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Alteration of microbial properties and community structure in soils exposed to napropamide

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Abstract

The effect of pesticide napropamide (*N,N*-diethyl-2-(1-naphthalenyloxy) propanamide) on soil microorganisms for long-term (56 d) was assessed by monitoring changes in soil microbial biological responses. Soils were treated with napropamide at 0, 2, 10, 20, 40, and 80 mg/kg soil and sampled at intervals of 1, 3, 7, 14, 28, 42, and 56 d. The average microbial biomass C declined in napropamide-treated soils as compared to control. The same trend was observed on microbial biomass N after napropamide application. We also determined the basal soil respiration (BSR) and observed a high level in soils treated with napropamide during the first 7 d of experiment. But with the passage of incubation time, BSR with napropamide decreased relatively to control. Application of napropamide at 2–80 mg/kg soil had inhibitory effects on the activity of urease and invertase. Activity of catalase was enhanced during the initial 7 d of napropamide application, but soon recovered to the basal level. The depressed enzyme activities might be due to the toxicity of napropamide to the soil microbial populations. To further understand the effect of napropamide on microbial communities, a PCR-DGGE-based experiment and cluster analysis of 16S rDNA community profiles were performed. Our analysis revealed an apparent difference in bacterial-community composition between the napropamide treatments and control. Addition of napropamide apparently increased the number of bands during the 7–14 d of incubation. These results imply that napropamide-induced toxicity was responsible for the disturbance of the microbial populations in soil.

Key words: napropamide; microbial respiration; microbial biomass; soil enzyme; PCR-DGGE; bacterial community

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Introduction

In modern agriculture, pesticides have been used in large quantities for controlling pests and weeds, and thus greatly improve food production. However, intensive use of common pesticides can lead to the toxicity to soils, vegetables and contamination to aqueous systems. For instance, when pesticides are applied to soils, they may interact with non-target soil microorganisms and have a chronic adverse effect on soil health (Omar and Abdel-Sater, 2001). Due to their high degree of toxicity, some pesticides, particularly those persistent in soils, constitute a very important group of contaminants. Although these pesticides have been restrictively used or even banned for several years, their persistence and bioaccumulation can still be found in many soils and plants (Viñas *et al*., 2002). As a result, the residues of these pesticides in soils have become a great concern.

Napropamide (*N,N*-diethyl-2-(1-naphthalenyloxy) propanamide) is a selective systemic herbicide used for the pre-emergence control of annual grasses and broad-leaved weeds (Zhang *et al*., 2000). Due to its broad spectrum of activity, napropamide is widely used on a variety of fruits, vegetables and crops. It is a relative stable and has a rather long half-life in soil (Wauchope *et al*., 1992; Liu and Zhu, 2000). Over-application of napropamide has already caused wide presence of its residues in soils, waters as well as agricultural products (Antonious *et al*., 2005). Thus, it is required to estimate soil biological responses to the pesticide, and to determine the concentration level of its residues. To date, many efforts have been made to understand the effect of pesticides on soil enzyme activity (Sannino and Gianfreda, 2001), but little is known about the effect of napropamide on soil enzymes and other soil biological responses.

It is well known that soil is an open but self-regulating ecosystem with a large variety of microbial populations (Kizilkaya *et al*., 2004). The living dynamic nature is one of the important features for soil quality and often used as a bio-indicator for soil health (Gianfreda *et al*., 2005; Sukul, 2006). Soil enzymes, that represent the major living organism activity, are involved in catalyzing various reactions necessary for organic matter metabolism, nutrient cycling, energy transfer and crop productivity (Kizilkaya *et al*., 2004). Since soil enzymes are frequently exposed to changeable circumstances (Gianfreda *et al*., 1995), it is

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not surprising that soil activities are affected directly or indirectly by physical or chemical factors (e.g., pH, temperature, drought, organic carbon), biological disturbance (e.g., populations) and environment pollutants (e.g., heavy metal, xenobiotics). The basal soil respiration (BSR) is a good index of microbial activity and an ideal indicator of mineralizable substrates (Anderson, 1982). The microbial biomass plays a central role in the cycling of nutrients and mineralization of organic carbon and nitrogen of soil ecosystem. Among soil pollutants, pesticides are the major organic contaminants due to their entry into soil environment in recent years (Sannino and Gianfreda, 2001; Gianfreda *et al*., 2005).

