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Effects of Repeated Applications of Chlorimuron-Ethyl on the Soil Microbial Biomass, Activity and Microbial Community in the Greenhouse

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Abstract The impacts of repeated chlorimuron-ethyl applications on soil microbial community structure and function were studied under greenhouse conditions. Chlorimuron-ethyl was applied to soil samples at three different doses [1-,10-,100-fold of recommended field rate (T1, T10, T100)] for 3 years. The half-lives of chlorimuron-ethyl were 37.1–54.6 days. The soil microbial biomass (microbial biomass carbon and total phospholipid fatty acid), the microbial activity (basal respiration and average well color development), the ratio of Gram-negative/Gram-positive bacteria and Shannon index were stimulated by chlorimuron-ethyl during the initial period. Except for T100, the other treatments recovered to the untreated level. The ratio of fungi/bacteria decreased during the initial period and then recovered in the end. Principal component analysis of phospholipid fatty acid showed that chlorimuron-ethyl altered the microbial community structure. Except got T100, T1 and T10 were not different from the control at the end of experiment. These results suggested a dosage effect of chlorimuron-ethyl on the living microbial biomass and the microbial community.

Keywords Chlorimuron-ethyl · Repeated application · Soil activity · Soil microbial community

Chlorimuron-ethyl [ethyl 2-(((((4-chloro-6-methoxypyrimidin-2-yl) amino) carbonyl) amino) sulfonyl) benzoate] is a member of sulfonylurea herbicides, characterized by broad-spectrum weed control at very low use rate, good crop selectivity and relatively high persistence in soil (Brown 2006). This herbicide has been applied widely for weed control in farmland, especially in soybean fields in China over the past decades. Only in Heilongjiang Province, an important soybean production base of China, 400 tons of chlorimuron-ethyl was used each year to control broadleaf weeds in soybean fields (Zhao and He 2007).

Soil microbes are the basic components of the soil ecosystem and are vital for soil fertility, the degradation of organic matter and pollutants in soils, which are often more sensitive to environment change and stressors than plants and animals (Winding et al. 2005; Sharma and Singh 2012). Soil microbial properties can serve as indicators of soil health (Hernández-Allica et al. 2006). Herbicides are usually applied when crops are absent or at early growth stages. The intensive use of herbicides has become a matter of environmental concern, particularly because of the adverse effects on soil microbes. Repeated applications of pesticides might alter their effects on soil microbial community, thus posing a serious threat to soil health (Wang et al. 2012; Wu et al. 2012). To the best of our knowledge, most of reported results were obtained with a single application of chlorimuron-ethyl (Teng and Tao 2008; Zawoznik and Tomaro 2005). The consequences of successive applications of chlorimuron-ethyl to soil may differ from the effects of a single application. Therefore, it is necessary to assess the impacts of repeated applications of chlorimuron-ethyl on soil microbial biomass, activity, microbial community structure and function.

Phospholipid fatty acid (PLFA) analysis offers a quantitative approach towards assessment of the microbial community structure through evaluating microbial biomass and microbial groups, and has been successfully applied in

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research on diversity and structure of soil microbial communities (Xue et al. 2005; Xu et al. 2013). Community-level catabolic profile (CLCP) analysis evaluates the catabolic characteristic of a microbial community by average well color development (AWCD), and is often used to characterize soil microbial communities under different chemical stresses (Braun et al. 2006; Sojka et al. 2006).

In this study, an experiment was conducted to examine the effects of continuous inputs of chlorimuron-ethyl at three different rates for 3 years on the soil microbial community under greenhouse condition through characterizing the soil microbial activity (basic respiration), soil microbial biomass (microbial biomass carbon, total PLFA, and the AWCD), and community structure and functional diversity (Gram-negative/Gram-positive bacteria, fungi/bacteria, principal component analysis of PFLA, and Shannon Index). The aims of this study were to (1) examine whether and how continuous chlorimuron-ethyl inputs affect the soil microbial activity, the soil community structure and functional diversity; (2) compare the effects of chlorimuron-ethyl at different rates on the soil microbes.

Materials and Methods

Chemicals and Soil Samples

Chlorimuron-ethyl (purity, 96 %) was purchased from the Dikma Technologies Inc. (Beijing, China). The soil was collected from the soybean field of Xiangfang Farm in Heilongjiang Province. The soil properties were as follows: sand 16.75 %, clay 32.12 %, organic matter content 2.81 %, water holding capacity (WHC) 56.5 %, total nitrogen 0.198 % and pH 6.92. The soil was a loamy soil that had been subjected to conventional-tillage and soybean planting over the last 2 years and not treated with pesticide during the last 2 years. Soil from the top 0–10 cm was collected and taken to the laboratory in coolers (4–8°C), then mixed, sieved with 2 mm mesh and preserved at low temperature before use.

