from the same direction of diagonal focus were mixed thoroughly again and one half was taken. Namely, there were 3 composite samples per plot. The soil samples were placed into separate plastic resealable bags, sealed gently, transported to the laboratory in a cooling box, stored at 4 °C and extracted within 1 week after sampling.

2.3. Nematode extraction and identification

Nematodes were extracted from 100 g of dry soil from each pooled sample using a modified cotton–wool filter method (Oostenbrink, 1960; Townshend, 1963; Liang et al., 2009). After a 48 h extraction at room temperature, nematodes were heat-killed for 2 min at 60 °C and preserved in 4% (v/v) formaldehyde. The total number of the nematodes was determined under low magnification (50×) with a dissecting microscope. Subsequently, at least 100 specimens per sample were randomly selected and identified to genus or species level according to Bongers (1988) under higher magnification (200×) with an inverted compound microscope. Concurrently, all identified nematodes were placed into feeding groups according to Yeates et al. (1993a).

2.4. Measurement of community indices of soil nematodes

To characterize the nematode fauna from *Bt* and non-*Bt* cotton fields, the following indices were calculated: (1) Species richness *S*, the number of taxa represented in a set or collection of individuals; (2) the Shannon-Weaver diversity index ($H' = -\Sigma P_i \times \ln P_i$, where P_i is the proportion of individuals in the *i*th taxon); (3) the Simpson dominance index, $\lambda = \Sigma(P_i)^2$; (4) the Shannon evenness measure ($J' = H'/\ln S$) (Magurran, 2004), which is determined from the Shannon-Weaver diversity index as $J' = H'/H'_{max}$, and $H'_{max} = \ln S$, where S is the total number of genera; (5) Margalef's richness, $SR = (S-1)/\ln N$, where N is the number of individuals identified (Yeates and Bongers, 1999); (6) the maturity index (MI), $MI = \Sigma[v(i) \times f(i)]$, where $v(i)$ is the colonizer-persister (c-p) value of taxon *i*, and $f(i)$ is the frequency of taxon *i* in a sample (Bongers, 1990); (7) the plant parasite index (PPI), $PPI = \Sigma[v(i) \times f'(i)]$, where $v(i)$ is the colonizer-persister (c-p) value of taxon *i*, and $f'(i)$ is the proportion of taxon *i* in a sample (Bongers, 1990). The MI and PPI are calculated using a c-p value that ranges from colonizer (c-p = 1 or 2) to persister (c-p = 5), with the index values representing life-history characteristics associated with r- and K-selection, respectively; (8) MI/PPI; (9) the Nematode Channel Ratio ($NCR = B/(B+F)$), where *B* and *F* are abundances of bacterial- and fungal-feeders (Yeates, 2003); (10) *B*/*F*, where *B* and *F* are abundances of bacterial- and fungal-feeders (Twinn, 1974); (11) the Wasilewska index (WI), $WI = (F+B)/P$ (Wasilewska, 1997), where *F*, *B* and *P* are the abundance of bacterial-feeders, fungal-feeders and plant parasites, respectively; (12) MI2-5, the MI value of nematodes excluding the c-p1 enrichment opportunists; (13) the Enrichment Index (EI), $EI = 100 \times e/(e+b)$, where $e = (Ba_1 \times W_1) + (Fu_2 \times W_2)$, $b = (Ba_2 + Fu_2) \times W_2$, $W_1 = 3.2$ and $W_2 = 0.8$ (Ferris and Bongers, 2009); (14) the structure index (SI), $SI = 100 \times s/(s+b)$, where $s = Ba_n \times W_n + Ca_n \times W_n + Fu_n \times W_n + Om_n \times W_n$, $n = 3-5$, $W_3 = 1.8$, $W_4 = 3.2$, $W_5 = 5.0$. In general, the species with a c-p value of 1 (for MI) or 2 (for PPI) are r-selected or colonizers. Because of

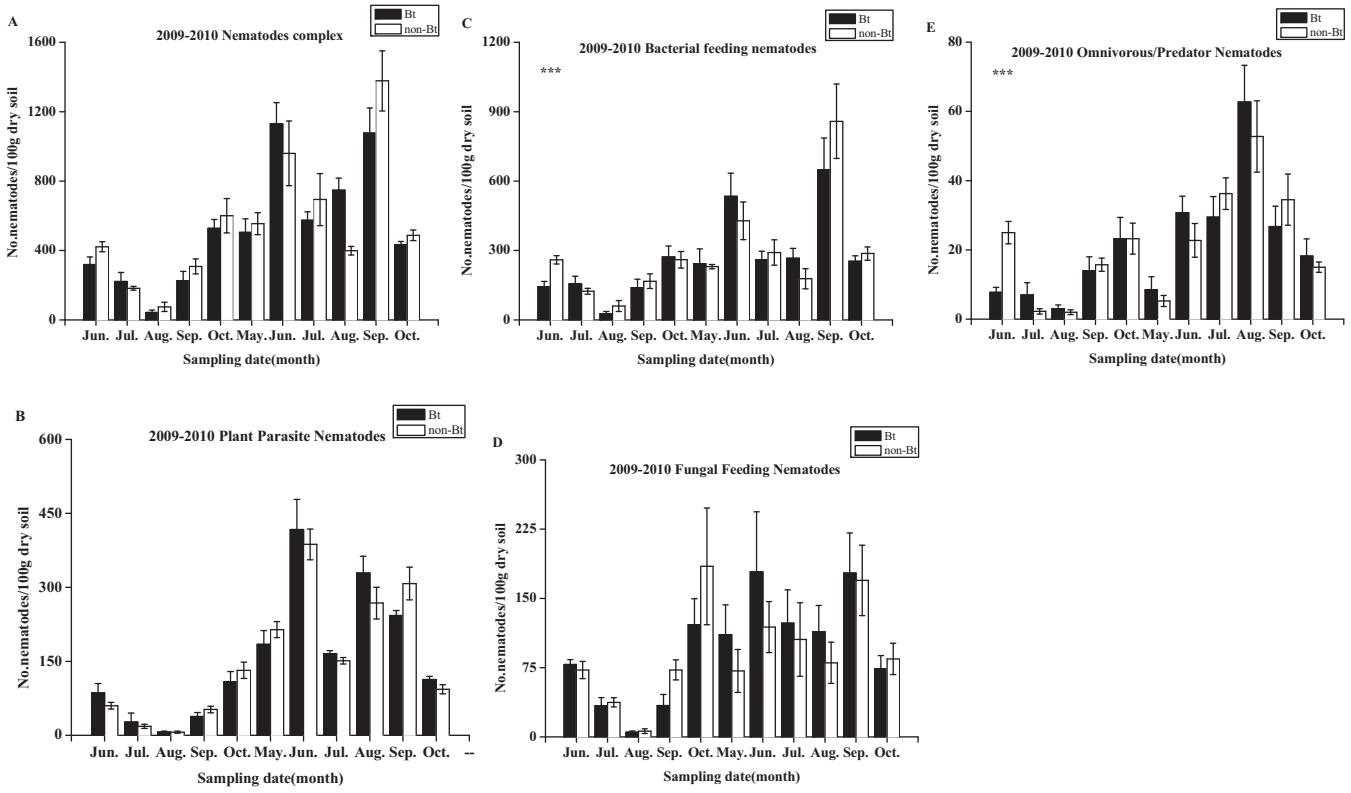

Fig. 1. Seasonal dynamics of abundance of (A) total nematode complex, (B) plant parasites, (C) bacteria feeders, (D) fungal feeders and (E) omnivores/predators in *Bt* vs. non-*Bt* cotton fields of northern China in 2009 and in 2010.

their short generation times, large population fluctuations, and high fecundity, these species are very tolerant of pollutants and other disturbances. Those with a c-p value of 5 are K-selected or persisters, with a long life span, low reproductive rates, low metabolic activity and slow movement; thus, they are very sensitive to pollutants and other disturbances. The c-p values in present study were obtained from Ferris (2010) and http://plpnemweb.ucdavis.edu/nemaplex/IndexFiles.

