

Diversity of arthropod community in transgenic poplar-cotton ecosystems

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ABSTRACT. Poplar-cotton agro-ecosystems are the main agricultural planting modes of plain cotton fields in China. Here, we performed a systematic survey of the diversity and population of arthropod communities in four different combination of poplar-cotton ecosystems, including I) non-transgenic poplar and non-transgenic cotton fields; II) non-transgenic poplar and transgenic cotton fields [*Bacillus thuringiensis* (Bt) cotton]; III) Bt transgenic poplar (high insect resistant strain Pb29) and non-transgenic cotton; and IV) transgenic poplar and transgenic cotton fields, over a period of 3 years. Based on the statistical methods used to investigate community ecology, the effects of transgenic ecosystems on the whole structure of the arthropod community, on the structure of arthropods in the nutritive layer, and on the similarity of arthropod communities were evaluated. The main results were as follows: the transgenic poplar-cotton ecosystem has a stronger inhibitory effect on insect pests and has no impact on the structure of the arthropod community, and therefore, maintains the diversity of the

arthropod community. The character index of the community indicated that the structure of the arthropod community of the transgenic poplar-cotton ecosystem was better than that of the poplar-cotton ecosystem, and that system IV had the best structure. As for the abundance of nutritional classes, the transgenic poplar-cotton ecosystem was also better than that of the non-transgenic poplar-cotton ecosystem. The cluster analysis and similarity of arthropod communities between the four different transgenic poplar-cotton ecosystems illustrated that the structure of the arthropod community excelled in the small sample of the transgenic poplar-cotton ecosystems.

Key words: Arthropod community; *Bacillus thuringiensis* transgene; Poplar-cotton ecosystem

INTRODUCTION

Crops generated by genetic modifications that confer pest or disease resistance play an important role in crop production (Perlak et al., 1990). Transgenes that confer insect resistance rely on the transformation of insecticidal toxin genes from microbes to the target organism, among which the *Bacillus thuringiensis* (Bt) gene is the most widely used. The Bt endotoxin is encoded by various *Cry* genes and has specific activities against insect species of different orders, and its main mechanism of action is to paralyze the digestive tract of ingested pests (Gill et al., 1992).

With a variety of transgenic organisms, such as plants, and related products entering large-scale commercialization, the effects they might have on human health and the ecological environment have become of interest (Bawa and Anilakumar, 2013). Insect resistant forms of major crop varieties such as cotton, rice, maize, soybean, and wheat have been developed, and some of these have passed field and environmental release trials and are now widely cultivated on a commercial scale (Paul et al., 2006). At the same time, the potential ecological risk of transgenic plants released into the environment is receiving more attention. Biological safety is the main issue that hinders the large-scale plantation of transgenic plants. It is generally agreed that the main concerns about transgenic plants include: the development of drug resistance in the target organisms, the genes escaping and polluting the environment, the loss of biodiversity, and indirect effects on non-target organisms and the destruction of ecological balance (Conner et al., 2003; Andow and Zwahlen, 2006; Warwick et al., 2009). Presently, researchers have extensively researched the effects of gene-escaping pollution of the transgenic plants, the adaptation and control of pest resistance, and the adverse effects on natural predators (Chen et al., 2006; Gassmann, 2012; Tabashnik et al., 2012).

Genes that confer insect-resistance enable the host plant to kill the target pest, and some genes have an additional broad-spectrum effect on non-target insects. Therefore, in plantations, these genes are likely to induce certain effects on individual insects, populations, and on the entire insect community, and, consequently, might affect the functioning of the entire ecological system (Xu et al., 2011; Bai et al., 2012). Therefore, this is an important research topic for ecological risk assessments, the utility of using the theory of community ecology

to analyze transgenic plants, and to monitor their effects on the regularity and mechanism of target insect and non-target insect community, and thus, determine the evaluation index. Many field studies have investigated the changes in diversity and abundance of target and non-target taxa using different transgenic crops. Some studies have also presented a number of possible mechanisms through which Bt plants might affect the insect community from the level of food webs, including predator/prey and host/parasitoid systems (Von Burg et al., 2011; Xu et al., 2012). However, most of the studies performed to date have used single farmland or forestland as the subject area, while comparatively less attention has been focused on defining the effects of intercrops and agro-ecosystems on insect populations. Therefore, it is important to analyze the potential impacts of transgenic plants on insect communities and to assess the ecological risk resulting from these impacts.

An agroforestry system refers to the artificial arrangement of perennial woody plants (such as arbors, shrubs, and bamboos), other cultivated plants (such as crops, medicinal plants, and economical important plants), and animals together in the same land management unit and in the same space or time with the principle of ecological economics to form a multi-group, multi-level, multi-product, multi-benefit artificial ecosystem. Studies have been carried out on the arthropod community of the compound ecological system, but most of these studies take the land or farmland as the research subject, while the woodland and farmland in areas with agroforestry ecosystem status get less attention. Liu et al. (2001) conducted a comparative study on the arthropod community between simple and complex litchi orchards in Dongguan, Guangdong, from September 1997 to September 1998. They showed that the complex litchi-herbage orchard had an increasing number of arthropod communities, species, uniformity, and enhanced diversity, compared with those in single orchard farmland. Zeng et al. (2008) investigated the arthropod community of the jujube-wheat ecosystem, the jujube-grass ecosystem, and the pure wheat system and found that the plant community diversity indices of the agro-forestry ecosystems were higher than those of the pure agriculture system and that the difference in functional groups among the three samples was not significant.

