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## Temporal variation of soil friedelin and microbial community under different land uses in a long-term agroecosystem

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## ABSTRACT

Despite increasing knowledge of soil microbial community dynamics involved in various factors, relatively little is known about plant-derived allelochemicals and their impact on the development of soil microbial community either independently or synergistically with the other factors. Here we examined an allelochemical friedelin and its relation to microbial community in soils from a long-term agroecosystem under different land uses and seasons. Four land uses (paddy field, maize field, barren and fallow) in an eight-year old continuous establishment were selected to conduct the experiments. Soil samples were taken in spring, summer, autumn and winter at different depths. Friedelin was quantified by gas chromatography (GC) and microbial communities were characterized by phospholipid fatty acid (PLFA) analysis. Subsequently, friedelin was found in all soils but its concentration varied with land uses, seasons and soil depths. The largest observed concentrations always occurred in surface soils and winter samples of all four land uses. Compared with tillage fields, barren and fallow contained a greater amount of friedelin. Both soil microbial community and friedelin varied seasonally, and there were positive relationships between friedelin and microbial community. The signature lipid biomarkers of soil bacteria and fungi, and soil microbial community structure were affected under friedelin application. The results suggest that friedelin may be one of the factors involved in microbial community dynamics under different land-use scenarios and seasonal variations, and friedelin-specific influences in the corresponding microbial community composition result in changes in the microbial community structure in soils from a given agroecosystem.

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## 1. Introduction

Soil microorganisms play important roles in ecosystem function and sustainability (Paul and Clark, 1989). Soil microbial community in agroecosystems varies greatly with biotic and abiotic factors, such as crop plant, seasonal variation, land-use scenario, cultivation and management intensity (Bossio et al., 1998; Bardgett et al., 1999; Buckley and Schmidt, 2003; Tian et al., 2012; Zhang et al., 2012; Bini et al., 2013). In particular, different land uses and long-term tillage practices have the lasting impact on soil microbial community composition (Buckley and Schmidt, 2001; Jangid et al., 2010, 2011; Yu et al., 2011). Any change to soil microbial community is likely to alter the nutrient availability and fertility management, and subsequently on crop productivity in agroecosystems.

Patterns in soil microbial communities are often found to be associated with plant species composition, richness and biomass (Saetre, 1999; Pietikainen et al., 2007). Many studies have shown that aboveground vegetation is the dominant factor governing the soil microbial community, and thus plant-driven selection of microbes occurs in various natural and managed ecosystems (Buckley and Schmidt, 2003; Carney and Matson, 2006; Hartmann et al., 2009; Bach et al., 2010). However, most studies of aboveground vegetation impact on soil microbial community composition and structure have focused on the contribution of plant-derived carbon to soil microorganisms (Lu et al., 2002; Farrar et al., 2003; Ushio et al., 2013; Zhang et al., 2013). Relatively less attention has been paid to an enormous variety of potentially valuable low-molecular mass phytochemicals, such as allelochemicals released from litter decomposition and root exudation, and their effect on soil microbial community composition and structure. An increasing number of studies have shown that plant-derived allelochemicals not only provide carbon substrate for soil microorganism consumption but also restrict or direct the development of certain soil microbial

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species, resulting in changes in soil microbial community composition and structure (Bais et al., 2006; Kong et al., 2008b; Guo et al., 2011; Lorenzo et al., 2013).

Friedelin (friedooleanan-3-one), a penta-cyclic triterpene, occurs in many plant families (Moiteiro et al., 2006; Alarcon et al., 2008; Kuete et al., 2010). Friedelin has been described as having many bioactivities, such as allelopathic action (Santos et al., 2008), antiinflammatory and antimicrobial activities (Queiroga et al., 2000; Tamokou et al., 2009; Kuete et al., 2010; Tchakam et al., 2012). A few studies have shown that friedelin may act as a geochemical indicator and biomarker for higher land plants into the sediments in aquatic systems (Hanisch et al., 2003; Simoneit et al., 2009; Itoh and Hanari, 2010; Pisani et al., 2013). A previous study found the occurrence of friedelin in the natural evergreen broadleaf forest and Chinese fir tree plantation soils. The concentration of friedelin in the forest soils ranged from 3.14 nmol g<sup>-1</sup> dry soil to 43.69 nmol g<sup>-1</sup> dry soil (Kong et al., 2008a). However, potential actions and implications of friedelin in the soils remain obscure. In particular, the interactions between friedelin and soil microorganisms are largely unknown. In addition, it is unclear whether the occurrence of friedelin in soil is a general phenomenon or is restricted in tree ecosystems. Accordingly, the present study examined the occurrence and distribution of friedelin in relation to the microbial community in agricultural soils from a long-term agroecosystem under different land uses and seasons. Furthermore, the changes in the soil microbial community in an incubation experiment involving the addition of friedelin were evaluated at varying periods. Thus, we aimed at further enhancing the understanding of the presence of allelochemical friedelin in soils and the relative impact on the corresponding soil microbial communities.

