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Seasonal Effects on Soil Microbial and Biochemical Characteristics of an Invasive Weed, *Mikania micrantha* in South China

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***Mikania micrantha*, a perennial fast-growing climber and creeper, has widely invaded secondary forests and natural ecosystems in southern China. The understanding of seasonal effects on soil microbial community would help to clarify the invasive mechanism. The present study examined the relationships between soil characteristics, microbial community structure (as indexed by phospholipid fatty acid (PLFA) profiles) and function (as indexed by enzymatic activities) where *M. micrantha* was invading a native forest community at Shawan-Hill in Shenzhen, South China. Samples were collected four times within one year to study seasonal trends. The results indicated that soils undergoing *M. micrantha* invasion had significantly different physico-chemical properties, PLFAs and enzymatic activities than soils not yet invaded with *M. micrantha*. Significant fluctuations in soil microbial community composition and enzymatic activity occurred between seasons. Four enzymes, bacteria, and the ratios of mono/sat and 18:1É9t/ 18:1É9c exhibited the similar trends of higher value in warm months, whereas the abundance of fungi was higher in cold months. Differences in the structural variables were significantly correlated to differences in the functional variables, as determined by Pearson's correlation analysis. However, *Mikania* invasion was found to have more pronounced effects than season on soil physico-chemical characteristics and soil microorganisms. *Mikania* appears to increase N and P availability, alter microbial community structure and function, which might be the key factors for successful invasion.**

Keywords: Physico-chemical properties; Soil enzyme; Phospholipid fatty acid; Seasonality; *Mikania micrantha*

Invasions of exotic species are among the most pervasive and significant threats to nature ecosystems worldwide and have been of interest for several decades. Recent studies on exotic plants have proved a wide variety of plant-soil interactions that might lead to enhanced plant invasiveness in the new range. It has been demonstrated that exotic plants are likely to be able to create soil conditions that are more suitable for exotics than native plants by actively changing the composition and functioning of the soil microbial community (Callaway *et al*. 2004; Klironomos 2002; Marler *et al*. 1999; Richardson *et al*. 2000). However, these interactions between invasive exotic plants and soil microbes have been overlooked as a factor explaining the successful invasion process(Inderjit and van der Putten 2010; Reinhart and Callaway 2006).

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Mikania micrantha H.B.K (hereafter referred to as *Mikania*), commonly known as mile-a-minute weed, is a scrambling perennial vine native to Central and South America and is considered one of the 10 worst invasive weeds in the world (Holm *et al*. 1977). *Mikania* spread to the coastline of Guangdong, China from Hong Kong in the late 1980s (Zhang *et al*. 2004). It is widely distributed in southern China and causes serious damage to secondary forests and natural ecosystems as well as direct economic losses in the farming, forestry, and fruit industries (Feng *et al*. 2002). Most of the studies over the past 2 decades have focused on the invasion-related characteristics (Huang *et al*. 2000; Yang *et al*. 2005), allelopathy (Chen *et al*. 2009a; Chen *et al*. 2009b; Zhao and Peng 2009), effects on plant species richness(Kaur *et al*. 2012), and control methods and control effectiveness (Hou *et al*. 2011; Yu *et al*. 2009; Zhang *et al*. 2006), but neglected *Mikania*'s effects on soil microbes. Until recently, only a few soil microorganism-related papers were published that studied the changes in soil microbial biomass, respiration, community-level physiological profiles (CLPP), microbial community structure (PLFAs) and enzymatic activity (Li *et al*. 2007; Li *et al*. 2006) and the effects of root extracts of *M. micrantha* (Ni *et al*. 2006) on the soil microbial community. However, all of those studies were based on a single-day sampling period, and none investigated the seasonal effects on soil microbial dynamics to *Mikania* invasion. Several studies have indicated that the seasonal effects of invasive species on soil biochemical properties and soil microorganisms would be considerable (Bastida *et al*. 2008; Herr *et al*. 2007; Sugihara *et al*. 2010; Waldrop and Firestone 2006). It is also possible that the impacts of *Mikania* invasion on soil microorganisms vary throughout the year because of climatic conditions and the phenological characteristics of *Mikania*. An improved understanding of the seasonal effects of *Mikania* invasion on soil microorganisms could help to find the key factors in enhancing the invasiveness, which would benefit control and management efforts.

The present study investigates the physico-chemical properties, microbial structure and enzymatic activities in the rhizosphere soil of invasive exotic *Mikania* at four different seasons. The study also evaluates the relationships between the soil physico-chemical and microbial parameters, aiming to have a better understanding of the nutrient cycling and to find the key factors in enhancing the successful invasion.