2.5. Statistical analysis

Because the questions of interest were related to overall changes in abundance and community composition in *Bt* cotton relative to non-*Bt* cotton fields, species were pooled into higher trophic groups (plant parasites, bacteria feeders, fungal-feeders and omnivores/predators). To avoid pseudo-replication, all analyses were performed on the mean values for each plot. Abundances were $\log_{10}(x+1)$ transformed prior to analysis to meet assumptions of normality. To examine the effects of plant genotype, sampling date and the interaction of these factors on the abundance and community characteristics of nematodes in each season, we analyzed our data with repeated measures ANOVA ('proc mixed' procedure in SAS) with cotton genotype, sampling date as a fixed factor and plot as a random factor. All results are expressed as the Means ± Standard Error (SE), and untransformed data are presented in all tables and figures.

A graphical representation of the temporal response of the nematode community to the planting of *Bt* cotton was obtained using multivariate ordination Principal Response Curves (PRC) (Van den Brink and Ter Braak, 1998, 1999). The PRC method modifies Redundancy Analysis (RDA) to accommodate repeated measures data and to visualize the community composition vs. a control through time. In our analysis, the non-*Bt* was assigned as control. The data for the four plots of each genotype are combined in the calculation of PRC values so that in the resulting diagram each genotype is represented by a single point. The significance of the canonical axes of RDA model was tested against 999 unrestricted Monte Carlo permutations and yielded one significant axis. The result is a diagram showing the sampling periods on the *x*-axis and the first Principal Component of the variance explained by treatment on the *y*-axis. The abundance of the nematodes taxa were $\ln(x+1)$-transformed prior to the analysis. To examine whether the plots had diverged with time, variances were calculated each year for the sample scores of the RDA axes from the 8 plots and tested for homogeneity.

For the RDA and PRC, CANOCO 4.5 (Microcomputer Power, Ithaca, NY, USA) was used. All other statistical tests were performed using SAS for Windows 9.2 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Seasonal changes in soil nematode abundance during cotton growth stages

Within each growing season, the temporal dynamics of soil nematode abundance showed similar trends for *Bt* and non-*Bt* cotton fields; with no significant effect of the cotton genotype on either all nematode abundance or nematode abundance of each trophic group (Fig. 1A–E; Table 1). In addition, the temporal dynamic of nematode abundance from both *Bt* and non-*Bt* cotton fields showed a great variation across sampling dates, whether for total nematodes (Fig. 1A; Table 1), plant parasites (Fig. 1B; Table 1), fungal feeders (Fig. 1D; Table 1) and omnivores/predators (Fig. 1E; Table 1). However, there was a significant interaction between genotype and sampling date on the abundance of bacteria feeding nematodes in 2009 (Table 1).

Table 1
F- and *P*-Values of the effects of cotton genotype (*Bt* vs. non-*Bt*) and sampling date on the abundance of nematodes complex and separate trophic group in cotton fields of northern China.

Year	Factor	DF	Nematode complex		Herbivores		Bacterivores		Fungivores		Omnivores/Predators	
			F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
2009	Genotype	1, 3	3.0	0.182	0.2	0.711	3.5	0.159	0.7	0.462	0.03	0.873
	Date	4, 24	31.4	<0.001	44.7	<0.001	17.5	<0.001	59.1	<0.001	1.1	0.371
	Genotype × Date	4, 24	0.9	0.505	1.5	0.226	4.1	0.011	1.4	0.272	2.2	0.103
2010	Genotype	1, 3	2.9	0.189	0.2	0.682	0.03	0.883	0.6	0.505	0.03	0.881
	Date	5, 30	35.4	<0.001	48.2	<0.001	14.5	<0.001	4.0	0.006	149.6	<0.001
	Genotype × Date	5, 30	1.3	0.292	1.8	0.147	1.2	0.338	0.7	0.631	0.9	0.496

3.2. Soil nematode community composition changes at the genus level

In 2009, 11,683 specimens were collected during our study periods, thirty-three and thirty-one genera of nematodes were identified in *Bt* and non-*Bt* cotton fields, respectively. *Acrobeloides*, *Eucephalobus*, and *Aphelenchus* were eu-dominant genera in both *Bt* and non-*Bt* cotton fields, but no detectable differences in either density or dominance were found between the two genotypes ($P > 0.05$). However, the total densities of *Mesorhabditis*, *Monhystera*, and *Diphtherophora* in *Bt* cotton field were significantly higher than those in non-*Bt* cotton fields. The abundance of *Mesorhabditis* increased by 127% from 37 to 84 Ind./100 g soil in the Bt cotton field as compared to the control. The abundance of *Monhystera* increased by 400% from 1 to 5 Ind./100 g soil in the Bt cotton field as compared to the control. The *Diphtherophora* occurred in *Bt* cotton field, but not in non-*Bt* cotton field. Meanwhile, the dominances of *Monhystera* and *Diphtherophora* in *Bt* cotton fields were higher than those in non-*Bt* cotton fields.

In 2010, 36,255 specimens were collected during our study periods, forty-four and forty-two genera of nematodes were identified in *Bt* and non-*Bt* cotton fields, respectively. *Acrobeloides*, *Enchodelum*, and *Aphelenchoides* were eu-dominant genera in both of the *Bt* and non-*Bt* cotton fields, and no detectable difference in density of these genera were found between the two genotypes ($P > 0.05$), while the dominance of *Acrobeloides* in *Bt* fields was significantly higher than in non-*Bt* fields ($P = 0.043$). The total densities of *Protorhabditis*, *Alaimus*, and *Criconemoides* in *Bt* fields were higher than in non-*Bt* fields (Table 2), their abundance in *Bt* field increased by 350% (from 4 to 18 Ind./100 g soil), 167% (from 9 to 24 Ind./100 g soil) and 350% (from 2 to 9 Ind./100 g soil) respectively compared to the control. The cumulative densities of *Enchodelum* and *Psilenchus* markedly reduced in *Bt* cotton field compared with those of the non-*Bt* field (Table 2). The density of *Enchodelum* in *Bt* cotton field reduced by 67% (from 3 to 1 Ind./100 g soil) compared with the control. *Psilenchus* only occurred in the non-*Bt* field. In addition, the dominances of *Protorhabditis*, *Acrobeles*, *Alaimus*, *Tylenchus*, *Dorylaimidae*, and *Criconemoides* showed detectable differences between the two genotypes (Table 2).

