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Mobility and Microbial Activity of Allelochemicals in Soil

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ABSTRACT: The action of allelochemicals in soil needs their presence in the vicinity of the target plants. Using a soil TLC combined with bioassay approach, the mobility of 10 typical allelochemicals was evaluated. Ferulic, *p*-hydroxymandelic, *p*-hydroxybenzoic, and vanillic acids always had the lowest mobility ($Rf < 0.1$), whereas phenolic aldehyde and lactone (vanillin and coumarin) showed the highest mobility ($Rf > 0.5$). The *Rf* values of daidzein, 1α-angeloyloxycarotol, DIMBOA, and *m*-tyrosine ranged from 0.24 to 0.32. Binary mixtures of these allelochemicals led to an increase in mobility factors for selected combinations. Phospholipid fatty acid profiling indicated that there were different soil microbial communities in the segments containing allelochemicals residues in the developed TLC soil layer. A difference in microbial community structure occurred between two nitrogenous DIMBOA and *m*-tyrosine and another eight allelochemicals. The results suggest that the soil activity of allelochemicals on bioassay species and microbial communities depends on their mobility in soil.

KEYWORDS: *allelopathy, mobility factor, soil TLC, joint action, phospholipid fatty acid, soil microbial community*

INTRODUCTION

Plant−plant allelopathic interactions are mediated by allelochemicals that release from plants into the environment, mostly into the soil. The action of allelochemicals needs their presence in soil at phytotoxic levels in the vicinity of the target plants.[1,2] Once allelochemicals are introduced into the soil environment, a series of interactions among allelochemicals with soil factors take place.[3,4] It is complex and poorly understood that allelochemicals endure under soil processes. In most cases, however, allelochemicals are adsorbed and desorbed on soil solids, transported with water, and biotransformed by soil microorganisms.[5−7] Of these processes, the mobility of allelochemicals and their persistence in soil would likely be crucially important factors for the assessment of the action of allelochemicals.[2,4,8] Any retention and microbial degradation during the movement in soil should affect the concentration and final destination of the allelochemicals in the soil environment and, subsequently, their allelopathic activities.

The mobility and persistence of allelochemicals through the soil environment is an important process comprising interactions between soil abiotic and biotic factors.[3,4] In particular, soil microorganisms affect the persistence of allelochemicals at significant rates.[9,10] Such interactions lead to the concentration and bioavailability of allelochemicals in the vicinity of the target plants. An increasing number of studies have shown that allelochemicals released or applied into soil change the microbial community structure to provide specific microbial activity and bioavailability of allelochemicals.[3−5,11−13] A few studies characterize the mobility of several allelochemicals, such as phenolic acids and flavonoids.[3,6,14−16] Besides these allelochemicals, however, studies are lacking for almost all other major allelochemicals occurring in managed and natural ecosystems. In particular, relatively little is known about their mobility and interactions with the soil microbial community.

Studying the mobility of allelochemicals in soil has been very difficult in the past due to methodological limitations. A quick and reproducible method to assess the mobility of allelochemicals through the soil environment is lacking. Actually, soil is a natural chromatograph. The movement of any chemicals in soil can be eluted by rainfall under natural conditions. Therefore, soil thin-layer chromatography (TLC) with water as solvent allows observation and measurement of the mobility of chemicals, especially pesticides, through soil microstructures.[17] Soil TLC is analogous to conventional TLC, with the use of soil instead of silica gel as the adsorbent phase. It is a qualitative screening tool suitable for obtaining an estimate of the mobility of chemicals through the soil environment and offers many desirable features.[18,19] There is a wealth of information on using a soil TLC approach for pesticide mobility.[17−21] However, when it comes to the mobility of allelochemicals in the soil environment, there is a lack of data, which calls for further studies.

Soil TLC was originally designed for use with radiolabeled pesticides.[17,19,20] Although a few allelochemicals, such as sorgoleone released from the root hairs of sorghum (*Sorghum bicolor*), were radioactively labeled and used to study mineralization in soil,[8] it is extremely difficult to unambiguously radiolabel a wide variety of allelochemicals identified from different plant species. Thus, the method has relative limitations in using for allelochemicals' evaluation. An effort should now be directed toward assessing the mobility of allelochemicals using a soil TLC approach. Fortunately, allelochemicals always demonstrate their phytotoxicity in soil. Instead of radiolabel, therefore, a sensitive plant species such as lettuce (*Lactuca sativa*) as an indicator of phytotoxic location would be incorporated into the soil TLC for determining allelochemical migration. A previous study clearly showed that the mobility of two flavone *O*-glycosides and their aglycones could be evaluated by soil TLC incubated with lettuce.[16] In the present study, the mobility of 10 allelochemicals with a wide variety of structural types and their mixtures was

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investigated by a combination of two methods of soil TLC and bioassay. Furthermore, the microbial community structure in the segments containing allelochemical residues in the developed TLC soil layer was determined using phospholipid fatty acid (PLFA) analysis,[22] with an attempt to further our understanding of the action of allelochemicals in the soil environment.

MATERIALS AND METHODS

Allelochemicals and Soil. Ten allelochemicals were used in this study. These allelochemicals involve a wide variety of structural types including phenolic compounds (acid, aldehyde, lactone, flavonoid), terpenoid, benzoxazinoid, and nonprotein amino acid (Table 1). Their

Table 1. Mobility Factor (*Rf*) of Allelochemicals in Soil TLC with Different Detection Methods[a]

	detection methods	
allelochemical (type)	bioassay	segment quantification
ferulic acid (phenolic acid)	0.096 ± 0.004a	0.095 ± 0.002a
p-hydroxymandelic acid (phenolic acid)	0.084 ± 0.004a	0.084 ± 0.001a
p-hydroxybenzoic acid (phenolic acid)	0.086 ± 0.004a	0.088 ± 0.004a
vanillic acid (phenolic acid)	0.092 ± 0.005a	0.090 ± 0.004a
vanillin (phenolic aldehyde)	0.718 ± 0.025f	0.715 ± 0.022f
coumarin (phenolic lactone)	0.580 ± 0.016e	0.576 ± 0.014e
daidzein (isoflavone)	0.315 ± 0.017d	0.320 ± 0.010d
1α-angeloyloxycarotol (terpenoid)	0.235 ± 0.020b	0.238 ± 0.016b
DIMBOA (benzoxazinoid)	0.305 ± 0.013cd	0.302 ± 0.008cd
m-tyrosine (nonprotein amino acid)	0.270 ± 0.008bc	0.266 ± 0.012bc
2,4-D (herbicide)	0.905 ± 0.013g	0.902 ± 0.015g