## 2. Materials and methods

### 2.1. Site description

The field experimental site was located at Shenyang Experimental Station of Chinese Academy of Sciences (Liaoning Province, China, 41°31' N, 123°24' E). The experimental station was built in 1987, in which represents the typical soil and climate types in Northeast China. Soil is classified as a Hapli-Udic Cambisol (FAO Classification). As in a continental monsoon climate zone, the experimental station is dry and cold in winter and warm and humid in summer, with a mean annual air temperature of 7.5 °C and an annual precipitation of 770 mm, of which 70% falls in July and August. Average air temperatures in January and July are -12.5 °C and 25.6 °C, respectively. The frost-free period is between 140 and 160 days in length, with an early frost in November and late frost in March, and soil is frozen from December to February next year. Maize (*Zea mays*), soybean (*Glycine max*) and rice (*Oryza sativa*) are major crop plants in this experimental station.

### 2.2. Field design

Four different land uses, paddy field, maize field, barren and fallow, were selected to conduct the experiments in this study. A farmland was randomly selected from the experimental station described above in 2004. The farmland was divided into two fields (20 m × 30 m). Each field was separated by trenches with at least 5 m buffer strips on each side. One set of two fields has never been planted with any crop plants and no tillage practices, resulting in a fallow plot in which a heavy infestation of weeds, such as *Cyperus rotundus*, *Bromus japonicus* and *Alopecurus aequalis*, covered the surface year by year. A second set has never been planted with any crop plants but the infesting weeds were removed by hand during

the growing seasons every year, resulting in a barren plot. In addition, a maize field and a paddy field at the experimental station were selected in 2004, respectively.

The maize field has been planted with maize under conventional tillage. Maize was sown at a rate of 49,000 seeds ha<sup>-1</sup> in 75 cm row width. Conventional tillage involved soil disturbance with one moldboard plowing to a depth of 20 cm in early November after maize harvest, one disking (7–10 cm depth) and field cultivation in late April prior to planting. All aboveground maize residues were removed before plowing in the field. Each year, 99 kg ha<sup>-1</sup> urea, 163 kg ha<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, and 125 kg ha<sup>-1</sup> KCl were applied to the maize as a starter fertilizer during the planting, and 375 kg ha<sup>-1</sup> urea was used as the top dressing for maize at the 6-leaf stage.

The paddy field has been planted with rice by means of transplanting. A fertilizer treatment of 145 kg ha<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 150 kg ha<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, and 100 kg ha<sup>-1</sup> KCl was applied before the paddy field was saturated with water. Rice seedlings at the 3-leaf stage were transplanted into the paddy field at a density of 3.0 × 10<sup>5</sup> plants ha<sup>-1</sup>. After transplanting, seedling growth was carried out with a 5 cm flooded depth and 10 days flooded duration. Nitrogen was applied at a level of 315 kg ha<sup>-1</sup> at 15 days after transplanting. All other field management was performed on the basis of the rules of the rural administration in Liaoning Province, China. Rice was planted in early May and harvested in late October every year.

### 2.3. Soil sampling

Soil samples were each collected from four land uses, i.e. paddy field, maize field, barren plot and fallow plot described above at the initiation of the experiments and at four time points over a crop growing season in 2012. The sampling date on March 20 was at the initiation of the experiments when the experimental station was opened to start a crop growing season. The four sampling dates on April 20 (spring), July 20 (summer), September 20 (autumn), and November 20 (winter) allowed a limited seasonal comparison. Six soil cores (2.5 cm in diameter) in a completely randomized design were sampled from per field or plot of four land uses at the 0–40 cm depth with a hand auger, in which each core represented a replication. The soil cores were cut into three segments of 0–10 cm, 10–20 cm and 20–40 cm, resulting in soil samples on a depth basis. Each of the soil samples was passed through a 2 mm sieve, homogenized and then divided into two subsamples. The subsamples were stored separately based on the methodological requirements of the procedures for the quantification of friedelin and microbiological analysis as described below.

