African Journal of Agricultural Research Vol. 5(20), pp. 2787-2794, 18 October, 2010
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X ©2010 Academic Journals

Full Length Research Paper

Effect of acetochlor and carbofuran on soil nematode communities in a Chinese soybean field

Jingnan Zhang[1,2], Qi Li[1]* and Wenju Liang[1]

[1]Liaoning Key Laboratory of Soil Environmental Quality and Agro-product Safety, Institute of Applied Ecology, Chinese Academy of Science, Shenyang 110016, China.
[2]Graduate School of the Chinese Academy of Sciences, Beijing 100049, China.

Accepted 23 August, 2010

A field experiment was conducted at the Shenyang experimental station of ecology, Northeast China, to determine the response of soil nematode communities to agrochemical applications. Four treatments were compared including the control (CK, no agrochemicals applied), herbicide acetochlor (A), nematicide carbofuran (F) and the combination of acetochlor and carbofuran (AF). The obtained results indicated that the agrochemical applications could influence the soil nematode communities. In the treatments with acetochlor and/or carbonfuran, the abundances of total nematodes and plant-parasitic nematodes were reduced, and those of bacterivores decreased obviously at the 8th week. Forty-two nematode genera were identified, and *Acrobeloides*, *Pratylenchus* and *Helicotylenchus* were dominant and demonstrated different susceptibilities to agrochemical treatments. No significant sampling date and treatment effects were observed in the values of enrichment index (EI) and channel index (CI). However, the basal index (BI) and structural index (SI) were more suitable for indicating the soil conditions with different agrochemicals in this study, and higher BI and lower SI could indicate poor ecosystem health.

Key words: Soil nematode, community structure, acetochlor, carbofuran, soybean field.

INTRODUCTION

Soybean (*Glycine max*) is globally important economic crops for its food and industrial uses. Nowadays, in order to increase soybean productivity, many agrochemicals has been applied to control disease, pest and weed problems. Therefore, agrochemical applications not only cause soil and water pollution, but also have a negative impact on non-target species in soybean field (Correa et al., 2009). Due to widespread use of agrochemicals in agriculture, contaminations of food, water and air have become serious, and consequently adverse health effects are inevitable on vertebrates and invertebrates (Yoon et al., 2001; Chen et al., 2003; Wada and Toyota, 2008). Among soil invertebrates, nematodes posses many characteristics that make them ideal as bioindicators for soil health assessment (Bongers and Ferris, 1999), and their fauna composition, together with its ecological indices, has emerged as a useful monitor of environmental conditions and soil ecosystem function (Neher, 2001).

Acetochlor and carbofuran were two kinds of agrochemicals extensively used in soybean fields in Northeast China. Acetochlor, namely harness, is a kind of selective herbicide before sprout, and used for control of most annual grasses and certain broadleaf weeds, with a half life of two months in soil. Carbofuran is a carbamate pesticide that has wide applications as a nematicide to be typically used to control plant-parasitic nematodes but is persistent and non-target, with a half life of 30 to 60 days in soil.

The results gained from both in field and laboratory toxicity experiments have proved that carbofuran is effective on controlling plant-parasitic nematodes (Todd et al., 1992; Waliyar et al., 1992; Shukla and Haseeb, 1996; Al-Rehiayani, 2008; Shakil et al., 2008). In recent years, there is a growing concern over the impacts of such pesticides on non-target free-living nematodes. Yardim and Edwards (1998) studied the responses of nematode communities to mixtures of insecticides (carbaryl, endosulfan and esfenvalerate), fungicide (chlorothalonil) and

*Corresponding author. E-mail: liq@iae.ac.cn. Tel: +86-24-83970359. Fax: +86-24-83970300.

herbicides (trifluralin and paraquat) in a tomato field, and found that the temporal community dynamics of different nematode trophic groups differed with the pesticide applications. Yeates et al. (1999) reported that the diversity of nematode community was increased and bacterivores were consistently low in herbicide-treated plots over a seven-year period. Chen et al. (2003) monitored the effect of acetochlor on the nematode community structure in a soybean field.