Overall, the composition of soil nematodes became more complex with the planting of cotton, either for *Bt* or non-*Bt* treatment. *Acrobeloides* was the eu-dominant genus in both *Bt* and non-*Bt* cotton fields for the two seasons. The dominance of *Acrobeloides* in *Bt* and non-*Bt* cotton fields was comparable in 2009, and the difference became significant in 2010. Although some genera found in the first season disappeared in the second year, but new genera occurred during the second growing season.

3.3. Soil nematode community composition changes at the trophic and c-p group level

In general, most of the nematodes belonged to the c-p 2 and c-p 3. The percentage of cp-3 group in *Bt* cotton fields was significantly higher than that of non-*Bt* cotton plots in June and in August during the growing season of 2009, and in August and in October during the growing season of 2010 (Table 3). There was no significant difference between the two genotypes at other sampling dates, except for July in 2009. No marked difference in any other c-p groups between the two genotypes was found (Table 3).

In terms of the trophic group, the nematodes were dominated by bacteria feeders, plant parasite nematodes and fungal feeders. The bacteria feeder group was the group with the most individuals at each sampling event. The cotton genotype significantly affected the dominance of each group in June 2009 (Table 3). There were more plant parasites and fungal feeders in the *Bt* cotton than in non-*Bt* but fewer bacterial feeders and omnivorous/predators in *Bt* cotton compared to non-*Bt*. Significant differences in the dominances of the fungal feeders and bacteria feeders between the two genotypes were also found on August, 2009 (Table 3). The dominance of the bacteria feeders in *Bt* cotton were less than in non-*Bt*, while the dominance of the fungal feeders was higher in *Bt* fields. However, these phenomena were transient because there was no significant difference between the two genotypes at other sampling dates in 2009, nor for any sampling date in 2010 (Table 3).

3.4. Nematode community indices

For all of the routine diversity indices, the values changed significantly across the sampling dates within each season, except for the Shannon evenness index. However, cotton genotype was not an important factor in determining the ecological indices of soil nematodes (Table 4). In addition, the interactions between the genotype of cotton and the sampling date were not pronounced for all tested variables, except for the Margalef's richness ($F = 3.14$, $df = 4, 24$, $P = 0.033$, Table 4).

During any growing season, most of the functional indices changed with the sampling date (Table 5), while cotton genotype failed to affect any of the functional indices of the nematode community. In addition, there were significant interactions between genotype and sampling date on fungivores to bacterivores ratio (*F*/*B*), enrichment index (EI), nematode channel ratio (NCR), plant parasite index (PPI), ratio of plant parasite nematodes to free-living nematodes (PPI/MI), and Wasilewska index (WI) in 2009 (Table 5).

3.5. Nematode community response

The response of the nematode communities and each taxon to *Bt* cotton cultivation over its growth stages and through the 2-yr study period is best represented by PRC. In the PRC_1 (Fig. 2), positive values of the curve indicate a positive effect of *Bt* cotton on genera, whereas genera with negative scores have the opposite pattern and are therefore expected to decrease in relative abundance. The higher the value on the curve (positive and negative), the larger the deviation is from the control (*non-Bt*

Table 2
The trophic position (Ba = bacterivores, Fu = fungivores, Om = omnivores/predators, Pp = plant parasites), number of identified individuals (N), c-p group and density (mean ± SE, $n = 4$) of the nematode genera in *Bt* vs. non-*Bt* cotton fields in northern China in 2009 and in 2010 (statistically significant differences between the *Bt* and non-*Bt* cotton plots are shown in bold type).

