

## **Influence of *Glomus etunicatum*/*Zea mays* mycorrhiza on atrazine degradation, soil phosphatase and dehydrogenase activities, and soil microbial community structure**

Huang, H. L., Zhang, S. Z., Wu, N. Y., Luo, L., & Christie, P. (2009). Influence of *Glomus etunicatum*/*Zea mays* mycorrhiza on atrazine degradation, soil phosphatase and dehydrogenase activities, and soil microbial community structure. *Soil Biology and Biochemistry*, 41(4), 726-734. DOI: 10.1016/j.soilbio.2009.01.009

**Published in:**  
Soil Biology and Biochemistry

**Queen's University Belfast - Research Portal:**  
[Link to publication record in Queen's University Belfast Research Portal](#)

### **General rights**

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

### **Take down policy**

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact [openaccess@qub.ac.uk](mailto:openaccess@qub.ac.uk).



## Influence of *Glomus etunicatum*/*Zea mays* mycorrhiza on atrazine degradation, soil phosphatase and dehydrogenase activities, and soil microbial community structure

Honglin Huang<sup>a</sup>, Shuzhen Zhang<sup>a,\*</sup>, Naiying Wu<sup>a</sup>, Lei Luo<sup>a</sup>, Peter Christie<sup>b</sup>

<sup>a</sup> State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, PO Box 2871, Beijing 100085, China

<sup>b</sup> Agri-Environment Branch, Agri-Food and Biosciences Institute, Newforge Lane, Belfast, BT9 5PX, UK

### ARTICLE INFO

#### Article history:

Received 18 June 2008

Received in revised form

24 December 2008

Accepted 11 January 2009

Available online 5 February 2009

#### Keywords:

Arbuscular mycorrhiza

Atrazine dissipation

Maize

PLFA profiles

Soil enzymes

### ABSTRACT

The effects of an arbuscular mycorrhizal (AM) fungus (*Glomus etunicatum*) on atrazine dissipation, soil phosphatase and dehydrogenase activities and soil microbial community structure were investigated. A compartmented side-arm ('cross-pot') system was used for plant cultivation. Maize was cultivated in the main root compartment and atrazine-contaminated soil was added to the side-arms and between them 650 or 37  $\mu\text{m}$  nylon mesh was inserted which allowed mycorrhizal roots or extraradical mycelium to access atrazine in soil in the side-arms. Mycorrhizal roots and extraradical mycelium increased the degradation of atrazine in soil and modified the soil enzyme activities and total soil phospholipid fatty acids (PLFAs). Atrazine declined more and there was greater stimulation of phosphatase and dehydrogenase activities and total PLFAs in soil in the extraradical mycelium compartment than in the mycorrhizal root compartment when the atrazine addition rate to soil was 5.0  $\text{mg kg}^{-1}$ . Mycelium had a more important influence than mycorrhizal roots on atrazine degradation. However, when the atrazine addition rate was 50.0  $\text{mg kg}^{-1}$ , atrazine declined more in the mycorrhizal root compartment than in the extraradical mycelium compartment, perhaps due to inhibition of bacterial activity and higher toxicity to AM mycelium by atrazine at higher concentration. Soil PLFA profiles indicated that the AM fungus exerted a pronounced effect on soil microbial community structure.

© 2009 Elsevier Ltd. All rights reserved.

### 1. Introduction

Arbuscular mycorrhizal (AM) fungi are among the most ubiquitous soil microorganisms and they form mutualistic associations with 80–90% of vascular plant species in ecosystems throughout the world (Harrison, 1997; Smith and Read, 1997). Most previous studies have found that AM fungi have positive effects on the dissipation of organic contaminants such as atrazine (Huang et al., 2007), PAHs (Joner et al., 2001; Joner and Leyval, 2003; Xu et al., 2006; Wu et al., 2008a), DDT (Wu et al., 2008b) and weathered *p,p*-DDE in soils (White et al., 2006), although no impact of AM fungi on PAH dissipation was observed by Binet et al. (2000) and a depression in PAH dissipation in the presence of ectomycorrhizas (Joner et al., 2006; Genney et al., 2004) has also been reported. AM fungi

may therefore play a critical role in the degradation of organic contaminants in soils.

The mechanisms involved in interactions between AM fungi and organic contaminants in soil remain unclear. It is reasonable to expect that soil microbial activity enhanced and soil microbial communities modified by AM fungi play a key role in the degradation of organic contaminants. Once arbuscular mycorrhizal association has developed, AM hyphae influence the surrounding soil which has been termed the mycorrhizosphere (Linderman, 1988), resulting in the development of distinct microbial communities in the rhizosphere and bulk soil (Andrade et al., 1997; Söderberg et al., 2002; Cheng and Baumgartner, 2006; Purin and Rillig, 2008). Phospholipid fatty acid (PLFA) analysis has revealed an important qualitative difference in microbial community structure in mycorrhizosphere soil as affected by AM fungi in PAH-spiked soil (Joner et al., 2001). Furthermore, the AM fungal hyphosphere, the zone of soil affected by the extraradical hyphae (Marschner, 1995), may support a distinct microbial community within the mycorrhizosphere and exert effects on degradation of organic compounds

\* Corresponding author. Tel.: +86 10 62849683; fax: +86 10 62923563.  
E-mail address: [szzhang@rcees.ac.cn](mailto:szzhang@rcees.ac.cn) (S. Zhang).

in soil. There have been no published reports on attempts to distinguish the specific role of extraradical hyphae as well as to separate the roles of arbuscular mycorrhizal roots, extraradical mycelium and non-mycorrhizal roots on the dissipation of organic contaminants in soil.

Studies have indicated that AM fungi can increase the activities of soil enzymes such as phosphatase and dehydrogenase (Dodd et al., 1987; Kothari et al., 1990; Vázquez et al., 2000). Dehydrogenase, a soil oxidoreductase, is an intracellular enzyme catalyzing oxidation reactions of organic compounds. Several studies have demonstrated that the dehydrogenase enzyme activity of microorganisms is one of the most sensitive parameters available for toxicity evaluation and alkaline phosphatase is involved in the process of phosphate acquisition in mycorrhizal plants (Gianinazzi et al., 1992). Both of these enzymes are considered to play key metabolic roles in mycorrhizal function (Vivas et al., 2003; López-Gutiérrez et al., 2004). However, there have been few reports that deal with the effects of AM inoculation on soil enzymes and microbial community structure in soils containing organic contaminants.

Atrazine is one of the agricultural herbicides most frequently detected in soils and waters (Kazemi et al., 2008). It has been reported that in rhizosphere soil atrazine degradation is associated with higher dehydrogenase (Seibert et al., 1991; Singh et al., 2004) and phosphatase activities (Perucei et al., 1988; Bielinska and Pranağal, 2007), and atrazine can influence the populations of certain microbial groups (Ros et al., 2006). We observed in our previous studies that atrazine dissipation in soil was enhanced by AM inoculation (Huang et al., 2006). We therefore hypothesized that increased dissipation of atrazine in soil might contribute to: (1) specific effects of mycorrhizal roots and extraradical mycelium on atrazine dissipation; and/or (2) modification of the effects on soil enzyme activities and microbial community structure resulting from mycorrhizal inoculation. The present study was therefore carried out to identify the specific effects of mycorrhizal roots and extraradical mycelium on dissipation of atrazine in soil using a compartmented cultivation system. Phosphatase and dehydrogenase activities and microbial community structure in soils under the influence of mycorrhizal roots and extraradical mycelium were examined in order to elucidate the interaction between atrazine degradation and soil enzymes and microbial communities.