The poplar-cotton ecosystem is the main agricultural planting mode in plain cotton regions of China. Transgenic cotton that produce the Bt toxin Cry1Ac have been planted in the USA since 1996, and have been commercially available in China since 1997 (James, 2006). Transgenic poplars that contain the Bt toxin gene have also been developed and commercialized (Rao et al., 2001). Following the introduction of transgenic poplar and cotton into the fields, these plants co-existed and interacted in this single ecosystem. The bioefficacy and biosafety of this system need to be determined because of the diverse performance and interactions of transgenic crops in different ecosystems. Moreover, the status of combined forestland and farmland in this regard has attracted less attention in agroforestry ecosystems. Referring to the development of resistance in target insects, many countries have proposed strategies to decelerate the development of insect resistance. For example, some countries take "refuge" countermeasures to manage or mitigate the development of resistance to the cotton bollworm. Bt crops constitutively express toxin, but the rotation and intercropping of the Bt crop (namely, crops with different Bt toxin genes) and non-transgenic crops will contribute to delaying pest resistance. Chinese scholars have also accumulated data on the management of transgenic cotton resistance, for example, the presence of corn and castor in the field could play a role in blocking and refuge. However, studies on the application of an agroforestry system that is used to delay the development of pest resistance are still lacking. The present study

on arthropod communities in the transgenic Bt poplar-cotton ecosystem will be helpful for the reasonable promotion and application of transgenic plant safety and will also contribute to the development of a transgenic compound ecological system for long-term monitoring, tracking, and study of insect-resistant transgenic trees and possible effects of these agricultural crops on the ecosystem.

MATERIAL AND METHODS

Materials

Non-transgenic poplar 741 (abbreviated as CK poplar), a strain of 741 poplar with high insect-resistance Pb29 (abbreviated as high-resistant poplar Pb29), non-transgenic cotton-Xinluzao No. 36 (CK cotton as abbreviation), and transgenic cotton-Handan 109 (Bt cotton) were provided by the College of Forestry, Agricultural University of Hebei. The age of the poplar trees was 7 to 8 years.

Poplar 741 was cultivated as a superior white poplar hybrid clone by the Agricultural University of Hebei. The hybrid combination was [*Populus alba* L. x (*P. davidiana* Dode. + *P. simonii* Carr.)] x *P. tomentosa* Carr. (Jiang et al., 1991). Transgenic hybrid poplar 741 was obtained by transformation of Bt insecticidal protein gene (*BtCryIAc*) and the arrowhead proteinase inhibitor (*API*) gene into clones of *Populus tomentosa* 741 using *Agrobacterium* (Yang et al., 2006). The Pb29 used in this study was a high insect-resistant strain of transgenic hybrid poplar 741.

Xinluzao No. 36 (Xinshi K8) was used as the female parent and is a hybrid of the self-fertile and early-maturing high-yield strain 1304. The disease-resistant strain BD103 from the Plant Protection Institute, Chinese Academy of Agricultural Science, was used as the male parent, and was hybridized by Xinjiang Shihezi Cotton Research Institute in 1997 through sexual hybridization breeding. Handan 109 is a new variety of disease-resistant cotton with high-yield and good quality that is corporately bred by the Handan Academy of Agricultural Science and the Biotechnology Research Institute of Chinese Academy of Agricultural Science (Ban, 2005).

Study area

The experimental release field was approved by the State Forestry Bureau of the Biological Genetic Engineering Safety Committee. The experimental forest comprised approximately 1 hm² of the national forest cultivation area in Zhuozhou city, Hebei Province, China (39°21'-39°36' N, 115°44'-116°15'E). This area is a warm temperate semi-humid monsoon region with a typical continental monsoon climate. The annual average temperature is around 11.6°C. The annual average ground temperature is 14.2°C and the annual average depth of frozen soil is 40 cm. The area receives an annual average of 2569 h of sunshine, the annual average rainfall is 617 mm, and the frost-free season lasts approximately 178 days. CK poplar and highly resistant poplar Pb29 were planted in approximately 25 x 100 m regions and the interval zone between samples was 100 x 100 m. Next to each poplar field, CK and Bt cotton were planted in about 25 x 50 m regions with oriental arborvitae as the interval zone between different types of cotton fields. Thus, the test field was divided into four distinct agro-

ecosystems: CK poplar and CK cotton; CK poplar and Bt cotton; highly resistant Pb29 poplar and CK cotton; and highly resistant Pb29 poplar and Bt cotton. The corresponding sample numbers assigned to the four systems were I, II, III, and IV (Figure 1). Normal agronomic practices were followed for raising the crop and no insecticides were applied during the cropping season.