Year	Genus	Family	Trophic	c-p	*N*	Density (Individuals/100 g dry soil)	
						Bt	*non-Bt*
2009	*Diploscapter*	Rhabditidae	Ba	1	116	10.0 ± 2.3	18.8 ± 8.5
	Rhabditidae spp.	Rhabditidae	Ba	1	629	84.0 ± 22.4	73.0 ± 12.1
	Mesorhabditis	Rhabditidae	Ba	1	485	**37.3 ± 8.4**	**84.3 ± 20.7**
	Protorhabditis	Rhabditidae	Ba	1	2	0.3 ± 0.3	0.3 ± 0.3
	Pseudodiplogasteroides	Diplogasteridae	Ba	1	23	3.3 ± 1.7	2.5 ± 1.2
	Diplogaster	Diplogasteridae	Ba	1	3	0.3 ± 0.3	0.5 ± 0.5
	Acrobeloides	Cephalobidae	Ba	2	2443	292 ± 28.1	319 ± 21.1
	Acrobeles	Cephalobidae	Ba	2	90	9.8 ± 4.0	12.8 ± 4.2
	Cephalobus	Cephalobidae	Ba	2	682	80.8 ± 8.8	89.8 ± 6.8
	Eucephalobus	Cephalobidae	Ba	2	1394	162 ± 40.1	187 ± 22.1
	Plectus	Plectidae	Ba	2	333	34.3 ± 4.3	48.8 ± 2.9
	Monhystera	Monhysteridae	Ba	2	26	**5.3 ± 1.7**	**1.3 ± 0.6**
	Prismatolaimus	Prismatolaimidae	Ba	3	86	7.0 ± 2.4	14.8 ± 2.6
	Metateratocephalus	Teratocephalidae	Ba	3	47	3.8 ± 1.7	8.3 ± 2.7
	Aulolaimus	Rhabdolaimidae	Ba	3	1	0.3 ± 0.3	0
	Alaimus	Alaimidae	Ba	4	80	9.0 ± 2.1	11.0 ± 2.0
	Dorylaimus	Dorylaimidae	Om	4	43	6.8 ± 2.1	3.8 ± 0.9
	Eudorylaimus	Dorylaimidae	Om	4	313	33.8 ± 3.2	44.8 ± 5.1
	Aporcelaimus	Dorylaimidae	Om	4	163	16.8 ± 5.3	24.0 ± 1.6
	Aphelenchus	Aphelenchidae	Fu	2	1824	182 ± 18.0	275 ± 53.9
	Diphtherophora	Diphtherophoridae	Fu	3	4	**1.0 ± 0.4**	**0**
	Tylencholaimus	Tylencholaimidae	Fu	4	57	7.0 ± 1.5	7.5 ± 1.2
	Aphelenchoides	Aphelenchoididae	Pp	2	647	71.5 ± 26.7	90.3 ± 22.5
	Ditylenchus	Anguinidae	Pp	2	47	10.5 ± 5.5	1.3 ± 0.8
	Filenchus	Tylenchidae	Pp	2	307	38.5 ± 9.1	37.8 ± 1.7
	Tylenchus	Tylenchidae	Pp	2	6	1.0 ± 1.0	0.5 ± 0.5
	Boleodorus	Tylenchidae	Pp	2	493	61.0 ± 14.9	62.5 ± 9.4
	Psilenchus	Tylenchidae	Pp	2	13	1.3 ± 0.8	1.8 ± 1.1
	Tylenchorhynchus	Anguinidae	Pp	2	66	7.5 ± 0.9	9.5 ± 1.3
	Pratylenchus	Pratylenchidae	Pp	2	72	8.3 ± 1.4	10.3 ± 2.5
	Brachydorus	Dolichodoridae	Pp	3	446	42.0 ± 7.7	69.5 ± 10.8
	Helicotylenchus	Hoplolaimidae	Pp	3	717	107 ± 26.8	72.8 ± 16.4
	Heterodera	Heteroderidae	Pp	3	22	1.0 ± 0.4	4.8 ± 2.8
	Unknown				5	0.3 ± 0.3	1.0 ± 0.4
	Total				11,683	1334 ± 143	1587 ± 109
2010	*Rhabditidae* spp.	Rhabditidae	Ba	1	450	57.3 ± 26.2	55.3 ± 35.4
	Rhabditis	Rhabditidae	Ba	1	101	14.0 ± 2.6	11.3 ± 3.8
	Mesorhabditis	Rhabditidae	Ba	1	1846	203 ± 43.1	259 ± 115.2
	Protorhabditis	Rhabditidae	Ba	1	88	**18.0 ± 4.7**	**3.8 ± 1.0**
	Diploscapter	Diploscapteridae	Ba	1	941	156 ± 58.5	79.3 ± 18.0
	Diplogaster	Diplogasteridae	Ba	1	2	0.5 ± 0.5	0
	Acrobeloides	Cephalobidae	Ba	2	7302	838 ± 69.4	987 ± 90.7
	Eucephalobus	Cephalobidae	Ba	2	3446	421 ± 105.9	441 ± 105.1
	Acrobeles	Cephalobidae	Ba	2	633	98.8 ± 14.5	59.8 ± 3.2
	Cephalobus	Cephalobidae	Ba	2	922	109 ± 19.5	121 ± 13.0
	Plectus	Plectidae	Ba	2	589	78.3 ± 8.6	69.3 ± 22.7
	Wilsonema	Plectidae	Ba	2	51	10.3 ± 2.8	2.3 ± 1.7
	Monhystera	Monhysteridae	Ba	2	28	3.0 ± 1.8	4.0 ± 4.0
	Pseudoaulolaimus	Aulolaimidae	Ba	3	3	0.8 ± 0.8	0
	Aulolaimoides	Aulolaimidae	Ba	3	19	3.5 ± 1.6	1.5 ± 1.5
	Prismatolaimus	Prismatolaimidae	Ba	3	361	50.3 ± 14.6	40.3 ± 5.6
	Metateratocephalus	Teratocephalidae	Ba	3	361	43.8 ± 8.9	46.3 ± 14.6
	Alaimus	Alaimidae	Ba	4	129	**23.5 ± 1.3**	**9.3 ± 3.5**
	Seinura	Aphelenchoididae	Om	2	22	1.0 ± 0.7	4.5 ± 0.8
	Tripyla	Tripylidae	Om	3	3	0.5 ± 0.5	0.3 ± 0.3
	Dorylaimus	Dorylaimidae	Om	4	48	7.8 ± 3.6	4.3 ± 1.4
	Eudorylaimus	Qudsianematidae	Om	4	740	89.8 ± 16.3	95.3 ± 12.7
	Discolaimus	Discolaimidae	Om	5	7	1.5 ± 1.0	0.3 ± 0.3
	Panagrolaimus	Swangeriidae	Om	5	575	62.0 ± 37.2	81.8 ± 33.3
	Disclaimum	Discolaimidae	Om	5	208	19.5 ± 6.0	32.5 ± 22.5
	Dorylaimidae spp.	Dorylaimidae	Om	4	113	14.0 ± 5.9	14.0 ± 2.0
	Campydora	Campydoridae	Om	4	24	5.0 ± 2.0	1.0 ± 0.4
	Enchodelum	Nordiidae	Om	4	15	**0.5 ± 0.5**	**3.3 ± 0.3**
	Aporcelaimus	Aporcelaimidae	Om	5	343	47.3 ± 7.0	38.5 ± 5.7
	Aphelenchus	Aphelenchidae	Fu	2	3843	528 ± 164.7	433 ± 91.4
	Diphtherophora	Diphtherophoridae	Fu	3	31	6.8 ± 5.5	0.8 ± 0.8
	Tylenchus	Tylenchidae	Pp	2	1	0	0.3 ± 0.3
	Filenchus	Tylenchidae	Pp	2	1047	132 ± 31.1	130 ± 24.1
	Psilenchus	Tylenchidae	Pp	2	10	**0.3 ± 0.3**	**2.3 ± 0.5**
	Boleodorus	Tylenchidae	Pp	2	882	97.3 ± 28.4	124 ± 17.7
	Nothotylenchus	Anguinidae	Pp	2	1715	243 ± 39.5	189 ± 33.5

Table 2 (*Continued*)

Year	Genus	Family	Trophic	c-p	*N*	Density (Individuals/100 g dry soil)	
						Bt	*non-Bt*
	Aphelenchoides	Aphelenchoididae	Pp	2	703	97.0 ± 40.5	78.8 ± 23.2
	Tylenchorhynchus	Dolichodoridae	Pp	3	1286	194 ± 31.6	127 ± 51.7
	Pratylenchus	Pratylenchidae	Pp	3	4473	554 ± 85.0	565 ± 122
	Rotylenchus	Hoplolaimidae	Pp	3	2592	359 ± 90.7	289 ± 54.8
	Scutellonema	Hoplolaimidae	Pp	3	27	5.5 ± 4.3	1.3 ± 0.8
	Tylencholaimus	Dolichodoridae	Pp	3	128	18.0 ± 3.7	14.0 ± 2.5
	Brevibucca	Brevibuccidae	Pp	3	8	2.0 ± 2.0	0
	Criconemoides	Criconematidae	Pp	3	45	**9.0 ± 3.5**	**2.3 ± 0.6**
	Belondira	Belondiridae	Pp	5	38	5.5 ± 1.2	4.0 ± 1.6
	Unknown				53	10.5 ± 6.4	2.8 ± 0.6
	Total				36,255	4637 ± 285	4427 ± 178

Table 3
Percentage of the nematode complex individual (mean ± SE, $n = 4$) belonging to the c-p groups and trophic groups in field plots with *Bt* vs. non-*Bt* cotton production in northern China in 2009 and in 2010 (statistically significant differences between the *Bt* and non-*Bt* cotton plots are shown in bold type).