## 2. Materials and methods

### 2.1. Host plants and AM fungus

Seeds of maize (*Zea mays* L. cv. Nongda 108) were surface sterilized in a 10% (v/v) solution of hydrogen peroxide for 10 min, rinsed with sterile distilled water, and soaked in a 3 mM solution of  $\text{Ca}(\text{NO}_3)_2$  for 4 h in the dark. Then they were germinated on moist filter paper for about 48 h. Seedlings of uniform size were selected and were ready for sowing.

Original inoculum of the AM fungus *Glomus etunicatum* (BGC USA01), which was kindly provided by the Institute of Plant Nutrition and Fertilizers, Beijing Academy of Agronomy and Forestry, was propagated in pot culture on sorghum for 10 weeks in a zeolite–sand mixture in a greenhouse. The inoculum, a mixture of spores, mycelium, sand and root fragments, was air-dried and sieved (<2 mm). For the extraction of spores and sporocarps, 20 mL of inoculum were treated by the wet sieving and decanting method (Gerdemann and Nicolson, 1963). The resulting material was centrifuged with 80% saccharose (Walker et al., 1982). Quantification was carried out in 9-cm-diameter Petri dishes with gridlines of 1 cm per side under a stereoscopic microscope at  $\times 50$  magnification. The inoculum contained about 2200 spores per 20 mL.

### 2.2. Compartmented cultivation system

The compartmented cultivation system ('cross-pots') (Chen et al., 2007; Joner and Leyval, 1997; Joner et al., 2000) comprised a vertical root compartment (PVC tube, 6.0 cm diameter  $\times$  25 cm long) for plant growth and two symmetrical horizontal side-arm compartments (PVC tube, 4.5 cm diameter  $\times$  9.0 cm long). The side-arm compartments were separated from the main root compartment by 37- or 650- $\mu\text{m}$  nylon mesh, the fine mesh to prevent the entry of roots and only allow free passage of mycelium into the side compartment and the coarse mesh to allow the passage of mycorrhiza together with any extraradical mycelium.

### 2.3. Experimental design

The experiment was a  $2 \times 2 \times 3$  factorial design with mycorrhizal colonization (+M/–M), coarse mesh/fine mesh separating the main root compartment from the side-arm compartment, and atrazine-free and two addition levels of atrazine (5.0 and 50.0 mg  $\text{kg}^{-1}$ ) in the side-arm compartment soil. The two atrazine addition levels were selected according to the range of contamination levels in soil (0.017 mg  $\text{kg}^{-1}$  – 482.7 mg  $\text{kg}^{-1}$ ) reported by Grigg et al. (1997) as well as its limited phyto-availability because only the roots penetrating through the mesh would be exposed to atrazine in the side-arm compartment. Four replicate pots of each treatment were set up.

### 2.4. Growth medium

A brown soil (Alfisol containing 1.35% organic matter) was collected from the top 15 cm of the soil profile in an experimental field at Beijing Academy of Agriculture and Forestry. The soil was air-dried, ground, and passed through a 2-mm nylon sieve. A 1:1 (v/v) mixture of sand (1–2 mm) and soil was used as the growth medium in order to enhance the permeability of the soil (Biermann and Linderman, 1983) and the mixture was sterilized by  $\gamma$ -radiation (10 kGy, 10 MeV  $\gamma$ -rays) to inactivate the native AM fungi. The soil mixture (henceforth referred to as the soil) had a pH of 7.89 (1:2.5 soil/water) and a 0.5 M  $\text{NaHCO}_3$ -extractable P content of 9.98 mg  $\text{kg}^{-1}$ . P was added to the soil at a rate of 50.0 mg  $\text{kg}^{-1}$  and portions of the soil were then artificially dosed with atrazine. HPLC grade atrazine (Sigma Chemical Co.) was dissolved in reagent grade acetone and added to portions of the soil at concentrations of 0, 5.0 and 50.0 mg  $\text{kg}^{-1}$  (dry matter basis). The soil mixtures were then allowed to dry in a fume hood until the acetone had volatilized completely and were shaken, homogenized, and incubated for 4 weeks to allow the contaminant to equilibrate.

### 2.5. Pot experiment

Each side-arm of the cross-pot was filled with 135 g sterile atrazine-contaminated soil and then with 45 g atrazine-free soil to establish a buffer layer to minimize possible movement of atrazine from the side-arm compartment to the main root compartment. Deionized water was added to the side-arm compartment soil to adjust the moisture content to 60% of water holding capacity (WHC). The side-arm tubes were then sealed with nylon mesh and allowed to stand overnight. Soil amended with basal nutrients was placed in the main root compartment to support plant growth. For mycorrhizal treatments, 60 g inoculum was thoroughly mixed with 850 g soil and each pot contained about 6600 spores. Above the side-arm compartment a further 10 g inoculum was placed on the soil. The side-arm compartments were fixed tightly to the main root compartment which was then sealed at the bottom with a plastic bag. Non-mycorrhizal treatments received the same

amount of sterilized inoculum (70 g) together with a 2-mL aliquot of a filtrate (<20 µm) of the AM inoculum to provide a general microbial population free of AM propagules. Four pre-germinated maize seeds were sown in each pot and five days after emergence the seedlings were thinned to two of uniform size. All pots were lined with polyethylene bags to avoid cross-contamination and water loss and the surface of each pot was covered with a black plastic bag to minimize algal growth. The experiment was conducted in a controlled-environment growth chamber that maintained a daily 14-h light period at a light intensity of 250 µmol m<sup>-2</sup> s<sup>-1</sup> provided by supplementary illumination. The day/night temperature regime was 25/20 °C. The relative humidity was maintained at 70%. The plants grew for 8 weeks. Deionized water was added as required to maintain soil moisture content at 60–70% WHC.

## 2.6. Harvest and analysis

### 2.6.1. Sample preparation

At harvest the side-arm compartments were separated from the main root compartment and the buffer soil layer was discarded. Soil samples were collected from the main root and the side-arm compartments, freeze-dried and stored at 4 °C. Soil PLFA, dehydrogenase activity and phosphatase activity were determined within 1 month. Plant shoots and roots were harvested separately. Root samples were first carefully washed with tap water to remove any adhering soil particles. Then the shoot and root samples were thoroughly rinsed with distilled water, wiped with tissue paper, and immediately weighed. A subsample of fresh roots was taken from each treatment for the determination of the proportion of root length colonized by the AM fungus. The remaining plant samples were freeze-dried, weighed, and stored at 4 °C.

### 2.6.2. Determination of root colonization

The proportion of root length colonized by the fungus was estimated by randomly taking a subsample of 1.0 g of fresh roots and cutting them into 0.5–1.0-cm pieces. Root segments were cleared in 10% KOH for 10 min at 90 °C in a water bath, rinsed in water, and then stained with 0.1% Trypan Blue for 3–5 min at 90 °C in a water bath. Percentage root length colonization and total root length were determined by the gridline intersect method (Giovannetti and Mosse, 1980).

### 2.6.3. Dehydrogenase activity

Twenty grams of freeze-dried soil were thoroughly mixed with 0.2 g CaCO<sub>3</sub> and three replicate samples of 5.0 g soil were placed in three test tubes. Three milliliters of 1% 2,3,5-triphenyltetrazolium chloride (TTC) and 2.0 mL of distilled water were added to each tube. Samples were then incubated at 37 °C for 24 h with constant shaking at medium speed. Afterward, 10 mL of methanol were added to each tube and the samples were vortexed. The soil suspensions were filtered through glass funnels plugged with absorbent cotton. The filtrates were diluted with methanol to 100 mL volume and the intensity of the reddish color was measured at 485 nm using a spectrophotometer. Dehydrogenase activities in the samples were calculated by using calibration graphs prepared from 500, 1000, 1500 and 2000 mg triphenyl formazan (TPF) per 100 mL standards. Results are presented as mg TPF kg<sup>-1</sup> soil.