When applied to soils, pesticides may have different fates. Its sorption by natural soil complexes often limits their bioavailability, thus affects their microbial degradation rate (Grosser *et al*., 2000; Gianfreda *et al*., 2005). On the other side, desorption of pesticides that have already been absorbed on soils is the main processes that influence pesticide transport and bioavailability (Yang *et al*., 2005). The presence of pesticides in soils affects biochemical processes through microbial proliferation and enzyme activities (Andreoni *et al*., 2004; Antonious *et al*., 2005; Singh and Singh, 2005). Pesticides inhibit enzyme activities by masking catalytically active groups, having denaturing effects on the conformation of proteins, or competing with the metal ions involved in the formation of enzyme-substrate complexes (Gil-Sotres *et al*., 2005).

Changes of soil quality can be monitored by many bioindicators in soil because of its living dynamic nature (Pandey and Singh, 2006; Gianfreda *et al*., 2005; Sukul, 2006). Microbial biomass, respiration and soil enzymatic activity can be utilized as indicator for changes in soil quality (Giller *et al*., 1998; Albiach *et al*., 2000). Recently, several molecular techniques have been developed to study natural samples (Muyzer *et al*., 1993; Borneman and Triplett, 1997; Tiquia *et al*., 2002). These molecular techniques identify bacteria without isolation and reveal the enormous extent of microbial diversity. Specifically, denaturing gradient gel electrophoresis (DGGE) has emerged as a powerful tool to explore microbial diversity and to identify microorganisms (Muyzer and Ramsing, 1995; Muyzer and Smalla, 1998). Due to the fact that the use of napropamide may have side-effects on soil health and environmental quality, it is extremely important to gain knowledge about the effects of napropamide on soil chemical, biological and physical properties. Therefore, the objective of this work is to study the effects of continued application of napropamide on the microbial biomass C and N, basal respiration, enzymatic activity, and bacterial genetic diversity (based on DGGE analysis of 16S rDNA) in soils.

1 Materials and methods

1.1 Chemicals and soils

Napropamide (20% emulsifiable concentrate) was obtained from Rudong Pesticide Institute, Jiangsu, China. Field-moist yellow-brown soils, without any pre-history of napropamide application for last 5 years, were collected from an upland soil within top 20 cm at the Experimental Station of Nanjing Agricultural University, Nanjing, China. Some chemical properties of the soil were listed as follows: pH 7.65; organic matter 1.40%; total N 1.26 g/kg; available P 34.3 mg/kg; and available K 91.5 mg/kg. The soil samples were manually and gently crumbled, air-dried at room temperature and sieved through a 2-mm mesh to remove root residues and small rocks (Bending *et al*., 2006). After mixed thoroughly, the physico-chemical properties of soil samples were analyzed immediately and soil samples were stored at 4°C.

1.2 Soil treatment

The recommended dosage of napropamide is 150 kg a.i./km^2. According to the parameters (5 cm soil in depth, 1.5 g/cm^3 bulk in density), this recommended dosage is equivalent to 2 mg napropamide/kg dry soil. Different volumes 20% napropamide emulsible concentrate was added to above soil and mixed thoroughly. Experiments were conducted with the following treatments: without napropamide, recommended dosage (2 mg/kg), 5-fold (10 mg/kg), 10-fold (20 mg/kg), 20-fold (40 mg/kg) and 40-fold (80 mg/kg) of recommended dosage of napropamide. The moisture content was adjusted to 60% of maximum water holding capacity.

In order to determine soil microbial biomass, enzymatic activities and bacterial community structure in a time-course manner, 900 g dry soils for each treatment (napropamide at 0, 2, 10, 20, 40, and 80 mg/kg) were placed in a plastic beaker (1 L). Each treatment was repeated in triplicates. For the assessment of basal soil respiration, 100 g of oven-dry equivalence of the moist soil were weighed into a container and incubated in a 500-mL airtight jar. Soils were incubated in the dark at 25 ± 1°C for 56 d. Soil microbial properties were measured at the time intervals of 1, 3, 7, 14, 28, 42, and 56 d, and bacterial community structure was determined at 7 and 14 d. Throughout the incubation period, the water content was held constantly by the addition of distilled water.

1.3 Soil physico-chemical analysis

The physicochemical analysis of soil was carried out using air-dried soil samples stored at room temperature. soil particle size distribution analysis was conducted by hydromerter methods (Lu, 1999), cation exchange capacity (CEC) was measured by the ammonium chloride (pH 7.0) method (Bao, 1999), organic C was determined by the wet oxidation method with $K_2Cr_2O_7$ (Rowell, 1996), total nitrogen (TN) was determined according to Kjeldahl method (Bremner and Mulvaney, 1982), available phosphate with the molybdate reagent (Bao, 1999) and potassium using the ammonium acetate method (Knudsen *et al*., 1982), soil pH was measured using a pH meter (Thermo Orion 250, Orion Research, Inc., USA) at a soil to solution ratio of 1:2.5 in deionized water.