MATERIALS AND METHODS

Study sites

The study site, approximately 13 ha in size, was located east of Shawan Hill (E 114°08', N 22°35') in the city of Shenzhen, Guangdong Province, China, covering a total area of approximately 1,000 ha. The area has a lower-subtropical oceanic monsoon climate and an annual mean temperature of 22.4°C with a maximum of 36.6°C in July or August and a minimum of 1.44°C in January. The mean annual precipitation and daily solar irradiance are 1926.4 mm and 2,209 h, respectively, with a clear alternation between arid and humid seasons.

The vegetation type of the study site was evergreen broadleaved forest dominated by the trees *Cratoxylon ligustrinum* (Spach.) Bl., *Rhus succedanea* L. and *Microcos paniculata* L., the native shrubs *Uvaria macrophylla* Roxb. and *Melastoma candidum* D. Don and some native lianas, such as *Tetracera asiatica* (Lour.) Hogl. *Mikania* was the only exotic species in the study site. The trees were approximately 2-4 m high with diameters at a breast height of approximately 20-45 cm. The forest was growing vigorously with a high proportion of shrubs and grass and few trees. The site had experienced invasion by *Mikania* since 2002, and approximately 45% of the forest was covered with *Mikania*. The site was thus defined as the *Mikania*-invaded area (EXOT) and the adjacent site which had no *Mikania* growing was defined as the native community area (NATV) in our study.

Soil sampling

Five sample plots were established in the focal areas of 10,000 m^2 (100 m×100 m) as X-transect from the centre to the 4r corners (the 4 corners and the centre, 10 m×10 m each). Each plot was then divided into 5 subplots (circles of approximately 1 m^2). In EXOT, all *Mikania* plants (0-10cm) were excavated, and the rhizosphere soil was collected. The 5 subplots for each plot were combined into a mixed soil sample. And in NATV with no *Mikania* growing, soil samples were taken to a depth of the

surface 10 cm of native community understory soil after any litter and organic matter were removed.

Soil samples were collected from these sites in four seasons, i.e. spring (April 2008), summer (July 2008), fall (October 2008) and winter (January 2009). All soil samples were collected at 9:00 A.M. on a sunny day. In each plot, a total of approximately 1000 g of soil were taken with a core (diameter 7.5cm) from the 5 sub-plots and transferred in sealed plastic bags to laboratory under cooled conditions as soon as possible. In the laboratory, the soil samples were divided into 3 parts after being ground and sifted through a 2 mm sieve: one part was air-dried for physico-chemical analysis, the second part was stored at 4°C for enzymatic analysis, and the third was freeze-dried at -80°C for PLFA analysis.

Soil physico-chemical characteristics

The soil moisture content was measured after drying at 60°C for at least 48 h and given on a gravimetric basis. Soil pH was determined with a glass electrode in a soil-water suspension (soil: distilled water ratio 1:1).

The organic matter content was assayed using the $K_2Cr_2O_7$-H_2SO_4 oxidation method. Briefly, 0.2 g of air-dried soil was mixed with 10 ml of 0.4 M $K_2Cr_2O_7$-H_2SO_4 and then heated to the boiling point in a 170-180°C oil bath pot for 5 min. After cooling, the organic carbon was titrated with standard $FeSO_4$ using phenanthroline as an indicator. The organic carbon was converted into organic matter with the correction factors of 1.10 and 1.714, accounting for the facts that only 90% of the organic carbon could be oxidized and that an average of 58% of the organic matter was carbon, respectively.

The total N was determined using the Kjeldahl method. Briefly, 1.0 g of air-dried soil was mixed with 1.8 g of catalyst (mixture of K_2SO_4, $CuSO_4.5H_2O$ and Se, 100: 10: 1, m/m/m) and 5 ml of 98% H_2SO_4 in a Kjeldahl digestion flasks. The Kjeldahl flasks were heated to 400°C until clearing of the solution. After cooling, the solution was transferred to the distillation chamber. Distilled NH_3 was absorbed by a 2% boric acid solution and then determined by titration with 0.05 M H_2SO_4 using bromcresol green- methyl as an indicator.

The NH_4-N was measured using Nessler's reagent spectrophotometric method at 460 nm on the 2M KCl extracts. The NO_3-N was extracted with a $CaSO_4$ solution and analysed using the phenol disulfonic acid spectrophotometric method at 420 nm. The available P was extracted with a solvent consisting of a mixture of 0.025M HCl and 0.03M NH_4F (5:3, v/v) and colorimetrically determined at 700 nm.

Soil microbial community structure

The microbial community structure was expressed using the PLFA analysis method (Frostegard *et al*. 1993a). Before PLFA extraction, glassware was washed with detergent, dried, and heated at 550°C for 4 h. A clean Teflon tube was first washed with methane and then rinsed with chloroform.