The results showed that the soil nematode community structure could be greatly influenced by acetochlor, and the numbers of total nematode and trophic groups were reduced, and richness (SR) was effective in distinguishing the differences between the acetochlor plots and the control plots. Pen-Mouratov and Steinberger (2005) investigated the effect of two pesticides, Nemacur and Edigan, on soil nematodes in a desert system, and the results indicated that the numbers of total nematodes, fungivores and bacterivores were decreased in the pesticides treatments.

Wada and Toyota (2008) conducted a pot test to evaluate the toxicity of imicyafos and fosthiazate to non-target organisms, and reported that the two organophosphorous nematicides effectively suppressed a plant-parasitic nematode *Pratylenchus penetrans*, but had little impact on free-living nematodes and soil microbial community. And so far, little is known about the effects of acetochlor and carbofuran applied at a recommended dose on the nematode community structure in Northeast China. Our objectives were to determine the responses of soil nematode communities to the application of acetochlor, carbofuran and their combination at a recommendation dose, and to evaluate potential effects of these agro-chemicals on non-target organism in soil ecosystems.

MATERIALS AND METHODS

The study was conducted at the Shenyang experimental station of ecology (41°22′N, 123°32′E), Chinese Academy of Sciences, a Chinese Ecosystem Research Network (CERN) site in the Lower Reaches of Liao River plain in Northeast China. The station is located in the continental temperate monsoon zone. The annual mean temperature is 7.0 to 8.0°C, annual precipitation averages 650 to 700 mm, and annual non-frost period is 147 to 164 days. The soil at the study site is classified as an aquic brown soil (silty loam Hapli-Udic Cambosols in Chinese Soil Taxonomy) (Liang et al., 2005), with 21.4% sand, 46.5% silt and 32.1% clay at the 0 to 20 cm depth. The pH varied from 5.2 to 6.1 across the field.

The experiment field (17 × 17 m^2) with a randomized block design was divided into 16 plots of 3 × 3 m^2 each, involving four treatments with four replicates. A hundred soybean seeds, variety Tiefeng 29, were sown equidistantly in each plot on ridges in 10 rows on May 5th 2008. Four treatments, i.e. CK (control, no agrochemicals were applied), A (application of acetochlor, 0.18 mL m^{-2}), F (application of carbofuran, 4.5 g m^{-2}) and AF (combined application of acetochlor (0.18 mL m^{-2}) with carbofuran (4.5 g m^{-2}).

50% EC acetochlor and carbofuran 3G used in this study were purchased from Dalian Regar Pesticides of China and FMC of the US (encapsulation in China), respectively. Carbofuran was applied at the time of sowing, while acetochlor was insufflated one week late.

During the soybean growing season, five samplings were taken in 0, 2nd, 4th, 8th and 16th week after sowing in 2008. Each soil sample, collected from topsoil (0 to 20 cm) of each plot, was comprised of 5 cores, thoroughly mixed, and placed in plastic bags stored in cold storage at 4°C until processed.

Nematodes were extracted from 100 g soil sample (fresh weight) using cotton-wool filter method with minor modifications (Oostenbrink, 1960; Townshend, 1963). In each sample, all extracted nematodes were counted, and 100 nematodes were randomly selected and identified to genus level using an inverted compound microscope. The ecological indices of enrichment index (EI), structure index (SI), basal index (BI) and channel index (CI) were calculated as: $EI = 100 \times (e/(e + b))$, $SI = 100 \times (s/(b + s))$, $BI=(b/(b + e + s))$, $CI = 100(0.8Fu_2)/(3.2Ba_1 + 0.8Fu_2)$, where e is the abundance of individuals in guilds in the enrichment component weighted by their respective k_e values, b is the abundance of individuals in the basal component weighted by their k_b values, and s is the abundance of individuals in the structural component weighted by their k_s values. k_e is the weighting assigned to guilds Ba_1 and Fu_2, k_b is the weighting assigned to guilds Ba_2 and Fu_2, and k_s is the weighting assigned to guilds Ba_3-Ba_5, Fu_3-Fu_5, Om_4-Om_5 and Ca_2-Ca_5 (Ferris et al., 2001). Ba_x, Fu_x, Ca_x, Om_x and H_x (where x = 1 to 5) represent the functional guilds of nematodes that are bacterivores (Ba), fungivores (Fu), predators (Ca), omnivores (Om) or plant parasites (H) (Yeates et al., 1993) where the guilds have the character indicated by x on the colonizer-persister (*cp*) scale (1-5) according to the *r* and *K* characteristics following Bongers (1990) and Bongers and Bongers (1998).