[a]Mean ± standard error (SE) from three independent experiments for each determination is shown. There are no differences between the bioassay and segment quantification for any allelochemicals. Data in a column followed by the same letter among allelochemicals are not significantly different at $P < 0.05$, analysis of variance (ANOVA) followed by Tukey's honestly significant difference tests.

allelopathic actions were exhibited in several studies.[6,11,12,15,23−25] Among these allelochemicals, ferulic acid, *p*-hydroxymandelic acid, *p*-hydroxybenzoic acid, vanillic acid, vanillin, coumarin, daidzein, and *m*-tyrosine were purchased from Sigma-Aldrich Co. The benzoxazinoid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) was isolated from maize (*Zea mays*) seedlings according to the procedure of Larsen and Christensen.[26] 1α-Angeloyloxycarotol, a carotene-type sesquiterpene, was obtained from *Ambrosia trifida*-infested soils as described by Kong et al.[24] 2,4-D with 98% purity, as a comparison of herbicide, was provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (Beijing, China).

Soil was collected from a field at Shangzhuang Experimental Station of China Agricultural University (Beijing, China). The field had previously been planted with wheat (*Triticum aestivum*) and had not been planted with any crop plants since 2010. Three control plots (1 m × 1 m) were randomly selected from the field in 2011. If any plant species occurred in the control plots, they were uprooted by hand. A soil core, 2.5 cm in diameter by 10 cm depth, was obtained from a central location on each plot. Soil samples were air-dried, mixed, and then sieved (2 mm mesh) to remove plant tissues. The soil is a Hapli-Udic Cambisol (FAO classification) with clay, 14.30%; sand, 52.60%; silt, 33.10%; and cation exchange capacity, 16.00 cmol(+)/kg. The soil had a pH of 6.52, an organic matter content of 1.65%, and a fertility status of available N of 71.41 mg/kg, available P of 70.12 mg/kg, and available K of 94.51 mg/kg. The soil samples were divided into three groups. The first group received no treatment. The second group was adjusted with 1 N HCl or NaOH, resulting in three soil subsamples with pH 2.5, 7.0, and 9.5. The third group was treated with 10 or 30% H_2O_2, which lowered the soil organic matter content from 1.65 to 1.34 and 0.97%, respectively.

Assay for Seed Germination. Lettuce (*L. sativa*) acts as a sensitive plant indicator for the phytotoxic activity of allelochemicals,[27] and the results were used to identify the lowest concentration required to have an observable inhibition of seed germination for later soil TLC combined with bioassay approach. A randomized, complete-block design was used with three replicates. A total of 50 presterile lettuce seeds were uniformly sown in a Petri dish (diameter, 9 cm) containing 100 g of soil without any treatment as described above. After the soil was moistened with 10 mL of distilled water, allelochemicals tested at a concentration of 100 or 200 μg/g were added to each of the treated dishes. The control dishes received water only. All dishes were placed in an environmental chamber at a relative humidity of 70% with a temperature of 25 °C. Dishes were randomized once at 12 h intervals. After 48 h, emergence of lettuce (radicle length, >2 mm) was counted. Percentage of inhibition at different concentrations was obtained from the comparison of emergence numbers between the treated and control dishes.

Soil TLC. Soil TLC was conducted using the method developed by Ravanel et al.[17] with some modifications. The soils with or without H_2O_2 or pH treatments as described above were ground and sieved to 125 μm. Soil was suspended in a dioxane/water (1:1, v/v) solvent to make a slurry, which was then spread as a 0.7 mm thick layer on 10 cm × 20 cm glass plates. The plates were air-dried at room temperature (20−25 °C) and stored in a desiccating chamber until used for chromatographic tests. Various allelochemicals or their mixtures (1:1, w/w) at a concentration of 200 μg/g were each sampled with a microsyringe at 2.5 cm from the bottom edge of the plates. Distilled water in sampling served as the control. After the spots had been deposited, the plates were allowed to develop in a closed glass chamber using distilled water as solvent. A sheet of filter paper dipping into the developing water fed water continuously to the substrate at the base of the plate, thus leading to a relatively uniform flow. During the development with water, the whole device was held in a horizontal position. Water migration occurred at a distance 17.5 cm from the baseline. The plates were then dried at room temperature. The migration lasted between 1 and 5 h according to the different treatments. The movements of various allelochemicals or their mixtures were detected using bioassay and/or segment quantification described below.

Bioassay. The dried surface of the developed soil TLC plates described above was uniformly sown with lettuce seeds and then was saturated with distilled water. The plates were cultured in a constant humidity (70%) cabinet at a temperature of 25 °C. The germination of lettuce was observed after 24 h. The location with the largest observed inhibition (percent of the control) of lettuce germination was regarded as the migration distance of allelochemicals (R_1). The distances covered by the allelochemicals on the thin layer compared to that covered by water, that is, the mobility factor (*Rf*) value, were calculated as $Rf = R_1/R_2$ (R_2 is the water migration distance).[16,17] All manipulations as described above were conducted three times for each determination under identical conditions.

Segment Quantification. After the developed soil TLC plates without H_2O_2 and pH treatments described above were dried at room temperature, the dried soil layer of the developed TLC plates with three replicates for each allelochemical was cut into segments of 1.5 cm each. To avoid microbial degradation and transformation, the allelochemical residue in each segment was quantified immediately by ultraperformance liquid chromatography (UPLC) as described below. The mobility factor (*Rf*) value of each allelochemical was calculated according to the formula $Rf = \sum R_i \times M_i/R_w \times \sum M_i$,[18] where R_w is the remove distance of water from the starting point, i is the number of segments, R_i is the distance of segment i from the starting point, and M_i is the allelochemical content in segment i.

