2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-Methoxy-benzoxazolin-2-one (MBOA) Levels in the Wheat Rhizosphere and Their Effect on the Soil Microbial Community Struc...

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · NOVEMBER 2010

Impact Factor: 2.91 · DOI: 10.1021/jf1032608 · Source: PubMed

6 AUTHORS, INCLUDING:

Chui-Hua Kong
China Agricultural University
96 PUBLICATIONS **1,181** CITATIONS

SEE PROFILE

DOI:10.1021/jf1032608

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-Methoxy-benzoxazolin-2-one (MBOA) Levels in the Wheat Rhizosphere and Their Effect on the Soil Microbial Community Structure

Ke-Jing Chen,[†] Yong-Quan Zheng,[‡] Chui-Hua Kong,*[,†] Song-Zhu Zhang,[†] Jing Li,[‡] and Xing-Gang Liu[‡]

[†]College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China, and [‡]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Despite increasing knowledge of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-methoxy-benzoxazolin-2-one (MBOA) as allelochemicals involved in the defense of wheat against pests, relatively little is known about their levels in the rhizosphere and interactions with the soil microbial community. This study quantified DIMBOA and MBOA in the wheat rhizosphere and analyzed the soil microbial community structure. MBOA rather than DIMBAO was found in the wheat rhizosphere, and its concentration varied with cultivars, plant densities, and growth conditions. Wheat could detect the presence of competing weeds and respond by increased MBOA in the rhizosphere. There was a linear positive relationship between the MBOA level in the wheat rhizosphere and soil fungi/bacteria. When DIMBOA was applied to soil, yielding MBOA increased soil fungi. There were different phospholipid fatty acid (PLFA) patterns in soil incubated with DIMBOA and MBOA. These results suggested that DIMBOA and MBOA could affect the soil microbial community structure to their advantage through the change in fungi populations.

KEYWORDS: *Triticum aestivum*; benzoxazinoids; rhizosphere; weed competition; soil fungi; microbial community structure

INTRODUCTION

2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) is the dominant compound in benzoxazinoids found in wheat (*Triticum aestivum*) roots (*1*), which is involved in the defense of wheat against various pests, most notably in allelopathy, affecting the germination and growth of weeds associated with wheat crop (*2*). DIMBOA has been identified as an important allelochemical in wheat (*3*−*5*). In particular, a holistic approach on research into allelopathy was launched in the FATEALLCHEM project (*6*). DIMBOA and other benzoxazinoids from wheat were the main group of allelochemicals studied in the project (*1*, *6*−*9*).

6-Methoxy-benzoxazolin-2-one (MBOA) is an intermediate in the degradation pathway from DIMBOA to 2-amino-7-methoxy-3*H*-phenoxazin-3-one. When leached and released to the soil, DIMBOA is rapidly transformed to MBOA, which is more resistant toward degradation in soil (*3*). MBOA has a relatively broad-spectrum activity on weeds in a dose-dependent manner and specificity often occurs in cropping systems (*10*). There is a wealth of information on the herbicidal potential and soil transformation of DIMBOA and MBOA (*3*, *7*−*9*, *11*). However, when it comes to the effect on the soil microbial community, there is a lack of data, which calls for further studies.

*To whom correspondence should be addressed. Telephone: +86-10-62732752. Fax: +86-10-62731016. E-mail: kongch@cau.edu.cn.

Roots release allelochemicals to the soil at rates of significance to interact with soil microorganisms (*12*). Roots are able to exert an effect on soil microorganisms through the release of allelochemicals. In turn, soil microorganisms consume and decompose allelochemicals and are an important determinant of allelopathic activity (*13*, *14*). A few studies have clearly shown that DIMBOA and MBOA together with some degradation products released from wheat residues or living roots can affect soil microorganisms, in particular, pathogenic fungi (*15*−*17*). However, it remains obscure whether and how the microbial community structure was affected by exudation and addition of DIMBOA and MBOA to the soil.