Nematode abundances and relevant abundances were ln(x + 1) transformed prior to statistical analysis and expressed as numbers per 100 g dry soil. All the data obtained in the study were subjected to statistical analysis of variance (two-way ANOVA) by SPSS software package, and differences at the $P < 0.05$ level were considered significant.

RESULTS

During the study period, the number of total nematodes fluctuated among different treatments (Figure 1), with the highest value (743 individuals 100 g^{-1} dry soil) in the F treatment at the 2nd week, and the lowest (284 individuals 100 g^{-1} dry soil) in the CK at 0 week, respectively. Significant sampling date effects were found in the total nematodes ($P < 0.05$), but no obvious treatment effects were observed. The numbers of total nematodes in the CK increased gradually during the whole study period. However, obvious reductions in the numbers of total nematodes occurred in the agrochemicals treatments with lower values observed in the AF treatment after the 2nd week and in the A and F treatments after the 8th week in comparison with the CK.

Different trophic groups of soil nematodes responded differently to agrochemical applications (Figure 2), and significant sampling date and/or treatment or their interaction effects were observed in the bacterivores, plant parasites and omnivores-predators ($P < 0.05$) (Table 2). The numbers of bacterivores were significantly lower in the A, F and AF treatments than in the CK at the 8th week ($P < 0.05$). The numbers of plant parasites in the CK increased with the sampling dates, and were lower in the A and F treatments than in the CK at the 8th and 16th

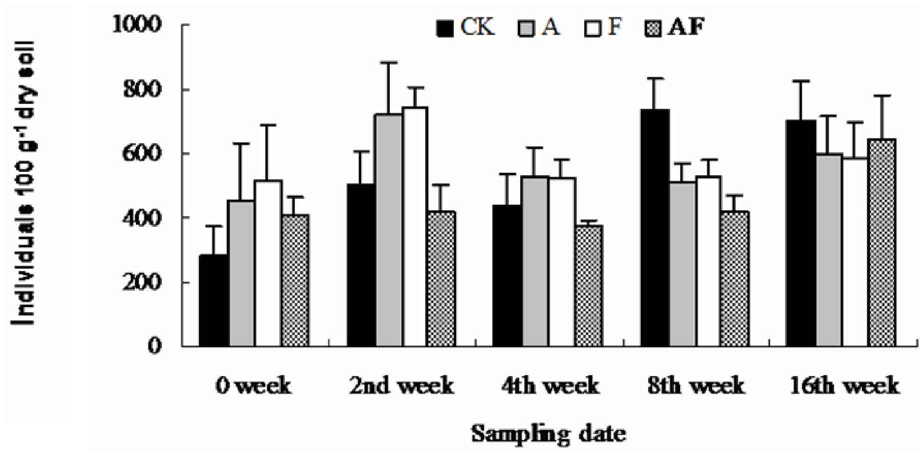

Figure 1. Responses of total nematodes to agrochemical applications (Bars indicate the standard error; CK, control; A, herbicide acetochlor; F, nematicide carbofuran; AF, combined application of acetochlor with carbofuran).