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1.4 Soil microbial biomass

The microbial biomass C in the soil samples was measured according to the chloroform fumigation-extraction method (Vance *et al*., 1987). Triplicate sub-samples of 12.5 g of soil (dry weight equivalent) were fumigated with $CHCl_3$ for 24 h at 20°C. Fumigated and non-fumigated samples were extracted with 0.5 mol/L K_2SO_4 (30 min at 200 r/min), then filtered using a 0.45-μm cellulose ester filter (Millipore, USA). The total organic carbon was analysed using an automated TOC analyzer (TOC-500, Shimadzu, Japan). Microbial C was calculated from the equation: SMB C = 2.22 (C in fumigated soil extract-C in unfumigated soil extract).

Soil microbial biomass N was determined by the method described previously (Turner *et al*., 2001). The UV absorbance of the extracts of fumigated and unfumigated soils filtered by 0.5 mol/L K_2SO_4 was measured at 280 nm using a UV-1700 spectrophotometer (Shimadzu, Japan). The results for UV absorbance are expressed as the increase in absorbance at 280 nm after fumigation on a dry soil basis. All biomass measurements were carried out in triplicate.

1.5 Basal soil respiration

BSR at field capacity (CO_2 production at 25°C without any addition of substrate) was measured. After the incubation, excess $BaCl_2$ was added to the NaOH solution to precipitate carbonate and the remaining NaOH was titrated with HCl using phenolphthalein as an indicator. Three replicates of each sample were tested. Data are expressed as mg CO_2/100 g dry soil (Wang *et al*., 2003).

1.6 Soil enzyme activity

The catalase (EC 1.11.1.6) activity was measured by back-titrating residual H_2O_2 with $KMnO_4$ (Yao *et al*., 2006). Soil samples (2 g) were added to a tube containing 40 mL distilled water and 5 mL of 0.3% hydrogen peroxide solution. The mixture was incubated at 25°C, shaking at 120 r/min for 20 min. After incubation, the reaction was terminated by the addition of 5 mL of 1.5 mol/L sulfuric acid. The mixture was filtered and titrated using 5 mmol/L $KMnO_4$. Consumed amount of 5 mmol/L $KMnO_4$ per gram dried soil was used to express the activity of catalase.

The urease (EC 3.5.1.5) activity was assayed according to the previous method (Kizilkaya *et al*., 2004) with some modifications. Briefly, 5 g soil was mixed with 1 mL methylbenzene, 10 mL of 0.1 mol/L citrate buffer (pH 6.7) and 5 mL of 10% (*W/V*) urea substrate solution in a reaction flask. Subsequently, the samples were incubated at 37°C for 24 h and the volume was made up to 50 mL with 38°C distilled water. Following filtration through a filter paper, 1 mL of filtrate was diluted to 10 mL with distilled water, and 4 mL of sodium phenolate (12.5% (*W/V*) phenol + 5.4% (*W/V*) NaOH) and 3 mL of 0.9% (*W/V*) sodium hypochloride were added. Afterward, the released ammonium was determined spectrophotometrically at 578 nm. Results were expressed as μg NH_3-N/g dried soil.

The determination of invertase (EC 3.2.1.26) activity was carried out based on the previous method (Guan, 1986). The soil (5.0 g) with 5 mL phosphate buffer (pH 5.5) and 15 mL of 8% (*W/V*) sucrose substrate solution was incubated at 37°C for 24 h. The invertase activity was determined spectrophotometrically at 540 nm in a UV-Vis spectrophotometer using the 3,5-dinitrosalicylic acid and calculated according to the standard curve of glucose equivalents. The invertase activity was expressed as mg glucose/g dried soil in 24 h.

1.7 Bacterial community structure

Total DNA was extracted by a SDS-based method from the bulk soil (Porteous *et al*., 1997) and purified with Biospin gel extraction kit (Bio-Rad, USA). The PCR amplification of the 16S rDNA was performed on the purified DNA, using bacterial universal primers F338 and R518 (Wang *et al*., 2007). PCR reaction for V3 amplification was performed according to the method described by Wang *et al*. (2007). For DGGE analysis of the PCR products, a 40-bp GC-rich sequence (GC-clamp) was attached to the 5' end of primer F338 (Muyzer *et al*., 1993). V3 PCR products from soil were characterized by a DGGE run on a vertical acrylamide gel in the Bio-Rad D-Code System.

DGGE was performed with 8% (*W/V*) polyacrylamide gels in TAE buffer (20 mmol/L Tris-acetate pH 7.5, 10 mmol/L sodium acetate, 0.5 mmol/L Na_2-EDTA) with a linear chemical gradient ranging from 35% to 60%. Denaturant solutions were prepared by mixing the appropriate volumes of two (0–100)% denaturant stock solutions (7 mol/L urea, and 40% *V/V* formamide). Gels were run at a constant voltage of 85 V for 16 h at 60°C. After electrophoresis, the gels were stained with silver staining (Sanguinetty *et al*., 1994).