### 2.4. Soil incubated with friedelin

A series of 150 ml vials were filled with 100 g of dry soil collected from a depth of 20–40 cm at the initiation of the experiments. Friedelin was added into the treated vials at a concentration of 25 µg g<sup>-1</sup>. The control vials received distilled water only. The vials were airtight with lids and then placed in an environmental chamber with a temperature of 28 °C. The vials were taken out from the chamber randomly after different incubation periods (1, 7, or 28 days), and the soils were taken for phospholipid fatty acid (PLFA) analysis as described below.

### 2.5. Quantification of friedelin

Quantitative analysis of friedelin was carried out with gas chromatography (GC) (Hanisch et al., 2003; Kong et al., 2008a). Dry soils (10 g) were ultrasonically extracted with MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1:3, v/

v), MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1:9, v/v) and finally CH<sub>2</sub>Cl<sub>2</sub> until the extracts were colorless, and then centrifuged at 1000 g for 30 min. The soil extracts were evaporated to dryness individually with nitrogen gas, and the residues were dissolved with 1 ml CHCl<sub>3</sub>. The samples were subjected to GC for quantitative analysis.

GC analysis was performed on an Agilent 7890 instrument with a split injector at 280 °C and a flame ionization detector (FID) at 300 °C. The injection volume was 1 µl, and the split ratio was 1:10. Nitrogen was employed as the carrier gas, and the flow rate was 1.5 ml min<sup>-1</sup>. The HP-5 capillary column was 30 m × 0.32 mm with a 0.25 µm film of a cross-linked methyl (95%) phenyl (5%) polysiloxane stationary phase. The column temperature was programmed from 200 °C at 25 °C min<sup>-1</sup>–300 °C and maintained for 25 min. Friedelin peak was identified by its retention time (21.8 min) and coelution with authentic friedelin (Sigma–Aldrich Co., USA). Friedelin was quantified by comparison of the peak areas of samples with those of authentic standard. Working standard solutions were prepared by dilution to establish a calibration curve ( $Y = 33.99 + 3.37X$ ,  $r^2 = 0.998$ ). The quantification was achieved by regression analysis of the peak areas against standard concentrations. The mean recovery of known amounts of friedelin added into soil were 60.0%, which was used to correct the concentrations determined.

## 2.6. Microbiological analysis

Soil microbial biomass C (MBC) was determined by the chloroform fumigation–extraction method (Vance et al., 1987). An automated TOC analyzer (Multi 208 N/C 3000, German) was used to determine the amount of extractable carbon. The MBC content was calculated by  $MBC = Ec/Kc$ , where  $Ec$  = (C extracted from fumigated soil) – (C extracted from nonfumigated soil) and  $Kc$  with value of 0.38 is a calibration factor.

PLFA analysis was performed by a combination of two methods in the literature (Frostegard et al., 1993; Baath et al., 1995) with minor modifications. Briefly, triplicate 5 g subsamples of milled and freeze-dried soils were extracted with mixture of CHCl<sub>3</sub>/MeOH/citrate buffer (1:2:0.8, v/v/v) and the phospholipids were separated from other lipids on silica gel-filled solid-phase extraction cartridges (0.50 g of Si; Supelco Inc., USA). The samples were then subjected to mild alkaline methanolysis, and the resulting fatty acid methyl esters (FAMES) were separated before being quantified and identified by GC. Identification of FAMES was based on retention time comparisons to FAME controls (Supelco Inc., USA). Quantification was carried out by calibration against standard solutions of nonadecanoate methyl ester (C19:0), which was also used as the internal standard.

A total of 22 PLFAs were identified in the soil samples, and fatty acids present in proportions >0.5% were used in the analysis. The sum of 12 fatty acids (i15:0, a15:0, 15:0, i16:0, 16:0, 16:1ω7c, i17:0, 17:0, 18:1ω7c, 18:0, cy19:0 and 20:0) was used to assess bacterial biomass. Among them i15:0, a15:0, i16:0 and i17:0 were considered representative of Gram (+) bacteria, and 16:1ω7c, cy17:0, 18:1ω7c and cy19:0 were considered representative of Gram (–) bacteria, while 16:1ω7c and 18:1ω7c for aerobic bacteria and cy17:0, cy19:0 for anaerobic bacteria were used (Vestal and White, 1989). Fungal biomass was assessed by quantifying 18:1ω9c and 18:2ω6,9c, while 10Me18:0 was used as biomarker of actinobacterial biomass.

