Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2010, 22(8) 1225–1231
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Microbial community variation in phytoremediation of triazophos by *Canna indica* Linn. in a hydroponic system

Huiping Xiao[1,2], Shuiping Cheng[1,*], Zhenbin Wu[1]

1. State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan 430072, China. E-mail: xiaohuiping422@sina.com
2. Graduate University of Chinese Academy of Sciences, Beijing 100039, China

Received 20 October 2009; revised 14 December 2009; accepted 12 January 2010

Abstract

Phytoremediation of triazophos (*O,O*-diethyl-*O*-(1-phenyl-1,2,4-triazole-3-base) sulfur phosphate, TAP) pollution by *Canna indica* Linn. in a hydroponic system has been well studied, whereas the microbial mechanism on TAP degradation is still unknown. The variation in microbial community compositions was investigated by analyzing phospholipid fatty acids (PLFAs) profiles in microbes under TAP exposure. The TAP exposure resulted in an increase in proportions of fatty acid 16:0 and decrease in fatty acid 18:2ω9,12c, indicating that TAP may stimulate the reproduction of microorganisms and inhibit the growth of fungi to some degree. Significant correlation was found between the ratio of fungi to bacteria and TAP removal (r^2 = 0.840, p < 0.01). In addition, the microbial community in the phytoremediation system with *C. indica* was dominated by Gram negative bacteria, which possibly contributed to the degradation of TAP. These results indicated that TAP might induce the colonization of bacteria in the hydroponic system planted with *C. indica*, and lead to a discrimination of microbial community, which might be one of the mechanisms on TAP dissipation in phytoremediation system.

Key words: microbial community; phospholipid fatty acids; triazophos; phytoremediation; *Canna indica* Linn.
DOI: 10.1016/S1001-0742(09)60242-4

Introduction

Triazophos (*O,O*-diethyl-*O*-(1-phenyl-1,2,4-triazole-3-base) sulfur phosphate, TAP) is an organophosphorous pesticide, commonly used for insects control in many developing countries. For past several years, the overuse of TAP in Southeast China has brought severe pollution in surface water, soil, food and biota (Shen et al., 2005; Zhong et al., 2006). According to the International Union of Pure and Applied Chemistry (IUPAC), TAP may harm non-target organisms as an acetyl cholinesterase inhibitor and neurotoxicant, even causing threats to human health through food chains. It also damages the metabolism enzyme, immune related genes, and membrane proteins of non-target organisms (Zhong et al., 2005; Ma et al., 2007). Therefore, studies on the residues and dissipation of TAP have been of great environmental concern (Rani et al., 2001; Wang et al., 2005). TAP could be easily uptaken and degraded by plants (Li et al., 2008). This characteristic may be valuable when using phytoremediation to eliminate TAP pollution from the environment.

Phytoremediation technology, which utilizes plants to uptake, transform, stabilize, evaporate, and degrade pollutants (Schnoor et al., 1995), has proved to be a useful method to transport and transform pollutants from the environment (Yu and Gu, 2006; Petroutsos et al., 2008). In addition to research on the role of plants in phytoremediation, degradation of organic pollutants in rhizosphere has also been investigated. It is commonly accepted that exudates released by plants, as well as the addition of organic contaminants, could promote the activities and reproduction of microbes (Fan et al., 2007; Lin et al., 2008), and result in an acceleration of contaminants degradation by providing carbon and/or energy source to microbes in ambient environment, thereby subsequently affect the mineralization of pollutants (Nakamura et al., 2004).

Our previous study determined the TAP degradation in a hydroponic system with *Canna indica* Linn., in which 11% of TAP dissipation was contributed by microbes, and microbes played an important role in TAP phytoremediation (Cheng et al., 2007). But the microbial community structure and its variation were unknown. It is necessary to evaluate shifts in microbes during phytoremediation, to help us better understand the microbial mechanism involved. To analyze the phospholipid fatty acids (PLFAs) profile of microbes is an emerging technology that recently

* Corresponding author. E-mail: shpcheng@ihb.ac.cn; shpcheng@public.wh.hb.cn

replaces the traditional bacterial diversity techniques, to divulge the fingerprint of microbial community structure at the phenotypic level (Kaur et al., 2005). It has been commonly used to monitor microbes in contaminated soils (Frostegård et al., 1993). PLFAs analysis can reflect microbial community changes that may contribute to the phytoremediaiton of nonlinear degradation of pentachlorophenol (PCP) by *Lolium perenne* L. (He et al., 2007). Antizar-Ladislao et al. (2008) also analyzed the PLFAs to identify Gram positive (GP) bacteria and showed GP dominated the mineralization of polycyclic aromatic hydrocarbons (PAHs). Therefore, the analysis of PLFAs profiles of microbes was employed in this study to investigate the microbial variation in the phytoremediation system, and the relationship between TAP dissipation and microbes was also evaluated.