1.8 Analysis of DGGE patterns

DGGE analyses were performed to compare the bacterial community structures of soils. Band patterns of triplicate DGGE profiles were analysed by the Diversity Database Fingerprinting software (Bio-Rad, USA). The similarity dendrograms for different treatment soil samples were generated using a clustering method based on the Ward method (Rademaker *et al*., 1999).

1.9 Statistical analysis

All the experiments were performed at least three repetitive independent treatments. The values are expressed as means ± SD. The significance of the differences among the means was calculated by Duncan's multiple range test. Statistical significance was set at $P < 0.05$.

2 Results

2.1 Effect of napropamide on microbial biomass

Table 1 shows the responses of microbial biomass C content to the napropamide treatments. The microbial biomass C content in all samples was lower than that of the control within the initial 42 d after application of napropamide. Significant differences (P <

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Table 1 Dynamic responses of soil microbial biomass carbon to napropamide treatments at various levels

Incubation time (d)	C_{mic} (μg C/g soil)					
	0 mg/kg	2 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg
1	159.32 ± 4.6 a	147.84 ± 3.8 b	132.03 ± 3.1 c	104.46 ± 3.0 e	101.11 ± 2.9 f	108.9 ± 2.7 d
3	126.61 ± 3.0 a	113.52 ± 3.5 b	101.36 ± 2.8 c	93.80 ± 2.8 e	95.36 ± 2.9 d	98.24 ± 3.0 d
7	111.51 ± 3.2 a	110.46 ± 3.1 a	103.70 ± 2.7 b	97.97 ± 2.8 c	98.88 ± 2.6 bc	99.21 ± 2.9 bc
14	91.71 ± 2.8 a	80.71 ± 2.6 bc	78.02 ± 2.2 c	59.03 ± 1.8 e	81.71 ± 2.6 b	64.31 ± 1.9 d
28	90.93 ± 2.1 a	88.84 ± 2.4 ab	74.74 ± 2.3 d	60.62 ± 2.0 e	85.17 ± 2.4 c	88.31 ± 2.5 bc
42	80.34 ± 2.8 a	72.50 ± 2.5 bc	60.01 ± 2.2 d	73.02 ± 2.4 b	72.49 ± 2.9 b	69.89 ± 2.0 c
56	86.72 ± 2.7 d	106.5 ± 2.8 b	109.72 ± 3.5 a	97.71 ± 2.5 c	75.24 ± 2.0 e	68.46 ± 2.1 f
Mean	106.73	102.92	94.23	83.80	87.14	85.33

Data in a row by the same letter are not significantly different at $P > 0.05$ according to Duncan's multiple range test.

0.05) were found in the microbial biomass C between the napropamide-treated soil samples and control. At day 56, the high concentration napropamide treatments (40–80 mg/kg) inhibited the accumulation of microbial biomass C, although treatments with low concentrations of napropamide (2–20 mg/kg) stimulated the microbial biomass C. The average microbial biomass C content in soils decreased by 3.57%, 11.71%, 21.48%, 18.35%, and 20.05%, respectively, when napropamide was added to the soil at the concentration of 2, 10, 20, 40, and 80 mg/kg soil, respectively.

Similar to the variation of the soil microbial biomass C, application of napropamide also generally decreased the microbial biomass N content in soils (Table 2). Dynamic responses of microbial biomass N in soils treated with napropamide were parallel to those of microbial biomass C during the incubation time.

2.2 Effect of napropamide on soil respiration

Total CO_2 released from soil microorganisms changed over incubation time (Table 3). At the initial stage (0–7 d), total CO_2 released from soils treated with napropamide was higher than that of the control. With the prolonged incubation time, total released-CO_2 with high levels of napropamide (40–80 mg/kg) declined relative to the control, indicating that treatment with high concentrations of napropamide had distinct influence on soil respiration.

2.3 Effect of napropamide on soil enzymatic activities

Treatments of soils with napropamide at 2–80 mg/kg had an inhibitory effect on invertase activity over the time (Fig. 1a). The difference of invertase activity between the napropamide treatment and control was significant ($P < 0.05$). But the results of multiple comparisons showed no significant ($P > 0.05$) differences in the invertase activity

Table 2 Dynamic responses of soil microbial biomass N to napropamide treatments at various levels