Although distribution and exudation of DIMBOA in wheat are variety-specific, a few wheat varieties do release considerable amounts of DIMBOA into their growing medium (*3*−*5*, *18*). Much efforts have been performed to identify and quantify DIMBOA and MBOA in wheat tissues and growth media (*1*, *3*−*5*, *18*), but there are few studies on the quantification in the rhizosphere. The rhizosphere encompasses the millimeters of soil surrounding a wheat root, where complex biological and ecological processes occur (*19*). Wheat roots contain several benzoxazinoids, including DIMBOA, MBOA, 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), and benzoxazolin-2-one (BOA), but the dominant compound is DIMBOA (*1*). Moreover, there is more DIMBOA in wheat roots than in wheat shoots (*5*). Therefore, the concentration of DIMBOA root-exuded and/or MBOA could be locally much higher in

 Published on Web 11/18/2010

the wheat rhizosphere. In this manner, DIMBOA and MBOA would likely affect the soil microbial community structure.

The soil microbial community plays an essential role in the interactions between allelochemicals and microorganisms (*12*,*14*). Studying the composition of the soil microbial community has been very difficult in the past because of methodological limitations. However, new technologies, such as phospholipid fatty acid (PLFA) analysis, polymerase chain reaction (PCR)−denaturing gradient gel electrophoresis (DGGE), and physiological profiles in microplates, have greatly helped the study of the soil microbial community (*20*). In the present study, we quantified DIMBOA and MBOA in wheat rhizosphere using liquid chromatography−electrospray ionization−tandem mass spectrometry (LC−ESI−MS/MS) and analyzed the soil microbial community using PLFA profiles. Furthermore, the changes in the microbial community structure following DIMBOA and MBOA application to the soil were evaluated, with an attempt to help our further understanding of the allelochemical interference of wheat with soil microorganisms.

MATERIALS AND METHODS

Plant Materials, Soil, and Chemicals. Six winter wheat cultivars (Duokang1, Jing411, Lumai168, Nongda211, Zhongbeizhong39, and Zhongfu9507) were used in this study (**Table 1**). These cultivars were selected on the basis of their commercial importance and popularity in the local wheat industry. Seeds of three weeds, crabgrass (*Digitaria sanguinalis*), wild oat (*Avena fatua*), and barnyard grass (*Echinochloa crus-galli*), used in this study were collected from wheat fields during their growing seasons in 2008. The seeds were dried under sunlight and then stored in sealed glass jars. All of seeds were sterilized with 5% NaClO for 10 min and rinsed with distilled water before use.

Soil was collected from a field at the experimental station of China Agricultural University (Beijing, China). Soil was air-dried, mixed, and then sieved (2 mm mesh) to remove plant tissues. Soil was a silty loam with a pH of 6.92, organic matter content of 20.5 g/kg, and fertility status of available N, 102.4 mg/kg; available P, 16.5 mg/kg; and available K, 206.2 mg/kg.

DIMBOA was isolated from maize (*Zea mays*) seedlings by the method developed in Larsen and Christensen' laboratory (*21*). Authentic MBOA was obtained from Alfa Aesar Co., Ltd., while other organic solvents and chemicals were purchased from a local market in Beijing, China, and were of the highest purity available.

Field Trials. A wheat field at the experimental station described above was divided into numerous plots (2 × 3 m) that were in a completely randomized design, with three replicates for each wheat cultivar tested. Each plot was separated by trenches with at least 20 cm on each side. Six wheat cultivars were each sown into the plots at a density of 450 seeds/m^2 in October, 2009. Before sowing, the seed viability of all cultivars was examined using germination percentage (>98%). Field management was carried out according to the rules of the local rural administration. In March, 2010, wheat seedlings of each cultivar were harvested to collect their rhizosphere soils, as described below.

Greenhouse Experiments. Three experiments were carried out in a greenhouse with 20−30 °C night and daytime temperatures and 65−90% relatively humidity maintained. The experiments were conducted with five replications and repeated three times under identical conditions.

The first experiment was for the growth of six wheat cultivars tested. A total of 10 pre-germinated seeds of each cultivar were uniformly sown in the plastic pots (10 × 13 cm) containing 800 g of soil collected from the experimental station described above. The second experiment was for the growth of Jing411, a MBOA-rich wheat cultivar (**Table 1**), with varying plant density. Pre-germinated Jing411 seeds were uniformly sown in the plastic pots (10 × 13 cm) containing 800 g of soil at a density of 0, 5, 10, 20, 40, or 80 plants/pot, respectively.