Quantification of Allelochemicals. The quantification of allelochemicals in each segment on the developed soil layer described above was performed by a liquid extraction−solid-phase extraction followed by UPLC. Soil samples (5 g) were each extracted with 3 × 10 mL of the MeOH/Me_2CO (3:5, v/v) mixture, agitated for 6 h at room temperature and then centrifuged at 1200*g* for 10 min. The extracts were

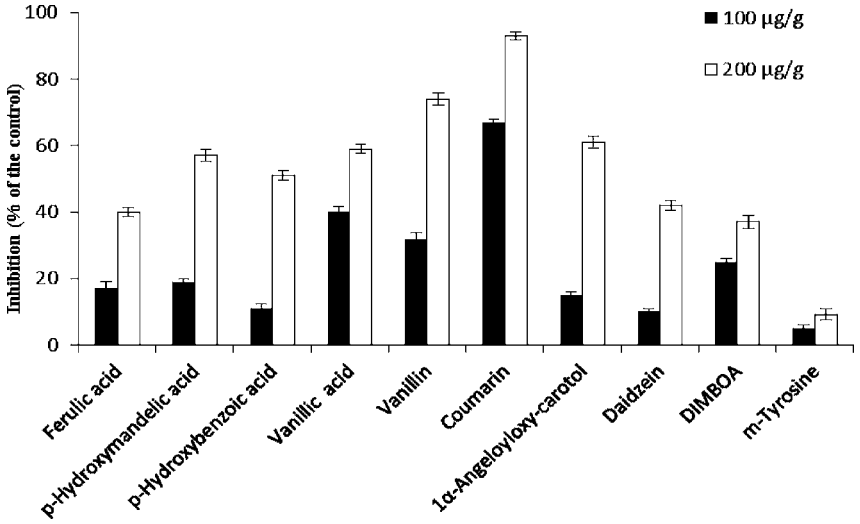

Figure 1. Inhibition of 10 allelochemicals tested on the germination of lettuce in soil. Mean ± standard error (SE) from three independent experiments for each determination is shown.

evaporated under vacuum. The residues were respectively dissolved in distilled water (5 mL) and loaded onto reversed phase C_{18} Sep-Pak cartridges (Waters Co.). The cartridge was eluted with 50% aqueous MeOH (5 × 3 mL) and then MeOH (3 × 3 mL), and the MeOH fraction was concentrated with nitrogen gas to obtain the concentrate (100 μL) for quantitative analysis.

Quantitative analysis of each allelochemical was conducted with a Waters ACQUITY UPLC quipped with a BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm) with a UV detector at a temperature of 45 °C. The injection volume of the samples was 5 μL. The detection wavelength was 260 nm. Elution was performed with a binary solvent system gradient consisting of H_2O (solvent A) and MeCN (solvent B) at a constant flow rate of 300 μL/min. A linear gradient profile with the following proportions (v/v) of solvent B was applied (0−15.0 min, linear gradient 20−80% B; 15.0−15.1 min, linear gradient 80−20%). The peaks of allelochemicals were each identified by their retention time and coelution with authentic allelochenicals described above. Working standard solutions ranging from 0.1 to 200 μg/g were prepared to establish a calibration curve. Regression analysis of the peak areas against standard concentrations was used to quantify the allelochemicals.

Similar manipulations as described above were conducted to examine the occurrence and level of 10 allelochemicals and their recoveries in soil samples. *p*-Hydroxybenzoic acid only at a concentration of 0.84 ± 0.02 μg/g was detected, whereas the other nine allelochemicals were not found in soil samples. The mean recoveries of known amounts of allelochemicals added into soil were 80.2% (ferulic acid), 76.5% (*p*-hydroxymandelic acid), 79.8% (*p*-hydroxybenzoic acid), 81.4% (vanillic acid), 77.8% (vanillin), 81.3% (coumarin), 77.2% (daidzein), 82.4% (1α-angeloyloxycarotol), 86.2% (DIMBOA), 75.4% (*m*-tyrosine), and 89.6% (2,4-D). These mean recoveries and *p*-hydroxybenzoic acid concentration were used to correct the concentrations determined in soil samples.

PLFA Analysis. The soil samples were collected from the dried and developed TLC plates without lettuce seeds, H_2O_2, and pH treatments as described above. The segments containing each allelochemical residue were fully mixed, and their soils were added into a series of vials, respectively. The water content of the soils in vials was maintained at 70% of water-holding capacity. The vials were sealed with airtight lids to prevent drying and then placed in an environmental chamber at a temperature of 25 °C. The vials were removed from the chamber after 48 h for PLFA analysis. PLFA analysis was conducted by a combination of two methods in the literature,[12,22] with minor modifications. Briefly, triplicate 5 g samples of freeze-dried and milled soil were extracted with a mixture of $CHCl_3$/MeOH/citrate buffer (1:2:0.8, v/v/v), and the phospholipids were separated from other lipids on a silica gel-filled solid-phase extraction cartridge (0.50 g Si, Supelco, Inc.). The samples were then subjected to mild alkaline methanolysis, and the resulting fatty acid methyl esters (FAME) were separated before being quantified and identified by GC-MS. The identification of FAME was based on retention time comparisons with FAME controls (Supelco, Inc.). Quantification was performed by calibration against standard solutions of nonadecanoate methyl ester (C19:0), which was also used as internal standard.

A total of 36 PLFAs were identified in the soil samples. Among them, the fatty acids present in proportions of >0.5% were used in the analysis. The sum of 18 fatty acids (14:0, 2OH14:0, i15:0, a15:0,15:0, i16:0, 16:0, 10Me16:0,16:1ω9,16:1ω7c, 2OH16:0, i17:0,17:0, cy17:0, 18:1ω7,18:0, cy19:0, and 20:0) was used to assess bacterial biomass. Among them, i15:0, a15:0, i16:0, and i17:0 were considered to be representative of Gram negative (−) bacteria, whereas 16:1ω7c, cy17:0, 18:1ω7c, and cy19:0 were considered to be representative of Gram positive (+) bacteria. Fungal biomass was assessed by quantifying 18:2ω6,9c and 18:1ω9. Before being subjected to principal component analysis (PCA), the results were expressed as a percentage of the total PLFA. PCA was performed by using the STATISTICA software package, version 6.0 (Statsoft Inc., Tulsa, OK, USA), applied separately to PLFA proportion, and showed relationships among different samples that contain multiple variables. PCA is useful for discerning patterns within the PLFA data itself and describes the axes of maximum variability in the multivariate data set.[28] Data were presented as a 2D plot to better understand the relationships among different samples.