Incubation time (d)	Increment in absorbance (abs 10 /g dry soil)					
	0 mg/kg	2 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg
1	0.058 ± 0.002 a	0.046 ± 0.002 b	0.035 ± 0.001 c	0.029 ± 0.001 d	0.023 ± 0.001 c	0.031 ± 0.001 d
3	0.047 ± 0.002 a	0.036 ± 0.002 b	0.028 ± 0.001 c	0.017 ± 0.001 f	0.021 ± 0.001 e	0.024 ± 0.001 d
7	0.035 ± 0.002 a	0.034 ± 0.001 a	0.029 ± 0.001 b	0.019 ± 0.001 d	0.020 ± 0.001 d	0.025 ± 0.001 c
14	0.015 ± 0.001 a	0.010 ± 0.0006 b	0.009 ± 0.0005 b	0.008 ± 0.0004 b	0.010 ± 0.0005 b	0.007 ± 0.0003 b
28	0.014 ± 0.001 a	0.013 ± 0.0005 a	0.008 ± 0.0004 b	0.007 ± 0.0004 b	0.012 ± 0.0006 a	0.013 ± 0.0007 a
42	0.011 ± 0.0006 a	0.010 ± 0.0004 ab	0.007 ± 0.0004 c	0.008 ± 0.0003 abc	0.007 ± 0.0004 bc	0.006 ± 0.0003 c
56	0.016 ± 0.0007 a	0.029 ± 0.0009 ab	0.030 ± 0.001 c	0.018 ± 0.0009 abc	0.008 ± 0.0005 bc	0.007 ± 0.0003 c
Mean	0.028	0.025	0.020	0.025	0.014	0.016
Mean variation (%)*	–	10.71	28.57	10.71	50.00	42.86

* Mean variation of increment in absorbance. Data in a row by the same letter are not significantly different at $P > 0.05$ according to Duncan's multiple range test.

Table 3 Effect of napropamide on basal soil respiration

Incubation time (d)	Total CO_2 released (mg/100 g soil)					
	0 mg/kg	2 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg
0–1	29.56 ± 1.1 d	32.02 ± 1.3 c	32.26 ± 1.0 c	32.87 ± 0.9 b	34.72 ± 1.4 a	32.63 ± 1.3 b
1–3	19.21 ± 1.0 f	24.18 ± 1.1 c	25.60 ± 0.9 b	26.32 ± 0.7 a	23.75 ± 0.5 d	21.34 ± 0.6 e
3–7	25.89 ± 1.2 c	26.56 ± 1.2 b	26.04 ± 1.2 c	31.81 ± 1.4 a	26.91 ± 1.1 b	25.96 ± 1.1 c
7–14	30.66 ± 1.3 a	29.52 ± 1.1 c	29.63 ± 1.2 bc	29.95 ± 0.9 b	28.73 ± 0.7 d	28.42 ± 0.6 d
14–28	44.11 ± 1.5 a	39.42 ± 1.2 c	39.69 ± 1.3 c	40.21 ± 1.5 b	38.47 ± 1.2 d	38.11 ± 1.0 d
28–42	41.72 ± 1.3 a	37.51 ± 1.4 b	33.64 ± 1.2 c	31.82 ± 1.3 d	29.66 ± 1.1 e	27.20 ± 1.0 f
42–56	30.14 ± 1.2 c	32.33 ± 0.8 a	31.54 ± 0.8 b	28.91 ± 0.9 d	24.88 ± 1.0 e	21.02 ± 0.5 f
0–56	221.29	221.17	218.77	221.89	207.12	194.71
Mean	3.952	3.950	3.901	3.962	3.698	3.477

Data in a row by the same letter are not significantly different at $P > 0.05$ according to Duncan's multiple range test. For each treatment, mean values (n = 3) with the same letters are not significantly different ($P > 0.05$).

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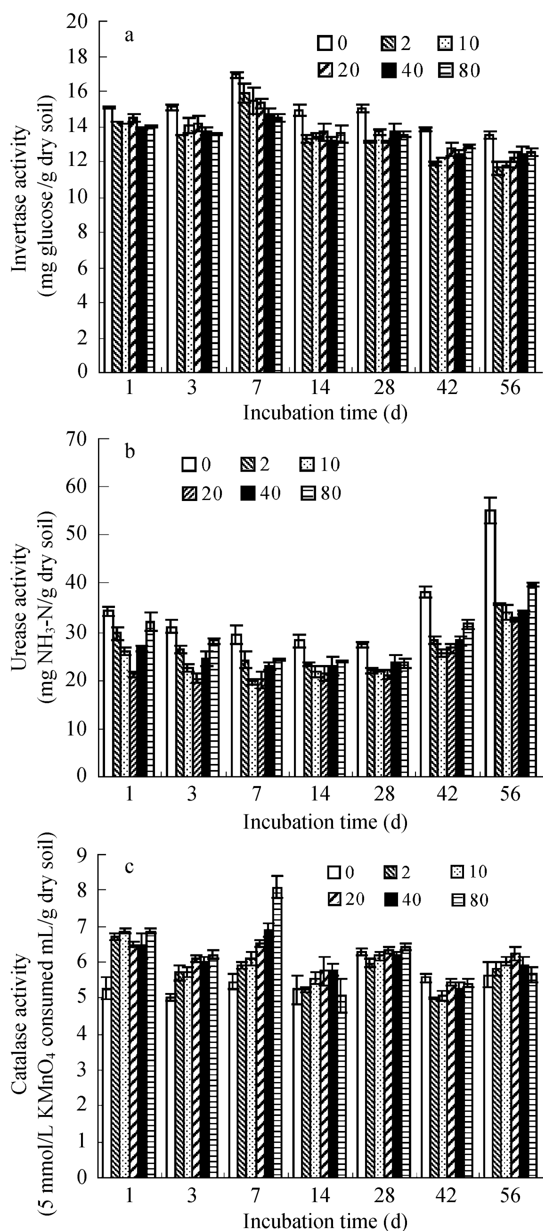

