



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



Hong-Yun Dong^a, Chui-Hua Kong^{a,*}, Peng Wang^b, Qi-Liang Huang^c

^a College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China

^b State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

^c Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

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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

* Corresponding author. Tel.: +86 10 62732752; fax: +86 10 62731016.

E-mail address: kongch@cau.edu.cn (C.-H. Kong).

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⁻¹ dry soil to 43.69 nmol g⁻¹ 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⁻¹ 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⁻¹ urea, 163 kg ha⁻¹ (NH₄)₂HPO₄, and 125 kg ha⁻¹ KCl were applied to the maize as a starter fertilizer during the planting, and 375 kg ha⁻¹ 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⁻¹ (NH₄)₂SO₄, 150 kg ha⁻¹ (NH₄)₂HPO₄, and 100 kg ha⁻¹ 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 × 10⁵ plants ha⁻¹. 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⁻¹ 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⁻¹. 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/CH₂Cl₂ (1:3, v/

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