Lipids were extracted with a modified single-phase mixture of chloroform-methanol-citrate buffer (1:2:0.8, v/v/v). Briefly, 2 g of freeze-dried soil from each sample was extracted with 10 ml of the solvent in a 50-ml Teflon tube. After shaking for 5 min, the samples were extracted overnight at room temperature. The samples were then centrifuged at 1800 rpm for 15 min, and the supernatant was removed to a new clean tube. The remaining soil was re-extracted with 5 ml of the same extraction solvent for 2 h. The supernatant was removed after centrifuging, and the combined extract was then evaporated under a stream of nitrogen (N_2) on a heating block at 40°C. The resulting lipid material was fractionated into neutral lipids, glycolipids and polar lipids by a silica-boned phase column (strata SPE-Si, 500 mg/3 ml, Phenomenex, USA). The polar lipid fraction was eluted with 10 ml of methanol and evaporated to dryness under N_2. The polar lipids were trans-esterified to fatty acid methyl esters by a mild alkaline methanolysis. The final phospholipids in 100 *u*l of hexane were identified and quantified by chromatographic retention time and mass spectral comparison on an Agilent 6890-5975GC-MSD, using nonadecanoic acid methyl ester (19:0) as the internal standard (Sigma-Aldrich). The oven temperature was set at 80°C for 1 min, increased to 170°C at 30°C min^{-1}, held for 3 min, increased to 210°C at 10°C min^{-1}, held for 10 min, and then increased to 280°C at 5°C min^{-1} and held for 5 min. The detection mode was SIM. The analytical quality was confirmed by a 26-component bacterial acid methyl esters mix (FAME, C11-C20, R189-19, Supelco). The fatty acid nomenclature in which the number preceding the colon represents the

number of carbons in the hydrocarbon chain and the number following the colon indicates the number of double bonds in the hydrocarbon chain, was used (Frostegard *et al*. 1993b). Diagnostic fatty acids were used to classify different microbial groups, and several indices were calculated as follows: G-(16:1É9ÿcy17:0ÿ18:1É9tÿ18:1É9cÿcy19:0) to G+ (i15:0, a15:0, i16:0, i17:0), fungi (18:2É6,9) to bacteria (15:0ÿi15:0ÿa15:0ÿ i16:0ÿ16:1É9ÿ17:0ÿ i17:0ÿ cy17:0, 18:1É9tÿ18:1É9c, cy19:0), 18:1É9t/18:1É9c and mono/sat ratios (Kourtev *et al*. 2002).

Enzymatic activities

Dehydrogenase activity was determined by the reduction of triphenylterazolium chloride to triphenylformazone following a modified method of the Institute of Soil Science, Chinese Academy of Sciences (Institute of Soil Science Chinese Academy of Sciences 1985). A 5-g soil sample was incubated in 5 ml of 5 g l^{-1} triphenylterazolium chloride and 2 ml of 0.1 M glucose at 37°C for 1 h. The reactions were terminated by adding 0.25 ml of 98% H_2SO_4, and the products were extracted with 5 ml of toluene for 30 min on a shaker. After centrifugation, the triphenylformazone dissolved in toluene was assayed at 492 nm.

The determination of phosphatase activity was based on the determination of released *p*-nitrophenol (Sannino and Gianfreda 2001). A soil sample of 1.0 g wet weight was mixed with 4 ml of a 0.1 M maleate buffer (pH 6.5), 0.25 ml of toluene and 1 ml of substrate (0.05 M), and incubated at 37°C for 1 h. After incubation, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added. The *p*-nitrophenol released was measured colorimetrically at 400 nm.

The activity of protease was analysed using copper salt Colorimetry (Guan 1986). Two ml of toluene and 20 ml of L-arginine solution (1% w/v) were added to 5 g of fresh soil. The samples were incubated at 37°C for 24 h. After incubation, glycine was determined photometrically at 650nm using the copper-phosphate solution (mixture of $CuCl_2$, Na_2HPO_4 and $Na_2B_4O_7$ (1:2:2, v/v/v)).

Sodium phenolate colourimetry was used to measure urease activity (Xu 1986). Briefly, 5 g of air-dried soil was incubated with 1 ml of toluene, 10 ml of urea solution (10% w/v) and 20 ml of citric buffer (pH 6.7) at 37°C for 24 h. After incubation, ammonium concentration was measured photometrically at 578 nm with the mixture of phenol and sodium hypochlorite.