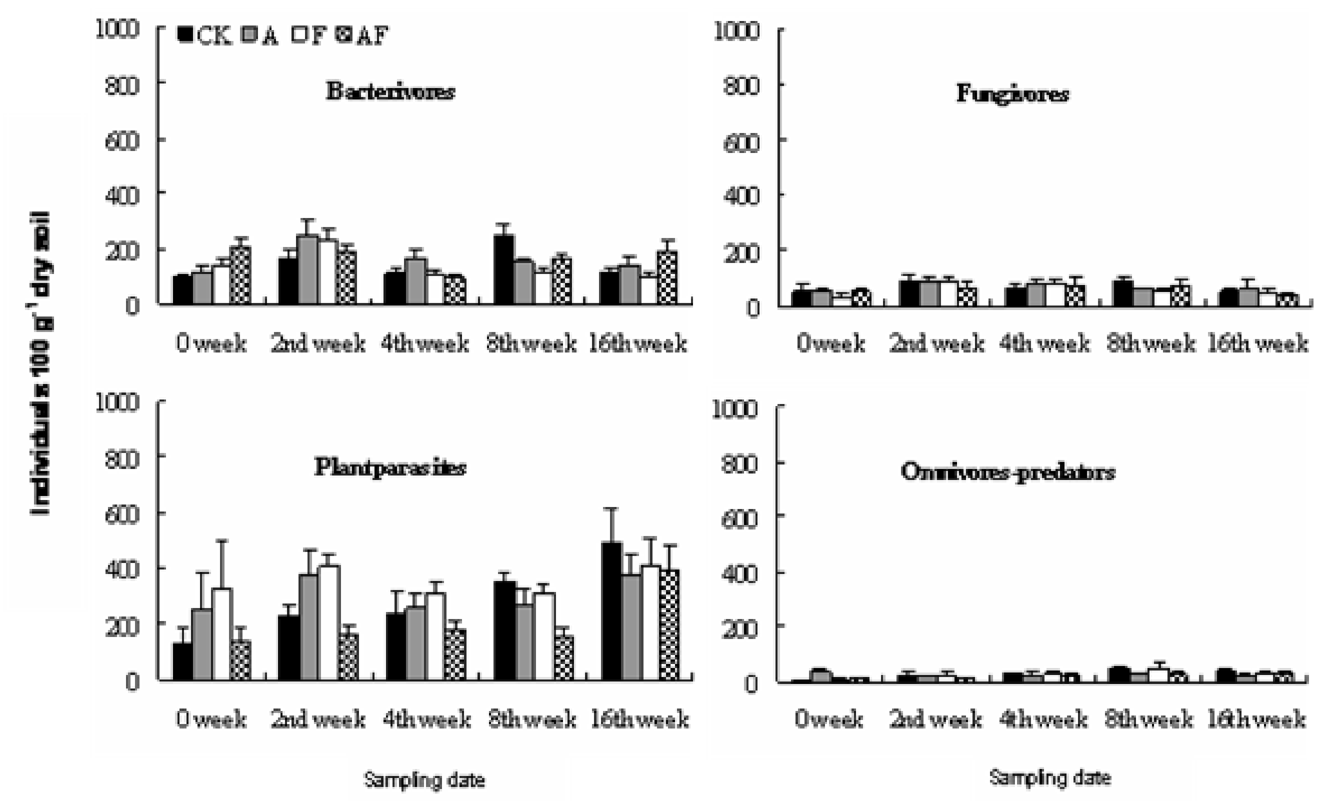

Figure 2. Responses of nematode community composition to agrochemical applications (Bars indicate the standard error; CK, control; A, herbicide acetochlor; F, nematicide carbofuran; AF, combined application of acetochlor with carbofuran).

weeks ($P < 0.05$). The least number of plant parasites was presented in the AF treatment during the study period. The numbers of fungivores and omnivores-predators fluctuated among sampling dates and treatments, but no significant treatment effects were observed.

Forty-two nematode genera belonging to twenty-four families were identified during the study period (Table 1).

Table 1. The relative abundance (%) of soil nematode genera under different agrochemical treatments in a soybean field.