RESULTS AND DISCUSSION

Ten allelochemicals inhibited the germination of lettuce in soil, but their inhibition varied greatly with structural types and concentrations. The lowest observed inhibition occurred in a nonprotein amino acid *m*-tyrosine (Figure 1). *m*-Tyrosine was identified as the major allelochemical from fine leaf fescue cultivar Intrigue (*Festuca rubra* spp. *commutata*), which strongly inhibited the germination and growth of lettuce.[25] However, the bioassays in that study were carried with filter paper rather than soil medium. A recent study indicated that soil microbial communities could obviate allelopathic effects of *m*-tyrosine.[29] In fact, the effect on evaluating allelochemicals without soil has been criticized because soil interactions radically alter the environment and give a much better indication of real effects.[4] A lot of events may occur when allelochemicals are administered through the soil such as adsorption, microbial degradation, and transformation.[1−3,7] In particular, soil microorganisms are an important determinant of allelopathic activity.[5,9,10] Regardless of allelocehemicals, there always was much stronger inhibition at a

Table 2. Changes and Correlation in the Mobility Factor (*Rf*) for Allelochemicals Chromatographed on Soil Thin-Layer Plates with Different pH Values and Organic Matter Contents[a]

	pH				organic matter content		
allelochemical	2.5	7.0	9.5	*r*	1.34%	0.97%	*r*
ferulic acid	0.073 ± 0.005aA	0.094 ± 0.004aA	0.203 ± 0.015bB	0.860	0.123 ± 0.003abA	0.213 ± 0.015bB	−0.976
p-hydroxymandelic acid	0.068 ± 0.002abA	0.085 ± 0.004aB	0.284 ± 0.005dC	0.762	0.140 ± 0.030bA	0.247 ± 0.006cB	−0.995
p-hydroxybenzoic acid	0.088 ± 0.003aA	0.084 ± 0.004aA	0.121 ± 0.011aB	0.933	0.097 ± 0.002aA	0.220 ± 0.010hbB	−0.938
vanillic acid	0.084 ± 0.001aA	0.093 ± 0.006aA	0.124 ± 0.003aB	0.852	0.736 ± 0.005gA	0.803 ± 0.006hB	−0.921
vanillin	0.600 ± 0.010eA	0.707 ± 0.015fB	0.804 ± 0.012hC	0.857	0.119 ± 0.001abA	0.144 ± 0.030aB	−0.965
coumarin	0.507 ± 0.015dA	0.573 ± 0.012eB	0.607 ± 0.015gC	1.000	0.645 ± 0.013fA	0.762 ± 0.007gB	−0.997
daidzein	0.263 ± 0.006cA	0.320 ± 0.017dB	0.513 ± 0.021fC	0.893	0.330 ± 0.010eA	0.362 ± 0.007fB	−0.992
1α-angeloyloxycarotol	0.126 ± 0.007bA	0.228 ± 0.019bB	0.238 ± 0.007cB	0.961	0.279 ± 0.009cdA	0.327 ± 0.015eB	−0.998
DIMBOA	0.263 ± 0.006cA	0.300 ± 0.010cdB	0.320 ± 0.010eB	0.879	0.313 ± 0.006deA	0.347 ± 0.006efB	−0.946
m-tyrosine	0.260 ± 0.010cA	0.270 ± 0.010cB	0.320 ± 0.012eB	0.912	0.273 ± 0.006cA	0.293 ± 0.006dB	−0.953
2,4-D	0.727 ± 0.040fA	0.910 ± 0.010gB	0.883 ± 0.006iB	0.857	0.913 ± 0.015hA	0.917 ± 0.006iA	−0.963

[a]Mean ± standard error (SE) from three independent experiments for each determination is shown. Data in a row followed by the same upper case letter between pH values or organic matter contents and data in a column followed by the same lower case letter among allelochemicals are not significantly different at $P < 0.05$, analysis of variance (ANOVA) followed by Tukey's honestly significant difference tests. *r* indicates Spearman rank correlation at $P < 0.001$.

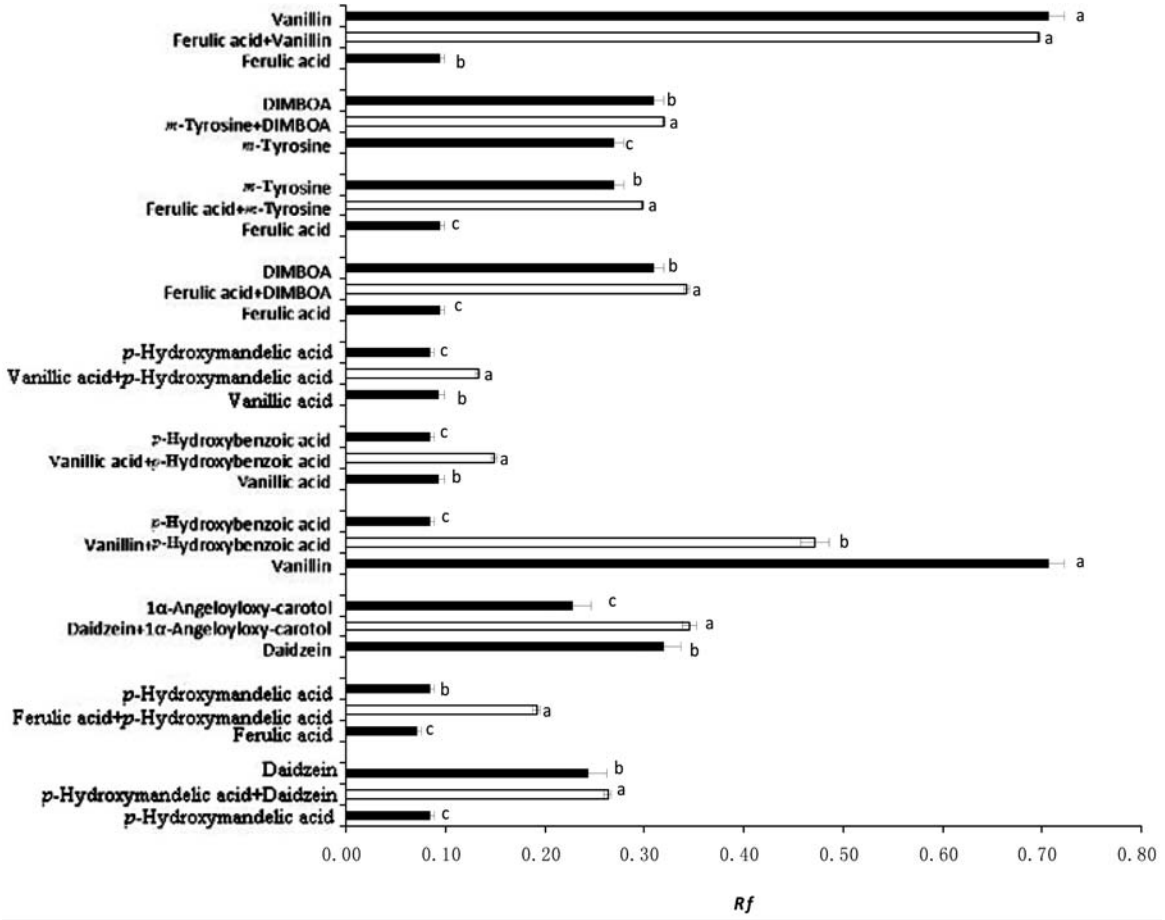

Figure 2. Joint action of various allelochemicals at 1:1 mixture proportion on the mobility factors. Mean ± standard error (SE) from three independent experiments for each determination is shown. Columns with different letters indicate significant differences between individual allelochemicals and their mixture at $P < 0.05$, analysis of variance (ANOVA) followed by Tukey's honestly significant difference test.

concentration of 200 μg/g than at 100 μg/g. *m*-Tyrosine was required to have an observable inhibition at a concentration of 200 μg/g (Figure 1). Therefore, subsequent soil TLC combined with the bioassay approach was conducted at this concentration.