1 Materials and methods

1.1 Materials and treatments

Field-scale experiments were set in the experimental base of Institute of Hydrobiology, Chinese Academy of Sciences, China. *C. indica* was selected for its nice resistance to heavy metal stress (Cheng et al., 2002), as well as the potential to degrade TAP (Cheng et al., 2007). Seedlings of *C. indica* were pre-cultivated in 1/10 Hoagland nutrient solution for 7–10 days and then placed in concrete tanks (50 cm × 50 cm × 50 cm) containing 50 L nutrient solution. A total of (926 ± 33) g fresh weight of *C. indica* seedlings were grouped and cultivated in tanks at 25–35°C and natural illumination (14 hr day/10 hr night). Water loss from evaporation and transpiration by plants was compensated by adding tap water every other day.

TAP (EC, 80%, Deshijia Pesticide Consultation Center, Shandong, China) was used for the treatment. Three treatments were set, and each with 3 replicates, including the control without TAP, but with *C. indica* planted; the treatment (T) with 1 mg/L TAP, but without plants; and the treatment (TP) with 1 mg/L TAP and with *C. indica* planted.

1.2 Sample analysis

Water samples were collected on day 0, 7 and 15 for TAP analysis. They were pretreated by the methods of Zhang et al. (2005). Samples were centrifuged at 8000 r/min at 25°C for 30 min and the concentrations of TAP in the supernatants were measured by high-performance liquid chromatography (1100 serial HPLC, Agilent, USA) using a RP-C18 column (Waters XTerra, 150 mm × 3.90 mm × 5.00 μm, Waters Co., USA), and a mixture of water-methanol (3:7) was as a fluent. The diode-array detector wavelength was 246 nm, and the retention time of TAP was 3.26 min. The standard deviation (SD) of ten times analysis of the same concentration was 0.081, and the coefficient of variance (CV = SD/$\bar{x}$) was 1.50%, which was involved the error limitation.

PLFAs in microbes from water samples were collected and pretreated following the method of Brinis et al. (2004). Ten litters of water sample from each tank was collected on day 7 and 15. In order to eliminate large zooplankton, water samples were pre-filtered on a plankton-net (64 μm pore size) prior to filtrations on a fiber filtration film (0.22 μm pore size, 50 mm diameter). The filtration films were then preserved in 10 mL phosphate buffers at –4°C before extraction that followed the method provided by Frostegård et al. (1993).

Analysis of fatty acid methyl esters was performed on a gas chromatograph using flame ionization detection (GC-2014, Shimadzu, Japan), which was fitted with an manual injector. A polar capillary column (0.25 μm d.f. × 30 m length × 0.25 mm i.d., Wonda CAP 5, Shimadzu, Japan) was used to separate PLFA, with spitless mode. The temperature program used was 80°C for 1 min then increasing to 160°C at 20°C/min, and to 280°C at 4°C/min, held for 5 min. The concentrations (mole percentage) of individual fatty acids in water samples were determined from the peak areas of each fatty acid relative to the peak area of the internal standard.

1.3 Signature PLFAs analysis

PLFAs are typical components of cell membranes, thus the PLFAs analysis are usually subjected to quantify viable bacterial biomass. According to Rajendran et al. (1992, 1997), the ratio of *trans*/*cis* monounsaturated fatty acids (MUFAs) (the mol% of 18:1ω9t to that of 18:1ω9c) is commonly used to indicate the environmental stress on microorganisms, whereas the ratio of MUFAs (the sum mol% of 18:1ω9c, 18:1ω9t and 16:1ω9c) to branched fatty acids (the sum mol% of i15:0, a15:0, i16:0 and i17:0) is subjected to relative abundance of aerobic to anaerobic microbes. The ratio of fungal fatty acid (the mol% of 18:2ω9) to bacterial fatty acids (the sum mol% of i15:0, i16:0, a15:0, cy17:0, cy19:0, 15:0, 17:0, 16:1ω9c, i17:0, and 18:0) reflects their biomass (Frostegård et al., 1993). And fatty acids i15:0, a15:0, i16:0 and i17:0 are typical for GP bacteria, while 16:1ω9c, 18:1ω9c, 18:1ω9t, cy17:0 and cy19:0 represent Gram negative (GN) bacteria (Zelles, 1999).

1.4 Statistical analysis

Present data were the mean values with standard deviation of three replicates. The differences of TAP removal percentages in T and TP treatments were analyzed by independent-samples *T* test. The mole percentages of individual PLFA and signature fatty acids in different treatments were compared using analysis of variance (ANOVA). The mean values were compared using least significant difference (LSD) test. In addition, simple correlation was calculated to study the relationship between PLFAs and TAP removal efficiencies. The above analyses were performed using the Statistical Package for Social Sciences (SPSS 11.5 for Windows) (SPSS Inc., USA) at 0.05 levels.

