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Responses of butachlor degradation and microbial properties in a riparian soil to the cultivation of three different plants

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Abstract

A pot experiment was conducted to investigate the biodegradation dynamics and related microbial ecophysiological responses to butachlor addition in a riparian soil planted with different plants such as *Phragmites australis*, *Zizania aquatica*, and *Acorus calamus*. The results showed that there were significant differences in microbial degradation dynamics of butachlor in the rhizosphere soils among the three riparian plants. *A. calamus* displays a significantly higher degradation efficiency of butachlor in the rhizosphere soils, as compared with *Z. aquatica* and *P. australis*. Half-life time of butachlor degradation in the rhizospheric soils of *P. australis*, *Z. aquatica*, and *A. calamus* were 7.5, 9.8 and 5.4 days, respectively. Residual butachlor concentration in *A. calamus* rhizosphere soil was 35.2% and 21.7% lower than that in *Z. aquatica* and *P. australis* rhizosphere soils, respectively, indicating that *A. calamus* showed a greater improvement effect on biodegradation of butachlor in rhizosphere soils than the other two riparian plant. In general, microbial biomass and biochemical activities in rhizosphere soils were depressed by butachlor addition, despite the riparian plant types. However, rhizospheric soil microbial ecophysiological responses to butachlor addition significantly ($P < 0.05$) differed between riparian plant species. Compared to *Z. aquatica* and *P. australis*, *A. calamus* showed significantly larger microbial number, higher enzyme activities and soil respiration rates in the rhizosphere soils. The results indicated that *A. calamus* have a better alleviative effect on inhibition of microbial growth due to butachlor addition and can be used as a suitable riparian plant for detoxifying and remediating butachlor contamination from agricultural nonpoint pollution.

Key words: riparian wetland; butachlor; rhizospheric degradation; soil microflora; enzymatic activities; basal respiration

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Introduction

Butachlor (2-chloro-2,6-diethyl-N-(butoxymethyl)-acetanilide) is a pre-emergence chloroacetanilide herbicide widely used to control undesired weeds of rice fields in Asia (Kumar et al., 2009). As one of the three herbicides heavily used in China, the yearly application of butachlor reaches 8000 tons (Yu et al., 2003). As a persistent pollutant in agricultural soil (Debnath et al., 2002; Xu et al., 2005), butachlor can influence soil microbial populations and enzyme activities. Fang (2009) concluded that butachlor sometimes adversely affected the growth and activities of beneficial microorganisms in soils. Many reports showed stimulatory and inhibitory effects on nitrogen fixation by butachlor application in rice rhizosphere (Suseel, 2001; Min et al., 2002; Chen et al., 2009). Moreover, some researchers have demonstrated that butachlor is highly toxic to some aquatic organisms such as green algae (Junghans et al., 2003) and fish (Farah et al., 2004), which brings a large water environment risk. Especially, after heavy application to paddy field, butachlor may be transferred into surrounding water easily through drainage, irrigation, farmland runoff and so on, which, consequently, causes contamination risk to water and aquatic ecosystem health.

Microbial transformation/mineralization is the most important route for pesticide degradation in soils (Yu et al., 2003; Luo et al., 2008; Chai et al., 2010). The size and the activity of the soil microbial biomass affect the rate of pesticide degradation (Beulke et al., 2005; Triky-Dotan et al., 2010). Plants sustain large microbial populations in the rhizosphere by secreting substances, such as carbohydrates and amino acids (Bowen and Rovira, 1991; Turpault et al., 2007), which provide important sources of nutrients for microorganisms in the rhizosphere. Larger microbial populations can, therefore, exist in rhizosphere soil than in bulk soil, and these larger populations have been shown to increase the degradation of organic chemicals including pesticides (Ye et al., 2002; Joner et al., 2003; Chen et al., 2003).