## 2.7. Data analysis

The data were analyzed using Student's *t*-test or analysis of variance (ANOVA) with Tukey's honestly significant difference test. ANOVA and multiple comparisons were carried out with the SPSS 10.0 program. Repeated measures analysis of variance (RMANOVA)

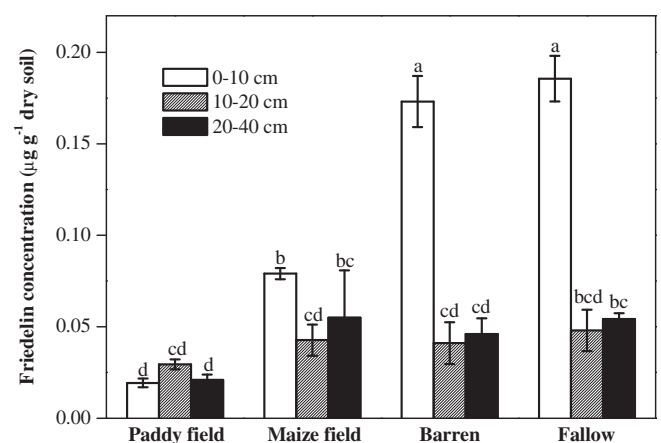
was used to determine the effect of friedelin and soil microbial community composition under different land uses and seasons. Pearson correlation analysis used bivariate correlation coefficients was carried out with the SPSS16.0 program. Principal component analysis (PCA) and discriminant analysis (DA) were applied separately to PLFA proportion to show relationships among different soil samples that contain multiple variables. Significance differences between means in the ordination space were tested by multivariate analysis of variance (MANOVA) on the principal component scores. PCA and DA are performed with the STATISTICA software package, version 6.0 (Statsoft Inc., USA).

## 3. Results

### 3.1. Occurrence and variation of soil friedelin under different land uses and seasons

Significant differences were observed in soil parameters including organic matter content, microbial biomass C, available N, available P and available K among paddy field, maize field, barren and fallow at the initiation of the experiments (Table S1). Although friedelin was found in all of soil samples collected from paddy field, maize field, barren and fallow, its concentrations varied greatly with land-use scenarios and soil depths at the initiation of the experiments (Fig. 1). The concentrations of friedelin at the 0–10 cm soil depth were much greater in fallow and barren than in maize field and paddy field. Furthermore, greater amounts of friedelin were detected at the 0–10 cm soil depth than at the 10–20 cm and 20–40 cm soil depths with an exception of paddy field. However, there were no significant differences in friedelin concentrations at the 10–20 cm and 20–40 cm soil depths among four land uses (Fig. 1). Therefore, further experiments were carried out with a 0–10 cm soil depth.

Seasonal variations of soil friedelin occurred in paddy field, maize field, barren and fallow (Fig. 2). The concentration of friedelin in fallow and barren at the 0–10 cm soil depth was slightly higher in spring, but its amounts declined in summer and then increased rapidly in autumn and winter. Such a trend was not observed in maize field and paddy field. However, the largest concentrations of friedelin were consistently observed in soil surface in winter for all land-uses (Fig. 2).



**Fig. 1.** Friedelin concentrations at different soil depths involved in four land uses at the initiation of the experiments. Means ± standard error (SE) from six replications for each determination are shown. Columns identified with common letters are not significantly different from each other at  $P < 0.05$ , one-way ANOVA, followed by the Tukey's honestly significant difference test.



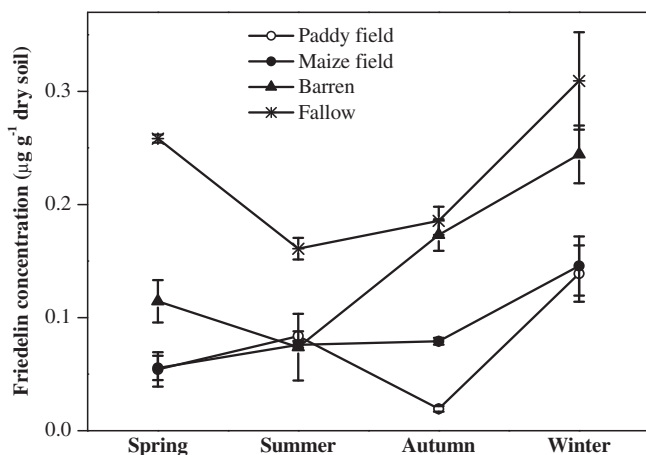


Fig. 2. Seasonal variation of friedelin at the 0–10 cm soil depth under four land uses. Means  $\pm$  standard error (SE) from six replications for each determination are shown.