### 2.6.4. Phosphatase activity

The assay of phosphatase activity was determined by measuring the *p*-nitrophenol released by phosphatase activity when soil was incubated with buffered (pH 9.4) sodium *p*-nitrophenyl phosphate solution and toluene at 37 °C for 24 h using the modified method proposed by Tabatabai and Bremner (1969). The *p*-nitrophenol

formed was determined using a spectrophotometer at 660 nm. Controls were prepared in the same way.

### 2.6.5. PLFA analysis

Fine root fragments were teased out and then the soil samples were sieved (2.0 mm mesh). Five grams of freeze-dried fresh soil were transferred to a test tube for lipid extraction according to the method of Frostegård et al. (1993). The samples were extracted with a one-phase mixture of chloroform, methanol and citrate buffer (0.15 M, pH 4) (1:2:0.8 v/v/v). The phases were separated after adding 3.0 mL of chloroform and 3.0 mL of the buffer. The lower phase was collected, dried and used in lipid fractionation. The dried lipid extract was dissolved in 100 mL chloroform and fractionated on pre-packed columns with 100 mg silicic acid (Bond Elut Extraction Cartridges, Varian, USA). Phospholipid fractions were collected and methyl nonadecanoate was added as the internal standard and then transesterified by a mild alkaline methanolysis (Dowling et al., 1986). Blanks without soil were subjected to the same lipid extraction protocol in order to detect possible contamination errors. The fatty acid methyl esters (FAMES) 16:1*cis*11 are often used as a biomarker for AM fungi in roots and soils (Olsson and Johansen, 2000; Olsson, 1999), *i*-15:0, *a*-15:0, *i*-16:0, *i*-17:0, and *a*-17:0 for Gram-positive bacteria (O'Leary and Wilkinson, 1988), 18:1*ω*7, *cy*-17:0, and *cy*-19:0 for Gram-negative bacteria (Wilkinson, 1988), and 18:2*ω*6,9 for fungi (Federle, 1986).

### 2.6.6. Atrazine analysis

Five grams of the soil samples (dry matter basis) were extracted twice with 50.0 mL of 80% aqueous methanol by shaking the suspension on a reciprocating shaker for 48 h. The extracts were filtered and combined and then extracted successively with 50 mL of petroleum ether/dichloromethane (65:35, v/v) three times. Supernatants were passed through anhydrous Na<sub>2</sub>SO<sub>4</sub> columns and collected. The volumes of the eluates were reduced to 1–2 mL. Then they were solvated with 30 mL of petroleum ether and re-extracted three times with 20 mL of acetonitrile. The acetonitrile fractions were combined, concentrated, and evaporated off. The residues were solvated with petroleum ether and cleaned with Florisil columns. The concentrations of atrazine in extracts were analyzed with an Agilent 6890 gas chromatograph equipped with a detector of NPD using a HP-5 capillary column (0.32 mm × 30 m, 0.25 µm film thickness). The column oven was programmed from an initial temperature of 70 °C for 2 min to 220 °C at a rate of 20 °C min<sup>-1</sup>, held for 1 min, and then ramped at a rate of 4 °C min<sup>-1</sup> to 240 °C with a final hold time of 10 min. The detector and injector were maintained at 300 and 250 °C, respectively. The injector was in the splitless mode for nitrogen–phosphorus detection. To determine analytical recovery, aliquots of soil were spiked with pesticides. Recoveries ranged from 85 to 90% (RSD = 6.8%, *n* = 5).

## 2.7. Statistical analysis

The data were analyzed by three-way analysis of variance using the SPSS version 11.5 software package. Means and standard errors of four replicates were calculated. The data were examined for the significance of AM treatment, atrazine application and coarse mesh/fine mesh separation between the main root compartment and the side-arm compartment. A 95% confidence limit (*P* < 0.05) was chosen to indicate differences between samples and least significant differences (LSD) were calculated when samples were significantly different. The PLFA data (24 distinct PLFAs identified) were subjected to principal component analysis (PCA) to examine patterns of compartment and inoculation combinations. PCA scores were subjected to three-way analysis of variance (ANOVA) to test the significance of the effects of AM treatment, atrazine application



and mesh separation and their interactions on microbial community structure.

### 3. Results

#### 3.1. Colonization of roots by *Glomus etunicatum* and plant biomass

Roots of inoculated plants were extensively colonized by *Glomus etunicatum* but non-inoculated controls remained non-mycorrhizal (Table 1). The percentage of root length colonized in the main root compartment ranged from 45 to 64% across all the inoculated treatments. Rate of root colonization was higher when the side-arm compartment was separated from the main root compartment by the 37- $\mu\text{m}$  nylon mesh than by the 650- $\mu\text{m}$  nylon mesh ( $P < 0.05$ ) and decreased with increasing atrazine application rate in the side-arm compartment. Atrazine application did not significantly affect the dry weights of shoots and roots ( $P > 0.05$ ). Colonization significantly increased the root dry weight ( $P < 0.001$ ) but did not affect the shoot dry weight.

#### 3.2. Residual atrazine in soil and accumulation in maize

After plant harvest the atrazine concentration in the side-arm compartment soils decreased by 26.4 to 77.3% compared with the initial concentrations (Table 1). Inoculation significantly decreased the amount of residual atrazine in the side-arm soil ( $P < 0.05$ ). The residual atrazine concentration decreased by 22.7 and 36.1% in the mycorrhizal root compartment soil and in the mycelium compartment soil respectively when the application rate of atrazine to soil was 5.0 mg kg<sup>-1</sup>, with corresponding values of 53.4 and 46.2% when the atrazine was applied at 50.0 mg kg<sup>-1</sup>. No atrazine was found in the main root compartment soil when the application rate was 5.0 mg kg<sup>-1</sup> and only a very small amount was detected at the application rate of 50.0 mg kg<sup>-1</sup> (data not shown). No atrazine was detected in either the roots or shoots of maize.

#### 3.3. Soil phosphatase activity

Phosphatase activities in soils in both the side-arm and the main root compartments were enhanced by AM inoculation ( $P < 0.05$ ) (Fig. 1). Compared with the non-mycorrhizal control, the soil phosphatase activity in the inoculation treatment increased by 27.1–71.1% and 8–111% in the main root compartment with mesh separation of 650 and 37  $\mu\text{m}$  ( $P < 0.05$ ), respectively. Soil phosphatase activity increased by 35.8–70.1% and 90.2–179% in the mycorrhizal and mycelium side-arm compartments ( $P < 0.05$ ), respectively. Application of atrazine activated the phosphatase activities in the side-arm compartment soil. No consistent effect of atrazine application on phosphatase activity in the main root compartment soil was observed.

#### 3.4. Soil dehydrogenase activity

When no atrazine was applied, AM inoculation had no significant effect on dehydrogenase activity in the control main root compartment soil ( $P > 0.05$ ; Fig. 2). When atrazine was applied at 5.0 mg kg<sup>-1</sup> the dehydrogenase activity in the main root compartment soil decreased by 17.0 and 19.4% by inoculation in the coarse and fine mesh treatments ( $P < 0.05$ ), respectively. When atrazine was applied at 50.0 mg kg<sup>-1</sup> the dehydrogenase activities in the main root compartment soil increased by 20.0 and 12.9% by inoculation in the coarse and fine mesh treatments ( $P < 0.05$ ). Dehydrogenase activity was higher in soil in the mycelium compartment than in the mycorrhizal root compartment when atrazine was applied at 5.0 mg kg<sup>-1</sup> ( $P < 0.05$ ), the opposite trend to that when the atrazine application rate was 50.0 mg kg<sup>-1</sup> ( $P < 0.05$ ). Dehydrogenase activity in the side-arm compartment soil was decreased by 6.0–22.9% comparing the treatment with atrazine application to the atrazine-free control treatment including both mesh treatments and atrazine application levels.