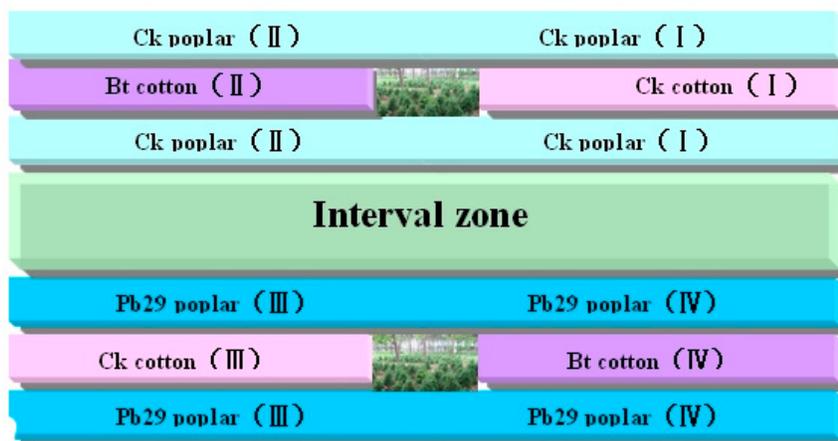


Figure 1. Profile of the mixed planting pattern. Poplars were planted at approximately 25 x 100 m region, the interval zone between samples was 100 x 100 m. Neighboring each poplar field, cotton was planted in a region of 25 x 50 m with oriental arborvitae as the interval zones between different types of cotton fields. Hence, the test field is classified into four distinct agro-ecosystems: CK poplar and CK cotton (I); CK poplar and Bt cotton (II); high-resistant Pb29 poplar and CK cotton (III); and high-resistant Pb29 poplar and Bt cotton (IV).

Research methodology

A systematic investigation into the arthropod community of a poplar-cotton ecosystem in the sample area was carried out for 3 years from June to October in 2009 to 2011. A sample survey and a detailed record were conducted every 10 days. Any unknown insect types were collected in a unified label and taken to the lab for identification (Yuan et al., 1996; Song et al., 2011).

Survey of the poplar grove arthropod community

Survey on the plant arthropod community

Five strains of tree were randomly selected from the CK poplar and highly resistant Pb29 sample areas, and 15-cm long branches were selected from the upper, middle, and lower layers, which corresponded to the south, east, north, and west. The composition of the arthropod community on the above samples was recorded. The species composition and quantities of the arthropod community on the trunk were calculated at around 5 m from the ground surface. For CK and Bt cotton, five 1 x 1-m sample areas were randomly selected, and about 5 to 6 strains of cotton from each sample area were investigated and the species composition of the

arthropod community was recorded. Aphids and mites were collected from the third branch leaves from the top of the specimen and counted. Any insects that could not be identified were placed into test tubes filled with alcohol or into a poison bottle with ethyl acetate and taken to the lab for further identification.

Survey on the shrub layer arthropod community

An insect net was used to sample the flying arthropod community by randomly sweeping 50 times in various layers of shrub. Next, the insects were euthanized by ethyl acetate for 10 min and were then marked for identification. Finally, the composition of the arthropod community in the shrub layer was determined.

Survey on the surface layer arthropod community

Five 0.5 x 0.5-m samples were randomly chosen from layers of various sample areas, and the types and quantities of the arthropod community on the surface were investigated and recorded.

Classification of the nutritional layer and the functional groups of the arthropod community

According to the feeding habits of different arthropod species, the arthropod communities are divided into three trophic levels: basal species, which do not prey on other species but are preyed upon, consisting mainly of herbivorous pests and a variety of neutral insects (including mosquitoes, springtails, and saprophytic flies); intermediate species, which prey on other species and are preyed upon on, consisting mainly of small carnivorous species (such as *Erigone*, predacious bugs, ladybirds, lacewings, and parasitic wasps); and top species, which prey on other species and are not preyed upon, consisting mainly of ferocious feeders and large- and medium-sized migratory predators (such as mantis, jumping spiders, tarantulas, and crab spiders) (Adans, 1985; Blondel, 2003). Based on the concept of functional groups proposed by Root (1967), similar communities that take advantage of the same level of habitat resources were divided into multiple aggregates with similar feeding behavior in terms of resource use and habitat selection. This paper focuses mainly on the use of system classification, space distribution, and similar feeding habits to characterize the functional group. All the living organisms in the arthropod community of the poplar-cotton ecosystem are divided into different functional groups as required.

Data processing method (Hill, 1973; Gema et al., 2008)

Calculation of the characteristic index of the arthropod community

Richness (S): the number of the species in the community. The number of individuals:

$$N = \sum_{i=1}^s n_i \quad (\text{Equation 1})$$

where, n_i = the number of the kind i species, N = the total number of all the species diversity index:

$$H = -\sum_{i=1}^s P_i \ln P_i, P_i = n_i/N \quad (\text{Equation 2})$$

evenness: $J = H/\ln S$, H = Shannon's diversity index, S = the number of species dominant concentration (Simpson index):

$$N = \sum_{i=1}^s P_i^2, P_i - \text{relative abundance} \quad (\text{Equation 3})$$

Calculation of the similarity index of the arthropod community

Community similarity coefficients (C_{ij}): $C_{ij} = 2 \times \sum \text{Min}(x_{ik}, x_{jk}) / \sum (x_{ik} + x_{jk})$, ($i, j = 1, 2, 3, \dots, n$), s is the number of species; where x_{ik}, x_{jk} indicate different departments of the number of the K species.

Community dissimilarity coefficients = $1 - C_{ij}$.

Clustering analysis of the arthropod community

Clustering analysis uses the similarity coefficient method to determine the relationship among clustering indicators. After determining the similarity coefficient among the indicators, they were clustered by the average class method of the system clustering method. The calculation was analyzed using the DPS v7.05 software.

RESULTS AND DISCUSSION

Total arthropod community composition of the transgenic poplar-cotton ecosystem

Samples were obtained on 30 occasions from June to October during 2009 to 2011 (10 times per year) in the experimental field of the national forest cultivation area of the Zhuozhou City, Hebei Province. The arthropod community was systematically investigated and overall, 179,599 arthropods were collected. Among these, 176,971 individuals belonged to Insecta (Homoptera, Hymenoptera, Hemiptera, Lepidoptera, Thysanoptera, Coleoptera, Orthoptera, Diptera, Neuroptera, Mantodea, and Odonata), 679 belonged to Acarina of Araneae, 1806 belonged to Arachnoidea, and 143 belonged to Myriapoda.