### 3.2. Soil microbial community dynamics under different land uses and seasons

PLFA profiling indicated seasonal dynamics of soil microbial community under four land uses (Fig. 3). The signature lipid biomarkers of bacteria and fungi, total PLFAs and fungi/bacteria ratios varied seasonally and affected by land-use scenarios. There were similar seasonal variations between bacterial and total PLFAs under four different land uses. The largest observed concentration of bacterial and total PLFAs occurred in autumn with the exception of maize field in spring. The largest concentration of fungal PLFAs was consistently observed in autumn for all land-uses. However, values of bacteria/fungi ratios were lowered in autumn under four land uses. Furthermore, the concentrations of bacterial, fungal, total PLFAs were sharply decreased from autumn to winter. Regardless of seasons, there always were a greater concentration of bacterial, fungal, total PLFAs in fallow than in paddy field, maize field and barren. In contrast, fallow always had a minimum of bacteria/fungi ratios in response to seasonal variations (Fig. 3).

There were different microbial communities in the soils with varying seasons and land uses. Changes in soil microbial community compositions were observed in the seasons depending on different land-use scenarios. PCA scores for the PLFA extracted from soil samples were clearly distinguished by the composition of total PLFA of soil samples representing different seasons of four land-uses (Fig. 4). The soil microbial communities were divided into four groups: four land uses in winter alone, paddy field and maize paddy in autumn together, fallow in three seasons (spring, summer and autumn) alone, and paddy field and maize paddy in spring and summer and barren in three seasons (spring, summer and autumn) together. Each group occupied a distinct ordination space. The first principal component (PC1 = 57.96%) and second principal component (PC2 = 13.27%) together accounted for 71.23% of the total variation. This analysis revealed a remarkable difference in microbial community between land uses and seasons. In particular, the microbial community of winter sampling separated clearly from that of the other three seasons sampling (Fig. 4).

### 3.3. Relationships between soil friedelin and microbial community

Repeated measures analysis of variance (RMANOVA) for friedelin and soil microbial community composition showed the inherent differences between land use and season (Table S2). A significant effect of land use ( $P < 0.001$ ) and season ( $P < 0.001$ ), and

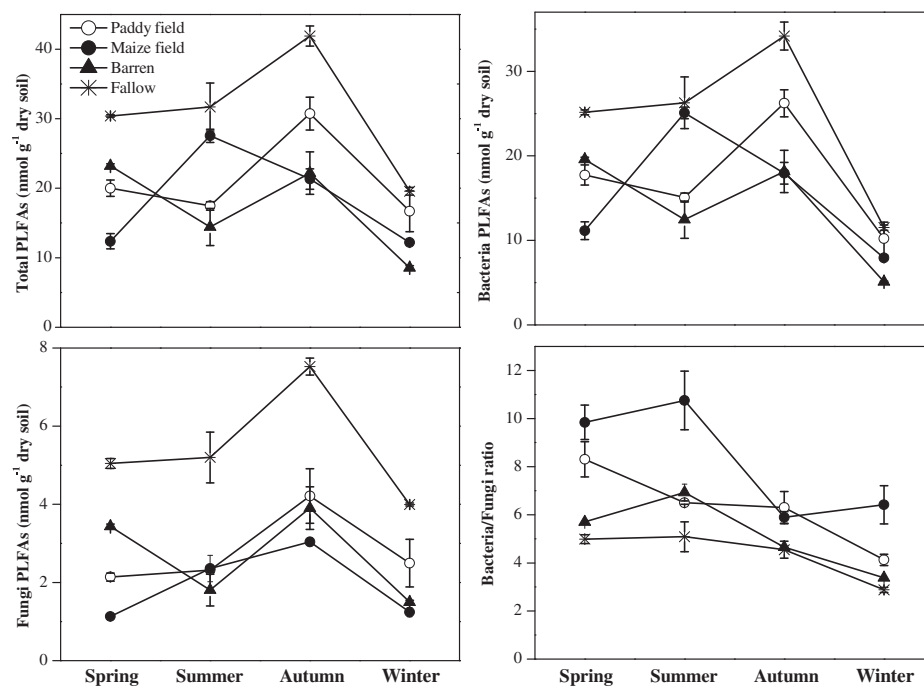
a significant effect of land use  $\times$  season interactions ( $P < 0.001$ ), were found for friedelin in most parameters of microbial community compositions. However, the values of Gram (–)/Gram (+) ratios in land use ( $P = 0.118$ ) and Gram (+) bacteria in season ( $P = 0.275$ ) showed no significant effects (Table S2). Pearson correlation coefficient indicated that there were positive relationships between friedelin and bacteria, actinobacteria, fungi and total PLFAs under seasonal variation, and significant positive relationships occurred in anaerobe in spring ( $P < 0.05$ ), fungi ( $P < 0.01$ ) and Gram (+) bacteria ( $P < 0.05$ ) in summer, and monounsaturated PLFA/saturated PLFAs ( $P < 0.01$ ) in winter. However, negative relationships were observed in bacteria/fungi ratios in all seasons, especially a significant effect ( $P < 0.01$ ) in autumn (Table S3).