Soil Treatments

The experiments were conducted in a greenhouse. The soil was contained in plastic pots which were 60 cm length, 50 cm width and 25 cm deep, and filled with 25 kg of soil. The chlorimuron-ethyl input began in June 2010 and ended in June 2012, and applied once a year. Four treatments were assigned, including no chlorimuron-ethyl control (CK), the recommended field rate (T1), 10-fold recommended field rate (T10) and 100-fold recommended field rate (T100). T1 corresponded approximately to the highest recommended field application rate of chlorimuron-ethyl for soybean (22.5 g a.i./ha; China Pesticide Information Network 2010). T10 (10-fold) and T100 (100-fold) were chosen following standard ecotoxicological practice for establishing possible negative effects of a substance in the environment (Chen and Edwards 2001; Cycon et al. 2006; Guidelines on environmental safety assessment for chemical pesticides in China, 2012). Three replications of each treatment were included. Each pot was watered regularly to keep close to 40 % of the field water-holding capacity. Samples were collected at 1, 7, 15, 30, 60, 90 days after the third application. During the experiment, the average temperature ranged from 16 to 38°C, the average relative air humidity ranged from 55 % to 75 %.

Determination of Chlorimuron-Ethyl

10 g of soil sample was extracted by 5 mL 2 % formic acid and 10 mL acetonitrile in a 50 mL Teflon centrifuge tube. The tube was vortexed vigorously for 3 min at maximum speed, 6 g NaCl was added and immediately vortexed vigorously for 1 min and then centrifuged for 5 min at 2,077 g. Then, a 1.5 mL of the upper layer (acetonitrile) was transferred into the dispersive-SPE tube containing 150 mg anhydrous $MgSO_4$ and 50 mg primary secondary amine (PSA), and vortexed for 1 min, then centrifuged for 3 min at RCF 2,077g. The resulting supernatant was filtered through 0.22 μm Nylon syringe filter for Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) analysis. A Waters UPLC–MS/MS equipped with an electrospray ionization (ESI) source was used for determining concentration of chlorimuron-ethyl. A Waters BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm) was used. The mobile phase consisting of methanol (solvent A) and 0.2 % (v/v) formic acid in water (solvent B) was pumped at a flow rate of 0.3 mL/min. The gradient elution program was: 0–2 min, 10 %–90 % A; 2–3.5 min, 90 % A; 3.5–3.6 min, 90 %–10 % A; 3.6–5.0 min, 10 % A. The sample volume injected was 5 μL. Multi-reaction monitoring mode (MRM) was selected as the scan mode. The conditions were as follows: the capillary voltage and cone voltage were 3.0 kV and 30 V; the source and desolvation temperature were 120 and 350°C. 414.8 (*m/z*) was the precursor ion, and its quantitative and qualitative product ions were 212.9 (*m/z*) and 185.9 (*m/z*). The collision energy was 15 V. The retention time was 2.3 min.

Calibration was performed with external matrix-matched standards. The calibration curve obtained for chlorimuron-ethyl (0.005, 0.05, 0.5, 1, 2 mg/L) in soil matrix was $y = 51471x + 414.65$, $R^2 = 0.9997$. Average recoveries at three levels (0.01, 0.5, 2 mg/kg) ranged from 86.5 % to 95.1 %, with relative standard deviations (RSD) from 2.2 % to 7.6 %. The limit of detection (LOD) is 0.001 mg/kg. The initial concentrations of chlorimuron-

ethyl were 0.07, 0.21 and 1.13 mg/kg in T1, T10 and T100, respectively.

Respiratory Activity and Microbial Biomass Carbon (MBC)

Soil respiration rate was measured based on Wang et al. (2007) with minor modification. Each soil sample was divided into two portions, each containing 20 g of dry soil. One portion was fumigated with ethanol-free chloroform for 24 h in the dark at 25°C, while the other was left untreated. Both fumigated and unfumigated soil samples were extracted with 40 mL 0.5 mol/L K_2SO_4 for 60 min on a shaker. The supernatant was filtered and then 10 mL of filtrate was mixed with 5 mL 0.2 mol/L $K_2Cr_2O_7$ and 5 mL H_2SO_4. The mixture was boiled for 10 min at 170–180°C before cooling and then titrating with ferrous sulfate (Fe_2SO_4, 0.05 mol/L), in which phenanthroline ($C_{12}H_8N_2$) was the indicator. When the solution changed from yellow to cyan to brownish-red, titration was stopped. The MBC was calculated by MBC = Ec/Kc, where Ec = (C extracted from fumigated soil) – (C extracted from unfumigated soil), with C representing carbon and Kc is a calibration factor of 0.38 (Wu et al. 2006).