Genus		Treatment			
		CK	A	F	AF
Bacterivores		29.6	30.5	24.9	39.4
Mononchoides	Ba_1	0.4	0.2	0.8	0.7
Panagrolaimus	Ba_1	0.7	0.4	0.6	0.5
Rhabditidae	Ba_1	5.0	4.0	4.0	3.9
Acrobeles	Ba_2	0.4	0.3	0.3	0.4
Acrobeloides	Ba_2	14.0	16.4	11.8	22.9
Cephalobus	Ba_2	2.6	2.4	2.0	3.7
Cervidellus	Ba_2	0.2	0.1	0.0	0.3
Chiloplacus	Ba_2	3.6	3.7	3.0	4.7
Eucephalobus	Ba_2	1.1	0.9	1.1	0.9
Heterocephalobus	Ba_2	0.1	0.2	0.4	0.5
Plectus	Ba_2	0.4	0.6	0.3	0.3
Prismatolaimus	Ba_3	0.6	1.1	0.3	0.6
Rhabdolaimus	Ba_3	0.3	0.3	0.3	0.1
Fungivores		13.2	12.5	10.5	13.5
Aphelenchoides	Fu_2	4.3	3.3	3.9	6.9
Aphelenchus	Fu_2	4.2	4.0	3.7	2.8
Dorylaimoides	Fu_4	1.0	1.2	0.6	0.6
Tylencholaimus	Fu_4	3.7	4.0	2.3	3.3
Plant parasites		52.2	52.9	59.9	42.7
Basiria	H_2	0.6	0.9	0.5	0.8
Boleodorus	H_2	1.6	1.1	0.7	1.3
Filenchus	H_2	1.7	2.2	1.3	2.1
Malenchus	H_2	0.2	0.3	0.0	0.1
Paratylenchus	H_2	9.0	7.4	8.7	4.4
Psilenchus	H_2	0.1	1.0	0.5	0.4
Amplimerlinius	H_3	0.6	0.7	1.3	0.6
Geocenamus	H_3	0.1	0.2	0.2	0.1
Helicotylenchus	H_3	19.9	21.4	28.1	15.8
Heterodera	H_3	1.9	1.5	1.2	1.2
Pratylenchus	H_3	11.5	9.1	8.7	7.0
Rotylenchus	H_3	3.0	4.9	6.1	6.9
Trophurus	H_3	0.2	0.5	0.5	0.3
Axonchium	H_5	0.2	0.3	0.7	0.5
Dorylaimellus	H_5	1.5	1.4	1.4	1.2
Omnivores/predators		5.0	4.0	4.7	4.3
Epidorylaimus	Om_4	1.2	1.4	0.5	0.9
Eudorylaimus	Om_4	0.8	0.5	0.5	0.5
Kochinema	Om_4	0.6	0.3	0.6	0.3
Microdorylaimus	Om_4	0.8	0.7	1.0	0.9
Thonus	Om_4	0.3	0.5	0.3	0.5
Laimydorus	Om_5	0.1	0.1	0.2	0.0
Mesodorylaimus	Om_5	0.4	0.0	0.1	0.6
Seinura	Ca_2	0.0	0.0	0.1	0.1
Aporcelaimellus	Ca_5	0.5	0.3	0.8	0.4
Discolaimus	Ca_5	0.2	0.3	0.6	0.2

Ba_x, Fu_x, Ca_x, Om_x, Pl_x (where x=1-5) represent the functional guilds of nematodes that are bacterivores (Ba), fungivores (Fu), predators (Ca), omnivores (Om), plant parasites (H) (Yeates et al., 1993) where the guilds have the characters indicated by x on the colonizer-persister (cp) scale (1-5) according to their r and k characteristics following Bongers (1990) and Bongers and Bongers (1998). CK, control; A, herbicide acetochlor; F, nematicide carbofuran; AF, combined application of acetochlor with carbofuran.

Table 2. ANOVA of different agrochemical treatments in a soybean field.