There were significant differences in the mobility factor among allelochemicals tested. Ferulic acid, *p*-hydroxymandelic acid, *p*-hydroxybenzoic acid, and vanillic acid had very poor mobility ($Rf < 0.1$). Substantially differently from these four phenolic acids, however, phenolic aldehyde and lactone (vanillin and coumarin) showed excellent mobility ($Rf > 0.5$) in soil (Table 1). Similar to vanillin and coumarin, daidzein, 1α-angeloyloxycarotol, DIMBOA, and *m*-tyrosine showed good

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Table 3. Soil PLFA Concentrations (Nanomoles per Gram Dry Soil) and Selected Microbial Community Characteristics in the Segments Containing Allelochemical Residues in the Developed TLC Soil Layer[a]

PLFA	CK	ferulic acid	*p*-hydroxymandelic acid	*p*-hydroxybenzoic acid	vanillic acid	vanillin	coumarin	1α-angeloyloxycarotol	daidzein	DIMBOA	*m*-tyrosine
total PLFA	29.18 ± 0.13e	25.77 ± 0.37bc	27.25 ± 1.00d	27.45 ± 0.96d	24.54 ± 0.76b	19.63 ± 0.46a	27.62 ± 0.33d	29.80 ± 0.65e	30.35 ± 0.97e	25.18 ± 0.35b	29.08 ± 0.67e
18:2ω6,9c	2.50 ± 0.01d	2.55 ± 0.03d	2.28 ± 0.10c	2.89 ± 0.16e	1.92 ± 0.14b	1.71 ± 0.01b	2.80 ± 0.06e	2.08 ± 0.02c	2.52 ± 0.11d	1.31 ± 0.72a	1.25 ± 0.02a
18:1ω9c	2.11 ± 0.01a	1.69 ± 0.05a	2.11 ± 0.06a	1.85 ± 0.07a	1.93 ± 0.17a	1.61 ± 0.03a	2.42 ± 0.04ab	1.76 ± 0.05a	2.14 ± 0.05a	4.34 ± 0.47b	2.71 ± 0.02ab
fungi	5.65 ± 0.05d	5.29 ± 0.09c	5.16 ± 0.20c	5.12 ± 0.28c	4.80 ± 0.35ab	4.11 ± 0.22a	5.10 ± 0.11c	4.76 ± 0.12ab	5.14 ± 0.21c	5.04 ± 0.20c	4.14 ± 0.02a
i15:0	0.98 ± 0.01c	0.82 ± 0.04b	0.80 ± 0.05b	1.08 ± 0.07c	0.76 ± 0.06b	0.72 ± 0.01b	1.04 ± 0.03c	1.09 ± 0.03c	1.29 ± 0.07d	0.24 ± 0.03a	1.74 ± 0.02e
i16:0	2.69 ± 0.01d	2.57 ± 0.07 cd	2.36 ± 0.08c	2.71 ± 0.16d	2.34 ± 0.20c	1.93 ± 0.07b	2.84 ± 0.03d	3.14 ± 0.10e	3.55 ± 0.10f	0.72 ± 0.02a	0.81 ± 0.01a
16:1ω9	2.54 ± 0.01e	1.79 ± 0.04bc	2.24 ± 0.11de	2.30 ± 0.11de	2.11 ± 0.17cd	1.83 ± 0.01bc	2.07 ± 0.04cd	3.35 ± 0.07f	2.40 ± 0.10de	1.60 ± 0.35ab	1.37 ± 0.04a
cy19:0	1.12 ± 0.00f	1.05 ± 0.02ef	0.91 ± 0.01cd	0.99 ± 0.04de	0.95 ± 0.07d	0.82 ± 0.01bc	0.95 ± 0.01d	1.06 ± 0.02ef	1.12 ± 0.03f	0.73 ± 0.04b	0.49 ± 0.02a
12:00	0.76 ± 0.01abc	0.85 ± 0.03bc	0.87 ± 0.05cd	1.19 ± 0.09de	0.52 ± 0.05ab	0.48 ± 0.02a	0.93 ± 0.06cd	0.51 ± 0.02a	0.46 ± 0.01a	0.16 ± 0.01a	1.49 ± 0.37e
13:00	0.63 ± 0.01c	0.52 ± 0.04b	1.00 ± 0.03e	0.74 ± 0.03d	0.57 ± 0.06bc	0.52 ± 0.01b	0.78 ± 0.04d	0.57 ± 0.01bc	0.35 ± 0.01a	0.32 ± 0.01a	0.35 ± 0.03a
14:00	2.07 ± 0.02b	1.99 ± 0.04b	2.41 ± 0.13b	1.63 ± 1.20b	2.08 ± 0.17b	0.02 ± 0.00a	1.81 ± 0.00b	1.83 ± 0.04b	2.04 ± 0.06b	1.84 ± 0.04b	4.31 ± 0.21c
15:00	1.07 ± 0.03c	1.00 ± 0.05bc	0.82 ± 0.05bc	1.11 ± 0.06c	0.81 ± 0.07bc	0.72 ± 0.03b	0.93 ± 0.02bc	0.93 ± 0.04bc	0.94 ± 0.02bc	1.73 ± 0.33d	0.39 ± 0.04a
16:00	4.94 ± 0.04b	4.41 ± 0.12ab	4.08 ± 0.03a	4.28 ± 0.20a	4.23 ± 0.36a	3.91 ± 0.16a	4.28 ± 0.04a	5.56 ± 0.14c	6.68 ± 0.09d	6.88 ± 0.40d	4.23 ± 0.23a
17:00	2.54 ± 0.01f	1.79 ± 0.04b	2.24 ± 0.01de	2.30 ± 0.11def	2.11 ± 0.17d	1.83 ± 0.01bc	2.07 ± 0.04cd	3.35 ± 0.07h	2.40 ± 0.10ef	0.68 ± 0.03a	2.96 ± 0.13g
18:00	2.59 ± 0.0bc1	1.96 ± 0.07ab	2.71 ± 0.07bc	1.97 ± 0.06ab	2.07 ± 0.16ab	1.72 ± 0.04a	2.26 ± 0.10ab	2.26 ± 0.10ab	2.35 ± 0.02ab	2.51 ± 0.15b	3.35 ± 0.43c
20:00	0.47 ± 0.01b	0.66 ± 0.02c	0.52 ± 0.02b	0.69 ± 0.04c	0.29 ± 0.03a	0.27 ± 0.04a	0.53 ± 0.01b	0.54 ± 0.02b	0.32 ± 0.02a	0.26 ± 0.02a	0.67 ± 0.09c
bacteria	8.38 ± 0.06e	7.27 ± 0.07c	7.08 ± 0.30c	7.96 ± 0.44d	7.06 ± 0.54c	6.08 ± 0.23b	7.79 ± 0.11d	9.55 ± 0.04f	9.24 ± 0.34f	4.73 ± 0.37a	9.79 ± 0.13f
Gram(+)	3.67 ± 0.01c	3.29 ± 0.06b	3.16 ± 0.13b	3.79 ± 0.23c	3.10 ± 0.26b	2.64 ± 0.07a	3.89 ± 0.05cd	4.23 ± 0.12e	4.84 ± 0.16f	2.66 ± 0.01a	4.18 ± 0.07e
Gram(−)	3.60 ± 0.06e	2.83 ± 0.07cbcd	3.01 ± 0.15cd	3.18 ± 0.19de	3.02 ± 0.21cd	2.62 ± 0.19bc	2.96 ± 0.05cd	4.27 ± 0.12f	3.28 ± 0.15de	2.33 ± 0.42b	2.12 ± 0.06a