Group	Genotype	2009					2010					
		June	July	August	September	October	May	June	July	August	September	October
cp-1	*Bt*	9.0 ± 0.8	48.3 ± 0.8	40.4 ± 3.3	35.1 ± 2.0	24.1 ± 2.2	6.2 ± 2.0	15.7 ± 3.7	4.2 ± 0.9	7.6 ± 2.6	9.7 ± 1.7	4.2 ± 1.5
	non-*Bt*	14.9 ± 2.6	40.5 ± 2.6	53.2 ± 9.5	28.0 ± 2.8	21.5 ± 1.8	9.7 ± 2.0	8.9 ± 1.6	3.2 ± 0.4	6.7 ± 1.5	13.3 ± 5.5	6.2 ± 2.0
cp-2	*Bt*	67.3 ± 2.8	47.4 ± 5.8	44.0 ± 4.2	44.1 ± 3.8	57.7 ± 3.5	61.4 ± 2.2	50.6 ± 3.4	63.9 ± 3.3	43.7 ± 5.1	62.8 ± 2.7	71.3 ± 3.4
	non-*Bt*	67.8 ± 3.3	52.2 ± 3.4	35.6 ± 8.0	54.0 ± 4.4	56.9 ± 3.5	56.6 ± 0.3	50.7 ± 2.6	60.5 ± 2.2	56.6 ± 2.2	64.6 ± 7.1	73.1 ± 4.3
cp-3	*Bt*	**17.1 ± 2.5**	**1.9 + 0.7**	**9.4 + 0.9**	15.0 + 3.9	11.5 + 2.5	31.2 ± 1.1	30.7 ± 3.4	25.6 ± 2.7	**37.9 ± 4.1**	19.4 ± 2.7	**17.4 ± 1.6**
	non-*Bt*	**6.8 ± 0.9**	**5.9 + 0.8**	**4.7 + 1.0**	12.6 + 1.4	14.2 + 3.8	32.8 ± 2.0	37.8 ± 4.0	30.2 ± 1.2	**24.1 ± 3.4**	13.6 ± 2.5	**11.4 ± 0.7**
cp-4	*Bt*	6.5 ± 1.3	2.4 ± 0.7	6.2 ± 2.1	5.8 ± 1.5	4.9 ± 0.6	0.7 ± 0.4	2.1 ± 0.5	3.9 ± 0.6	7.6 ± 2.6	2.2 ± 1.1	2.5 ± 0.7
	non-*Bt*	10.4 ± 1.5	1.4 ± 0.4	6.5 ± 2.1	5.5 ± 0.5	4.4 ± 0.7	0.9 ± 0.3	1.6 ± 0.6	4.8 ± 0.9	9.3 ± 1.1	2.0 ± 0.2	2.2 ± 0.3
cp-5	*Bt*	0	0	0	0	0	0.6 ± 0.4	0.9 ± 0.1	2.2 ± 0.6	2.1 ± 0.2	5.9 ± 2.1	1.5 ± 0.5
	non-*Bt*	0	0	0	0	0	0	0.9 ± 0.2	1.1 ± 0.6	3.0 ± 0.9	6.6 ± 2.0	1.0 ± 0.2
Pp	*Bt*	**26.0 ± 2.2**	10.6 ± 5.4	15.2 ± 2.4	18.4 ± 2.4	20.6 ± 3.2	36.5 ± 0.2	39.5 ± 8.4	29.7 ± 3.5	44.4 ± 3.6	23.7 ± 2.9	26.4 ± 2.2
	non-*Bt*	**14.1 ± 0.9**	10.0 ± 2.1	9.6 ± 3.0	17.1 ± 0.8	23.4 ± 4.4	39.4 ± 2.7	44.9 ± 8.6	24.8 ± 5.0	69.3 ± 11.5	23.8 ± 4.1	19.4 ± 2.2
Ba	*Bt*	**45.0 ± 1.9**	72.5 ± 5.5	**63.2 ± 1.8**	59.7 ± 3.8	50.9 ± 4.1	48.6 ± 2.2	46.8 ± 5.3	44.9 ± 3.1	35.1 ± 3.6	58.3 ± 5.6	59.1 ± 6.9
	non-*Bt*	**61.7 ± 1.4**	67.7 ± 4.0	**78.3 ± 4.8**	53.5 ± 3.9	44.2 ± 4	42.7 ± 3.3	45.0 ± 3.0	42.8 ± 1.3	44.4 ± 9.8	62.5 ± 8.1	58.4 ± 2.4
Fu	*Bt*	**25.5 ± 2.4**	14.7 ± 0.8	**13.1 ± 1.0**	15.8 ± 3.4	23.9 ± 5.7	23.0 ± 7.5	16.6 ± 5.6	25.3 ± 8.4	13.6 ± 3.3	21.3 ± 5.7	17.8 ± 4.7
	non-*Bt*	**17.0 ± 1.3**	21.1 ± 3.2	**7.4 ± 2.0**	24.2 ± 4.0	28.4 ± 5.2	13.9 ± 5.5	10.6 ± 2.8	17.4 ± 6.4	12.0 ± 4.9	15.0 ± 3.6	20.4 ± 4.6
Om	*Bt*	**2.4 ± 0.2**	2.6 ± 0.9	6.4 ± 0.4	8.1 ± 3.0	4.3 ± 0.9	1.8 ± 0.8	2.9 ± 0.6	5.4 ± 1.2	9.0 ± 2.4	2.5 ± 0.5	4.3 ± 1.3
	non-*Bt*	**6.0 ± 1.1**	1.2 ± 0.4	4.1 ± 1.4	5.2 ± 0.3	4.0 ± 0.8	0.9 ± 0.2	2.7 ± 0.6	6.1 ± 1.8	13.1 ± 2	2.4 ± 0.3	3.1 ± 0.3

Table 4
F- and *P*-Values for the effects of cotton genotype (*Bt* vs. non-*Bt*) and sampling date on the diversity indices of soil nematode communities in cotton fields of northern China (λ = Simpson dominance index, H' = Shannon-Weaver diversity index, J' = Shannon evenness index; S = total number of taxa; SR = Margalef's richness).

Year	Factor	DF	S		SR		H'		λ		J'	
			F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
2009	Genotype	1, 3	0.9	0.415	6.4	0.086	0.9	0.409	1.2	0.356	0.5	0.537
	Date	4, 24	9.4	<0.001	6.0	0.002	7.1	0.001	5.5	0.003	2.5	0.073
	Genotype × Date	4, 24	1.0	0.412	3.1	0.033	2.5	0.071	1.4	0.282	1.1	0.369
2010	Genotype	1, 3	2.6	0.207	0.4	0.553	5.7	0.097	4.0	0.138	3.1	0.178
	Date	5, 30	11.1	<0.001	29.7	<0.001	6.0	0.001	2.9	0.031	5.7	0.001
	Genotype × Date	5, 30	0.8	0.557	2.5	0.056	1.3	0.276	1.3	0.306	0.6	0.670

Table 5
F-Values of the effects of cotton genotype (*Bt* vs. non-*Bt*) and sampling date on the functional indices of soil nematode communities in cotton fields in northern China.

Year	Factor	DF	*F*/*B*	EI	MI	MI2-5	NCR	PPI/MI	PPI	SI	WI
2009	Genotype	1, 3	0.08^{ns}	0.86^{ns}	1.25^{ns}	0.48^{ns}	0.21^{ns}	2.88^{ns}	3.90^{ns}	0.02^{ns}	0.59^{ns}
	Date	4, 24	14.74^{***}	11.73^{***}	8.37^{***}	4.02^{**}	13.12^{***}	8.80^{***}	10.44^{***}	24.65^{***}	15.61^{***}
	Genotype × Date	4, 24	5.31^{**}	6.12^{**}	1.85^{ns}	0.93^{ns}	6.69^{***}	3.47^{**}	4.98^{**}	1.18^{ns}	6.57^{***}
2010	Genotype	1, 3	0.57^{ns}	0.12^{ns}	0.80^{ns}	0.00^{ns}	0.12^{ns}	1.09^{ns}	0.00^{ns}	0.01^{ns}	0.00^{ns}
	Date	5, 30	2.01^{ns}	11.40^{***}	9.42^{***}	5.47^{***}	1.85^{ns}	22.92^{***}	34.46^{***}	6.52^{**}	5.71^{***}
	Genotype × Date	5, 30	0.53^{ns}	1.69^{ns}	1.65^{ns}	1.37^{ns}	0.39^{ns}	3.33^{**}	0.15^{ns}	1.18^{ns}	1.52^{ns}

Note: *F*/*B*: fungivores to bacterivores ratio, EI: enrichment index, MI: maturity index for free-living nematodes, MI2-5: maturity index for free-living and plant parasite nematodes, NCR: nematode channel ratio, PPI: plant parasite index, PPI/MI: the ratio of plant parasite nematodes to free-living nematodes, WI = Wasilewska index, SI = structure index; ns: not significant.
$^{*}P < 0.05$.
$^{**}P < 0.01$.
$^{***}P < 0.0001$.