**Table 1**

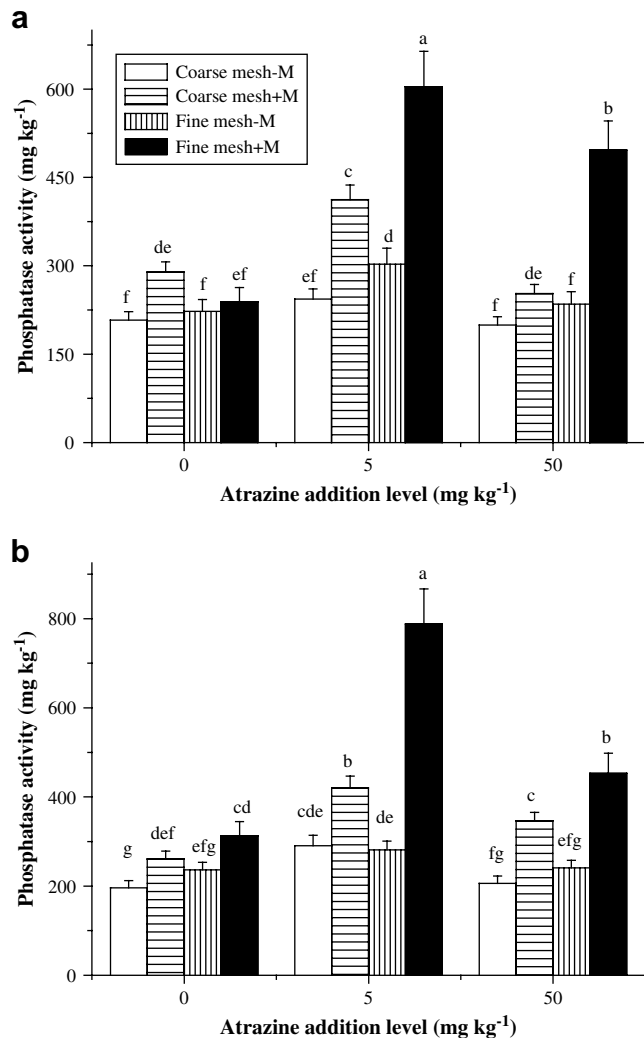
Biomass of maize (dry matter basis), mycorrhizal colonization rates of roots and residual atrazine in the side-arm compartment soil in association with *Glomus etunicatum* as influenced by different sizes of mesh and various concentrations of atrazine added to the side-arm compartment soil.

Mycorrhiza inoculation treatment	Type of nylon mesh	Added ATR <sup>a</sup> (mg kg <sup>-1</sup> )	Dry matter (g)		Root colonization (%)	Residual ATR (mg kg <sup>-1</sup> )
			Roots	Shoots		
Non- inoculated	Coarse mesh	0	1.26 (0.24)cde	7.50 (0.59)a	0	0
		5	1.22 (0.17)de	7.61 (0.86)a	0	3.15 (0.14)f
		50	1.18 (0.26)e	7.37 (0.68)a	0	21.18 (1.70)b
	Fine mesh	0	1.38 (0.07)bcde	5.67 (1.18)b	0	0
		5	1.46 (0.32)abcde	6.86 (1.02)a	0	3.68 (0.15)e
		50	1.23 (0.16)de	7.41 (0.21)a	0	26.59 (2.12)a
Inoculated	Coarse mesh	0	1.65 (0.14)abc	7.31 (0.30)a	64 (3)ab	0
		5	1.72 (0.25)ab	7.02 (0.02)	56 (2)cd	2.67 (0.35)g
		50	1.58 (0.16)abcde	7.52 (0.14)a	45 (5)e	11.34 (0.91)d
	Fine mesh	0	1.81 (0.34)a	7.24 (0.43)a	67 (5)a	0
		5	1.62 (0.14)abcd	6.78 (0.44)a	58 (3)bc	2.31 (0.10)h
		50	1.49 (0.10)abcde	6.90 (0.25)a	48 (3)de	18.06 (1.54)c
Significance of <sup>b</sup>						
Inoculation (I)			***	NS	***	***
Mesh type (M)			*	**	*	*
ATR addition (A)			NS	NS	***	*
I × M			***	**	**	**
I × A			**	NS	***	*
M × A			NS	*	*	*
I × M × A			**	NS	***	**

Values denote standard error of the mean (SEM) in parentheses;  $n = 4$ . Means within each column with the same letter are not significantly different at the 5% level. The root colonization data of non-inoculated controls were excluded from the statistical analysis.

<sup>a</sup> ATR, atrazine.

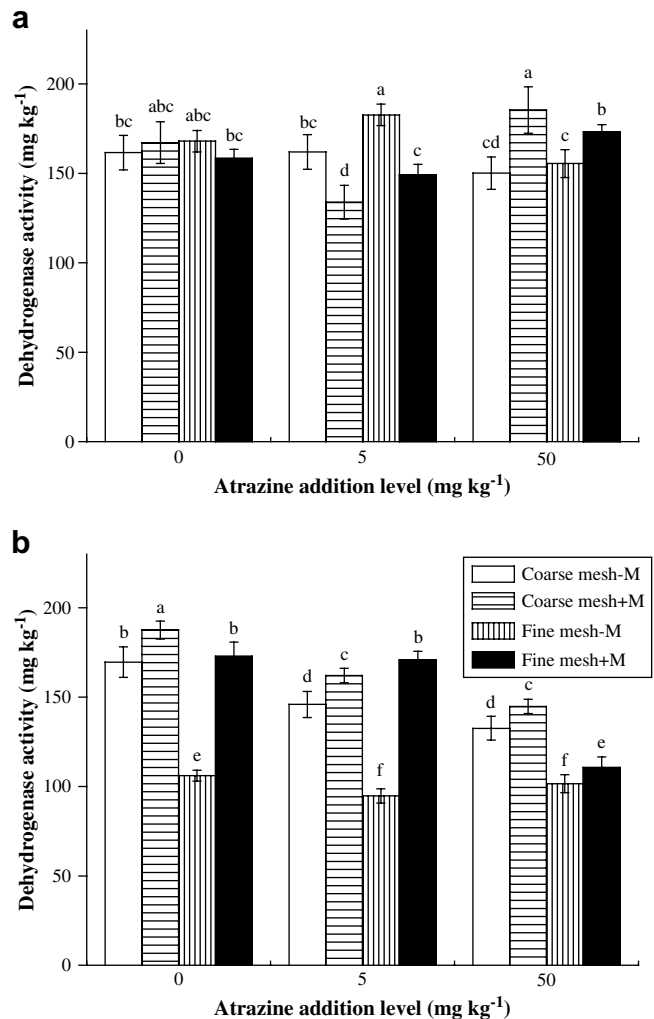
<sup>b</sup> By analysis of variance, \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; NS, not significant.



**Fig. 1.** Phosphatase activity in (a) the main root compartment soils and (b) the side-arm compartment soils with mycorrhizal and non-mycorrhizal (+M/-M) treatments as influenced by coarse or fine mesh and concentrations of atrazine added to the side-arm compartment soil. Different letters indicate significantly different values at  $P < 0.05$ . Error bars denote standard error of the mean (SEM).