[a]Mean ± standard error (SE) from three independent experiments for each determination is shown. Data in a row followed by the same letter among allelochemicals are not significantly different at $P < 0.05$, analysis of variance (ANOVA) followed by Tukey's honestly significant difference tests. CK is a nontreated soil control.

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mobility when compared with the four phenolic acids tested. Their *Rf* values ranged from 0.24 to 0.32 (Table 1). The differentiation in mobility factor among the allelocehmicals results from preferential sorption on soil colloids. High polar allelochemicals, such as phenolic acids, are readily adsorbed in soil matrices, and thus they could not easily be moved from the spots deposited or rhizosphere to the water front or bulk soil.[3,6,15] Furthermore, no significant changes of the mobility factor (*Rf*) were observed in two detection methods between bioassay and segment quantification (Table 1). It appeared from the results that the mobility factor was dependent on the structural specificity of allelochemicals themselves rather than detection methods. Segment quantification or radioactively labeled allelochemicals in the developed TLC soil layer are complex manipulations,[17,19] whereas using sensitive plant species may quickly indicate the phytotoxic location of allelochemicals in the developed TLC soil layer.[16] This study highlights that a combination of soil TLC and bioassay methods is feasible to assess the mobility of allelochmicals in soil.

The mobility factor was influenced by soil pH and organic matter. There were positive relationships between *Rf* and pH values. In contrast to pH, there were negative relationships between *Rf* values and organic matter contents (Table 2). Ionized chemicals depend on the pH value of the matrix, whereas the organic matter content of soil is often an important factor in the sorption of nonionic chamicals.[17−19] Interestingly, the mixtures of various allelochemicals had much better mobility than those applied alone. With the exception of vanillin, the mobility factor of individual allelochemicals was increased significantly by mixing with other allelochemicals. In particular, a mixture of phenolic acids led to a greater increase in *Rf* values when compared with individual phenolic acids (Figure 2). Joint action of phenolic acid mixtures on the phytotoxic activity was observed several years ago.[30] It is thought that the concentration of an individual allelochemical is generally below its inhibition threshold, and the mixture of allelochemicals may synergistically result in adverse effects on the establishment of plants. This study shows that the joint action of allelochemical mixtures may be attributed to their mobility in the soil environment. Actually, there always is a diversity of allelochemicals in natural ecosystems, and the overall allelopathic role will be the net effects of all allelochemicals present in the soil. Through the joint action of allelochemical mixtures on the concentration and mobility, allelopathic plant species may regulate effectively the establishment of competitors in their immediate vicinity.

There were different soil microbial biomass and community structure in the segments containing allelochemical residues in the developed TLC soil layer. The PLFA profile of the effect of 10 allelochemicals in soil was observed for the signature lipid biomarkers of fungi and bacteria (Table 3). In comparison to the controls, ferulic acid, *p*-hydroxymandelic acid, *p*-hydroxybenzoic acid, vanillic acid, vanillin, coumarin, and DIMBOA led to a significant decrease in the total PLFA concentrations, fungal biomass, and bacterial biomass. The largest reduction occurred in vanillin with maximal mobility factor ($Rf > 0.7$) among the 10 allelochemicals tested. 1α-Angeloyloxycarotol, daidzein, and *m*-tyrosine had no significant effect on the total PLFA concentrations, but resulted in the decrease of the fungal biomass and the increase of bacterial biomass (Table 3). PLFA is a good indicator of the living microbial biomass and is closely correlated with microbial biomass C.[31] PLFA data generated in this study indicated that application of allelochemicals could have different effects on soil microbial biomass. Ferulic acid, *p*-hydroxymandelic acid, *p*-hydroxybenzoic acid, vanillic acid, vanillin, coumarin, and DIMBOA had inhibitory effects on the soil bacteria and fungi, whereas 1α-angeloyloxycarotol, daidzein, and *m*-tyrosine inhibited the soil fungi but stimulated the soil bacteria.