Data analysis

The results were calculated based on oven-dry soil weight (DW) and represent the arithmetic means ± standard deviations (s.d.) of the five field replications for each area. The repeated-measures ANOVA was used to assess the effects of *Mikania* invasion and seasonal variations on soil enzymatic activity, microbial biomass and soil physico-chemical variables and was followed by a pair-wise Fisher's least significant difference (LSD) test when significant differences were found. Pearson's correlations were used to examine the relationships between soil properties and microbial variables. The composition of the soil microbial community was tested using a principle component analysis (PCA) of the relative abundances of PLFAs in each sample. Discriminant analysis (DA) was also applied to the PLFA profiles and enzymatic activities to detect patterns in the responses to *Mikania* invasion and seasonal change. Discriminant function (DF) scores were plotted to observe how *Mikania* invasion and seasonal effects clustered. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) 13.0.

RESULTS

Soil physico-chemical properties

The soil chemical properties of the studied fields varied with the sampled areas and with different seasons (Table 1). In the same reason, the soil moisture content, NO_3-N concentration, available P concentration and organic matter content in EXOT were nearly 2 times higher than the values recorded for NATV. The pH values in NATV were neutral-alkaline, whereas those in EXOT were neutral-acidic.

From the repeated-measures ANOVA results, the effect of seasonal change was relatively smaller than the effect of *Mikania* invasion. Seasonal effects on soil moisture content, pH value, total N concentration, and available P concentration showed the same trends with insignificant difference ($p>0.05$). However, the trends in the seasonal effects on soil organic matter, NH_4-N and NO_3-N were not consistent. The NH_4-

Table 1. Physico-chemical properties of the studied fields (mean±s.d., n=5)

Season	Soil moisture content (%)		pH		Organic matter (g kg^{-1})		Total N (g kg^{-1})		NH_4-N (mg kg^{-1})		NO_3-N (mg kg^{-1})		Available P (mg kg^{-1})	
	NATV	EXOT	NATV	EXOT	NATV	EXOT	NATV	EXOT	NATV	EXOT	NATV	EXOT	NATV	EXOT
April 2008	12.04±0.97	23.75±0.43	7.69±0.07	7.94±0.14	20.49±0.48	25.18±0.45	2.51±0.05	3.21±0.05	136.86±15.11	109.67±4.77	6.35±0.94	12.79±0.66	12.23±2.88	24.28±2.66
July 2008	10.69±1.08	21.33±1.46	7.38±0.16	6.86±0.07	23.39±0.83	64.21±11.65	2.52±0.16	3.39±0.17	137.46±1.54	91.23±1.56	6.19±0.49	13.89±0.28	20.48±0.84	35.25±1.01
October 2008	11.24±1.07	21.85±1.84	7.26±0.05	6.77±0.06	25.83±1.34	62.42±2.67	2.78±0.11	3.67±0.13	117.89±6.88	95.52±4.34	6.24±0.51	14.07±1.60	29.19±0.90	53.01±8.37
January 2009	11.09±0.99	21.25±11.32	7.35±0.21	6.94±0.07	26.01±1.07	54.92±3.92	2.69±0.06	3.65±0.09	116.98±2.31	96.84±3.05	6.36±0.63	15.02±0.96	28.05±3.55	54.27±2.02
p values														
Season (df=3)	NS	NS	*	NS	*	**	**							
Field (df=1)	***	*	***	***	**	***	***							
Season*field (df=3)	*	NS	*	*	*	**	**							

NS, not significant (p>0.05); *P<0.05; **p<0.01; *** p<0.001

N level in EXOT was significantly highest in spring (April 2008), whereas in NATV the NH_4-N level in spring was nearly equal to that in summer. The organic matter content was highest in summer in EXOT, and in NATV the highest value was found in winter. The season had no significant effects on NO_3-N in NATV, while in EXOT the seasonal effect was significant, with the highest level in winter (January 2009) and the lowest level in spring (April 2008).

Soil microbial community structure

A total of 26 fatty acids were identified in our experiment, dominated by bacterial fatty acids. Principle component 1 (PC1) (eigenvalue = 9.76) based on the 26 fatty acids explained 61.01% of the variability in the data dominated by the fatty acids 16:0, 20:0, and 18:0, whereas PC_2 (eigenvalue = 2.00) explained 12.52% of the total variance and was dominated by the fatty acids 15:1, 17:1, and 18:2É6,9.

Within each sample time, the content of bacteria fatty acids was higher in EXOT than in NATV, whereas the fungi content was the inverse, with a higher value in NATV (Table 2). In both EXOT and NATV, the bacterial fatty acids were significantly more abundant in the summer and fall than in the other two seasons, whereas the fungal fatty acids exhibited the opposite pattern, with higher values in cold seasons (October and January).