### 3.4. The effect of friedelin application on soil microbial community

On the basis of the relationships between friedelin and microbial community composition under different land uses and seasons, pure friedelin was added into the soil to examine its impact on the corresponding soil microbial communities. A comparison of PLFA patterns of the soils with friedelin showed that differences in PLFA profiles between incubation periods were significant (Fig. 5). Of the 15 PLFAs (20:0, 18:2 $\omega$ 6,9c, 18:1 $\omega$ 9c, 18:1 $\omega$ 7c, 18:0, i17:0, cy17:0, 17:0, i16:0, 16:1 $\omega$ 7c, 16:0, 15:0, i15:0, a15:0, 10Me18:0) used in assessing microbial community composition, four PLFAs (15:0, i15:0, a15:0 and 16:1 $\omega$ 7c) displayed significant difference between friedelin and control (distilled water) at the first incubation day, while 7 PLFAs displayed significant differences at the 7th incubation day. The concentrations of Gram (+) bacterial PLFAs (i16:0 and i17:0) and Gram (–) bacterial PLFAs (cy17:0 and 18:1 $\omega$ 7c) were significantly greater in the samples of the 7th incubation day sampling than those of the control. Similarly, friedelin application significantly increased the concentrations of fungal PLFAs (18:1 $\omega$ 9c and 18:2 $\omega$ 6,9c) and actinobacterial 10Me18:0 of the samples at the 7th incubation day. However, such PLFA profiles were not observed during late incubation periods. Compared with the control, only a fungal PLFA (18:1 $\omega$ 9c) displayed significant difference while no significant differences occurred in the other 14 PLFAs at the 28th incubation day (Fig. 5). Furthermore, the 15 fatty acids selected were subjected to a stepwise discriminant analysis (DA). Subsequently, different microbial communities were identified for friedelin application along with incubation periods (Fig. 6). DA clearly differentiated the samples incubated with the friedelin and the control samples. The first discriminant function (DF1) accounted for 61.60% of the variance ( $\chi^2 = 88.17$ ,  $P < 0.001$ ), and the second discriminant function (DF2) accounted for 35.31% of the variance ( $\chi^2 = 34.09$ ,  $P < 0.001$ ). The PLFA profile of friedelin incubated samples changed significantly with incubation periods. However, the PLFA pattern of the control samples kept unchanged (Fig. 6).

## 4. Discussion

Soil microbial communities are affected by multiple factors. In particular, land-use history has a stronger impact on soil microbial community composition than aboveground vegetation, soil properties and seasonal variation (Jangid et al., 2011; Yu et al., 2011). Although the relative impacts of these different factors on the development of soil microbial communities are still being elucidated, these factors influence microbial communities that may act either independently or synergistically with the other soil characteristics. The data generated in this study show that soil microbial community not only is affected by land-use scenario and seasonal variation but also correlates with the temporal variation of an allelochemical friedelin in soils from a long-term agroecosystem.



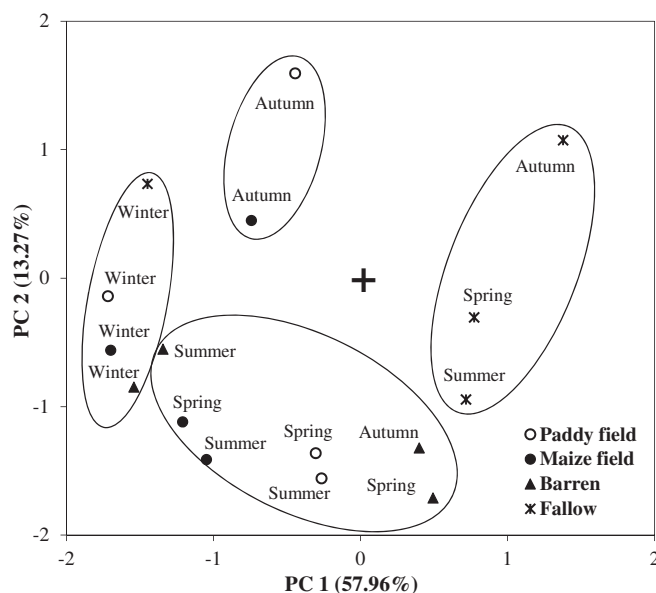
**Fig. 3.** Seasonal dynamics of microbial community at the 0–10 cm soil depth under four different land uses. Means  $\pm$  standard error (SE) from six replications for each determination are shown.