Community-Level Catabolic Profile Analysis (CLCP)

BIOLOG Ecoplates (BIOLOG Inc., Hayward, USA) were used to study community-level catabolic profile analysis (CLCP) of soil microbial communities as described by Girvan et al. (2003). Ecoplates contained three replicate wells of 31 different carbon sources and a control well without any carbon source. Briefly, 10 g (dry weight equivalent) of fresh soil was added to 90 mL sterile physiological saline solutions (0.85 % NaCl, m/v), shaken at 25°C, and at 200 rpm) for 60 min. Suspensions were allowed to settle for 30 min, and was then diluted stepwise to 10^{-3} in the sterile physiological saline solutions. A 150 µL of the diluted microbial suspension was added to each well of the Biolog Ecoplates. The plate was incubated at 25°C in the dark for 7 day Absorbance of the wells at 590 nm was measured every 24 h using a BIO-TEK Elx808 automated microplate reader (Biolog, Hayward, CA, USA). The absorbance values at 96 h were used to calculate average well-color development (AWCD). Microbial activity in each microplate, expressed as AWCD was determined as follows:

$$\mathrm{AWCD} = \sum \mathrm{OD_i}/31$$

where, ODi is the optical density value from each well by water blank subtraction. H′ was calculated as follows:

$$H\prime = -\sum P_i \times \ln(P_i)$$

where, pi is the ratio of the activity on each substrate (ODi) to the sum of activities on all substrates $\sum \mathrm{OD_i}$.

Determination and Analysis of PLFA

Phospholipid fatty acid analysis was based on previous reports (Zhang et al. 2010a, b; Xu et al. 2013). A 5 g freeze-dried soil was extracted by 19 mL mixture of $CHCl_3$/CH_3OH/citric acid buffer (0.15 mol/L, pH 4) (1:2:0.8, v/v/v) for 2 h. After centrifugation, the supernatant was decanted into a separatory funnel. The soil was vortexed and re-extracted with an additional 9.5 mL extractant, which was added to the first supernatant. A total of 7 mL of citric acid buffer and 8 mL of $CHCl_3$ were added. Samples were shaken and separated. The lower $CHCl_3$ layer was then collected and dried under N_2 for lipid fractionation. The extracted lipids were separated on silica gel columns (Supelco, Inc. USA) into glycol lipids, neutral lipids, and polar lipids. The polar lipids were then transesterified with methanolic KOH to recover the PLFAs as methyl esters through methanolysis in hexane. Finally, the hexane supernatant containing the resultant fatty acid methyl esters (FAMEs) was separated, quantified and identified by gas chromatography mass spectrometry (GC–MS). Methyl nonadecanoate (19:0) was used as an internal standard to calculate the individual fatty acid concentration.

A Polaris Q ion-trap GC–MS (Thermo Fisher Scientific, Inc. USA) with a HP-5 ms column (60 m × 0.25 mm inner diameter, 0.25 µm film thickness) was used for FAME identification. The microbial biomass was assessed by 19 fatty acids, 14:0, i15:0, a15:0, 15:0, 16:0, i16:0, 16:1ω7c, 17:0, i17:0, cy17:0, 18:1ω9c, 18:1ω9t, 18:2ω6, 9, 18:0, 10Me18:0, cy19:0, 20:0, 21:0, and 22:0. The branched phospholipids i15:0, a15:0, i16:0 and i17:0 were used as indicators of Gram-positive (GP) bacteria, while the PLFAs 16:1ω7c, cy17:0 and cy19:0 were indicative of Gram-negative (GN) bacteria. Fungal biomass was assessed by quantifying 18:1ω9c, 18:1ω9t, 18:2ω6, 9 (Zak et al. 2006). The total PLFAs was the sum of all microbial phospholipids mentioned. Prior to principal component analysis (PCA), the results were expressed as a percentage of the total PLFA.

Data Analysis

The degradation of chlorimuron-ethyl followed the first-order kinetics, $C_t = C_0 e^{-kt}$, where C_t represents the concentration of the pesticide residue at time t, C_0 represents the initial concentration and k is the rate constant in days^{-1}. Basal respiration, MBC, CLCP, total PLFA, the ratio of Gram-negative/Gram-positive bacteria (GN/GP) and fungi/bacteria were analyzed according to one-way

ANOVA. Soil microbial properties affected by chlorimuron-ethyl concentration, incubation time and their interaction were analyzed according to two-way ANOVA. Significant differences were accepted at $p < 0.05$*, $p < 0.01$** or $p < 0.001$***. All values reported are the mean ± standard error (SE), and two values in a column followed by the same letter are not significantly different. The principal component analysis (PCA) was applied to examine the PLFA community structure. Statistics were calculated using SAS 9.1.

Results and Discussion

Chlorimuron-Ethyl Degradation

The degradation rates of chlorimuron-ethyl were dependent on chlorimuron-ethyl concentrations, with half-lives of 37.1, 44.1 and 54.6 days for T1, T10 and T100, respectively (Table 1). After 90 day of incubation, 83.8, 76.5 % and 66.6 % of the initial chlorimuron-ethyl degraded for T1, T10 and T100, respectively.