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As an interface and transition zone between terrestrial and aquatic ecosystems, riparian wetlands and associated microbial, plant and fauna processes have multiple ecological and environmental functions such as riverside protection, water purification and non-point pollution control (Cornell et al., 2008; Woodward et al., 2009; Fischer et al., 2010). Especially, in agricultural watershed, riparian zone can effectively intercept, absorb and degrade various agricultural pollutants from surrounding agricultural runoff and irrigation by riparian plants and soils, thus, effectively control agricultural non-point source pollution (Zhao et al., 2009; Gorsevski et al., 2008; Schoonover et al., 2005). At present, the ecological and environmental functions of riparian zone has been well reported, and the most of studies focused on the nitrification and the denitrification in agricultural non-point source pollution (Mankin et al., 2007; Eva et al., 2009). However, little research is reported on the ecophysiological responses of riparian rhizospheric microorganisms to agricultural organic chemicals and their degradation dynamics.

Riparian plants can effectively trap non-point source pollution of herbicides by their powerful roots and their structure. It is likely that the rhizosphere has the potential to accelerate biodegradation of organic pesticides. The main objective of this current research was to investigate biological degradation dynamics of butachlor and microbial ecophysiological characteristics in a riparian soil planted with different riparian plants in Chongming Island, such as *Phragmites australis, Acorus calamus* and *Zizania aquatica*. These findings will provide theoretical basis for ecological control of butachlor in agricultural watershed.

1 Materials and methods

1.1 Collection and preparation of tested riparian soil and plants

A riparian soil was collected from a typical riparian zone away from farmland in Chongming Island, Shanghai, China. The riparian soil is generally stratified sandy clay loam over sandy mixed calcareous alluvium. The selected physical and chemical parameters of the tested soil are shown in Table 1. After removing the roots and stones from the fresh soils, the riparian soil was air-dried and then sieved through 5 and 2 mm mesh sieves for incubation experiment and chemical analysis, respectively.

A certain dose of herbicide butachlor was dissolved in methanol, and reached the concentration of 1000 μg/mL. The butachlor solution was then spiked to the tested riparian soil, and stirred to well mixing. After removal of the methanol by volatilization at room temperature, the parent soil with butachlor at the initial concentrations of 6 mg/kg dry soil was well prepared.

Table 1 Selected physical and chemical parameters of the tested riparian soil

Sand	Silt	Clay	pH (H_2O, 1:1)
73.5%	18.2%	8.3%	7.97
CEC (cmol/kg)	OM (g/kg)	TN (g/kg)	TP (g/kg)
4.41	6.53	1.09	0.74

Three typical riparian plant species, *P. australis, A. calamus* and *Z. aquatica* were collected at seedling stage in the riparian zone similar to the riparian soil collection. The roots of riparian plants were gently washed by deionized water before transplanting.

1.2 Pot incubation experiment

The incubation experiment was carried out with rhizobag system using 3.5 L plastic pots. Five kilograms prepared parent soil was filled in each plastic pot, of which 1 kg soil was placed into 20 μm nylon mesh cloths. In this system, the surfaces of soils inside and outside the rhizobag were level. The investigated three riparian plants were transplanted into rhizobags, respectively. The treatment with no butachlor addition, following the same procedures served as a control soil. All the treatments and controlled soil were spiked with carbamide and monopotassium phosphate as base fertilizers. The pot experiments were set up with four replicate per treatment. The water content in the pots was adjusted by distilled water, which involved flooding (approximately 3 cm above the soil surface) for two weeks after riparian plant transplanted, followed by alternate 10 days periods of drainage, and then re-flooding. When drying, pots were maintained at field capacity to ensure that plants did not suffer any water stress. Thus, the pots were kept under intermittently flooded conditions until experiment finished. Every pot was hided by silver paper to decrease the effects of photodegradation of butachlor. The pot experiment ran for 35 days.

1.3 Soil sampling and preparation

Riparian soil sampling was conducted at intervals of 1, 7, 14, 21, 28, 35 and 45 days. About 50 grams soil from inside the rhizobag (rhizosphere soil) or outside the rhizobag (bulk soil) at the similar depth were collected, mixed and wet-sieved through a 2 mm sieve, and the moisture contents were determined. Soil samples were divided into two parts. One fraction was stored at 4°C for analyzing microbial parameters, and the other fraction of soil samples was freeze-dried for determination of the residual butachlor.

