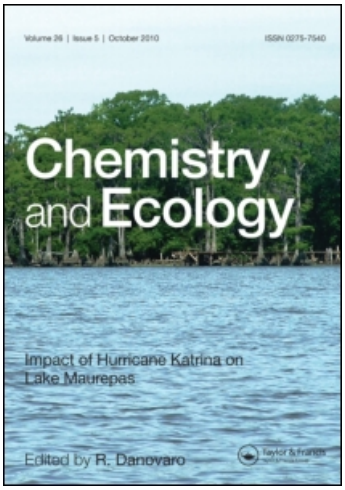

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

The effect of imazethapyr on soil microbes in soybean fields in northeast China

Changpeng Zhang[a]; Xingang Liu[a]; Fengshou Dong[a]; Jun Xu[a]; Yongquan Zheng[a]
[a] Key Laboratory of Pesticide Chemistry and Application, Chinese Academy of Agricultural Sciences, Beijing, China

Online publication date: 13 May 2010

To cite this Article Zhang, Changpeng , Liu, Xingang , Dong, Fengshou , Xu, Jun and Zheng, Yongquan(2010) 'The effect of imazethapyr on soil microbes in soybean fields in northeast China', Chemistry and Ecology, 26: 3, 173 — 182
To link to this Article: DOI: 10.1080/02757541003785817
URL: http://dx.doi.org/10.1080/02757541003785817

Chemistry and Ecology
Vol. 26, No. 3, June 2010, 173–182

Taylor & Francis
Taylor & Francis Group

The effect of imazethapyr on soil microbes in soybean fields in northeast China

Changpeng Zhang, Xingang Liu, Fengshou Dong, Jun Xu and Yongquan Zheng*

Key Laboratory of Pesticide Chemistry and Application, Chinese Academy of Agricultural Sciences, Beijing, China

(*Received 24 August 2009; final version received 9 February 2010*)

Large quantities of herbicides are used on agricultural soils, but the effects of herbicides on the structure of the soil microbial community have not been well investigated. In this study, soil from three soybean fields was investigated. The herbicide imazethapyr was applied in one year to soil 1 and in two sequential years to soil 2. Control soil received no imazethapyr. Microbial biomass and community structure were characterised using chloroform fumigation–extraction and phospholipid fatty acid (PLFA) determination. The imazethapyr residue was 1.62 $\mu g \cdot kg^{-1}$ in soil 1 and 1.79 $\mu g \cdot kg^{-1}$ in soil 2. The microbial biomass carbon and total PLFAs for soil 2 were much higher than for the other soils. PLFA profiles showed that fatty acids for Gram-negative and Gram-positive bacteria, as well as total bacteria and total fungi in soil 2 were higher than in other samples. Principal component analysis of the PLFAs showed that the structure of the microbial community differed substantially among the three different soybean field soils. Application of the herbicide imazethapyr to soybean fields clearly changed the soil microbial biomass and shifted the structure of the microbial community.

Keywords: imazethapyr; herbicide residue; soil microbial biomass; phospholipid fatty acid; microbial community structure

1. Introduction

A wide variety of pesticides are used in modern agriculture, even though the long-term environmental impact of their widespread application is unknown [1]. Therefore, if current agricultural practices are to be environmentally sustainable, the fate and action of herbicides in agricultural soils must be monitored and studied [2]. The northeast China Plain is a prime agricultural area of China, where the soil is under intensive agricultural use, with one crop per annum, which necessitates the use of agrochemicals [3]. Imazethapyr, a selective herbicide in the the imidazolinone class, is used to control a wide spectrum of broadleaf weeds in the cultivation of soybean and other leguminous crops [4]. In northeast China, 3000–6000 tons of imazethapyr preparations are used each year to control weeds in soybean fields [5]. Intensive use of the pesticide has caused serious pesticide injury to crops, and has also become concentrated in the environment because of the contamination of nontarget sites. Because pesticides can potentially be toxic to organisms

*Corresponding author. Email: yqzheng@ippcaas.cn

ISSN 0275-7540 print/ISSN 1029-0370 online

DOI: 10.1080/02757541003785817
http://www.informaworld.com

other than their intended target, determining their impact on nontarget organisms within the soil is of considerable interest [6]. Several studies have determined the effects of imazethapyr and other pesticides on soil microbes [7–9], including the microbial biomass, enzyme activity and microbial diversity. However, few studies have examined the effect on the structure of the soil microbial community in a soybean field after different times of imazethapyr application.

Soil microbes are a key component of soil ecosystems. They are critical for nutrient element cycling and play a major role in maintaining soil quality [10]. Soil microbes are an integral component of soil quality and a better understanding of their metabolic processes is needed [11]. Pesticides have the potential to influence the population and function of a diverse range of soil micro-organisms and thus alter soil fertility [12]. Characterisation of both pesticide residues and microbial degrader populations is necessary for a better understanding of how pesticides and microbial community structures interact. Lipid biomarkers, analysed as phosphoplipid fatty acids (PLFA), have been used successfully as a molecular tool to characterise microbial communities in soil. PLFAs are a powerful tool for assessing active microbial populations in environmental samples because they are highly sensitive to changes in the microbial community structure and can be quantitated to evaluate microbial biomass and microbial groups [13–15].