Impact on Soil Microbial Biomass Carbon (MBC) and Respiration

The MBC ranged from 135.4 to 354.9 mg/kg (Fig. 1a). A significant impact on the MBC was observed ($F_{\text{treatment}} = 67.11$, $p < 0.001$; Fig. 1a; Table 3), which was increased by chlorimuron-ethyl in all treatments. The MBC in T1 and T10 recovered to the control level by the 90th days. However, the MBC in T100 did not recover. The basal respiration in three treatments was higher than that in the untreated control during the first 30 days, which was stimulated by chlorimuron-ethyl ($p < 0.01$; Fig. 1b). Then, the basal respiration in all of the treatments recovered at the 60th days.

Chlorimuron-ethyl augmented basal respiration and MBC. Some soil microbes may tolerate and adapt to this herbicide after repeated applications, and these soil microorganisms may degrade chlorimuron-ethyl which would promote the growth. But after a single application of chlorimuron-ethyl, the soil respiration was slightly inhibited by the low concentration of chlorimuron-ethyl (0.01, 0.1, 1, 10 mg/kg) and was stimulated by high concentration of chlorimuron-ethyl (100 mg/kg; Teng and Tao 2008).

Table 1 Half-life for chlorimuron-ethyl dissipation in soil at three different application rates

Application rates	Regression equation	Correlation coefficient	Half-life (days)
T1	$y = 0.0465e^{-0.0187x}$	0.8245	37.1
T10	$y = 0.1655e^{-0.0157x}$	0.9141	44.1
T100	$y = 1.0765e^{-0.0127x}$	0.9691	54.6

T1, T10, T100 mean 1-,10-,100-fold of recommended field rate

Impact on Soil Microbial Community Functional Diversity

The soil microbial functional diversity was estimated with Biolog Ecoplates. The AWCD and Shannon index (H′) calculated using the data from the 96 h incubation readings are listed in Table 2. AWCD reflects the oxidative capacity of soil microorganisms developing in the Biolog microplates, and is usually used as an indicator of overall microbial activity (Rodriguez and Toranzos 2003). The AWCD in T10 was higher than that in control in the first 7 day, and the AWCD in T100 was higher than that in control during the initial 30 days. And then they all recovered to the control level at the 15th day and the 60th day, respectively. The AWCD values in T1 and control were not significantly different during the experiment. The effects of chlorimuron-ethyl on H′ were transient. The H′ in T10 and T100 was increased by chlorimuron-ethyl at the 1st day, and then recovered at day 7 and day 30, respectively. There was not a significant difference in the H′ between T1 and control during the whole experiment.

Community-level physiological profile provided an indication of the catabolic function diversity of heterotrophic cultivable microbes. The increase of AWCD in chlorimuron-ethyl treatments were in accordance with the enhancement of microbial biomass carbon. The soil microbes may tolerate and adapt to the pesticide after repeated applications due to the selective pressure of the chemicals, and these soil microorganisms may degrade chlorimuron-ethyl which would promote the growth. It has been reported that repeated applications of triadimefon, bensulfon-methyl, methamidophos and glyphosate resulted in the adaptation of soil microbes which would degrade these pesticides as a source of carbon and energy (Fang et al. 2012; Xie et al. 2004; Wang et al. 2006; Sarah et al. 2010).

Impact on the Microbial Community Structure Diversity

The soil microbial community structure diversity was estimated by PLFA. The significant effects on the total PLFA concentrations were observed in response to the chlorimuron-ethyl treatments ($F_{\text{treatment}} = 57.58$, $p < 0.001$; Fig. 2a; Table 3). Chlorimuron-ethyl tended to increase the total PLFA within the 60 day. The total PLFA in T1 and T10 was not different from that of the untreated control at the end of experiment. The total PLFA in T100 was significantly