Microbial community structure reflected by PLFAs patterns were analyzed by principal component analysis by Statistica 6.0 (StatSoft Inc., USA).

2 Results and discussion

2.1 TAP degradation

After 7 and 15 days exposure, TAP concentrations in unplanted (T) and planted (TP) treatments are shown in Table 1. Statistical analysis revealed that TAP levels in TP treatment were remarkably lower than those in T treatment on day 7 ($p < 0.05$) and on day 15 ($p < 0.01$). The degradation rate constant of TAP in the T and TP treatments was 0.04 and 0.12, and its half-life was 17.3 and 5.8 days, respectively. TAP degradation was much more rapidly than that reported by Cheng et al. (2007), which maybe ascribed to field conditions with higher temperatures.

2.2 Microbial community reflected by PLFAs

From day 7 to day 15, a total of 24 kinds of PLFAs, ranging from C11 to C20, were identified (Table 2). All samples were mainly characterized by unsaturated fatty acids (39%–53%), saturated fatty acids (29%–44%), branched and cyclic fatty acids (16%–25%), as well as hydroxyl fatty acids (< 1%). The major PLFAs 16:0, 18:1ω9t, 18:1ω9c and i15:0 of microorganisms in control constituted approximately 65% of the total PLFAs on day 7, while those in T and TP treatments were 82% and 68%, respectively. However, the components of fatty acids in treatments spiked with TAP changed on day 15, and the abundance of major PLFAs were 75% of the total PLFAs in T treatment and 70% in TP treatment, respectively.

As shown in Table 2, the percentage of individual PLFA varied under TAP exposure. Fatty acid 16:0 concentrations were significantly higher in T treatment than those in control ($p < 0.05$). While fatty acid 18:2ω9,12c concentration was extremely lower in T treatment than that in control on day 15 ($p < 0.01$). Fatty acid 18:2ω9,12c is a typical biomarker for fungi (Rajendran et al., 1997). The reduction of 18:2ω9,12c may result from the toxicity of organophosphorus pesticide to fungi (Tu, 1970). Meanwhile, individual PLFA such as saturated fatty acids 11:0, 17:0 and 18:0, as well as cyclic fatty acid cy19:0 exhibited different concentrations in both TAP treatments, compared to those in control (Table 2), whereas branched fatty acids i16:0 on day 15 and i17:0 on day 7 were more abundant in TP treatment ($p < 0.05$). Moreover, fatty acid 19:0 was

Table 1 Triazophos (TAP) concentration and removal percentage in the treatment without plant (T) and with plant (TP)

Treatment	Initial TAP conc. (mg/L)	Day 7		Day 15	
		TAP conc. (mg/L)	Removal (%)	TAP conc. (mg/L)	Removal (%)
T	0.986 (0.041)[a]	0.765 (0.055)	22.4	0.528 (0.05)	46.5
TP	1.030 (0.053)	0.545 (0.070)*	47.1	0.174 (0.056)**	83.1

[a] Standard deviations in parenthesis.
* Significant difference at 0.05 level; ** significant difference at 0.01 level.

Table 2 Phospholipid fatty acids (PLFAs) concentrations in triazophos (TAP) treatments and control (C) (mol%)