Fig. 1 Effect of napropamide on the activity of invertase (a), urease (b), catalase (c) in yellow-brown clay soil. Napropamide was added to the soil at concentrations of 0, 2, 10, 20, 40, and 80 mg/kg dry soil and incubated in the dark at 25 ± 1°C. Then, the treated soils were collected at the indicated time intervals and the enzyme activity was assayed. Vertical bars represent standard deviation of the mean (n = 3).

between 40 and 80 mg/kg treatments at the time intervals. Also, no dose-response change in invertase activity in napropamide-treated soils was found except for the time point of day 7, when the invertase activity decreased with the increasing napropamide concentration applied, showing a clear negative correlation between the invertase activity and napropamide concentration of soils (R = –0.8480).

Since urease is one of the major soil enzymes that are the most sensitive to pollutants such as heavy metals and organic contaminants (Gianfreda *et al*., 2005), activities of urease in napropamide-treated soils were measured. Urease activities in soils with napropamide at all range of concentrations were depressed compared to the control (Fig. 1b). The significant difference between the treatments and control was observed throughout the incubation time ($P < 0.05$). The inhibitory effect of napropamide on urease activities was more pronounced in those treatments with long periods (56 d).

The variation of catalase activity was observed. During the initial 7 d of treatment, there were significant increases in activities in the napropamide-treated soils as compared to the control (Fig. 1c). Following that time, however, no significant change of catalase activities was detected in the treatments and control. Although in the most cases, there were no dose-responses of catalase activities to napropamide exposure, at day 7 the catalase activity in napropamide-treated soils was gradually promoted with increasing concentrations of napropamide applied. Indeed, significant positive correlations were found between the catalase activity and napropamide concentrations applied (R = 0.9796).

2.4 PCR-DGGE-based community analysis

Using PCR-DGGE, the genetic fingerprinting of bacterial 16S rDNA was analyzed in soils exposed to napropamide for 7 and 14 d. The result showed that there were certain differences in bacterial community diversity in soils treated with different concentrations of napropamide (Figs. 2a and 2b). Also, microbial diversity in soils treated with napropamide changed with time. Profiles of rDNA from the same treatments were generally similar. The number of bands corresponding to the samples with napropamide treatments was more than that of the control. Within the napropamide treatments, the number and position of bands were similar. Cluster analysis was performed to infer similarities among the different banding patterns and present the resulting dendrogram (Figs. 2c and 2d). The similarities between the treatments and control were more than 90%, indicating that bacterial community structure were highly unanimous. At day 14, the predominant bands for soil samples treated with various napropamide concentrations were increased as compared to those at day 7, and the similarities between the treated soil samples and control were less than 40%. These results suggested that application of napropamide could affect the composition of microbial community in soils.

3 Discussion

Because of their high sensitivity to soil quality changes (Powlson *et al*., 1987; Vig *et al*., 2003), soil microbial biomass has been proposed as a useful measure of pollutants like pesticide/herbicide, heavy metals and their residues in soil (Brookes, 1995; Klose and Ajwa, 2004; Sukul, 2006). We tested napropamide, one of the most prevalent herbicides, and observed that microbial biomass carbon was reduced in napropamide-treated soils as compared to the control (Table 1). The same trend was observed on microbial biomass nitrogen after napropamide application (Table 2). The reason for it might be associated with the toxic effect of the pesticide, and that microorganisms in soil under napropamide stress divert energy