1.4 Extraction and determination of soil butachlor

Butachlor was extracted with acetone and detected by gas chromatography. Briefly, 30 mL of deionized water and 50 mL of acetone were added to the soil sample. The mixture was shaken for 1 hr on a rotary shaker and then filtered. The filter cake was washed twice with a mixture of acetone-water (2:1, *V*/*V*). All filtrates were combined and evaporated on a rotary evaporator to remove acetone. One gram of celite 545 and 5 mL of NH_4Cl-H_3PO_4 were added into the evaporated solution to precipitate impurity, and then filtered to remove it. The filtrate was extracted three times with 50, 30, and 30 mL of distilled petroleum (30–60°C) respectively. After dehydration with anhydrous Na_2SO_4, the extracts were collected in a round-bottom flask, concentrated on a rotary evaporator to 10 mL, and

finally analyzed by ECD-GC. ECD gas chromatography (Shimadzu GC-19A, Japan) fitted with a DB-17 glass capillary column (25 m × 0.25 mm i.d.) were employed. Operating temperatures were as follows: injection port, 230°C; column, 200°C; detector, 230°C. Nitrogen of 1.2 kg/cm^2 was used as carrier gas at a spilt at ratio of 1:20. The limit of detection (LOD) was 0.01 mg/kg. Average recoveries from soils fortified at levels of 0.01–1.0 mg/kg ranged between 82%–93% with relative standard deviations (RSDs) of 4%–6%.

1.5 Analysis of soil microbial parameters

The enumeration of the soil microflora was done by the dilution plate method (Xu and Zheng, 1986). The total colony forming units (cfu) of bacteria, fungi, actinomycetes were recorded on nutrient agar, Ken Knight and Munaier's agar, and Martin's rose bengal agar media, respectively. The plates were incubated at (28 ± 2)°C and microbial population was calculated and expressed as $\times 10^n$ cfu/g air dried soil, where 10^n was dilution factor.

Activities of soil dehydrogenase (DH-ase) and catalase were determined using the methodology described by Guan (1986). The activity of DH-ase in soils was determined by the method of triphenyl tetrazolium chloride (TTC), when TTC were deoxidized, the fat-soluble red product-triphenylformazan (TPF) could be formed. The activity of DH-ase was expressed as mg TPF/(g dry soil·24 hr). The method to determine catalase activity was measured the disappearance rate of H_2O_2. An air-dried soil sample (2 g) was mixed with 10 mL of distilled water and 5 mL of 0.3% H_2O_2. After the mixture had been shaken for 20 min, 5 mL of 1.5 mol/L H_2SO_4 was introduced to terminate the reaction. The mixture was then filtered, and the filtrate was titrated with 0.02 mol/L $KMnO_4$. Results were expressed as μmol $KMnO_4$/(g dry soil·min).

Soil basal respiration rate was determined by measuring CO_2 evolution in the aerobic condition (Ohlinger, 1995). The soil sample was humidified to 50% of its water holding capacity and incubated at 30°C in the dark for 24 hr. The CO_2 evolved was trapped in NaOH solution and titrated with HCl. Soil basal respiration rates were expressed as mg CO_2/(g dry soil·hr).

1.6 Data statistical analysis

All analyses were performed in four replicates. Data were analyzed statistically by analysis of variance (ANOVA). Duncan's New Multiple Range Test (DMRT) was employed to assess differences between the treatment means. The effects of plant types on butachlor degradation and rhizospheric soil microbial process were declared as significant at 5% probability levels. Standard errors were calculated for mean values of all determinations. Correlation analysis was performed using Pearson's coefficients at $P < 0.05$. All statistical analyses were performed with SPSS 12.0 software.

2 Results and discussion

2.1 Degradation of butachlor in rhizosphere soils planted with different riparian plants

The degradation dynamics of butachlor in the rhizosphere and bulk soils planted with the three different riparian plants are shown in Fig. 1. The degradation dynamics of butachlor in soil was described by a first-order kinetic model with the determination coefficient (R^2) > 0.98:

$$C_t = C_0 \times e^{-kt} \quad (1)$$

where, C_t (mg/kg) is the mean concentration of butachlor as a function of time (t, day), C_0 (mg/kg) is the initial butachlor concentration, k (day^{-1}) is the rate constant. The degradation kinetic of butachlor in both rhizosphere soils and bulk soils are shown in the Table 2.