### 3.5. Phospholipid fatty acid composition and total microbial biomass

Twenty-four PLFAs, including a variety of saturated, unsaturated, branched, cyclopropane, and hydroxyl fatty acids, were determined in the soils. Clear variation was observed among treatments in the PLFA composition (microbial community structure) in soil. Inoculation treatments generally spread towards the right along the axes while non-inoculation treatments spread to the left along the axes in the PCA plot (Fig. 3). The principal component analysis (PCA) of the PLFA signatures showed that the first two dimensions could explain altogether 80.4% ( $PC_1 = 70.5\%$ ,  $PC_2 = 9.9\%$ ) and 82.4% ( $PC_1 = 70.1\%$ ,  $PC_2 = 12.3\%$ ) of the total variance in the main root compartment and the side-arm compartment soils. Along axis  $PC_1$  microbial profiles were significantly affected by inoculation treatment and application of atrazine to soil ( $F_M = 5.597$ ,  $P_M = 0.025$ ;  $F_{ATR} = 4.498$ , and  $P_{ATR} = 0.020$ , respectively), and along axis  $PC_2$  microbial profiles were only significantly affected by inoculation treatment ( $F_M = 6.555$ ,  $P_M = 0.016$ ), and the microbial profiles were not affected by mesh or treatment interactions.



**Fig. 2.** Dehydrogenase activity in (a) the main root compartment soils and (b) the side-arm compartment soils. Error bars denote SEM.

Microbial biomass determined by the total PLFAs (Fig. 4) showed marked variation between the different mesh sizes as well as the inoculation treatments. This observation is consistent with Liu et al. (2008) and indicates a contribution to the differences in soil hyphal length density and root colonization rate in the central compartment when it was separated from the side compartment by fine or coarse mesh (Chen et al., 2007). The total amount of PLFAs was higher in the inoculated than in the non-inoculated soil ( $P < 0.05$ ), and was higher in the mycelium side-arm compartment than in the mycorrhizal root side-arm compartment ( $P < 0.05$ ). Microbial biomass was also affected ( $P < 0.05$ ) by the application of atrazine, but no general trend could be drawn between the atrazine concentration applied and the microbial biomass.

Fig. 5a shows the PLFA concentrations in the main root compartment soil indicative of different types of bacteria and fungi and they were in the following sequence: bacteria > Gram-positive bacteria > AM fungi > Gram-negative bacteria. The inoculation treatment increased PLFA concentrations indicative of the different types of microbial groups ( $P < 0.05$ ). Application of 5.0 mg kg<sup>-1</sup> atrazine increased the PLFA concentrations but application of 50.0 mg kg<sup>-1</sup> atrazine decreased them irrespective of microbial groups ( $P < 0.05$ ). PLFA concentrations in the side-arm compartment soils are given in Fig. 5b. Gram-negative bacteria in soil were higher in the mycelium compartment than in the mycorrhizal root

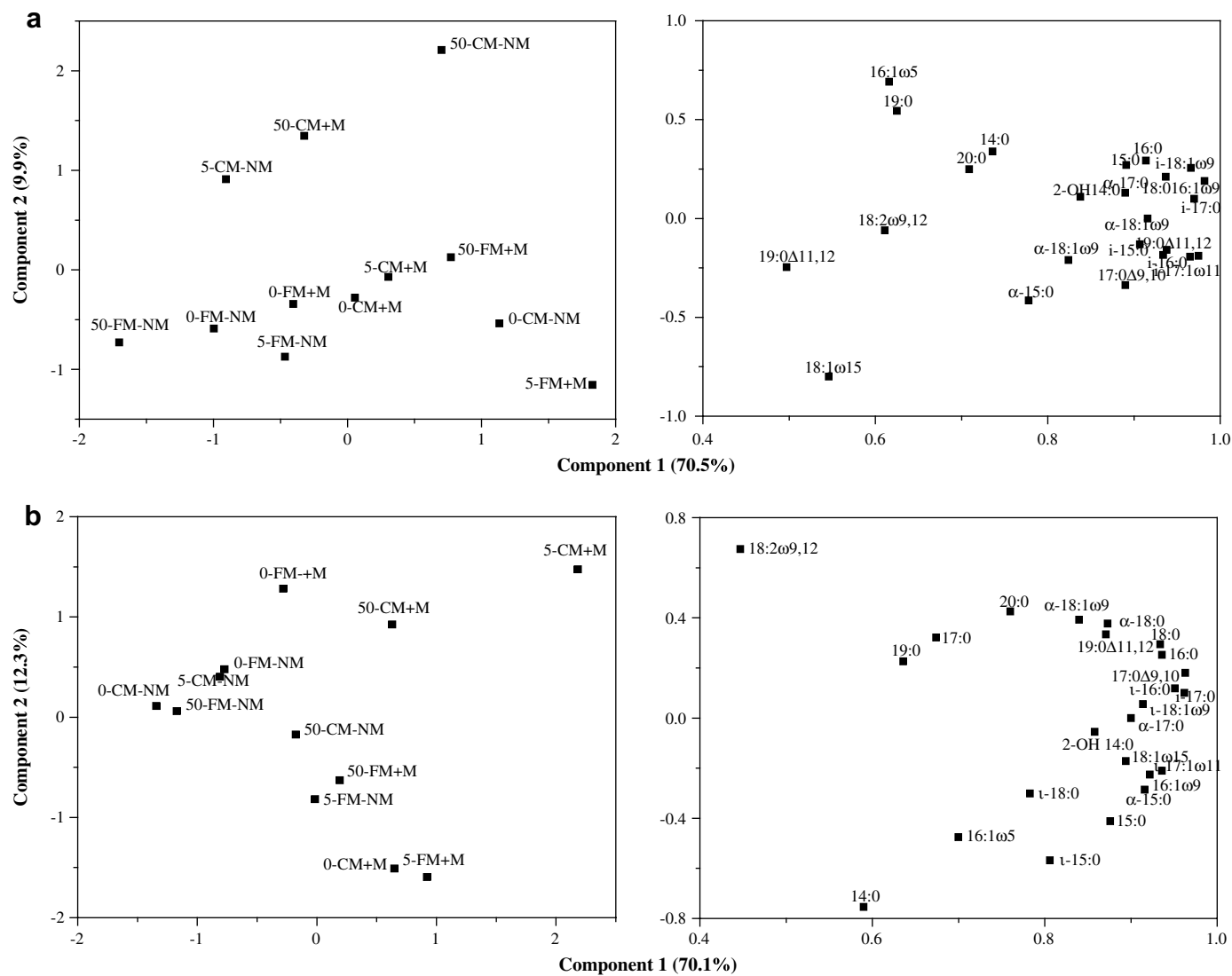


Fig. 3. Principal component analysis (PCA) of phospholipid fatty acids (PLFAs) in (a) the main root compartment soils and (b) side-arm compartment soils FM, fine mesh; CM, coarse mesh.

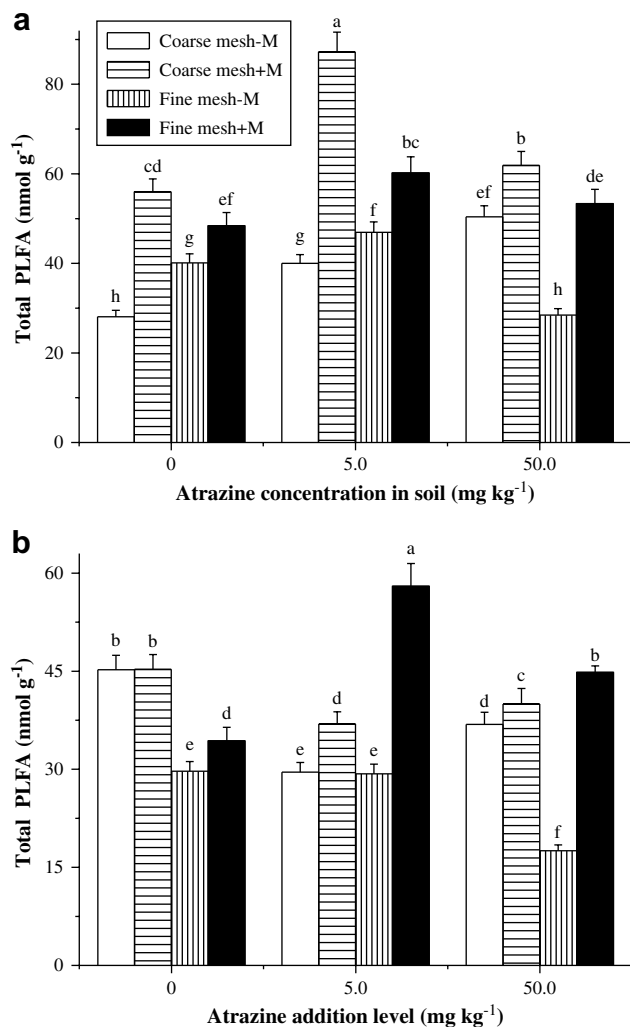


Fig. 4. Total PLFA concentrations in (a) the main root compartment soils and (b) side-arm compartment soils. Error bars denote SEM.