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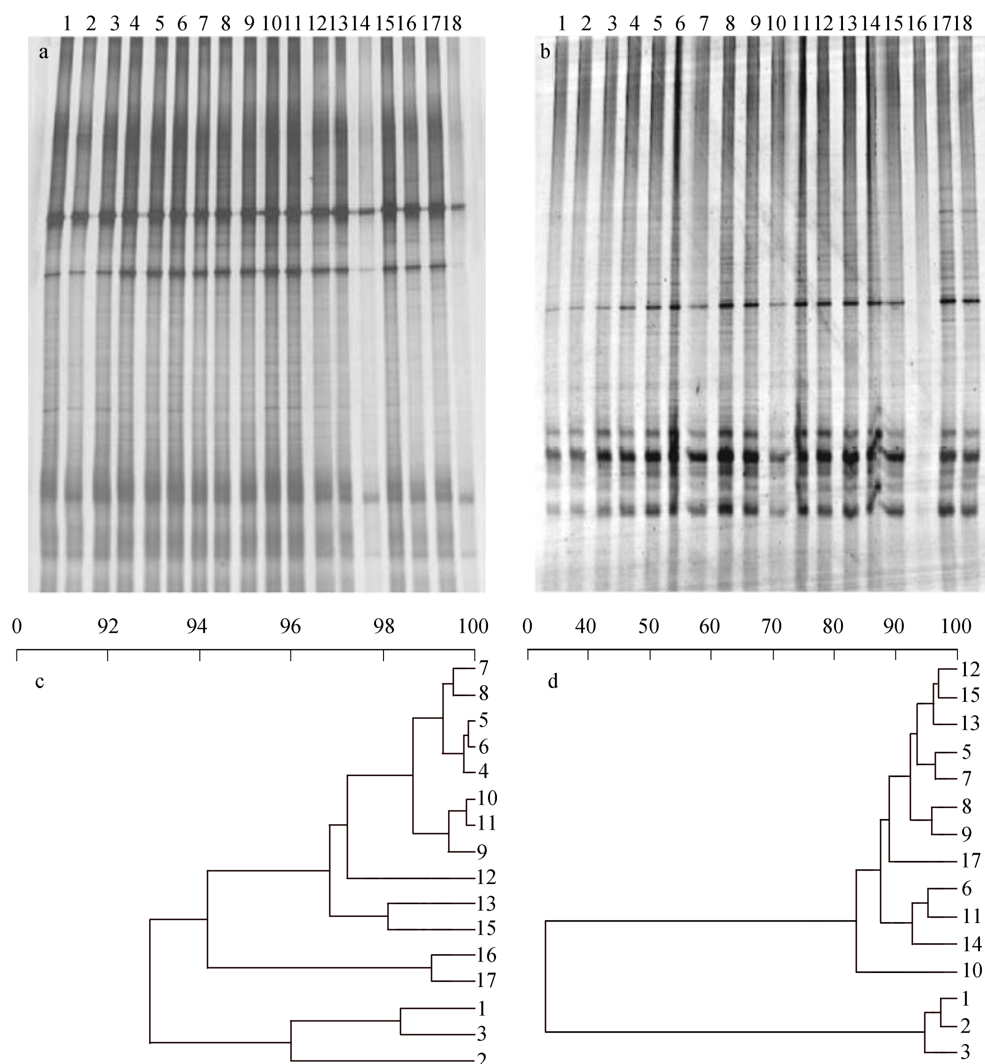

Fig. 2 DGGE patterns and similarity analysis of amplified 16S rDNAs. DGGE profiles of 16S rDNAs fragments from soil applied with napropamide in different concentrations for 7 (a) and 14 d (b). Similarity index of DGGE profiles of amplified 16S rDNA fragments from soil amended with napropamide in different concentrations for 7 (c) and 14 d (d). 1–3: control; 4–6: 2 mg/kg; 7–9: 10 mg/kg; 10–12: 20 mg/kg; 13–15: 40 mg/kg; 16–18: 80 mg/kg dry soil.

from growth to cell maintenance functions (Killham and Staddon, 2002). The increase in microbial biomass carbon or nitrogen with low concentrations of napropamide (2–20 mg/kg) and decrease at the high concentrations of napropamide (40–80 mg/kg) compared to the control at day 56 (Tables 1 and 2) might be due to improvement or stimulation in microbial biomass carbon or nitrogen of soil with low-concentration napropamide treatments (2–20 mg/kg) and strong toxic effect of high concentration napropamide treatments on microbial biomass carbon or nitrogen of soil during the previous incubation period (Dar, 1997). Unlike other amide pesticide, napropamide degradation by microorganisms is slow (Aguer *et al*., 1998), with a reported half-life of 56–84 d (Wauchope *et al*., 1992). This suggests that this pesticide was not substantially broken down at the initial period of incubation, whereas at day 56, the increased microbial biomass with low concentrations of napropamide might be attributed to reduced the toxicity napropamide or adaptation to it at the end of experiment.