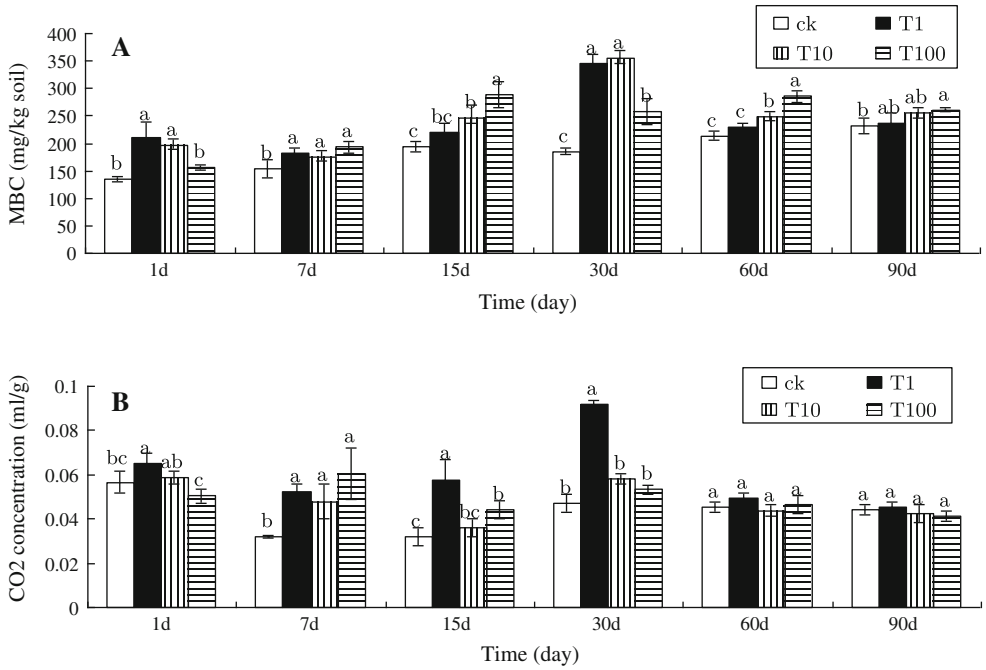

Fig. 1 Soil MBC (**a**), basal respiration (**b**) depending on treatments (CK, T1, T10, T100). *Different letters* indicate significantly different according to ANOVA with Duncan's multiple-range test ($p < 0.05$)

Table 2 Effects of chlorimuron-ethyl on CLPPs at different application rates. (CK, T1, T10 and T100)

Incubation time (day)	Chlorimuron-ethyl application rate			
	Control	T1	T10	T100
AWCD				
1	0.19 ± 0.006b	0.19 ± 0.008b	0.33 ± 0.033a	0.36 ± 0.028a
7	0.30 ± 0.017b	0.33 ± 0.013b	0.37 ± 0.030a	0.38 ± 0.018a
15	0.32 ± 0.029b	0.33 ± 0.031b	0.35 ± 0.031ab	0.36 ± 0.088a
30	0.36 ± 0.011b	0.34 ± 0.051b	0.35 ± 0.047b	0.40 ± 0.017a
60	0.17 ± 0.008a	0.16 ± 0.014a	0.17 ± 0.009a	0.18 ± 0.012a
90	0.16 ± 0.011a	0.15 ± 0.015a	0.15 ± 0.016a	0.15 ± 0.011a
Shannon index (H′)				
1	2.27 ± 0.124b	2.1 ± 0.170b	2.65 ± 0.091a	2.64 ± 0.194a
7	2.63 ± 0.229a	2.60 ± 0.096a	2.61 ± 0.228a	2.69 ± 0.153a
15	2.71 ± 0.146b	2.73 ± 0.014b	2.74 ± 0.053b	2.99 ± 0.119a
30	2.62 ± 0.104a	2.72 ± 0.125a	2.65 ± 0.199a	2.82 ± 0.060a
60	2.54 ± 0.025a	2.44 ± 0.153a	2.62 ± 0.294a	2.51 ± 0.251a
90	2.37 ± 0.077a	2.47 ± 0.054a	2.43 ± 0.104a	2.38 ± 0.045a

Different letters indicate significant differences ($p < 0.05$) among treatments at a given incubation time (ANOVA with Duncan's multiple range test). Mean values (n = 3) ± SE

higher than that in the control, which did not recover by the end of the experiment ($p < 0.05$). The total PLFA is a good indicator of the living microbial biomass (Baath and Anderson 2003), which is closely correlated with the microbial biomass carbon (MBC) in this study.

The ratio of Gram-negative/Gram-positive bacteria (GN/GP) was used to study changes in the microbial community. The GN/GP of all the treatments varied significantly throughout the entire experimental period ($F_{time} = 245.44$, $p < 0.001$), with significant effects observed in response to the chlorimuron-ethyl treatments ($F_{treatment} = 23.28$, $p < 0.001$, Table 3). The GN/GP tended to increase slightly in the herbicide treatments (T1, T10 and T100) compared to the control within the initial