PLFAs	Control		TAP treatment without plant		TAP treatment with plant	
	Day 7	Day 15	Day 7	Day 15	Day 7	Day 15
Saturated						
11:0	0.63 (0.28)[a]	0.31 (0.09)	0.13 (0.03)*	0.16 (0.05)	0.15 (0.04)*	0.14 (0.05)*
12:0	1.48 (0.40)	0.38 (0.09)	0.21 (0.03)*	0.34 (0.14)	1.09 (0.28)	0.53 (0.11)
13:0	0.50 (0.23)	0.27 (0.10)	0.11 (0.01)*	0.14 (0.01)	0.17 (0.02)	0.18 (0.12)
14:0	4.82 (1.91)	3.28 (0.55)	2.45 (0.20)	2.67 (0.76)	3.72 (0.22)	2.58 (0.32)
15:0	1.19 (0.19)	1.44 (0.08)	1.71 (0.49)	1.81 (1.21)	1.48 (0.50)	1.60 (0.23)
16:0	25.37 (3.29)	25.57 (1.30)	39.13 (4.51)*	35.52 (4.92)*	29.66 (5.37)	27.52 (1.03)
17:0	0.56 (0.03)	1.56 (0.27)	0.59 (0.17)	0.67 (0.24)*	0.41 (0.05)*	0.60 (0.10)*
18:0	4.55 (1.86)	5.75 (0.93)	1.11 (0.08)	1.82 (0.53)*	2.33 (0.82)	2.82 (0.15)*
19:0	0.48 (0.09)	0.35 (0.19)	n.d.	n.d.	n.d.	n.d.
20:0	0.39 (0.17)	0.36 (0.02)	n.d.	0.53 (0.43)	0.29 (0.14)	0.28 (0.07)
Unsaturated						
18:1ω9c	8.16 (3.20)	8.23 (1.66)	3.03 (2.20)	4.72 (2.37)	6.98 (1.79)	14.29 (7.14)
18:1ω9t	24.72 (3.60)	22.65 (4.43)	31.59 (5.38)	29.76 (5.51)	21.41 (1.65)	23.58 (5.57)
16:1ω9c	1.61 (0.33)	2.03 (0.99)	1.95 (1.74)	2.42 (1.43)	3.70 (1.05)	1.23 (0.26)
18:2ω9,12c	6.38 (2.27)	11.57 (1.27)	2.32 (1.59)	3.85 (1.37)**	5.50 (0.68)	8.66 (0.61)*
Branched and cyclic						
cy17:0	0.84 (0.22)	0.64 (0.10)	0.81 (0.03)	1.76 (1.06)	0.76 (0.27)	0.68 (0.08)
cy19:0	0.45 (0.11)	0.75 (0.19)	0.28 (0.35)*	0.56 (0.30)	0.31 (0.16)	0.39 (0.24)
i15:0	7.33 (2.10)	7.83 (0.98)	8.33 (1.99)	5.47 (4.81)	9.82 (1.52)	4.87 (4.21)
a15:0	5.27 (2.25)	3.24 (0.60)	2.70 (0.59)	3.88 (3.96)*	6.26 (0.56)	5.03 (3.79)
i16:0	3.96 (0.26)	2.37 (0.17)	2.21 (1.41)	1.94 (0.45)	3.43 (0.47)	2.90 (0.14)*
i17:0	0.58 (0.01)	0.93 (0.19)	0.87 (0.61)	1.89 (0.73)	1.63 (0.49)*	1.47 (0.34)
Hydroxyl						
12:0 (2OH)	n.d.	n.d.	0.14	n.d.	n.d.	n.d.
12:0 (3OH)	0.73 (0.09)	0.72 (0.30)	0.48 (0.36)	0.26 (0.10)	0.92 (0.42)	0.73 (0.57)
14:0 (3OH)	0.63	0.26	n.d.	n.d.	0.69 (0.15)	n.d.
16:0 (2OH)	n.d.	n.d.	n.d.	n.d.	0.94	n.d.

[a] Standard deviations in parenthesis; n.d.: not detectable.
* Significant difference at 0.05 level compared with control; ** significantly difference at 0.01 level compared with control.

absent in both TAP treatments. These results indicated a shift in the microbial community induced by TAP.

The relationship between the concentrations of individual fatty acid and the TAP removal percentages were analyzed. There was a negative relationship between the concentrations of fatty acid 16:0 and the TAP removal percentages (r^2 = –0.733, $p < 0.01$), while the relationship between the concentrations of fatty acid 18:1ω9c and the TAP removal percentages were positive (r^2 = 0.755, $p < 0.01$). Fatty acid 16:0, which is found in cell membranes of most microorganisms, is often used to represent the microbial biomass in soil conditions (Salomonavá et al., 2003). Our results indicated that fatty acid 16:0 concentrations in hydroponic system were significantly affected by the dissipation of TAP. Thus, TAP degradation under field condition seemed to be controlled by the population of microbes. Whereas the shift in fatty acid 18:1ω9c, which is commonly found in GN bacteria (Salomonavá et al., 2003), indicated that microbial community may have changed during the exposure.

2.3 Signature microbial community changes during cultivation

Signature PLFAs generally provide an estimation of certain groups of microorganisms, due to their specific component of cellular membrane (Kaur et al., 2005). Microbial community parameters, including distribution and growth index of microbes, are shown in Fig. 1.

The ratio of *trans*/*cis* monounsaturated fatty acids in microbes from all the treatments were investigated (Fig. 1A). The ratios were significantly higher in T treatment than those in control on day 15 ($p < 0.05$), whereas no difference was observed between the control and TP treatments, probably caused by the fact that *C. indica* may have weakened the stress of TAP on microbes by releasing and/or inducing phosphatase to degrade TAP (Cheng et al., 2007). Heipieper et al. (1996) ascribed this isomerization of *trans* to *cis* unsaturated fatty acids as one such adaptation mechanism that was induced by increased membrane fluidity in microbes owing to environmental stress, such as heavy metal stress (Frostegård et al., 1993).