The aims of this study were to assess the effects of applying imazethapyr for different lengths of time on soil microbes in soybean fields. Microbial biomass carbon (MBC) and microbial community structure were studied using the chloroform fumigation–extraction method and the culture-independent method of PLFA analysis.

2. Materials and methods

2.1. *Field sampling*

Soils were collected from the soybean field of Laolai Farm near Nehe city (48° 29′ N, 124° 51′ E), in Heilongjiang Province. The average temperatures in the region are −7.5 °C in winter and 22.7 °C in summer, with rainfall of ∼400–550 mm per year. The investigated field soils were classified as loam. Three soils with the same texture, but which had been treated with imazethapyr for 0, 1 or 2 years, were selected and designated control soil, soil 1 and soil 2. Imazethapyr was applied at 100 g ai·hm^{-2} (ai, active ingredient) to soil 1 on 30 May 2008 and 20 June 2008. Soil 2 received 100 g ai·hm^{-2} imazethapyr in May and June of 2007 and 2008. The herbicide was 5% imazethapyr aqueous solution, which was applied at 2000 g aqueous solution·hm^{-2} using a water spray. No pesticide was applied to the control soil, with weeds removed by pulling. Soil characteristics are given in Table 1.

Field samplings were processed according to Donald [16] and Niu [17]. The agricultural plot was divided into a grid and randomly sampled. Sample areas were 8× 8 m, the distance between

Table 1. Characteristics of the soybean field soils.

Soil property	Soybean field soil
Texture	Loam soil
Organic matter (%)	2.49
pH	8.48
NH_4^+-N (mg·kg^{-1})	138.24
Available P (mg·kg^{-1})	4.25
Available K(mg·kg^{-1})	139.03
Total N (%)	0.12
Total P (%)	0.02
Total K (%)	0.08
CEC (100 g soil)	12.22

samples was >20 m, and microhabitats and soil textures were same. Triplicate composite samples were collected for the three soil types in November 2008. After plants and other large items had been removed, soil samples of 0–15 cm were collected on nonsunny days, by taking 20 random, 3 cm diameter cores from each treatment plot, in replicates. Soil samples were fully mixed, packed, labelled and transported to the laboratory, where they were sieved through a 2 mm mesh and divided into three portions. The first was stored at −10 °C to determine imazethapyr residue. The second was maintained at 4 °C to determine soil MBC within one week, and the third was freeze-dried and stored at −70 °C to analyse the soil microbial community structure for PLFAs.

2.2. *Residue analysis*

Imazethapyr residue was determined according to Ma et al. [18] with modifications. A 20 g soil sample was weighed into a 50 mL tube and 25 mL 0.1 M NH_3-NH_4Cl buffer (pH 2) was added. The mixture was swirled for 1 min, extracted for 20 min by ultrasonication and centrifuged for 3 min at 6000 rpm. Supernatants (10 mL) were removed and acidified to pH 2.0. The residue was purified on a C_{18} cartridge (Cleanert C_{18}, 500 mg·6 mL^{-1}; Agela Technologies Inc.). The cartridge was preconditioned with 5 mL hexane, 5 mL CH_2Cl_2, 5 mL CH_3OH, 5 mL water and 5 mL 0.01 M HCl, before 5 mL of acidified supernatant was added. The cartridge was washed with 5 mL water and 5 mL hexane, and eluted with 10 mL CH_2Cl_2. The filtrate was dried under N_2 and resuspended with methanol to 2 mL. The residue was filtered through a 0.22 μm nylon syringe filter for chromatographic injection.

The Waters Acquity UPLC system (USA), consisted of a binary solvent manager and an Acuity column heater equipped with a Waters Acquity UPLC BEH C_{18} column (2.1 × 50 mm, 1.7 μm particle size) (Waters, Milford, MA, USA). Gradient UPLC elution was performed with methanol (LC grade) as mobile phase A and 0.2% formic acid in ultrapure water as mobile phase B. Separation of the analytes was performed at 45 °C at a flow rate of 0.3 mL·min^{-1} with an elution gradient of 90 to 10% B in 2.5 min, then 90% B in 0.1 min, holding at 90% B for 2 min. The temperature in the autosampler was set at 10 °C and the sample volume injected was 3 μL.

A triple-quadrupole mass spectrometer (Waters Corp.) equipped with an electrospray ionisation source was used for imazethapyr detection. MS/MS detection was performed in positive mode and the monitoring conditions were optimised for imazethapyr. Acquisition parameters were capillary voltage 3 kV, source temperature 120 °C and desolvation temperature 350 °C. The cone and desolvation gas flows were 50 and 400 L·h^{-1} nitrogen, respectively. The precursor ion was 290 (m/z) and its product quantitative ion was 245 (m/z), when the collision energy was set at 22 V. The multiple reaction monitoring (MRM) was in scan mode.