The 78,113 arthropod communities collected in 2009 belonged to 3 classes, 14 orders, 71 families, and 104 species; a total of 68,550 arthropod communities in 2010 belonged to 3 classes, 13 orders, 74 families, and 103 species; and 32,936 communities collected in 2011 belonged to 3 classes, 12 orders, 85 families, and 154 species.

There was no significant change in the number of species observed in the first 2 years. Compared to 2009, in 2010, the total number of individuals had reduced by 9563, and compared to that 2010, in 2011, the numbers had declined by 35,614. Comparing 3 years of data, Coleoptera, Diptera, Araneae, Lepidoptera, Hemiptera, and Homoptera appear to be in a dominant position in arthropod communities in terms of the number of families and species. However, Homoptera insects were dominant in terms of the number of individuals,

accounting for 52.08, 60.07, and 66.81% of the individuals sampled. In 2009, Hymenoptera and Hemiptera accounted for 16.28 and 15.49% of individuals, respectively. In 2010, Hymenoptera and Lepidoptera accounted for 23.48 and 7.72% individuals, respectively. In 2011, Hymenoptera and Hemiptera accounted for 17.62 and 6.60% of individuals, respectively. As can be seen from data obtained over 3 years, Homoptera account for a large proportion of individuals, followed by Hymenoptera, Hemiptera, and Lepidoptera, which were also present in high numbers.

Species composition of the arthropod community in the transgenic poplar-cotton ecosystem

Variation in the species composition is characteristic of insect communities. The composition and variation of different communities are reflective of the interactions between the arthropod community and the environmental condition of different poplar-cotton ecosystems.

As shown in Tables 1 to 3, the composition of the arthropod community in three different poplar-cotton ecosystems, systems I, II, and III, were found to be similar, but the number of species in system IV increased from 2009 to 2010 when four different kinds of species were compared. Between 2010 and 2011, the total number of species increased in all four systems. The results from all years showed that there were more Coleoptera species, that is, 21 species in 2009, 17 in 2010, and 18 in 2011, accounting for 25.61, 18.28 and 15.25% of the total species present in each system. The majority of Coleoptera insects were predatory groups living on plants, such as *Harmonia axyridis*, *Propylaea japonica*, and *Thinopinus pictus*, which are the major natural enemies of the pests, followed by some leaf-eating insects, such as leaf beetles, which were also found to occupy a certain proportion of the community, indicating that it has an important position in the arthropod community. As a special group, Araneae insects were also found to occupy a superior position in the arthropod community. These are predatory insects that live on the plants or on the ground, and are major natural enemies of insect pests. The dominant groups of Hemiptera are Tingidae, Pentatomidae, and the predatory natural enemy Anthocoridae. The dominant groups of Lepidoptera are Arctiidae, Notodontidae, and Tortricidae. Aphidoidea, Aleyrodidae, and leafhoppers are the dominant groups of Homoptera. The dominant groups of Diptera are Muscidae, followed by Culicidae, Calliphoridae, Drosophilidae, Sarcophagidae, and Syrphidae. Formicidae account for a large proportion of Hymenoptera, mainly due to their presence in large numbers, followed by Chalcididae and Eumenidae. The number of other species was fewer, and these accounted for a smaller proportion of the insect community.

In terms of the numbers of individuals comprising the community, there was no significant difference among the four poplar-cotton ecosystems in 2009. The individual number of insects in system IV was relatively low (13,701) in 2010. In 2011, the number of individuals of four kinds of poplar-cotton ecosystem decreased compared to that observed during the first 2 years, especially the number of individuals observed in system IV, which decreased by 6403. During the 3 years of the study, the numbers of Homoptera, Hemiptera, and Hymenoptera individuals accounted for a larger proportion of the arthropod community, while the numbers of Neuroptera, Acarina, and Mantodea individuals were relatively low.

Table 1. Number of species (NS) and individuals (NI) in the transgenic poplar-cotton systems (2009).

Insect group	I		II		III		IV	
	NS	NI	NS	NI	NS	NI	NS	NI
Homoptera	11	10,446	8	10,630	8	9650	9	9958
Hymenoptera	9	4004	8	2616	10	2881	8	3213
Hemiptera	8	1132	6	3621	10	3456	7	3888
Lepidoptera	10	1387	8	827	7	1884	8	710
Thysanoptera	2	548	2	513	2	596	2	778
Coleoptera	10	548	21	584	17	237	15	462
Orthoptera	5	314	5	273	7	162	6	230
Diptera	13	269	5	218	7	174	3	194
Acarina	2	89	2	108	2	228	2	152
Neuroptera	2	19	2	10	2	17	2	8
Mantodea	1	2	1	3	1	10	1	3
Odonata	0	0	1	1	0	0	0	0
Araneida	11	224	11	245	10	281	11	211
Myriapoda	2	50	2	39	1	8	2	2
Total	86	19,032	82	19,688	84	19,584	76	19,809

Table 2. Number of species (NS) and individuals (NI) in the transgenic poplar-cotton systems (2010).