The fungal/bacterial and G^+/G^- ratios for diagnostic fatty acids were lower in EXOT than in NATV; however, the mono/sat ratio was higher in EXOT (Fig. 1). The effect of season on G^+/G^- in EXOT was significant, and ratio values followed the order spring > fall > summer = winter; however, there were no significant effects of seasonal changes in NATV (Fig. 1(b)). The seasonal effects on the fungal/bacterial (Fig. 1(a)) and mono/sat (Fig. 1(c)) ratios were not similar: the fungal/bacterial ratio was significantly higher in cold months (January and April) than in warm months (July and October) in both EXOT and NATV. Mono/sat values were significantly higher in warm months (July and October) in EXOT, but no significant seasonal effects were found in NATV. The ratios of 18:1É9t/18:1É9c were significantly higher in warm months (July and October) than in cold months (January and April) (Fig. 1(d)).

Discriminant analysis based on the PLFA

Table 2. Classes of characteristic fatty acids in the studied fields (mean±s.d., n=5)

Season	Fungi (nmol g^{-1} dw)		Bacteria (nmol g^{-1} dw)		G+ (nmol g^{-1} dw)		G- (nmol g^{-1} dw)	
	NATV	EXOT	NATV	EXOT	NATV	EXOT	NATV	EXOT
April- 2008	1.57±0.21	1.42±0.01	23.15±2.39	28.54±0.11	3.83±0.61	4.03±0.30	1.54±0.22	2.26±0.12
July-2008	0.85±0.04	0.58±0.02	28.79±3.00	49.42±0.91	4.03±0.22	5.02±0.11	1.75±0.19	4.62±0.22
October- 2008	0.97±0.01	0.41±0.06	29.49±0.81	41.98±5.01	4.16±0.19	5.20±0.71	2.16±0.03	3.81±0.05
January- 2009	1.31±0.03	1.03±0.06	20.93±1.72	25.55±3.22	3.22±0.05	2.02±0.32	1.29±0.08	2.02±0.24
p values								
Season (df=3)	*	*	NS	NS				
Field (df=1)	*	**	*	*				
Season*field (df=3)	*	*	NS	NS				

NS, not significant (p>0.05); *P<0.05; **p<0.01

Table 3. Canonical correlations of the individual variables with the first two discriminant functions (DF) extracted from the discriminant analysis of the enzymatic activities

Enzyme	Canonical correlations with	
	DF_1	DF_2
Urease	0.858	0.254
Protease	0.951	-0.118
Dehydrogenase	0.979	0.182
Phosphatase	0.929	-0.362

profiles clearly separated the sample fields and sample seasons (Fig. 2 (a)). The first 2 discriminant functions (DF) explained 88.00% of the variation (DF_1 = 72.90%, p<0.001; DF_2 = 15.10%, p<0.001). DF_1 separated *Mikania*-invaded soil from the native soil, and DF_2 separated the four seasons.

Soil enzymatic activities

All of the 4 soil enzymatic activities in EXOT were significantly higher than those in NATV within the same season (p<0.05) (Fig. 3).

In NATV, there was no significant seasonal difference in dehydrogenase activity (p>0.05). In EXOT, however, dehydrogenase activity in fall (October) and winter (January) was significantly higher than in the other two seasons (p<0.05) (Fig. 3 (a)).

Urease activity in EXOT was significantly higher in fall (October) and winter (January) than in spring (April) and summer (July); the differences in urease activity between fall and winter and between spring and summer were not significant (Fig. 3 (b)).

Protease activity in EXOT followed the order summer = fall > winter > spring(Fig. 3 (c)). Phosphatase activity in EXOT was highest in summer and lowest in spring, and showed the significant seasonal effects (Fig. 3 (d)).

Discriminant analysis of the 4 soil enzymes separated the soils from sample fields at different seasons (Fig. 2 (b)). The first 2 discriminant functions (DF) explained 97.40% of the variation (DF_1 = 87.3%, χ^2 = 285.10, p<0.001; DF_2 = 10.10%, χ^2 = 135.18, p<0.001). DF_1 (eigenvalue = 92.98) separated the *Mikania*-invaded soil from the native soil, and DF_2 (eigenvalue = 10.73) separated spring, summer and winter/fall in both

Table 4. Correlation matrix between diagnostic fatty acids and enzymatic activities (n=40)

0	Fungi	Bacteria	Fungal /bacterial	G+	G-	G+/G-	Mono /sat	18:1ω9t/ 18:1ω9c
Protease	-0.956**	0.661**	-0.893**	0.311*	0.685**	-0.736**	0.925**	0.778**
Urease	-0.451**	-0.167	-0.266	-0.279	-0.140	-0.450**	0.268	-0.013
Dehydrogenase	-0.581**	0.001	-0.407**	-0.277	0.032	-0.702**	0.444**	0.168
Phosphatase	-0.820**	0.724**	-0.808**	0.246	0.744**	-0.847**	0.883**	0.810**