There was no difference on the ratio of MUFAs to branched fatty acids between both TAP treatments and the control on day 7 and day 15 ($p > 0.05$) (Fig. 1B). However, the ratio in the TP treatment increased remarkably from day 7 (1.53) to day 15 (2.80) ($p < 0.05$). Oxygen released by the root of plant would be the reason, and it simulated the reproduction of aerobic microorganisms (Shimp et al., 1993), which would facilitate the breakdown of TAP (Lin and Yuan, 2005).

The ratios of fungi to bacteria in all treatments ranged from 0.025 to 0.14 (Fig. 1C), which were within the range of 0.02–0.56 in the literature (Antizar-Ladislao et al., 2008). Lower proportions of fungi were found in T treatment, suggesting that TAP might induce the reproduction of bacteria and/or inhibit the growth of fungi to some degree. Similar result was also observed by Vig et al. (2008), who reported a decrease in fungi population in

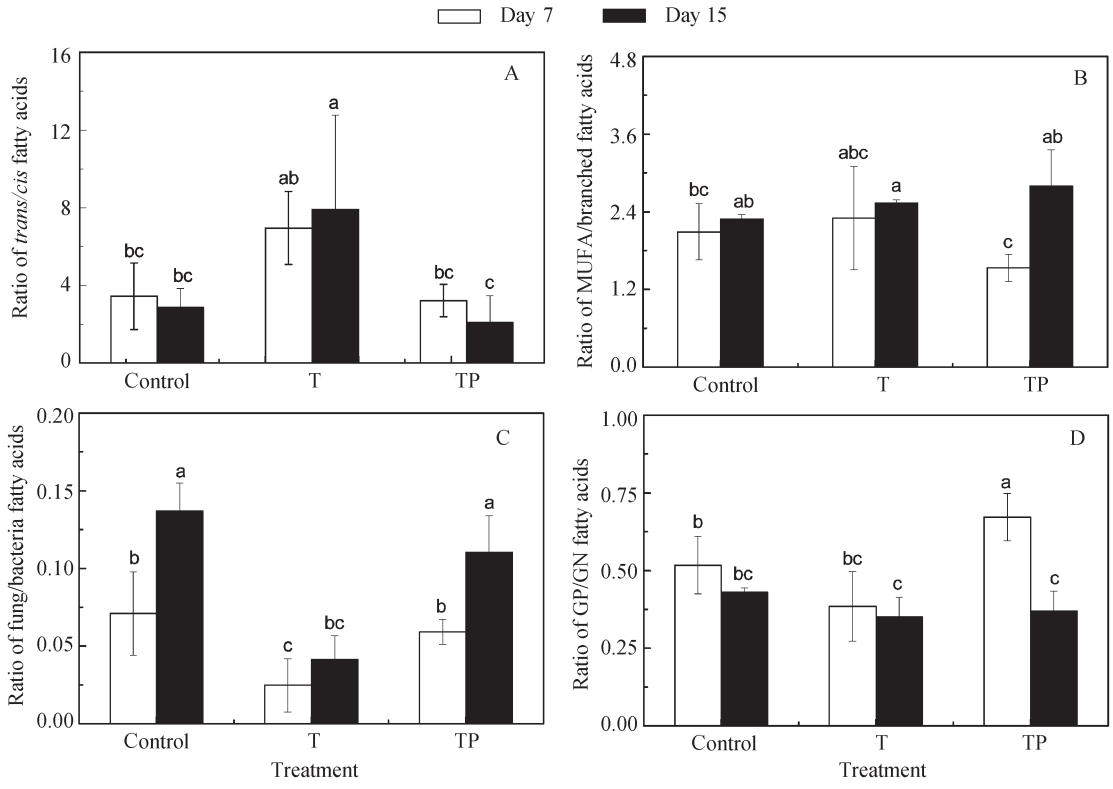

Fig. 1 Variation of signature PLFAs in TAP treatments (T and TP) and control on day 7 and 15. (A) *trans*/*cis* fatty acids; (B) MUFA/branched fatty acids; (C) fungi/bacteria fatty acids; (D) Gram positive/Gram negative (GP/GN) fatty acids in microbes. Error bars represent the standard deviation of three replicates. Different letters (a, b, c) above the bars indicate significant differences between treatments of the data at 5% level. C: control without TAP but with plant; T: 1 mg/L TAP without plant; TP: 1 mg/L TAP with plant.

soils under 600 g/ha of TAP dosage. Depressing effects on fungi were also documented after the application of some other organophosphorus insecticides (Tu, 1970). It appeared that organophosphorus pesticide may be more harmful to fungi than bacteria. The relationship between the ratios of fungi/bacteria and TAP removal percentages was significant (r^2 = 0.840, p < 0.01). There was little change in fungi/bacteria ratios in TP treatment, compared with those in control. *C. indica* seemed to decrease the TAP inhibition effect on fungi growth in this experiment.