Insect group	I		II		III		IV	
	NS	NI	NS	NI	NS	NI	NS	NI
Homoptera	11	12,149	8	11,803	8	10,331	10	6893
Hymenoptera	11	3695	13	3082	9	4853	9	4565
Lepidoptera	12	1497	9	1490	8	1504	11	801
Thysanoptera	2	547	3	389	3	248	3	229
Neuroptera	1	16	1	26	1	53	1	49
Diptera	8	286	8	231	10	289	9	239
Coleoptera	17	218	16	206	8	192	15	357
Orthoptera	6	233	5	238	6	240	7	228
Hemiptera	11	150	10	131	8	120	10	190
Acarina	2	21	2	10	2	8	2	20
Mantodea	0	0	1	3	1	2	1	1
Araneida	11	208	12	167	11	173	11	125
Myriapoda	1	8	1	14	1	18	1	4
Total	93	19,028	89	17,790	76	18,031	90	13,701

Table 3. Number of species (NS) and individuals (NI) in the transgenic poplar-cotton systems (2011).

Insect group	I		II		III		IV	
	NS	NI	NS	NI	NS	NI	NS	NI
Homoptera	16	4976	14	6511	14	6349	15	4167
Hymenoptera	16	1489	15	1294	14	1384	15	1636
Hemiptera	20	402	20	520	18	582	16	670
Diptera	16	252	17	281	18	335	17	435
Lepidoptera	9	121	10	37	10	100	9	34
Coleoptera	18	87	14	84	14	85	14	80
Orthoptera	6	84	6	95	5	81	5	104
Neuroptera	3	67	3	75	3	55	4	46
Araneida	11	56	10	36	11	36	7	44
Thysanoptera	1	39	1	56	1	17	2	71
Acarina	2	18	1	13	2	21	1	9
Mantodea	0	0	0	0	0	0	1	2
Total	118	7591	111	9002	110	9045	106	7298

Number of species and individual insects in the arthropod sub-community of the transgenic poplar-cotton ecosystem

The arthropod community can be divided into three groups according to its impact on food production for humans and lives: the pest sub-community, natural enemy sub-community, and neutral arthropod community. In this survey, little difference was observed in these three groups between the four ecosystems in 2009. However, the number of individual insects in the pest sub-community was lowest in system I (14,139) and the highest in system II (16,271), and this difference was due to the stinkbug of Hemiptera. Referring to the natural enemy sub-community, the number of individuals in system I (4643) was the largest, of which Hymenoptera and Araneida accounted for a large proportion. As for the sub-community in the neutral arthropod community, flies and mosquitoes were the reason for the numbers of individuals in systems I and II to be larger than those of systems III and IV.

The observations made in the sub-communities in 2010 were different from those observed in 2009. As for the pest sub-community, the number of species in system I (47) was higher than in system III for 11 species, and the number of individuals in system I (14,701) was higher than in system IV by 6323. The piercing-sucking group of insects was responsible for this difference. As for the natural enemy sub-community, the number of species in system IV (38) was higher than that in system II (36), but the numbers of individuals in systems III and IV (5236 and 5087, respectively) were relatively higher than those in systems I and II (4041 and 3425, respectively), presumably because both systems III and IV contain transgenic poplar. The number of species in the neutral arthropod community in systems II and IV in 2010 was higher than that in 2009 at over five kinds, and the number of individuals also increased.

The numbers of species making up the pest sub-community and the natural enemy sub-community in 2011 were significantly different between the four ecosystems. In the pest sub-community, the number of species in system I (65) was significantly higher than in the other three systems, and the number of individuals (5657) was lower than systems II (7243) and III (7165), but higher than system IV (5094). In the natural enemies sub-community, the number of species in system IV (1846) was higher than that in the other three systems (1740, 1549, and 1621, respectively), but there was no obvious difference in the numbers of individuals. In the neutral arthropod community, the number of species in the four ecosystems was not different, but the number of individuals in system IV (358) was greater than that in the other three systems (194, 210, and 259, respectively).

Comprehensive analyses of the data obtained during the first 2 years showed that the individual numbers making up the natural enemy sub-community and the neutral arthropods sub-community of poplar-cotton ecosystems II, III, and IV increased, while the individual numbers making up the pest sub-community declined. Thus, these results show that the transgenic poplar-cotton ecosystem was involved in reducing the number of pest sub-communities and in maintaining the stability of the arthropod community. In the third year, the numbers of species and individuals of the neutral arthropod community were unchanged compared to those of the previous 2 years, but the number of species in the pest and natural enemy sub-communities increased while the number of individuals declined significantly, which might be because the transgenic poplar-cotton ecosystem can effectively resist the proliferation of the pest sub-community. Meanwhile, this also showed that the transgenic poplar-cotton ecosystem would not affect the biological diversity of the three sub-communities mentioned above.

Structure characteristic index of the arthropod community in the transgenic poplar-cotton ecosystem

Characteristics of the community, such as the community richness, evenness, dominance, and diversity, could reflect the dynamic characteristics of insect species and the composition of a community, and could also determine linkages between the various communities to consequently reflect static and dynamic variation of communities.

Characteristic index of arthropod communities in transgenic poplar-cotton ecosystem

The characteristic indexes of arthropod communities in different poplar-cotton ecosystem in the 3 years of this study are shown in Table 4.

Table 4. Characteristic indexes of arthropod communities in the transgenic poplar-cotton systems.