In this study, the high values for basal soil respiration were observed in soil samples treated with napropamide during the first 7 d of incubation (Table 3), suggesting that the microorganisms tolerance to napropamide toxicity was able to utilize it as a carbon source. However, with the passage of incubation time, BSR with napropamide decreased relatively to the control, suggesting that herbicide napropamide may reduce the bioavailability of soil substrate for BSR by decreasing soil microorganisms during long incubation period (Insam *et al*., 1996; Liao *et al*., 2005).

Since soil enzyme activity has been considered as a very sensitive indicator, any disturbance due to biotic or environmental stresses in soil ecosystem may affect soil biological properties. Several important soil enzymes in matter cycle were selected to test in the study. Our analysis revealed that urease activity was depressed in napropamide-treated soils over experiment (Fig. 1b), suggesting that the enzyme is rather sensitive to napropamide. On the other hand, several reports indicated that urease

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was strongly adsorbed by clay and humus, thus, it is more resistant to environmental impact (Sannino and Gianfreda, 2001; Yao *et al*., 2006). These results indicate that differences in microbial community structure among soils, which vary in sensitivity to pesticide toxicity, may be an important factor in explaining discrepancies among studies (Kizilkaya *et al*., 2004). Urease is involved in hydrolysis of urea to carbon dioxide and ammonia, which can be assimilated by microbes and plants (Sannino and Gianfreda, 2001). Urea is incorporated into soil as fertilizers, animal excreta, or a breakdown product of nucleic acids (Sukul, 2006).

Similar to urease, invertase activity was constantly lower in napropamide-treated soils than in the control (Fig. 1a). Furthermore, invertase activities of all treatments showed a decreasing tendency with prolonged incubation time after 7 d. The relatively low activity of invertase might result from toxic effect of napropamide on soil microorganisms, which in turn produce invertase. Our results appeared not to be consistent with previous reports, in which it is demonstrated that pesticides stimulated invertase activity of soils (Sannino and Gianfreda, 2001; Srinivasulu and Rangaswamy, 2006). The inhibition of invertase activity by napropamide could be attributed to the properties of napropamide. Thus far, no information has been available about the influence of napropamide on the soil enzyme activity.

The biological function of catalase is involved in the removal of H_2O_2 in organisms because high level of H_2O_2 as one of the reactive oxygen species (ROS) is toxic to cells. In many plant species and microbial community, environmental stresses such as cold, heat, salty, drought and heavy metals can induce the increase in catalase activity (Wang and Yang, 2005; Renella *et al*., 2005; Song *et al*., 2007). In the present study, the catalase activity could be also promoted by the presence of napropamide at the early stage of treatment (Fig. 1c), suggesting that at the stage of incubation napropamide had an oxidative stress on soil microorganism. However, the alternation of catalase activity was short-lived, and in the most cases, the activity was recovered over the treatment time. The variation of catalase activity might reflect the adaptation of soil microorganism to napropamide over the incubation time.

To gain an insight into the effect of napropamide on the microbial composition, a PCR-DGGE based experiment was performed to monitor the bacterial community change at day 7 and 14 after napropamide was applied. The results have demonstrated an apparent difference in bacterial-community composition between the napropamide treatments and control (Fig. 2). Addition of napropamide apparently increased the number of bands during the 7–14 d of incubation. Indeed, with the passage of incubation time, some bacteria became dominant after the addition of napropamide. This suggests that some particular bacteria may be adapted to the napropamide environment. Further research may help the understanding of more detailed molecular response of bacteria to napropamide.

We also performed the cluster analysis of 16S rDNA community profiles based on a general bacterial primer pair. Our analysis revealed the complex profiles reflecting the high diversity of the microbial community among napropamide treatments. The bands in the DGGE profiles represent the dominant microbial populations (Muyzer *et al*., 1993). At day 7, the similarity indices between treatments and control were more than 90%. However, At day 14, the similarity indices decreased to 40%. This indicated that the bacterial communities changed with the addition of napropamide and increased with incubation time.

4 Conclusions

The present results have demonstrated that napropamide affected on the activities of microbial biomass and enzyme in soil. The average microbial biomass C and N in soil declined in napropamide-treated soils (2–80 mg/kg) as compared to the control over the incubation time (1–56 d). The total BSR with napropamide treated (2–80 mg/kg) decreased relatively to the control over the incubation time (0–56 d). The activities of urease and invertase in soil with naprapamide treated (2–80 mg/kg) were inhibited compared to the control at intervals (1–56 d). The PCR-DGGE-based experiment showed that the number of bands corresponding to the samples with napropamide treatments was more than that of the control at day 7 and 14 of incubation. The cluster analysis of 16S rDNA community profiles was revealed that the similarity indices between treatments and control were more than 90% at day 7, however, at day 14, the similarity indices decreased to 40%.

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