There was no significant difference between the ratios of GP/GN in T treatment and in control (p > 0.05) (Fig. 1D). However, microbes in TP treatment responded differently. The ratio in TP treatment on day 7 was 1.3 times of that in control and 1.8 times as high as that for T treatment, demonstrating a significant difference (p < 0.05) in proportions of GP and GN in TP treatment. The ratio of GP/GN after TP treatment decreased to the control level on day 15, suggesting a significant shift in the composition of GP and GN bacteria. The greater fractions of GN bacteria on day 15 may originate from the degradation of TAP, which was confirmed by the correlation analysis of fatty acid 18:1ω9c and TAP dissipation. The result also coincided with research on isolation and identification of some TAP-degrading bacteria. Wang et al. (2005) and Dai et al. (2005) confirmed that *Klebsiella* sp. E6 and *Ochrobactrum* sp. mp-4, GN bacteria, were capable to degrade TAP. Similar changes in GP/GN ratios were also found in response to other contaminants (Frostegård et al., 1993; Klamer and Bååth, 1998). As reported by Guckert et al. (1986), the greater survival of GN bacteria may be linked to the presence of cyclo fatty acids in the cell membrane, as well as the outer lipopolysacchride layer, which would better resist stress from the environment (Kaur et al., 2005). However, the ratio of GP/GN increased while PAHs concentration decreased in the composed mixtures, indicating that GP bacteria were probably responsible for PAHs degradation (Antizar-Ladislao et al., 2008). Different organic contaminates may result in different changes of GP/GN ratios.

2.4 Microbes community structure reflected by PLFAs patterns

Changes of PLFA patterns that are exemplified by the principal component analysis (PCA) loading plot help us to better understand the structure of the microbial community involved. The influence of TAP on PLFAs patterns were analyzed in separate PCA (Fig. 2).

The first principal component (PC1) and second principal component (PC2) explained in sum 47.6% of the variance and discriminated the samples between control, T and TP treatments. On day 7, the scattered points of T and TP treatments were clearly separated from those of the control along PC1, but not apart along PC2. However, after several days of cultivation (on day 15) the points in both T and TP treatments were close to those of the control along PC1, but separated from the control along PC2 (Fig. 2a). Considering PC1 accounted for larger proportions of the total variance than PC2, it might be concluded that microbial community structure in both TAP treatments were discriminated from that of control on day 7. Subsequently, the discrimination in microbial community structure weakened with the degradation of TAP in the hydroponic system (Table 1). Compared with T treatment, scattered points representing the microbial community structure in TP treatment were much closer to those of control, and the variation in microbial community structure between the three treatments on day 7 were more obvious than those on day 15. Both results indicated that the reproduction of microbes changed under the stress of TAP, and the difference was reduced with *C. indica* planted in TP treatment.

Figure 2b shows the distribution of microbial community structures in the three treatments. As seen from the plot, PLFAs 16:0 and 18:1ω9t on the right side accounted for much of the scores along PC1, and they were the more abundant fatty acids in all of the treatments as indicated by Table 2. On contrary, PLFAs 19:0, 11:0 and 13:0 located on the left side of the plot represented less abundant fatty acids in treatments. Additionally, PLFAs representing GN

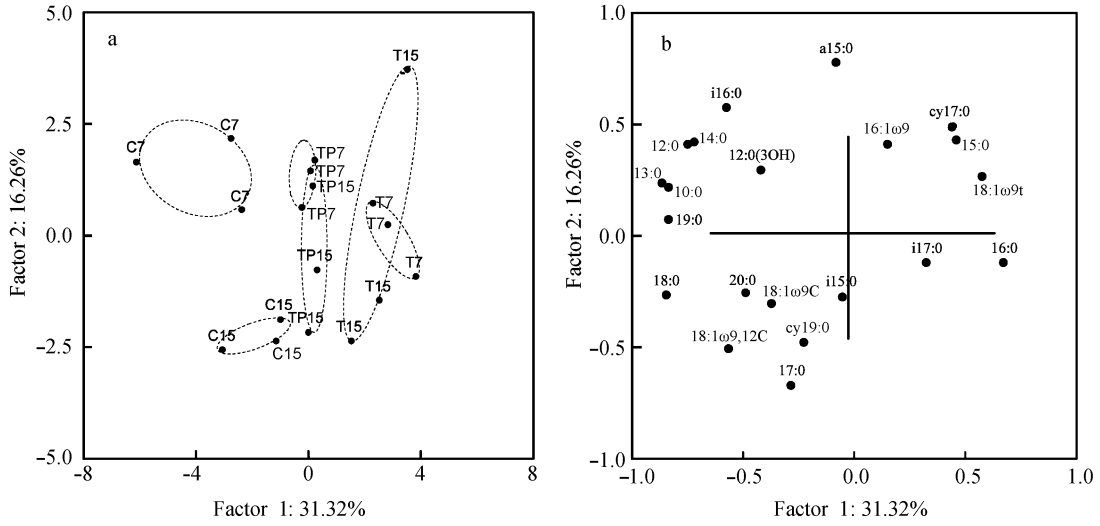

Fig. 2 Principal component analysis of PLFAs in the hydroponic system. (a) PLFA patterns (control: 7 and 15 represent the sampling day); (b) loading values of individual PLFAs.

bacteria were distributed mainly on the left-down and right-up side of the plot, whereas those of GP bacteria were located on the opposite side of the plot.