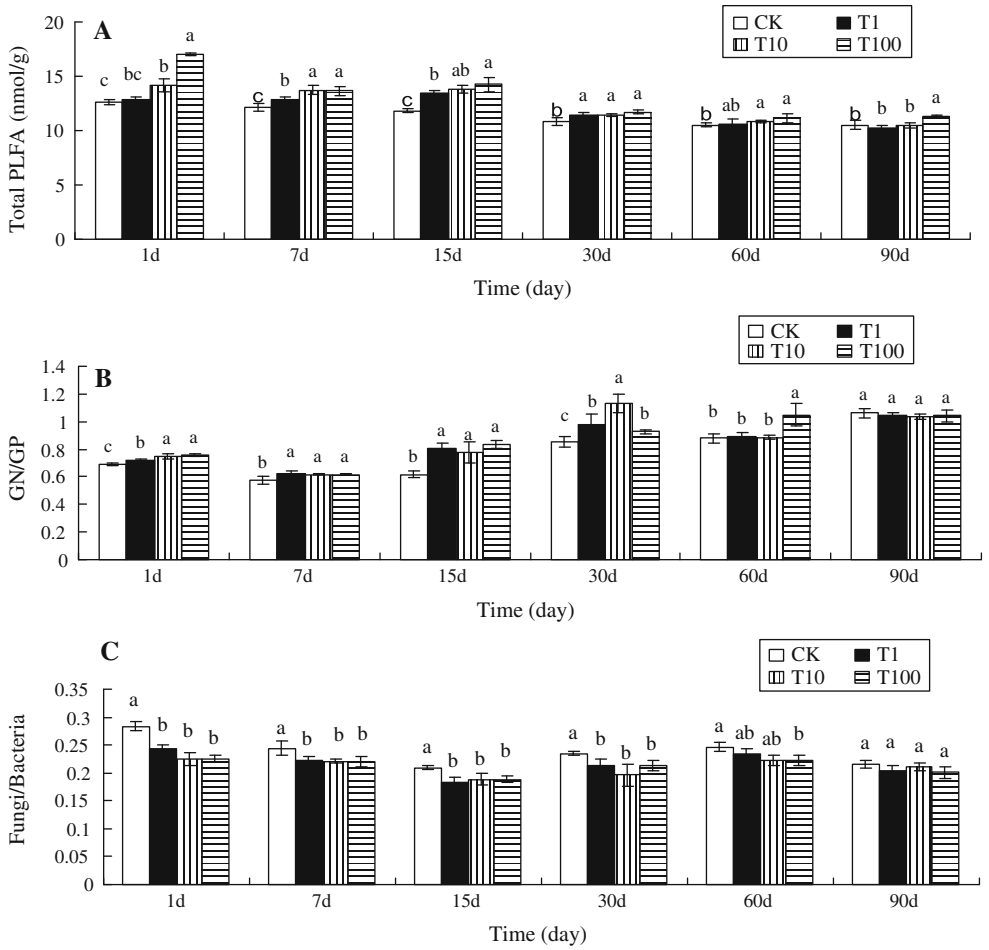

Fig. 2 Total PLFA (**a**), GN/G P (**b**), fungi/bacteria (**c**) depending on treatments (CK, T1, T10, T100). *Different letters* indicate significantly different according to ANOVA with Duncan's multiple-range test ($p < 0.05$)

Table 3 Two-way ANOVA for soil microbial properties affected by chlorimuron-ethyl concentration (Treatment), incubation time (Time) and their interaction (Treatment × time)

Factor	Basal respiration	MBC	AWCD	H′	Total PLFA	GN/GP	Fungi/bacteria
Treatment	45.13***	67.11***	33.89***	1.63*	57.58***	23.28***	143.16***
Time	37.58***	104.82***	275.17***	15.47**	133.02***	245.44***	109.06***
Treatment × time	11.01***	16.97***	8.84***	1.77	9.83***	8.88***	11.76***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

30 day (Fig. 2b). The GN/GP in T1 and T10 recovered at the 60th day, meanwhile the GN/GP in T100 recovered at the 90th day. Gram-negative bacteria (GN) are known to change significantly with variations in the environment, and increase rapidly when easily utilizable carbon sources are available (Byss et al. 2008). In this study, the GN/GP was higher in the treated samples during the initial 30 day. This may be because the herbicide acted as a carbon source for the Gram-negative soil bacteria. Similar results have

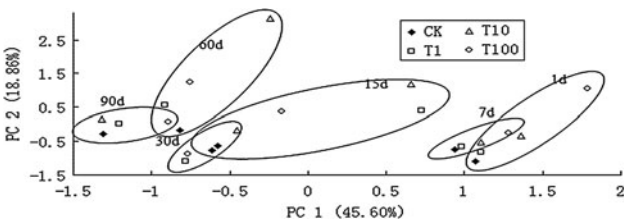

Fig. 3 PCA of PLFAs form microbial community structure for all treatments (CK, T1, T10, T100)

Table 4 PC scores in PCA analysis of soil microbe PLFAs after chlorimuron-ethyl application