ANOVA	*P*-values											
	TNEM	BF	FF	PP	OP	*Acr*	*Hel*	*Pry*	SI	EI	CI	BI
Time	0.02	0.01	0.07	<0.01	<0.01	<0.01	0.28	<0.01	0.02	0.08	0.26	0.03
Treat	0.26	0.23	0.86	<0.01	0.97	<0.01	0.03	0.36	0.06	0.12	0.70	<0.01
Time × treat	0.46	0.02	0.95	0.69	0.61	0.66	0.79	0.54	0.60	0.70	0.35	0.90

TNEM, total nematodes; BF, bacterivores; FF, Fungivores; PP, Plant parasites; OP, Omnivore-predators; *Acr*, *Hel* and *Pry* are abrreviations for *Acrobeloides*, *Helicotylenchus* and *Pratylenchus*, respectively; SI, structure index; EI, enrichment index; CI, channel index; BI, basal index.

Acrobeloides belonging to bacterivores of cp-2 guild was dominant in all treatments. Of the four genera infungivores, *Aphelenchoides* was predominant in the AF treatment (> 5%). Among six genera in plant parasites of cp-2 guild, *Paratylenchus* was dominant in the CK, A and F treatments. Among eight genera in the plant parasites of cp-3 guild, *Helicotylenchus* was predominant, followed by *Pratylenchus*. Statistical analysis was conducted on the dominant genera (relative abundance higher than 5% in all treatments).

Significant sampling date and treatment effects were found in the numbers of *Acrobeloides* ($P < 0.01$), and significant sampling date and treatment effects were observed in the numbers of *Pratylenchus* and *Helicotylenchus*, respectively ($P < 0.05$). Except the 0 week, the numbers of *Acrobeloides* were higher in the A and AF treatments than in the CK, and those of *Helicotylenchus* in the F treatment showed a similar trends except the 16th week (Figure 3), while those of *Pratylenchus* in the A and AF treatments except the 8th week, and in the F treatment excluding the16th week were lower than those in the CK.

The values of ecological indices (EI, SI, BI and CI) fluctuated across all sampling dates under different treatments (Table 3). Significant sampling date and treatment effects were observed in the values of BI ($P < 0.05$), and SI was the only index that responded to different sampling dates ($P < 0.01$), while no significant differences were gained in the values of EI and CI. Except the 2nd week and 8th week, the values of BI were significantly higher in the AF, F and A treatments than in the CK, and those of SI showed an opposite trend.

DISCUSSION

In this study, agrochemical applications could influence the soil nematode communities. During the half lives of the herbicide and the nematicide, the study results indicated that the acetochlor, carbofuran and their combination reduced the abundances of total nematodes and plant parasites. Agrochemical treatments caused obvious decreases in the numbers of bacterivores at the 8th week, while the numbers of fungivores and omnivores-predators were not significantly influenced. Waliyar et al. (1992) reported that application of a high dose of carbofuran (10 kg ha^{-1}) could reduce plant parasites in soil. Yeates et al. (1999) reported that the diversity of bacterivores were consistently low in herbicide-treated plots over a seven-year period. Chen et al. (2003) indicated that negative effects of acetochlor on the numbers of total nematode and trophic groups in a Chinese soybean field.

The susceptibility of nematodes depends on several factors such as species, strain, agrochemical formulations and application dose (Grewal, 2002). The abundance of *Helicotylenchus* was higher in the F treatment than in the CK except the 16th week. Todd et al. (1992) observed that carbofuran at rates of 11.1 and 22.2 kg ha^{-1} effectively reduced the densities of root-feeding nematodes, especially the dominant herbivorous genus *Helicotylenchus*. Our experiment did not support this result, and this difference may be attributed to the different application dose. During the study period, the numbers of *Pratylenchus* were lower in the A, F and AF treatments than in the CK except in the 8th and16th week. A similar result was also observed by Shukla and Haseeb (1996) that carbofuran at 3 kg ha^{-1} were effective in reducing the reproduction of *Pratylenchus thornei* on *Mentha citrate*. The numbers of *Acrobeloides* were higher in the A and AF treatments than in the CK during the study period. These results might imply that, as a dominant genus and a non-target organism, *Acrobeloides* was tolerant to agrochemicals and showed an increasing trend under the recommended dose of the two agrochemicals.