compartment when atrazine was applied at 5.0 mg kg<sup>-1</sup>, and the opposite trend was obtained for the atrazine application rate of 50.0 mg kg<sup>-1</sup> ( $P < 0.05$ ). Concentration of bacteria was consistent with that of Gram-negative bacteria. Gram-positive bacteria and AM fungi were respectively 1.48–1.71 and 1.21–1.49 fold in soil in the mycelium compartment compared with the mycorrhizal root compartment when atrazine had been applied to the soil.

#### 4. Discussion

No atrazine was detected in either maize roots or shoots due to the very limited uptake of atrazine by mycorrhizal roots or extraradical mycelium from the side-arm compartment soil in addition to a plant tissue dilution effect. There was a significant decrease in the residual atrazine concentration in the soil after plant harvest ( $P < 0.05$ ) and this became more evident with inoculation, which was consistent with our previous findings for atrazine by bulk cultivation (Huang et al., 2006). Atrazine in soil decreased more in the extraradical mycelium compartment than in the mycorrhizal root compartment when the atrazine addition rate to soil was 5.0 mg kg<sup>-1</sup>. The mycelium exerted a greater influence on atrazine degradation than did mycorrhizal roots. However, atrazine added at

50.0 mg kg<sup>-1</sup> decreased more in the mycorrhizal root compartment than in the extraradical mycelium compartment.

Inoculation significantly increased phosphatase activity in soil (Fig. 1) and followed: in the mycelium compartment (90.2–179.3%) > in the mycorrhizal root compartment (35.8–70.1%) > in the root compartment (27.1–71.1%), indicating that the extraradical mycelium can stimulate phosphatase activity in soil more than mycorrhizal roots can. Dehydrogenase activity was higher in soil in the mycelium compartment than in the mycorrhizal root compartment when atrazine was applied at 5.0 mg kg<sup>-1</sup> ( $P < 0.05$ ) and the opposite trend occurred at 50.0 mg kg<sup>-1</sup> ( $P < 0.05$ ) (Fig. 2b). Concentrations of Gram-positive bacteria and AMF were higher in soil in the mycelium compartment than in the mycorrhizal compartment for both atrazine application treatments. Concentrations of Gram-negative bacteria and bacteria were higher in the soil in the mycelium compartment than in the mycorrhizal root compartment when atrazine was applied at 5.0 mg kg<sup>-1</sup> ( $P < 0.05$ ), and the opposite trend was found when atrazine was applied at 50.0 mg kg<sup>-1</sup> ( $P < 0.05$ ) (Fig. 5). This demonstrates that with increasing atrazine concentration in soil the Gram-positive bacteria decreased less than did the Gram-negative bacteria or the fungi. Gram-positive bacteria are considered to be more stress-tolerant to atrazine, possibly due to their relatively thick cell walls and their ability to form endospores (Stainer et al., 1977).

The evidence of greater stimulation of phosphatase and dehydrogenase activities and total PLFAs in the mycelium compartment soils is consistent with the observation of more atrazine degradation in this soil compartment than in the mycorrhizal root compartment when the atrazine addition rate to soil was 5.0 mg kg<sup>-1</sup>. This suggests that extraradical mycelium is more effective than mycorrhizal roots at stimulating soil enzymes and bacterial activities, resulting in more atrazine dissipation in the mycelium compartment soil. However, the opposite trend occurred for both atrazine dissipation in soil and soil enzymes or bacterial activities when atrazine was applied at 50.0 mg kg<sup>-1</sup>. This may contribute to the inhibition of bacterial activity (Widenfalk et al., 2004) and higher atrazine toxicity at the higher application rate, particularly to the extraradical mycelium.

Exposure to atrazine may affect soil microbial activity and induce shifts in microbial community structure and function. Atrazine has been reported to significantly depress alkaline phosphatase activity in soil (Sannino and Gianfreda, 2001). However, we observed that both levels of atrazine that we used increased phosphatase activities in soils when AM inoculation was present. Application of atrazine had no consistent effect on dehydrogenase activity or soil microbial biomass. Previous studies have also shown conflicting results due to the difficulty in establishing the cause-and-effect relationship between organic pollutants and enzyme activities (Accinelli et al., 2002; Haney et al., 2002). Atrazine, which is an inhibitor of photosynthesis, has been reported to strongly depress cyanobacteria, to transiently affect the soil fungal microflora and to alter the denitrifying microflora (Isakeit and Lockwood, 1990; Martin-Laurent et al., 2003). In this study bacterial, Gram-negative bacterial, Gram-positive bacterial and arbuscular mycorrhizal fungal community composition showed significant ( $P < 0.05$ ) atrazine-induced changes. This is in line with the responses at various levels of microbial community organization reported by Seghers et al. (2003).

We can conclude from the above analysis that mycorrhizal roots and extramatrix mycelium selectively increased the degradation of atrazine in soil and modified the soil enzymes and total soil PLFAs. The greater decrease in atrazine observed in the mycelium compartment than in the mycorrhizal compartment when the atrazine application rate to soil was 5.0 mg kg<sup>-1</sup> is consistent with the conclusion that extraradical mycelium can