*P<0.05; **p<0.01

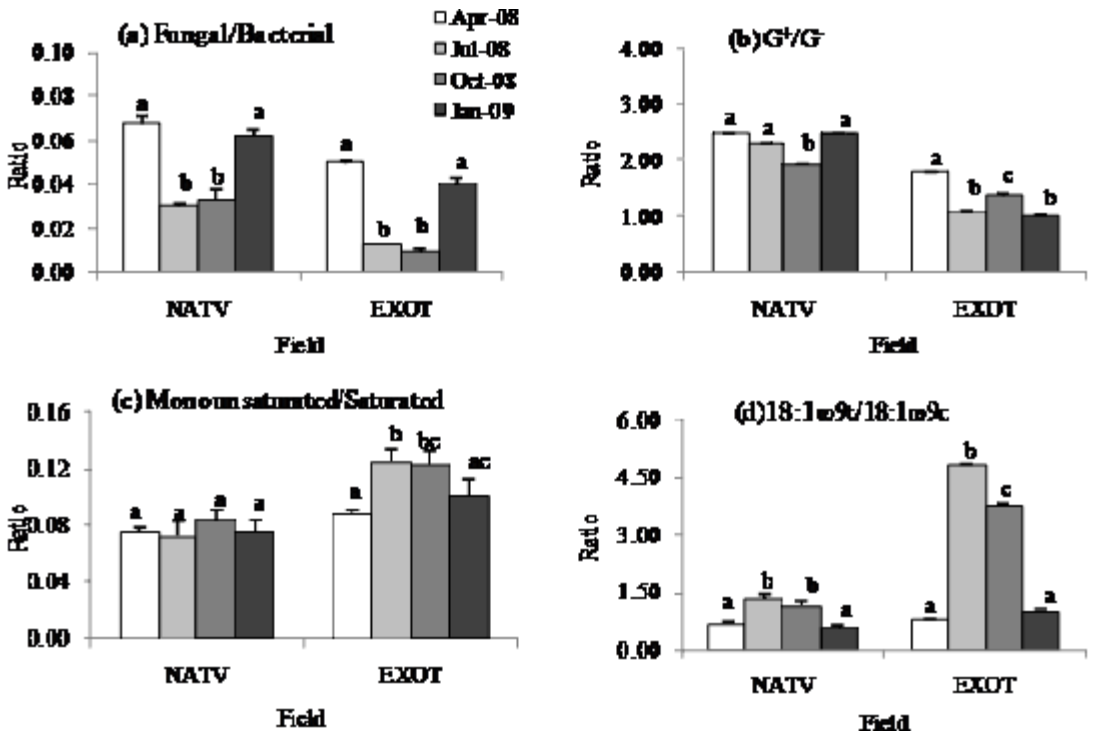

Fig. 1. Ratios of characteristic fatty acids in the studied fields. The mean and standard deviation values (Std) of 5 replicates are illustrated, and Std bars marked by the same letter are not significantly different among seasons for the same area (P>0.05).

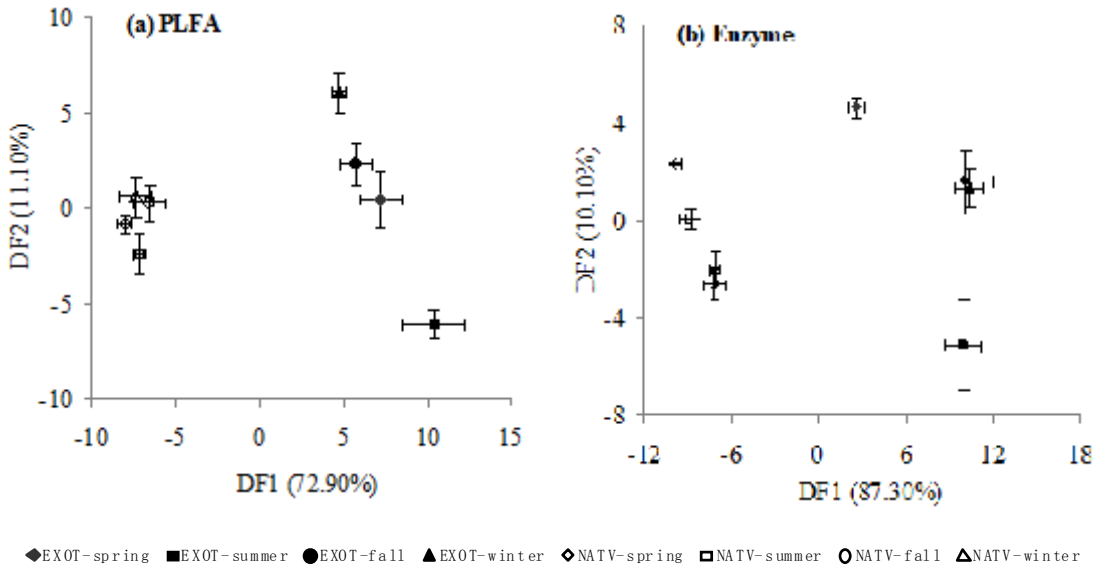

Fig. 2. Ordination plot of discriminant functions from the fatty acids and enzymes in the studied areas at different seasons.

sample fields. However, neither axis could separate the soil in the fall from that in winter, and the plots for winter and fall overlapped. All of the 4 studied soil enzymatic activities were positively correlated with DF_1 (Table 3).