2.3. *Microbiological analysis*

MBC was determined using the chloroform fumigation–extraction method [19,20] with modifications. Each soil sample was divided into two groups, each containing 20.0 g of dry soil. One group was fumigated with ethanol-free chloroform for 24 h in the dark at 25 °C, and another group was untreated. Both fumigated and unfumigated soils were extracted with 0.5 M K_2SO_4 solution for 30 min on a shaker (1 : 4 soil/solution w/w). The supernatant was filtered and 10.00 mL filtrate was mixed with 5.00 mL 0.2 M $K_2Cr_2O_7$ and 5 mL H_2SO_4. The mixture with zeolite was boiled for 10 min at 170–180 °C, before cooling and titrating with 0.05 M $FeSO_4$ $C_2H_8N_2$as an indicator. When the solution changed from yellow to cyan to brownish-red, titration was stopped. MBC was calculated as MBC = Ec/Kc, where Ec = (C extracted from fumigated soil) – (C extracted from nonfumigated soil), with C representing carbon, and Kc a calibration factor of 0.38 [21].

PLFA analysis was performed by a combination of two methods [22,23]. A 5 g sample of freeze-dried soil was extracted for 2 h in 19 mL of a one-phase extraction mixture containing $CHCl_3$/MeOH/citric acid buffer (1 : 2 : 0.8, v/v/v). After centrifugation, the supernatant was decanted to a separatory funnel. The soil was vortexed and re-extracted with an additional 19 mL of extractant, which was added to the first supernatant. A total of 10 mL of citric acid buffer and 10 mL of $CHCl_3$ were added. Samples were shaken and separated. The $CHCl_3$ layer was decanted and dried under N_2 at 30 °C. Phospholipids were separated from neutral and glycolipids on solid-phase 0.50 g Si extraction columns (Supelco, Inc.). The column was conditioned with 3 mL of $CHCl_3$, and lipids were transferred to the column with $CHCl_3$. Neutral lipids and glycolipids were eluted with 5 mL of $CHCl_3$, followed by 10 mL of acetone. Polar lipids were eluted with 5 mL of MeOH and dried under N_2 at 30 °C. Samples were subjected to mild alkaline methanolysis by dissolving in 1 mL of MeOH/toluene (1 : 1, v/v) and 1 mL of 0.2 M KOH and heating at 37°C for 15 min. A total of 2 mL of H_2O, 0.3 mL of 1.0 M acetic acid and 2 mL hexane were added. After centrifugation, the supernatant contained the resultant fatty acid methyl esters (FAMEs), which were separated, quantified and identified by gas chromatography mass spectrometry (GC-MS).

PolarisQ ion-trap GC-MS (Thermo Fisher Scientific, Inc.) with HP-5 ms column (60 m × 0.25 mm inner diameter, 0.25 μm film thickness) was used for FAME identification. Helium was used as the carrier gas (1.0 mL·s^{-1}). The oven temperature was 140 °C for 3 min, increased to 190 °C (held for 1 min) at 10 °C·min^{-1}, increased to 230 °C (held for 1 min) at 3 °C·min^{-1}, and then to 250 °C (held for 1 min) at 2 °C·min^{-1}. Subsequently, the temperature was increased at 10 °C·min^{-1} to 300 °C and held for 5 min. The injection volume was 1 μL. Quantification was by calibration against standard solutions of nonadecanoate methyl ester (C19 : 0), which were also used as the internal standard. A total of 37 PLFAs were identified in the soil samples, and fatty acids present at >0.5% were used in the analysis. The prefixes 'a' and 'i' indicate antiso- and isobranching, and 'cy' indicates a cyclopropane fatty acid. Microbial biomass was assessed by 18 fatty acids, 14:0, 2OH14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 16:1ω9c, 2OH16:0, i17:0, cy17:0, 10Me17:0, 10Me18:0, 18:1ω9c, 18:1ω9t, 18:2ω6,9, cy19:0 and 24:0. The branched phospholipids i15:0, a15:0 and i16:0 were used as indicators for Gram-positive bacteria, whereas the PLFAs 16:1ω7c and cy17:0 were indicative of Gram-negative bacteria. Fungal biomass was assessed by quantifying 18:1ω9c, 18:1ω9t and 18:2ω6,9 [16]. The total amount of PLFA was the sum of all microbial phospholipids mentioned. The Gram-negative/Gram-positive ratio was used as an indicator of changes in the relative abundance of these microbial groups [24]. The stress level (cyc/precursor) for the microbial community was calculated from the ratio (cyc17:0 + cyc19:0)/(16:1ω7c + 18:1ω7c) [22]. Before being subjected to principal component analysis (PCAs), results were expressed as a percentage of the total PLFA.