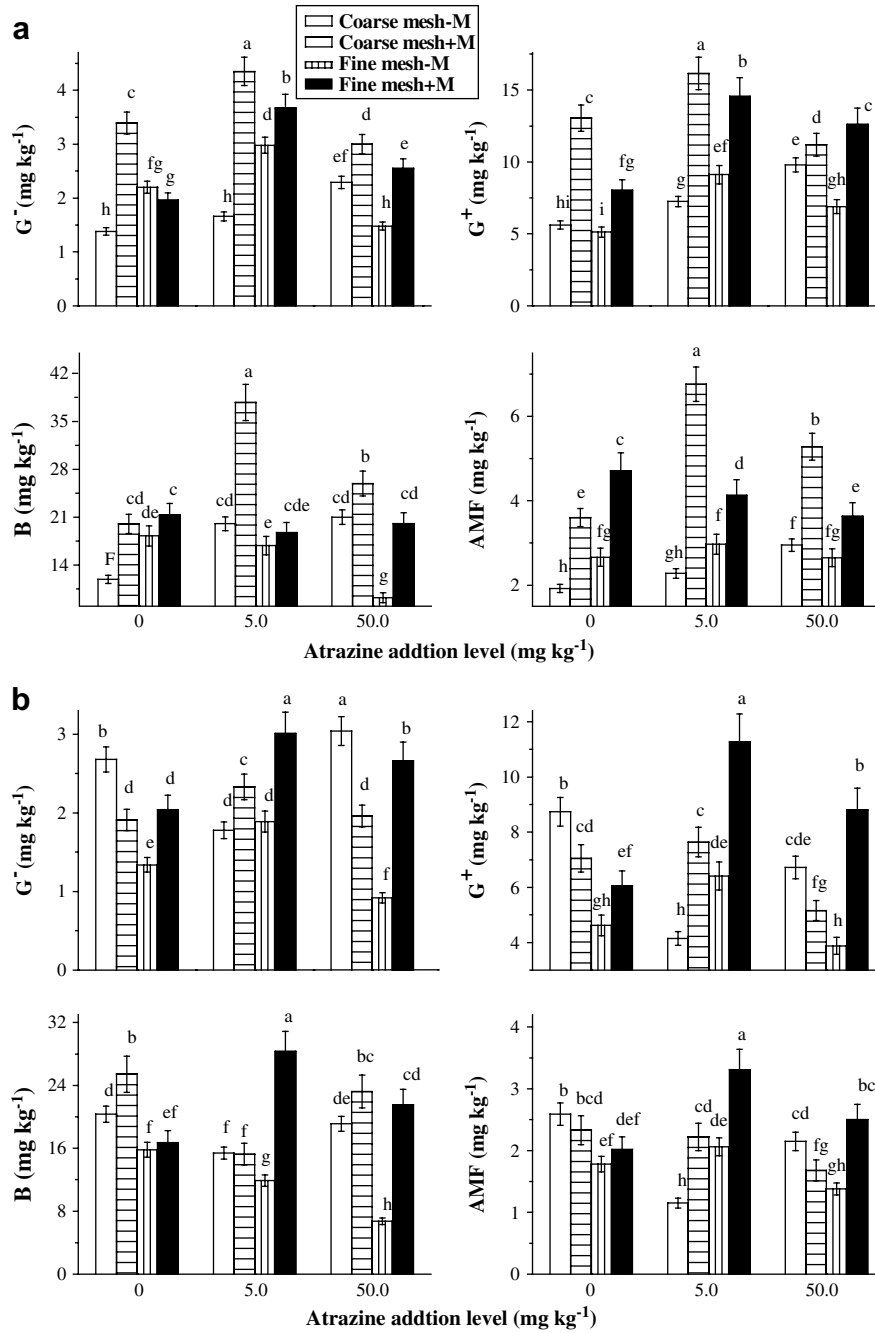


Fig. 5. PLFA concentrations indicative of Gram-negative bacteria ( $G^-$ ), Gram-positive bacteria ( $G^+$ ), bacterial (B) and arbuscular mycorrhizal fungi (AMF) in (a) the main root compartment soils and (b) side-arm compartment soils. Error bars denote SEM.

stimulate more phosphatase and dehydrogenase activity and total PLFAs in soil than can mycorrhizal roots. This implies that mycelium has a more important influence than mycorrhizal roots on atrazine degradation and this effect is related to microbial processes.

**Acknowledgements**

This work was funded by the National Natural Science Foundation of China (Projects 20677072, 40730740 and 20621703) and the Hi-tech Research and Development Program of China (Project 2006AA06Z349).

**References**

Accinelli, C., Screpanti, C., Dinelli, G., Vicari, A., Fosmgaard, I.S., 2002. Short time effects of pure and formulated herbicides on soil microbial activity and biomass. *International Journal of Environmental Analytical Chemistry* 82, 519–527.

Andrade, G., Mihara, K.L., Linderman, R.G., Bethlenfalvay, G.J., 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. *Plant and Soil* 192, 71–79.

Bielinska, E.J., Pranagal, J., 2007. Enzymatic activity of soil contaminated with triazine herbicides. *Polish Journal of Environmental Studies* 16, 295–300.

Biermann, B., Linderman, R.G., 1983. Effect of container plant growth medium and fertilizer phosphorus on establishment and host growth response to vesicular-arbuscular mycorrhizae propagules. *Journal of the American Society for Horticultural Science* 108, 962–971.

Binet, P., Portal, J.M., Leyval, C., 2000. Fate of polycyclic aromatic hydrocarbons (PAH) in the rhizosphere and mycorrhizosphere of ryegrass. *Plant and Soil* 227, 207–213.