Principal component	Incubation time(d)	Control	T1	T10	T100
PC1	1	1.069 ± 0.104b	1.110 ± 0.233b	1.360 ± 0.268ab	1.786 ± 0.126a
	7	0.936 ± 0.055b	0.986 ± 0.126b	1.110 ± 0.130ab	1.283 ± 0.121a
	15	−0.574 ± 0.114c	0.728 ± 0.156a	0.663 ± 0.169a	−0.164 ± 0.123b
	30	−0.614 ± 0.087a	−0.780 ± 0.130b	−0.449 ± 0.078a	−0.769 ± 0.112b
	60	−0.819 ± 0.125b	−0.912 ± 0.096b	−0.239 ± 0.037a	−0.756 ± 0.159b
	90	−1.309 ± 0.128b	−1.206 ± 0.255b	−1.314 ± 0.132b	−0.890 ± 0.031a
PC2	1	−1.083 ± 0.169c	−0.827 ± 0.182bc	−0.334 ± 0.256b	1.042 ± 0.133a
	7	−0.750 ± 0.137c	−0.657 ± 0.052bc	−0.532 ± 0.066b	−0.299 ± 0.096a
	15	−0.629 ± 0.121c	0.395 ± 0.171b	1.180 ± 0.247a	0.383 ± 0.124b
	30	−0.778 ± 0.148c	−1.099 ± 0.180a	−0.170 ± 0.092a	−0.866 ± 0.153ab
	60	−0.166 ± 0.128d	0.551 ± 0.172c	3.128 ± 0.251a	1.241 ± 0.084b
	90	−0.287 ± 0.168a	0.006 ± 0.289a	0.155 ± 0.323a	0.070 ± 0.148a

Different letters indicate significantly different according to ANOVA with Duncan's multiple-range test ($p < 0.05$). Mean values (n = 3) ± SE

been observed in other studies. Zhang et al. (2010a) reported that the GN/GP in the imazethapyr-applied soils was higher than that in the control. The ratio of fungi/bacteria was significantly affected by chlorimuron-ethyl ($F_{\text{treatment}} = 143.16$, $p < 0.001$, Table 3), which tended to decrease in the herbicide treatments (T1, T10 and T100) in the first 30 day, and then recovered at the 60th day (T1 and T10) and the 90th day (T100; Fig. 2c).

The results of PCA using all 19 individual fatty acids are shown in Fig. 3. Different community structures were identified in soils subjected to different treatments. PCA showed that the first principal component (PC1 = 45.60 %) and the second principal component (PC2 = 18.86 %) explained 64.46 % of the total variance from all the identified PLFAs between the samples treated with chlorimuron-ethyl and the control. In total, 9 PLFAs were strongly correlated ($|r| \geq 0.6$, $|r|$ is absolute value of correlation coefficient; Wang et al. 2008) to PC1, including i15:0, a15:0, i16:0, 16:1ω7c, i17:0, cy17:0, 18:1ω9c, 18:1ω9t and cy19:0, and 4 PLFAs were strongly correlated to PC2, including 15:0, 20:0, 21:0, and 22:0. Differences among the incubation times of control samples and the three treatments (T1, T 10 and T100) are shown in Table 4. The microbial community structure of the control was significantly different from T100 at day 1 and day 7. At the 15th day, distinct differences were observed between the chlorimuron-ethyl treated soils (T1, T10 and T100) and the control. At the 90th day, the microbial community structures among the filed rate treatment, the 10-fold filed rate treatment and the untreated control had no obvious difference. The 100-fold rate of chlorimuron-ethyl had the consistent effect on the microbial community structure, which did not recover at the end of incubation.

Conclusions

Repeated chlorimuron-ethyl inputs had a temporary stimulation on the soil activity, microbial biomass, GN/GP, and Shannon index. The application of chlorimuron-ethyl led to a shift in the microbial community structure during the incubation time. And then, these effects at the field rate and 10-fold field rate treatments became undetectable at the end of this experiment. Repeated chlorimuron-ethyl application at the highest rate (100-fold field rate) had the persistent effects on the microbial biomass carbon, total PFLA and soil microbial community structure to the end of experiment. There existed a dosage effect of chlorimuron-ethyl on the living microbial biomass and the microbial community structure.

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References

Baath E, Anderson TH (2003) Comparison of soil fungi/bacterial rations in a pH gradient using physiological and PLFA-based techniques. Soil Biol Biochem 35:965–963

Braun B, Bockelmann U, Grohmann E, Szewzyk U (2006) Polyphasic characterization of the bacterial community in an urban soil profile with in situ and culture-dependent methods. Appl Soil Ecol 31:267–279

Brown HM (2006) Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic Sci 29:263–281

Byss M, Elhottová D, Tříska J, Baldrian P (2008) Fungal bioremediation of the creosote-contaminated soil: influence of *Pleurotus ostreatus* on polycyclic aromatic hydrocarbons removal and soil microbial community composition in the laboratory-scale study. Chemosphere 73:1518–1523

Chen SK, Edwards CA (2001) A microcosm approach to assess the effects of fungicides on soil ecological processes and plant

growth: comparisons of two soil type. Soil Biol Biochem 33:1982–1991

China Pesticide Information Network (2010) http://www.chinapesticide.gov.cn/service/aspx/B3X.aspx?aiid=HECHL