2.4. *Statistical analyses*

A one-way analysis of variance (ANOVA) with Duncan's multiple-range test was used to compare the differences between measured MBC, total PLFA and relative abundance of PLFA. PCA was used to examine PLFA community structure among different samples that contained multiple variables. All values are mean ± SE of three replicates, and in the tables data in a column (or row) followed by different letters are significantly different at $p < 0.05$. Statistics were calculated using Statistica 6.0 (Statsoft, Tulsa, OK, USA).

3. Results and discussion

Imazethapyr and other imidazolinone herbicides interrupt the biosynthesis of branched-chain amino acids in plants by blocking acetolactate synthase, the first common enzyme of the

pathway [4]. Imazethapyr, which can persist for a long time in soils and has half-life of 53–122 days [25], was applied at the recommended field rate of 100 g ai·hm^{-2} in northeast China to remove weeds for soybean cultivation. Herbicide residue is thought to inflict injury on subsequent crops [26,27]. The initial residue level after application of the herbicide was 0.66 mg·kg^{-1} in soils. However, imazethapyr residue was 1.62 and 1.79 μg·kg^{-1}, respectively, in soil 1 and soil 2 (Table 2), indicating that this herbicide was decomposed almost completely.

Most studies on the effect of pesticides on soil microbes have been conducted in the laboratory [7,28,29], which does not completely reflect the field situation. However, this study was performed in a soybean field, so the results have more practical significance and reflect the real-life situation. The MBC (Figure 1) in soil 2 was clearly higher than in the other soils. Perucci et al. [8] and Lupwayi et al. [10] reported that imazethapyr had no adverse effects on soil MBC or microbial diversity, when applied at the field rate, but had a toxic effect when applied at higher rates. Similarly, total PLFAs (Table 3) ranged from 85.39 ± 1.02 nmol·g^{-1} in soil 1 to 101.57 ± 0.44 nmol·g^{-1} in soil 2, and total PLFAs were clearly higher in soil 2 than in control soil samples. Widenfalk et al. [29], however, reported that the total PLFAs in profundal sediment were not affected by glyphosate exposure. Thus, the result that two years of imzethapyr application may increase the soil MBC and the total PLFAs in soil, might indicate that the herbicide itself can provide a carbon source for soil microbes.

Microbial communities in the three soil samples differed significantly in composition (Figure 2). Of the 18 PLFAs used to assess microbial community composition, 16 displayed significant differences in the soils (Figure 2). PLFA profiles were dominated by the fatty acids 18:1ω9c, 16:0, a15:0, i15:0 and 16:1ω7c, which together accounted for >60% of total PLFAs. Both soils 1 and 2, which received imazethapyr applications, showed a significant decrease in the relative abundance of Gram-positive fatty acids (a15:0), bacterial fatty acids (3OH12:0, i17:0) and fungal

Table 2. Imazethapyr residue in the soybean field soils. Values are given as mean ± SE.

Treatment	CK	Soil 1	Soil 2
Imazethapyr (μg·kg^{-1})	ND	1.62 ± 0.33[a]	1.79 ± 0.244[a]

Notes: Values labelled with different letters are significantly different at $p < 0.05$, ANOVA with Duncan's multiple-range test. ND, Not detected.

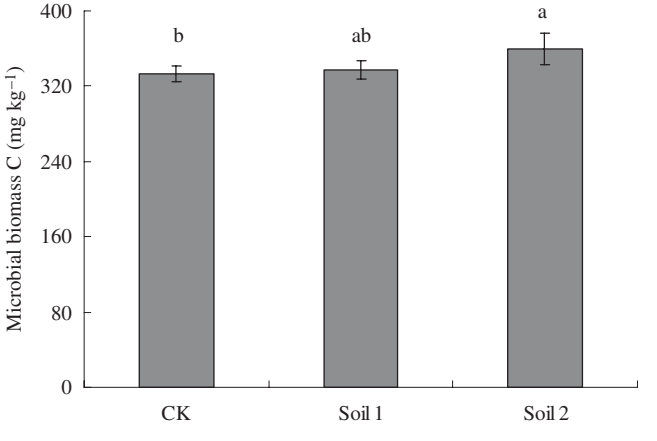

Figure 1. Microbial biomass carbon from soybean field soils after application of imazethapyr for 0, 1 or 2 years. Data are given as means ± SE. Values labelled with different letters are significantly different at $p < 0.05$, ANOVA with Duncan's multiple-range test.

Table 3. Total phospholipid fatty acid (PLFA) and PLFA of bacteria and fungi extracted from micro-oganisms from soybean field soil with imazethapyr application for 0, 1 or 2 years. Values are given as mean ± SE.