- Chen, B.D., Xiao, X.Y., Zhu, Y.G., Smith, F.A., Miao, X., Smith, S.E., 2007. The arbuscular mycorrhizal fungus *Glomus mosseae* gives contradictory effects on phosphorus and arsenic acquisition by *Medicago sativa* Linn. *Science of the Total Environment* 379, 226–234.
- Cheng, X.M., Baumgartner, K., 2006. Effects of mycorrhizal roots and extraradical hyphae on  $^{15}\text{N}$  uptake from vineyard cover crop litter and the soil microbial community. *Soil Biology & Biochemistry* 38, 2665–2675.
- Dodd, J.C., Burton, C.C., Burns, R.G., Jeffries, P., 1987. Phosphatase activity associated with the roots and the rhizosphere of plants infected with vesicular arbuscular mycorrhizal fungus. *New Phytologist* 107, 163–172.
- Dowling, N.J.E., Widdel, F., White, D.C., 1986. Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulphate-reducers and other sulphide-forming bacteria. *Journal of General Microbiology* 132, 1815–1825.
- Federle, T.W., 1986. Microbial distribution in soil: new techniques. In: Meguar, F., Gantar, M. (Eds.), *Perspectives in Microbial Biology*. Slovene Society for Microbiology, Ljubljana, Slovenia, pp. 493–498.
- Frostegård, Å., Bååth, E., Tunlid, A., 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied Environmental Microbiology* 59, 3605–3617.
- Genney, D.R., Alexander, I.J., Killham, K., Meharg, A.A., 2004. Degradation of the polycyclic aromatic hydrocarbon (PAH) fluorene is retarded in a Scots pine ectomycorrhizosphere. *New Phytologist* 163, 641–649.
- Gerdemann, J.W., Nicolson, T.H., 1963. Spores of a mycorrhizal *Endogone* species extracted from the soil by wet sieving and decanting. *Transactions of the British Mycological Society* 46, 235–244.
- Gianinazzi, S., Gianinazzi-Pearson, V., Tisserant, B., Lemoine, M.C., 1992. Protein activities as potential markers of functional endomycorrhiza in plants. In: Read, D.J., Lewis, D.H., Fitter, A.H., Alexander, I.J. (Eds.), *Mycorrhizas in Ecosystems*. CAB International Wallingford, Oxon, pp. 333–339.
- Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84, 489–500.
- Grigg, B.C., Assaf, N.A., Turco, R.F., 1997. Removal of atrazine contamination in soil and liquid systems using bioaugmentation. *Pesticide Science* 50, 211–220.
- Haney, R.L., Senseman, S.A., Hons, F.M., 2002. Effect of roundup ultra on microbial activity and biomass from selected soils. *Journal of Environmental Quality* 31, 730–735.
- Harrison, M.J., 1997. The arbuscular mycorrhizal symbiosis: an underground association. *Trends in Plant Science* 2, 54–60.
- Huang, H.L., Zhang, S.Z., Chen, B.D., Wu, N.Y., Shan, X.Q., Christie, P., 2006. Uptake of atrazine and cadmium from soil by maize (*Zea mays* L.) in association with the arbuscular mycorrhizal fungus *Glomus etunicatum*. *Journal of Agricultural and Food Chemistry* 54, 9377–9382.
- Huang, H.L., Zhang, S.Z., Shan, X.Q., Chen, B.D., Zhu, Y.G., Bell, J.N.B., 2007. Effect of arbuscular mycorrhizal fungus (*Glomus caledonium*) on the accumulation and metabolism of atrazine in maize (*Zea mays* L.) and atrazine dissipation in soil. *Environmental Pollution* 146, 452–457.
- Isakeit, T., Lockwood, J.L., 1990. Evaluation of the soil microbiota in producing the deleterious effect of atrazine on ungerminated conidia of *Cochliobolus sativus* in soil. *Soil Biology & Biochemistry* 22, 413–417.
- Joner, E.J., Leyval, C., 1997. Uptake of  $^{109}\text{Cd}$  by roots and hyphae of a *Glomus mosseae*/*Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. *New Phytologist* 135, 353–360.
- Joner, E.J., Leyval, C., 2003. Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza. *Environmental Science & Technology* 37, 2371–2375.
- Joner, E.J., Briones, R., Leyval, C., 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant and Soil* 226, 227–234.
- Joner, E.J., Johansen, A., dela Cruz, M.A.T., Szolar, O.J.H., Loibner, A., Portal, J.M., Leyval, C., 2001. Rhizosphere effects on microbial community structure and dissipation and toxicity of polycyclic aromatic hydrocarbons (PAHs) in spiked soil. *Environmental Science & Technology* 35, 2773–2777.
- Joner, E.J., Leyval, C., Colpaert, J.V., 2006. Ectomycorrhizas impede phytoremediation of polycyclic aromatic hydrocarbons (PAHs) both within and beyond the rhizosphere. *Environmental Pollution* 142, 34–38.
- Kazemi, H.V., Anderson, S.H., Goynes, K.W., Gantzer, C.J., 2008. Atrazine and alachlor transport in claypan soils as influenced by differential antecedent soil water content. *Journal of Environmental Quality* 37, 1599–1607.
- Kothari, S.K., Marschner, H., Romheld, V., 1990. Direct and indirect effects of VA mycorrhizae and rhizosphere microorganisms on mineral nutrient acquisition by maize (*Zea mays* L.) in a calcareous soil. *New Phytologist* 116, 637–645.
- Linderman, R.G., 1988. Mycorrhizal interactions with the rhizosphere microflora – the mycorrhizosphere effect. *Phytopathology* 78, 366–371.
- Liu, Q., Loganathan, P., Hedley, M.J., Grace, L.J., 2008. Effect of mycorrhizal inoculation on rhizosphere properties, phosphorus uptake and growth of pine seedlings treated with and without a phosphate rock fertilizer. *Journal of Plant Nutrition* 31, 137–156.
- López-Gutiérrez, J.C., Toro, M., López-Hernández, D., 2004. Arbuscular mycorrhiza and enzymatic activities in the rhizosphere of *Trachypogon plumosus* Ness. in three acid savanna soils. *Agriculture, Ecosystems & Environment* 103, 405–411.
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*. Academic Press, London, UK.
- Martin-Laurent, F., Piutti, S., Hallet, S., Wagschal, I., Philippot, L., Catroux, G., Soulas, G., 2003. Monitoring of atrazine treatment on soil bacterial, fungal and atrazine-degrading communities by quantitative competitive PCR. *Pest Management Science* 59, 259–268.
- Olsson, P.A., 1999. Mini review: signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology* 29, 303–310.
- Olsson, P.A., Johansen, A., 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. *Mycological Research* 104, 429–434.
- O'Leary, W.M., Wilkinson, S.G., 1988. Gram-positive bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.), *Microbial Lipids*. Academic Press, London, UK, pp. 117–201.
- Perucei, P., Scarponi, L., Monotti, M., 1988. Interference with soil phosphatase activity by maize herbicidal treatment and incorporation of maize residues. *Biology and Fertility of Soils* 6, 286–291.
- Purin, S., Rillig, M.C., 2008. Parasitism of arbuscular mycorrhizal fungi: reviewing the evidence. *FEMS Microbiology Letters* 279, 8–14.
- Ros, M., Goberna, M., Moreno, J.L., Hernandez, T., García, C., Insam, H., Pascual, J.A., 2006. Molecular and physiological bacterial diversity of a semiarid soil contaminated with different levels of formulated atrazine. *Applied Soil Ecology* 34, 93–102.
- Sannino, F., Gianfreda, L., 2001. Pesticide influence on soil enzymatic activities. *Chemosphere* 45, 417–425.
- Seghers, D., Verthe, K., Reheul, D., Bulcke, R., Siciliano, S.D., Verstraete, W., Top, E.M., 2003. Effect of long-term herbicide applications on the bacterial community structure and function in an agricultural soil. *FEMS Microbiology Ecology* 46, 139–146.
- Seibert, K., Fuehr, F., Cheng, H.H., 1991. Experiments on the degradation of atrazine in the maize-rhizosphere soil. *Theory and Practical Use of Soil Applied Herbicide*. European Weed Research Society Symposium, Paris, pp. 137–146.
- Singh, N., Megharaj, M., Kookana, R.S., Naidu, R., Sethunathan, N., 2004. Atrazine and simazine degradation in *Pennisetum* rhizosphere. *Chemosphere* 56, 257–263.
- Smith, S.E., Read, D.J., 1997. *Mycorrhizal Symbiosis*, second ed. Academic Press, London.
- Söderberg, K.H., Olsson, P.A., Bafafith, E., 2002. Structure and activity of the bacterial community in the rhizosphere of different plant species and the effect of arbuscular mycorrhizal colonization. *FEMS Microbiology Ecology* 40, 223–231.
- Stainer, R.Y., Adelberg, E.A., Ingraham, J.L., 1977. *General Microbiology*. Macmillan, London, UK.
- Tabatabai, M.A., Bremner, J.M., 1969. Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology & Biochemistry* 1, 301–307.
- Vázquez, M.M., César, S., Azcón, R., Barea, J.M., 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. *Applied Soil Ecology* 15, 261–272.
- Vivas, A., Marulanda, A., Gómez, M., Barea, J.M., Azcón, R., 2003. Physiological characteristics (SDH and ALP activities) of arbuscular mycorrhizal colonization as affected by *Bacillus thuringiensis* inoculation under two phosphorus levels. *Soil Biology & Biochemistry* 35, 987–996.
- Walker, C., Mize, W., McNabb, H.S., 1982. Populations of endogonaceous fungi at two populations in central Iowa. *Canadian Journal of Botany* 60, 2518–2529.
- White, J.C., Ross, D.W., Gent, M.P.N., Eitzer, B.D., Mattina, M.J.I., 2006. Effect of mycorrhizal fungi on the phytoextraction of weathered *p,p*-DDE by *Cucurbita pepo*. *Journal of Hazardous Materials B* 137, 1750–1757.
- Widenfalk, A., Goedkoop, W., Svensson, J.M., 2004. Effects of the pesticides captan, deltamethrin, isoproturon, and pirimicarb on the microbial community of a freshwater sediment. *Environmental Toxicology and Chemistry* 23, 1920–1927.
- Wilkinson, S.G., 1988. Gram-negative bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.), *Microbial Lipids*. Academic Press, London, UK, pp. 299–488.
- Wu, N.Y., Zhang, S.Z., Huang, H.L., Christie, P., 2008a. Enhanced dissipation of phenanthrene in spiked soil by arbuscular mycorrhizal alfalfa combined with a non-ionic surfactant amendment. *Science of the Total Environment* 394, 230–236.
- Wu, N.Y., Zhang, S.Z., Huang, H.L., Shan, X.Q., Christie, P., Wang, Y.S., 2008b. DDT uptake by arbuscular mycorrhizal alfalfa and depletion in soil as influenced by soil application of a non-ionic surfactant. *Environmental Pollution* 151, 569–575.
- Xu, S.Y., Chen, Y.X., Wu, W.X., Wang, K.X., Lin, Q., Liang, X.Q., 2006. Enhanced dissipation of phenanthrene and pyrene in spiked soils by combined plants cultivation. *Science of the Total Environment* 363, 206–215.