Year	Sample	Diversity index	Evenness	Dominant concentration
2009	I	2.7989	0.6175	0.0966
	II	2.6479	0.5842	0.1209
	III	2.6258	0.5753	0.1171
	IV	2.7638	0.6189	0.0952
2010	I	2.3941	0.5563	0.1412
	II	2.3403	0.5472	0.1449
	III	2.2675	0.5302	0.1608
	IV	2.2874	0.5235	0.1690
2011	I	2.7534	0.5772	0.1199
	II	2.6635	0.5656	0.1516
	III	2.6327	0.5601	0.1480
	IV	2.9127	0.6246	0.0918

Survey data collected over 3 years showed that variation in the characteristic index of the transgenic poplar-cotton ecosystem was very complicated, but the difference between different ecosystems in the same year was not significant. As shown in Table 4, there is no difference between the characteristic indexes of four ecosystems in 2009. However, the diversity and evenness index of systems I and IV were relatively higher, and the dominant concentration index of system IV was the lowest. Therefore, the structure of the arthropod community in transgenic poplar-cotton ecosystem IV was relatively better than the other three ecosystems.

In 2010, the difference between the characteristic indexes of different ecosystems was not significant. The diversity and evenness indexes of systems I and II were slightly higher than those of systems III and IV, while the dominant concentration index was relatively low. Compared to the results obtained in 2009, the diversity and evenness indexes of all four ecosystems declined, but the dominant concentration index increased (Table 4).

Table 4 also shows that the diversity and evenness indexes of four ecosystems increased significantly between 2010 and 2011. However, the dominant concentration index of all four ecosystems declined; among which the dominant concentration index of system IV decreased to a level lower than that observed in 2009, indicating that the structure of the arthropod community in the transgenic poplar-cotton ecosystem IV is relatively better than that in other ecosystems.

Characteristic index of the arthropod sub-community in transgenic poplar-cotton ecosystems

The diversity index, evenness, dominant concentration index of pest sub-community, the natural enemy sub-community, and the neutral arthropods sub-community of the four poplar-cotton ecosystems in the 3 years were calculated and are shown in Table 5.

Table 5. Characteristic index of arthropod communities' species troop in the transgenic poplar-cotton systems.

Year	Sample	Pest sub-community			Natural enemy sub-community			Neutral arthropod sub-community		
		Diversity index	Evenness	Dominant concentration	Diversity index	Evenness	Dominant concentration	Diversity index	Evenness	Dominant concentration
2009	I	2.4285	0.6343	0.1300	1.4149	0.4012	0.4161	2.1110	0.8230	0.1418
	II	2.2454	0.5899	0.1641	1.6696	0.4624	0.3323	1.6722	0.6974	0.2900
	III	2.2433	0.5734	0.1584	1.5320	0.4344	0.3831	1.7987	0.7238	0.2754
	IV	2.3923	0.6322	0.1274	1.6045	0.4550	0.3664	1.5247	0.6939	0.2805
2010	I	1.9138	0.5188	0.2095	1.4244	0.4275	0.3560	1.4360	0.8015	0.2928
	II	1.8336	0.5117	0.2121	1.5648	0.4647	0.3054	1.5935	0.8189	0.2453
	III	1.6838	0.4699	0.2708	1.3498	0.3969	0.3647	1.5944	0.8899	0.2264
	IV	1.6448	0.4522	0.3144	1.3946	0.3955	0.3724	1.4648	0.7528	0.2793
2011	I	2.1477	0.5145	0.1921	1.9748	0.5353	0.2502	1.9470	0.7591	0.2111
	II	2.0926	0.5132	0.2247	2.1746	0.5818	0.2022	1.7225	0.7481	0.2166
	III	2.0497	0.5027	0.2235	2.0096	0.5448	0.2383	1.9016	0.7930	0.1793
	IV	2.4119	0.5966	0.1401	1.6452	0.4556	0.3540	1.3556	0.5455	0.3652

The diversity and evenness indexes of systems I and IV were higher in the pest sub-community in 2009, but the dominant concentration index of system IV (0.1274) was at its lowest, indicating that the number of species in the pest sub-community in system IV was low and that these were evenly distributed. However, no significant differences were observed in systems II and III. As for the natural enemy sub-community, the diversity and evenness of systems II and IV were higher, whereas the dominant concentration was lower, indicating that the natural enemy sub-community structure was relatively stable. As for the neutral arthropod community, the structure of system I was better than that in other ecosystems, and there was no significant difference between the other systems.

In 2010, the diversity and evenness of the pest sub-community in systems I and II were higher, but the dominant concentration was lower. Additionally, no difference was noted in these parameters between systems III and IV. In the natural enemy sub-community, three characteristic indices of systems I and II showed that the quantities of this sub-community were less than those in systems III and IV and were evenly distributed.

There was no difference in the three sub-community indexes of system I, II and III, in the pest sub-community in 2011, but the diversity (2.4119) and the evenness (0.5966) of system IV were higher and the dominant concentration (0.1401) was lower than those of the other three ecosystems. This indicates that the number of species in the pest sub-community of system IV was low and evenly distributed. In the natural enemy sub-community, the diversity and evenness index of system II were the highest, which was followed by systems III and I, whereas that in system IV was the lowest; however, the dominant concentration index followed the opposite trend.

Effect of the transgenic poplar-cotton ecosystem on the nutritional structure of the arthropod community

The arthropod community in a transgenic poplar-cotton ecosystem is a network sys-

tem that coexists with a variety of pests and natural enemies. Introducing pest-resistance genes may directly or indirectly affect the bio-production of each nutritional layer in the community via interspecific relationships of plants or pests and natural enemies, which affects the stability and balance of the community.