PCA clearly distinguished the composition of total PLFA between DIMBOA or *m*-tyrosine and all eight other allelochemicals tested (Figure 3). The soil microbial community structures

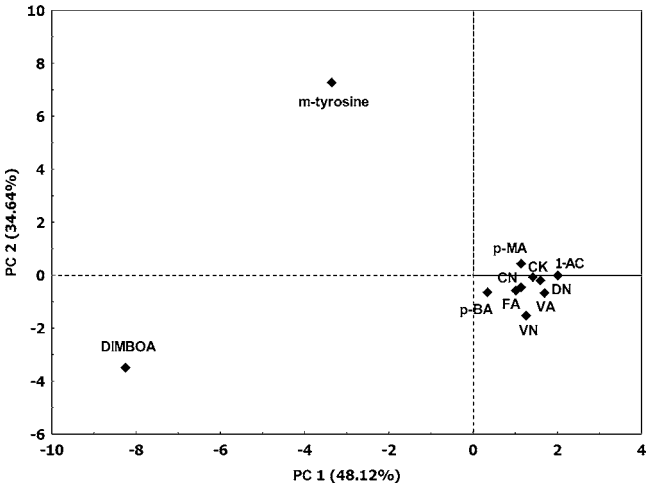

Figure 3. Principal component analysis using the relative abundance of individual PLFA in soils treated with all allelochemicals tested. PC indicates a principal component. CK, control; FA, ferulic acid; *p*-MA, *p*-hydroxymandelic acid; *p*-BA, *p*-hydroxybenzoic acid; VA, vanillic acid; VN, vanillin; CN, coumarin; 1-AC, 1α-angeloyloxycarotol; DN, daidzein.

were divided into three groups: DIMBOA alone, *m*-tyrosine alone, and phenolic compounds and α-angeloyloxycarotol together. Each group occupied a distinct ordination space. The first principal component (PC1 = 48.12%) and second principal component (PC2 = 34.64%) together accounted for 82.76% of the variation (Figure 3). This analysis revealed that allelochemicals tested could exert different effects on the soil microbial community structure. In particular, a remarkable difference occurred in DIMBOA and *m*-tyrosine. Substantially differently from phenolic compounds and α-angeloyloxycarotol, DIMBOA is a heterocyclic nitrogen compound and *m*-tyrosine is a nonprotein amino acid. Nitrogenous allelochemicals may provide organic N for the soil to change soil microbial communities.[32] Thus, both nitrogenous DIMBOA and *m*-tyrosine could have substantially different effects on the soil microbial community structure when compared with other non-nitrogenous allelochemicals tested in this study. Phenolic compounds, 1α-angeloyloxycarotol, and control occupied the same ordination space in all treatments (Figure 3), indicating that the microbial community structure resulted from similar chemical composition in soil. However, there were clearly differences among the treatments of the phenolic compounds, 1α-angeloyloxycarotol, and control once DIMBOA and *m*-tyrosine were separated from the other non-nitrogenous allelochemicals (Figure 4). The results imply that substantial changes in the soil microbial community structures depend on both the elemental and structural specificity of the allelochemicals tested.

Chemicals released from plant species have to diffuse and distribute to different locations through the soil environment

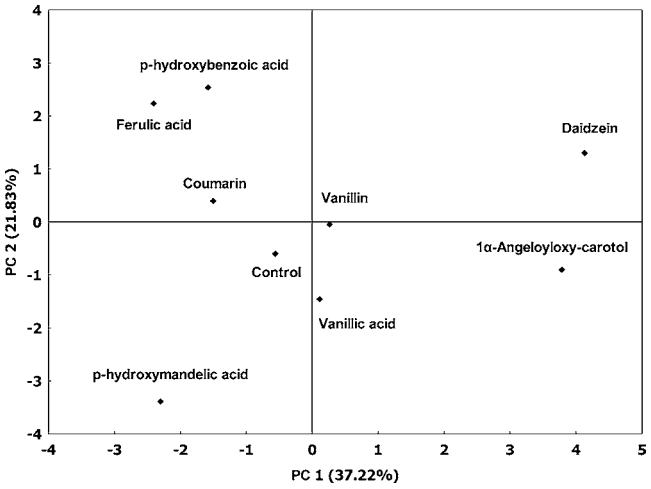

Figure 4. Plots of the soil microbial community structure in the treatments of 1α-angeloyloxycarotol and seven phenolic compounds by principal component analysis. PC indicates a principal component.

and then demonstrate their individual implications on neighboring species.[1,2,8] It is not appropriate to call them allelochemicals until they have been shown to be present in the vicinity of the target plants. On the basis of the results of this study showing the poor mobility of ferulic acid, *p*-hydroxymandelic acid, *p*-hydroxybenzoic acid, and vanillic acids ($Rf < 0.1$), it is unlikely that these phenolic acids would be transported far from their point of origin, limiting their allelopathic effects on the target plants.[6,15] For these phenolic acids to have allelopathic effects on plants, they would have to combine with other allelochemicals in the soil environment, resulting in an advantage through the joint action of allelochemicals to good mobility. Whereas vanillin, coumarin, daidzein, 1α-angeloyloxycarotol, DIMBOA, and *m*-tyrosine with good mobility could move from their point of origin to the vicinity of the target plants, exerting allelopathic effect independently.

Soil microorganisms play an important role in regulating the concentration of allelochemicals.[4,9] During the movement, soil microorganisms take advantage of allelochemicals as carbon substrates. In turn, efficient allelochemicals may regulate the soil microbial communities.[4,5,10,11] This study reveals that the action of allelochemicals in soil depends on their mobility and that moved allelochemicals affect the soil microbial community structure. The establishment of soil TLC combined with a bioassay approach, as well as its further application in a wide diversity of allelochemicals, may contribute to a better understanding of allelochemical behavior and process in the soil environment.

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Notes

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REFERENCES

(1) Macias, F. A.; Oliveros-Bastidas, A.; Marin, D.; Castellano, D.; Simonet, A. M.; Molinillo, J. M. G. Degradation studies on benzoxazinoids. Soil degradation dynamics of 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA) and its degradation products, phytotoxic allelochemicals from Gramineae. *J. Agric. Food Chem.* **2004**, *52*, 6402−6413.

(2) Kobayashi, K. Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biol. Manag.* **2004**, *4*, 1−7.

(3) Tharayil, N.; Bhowmik, P. C.; Xing, B. S. Bioavailability of allelochemicals as affected by companion compounds in soil matrices. *J. Agric. Food Chem.* **2008**, *56*, 3706−3713.

(4) Kong, C. H.; Wang, P.; Gu, Y.; Xu, X. H.; Wang, M. L. The fate and impact on microorganisms of rice allelochemicals in paddy soil. *J. Agric. Food Chem.* **2008**, *56*, 5043−5049.

(5) Fomsgaard, I. S.; Mortensen, A. G.; Idinger, J.; Coja, T.; Blumel, S. Transformation of benzoxazinones and derivatives and microbial activity in the test environment of soil ecotoxicological tests on *Poecilus cupreus* and *Folsomia candida*. *J. Agric. Food Chem.* **2006**, *54*, 1086−1092.