Correlations between soil microbial community structure and function

The four enzymatic activities were significantly negatively related to fungi abundance and to the fungal/bacterial and G^+/G^- ratios ($p<0.01$), whereas the relationship between the 4 soil enzymatic activities and the mono/sat ratio was significantly positive. The activities of protease and phosphatase were significantly correlated with bacterial PLFAs ($p<0.01$), whereas those of urease and dehydrogenase were not ($p>0.05$) (Table 4).

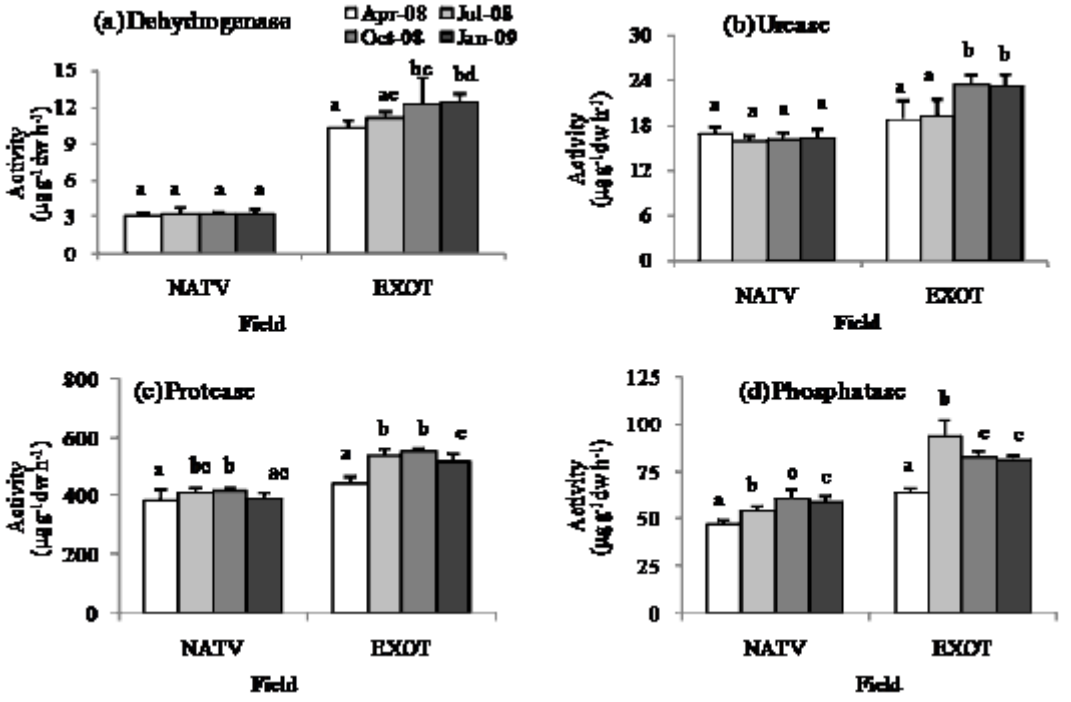

Fig. 3. Seasonal changes in soil enzymatic activities in the studied areas. The mean and standard deviation values (Std) of 5 replicates are illustrated, and Std bars marked by the same letter are not significantly different among seasons at the same area ($P>0.05$)

DISCUSSION

The repeated-measures ANOVA indicated that both *Mikania* invasion and season affected microbial community structure and function as well as physico-chemical parameters, although *Mikania* invasion had more pronounced effects than season.

In our study, significant increases in organic matter, total N and NO_3-N were observed in exotic *Mikania*-invaded soil compared with the native soil. Because *Mikania* often grows in a rich and wet environment, high levels of NO_3-N might be beneficial to its invasion by increasing N availability. However, the NH_4-N content was lower in *Mikania*-invaded soil. This was possibly attributable to a higher rate of nitrification and the fact that nitrifying bacteria convert more NH_4-N into NO_3-N, causing a higher NO_3-N concentration and lower pH in *Mikania*-invaded soil (Chen *et al*. 2009b). The result was different from Li *et al.* (2006) who reported that soil NH_4-N and NO_3-N content were lower in a *M. micranth* monoculture than in native communities. It was demonstrated that some exotic grasses had a positive effect on soil N and some had a negative effect (Kourtev *et al*. 2003; Hawkes *et al*. 2005). The different vegetation would be the main factor causing different soil NO_3-N

content; but further research should be done to elucidate the invasive mechanism on N cycling.