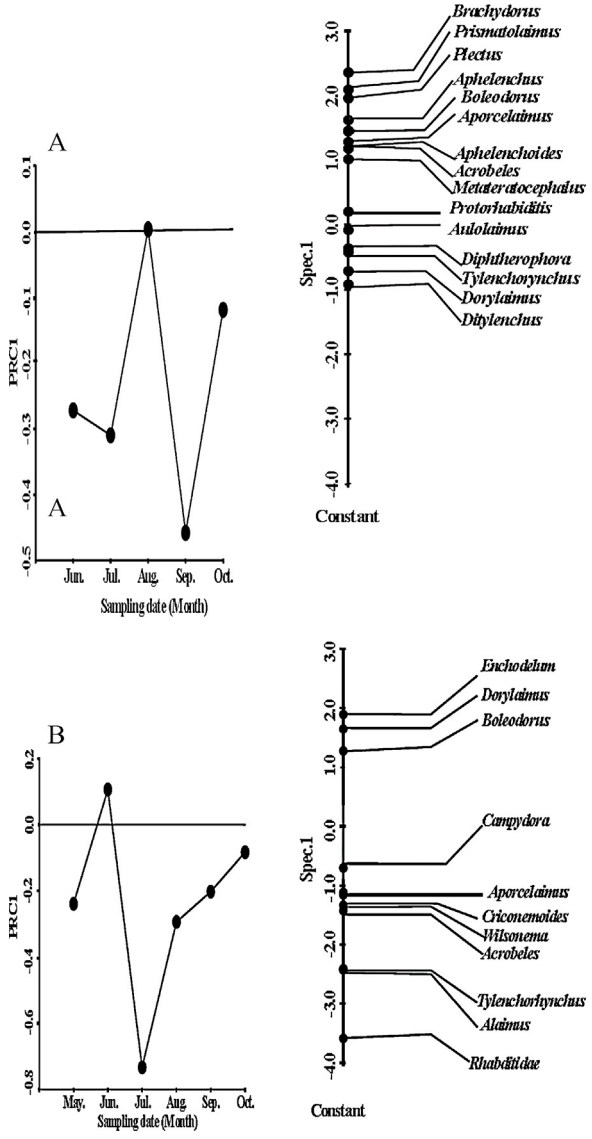

Fig. 2. Principal response curve diagrams of the response of the nematode community in *Bt* cotton plots in relation to that in non-*Bt* cotton plots (used as the control in the PRC analysis) for growing seasons (A) 2009 and (B) 2010. Genera with weights between −0.5 and 0.5 were removed for clarity.

cotton). In 2009, cotton genotype only accounted for 37.5% of the variation in community composition between *Bt* and non-*Bt* fields ($F = 2.268$, $P = 0.205$). The *Brachydorus*, *Prismatolaimus*, *Plectus*, *Aphelenchus*, *Boleodorus*, *Aporcelaimus*, *Aphelenchoides*, *Acrobeles*, and *Metateratocephalus* in *Bt* cotton field were slightly higher than that in non-*Bt* field, while the *Ditylenchus* and *Dorylaimus* are more likely to follow the opposite trends (Fig. 2a). In 2010, cotton genotype only accounted for 32% of the variation in community composition between *Bt* and non-*Bt* fields ($F = 1.921$, $P = 0.206$). The abundances of *Enchodelum*, *Dorylaimus*, and *Boleodorus* in *Bt* cotton field were slightly higher than that in non-*Bt* field, while the abundances of *Rhabditidae*, *Alaimus*, *Tylenchorhynchus*, *Acrobeles*, *Wilsonema*, *Aporcelaimus*, *Criconemoides* and *Campydora* decreased slightly in *Bt* fields, respectively. Finally, the density of *Boleodorus* in *Bt* cotton was a little higher than in non-*Bt* for both years. *Alaimus* densities in *Bt* cotton were comparable with or lower than in non-*Bt*, and the relative abundances of *Acrobeles* and *Aporcelaimus* fluctuated seasonally, ranging from positive to negative.

4. Discussion

The development of genetically modified cotton through *Bt* transformation significantly reduced the use of pesticides into the environment, and thus might alleviate the potential risk of exposure to pesticide for soil fauna. However, *Bt* proteins, such as *Cry1Ab* and *Cry1Ac*, can be released into the soil from cotton residues, root exudates and pollen during growth and after harvest (Gupta and Watson, 2004; Knox et al., 2007). As the growing cultivation acreage of transgenic *Bt* cotton, the environmental impact monitoring after commercial release has attracted increasing attention from public and the scientific community.

Soil nematodes community reflects changes in ecological structure and function of soils in ways more predictable and efficient than other soil flora or fauna. Consequently, any variable which characterized soil nematode community may be used as indicators of soil quality in agricultural ecosystems. *Bt* cotton cultivation in the present study did not affect the abundance of soil nematodes, whether for total taxa or separate trophic group. Our result is in agreement with Li and Liu (2013) who demonstrated the cultivation of transgenic insect-resistant cotton had no significant adverse effects on the abundance of soil nematodes. Other authors reported similar results for other soil organisms. For example, USEPA (2001) proposed no effect of *Bt* cotton on numbers of Collembolan, *Folsomia candida*. Rui et al. (2005) reported no effect on culturable functional bacteria. Shen et al. (2006) revealed no any direct adverse effect of cultivation of transgenic *Bt* cotton on functional bacteria populations or functional diversity of microbial communities in the rhizosphere soil. Mina (2011) found no apparent adverse effect of *Bt* cotton on the populations of soil nematodes, collembola and ants. Li et al. (2012) proposed that long-term cultivation of transgenic insect-resistant cottons had no significant effect on the abundance of soil invertebrates, namely Collembola, Acarina, Araneae, Coleoptera, Dermaptera, Diplura, Isoptera and Diptera.

Crop residues are the major source of carbon in the soil, and root exudates regulate organisms which reside in the rhizosphere. *Bt* cotton and non-*Bt* cotton were alike, except for the presence of the *Bt*-gene. Therefore, the expression, release of *Bt* toxins, the quality and quantity of root exudation, as well as unexpected changes in the chemical compositions of the transgenic cotton plants might be the main factors determining the interaction strength of *Bt* cotton and soil fauna. Head et al. (2002) reported that no *Cry1Ac* protein was detected in any soil samples collected from the fields experiencing 3–6 years continuous cultivation of *Bt* cotton. Additionally, Saxena et al. (2004) found no *Cry* proteins in the root exudates of *Bt* cotton. Finally, there was no apparent difference in chemical composition between *Bt* and non-*Bt* cotton. Coviella et al. (2000) showed no significant differences in N content of *Bt* cotton from that of non-*Bt* cotton. Lachnicht et al. (2004) found that there was no substantial difference in nutrient content of the materials between *Bt* cotton and non-*Bt* cotton. Based on these findings, it is reasonable for *Bt* cotton to exert no apparent adverse impact on soil fauna.