	Fatty acid concentration (nmol·g^{-1} dry soil)		
PLFA	Control	Soil 1	Soil 2
Gram negative	11.09 ± 0.14^{b}	11.07 ± 0.11^{b}	13.03 ± 0.09^{a}
Gram positive	22.05 ± 0.29^{b}	21.91 ± 0.21^{b}	25.79 ± 0.17^{a}
Gram negative/Gram positive	0.50 ± 0.00^{b}	0.51 ± 0.00^{a}	0.51 ± 0.01^{a}
Stress (cyc/precursor)	0.37 ± 0.01^{b}	0.39 ± 0.01^{a}	0.39 ± 0.01^{a}
Bacteria	67.65 ± 0.42^{b}	62.03 ± 0.83^{c}	74.66 ± 0.47^{a}
Fungi	20.87 ± 0.27^{b}	20.57 ± 0.23^{b}	23.82 ± 0.14^{a}
Actinomycetes	1.45 ± 0.02^{c}	2.10 ± 0.11^{b}	2.31 ± 0.06^{a}
Total PLFA	90.81 ± 0.70^{b}	85.39 ± 1.02^{c}	101.57 ± 0.44^{a}

Notes: Values labelled with different letters are significantly different at $p < 0.05$, ANOVA with Duncan's multiple-range test.

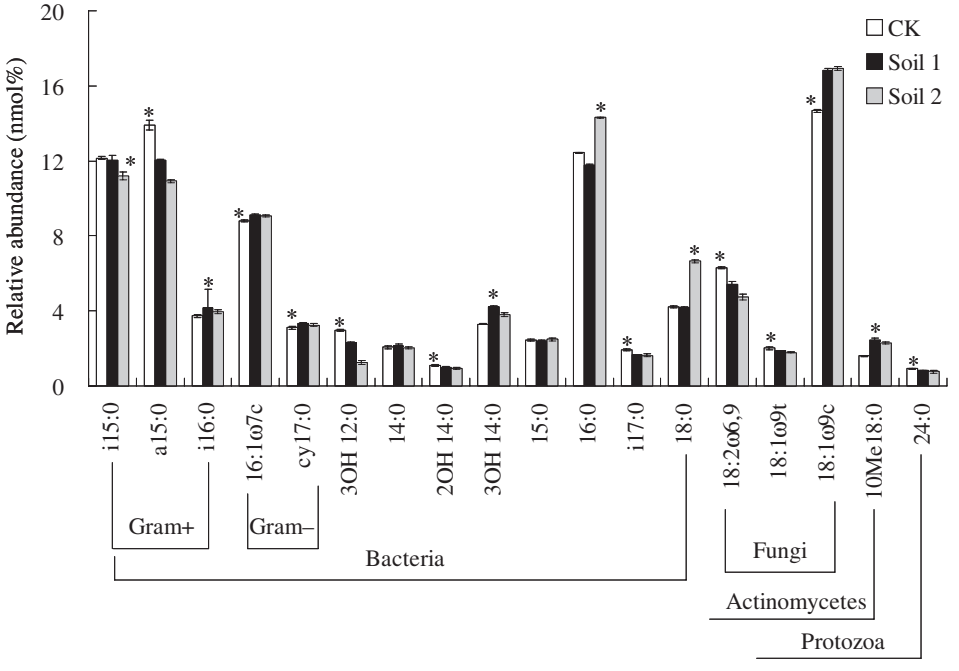

Figure 2. Relative abundance of bacterial, fungal, actinomycetal and protozoal phospholipid fatty acids (PLFAs) in soybean field soil. *$p < 0.05$.

fatty acids (18:2ω6,9, 18:1ω9t). By contrast, herbicide applications to soil 1 and 2 clearly increased the relative abundance of Gram-negative fatty acids (16:1ω7c and cy17:0), fungal fatty acids (18:1ω9c) and actinemycetal fatty acids (10Me18:0) (Figure 2).

The ratio Gram-negative/Gram-positive bacteria was used to study changes in the microbial community for the three different soil types [24]. Gram-negative bacteria are known to change significantly with variations in the environment, and increase rapidly when easily utilisable carbon sources are available [30]. In this work, the levels of Gram-negative bacteria (Table 3) were much higher in the soil 2 samples than in the others. This may be because the herbicide combined with soil acted as a carbon source for the Gram-negative soil bacteria. Similar results have been observed in other studies. Thompson et al. [31] and Blakely et al. [32] examined the effects of the organic

pollutants, 1,2-dichlorobenzen and polyclic aromatic hydrocarbons on soil microbial communities, and found that these chemicals both caused significant increases in Gram-negative bacteria. Gram-negative soil bacteria were significantly increased by methamidophos [11]. Because they have relatively thick cell walls and are able to form endospores, Gram-positive bacteria are considered stress tolerant [33], and levels of Gram-positive bacteria in samples of soil 2 were much greater than in other soils (Table 3). The ratio of Gram-negative to Gram-positive bacteria in the samples of soil 1 and soil 2 were higher than in the control soils, which demonstrated that the application of the herbicide imazethapyr for one and two years increased the proportion of Gram-negative bacteria.

In the samples of soil 1 and soil 2, application of imazethapyr affected the stress level of the microflora. The cyc/precursor ratio was larger after treatment with imazethapyr for one or two years (Table 3). The results suggested imazethapyr application in the soybean field had an impact on the microbial community. Similarly, Hammesfahr et al. [34] reported an increase in stress level under antibiotic sulfadiazine treatment of $100\,\mu g \cdot g^{-1}$.