3 Conclusions

The overall result indicated shifts in microbial community structure during TAP degradation. As an important contributor in TAP dissipation, microbes in T treatment suffered more stress than those in TP treatment, especially fungus in the system. It was evident that the hydroponic system was characterized by microbial communities dominated by GN bacteria. Additionally, there was discrimination in microbial community structure under the stress of TAP from the PCA analysis. Nevertheless, the cultivation of *C. indica* in TP treatment may have weakened the stress on microbes, to performance the degradation of TAP. Such result was explained as simultaneous interactions between microbes and plants in phytoremediation, which would facilitate the dissipation of organic chemicals.

Acknowledgments

This work was supported by the National Natural Science Fundation of China (No. 20877093) and the State Key Laboratory of Freshwater Ecology and Biotechnology (No. 2008FBZ11). The authors acknowledge the technical assistance by Profs. Renhui Li, Yongyuan Zhang, Facui Zhan, Baoyuan Liu and Dr. Qiaohong Zhou from Institute of Hydrobiology, the Chinese Academy of Sciences for their valuable guidance in the experiment.

References

Antizar-Ladislao B, Spanova K, Beck A J, Russell N J, 2008. Microbial community structure changes during bioremediation of PAHs in an aged coal-tar contaminated soil by in-vessel composting. *International Biodeterioration and Biodegradation*, 61(4): 357–364.

Brinis A, Méjanelle L, Momzikoff A, Gondry G, Fillaux J, Point V et al., 2004. Phospholipid ester-linked fatty acids composition of size-fractionated particles at the top ocean surface. *Organic Geochemistry*, 35(11-12): 1275–1287.

Cheng S P, Ren F, Grosse W, Wu Z B, 2002. Effects of cadmium on chlorophyll content, photochemical efficiency, and photosynthetic intensity of *Canna indica* Linn. *International Journal of Phytoremediation*, 4(3): 239–246.

Cheng S P, Xiao J, Xiao H P, Zhang L P, Wu Z B, 2007. Phytoremediation of triazophos by *Canna indica* Linn. in a hydroponic system. *International Journal of Phytoremediation*, 9(6): 453–463.

Dai Q H, Zhang R F, Jiang J D, Gu L F, Li S P, 2005. Isolation, identification and characterization of triazophos degrading bacterium mp-4. *Acta Pedologica Sinica*, 42(1): 111–115.

Fan S X, Li P J, Gong Z Q, Ren W X, He N, 2007. Promotion of pyrene degradation in rhizosphere of alfalfa (*Medicago sativa* L.). *Chemosphere*, 71(8): 1593–1598.

Frostegård A, Tunlid A, Bååth E, 1993. Phospholipid fatty-acid composition, biomass, and activity of microbial communities from 2 soil types experimentally exposed to different heavymetals. *Applied and Environmental Microbiology*, 59(11): 3605–3617.

Guckert J B, Hood M A, White D C, 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: Increases in the *trans*/*cis* ratio and proportions of cyclopropyl fatty acids. *Applied and Environmental Microbiology*, 52(4): 794–801.

He Y, Xu J M, Ma Z H, Wang H Z, Wu Y P, 2007. Profiling of PLFA: Implications for nonlinear spatial gradient of PCP degradation in the vicinity of *Lolium perenne* L. roots. *Soil Biology and Biochemistry*, 39(5): 1121–1129.

Heipieper H J, Meulenbeld G, Oirschot Q, Bont J A M, 1996. Effect of environmental factors on the *trans*/*cis* ratio of unsaturated fatty acids in *Pseudomonas putida* S12. *Applied and Environmental Microbiology*, 62(8): 2773–2777.

Kaur A, Chaudhary A, Kaur A, Choudhary R, Kaushik R, 2005. Phospholipid fatty acid – A bioindicator of environment monitoring and assessment in soil ecosystem. *Current Science*, 89(7): 1103–1112.

Klamer M, Bååth E, 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. *Federation of European Microbiological Societies (FEMS) Microbiology Ecology*, 27(1): 9–20.

Li W, Qiu S P, Wu Y J, 2008. Triazophos residues and dissipation rates in wheat crops and soil. *Ecotoxicology and Environmental Safety*, 69(2): 312–316.