There were significant ($P < 0.05$) differences in the degradation dynamics between the rhizosphere and bulk soil (Fig. 1). The degradation of butachlor in rhizosphere soils was enhanced with bigger rate constant and shorter half-life than that in the corresponding bulk soils, despite the riparian plant species (Table 2). For example, at the end of the incubation experiment, the residual concentrations of butachlor in *P. australis*, *Z. aquatica*, and *A. calamus* rhizosphere soils decreased by 36.5%, 28.5%, and 47.2%, respectively, compared to those in the corresponding bulk soils, suggesting that the rhizospheric effects on enhancing biodegradation of the herbicide butachlor were marked.

Figure 1 also shows that the differences in butachlor degradation rates between the three plants rhizosphere soils were significant ($P < 0.05$). *A. calamus* showed the highest butachlor biodegradation efficiency in the rhizosphere soil, followed by *P. australis* and *Z. aquatica*, respectively. The half life in *A. calamus* rhizosphere soil was significantly shorten compared to the other two riparian plants (Table 2). Consequently, the residual contents in the three tested riparian rhizosphere soils was 1.965 mg/kg for *P. australis*, 2.245 mg/kg for *Z. aquatica* and

Table 2 Degradation kinetics of butachlor in the rhizosphere and bulk soils

Riparian plants	Soil	Kinetic formular of degradation of butachlor	R^2	Half-life (day)
Z. aquatica	Rhizosphere	$C = 6.1749e^{-0.0537t}$	0.9972	7.5 b
	Bulk	$C = 6.3309e^{-0.0397t}$	0.9912	12.8 a
P. australis	Rhizosphere	$C = 6.1317e^{-0.0600t}$	0.9944	9.8 c
	Bulk	$C = 6.3354e^{-0.0498t}$	0.9887	13.6 a
A. calamus	Rhizosphere	$C = 6.2972e^{-0.0623t}$	0.9963	5.4 d
	Bulk	$C = 6.2953e^{-0.0291t}$	0.9872	12.9 a

Data followed by the different letter in a column are significantly different at P <0.05 by Duncan's multiple range test.