Plants produce and release an enormous variety of phytochemicals into the soil environment through litter decomposition and root exudation. The plant-derived chemicals interact with soil microorganisms at significant rates (Guo et al., 2011; Li et al., 2013; Ushio et al., 2013). Soil microbes are mostly heterotrophic organisms depending on the exogenous supply of carbon substrates as energy, as well as nutrient sources, for growth and development

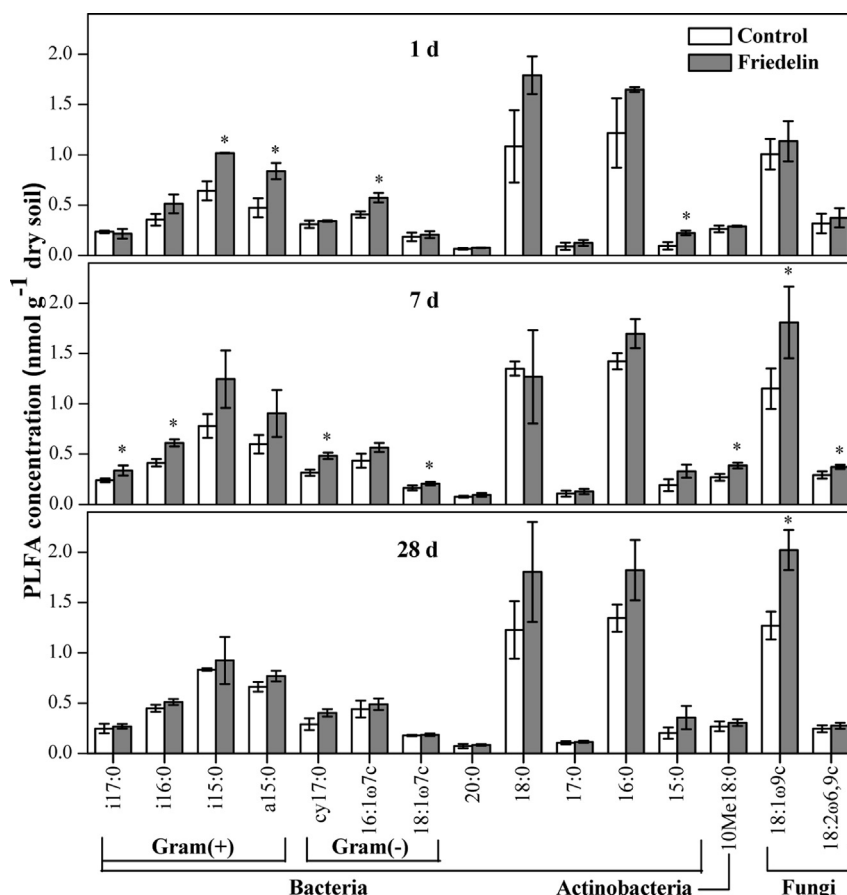
(Farrar et al., 2003; Hartmann et al., 2009). Plant-derived chemicals and metabolites increase soil organic matter, which favors microbe development and thus increases greatly soil microbial biomass and population (Lu et al., 2002; Bonilla et al., 2012). However, some potentially valuable phytochemicals, such as friedelin in this study, not only provide carbon for soil microorganism consumption but also have a series of interactions with soil microorganisms, resulting in the inhibition or stimulation of soil microbial biomass and population (Bais et al., 2006; Kong et al., 2008b; Lorenzo et al., 2013).

Similar seasonal changes in soil microorganisms and friedelin occurred in this given agroecosystem. Soil microorganisms in winter consume less plant-derived carbon, and thus there always are the largest observed concentrations of friedelin in winter regardless of land-use scenarios. Furthermore, there were positive relationships between friedelin and microbial community compositions under different land uses and seasons. In particular, soil microbial communities were altered under the friedelin application. The results indicated that the presence of friedelin in the soils had an impact on the corresponding microbial community composition, resulting in changes in soil microbial community structures. In this manner, friedelin may contribute to soil microbial community dynamics under different land uses and seasons.