We assumed the cultivation of *Bt* cotton would be detrimental to soil health and expected a simpler nematode community in *Bt* cotton field. Yet, species richness, Shannon-Weaver index, Simpson dominance index, evenness index and Margalef's richness showed a similar level in *Bt* and non-*Bt* cotton field in most cases. According to the proposal from Yeates (2003), larger, more diverse nematode assemblages reflect "more healthy" soils and are "desirable". This finding suggests the cultivation of transgenic *Bt* cotton has no harm to soil health. Similar conclusions also derive from the functional indices. Earlier studies demonstrated that ecological indices such as the Enrichment Index (EI), Structural Index (SI) and Channel Index (CI), which derived from the analysis of nematode fauna, provided useful indicators of the disturbance of the soil environment and

the condition of the soil food web (Ferris et al., 2001). If the cultivation of transgenic *Bt* cotton disrupt soil food web and/or biological properties and processes in the soil, the functional index of the soil nematodes in *Bt* cotton field should be significantly lower than that of non-*Bt* cotton field. We observed significant effects of *Bt* cotton on several functional indices, such as F/B, EI, NCR, PPI, PPI/MI and WI in 2009, yet these effects were not constant across sampling dates.

Finally, we observed significant differences in population shifts and community structure between the two years. Univariate ANOVA analysis showed distinct community composition between *Bt* cotton fields and non-*Bt* cotton fields based on the accumulative sampling of the whole season. However, PRC analysis suggested that planting *Bt* cotton did not alter the composition of soil nematodes from each sampling date. Considering the great fluctuation of the environmental variations, such as temperature and precipitation, parallel studies on other region is necessary. Given the fact that interactions between plants and soil organisms occur over a range of time scales from hours to seasons to millennia and are driven by root exudation, litter-fall and vegetation succession respectively, long-term study is badly needed. The comparable level of population densities and community diversity indices in *Bt* and non-*Bt* cotton fields within this study may be a transient phenomenon. Thus, studies on a large scale over a sufficiently long period to account for environmental variability are indispensable.

5. Conclusion

Analyses of the soil nematode community in the current study indicated that transgenic insect-resistant cotton expressing fusion protein of *Cry1Ac* and *Cry1Ab* from *B. thuringiensis* did exert no significant impacts on abundance, diversity or ecological function (measured as functional indices) of the soil nematodes. Although most of the tested parameters exhibited great variations across the sampling dates, only annual community composition of the soil nematodes demonstrated a detectable difference between the two genotypes. Yeates (2007) proposed that the abundance of soil nematodes reflects food resources and environmental conditions. Therefore, cultivation of *Bt* cotton exert no adverse impact on soil nematodes and related functions. However, the results presented here are just one case study of cotton belonging to the Guo-Kang series grown in northern China over a relative short time period. Long-term studies in multiple locations and with different cotton cultivars are needed to corroborate these findings.

Acknowledgments

We would like to thank Dr. Hai-feng Cao for statistics advice. We also thank two anonymous reviewers for their invaluable suggestions. This project is supported by the National Science and technology support program (no. 2012BAD19B05), National Nature Science Fund of China (no. 31030012) and the Special Program for New Transgenic Variety Breeding of the Ministry of Science and Technology, China (no. 2012ZX08011002).

References

Alphei, J., Bonkowski, M., Scheu, S., 1996. Protozoa, Nematoda and Lumbricidae in the rhizosphere of Hordelymus europaeus (Poaceae): faunal interactions, response of microorganisms and effects on plant growth. Oecologia 106, 111–126.

Blair, J.M., Bohlen, P.J., Freckman, D.W., 1996. Soil invertebrates as indicators of soil quality. In: Doran, J.W., Jones, A.J. (Eds.), Methods for Assessing Soil Quality. SSSA Special Publication No. 49. Soil Science Society of America, Madison, WI, pp. 283–301.

Bongers, T., 1988. De Nematoden van Nederland: Een identificatietabel voor de in Nederland aangetroffen zoetwater-en bodembewonende nematoden. Koninklijke Nederlandse Natuurhistorische Vereiniging.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14–19.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bio-indicator in environmental monitoring. Trends Ecol. Evol. 14, 224–228.

Bottjer, K.P., Bone, L.W., Gill, S.S., 1985. Nematoda: susceptibility of the egg to *Bacillus thuringiensis* toxins. Exp. Parasitol. 60, 239–244.

Coviella, C.E., Morgan, D.J.W., Trumble, J.T., 2000. Interactions of elevated CO_2 and nitrogen fertilization: effects on production of *Bacillus thuringiensis* toxins in transgenic plants. Environ. Entomol. 29, 781–787.

de Geode, R.N.G., Dekker, H.H., 1993. Effect of liming and fertilization on nematode communities in coniferous forest soils. Pedobiologia 37, 193–209.

Donegan, K.K., Seidler, R.J., 1999. Effects of Transgenic Plants on Soil and Plant Microorganisms. US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Western Ecology Division, pp. 415–424.

Ekschimitt, K., Bakonyi, G., Bongers, M., Bongers, T., Bostrom, S., Dogan, H., Harrison, A., Nagy, P., O'Donnell, A.G., Papatheodorou, E.M., Sohlenius, B., Stamou, G.P., Wolters, V., 2001. Nematode community structure as indicator of soil functioning in European grassland soil. Eur. J. Soil. Biol. 37, 263–268.

Ettema, C.H., Lowrance, R., Coleman, D.C., 1999. Riparian soil response to surface nitrogen input: temporal changes in dentrification, labile and microbial C and N pools, and bacterial and fungal respiration. Soil Biol. Biochem. 31, 1625–1628.

Ferris, H., 2010. Form and function: metabolic footprint of nematodes in the soil food web. Eur. J. Soil. Biol. 46, 97–104.

Ferris, H., Bongers, T., 2009. Indices Developed Specifically for Analysis of Nematode Assembleges. CABI, Wallingford, New York, pp. 124–145.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18, 13–29.

Fiscus, D.A., Neher, D.A., 2002. Distinguishing sensitivity of free-living soil nematode genera to physical and chemical disturbances. Ecol. Appl. 12, 565–575.

Freckman, D.W., Ettema, C.H., 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agric. Ecosyst. Environ. 45, 239–261.

Fu, S.L., Coleman, D.C., Hendrix, P.F., Crossley Jr., D.A., 2000. Responses of trophic groups of soil nematodes to residue application under conventional tillage and no-till regimes. Soil Biol. Biochem. 32, 1731–1741.

Gupta, V.V.S.R., Watson, S., 2004. Ecological Impacts of GM Cotton on Soil Biodiversity: Below-ground Production of Bt by GM Cotton and Bt Cotton Impacts on Soil Biological Processes. Australian Government Department of the Environment and Heritage, CSIRO Land and Water, pp. 1–72.