Cycon M, Piotrowska-Seget Z, Kaczynska A, Kozdrój J (2006) Microbiological characteristics of a sandy loam soil exposed to tebuconazole and l-cyhalothrin under laboratory conditions. Ecotoxicology 15:639–646

Fang H, Tang FF, Zhou W, Cao ZY, Wang DD, Liu KL, Wu XW, Yu YL (2012) Persistence of repeated triadimefon application and its impact on soil microbial functional diversity. J Environ Sci Health B 47(2):104–110

Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS (2003) Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl Environ Microbiol 69:1800–1809

Guidelines on environmental safety assessment for chemical pesticides in China, 2012

Hernández AJ, Becerril J, Zárate O, Garbisu C (2006) Assessment of the efficiency of a metal phytoextraction process with biological indicators of soil health. Plant Soil 281(1–2):147–158

Rodriguez RA, Toranzos GA (2003) Stability of bacterial populations in tropical soil upon exposure to Lindane. Int Microbiol 6(4):253–258

Sarah HL, Emily BH, Scott AS, Terry JG (2010) Effects of repeated glyphosate applications on soilmicrobial community composition and the mineralization of glyphosate. Pest Manag Sci 66:59–64

Sharma D, Singh SB (2012) Persistence of bifenthrin in sandy loam soil as affected by microbial community. Bull Environ Contam Toxicol 88:906–908

Sojka RE, Entry JA, Fuhrmann JJ (2006) The influence of high application rates of polyacrylamide on microbial metabolic potential in an agricultural soil. Appl Soil Ecol 32:243–252

Teng C, Tao B (2008) Effects of chlorimuron-ethyl on soil microorganism population and soil respiration intensity. Chin J Soil Sci 39:384–387

Wang MC, Gong M, Zang HB, Hua XM, Yao J, Pang YJ, Yang YH (2006) Effect of methamidophos and urea application on microbial communities in soils as determined by microbial biomass and community level physiological profiles. J Environ Sci Health B 41:399–413

Wang YP, Shi JY, Wang H, Lin Q, Chen XC, Chen YX (2007) The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. Ecotoxicol Environ Saf 67(1):75–81

Wang MC, Liu YH, Wang Q, Gong M, Hua XM, Pang YJ, Hu SJ, Yang YH (2008) Impacts of methamidophos on the biochemical, catabolic, and genetic characteristics of soil microbial communities. Soil Biol Biochem 40:778–788

Wang XG, Song M, Wang YQ, Gao CM, Zhang Q, Chu XQ, Fang H, Yu YL (2012) Response of soil bacterial community to repeated applications of carbendazim. Ecotoxicol Environ Saf 75:33–39

Winding A, Hund-Rinke K, Rutgers M (2005) The use of microorganisms in ecological soil classification and assessment concepts. Ecotoxicol Environ Saf 62:230–248

Wu J, Lin Q, Huang Q, Xiao H (2006) Soil microbial biomass-methods and application. China Meteorological Press (in Chinese), Beijing 12: 54-78

Wu XW, Cheng LY, Cao ZY, Yu YL (2012) Accumulation of chlorothalonil successively applied to soil and its effect on microbial activity in soil. Ecotoxicol Environ Saf 81:65–69

Xie XM, Liu WP, Abid S (2004) Rapid degradation of bensulfuron-methyl upon repeated application in paddy soils. J Environ Sci 16:49–52

Xu J, Guo LQ, Dong FS, Liu XG, Wu XH, Sheng Y, Zhang Y, Zheng YQ (2013) Response of the soil microbial community to imazethapyr application in a soybean field. J Environ Sci Health B 48:505–511

Xue K, Luo HF, Qi HY, Zhang HX (2005) Changes in soil microbial community structure associated with two types of genetically engineered plants analyzing by PLFA. J Environ Sci 17:130–134

Zak DR, Kling GW (2006) Microbial community composition and function across an arctic tundra landscape. Ecology 87: 1659–1670

Zawoznik MS, Tomaro ML (2005) Effect of chlorimuron-ethyl on *Bradyrhizobium japonicum* and its symbiosis with soybean. Pest Manag Sci 61:1003–1008

Zhang C, Xu J, Dong FS, Liu XG, Sheng Y, Zheng YQ (2010a) Impact of imazethapyr on the microbial community structure in agricultural soils. Chemosphere 81:800–806

Zhang CP, Liu XG, Dong FS, Xu J, Zheng YQ (2010b) The effect of imazethapyr on soil microbes in soybean fields in northeast China. Chem Ecol 26(3):173–182

Zhao CS, He FL (2007) Effects of long residue herbicides on agricultural development of Heilongjiang province. J Dongbei Agric Univ 38:136–139