Lin X, Li X J, Li P J, Li F, Zhang L, Zhou Q X, 2008. Evaluation of plant-microorganism synergy for the remediation of diesel fuel contaminated soil. *Bulletin of Environmental Contamination and Toxicology*, 81(1): 19–24.

Lin K D, Yuan D X, 2005. Degradation kinetics and products of triazophos in intertidal sediment. *Journal of Environmental Sciences*, 17(6): 933–936.

Ma G Y, Dong J W, Li Z G, Zhong W J, Xiao P, Yao X M, 2007. Experimental pathologic observation on carcinogenicity of pesticide triazophos in rats. *Journal of Environmental and Occupational Medicine*, 24(6): 592–595.

Nakamura T, Motoyama T, Suzuki Y, Yamaguchi I, 2004. Biotransformation of pentachlorophenol by Chinese chive and a recombinant derivative of its rhizosphere-competent microorganism, *Pseudomonas gladioli* M-2196. *Soil Biology and Biochemistry*, 36(5): 787–795.

Petroutsos D, Katapodis P, Samiotaki M, Panayotou G, Kekos D, 2008. Detoxification of 2,4-dichlorophenol by the marine microalga *Tetraselmis marina*. *Phytochemistry*, 69(3): 707–714.

Rajendran N, Matsuda O, Imamura N, Urushigawa Y, 1992. Variation in microbial biomass and community structure in sediments of eutrophic bays as determined by phospholipid ester-linked fatty acids. *Applied and Environmental Microbiology*, 58(2): 562–571.

Rajendran N, Matsuda O, Rajendran R, Urushigawa Y, 1997. Comparative description of microbial community structure in surface sediments of eutrophic bays. *Marine Pollution Bulletin*, 34(1): 26–33.

Rani S, Madan V K, Kathpal T S, 2001. Persistence and dissipation behavior of triazophos in canal water under Indian climatic conditions. *Ecotoxicology and Environmental Safety*, 50(1): 82–84.

Salomonavá S, Lamačová J, Rulík M, Polčík J, Čáp L, Bednář P et al., 2003. Determination of phospholipid fatty acids in sediments. *Acta Universitatis Palackianae Olomucensis Facultas Rerum Naturalium: Chemica*, 42: 39–49.

Schnoor J L, Wolfe N L, Licht L A, McCutcheon S C, Carreira L H, 1995. Phytoremediation of organic and nutrient contaminants. *Environmental Science and Technology*, 29A: 318–323.

Shen X H, Zhang J, Zhou X P, Shao G J, Jin L Z, Jin M C, 2005. Analyses of organic pesticide residues in vegetables and fruits of part areas of Zhejiang Province. *Chinese Journal of Public Health*, 21(2): 207–208.

Shimp J F, Tracy J C, Davis L C, Lee E, Huang W, Erickson L E, 1993. Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. *Critical Reviews in Environmental Science and Technology*, 23(1): 41–77.

Tu C M, 1970. Effect of four organophosphorus insecticides on microbial activities in soil. *Journal of Applied Microbiology*, 19(3): 479–484.

Vig K, Singh D K, Agarwal H C, Dhawan A K, Dureja P, 2008. Soil microorganisms in cotton fields sequentially treated with insecticides. *Ecotoxicology and Environmental Safety*, 69(2): 263–276.

Wang L H, Zhang L, Chen H L, Tian Q G, Zhu G N, 2005. Isolation of a triazophos-degrading strain *Klebsiella* sp. E6 effectively utilizing triazophos as sole nitrogen source. *FEMS Microbiology Letters*, 253(2): 259–265.

Yu X Z, Gu J D, 2006. Uptake, metabolism, and toxicity of methyl *tert*-butyl ether (MTBE) in weeping willows. *Journal of Hazardous Materials*, 137(3): 1417–1423.

Zelles L, 1999. Identification of single cultured micro-organisms based on their whole-community fatty acid profiles, using an extended extraction procedure. *Chemosphere*, 39(4): 665–682.

Zhang Z Y, He D J, Xu D M, Liu X J, Hong X Y, 2005. A study on residual determination and degradation of triazophos in different pH water. *Modern Agrochemicals*, 4(1): 25–27.

Zhong Z, Liu Q, Gu B Q, Liu S Z, Guo Y M, Chen X C, 2006. GC determine the triazophos pesticides residual in aquatic product. *Journal of Zhejiang Ocean University (Natural Science)*, 25(2): 196–199.

Zhong W J, Ying X P, Ma G Y, Xiao P, Dong J W, 2005. Differential genes expression analysis of liver in rats exposed to triazophos using oligonucleotide microarrays. *Journal of Environmental and Occupational Medicine*, 22(5): 395–398.