PCA of PLFAs showed clear distinctions in the microbial communities of the three soil samples (Figure 3), each of which occupied very different ordination space, indicating that the microbial community differed substantially among the three soil samples. PCA showed that the principal component axes PC1 and PC2 accounted for 85.31% of the variation among the three soil samples. Composite scores for the control soil occupied the positive portion of PC1, in which several bacterial PLFAs (2OH14:0, 24:0 and i17:0) received high positive weights (from 0.901 to 0.937). Several other PLFAs also received positive weights on this axis (18:1ω9t and 18:2ω6,9). The results demonstrated that the control soil correlated with the appearance of bacterial fatty acids (2OH14:0, 24:0 and i17:0) and fungal fatty acids (18:1ω9t and 18:2ω6,9). In composite scores for soil 1 for the positive portion of PC2, positive weights (0.612–0.834) were given to several bacterial PLFAs (15:0, 16:0 and 18:0). By contrast, composite scores for soil 2 occupied the negative portion of PC2, in which several bacterial PLFAs (3OH14:0, 14:0 and i16:0) received negative weights (from -0.580 to -0.705). This analysis clearly indicated that microbial communities were compositionally similar between sites within an ecosystem type, as evidenced by the close grouping of the replicate sites in ordination space, which indicates low within-ecosystem variation (Figure 3). This contrasts dramatically with the clear separation of microbial communities among the three different soil ecosystems (i.e. among-ecosystem variation; Figure 3). PCA of PLFAs

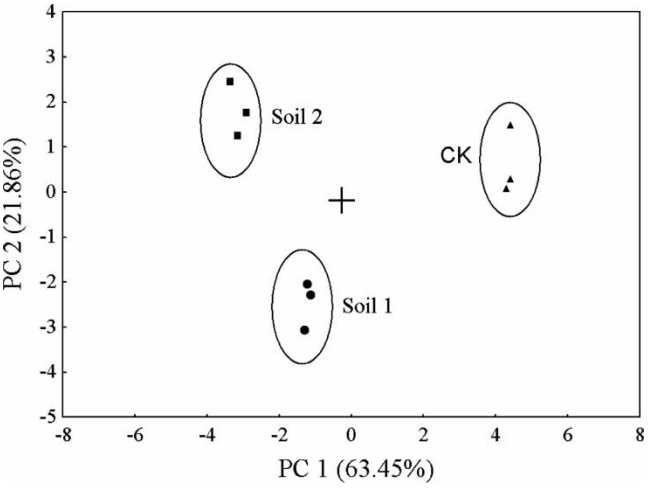

Figure 3. Principal components analysis plot of the microbial community structure of soybean field soils after application of imazethapyr for 0, 1 or 2 years. (+) indicates the (0, 0) point.

(Figure 3) showed that herbicide application shifted the soil microbial community, although this analysis does not provide profile details. These findings contribute significantly toward understanding the specific changes in soil microbial communities in response to long-term agricultural management practices. Any shift in community structure will have consequences for ecosystem function, if tolerant micro-organisms cannot compensate for the biogeochemical functions normally carried out by inhibited or eliminated microbial groups [29].

Soil bacteria are abundant, diverse and play important roles in the biogeochemical cycles that drive terrestrial ecosystems [35]. Soil microbial activity and diversity are influenced directly by environment fluctuation. Changes in soil type and field properties [36], soil water content [37], pH, plant diversity and composition [38] all influence the composition of soil microbial communities. Similarly, agricultural land management is a significant anthropogenic activity that greatly alters soil characteristics, including physical, chemical and biological properties and processes [39]. In this study, soils were collected from soybean fields in northeast China. Except for imazethapyr application in different years, farm operations were similar, including tillage practice, soybean variety grown and fertilisation, so the effects of imazethapyr applications for different periods on the soil micro-organisms could be deteremined. Soil microbes were studied in the MBC and in whole communities. Although the MBC and PLFA profile data did not correspond completely, variation in the soil microbial biomass and community structures could be distinguished among the three soil samples. Further studies will use PLFA analysis combined with other methods, such as community-level catabolic profiles (CLCPs) and amplified ribosomal DNA restriction analysis (ARDRA), to provide more comprehensive, exact information about the effect of the herbicide on the structure and function of microbial communities, and the implications on ecosystem-level processes.

4. Conclusions

The results presented here demonstrated that imazethapyr residue was $1.62\,\mu g{\cdot}kg^{-1}$ in soil 1 and $1.79\,\mu g{\cdot}kg^{-1}$ in soil 2. The MBC and total PLFAs in soil 2, which received two years' worth of herbicide application, were much higher than for the others. The PLFAs of bacteria, fungi, Gram-negative and Gram-positive bacteria in soil 2 were much higher than in other samples. The Gram-negative/Gram-positive ratio and stress levels were much higher in soil 1 and soil 2 than in controls. PCA of PLFAs clearly distinguished the microbial communities in soils that underwent different treatments. We conclude that application of the herbicide imazethapyr in soybean fields changed the soil microbial biomass and shifted the microbial community structure.