According to the classification of the nutritional layer and functional groups as described above, the composition and change of richness abundance and the functional groups of each nutritional layer in the arthropod community of a transgenic poplar-cotton composite system were investigated. Nutritional layers were divided into basal species, intermediate species, and top species. The basal species included pests of Lepidoptera, aphids, whiteflies, leafhoppers, spittlebugs, crickets, mole cricket, locusts, Chrysomelinae, Scarabaeoidea, weevils, stink bugs, thrips, mites, and neutral arthropods. The intermediate species consisted of ladybirds, ground beetles, Staphylinidae, tiger beetles, ants, parasitoids wasps, Eumenidae, Potter Wasps bees, predatory bugs, lacewings, Syrphidae, Katydids, ant spiders, dish spiders, garden spiders, round palate spiders, Agelenidae, flat belly spiders, and Linyphiidae. The top species included Tarantula, Salticidae, Thomisidae, Pholcidae, mantis, and dragonflies. Individual arthropods were counted and the percentage content and richness abundance were calculated (Table 6).

Table 6. Abundance of nutritional class functional groups in the transgenic poplar-cotton ecosystems.

Year	Nutritional layers	Functional group number	Richness abundance			
			I	II	III	IV
2009	Basal species	16	0.7560	0.8374	0.8214	0.8070
	Intermediate species	17	0.2348	0.1543	0.1684	0.1881
	Top species	6	0.0091	0.0083	0.0103	0.0048
2010	Basal species	16	0.7847	0.8066	0.7102	0.6297
	Intermediate species	18	0.2059	0.1856	0.2826	0.3631
	Top species	7	0.0094	0.0078	0.0072	0.0072
2011	Basal species	20	0.7708	0.8279	0.8208	0.7471
	Intermediate species	18	0.2272	0.1712	0.1776	0.2488
	Top species	4	0.0020	0.0009	0.0017	0.0041

In 2009, there were some differences in the nutritional layer and the number of functional groups of the four poplar-cotton ecosystems. The richness abundance of basal species in systems II, III, and IV was higher than that in system I by 8.14, 6.54, and 5.10% respectively, and this was mostly due to stinkbug (mainly lace bugs) outbreak in transgenic poplar-cotton ecosystems. The percentages of stinkbugs in systems II, III, and IV were 18.07, 16.89, and 19.52%, respectively, higher than the richness abundance of system I by 232.17, 210.48, and 258.82%. Lepidoptera accounted for 9.61% (III), 7.29% (I), 4.20% (II), and 3.58% (IV), of the community in the four poplar systems. System III took first place because of the larvae of *Hyphantria cunea* and *Notarcha derogata*, which occurred in poplar and cotton land. Other complex transgenic systems had stronger inhibitory effects on lepidopteran pests compared to the control system. The richness abundance of intermediate species in system I was higher than that of the other three systems, mainly due to the larger number of ants (20.89%) and Syrphidae (0.36%), while in the other insect community, the richness abundance of the transgenic ecosystem was higher than that of the control system. Comparing the number of functional groups, the dominant functional groups of top species and intermediate species were similar. However, the number of functional groups in the control ecosystem was quantitatively

lower than the number in the transgenic ecosystem, except for ants, Syrphidae, lacewings, and Salticidae.

In 2010, the number of nutritional layers and functional groups in four kinds of ecosystems changed differently compared to what was observed in 2009. The dominant functional groups of nutritional layers in each poplar-cotton ecosystem were similar to those found in 2009, but were quantitatively different. In the abundance of the basal species, only system II was 2.19% higher than that of the control system I. This was because the numbers of aphids (accounting for 54.19% of system II, and 44.19% of system I) were higher in systems I and II. With the exception of Trips, there were fewer basal species in control system I than in other transgenic ecosystems. For the intermediate species and top species, Eumenidae (0.05%), Thomisidae (0.60%), and Sphecidae (0.02%) were numerically superior in control system I. Thus, there were more functional groups in the transgenic ecosystems, especially in system IV.

In 2011, the four kinds of poplar-cotton ecosystems studied were similar to the previous 2 years in the number of functional groups, but the basal species were slightly higher and the top species were slightly lower than in the previous 2 years. The abundance of nutritional layers was different from that observed in the previous 2 years. Among them, the abundance of top species was significantly lower than that observed in the previous 2 years. Of the four kinds of poplar-cotton ecosystems, there was a significantly higher abundance of basal species in system II compared with the other three systems, and system IV contained the lowest, mainly because the individual numbers of aphids (59.04%) and leafhoppers (4.85%) were higher than in the other three systems. The abundance of intermediate and top species in the four systems followed an opposite trend to that observed for the basal species, namely, the abundance of system IV was the highest, followed by system I and systems III and II.

Comparative study of the similarity of the arthropod community in the transgenic poplar-cotton ecosystem

The measurement of community similarity is the basis of all number classifications used in the comparison between communities or community attributes, which is the quantitative indicator of the similarity index for the community composition including species kind and individual number. To a certain extent, it reflects the successive community succession change and the relationships between communities. To illustrate any differences between the composition and structure of the arthropod community in the transgenic poplar-cotton ecosystem, we used the similarity coefficient, C_{ij} , to compare the similarities and differences between the communities in the transgenic poplar-cotton ecosystems. The conclusion of the similarity coefficient analysis between the composite systems is shown in Table 7.

As shown in Table 7, the similarity coefficient between systems II and III was the largest, and that between control system I and the transgenic ecosystems was slightly lower, illustrating that there was a high degree of similarity among the transgenic poplar-cotton ecosystems II, III, and IV, which showed a greater difference from control system I.