(6) Tharayil, N.; Bhowmik, P. C.; Xing, B. S. Preferential sorption of phenolic phytotoxins to soil: implications for altering the availability of allelochemicals. *J. Agric. Food Chem.* **2006**, *54*, 3033−3040.

(7) Furubayashi, A.; Hiradate, S.; Fujii, Y. Adsorption and transformation reactions of L-DOPA in soils. *Soil Sci. Plant Nut.* **2005**, *51*, 819−825.

(8) Gimsing, A. L.; Blum, J.; Dayan, F. E.; Locke, M. A.; Sejer, L. H.; Jacobsen, C. S. Mineralization of the allelochemical sorgoleone in soil. *Chemosphere* **2009**, *76*, 1041−1047.

(9) Inderjit, S. Soil microorganisms: an important determinant of allelopathic activity. *Plant Soil* **2005**, *274*, 227−236.

(10) Cipollini, D.; Rigsby, C. M.; Barto, E. K. Microbes as targets and mediators of allelopathy in plants. *J. Chem. Ecol.* **2012**, *38*, 714−727.

(11) Chen, K. J.; Zheng, Y. Q.; Kong, C. H.; Zhang, S. Z.; Li, J.; Liu, X. G. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-methoxybenzoxazolin-2-one (MBOA) levels in the wheat rhizosphere and their effect on the soil microbial community structure. *J. Agric. Food Chem.* **2010**, *58*, 12710−12716.

(12) Guo, Z. Y.; Kong, C. H.; Wang, J. G.; Wang, Y. F. Rhizosphere isoflavones (daidzein and genistein) levels and their relation to the microbial community structure of mono-cropped soybean soil in field and controlled conditions. *Soil Biol. Biochem.* **2011**, *43*, 2257−2264.

(13) Teasdale, J. R.; Rice, C. P.; Cai, G. M.; Mangum, R. W. Expression of allelopathy in the soil environment: soil concentration and activity of benzoxazinoid compounds released by rye cover crop residue. *Plant Ecol.* **2012**, *123*, 1893−1905.

(14) Cecchi, A. M.; Koskinen, W. C.; Cheng, H. H.; Haider, K. Sorption-desorption of phenolic acids as affected by soil properties. *Biol. Fert. Soils* **2004**, *39*, 235−242.

(15) Inderjit, S.; Bhowmik, P. C. Sorption of benzoic acid onto soil colloids and its implications for allelopathy studies. *Biol. Fert. Soils* **2004**, *40*, 345−348.

(16) Kong, C. H.; Zhao, H.; Xu, X. H.; Wang, P.; Gu, Y. Activity and allelopathy of soil of flavone *O*-glycosides from rice. *J. Agric. Food Chem.* **2007**, *55*, 6007−6012.

(17) Ravanel, P.; Liegeois, M. H.; Chevallier, D.; Tissut, M. Soil thin-layer chromatography and pesticide mobility through soil microstructures: new technical approach. *J. Chromatogr., A* **1999**, *864*, 145−154.

(18) Li, S. N.; Sun, Y.; Yang, T.; Huangpu, W. G. Relationship between mobility factors (*Rf*) of two hydrophobic termiticides and selected field and artificial soil parameters. *Sci. Total Environ.* **2007**, *388*, 206−213.

(19) Sherma, J. Review of advances in the thin layer chromatography of pesticides: 2006−2008. *J. Environ. Sci. Health B* **2009**, *44*, 193−203.
(20) Strebe, T. A.; Talbert, R. E. Sorption and mobility of flumetsulam in several soils. *Weed Sci.* **2001**, *49*, 806−813.
(21) Williams, W.; Wehtje, G.; Walker, R. H. Quinclorac: soil behavior and foliar vs. root absorption by torpedograss (*Panicum repens*). *Weed Technol.* **2004**, *18*, 626−633.
(22) Marschner, P. Soil microbial community structure and function assessed by FAME, PLFA and DGGE; advantages and limitations. In *Modern Methods in Soil Microbiology*; Varma, A., Oelmueller, R., Eds.; Springer: New York, 2007; pp 181−200.
(23) Yamamoto, T.; Yokotani-Tomit, K.; Yamamura, S.; Yamada, K.; Hasegawa, K. Allelopathic substance exuded from a serious weed, germinating barnyard grass (*Echinochloa crus-galli* L.) roots. *J. Plant Growth Regul.* **1999**, *18*, 65−67.
(24) Kong, C. H.; Wang, P.; Xu, X. H. Allelopathic interference of *Ambrosia trifida* with wheat (*Triticum aestivum*). *Agric. Ecosyst. Environ.* **2007**, *119*, 416−420.
(25) Bertin, C.; Weston, L. A.; Huang, T. F.; Jander, G.; Owens, T.; Meinwald, J.; Schroeder, F. C. Grass roots chemistry: meta-tyrosine, an herbicidal nonprotein amino acid. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16964−16969.
(26) Larsen, E.; Christensen, L. P. Simple method for large scale isolation of the cyclic arylhydroxamic acid DIMBOA from maize (*Zea mays* L.). *J. Agric. Food Chem.* **2000**, *48*, 2556−2558.
(27) Fujii, Y.; Shibuya, T.; Yasuda, T. Method for screening allelopathic activities by using the logistic function fitted to lettuce seed germination and growth curves. *Weed Res.* **1990**, *35*, 353−361.
(28) Bossio, D. A.; Scow, K. M.; Gunapala, N.; Graham, K. J. Determinants of soil microbial communities: effect of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microb. Ecol.* **1998**, *36*, 1−12.
(29) Kaur, H.; Kaur, R.; Kaur, S.; Baldwin, I. T.; Inderjit, S. Taking ecological function seriously: Soil microbial communities can obviate allelopathic effects of released metabolites. *PLoS One* **2009**, *4*, e4700.
(30) Inderjit, S.; Streibig, J. C.; Olofsdotter, M. Joint action of phenolic acid mixtures and its significance in allelopathy research. *Physiol. Plant.* **2002**, *114*, 422−428.
(31) Baath, E.; Anderson, T. H. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biol. Biochem.* **2003**, *35*, 955−963.
(32) Wang, P.; Kong, C. H.; Sun, B.; Xu, X. H. Allantoin-induced change of microbial diversity and community in rice soil. *Plant Soil* **2010**, *332*, 357−368.