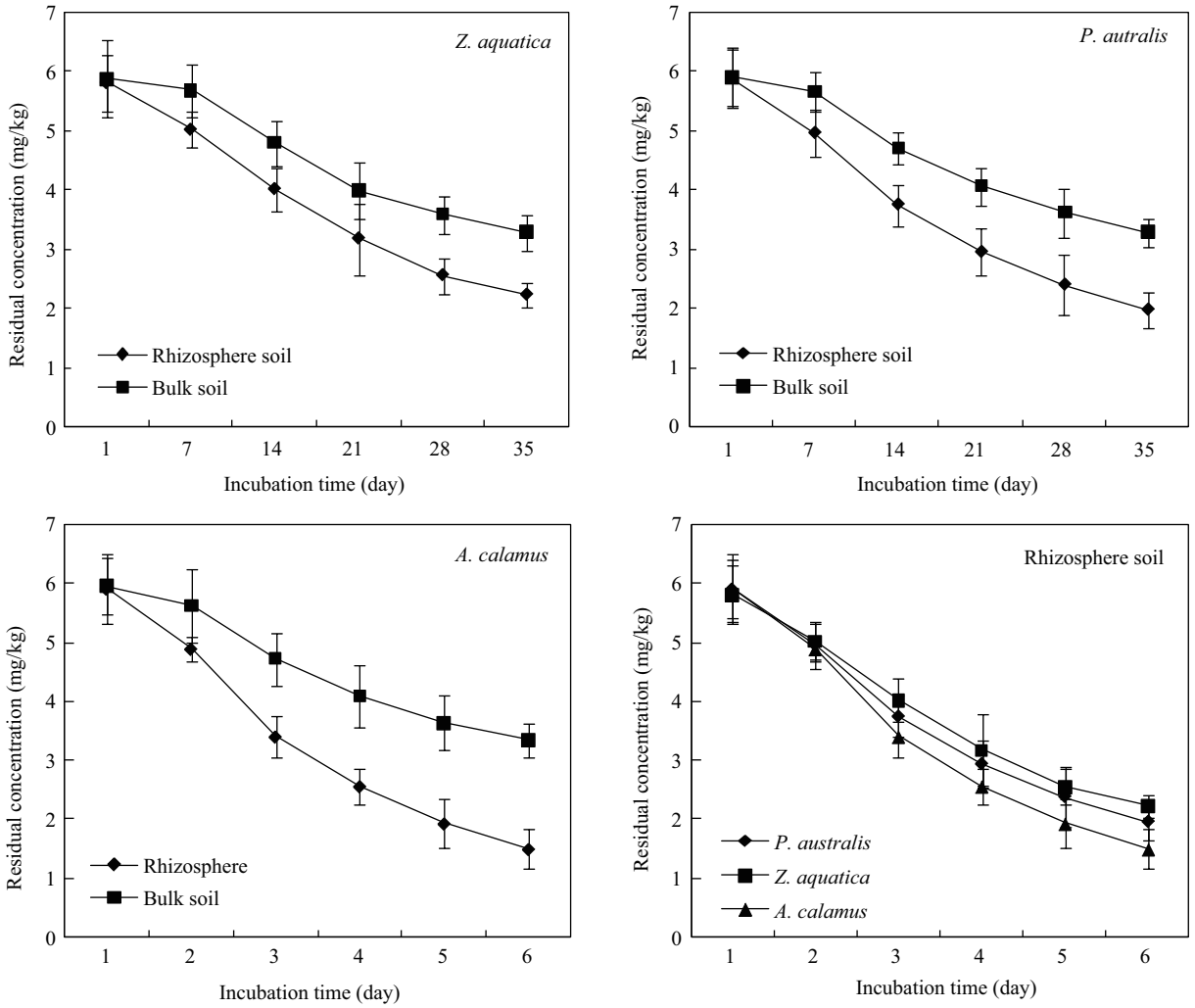

Fig. 1 Degradation of butachlor in the rhizosphere and bulk soils planted with different riparian plants. Error bars represent the standard deviation of three sampled pots.

1.573 mg/kg for *A. calamus*. The results indicate that plant species exert a significant improvement effect on rhizospheric degradation of butachlor in the riparian soil.

Microbial degradation is considered to be the primary mechanism for removing pesticides from soil (Pal et al., 2006). Dwivedi et al. (2010) reported that inoculation with butachlor-catabolizing bacterial strain could significantly improve the degradation of butachlor in agricultural soil. Vegetation significantly enhances the degradation of the herbicide butachlor in the soil. This is most likely the result of C exudation from plant roots into the rhizosphere, which supports an increased microbial population (Yu et al., 2003). Our present work showed a greater biodegradation efficiency of butachlor in *A. calamus* rhizosphere soil, as compared to the other two investigated riparian plants, which may be mainly contributed to relatively high microbial metabolic activities.

2.2 Riparian soil microflora dynamics as affected by butachlor addition

Rhizospheric microbial population dynamics in the riparian soils are showed in Table 3. As compared with the controls (without butachlor), butachlor addition decreased rhizosphere microbial number throughout the incubation period, despite riparian plant species. In the initial incubation period, the inhibition effects were more marked than those in the late incubation, in which, rhizosphere microbial number showed significant recovery (Table 3). This can be attributed to the carbon substrate supply by butachlor addition in the herbicide degradation process (Sunita et al., 2008; Fang et al., 2009). Averaged by the four sampling dates, inhibition rates of bacteria, fungi and actinomycete in rhizospheric soils were 38.9%, 24.4%, and 18.3%, respectively, indicating that bacteria community was more sensitive to butachlor addition than fungi and actinomycete, and can served as early indicators of butachlor contamination of riparian soils.

There existed significant ($P < 0.05$) differences in the inhibitory effects of butachlor addition on rhizosphere soil microbial populations between the investigated three riparian plants (Table 3). For example, after 21 days of incubation, inhibition rates of soil bacteria population in the rhizosphere of *P. australis*, *Z. aquatica*, and *A. calamus* were on average 35.1%, 30.1% and 17.2%, respectively, As a result, at the end of the incubation, rhizosphere of *A. calamus* showed the highest microbial populations, followed by *P. australis* and *Z. aquatica*, which indicates that the alleviative effectiveness of *A. calamus* to stress

Table 3 Dynamics of the microbial number in the rhizosphere and bulk soils with different riparian plants