Acknowledgements

This work was supported by National Key Technology R&D Program (Grant No. 2006BAD08A03), Science and Technology Project for Food Production (2006BAD02A16), National Key Project of Fundamental Research (The 973 Program, Grant No. 2009CB119006) and Central Public-interest Scientific Institute Basal Research Fund (2010).

References

[1] Z. Piotrowska-Seget, R. Engel, E. Nowak, and J. Kozdroj, *Successive soil treatment with captan or oxytetracycline affects non-target microorganisms*, World J. Microbiol. Biotechnol. 24 (2008), pp. 2843–2848.
[2] V. Cuadrado, L.J. Merini, C.G. Flocco, and A.M. Giulietti, *Degradation of 2,4-DB in Argentinean agricultural soils with high humic matter conten*, Appl. Microbiol. Biotechnol. 77 (2008), pp. 1371–1378.
[3] L. Jia, *Issues and some strategies of sustainable development of black soil resources in black soil zone in northeast China*, Chin. Agric. Sci. Bull. 21 (2005), pp. 351–354.
[4] D.L. Shaner and S.L. O'Connor, *The Imidazolinone Herbicides*, CRC Press, Boca Raton, FL, 1991.

[5] Z. Ana, G. Susana, M.S. Luisa, A.R. Luis, and R. Mercedes, *Oxidative stress is not related to the mode of action of herbicides that inhibit acetolactate synthase*, Environ. Exp. Bot. 59 (2007), pp. 150–159.
[6] W.Z. Li, D.S. Fan, and Y.B. Luan, *Current situation, problems and solutions of ethametsulfuron, chlorimuron-ethyl and imazethapyr*, Agrochemicals 47 (2008), pp. 781–789 [in Chinese].
[7] G.D. Bending, M.S. Rodriguez-Cruz, and S.D. Lincoln, *Fungicide impacts on microbial communities in soils with contrasting management histories*, Chemosphere 69 (2007), pp. 82–88.
[8] P. Perucci and L. Scarponi, *Effects of the herbicide imazethapyr on soil microbial biomass and various soil enzyme activities*, Biol. Fertil. Soils 17 (1994), pp. 237–240.
[9] P. Perucci, S. Dumontet, S.A. Bufo, A. Mazzatura, and C. Casucci, *Effects of organic amendment and herbicide treatment on soil microbial biomass*, Biol. Fertil. Soils. 32 (2000), pp. 17–23.
[10] N.Z. Lupwayi, K.N. Harker, G.W. Clayton, T.K. Turkington, W.A. Rice, and J.T. O'Donovan, *Soil microbial biomass and diversity after herbicide application*, Can. J. Plant Sci. 84 (2004), pp. 677–685.
[11] M.C. Wang, Y.H. Liu, Q. Wang, M. Gong, X.M. Hua, Y.J. Pang, S. Hu, and Y.H. Yang, *Impacts of methamidophos on the biochemical, catabolic, and genetic characteristics of soil microbial communities*, Soil Biol. Biochem. 40 (2008), pp. 778–788.
[12] P. Das, R. Pal, and A. Chowdhury, *Effect of novaluron on microbial biomass, respiration, and fluorescein diacetate-hydrolyzing activity in tropical soils*, Biol. Fertil. Soils 44 (2007), pp. 387–391.
[13] L. Zelles, *Fatty acids pattern of phospholipids and polysaccharides in the characterization of microbial communities in soil: a review*, Biol. Fertil. Soils 29 (1999), pp. 111–129.
[14] D.C. White and R.H. Findlay, *Biochemical markers for measurement of predation effects on the biomass, community structure, nutritional status, and metabolic activity of microbial biofilms*, Hydrobioligia 159 (1988), pp. 119–132.
[15] A. Frostegard, A. Tunlid, and E. Baath, *Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals*, Appl. Environ. Microbiol. 59 (1993), pp. 3605–3617.
[16] R.Z. Donald and W.K. George, *Microbial community composition and function across an arctic tundra landscape*, Ecology 87 (2006), pp. 1659–1670.
[17] H.B. Niu, W.X. Liu, and F.H. Wan, *Invasive effects of* Ageratina adenophora *Sprengel (Asteraceae) on soil microbial community and physical and chemical properties*, Ecol. Sin. Acta 27 (2007), pp. 3051–3060 [in Chinese].
[18] J.W. Ma, L. Chen, J.F. Wang, G.P. Jiang, and C.P. Pan, *Determination of imazethapyr residues in soil by SPE-HPLC*, Agrochemicals 46 (2007), pp. 843–845 [in Chinese].
[19] Y.P. Wang, J.Y. Shi, H. Wang, L. Qi, X.C. Chen, and Y.X. Chen, *The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter*, Ecotoxicol. Environ. Saf. 67 (2007), pp. 75–81.
[20] Q.M. Lin, Y.G. Wu, and H.L. Liu, *Modifiction of fumigation extraction method for measuring soil microbial biomass carbon*, Chin. J. Ecol. 18 (1999), pp. 63–66 [in Chinese].
[21] J.S. Wu, Q.M. Lin, Q.Y. Huang, and H.A. Xiao, *Soil Microbial Biomass – Methods and Application*, Weather Press, Beijing, 2006.
[22] D.A. Bossio, K.M. Scow, N. Gunapala, and K.J. Graham, *Determinants of soil microbial communities: effect of agricultural management, season, and soil type on phospholipid fatty acid profiles*, Microb. Ecol. 36 (1998), pp. 1–12.
[23] A. Frostegard, E. Baath, and A. Tulid, *Shifts in the structure of soil microbial communities in limes forests as revealed by phospholipids fatty-acid analysis*, Soil Biol. Biochem. 25 (1993), pp. 723–730.
[24] R.D. Bardgett, P.J. Hobbs, and A. Frostegard, *Changes in soil fungal: bacterial ratios following reductions in the intensity of management of an upland grassland*, Biol. Fertil. Soils 22 (1996), pp. 261–264.
[25] L.A. Avila, J.H. Massey, S.A. Senseman, K.L. Armbrust, S.R. Lancaster, G.N. Mccauley, and J.M. Chandler, *Imazethapyr aqueous photolysis, reaction quantum yield, and hydroxyl radical rate constant*, J. Agric. Food Chem. 54 (2006), pp. 2635–2639.
[26] Y.H. Zhao, Z.F. Zhang, and L. Sun, *Effect of imazethapyr residues in soil on subsequently planted rice*, Agrochemicals 45 (2006), pp. 189–190 [in Chinese].
[27] C.S. Zhao and F.L. He, *Effects of long residue herbicides on agricultural development of Heilongjiang province*, J. NE Agricult. Uni. 38 (2007), pp. 136–139 [in Chinese].
[28] F.A. Chinalia and K.S. Killham, *2,4-Dichlorophenoxyacetic acid (2,4-D) biodegradation in river sediments of northeast Scotland and its effect on the microbial communities (PLFA and DGGE)*, Chemosphere 64 (2006), pp. 1675–1683.
[29] A. Widenfalk, S. Bertilsson, I. Sundh, and W. Goedkoop, *Effects of pesticides on community composition and activity of sediment microbes-responses at various levels of microbial community organization*, Environ. Pollut. 152 (2008), pp. 576–584.
[30] B. Marius, E. Dana, T. Jan, and B. Petr, *Fungal bioremediation of the creosote-contaminated soil: influence of* Pleurotus ostreatus *and* Irpex lacteus *on polycyclic aromatic hydrocarbons removal and soil microbial community composition in the laboratory-scale study*, Chemosphere 73 (2008), pp. 1518–1523.
[31] I.P. Thompson, M.J. Bailey, E.M. Boyd, N. Maguire, A.A. Meharg, and R.J. Ellis, *Concentration effects of 1,2-dichlorobenzen on soil microbiology*, Environ. Toxicol. Chem. 18 (1999), pp. 1891–1898.
[32] J.K. Blakely, D.A. Neher, and A.L. Spongberg, *Soil invertebrate and microbial communities, and decomposition as indicators of polycyclic aromatic hydrocarbon contamination*, Appl. Soil Ecol. 21 (2002), pp. 71–88.
[33] H.L. Huang, S.Z. Zhang, N.Y. Wu, L. Luo, and P. Christie, *Influence of* Glomus etunicatum/Zea mays *mycorrhiza on atrazine degradation, soil phosphatase and dehydrogenase activities, and soil microbial community structure*, Soil Biol. Biochem. 41 (2009), pp. 726–734.