In our study, the values of EI and CI did not represent significant differences among sampling date and treatments. However, the results between BI and SI showed an opposite trends with higher values of BI and lower values of SI were observed in the agrochemical treatments in comparison with the CK. Berkelmans et al. (2003) concluded that BI and SI may be more suitable as general indicators for the health states of a soil, and a high BI would indicate poor ecosystem health, while a high SI would indicate a well-regulated healthy ecosystem.

The results obtained in this study showed that the soil

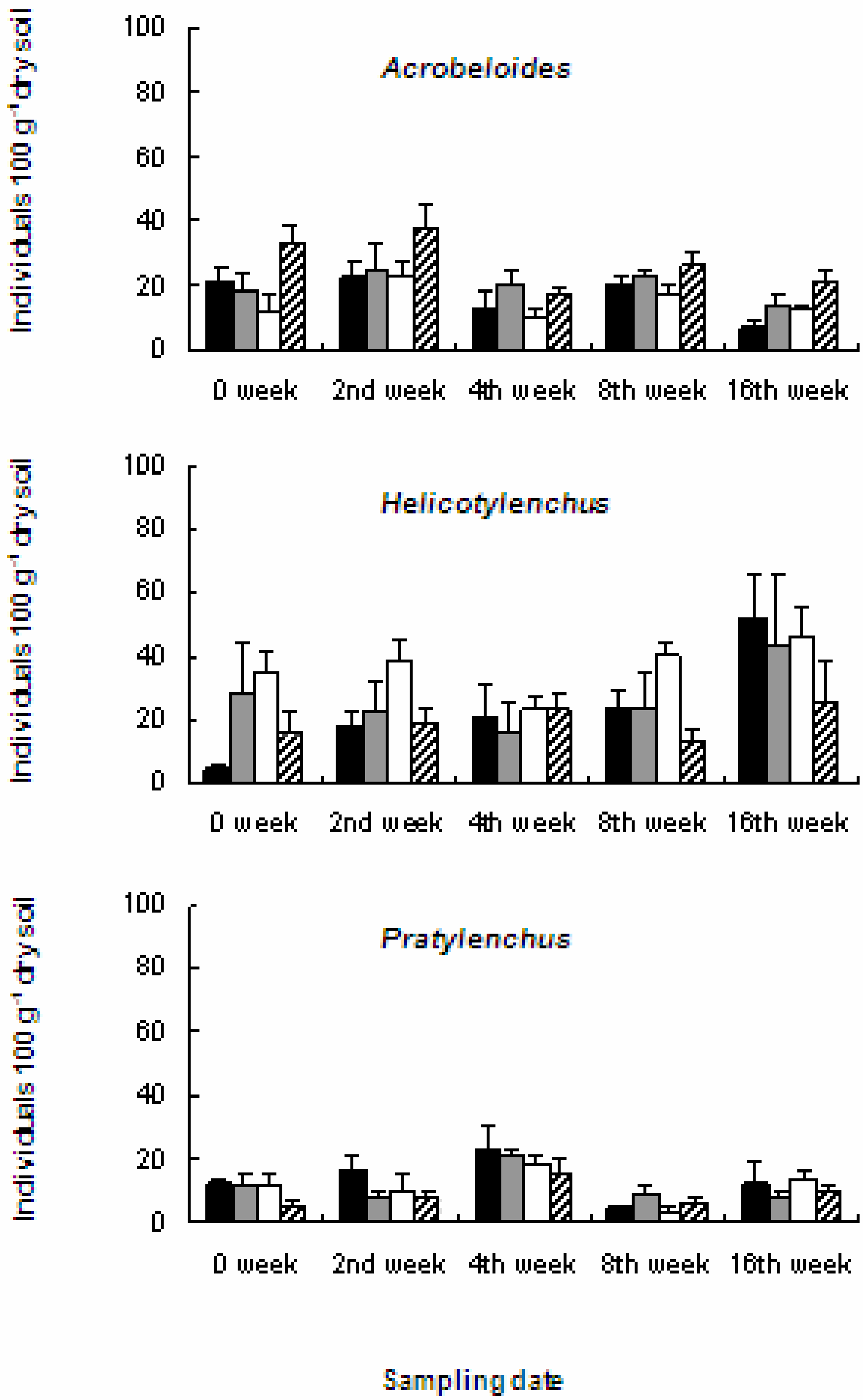

Figure 3. Responses of the dominant genera to agrochemical applications (Bars indicate the standard error; CK, control; A, herbicide acetochlor; F, nematicide carbofuran; AF, combined application of acetochlor with carbofuran).

nematode community structure could be influenced by agrochemical applications; BI and SI were useful indicators to assess the impact of agrochemicals on soil ecosystems.

ACKNOWLEDGEMENTS

This research was supported by the Knowledge Innovation Project of the Chinese Academy of Sciences

Table 3. Changes in the values of nematode ecological indices among different treatments at different sampling dates (means ± S.E.).

Ecological indices	Treatment	Sampling date				
		0 week	2nd week	4th week	8th week	16th week