This shows that, in 2009, the arthropod community was very similar in the transgenic poplar-cotton ecosystems II and III, and that the differences were the smallest; however, this was quite different to that observed between transgenic ecosystem IV and control ecosystem I. When the difference coefficient increased to 0.27, system I clustered into one group with the other three types of ecosystems. In 2010, the arthropod community was very similar in

systems I and III. As the two systems both contained the control cotton fields, it is speculated, therefore, that the control cotton fields had significant effects on these ecosystems. The presence of transgenic cotton fields might account for systems II and IV having low similarity with systems I and III. The results displayed in Figure 2 are consistent with the results shown in Table 7.

Table 7. Comparison of the similarity coefficient of the arthropod community in transgenic poplar-cotton ecosystems.

Time	Sample	I	II	III	IV
2009	I	1	0.758419	0.661021	0.759403
	II		1	0.80449	0.780616
	III			1	0.794049
	IV				1
2010	I	1	0.832173	0.879451	0.731196
	II		1	0.744295	0.719578
	III			1	0.803513
	IV				1
2011	I	1	0.826614	0.795985	0.783128
	II		1	0.886685	0.726503
	III			1	0.757388
	IV				1

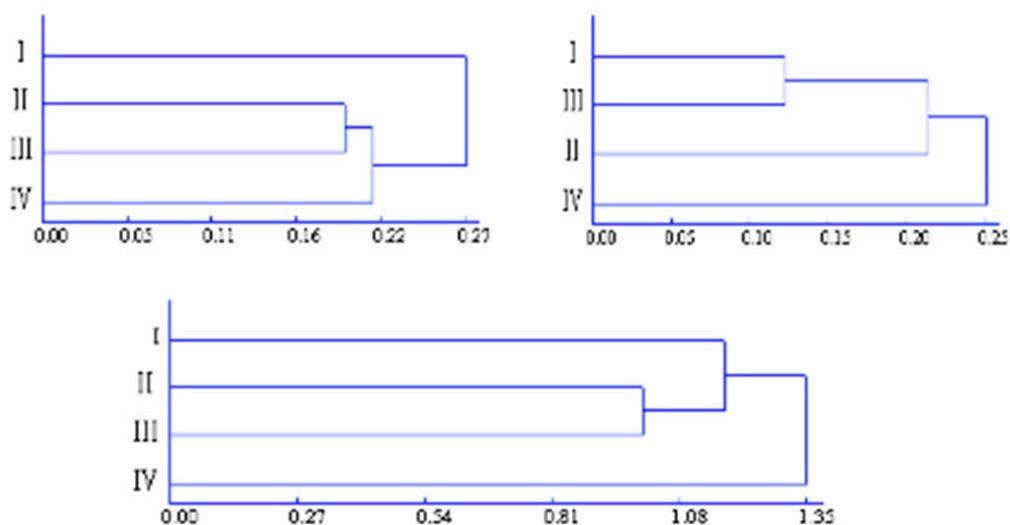


Figure 2. Cluster analysis of arthropod communities in the poplar-cotton systems (2009 on upper left; 2010 on upper right; 2011 on the below).

CONCLUSIONS

The arthropod community in transgenic poplar-cotton ecosystems is a complex network that involves mutual relationships and the coexistence of multiple herbivorous insects, natural enemies, and the neutral arthropod community through the flow of nutrients and chemical communication within the food chains. In this complex network, any composition of the

nutritional layer species, or change in the number of a certain species can directly or indirectly affect other nutritional layers through nutrient or information flow. Therefore, when the Bt gene is transformed into cotton, and the *BtCry1Ac* and *API* genes are transferred to poplar 741, their physical traits, nutrient substances, and volatile and non-volatile secondary metabolites may unexpectedly change, which may affect the target pests, non-target insects, beneficial insect species, the community structure, biodiversity, or even the whole ecological system. Furthermore, migration and proliferation of the arthropod community indirectly affect the composition and structure of the arthropod community (Priestley and Brownbridge, 2009; Bai et al., 2012). Understanding these effects is key to evaluating the ecological safety of the arthropod community in the transgenic poplar-cotton ecosystem.

To summarize, the survey data obtained in this study showed that the transgenic poplar-cotton ecosystem has some effects on the composition and structure of the arthropod community. Differences were observed in the data generated during the 3-year investigation. In some poplar-cotton ecosystems, a high proportion of pests and a low proportion of natural enemies were observed. These results may be due to the limited test area, free choice of spawning sites by Lepidopteran pests, or the random sampling used in the course of the investigation. Furthermore, it is possible that transgene expression is weaker in the fields than in the greenhouse; therefore, although the transgenic poplar significantly inhibits the growth and development of the target pests, the mortality rate did not decrease significantly. In addition, predator quantities in the cotton field next to the transgenic poplar are higher than those in the control field next to the non-transgenic poplar, suggesting that transgenic poplar in the ecosystem contributed partly to the increase in predator numbers in the cotton fields.

The effects of transgenic plants and ecosystems on the arthropod community require continuous monitoring over the long term. Due to time constraints, the present experiment involved only a 3-year community survey, so it is difficult to explain real changes in arthropod communities that occur in transgenic poplar-cotton ecosystems. Therefore, these results need further comprehensive study and analysis to provide a more profound basis for the rational application and promotion of transgenic poplar-cotton ecosystems.

Conflicts of interest

The authors declare no conflict of interest.

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