[34] U. Hammesfahr, H. Heuer, B. Manzke, K. Smalla, S.T. Bruhn, *Impact of the antibiotic sulfadiazine and pig manure on the microbial community structure in agricultural soils*, Soil Biol. Biochem. 40 (2008), pp. 1583–1591.
[35] J. Gans, M. Wolinsky, and J. Dunbar, *Computational improvements reveal great bacterial diversity and high metal toxicity in soil*, Science 309 (2005), pp. 1387–1390.
[36] T. Wu, D.O. Chellemi, J.H. Graham, K.J. Martin, and E.N. Rosskopf, *Comparison of soil bacterial communities under diverse agricultural land management and crop production practices*, Microb. Ecol. 55 (2008), pp. 293–310.
[37] D.A. Bossio and K.M. Scow, *Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns*, Microb. Ecol. 35 (1998), pp. 265–278.
[38] K.M. Carney and P.A. Matson, *The influence of tropical plant diversity and composition on soil microbial communities*, Microb. Ecol. 52 (2006), pp. 226–238.
[39] K. Jangid, M.A. Williams, A.J. Franzluebbers, J.S. Sanderlin, J.H. Reeves, M.B. Jenkins, D.M. Endale, D.C. Coleman, and W.B. Whitman, *Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems*, Soil Biol. Biochem. 40 (2008), pp. 2843–2853.