Land use is closely tied to soil disturbance, while soil disturbance is for sensitivity to the microbial community and occurrence of plant-derived chemicals in surface soil (Gil et al., 2011; Zhang et al., 2012; Li et al., 2013). In this case, the influence of friedelin on the soil microbial community is likely to vary based on soil disturbance. A significant reduction of soil friedelin was observed in paddy and maize fields where soils were disturbed frequently by cultivation and management intensity. In contrast, fallow without soil disturbance always had much greater amounts of friedelin than maize field and paddy field. Although the friedelin variations were not completely consistent with the microbial community dynamics, it is a fact that soil microbial community varies with friedelin seasonally under different land-use scenarios.



**Fig. 4.** Principal component plot of the soil microbial community under different land uses and seasons. PC indicates a principal component, and (+) indicates the origin. The ellipses around each cluster of data points are drawn based on the each point ordination (X Y Coordinate). Data used in the PCA plots are transformed using sample unit totals to represent relative abundance of each PLFA (mole percent of total PLFA).



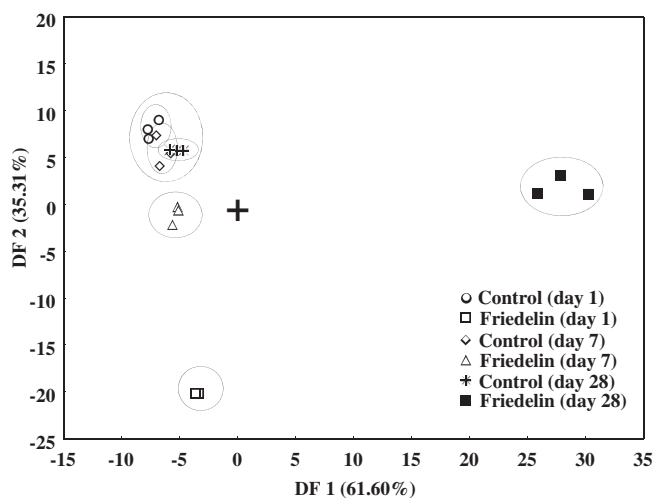
**Fig. 5.** PLFA concentrations of bacterial, actinobacterial and fungal importance extracted from microcosms of soil incubated with friedelin at different periods. Means  $\pm$  standard error (SE) from six replications for each determination are shown. The results of Student's *t*-test for the significance of the difference between the means of friedelin and control (distilled water) are represented by \* at *P* < 0.05.

Plant species, land-use scenarios, tillage practices and plant-derived chemicals may result in different impacts of microbial population and community structure in agricultural soils (Bossio et al., 1998; Buckley and Schmidt, 2001, 2003; Bonilla et al., 2012;

Zhang et al., 2012; Lorenzo et al., 2013). The impacts might be attributable to the interactions of all present factors in a given agroecosystem, but plant-derived chemicals and metabolites, especially allelochemicals, may be an important factor to interfere with soil microorganisms (Kong et al., 2008b; Guo et al., 2011; Lorenzo et al., 2013). A few studies have shown that friedelin has antimicrobial activities (Tamokou et al., 2009; Kuete et al., 2010; Tchakam et al., 2012). This study highlights that friedelin may act as an allelochemical interfering with the development of soil microbial community. However, this study did not clarify if soil friedelin had a direct regulation on microbial species, and which species are responsible for the dynamics of friedelin in the soil environment under different land-use scenarios and seasonal variation. A further clarification of the origin, fate, biotransformation and interaction of friedelin in agricultural soils is warranted in near future.

## 5. Conclusions

Plant-derived allelochemicals play an important role in regulating soil microbial community composition. In this study, an allelochemical friedelin was found in the soils from a long-term agroecosystem regardless of land-use scenarios and seasonal variation. There were positive relationships between friedelin and microbial community composition in the soils under different land uses and seasons. In particular, soil microbial communities were altered under friedelin application. The results suggest that friedelin may be one of the factors involved in soil microbial



**Fig. 6.** Plots of discriminant analysis of microbial community of control (distilled water) and the soil incubated with friedelin at different periods. DF indicates a forward stepwise discriminant analysis, and (+) indicates the origin. Data used in the DA plots are transformed using sample unit totals to represent relative abundance of each PLFA (mole percent of total PLFA).



community dynamics under different land uses in a given agroecosystem. The discovery of friedelin, as well as a further understanding of its potential implications and applications in agroecosystems, may lead to new insight into the interactions between plant-derived allelochemicals and microorganisms in agricultural soils.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2013.11.016>.

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