Head, G., Surber, J.B., Watson, J.A., Martin, J.W., Duan, J.J., 2002. No detection of *Cry1Ac* protein in soil after multiple years of transgenic *Bt* cotton (Bollgard) use. Environ. Entomol. 31, 30–36.

Huang, J.K., Pray, C.E., 2002. Economic impacts of Bt cotton in China. In: The 7th International Symposium on the Biosafety of Genetically Modified Organisms, Beijing, China, pp. 102–112.

Icoz, I., Stotzky, G., 2008. Fate and effects of insect-resistant *Bt* crops in soil ecosystems. Soil Biol. Biochem. 40, 559–586.

Knox, O.G., Gupta, V.V., Nehl, D.B., Stiller, W.N., 2007. Constitutive expression of Cry proteins in roots and border cells of transgenic cotton. Euphytica 154, 83–90.

Lachnicht, S.L., Hendrix, P.F., Potter, R.L., Coleman, D.C., Crossley Jr., D.A., 2004. Winter decomposition of transgenic cotton residue in conventional-till and no-till systems. Appl. Soil Ecol. 27, 135–142.

Li, X., Liu, B., 2013. A 2-year field study shows little evidence that the long-term planting of transgenic insect-resistant cotton affects the community structure of soil nematodes. PLOS ONE 8, e61670, http://dx.doi.org/10.1371/journal.pone.0061670.

Li, X., Liu, B., Wang, X., Han, Z., Cui, J., Luo, J., 2012. Field trials to evaluate effects of continuously planted transgenic insect-resistant cottons on soil invertebrates. J. Environ. Monit. 14, 1055–1063.

Liang, W.J., Lou, Y.L., Li, Q., Zhong, S., Zhang, X.K., Wang, J.K., 2009. Nematode faunal response to long-term application of nitrogen fertilizer and organic manure in Northeast China. Soil Biol. Biochem. 41, 883–890.

Magurran, A.E., 2004. Measuring Biological Diversity. Blackwell Science Ltd, USA.

Meadows, J., Gill, S., Leon, W., 1990. *Bacillus thuringiensis* strains affect population growth of the free-living nematode Turbatrix aceti. Invertebr. Reproduct. Dev. 17, 73–76.

Mina, U., Shakeel, A., Khan, S.A., Choudhary, R., Aggarwal, P.K., 2008. An approach for impact assessment of transgenic plants on soil ecosystem. Appl. Ecol. Environ. Res. 6, 1–19.

Mina, U., 2011. Effect of *Bt* cotton on enzymes activity and microorganisms in rhizosphere. J. Agric. Sci. 3, 96–104.

Oostenbrink, M., 1960. Estimating nematode populations by some selected methods. Nematology 6, 85–102.

Rui, Y.K., Yi, G.X., Zhao, J., Wang, B.M., Li, Z.H., Zhai, Z.X., He, Z.P., Li, Q., 2005. Changes of *Bt* toxin in the rhizosphere of transgenic *Bt* cotton and its influence on soil functional bacteria. World J. Microbiol. Biotechnol. 21, 1279–1284.

Sarkar, B., Patra, A.K., Purakayastha, T.J., Megharaj, M., 2009. Assessment of biological and biochemical indicators in soil under transgenic *Bt* and non-*Bt* cotton crop in a sub-tropical environment. Environ. Monit. Assess. 156, 595–604.

Saxena, D., Stewart, C.N., Altosaar, I., Shu, Q., Stotzky, G., 2004. Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B.*

thuringiensis corn, potato, and rice but not of *B. thuringiensis* canola, cotton, and tobacco. Plant Physiol. Biochem. 42, 383–387.
Shen, R.F., Cai, H., Gong, W.H., 2006. Transgenic Bt cotton has no apparent effect on enzymatic activities or functional diversity of microbial communities in rhizosphere soil. Plant Soil 285, 149–159.
Sochová, I., Hofman, J., Holoubek, I., 2006. Using nematodes in soil ecotoxicology. Environ. Int. 32, 374–383.
Sun, C.X., Chen, L.J., Wu, Z.J., Zhou, L.K., Shimizu, H., 2007. Soil persistence of *Bacillus thuringiensis*(*Bt*) toxin from transgenic *Bt* cotton tissues and its effect on soil enzymes activities. Biol. Fert. Soils 43, 617–620.
Tapp, L., Calamai, L., Stotzky, G., 1995. Insecticidal activity of the toxins from *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* adsorbed and bound on pure and soil clays. Appl. Environ. Microbiol. 61, 1786–1790.
Townshend, J.L., 1963. A modification and evaluation of the apparatus for the Oostenbrink direct cottonwood filter extraction method. Nematologica 9, 106–110.
Twinn, D.C., 1974. Nematodes. In: Dickinson, C.H., Pugh, G.J.F. (Eds.), Biology of Plant Litter Decomposition. Academic Press, London, pp. 421–465.
US Environmental Protection Agency (EPA), 2001. SAP Report No. 2000–07, Sets of Scientific Issues being Considered by the Environmental Protection Agency Regarding: Bt Plant Pesticides Risk and Benefits Assessments, http://www.epa.gov/scipoly/sap/2000/octoberfinal.pdf
Van den Brink, P.J., Ter Braak, C.J.F., 1998. Multivariate analysis of stress in experimental ecosystems by Principal Response Curves and similarity analysis. Aquat. Ecol. 32, 163–178.
Van den Brink, P.J., Ter Braak, C.J.F., 1999. Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. Environ. Toxicol. Chem. 18, 138–148.
Verschoor, B.C., de Goede, R.G.M., De Hoop, J.W., De Vries, F.W., 2001. Seasonal dynamics and vertical distribution of plant-feeding nematode communities in grasslands. Pedobiologia 45, 213–233.
Wasilewska, L., 1997. The relationship between the diversity of soil nematode communities and the plant species richness of meadows. Ekol. Pol. 45, 719–732.
Wu, K.M., Lu, Y.H., Feng, H.Q., Jiang, Y.Y., Zhao, J.Z., 2008. Suppression of cotton bollworm in multiple crops in China in areas with *Bt* toxin-containing cotton. Science 321, 1676–1678.
Yang, B., Liu, X.H., Chen, H., Ge, F., 2013. The specific responses of Acari community to *Bt* cotton cultivation in agricultural soils in northern China. Appl. Soil Ecol. 66, 1–7.
Yeates, G.W., 2003. Nematodes as soil indicators: functional and biodiversity aspects. Biol. Fert. Soils 37, 199–210.
Yeates, G.W., 2007. Abundance, diversity, and resilience of nematode assemblages in forest soils. Can. J. Forest. Res. 37, 216–225.
Yeates, G.W., Bongers, T., 1999. Nematode diversity in agroecosystems. Agr. Ecosyst. Environ. 74, 113–135.
Yeates, G.W., Bongers, T., de Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993a. Feeding habits in soil nematode families and genera: an outline for soil ecologists. J. Nematol. 25, 315–331.
Yeates, G.W., Wardle, D.A., Watson, R.N., 1993b. Relationships between nematodes soil microbial biomass and weed-management strategies in maize and asparagus cropping systems. Soil Biol. Biochem. 25, 869–876.