Riparian plant	Soil treatment	Soil	Bacteria ($\times 10^8$ cfu/g)				Fungi ($\times 10^4$ cfu/g)			
			7 days	14 days	21 days	35 days	7 days	14 days	21 days	35 days
P. australis	With butachlor	Rhizosphere	0.58 c	0.60 c	0.68 d	0.74 e	5.24 c	4.78 c	5.33 c	6.12 c
		Bulk	0.23 e	0.14 e	0.16 f	0.17 g	1.89 f	1.23 e	0.92 f	1.01 f
	Control	Rhizosphere	1.02 a	1.23 a	1.34 a	1.56 a	6.89 a	7.12 ab	7.46 a	8.17 a
		Bulk	0.34 d	0.42 d	0.28 e	0.24 f	2.12 e	2.45 d	2.13 e	1.85 e
Z. aquatica	With butachlor	Rhizosphere	0.52 b	0.62 c	0.67 d	0.71 e	4.23 d	3.23 d	3.56 d	4.12 d
		Bulk	0.26 e	0.17 e	0.14 f	0.18 g	2.10 e	1.44 e	0.89 f	1.10 f
	Control	Rhizosphere	0.97 a	1.02 a	1.07 b	1.23 c	6.12 b	6.87 b	7.23 a	7.78 ab
		Bulk	0.30 de	0.39 d	0.32 e	0.28 f	1.78 f	2.34 d	2.33 e	1.78 e
A. calamus	With butachlor	Rhizosphere	0.72 b	0.78 b	0.82 c	0.93 d	5.08 cd	4.97 c	6.56 b	7.23 b
		Bulk	0.27 e	0.17 e	0.16 f	0.14 g	2.05 ef	1.53 e	1.18 f	1.12 ef
	Control	Rhizosphere	0.95 a	1.12 a	1.24 a	1.28 b	6.33 ab	7.22 a	7.12 ab	7.98 a
		Bulk	0.32 d	0.41 d	0.35 e	0.27 f	2.42 e	2.78 d	2.55 e	1.92 e
Riparian plants	Soil treatment	Soil	Actinomycete ($\times 10^3$ cfu/g)							
			7 days	14 days	21 days	35 days				
P. australis	With butachlor	Rhizosphere	2.98 c	2.67 b	3.01 b	3.32 c				
		Bulk	1.23 f	0.87 e	0.74 e	0.48 f				
	Control	Rhizosphere	3.78 a	4.23 a	4.98 a	4.34 a				
		Bulk	1.44 f	1.86 d	1.73 d	1.35 e				
Z. aquatica	With butachlor	Rhizosphere	2.35 de	1.89 c	2.01 c	2.32 d				
		Bulk	1.25 f	0.82 e	0.76 e	0.53 f				
	Control	Rhizosphere	3.12 bc	4.12 a	4.44 a	3.98 bc				
		Bulk	1.37 f	1.58 d	1.84 d	1.46 e				
A. calamus	With butachlor	Rhizosphere	2.87 e	2.98 b	3.66 b	3.98 bc				
		Bulk	1.18 f	1.02 de	0.77 e	0.43 f				
	Control	Rhizosphere	3.56 ab	3.98 a	4.78 a	4.23 ab				
		Bulk	1.52 f	1.67 d	1.21 d	1.51 e				

Data followed by the different letter in a column are significantly different at $P < 0.05$ by Duncan's multiple range test.

of butachlor addition was greater than that of *Z. aquatica* and *P. australis*. This may be due to the different organic acids excreted from the roots of different riparian plants (Muratova et al., 2003).

2.3 Enzymatic activities in riparian rhizosphere soils

Enzymes are responsible for most of the transformations of natural and anthropogenic organic compounds which take place in soils (Wang et al., 2009). Key enzymatic activities in the rhizosphere soils planted with different riparian plants are shown in Table 4. At the initial incubation period, the dehydrogenase activity in rhizosphere soils treated with butachlor showed a significant decreased compared with the control (without butachlor), and exhibited a certain inhibition effect, despite the riparian plant species. However, butachlor addition displayed the obvious enhancing effect on dehydrogenase activity after 21 days of pot experiment, until the end of incubation. Catalase activities in rhizosphere riparian soils treated with butachlor were significantly lower than that without butachlor (control) through all the incubation period. Averaged with the four sampling, the inhibition percentages of dehydrogenase and catalase activity were 41.2% and 32.4%, respectively.