EI	CK	51.36 ± 9.20	48.96 ± 2.43	59.33 ± 2.81	45.39 ± 6.02	51.26 ± 7.44
	A	39.12 ± 0.54	44.81 ± 9.44	52.01 ± 5.67	41.91 ± 6.73	46.76 ± 7.08
	F	47.71 ± 13.04	49.65 ± 4.99	58.36 ± 6.38	31.76 ± 8.26	47.84 ± 4.14
	AF	28.41 ± 5.55	40.11 ± 3.76	45.40 ± 1.57	47.21 ± 8.90	42.55 ± 7.59
SI	CK	57.12 ± 13.17	48.46 ± 8.45	64.23 ± 4.89	54.45 ± 4.18	68.30 ± 3.72
	A	54.27 ± 13.38	57.04 ± 5.05	49.80 ± 11.24	53.35 ± 8.89	62.21 ± 5.76
	F	39.74 ± 11.69	45.70 ± 7.25	62.15 ± 2.84	64.86 ± 6.48	60.04 ± 5.26
	AF	36.77 ± 5.87	37.55 ± 5.21	59.50 ± 0.59	43.06 ± 5.86	54.28 ± 6.67
BI	CK	30.10 ± 9.70	33.34 ± 2.41	23.26 ± 2.51	32.25 ± 1.92	23.75 ± 3.32
	A	34.16 ± 7.44	32.55 ± 5.97	32.32 ± 6.29	33.21 ± 5.54	28.51 ± 4.56
	F	34.32 ± 1.28	35.06 ± 4.41	24.47 ± 3.19	29.20 ± 5.20	29.31 ± 3.83
	AF	50.71 ± 5.43	44.24 ± 4.18	30.30 ± 0.74	37.16 ± 6.45	34.95 ± 6.36
CI	CK	20.93 ± 4.65	29.55 ± 8.35	24.32 ± 5.42	29.55 ± 8.43	37.88 ± 12.88
	A	35.01 ± 2.54	23.75 ± 7.05	29.63 ± 10.73	32.55 ± 11.24	29.57 ± 12.02
	F	16.34 ± 1.92	25.86 ± 4.52	37.37 ± 12.08	57.92 ± 14.29	30.86 ± 6.12
	AF	42.26 ± 9.64	27.59 ± 9.13	47.47 ± 3.67	40.36 ± 12.35	19.34 ± 6.51

EI, enrichment index; SI, structure index; BI, basal index; CI, channel index; CK, control; A, herbicide acetochlor; F, nematicide carbofuran; AF, combined application of acetochlor with carbofuran.

(No. KZCX2-YW-408).

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