Mikania was found to have a high capacity to mobilise and store phosphorus (Swamy and Ramakrishnan 1987), and low pH could also increase P solubility (Herr *et al*., 2007), which would agree with a result of higher P and lower pH in *Mikania*-invaded soil. However, higher values of both pH and P concentration were found in EXOT compared with NATV in April. This phenomenon indicated that soil P concentration could be mediated by various mechanisms except for soil pH, such as production of phosphoesterases and symbiotic interactions.

PLFA, which can detect the majority of microbes in the soil, has been proven to be an effective approach to the study of soil microorganism community structure (Bossio *et al*. 1998). In our study, more bacteria and fewer fungi were found in *Mikania*-invaded soil compared with native soil in the same season, indicating more copiotrophic conditions with *Mikania* invasion (Ohtonen *et al*. 1999). Furthermore, more fungi and fewer bacteria were found in the cold seasons in EXOT. The available nutrients would be deficient because of low temperatures in spring and winter, and thus relatively more fungi were needed to decompose litters into soluble nutrients to satisfy the growth. Fungi exhibited a negative correlation with organic matter content (r^2 = -0.915, P<0.01), whereas bacteria exhibited a positive correlation (r^2 = 0.647, P<0.01), which further confirms the above results. The low fungal/bacterial ratio in *Mikania*-invaded soil might also be related to a higher moisture content (r^2=0.604, P<0.01) (Grayston and Prescott, 2005).

Soil enzymes play an important role in the processing and recovery of key nutrients from detrital inputs and accumulated soil organic matter (Caldwell 2005). The increase of the 4 soil enzymatic activities in the invaded soils indicated that *Mikania* invasion greatly enhanced nutrient cycling and facilitated *Mikania* invasion.

In this study, season was found to significantly affect soil physico-chemical parameters and microorganism communities, but the effects on soil microbes were more consistent, whereas on physico-chemical properties varied. The seasonal effects on soil physico-chemical properties were more dependent on the soil microbes and the capability of nutrient uptake (Hawkes *et al*. 2005). Physical variations that change according to season, such as soil moisture and temperature, might be important factors in the microbial community. Till now, no study found a winter maximum in microbial biomass (Wardle, 1992). It has been demonstrated that an increase in microbial biomass occurs in warm months, thereby increasing competition and allowing more dominant species to be detected in profiles of community structure and more active enzymes in microbial function (Grayston *et al*. 2001). In addition, the *Mikania* invasion has progressed over more than 5 years, it might well have adapted to the local climatic conditions. For the above reasons, the seasonal effects on soil microorganisms showed similar trends between *Mikania* and no-*Mikania* soils with more microbial biomass and higher enzymatic activity in most of the warmer months (July or October).

But for some functional microorganisms, the trends of seasonal effects between *Mikania* and no-*Mikania* soils were not the same. A higher value for the mono/sat ratio were found in *Mikania*-invaded soil in warmer months (summer and autumn), indicating a shift in the soil from oligotrophic to copiotrophic conditions, which facilitates *Mikania* invasion with less nutrient stress (Baath and Anderson 2003; Saetre and Baath 2000; Yao *et al*. 2000). *Mikania* grows most fast in July or August, and thus during the period a large amount of nutrient was needed, especially available P. Accordingly, the highest activity of phosphatase in EXOT was found in July, and the phosphatase would be one of the key enzymes in enhancing invasion.

In summary, it is likely that seasonal effects on soil microbial community and function, although present, are dependent on the local soil type and climatic conditions. Significantly, *Mikania* invasion was found to have more pronounced effects than season on soil physico-chemical characteristics and soil microorganisms. *Mikania* facilitates its own invasion by altering the attributes of native ecosystems, for example, by increasing NO_3-N and P availability, changing microbial community structure and activating enzymes, which might be accomplished by releasing water-soluble allelochemicals (Chen *et al*. 2009b; Wolfe and Klironomos 2005). Because the plant species

composition in a terrestrial ecosystem is the result of long-term interactions between plants and the abiotic environment, plant species frequently become well adapted to the original habitat (Cui and Song 2005). The new environment with greater nutrient availability induced by *Mikania* invasion might not support the stability of the native plant community, a possibility that must be taken into account for management practices. Further research is required to elucidate how seasonality and *Mikania* invasion impact microbial populations and to determine the key factors in controlling *Mikania* invasion.

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