By comparison, it was found that there were significant differences in enzymatic activities in rhizosphere soils with butachlor between three riparian plants (Table 4). Throughout the incubation periods, the rhizosphere soil of *A. calamus* showed higher enzymatic activities, followed by *P. australis* and *Z. aquatica*. Especially, at day 21 of the incubation, the differences in the soil enzymatic activities between the three rhizosphere soils was more significant (*P*

Table 4 Dynamics of key enzyme activities in the rhizosphere and bulk soils with different riparian plants

Riparian plants	Soil treatment	Soil	Dehydrogenase (mg TPF/(g·24 hr))				Catalase (μmol $KMnO_4$/(g·min))			
			7 days	14 days	21 days	35 days	7 days	14 days	21 days	35 days
P. australis	With butachlor	Rhizosphere	4.12 c	3.56 c	4.88 b	6.12 bc	5.72 c	4.84 c	4.78 d	4.12 d
		Bulk	1.76 f	1.21 f	0.56 e	0.62 g	2.45 f	1.89 f	1.12 g	1.23 f
	Control	Rhizosphere	5.17 ab	5.02 b	4.78 b	5.33 cd	8.85 b	9.78 a	8.45 a	9.32 a
		Bulk	2.10 e	2.34 e	1.78 d	1.63 f	3.01 e	3.45 e	3.15 e	2.78 e
Z. aquatica	With butachlor	Rhizosphere	3.05 d	2.37 d	3.22 c	4.75 d	4.72 d	4.12 d	3.68 e	2.85 e
		Bulk	1.82 f	1.33 f	0.62 e	0.54 g	2.48 f	2.10 f	1.45 g	0.89 f
	Control	Rhizosphere	3.63 cd	3.12 c	3.45 c	3.78 e	9.33 ab	9.82 a	8.10 ab	6.68 b
		Bulk	2.33 e	1.89 ef	1.63 d	1.67 f	3.78 e	4.12 d	3.03 f	2.67 e
A. calamus	With butachlor	Rhizosphere	4.87 bc	4.79 b	6.65 a	7.55 a	8.84 b	6.73 b	5.77 c	5.49 c
		Bulk	1.92 ef	1.18 f	0.70 e	0.51 g	2.87 ef	1.56 f	1.43 g	1.29 f
	Control	Rhizosphere	5.87 a	6.21 a	6.02 a	6.22 b	9.88 a	9.73 a	7.85 bc	6.39 b
		Bulk	2.12 e	2.45 e	2.12 d	1.74 f	3.34 e	4.13 d	3.14 ef	2.08 e

Data followed by the different letter in a column are significantly different at $P < 0.05$ by Duncan's multiple range test.

< 0.05). The results suggested that *A. calamus* can effectively alleviate the inhibitory effect of butachlor addition on microbial activity.

In comparison with bulk soil, the biochemical activity of microorganisms is enhanced as a result of exudation of compounds by the root (Sørensen, 1997; Raaijmakers et al., 2009). However, plant root exudates varied according to the kind of plant, and microorganisms also had a choice to use root exudates (Garbeva et al., 2008). In this study, the differences in both microbial number and enzymatic activity between three rhizosphere soils were significant (Tables 3 and 4), which is probably attributed to different physiological metabolic activities and root exudation patterns between the three riparian plants (Jude and Laurie, 2010).

2.4 Microbial basal respiration in ripatian rhizosphere soils

Soil basal respiration (SBR) is an important indicator of soil microbial activity. Microbial basal respiration rates in the rhizosphere soils planted with different riparian plants are shown in Fig. 2. Soil respiration rate was enhanced due to butachlor addition at day 7 of incubation, as compared with the control (without butachlor). This could be explained that short-term microbial physiological stress effect happened due to large dose of butachlor addition, which led to a sudden increase in microbial basal respiration process (Nwachukwu and Pulford, 2011). With the incubation duration, however, soil respiration rates were decreased due to butachlor addition (Fig. 2), relative to the control, despite the riparian plant species, indicating that the butachlor contamination posed an inhibitory effect on soil microbial metabolic activities.

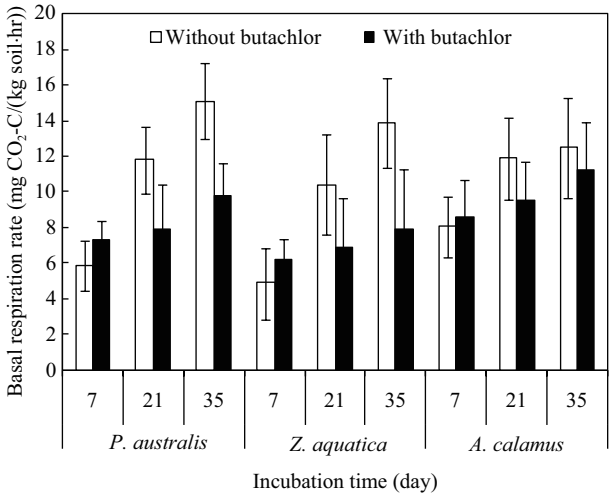

Fig. 2 Dynamics of basal respiration rate in the rhizosphere soils with different riparian plants. Error bars represent the standard deviation of three sampled pots.

By comparison, it was found that there was significant difference in basal respiration rate in the rhizosphere soils with butachlor between three riparian plants (Fig. 2). Especially, at day 21 of the incubation, the differences in the soil basal respiration rates between the three rhizosphere soils was more significant ($P < 0.05$). For rhizospheric soils without butachlor addition, the riparian plant *P. australis* showed the highest soil basal respiration rate among the three riparian plants. For rhizospheric soils with butachlor addition, however, the rhizosphere soil of *A. calamus* showed a higher soil basal respiration rate, as compared to the other two riparian plants. It indicates that microbial basal respiration in the *P. australis* rhizosphere soil is more sensitive to butachlor addition than that in the *A. calamus* and *Z. aquatica* rhizosphere soil.

2.5 Relationships between microbial number and activity and butachlor degradation rate

To determine the relationship between microbial number and biochemical indicators and butachlor degradation rates, correlations between these parameters were determined using Pearson's correlation. Data from rhizosphere soils with the three riparian plants during the entire incubation period were used, and the results are presented in Table 5. There were significant ($P < 0.05$) positive correlations between microbial parameters and the degradation rate constant (k) of butachlor in the riparian soils. Significant negative relationships were detected between microbial parameters and the half-life (DT_{50}) of the butachlor in the riparian soils. k was more closely related to the number of bacteria, DH-ase activity, and microbial basal respiration rate with the coefficients (r) of 0.736, 0.834, and 0.789, respectively. The results showed that these microbial parameters, in particular DH-ase activity and microbial basal respiration rate may be used as good indicators of butachlor degradation potential in the riparian soils.

3 Conclusions

On the basis of the results from the present pot experiment and data analysis, it is concluded that rhizosphere soil of *A. calamus* had markedly higher degradation rates and shorter half-life time as compare to *Z. aquatica* and *P. australis*. Consequently, the lowest butachlor residual concentration was detected in the rhizopshere soil planted with *A. calamus*, which can serve as a potential riparian plant for ecologically controlling and remediating non-point source pollution such as herbicides in agricultural watershed. In general, a relatively-high dose of butachlor significantly inhibited rhizospheric soil microbial growth, enzymatic activities and soil basal respiration rate, especially at initial

Table 5 Correlations of butachlor degradation to microbial parameters in ripatian rhizosphere soils

Butachlor degradation	Bacteria	Fungi	Actinomycete	Dehydrogenase activity	Catalase activity	Basal respiration rate
Half-life (DT_{50})	–0.612**	–0.587*	–0.523*	–0.685**	–0.643**	–0.605**
Degradation rate constant (k)	0.736***	0.514*	0.482*	0.834***	0.702**	0.789***

Statistical significance at: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$.

stages of incubation period. Throughout all the experiment period, the riparian plant *A. calamus* showed significantly larger microbial number, higher enzymatic activities and basal respiration rate in the rhizospheric soils with butachlor, as compared with *Z. aquatica* and *P. australis*. It demonstrated that *A. calamus* displayed a better alleviative effect on inhibition of microbial biochemical activities by butachlor addition. Correlations analysis indicates that DH-ase activity and microbial basal respiration rate may be good indicators of butachlor degradation potential in the riparian soils. Further research is needed to explore the specific mechanisms on riparian vegetation type effects on microbial community structure and its relation to biodegradation process of the herbicide butachlor.

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