The third experiment was for the growth of wheat−weed co-plantings without root contact. A series of plastic pots (10 × 13 cm) were used in this experiment. The center of the pots was placed a nylon net bag (6 × 10 cm, 300 mesh) containing 400 g of soil, and then the pots were filled with another 400 g of soil surrounding the nylon net bag outside. A total of 5 or 20 pre-germinated Jing411 seeds were uniformly sown in the nylon net bags, while 5 or 20 pre-germinated seeds of three weeds (crabgrass, wild oat, and barnyard grass) were sown surrounding the nylon net bags outside, respectively. The pots with 5 or 20 wheat plants rather than weeds surrounding the nylon net bags outside served as the controls. The nylon net bag blocked the contact of root systems between wheat and weed, but nutrients, chemicals, and microorganisms could exchange free in the pots.

Table 1. Soil Concentration (μg/g of Dry Soil) of DIMBOA and MBOA in the Rhizosphere of Wheat Seedlings with Varying Cultivars under Field Situations and Greenhouse Conditions[a]

	field		greenhouse	
wheat cultivar	DIMBOA	MBOA	DIMBOA	MBOA
Duokang1	ND[b]	0.16 ± 0.05 d	ND	1.05 ± 0.08 c
Jing411	ND	0.52 ± 0.08 a	ND	3.71 ± 0.12 a
Lumai168	ND	0.22 ± 0.04 c	ND	2.06 ± 0.08 b
Nongda211	ND	0.34 ± 0.06 b	ND	1.84 ± 0.96 b
Zhongbeizhong39	ND	0.36 ± 0.04 b	ND	3.19 ± 0.18 a
Zhongfu9507	ND	0.14 ± 0.02 d	ND	0.92 ± 0.06 c

[a] Different letters indicate significant differences among wheat cultivars at $p < 0.05$ [one-way analysis of variation (ANOVA), followed by Tukey's honestly significantly different (HSD) tests]. [b] ND = not detected.

All pots in three experiments described above were randomized and watered by tap once a day. Any plant species germinating in the pots other than wheat or test weed species were hand-removed soon after they were detected. At the three-leaf stage, wheat seedlings were harvested and their rhizosphere soils were sampled, as described below.

Rhizospere Soil. Rhizosphere soil was collected by pulling plants from the soil and shaking soil off from roots (*22*). Wheat seedlings at various experiments described above were collected from the plots or pots, respectively. The plants were excavated and air-dried. After the loosely adhering soil was shaken off, the roots were cut from plants and put inside glass tubes. The tubes were vigorously shaken, and soil that was still held to the roots was sampled. The rhizosphere soil was then sieved to 0.2 mm to remove visible root pieces. The soil samples were divided into several sub-samples, which were stored differently depending upon the quantification of DIMBOA and MBOA, and PLFA analysis, as described below, to be carried out.

Soil Incubated with DIMBOA and MBOA. A series of 150 mL vials was filled with 100 g of soil collected from the experimental station described above. DIMBOA or MBOA was added into the treated vials at a concentration of 5 μg/g of dry soil. This concentration was the maximal quantity of MBOA determined in the wheat rhizosphere (**Figure 1**). The control vials received distilled water only. The vials were sealed with airtight lids and then placed in an environmental chamber with a temperature of 26 °C. The vials were taken out from the chamber randomly after various incubation time intervals (1, 48, or 96 h), and the soils were taken for the quantification of DIMBOA and MBOA, and PLFA analysis, as described below.

Quantification of DIMBOA and MBOA. Quantification of DIMBOA and MBOA in soil samples was performed with a pressurized liquid extraction−solid-phase extraction followed by the LC−ESI−MS/MS method (*23*, *24*), with some modifications. Soil samples were freeze-dried and ground. An amount of 500 mg of the resulting powder was extracted with 10 mL of MeOH and then EtOAC, which was agitated for 4 h at 25 °C, and centrifuged for 10 min at 2800*g*. The supernatant combined with MeOH and EtOAC was dried under nitrogen gas to give residues. The residues were redissolved in 2.5 mL of 0.05% HOAc in MeOH−H_2O−CN (60:40) and then applied to the reversed-phase C_{18} Sep-Pak cartridge (Waters Co.). Analytes were eluted using 5 mL of acidified MeOH (1% HOAc).

Quantitative analysis of DIMBOA and MBOA was carried out using a Waters ACQUITY TQD tandem quadrupole UPLC−MS/MS system. A $BEHC_{18}$ (Waters, Milford, MA) column (50 × 2.1 mm, 1.7 μm) with a solvent flow rate of 300 μL/min was used. Acidified H_2O (0.2% HOAc) and MeOH were used as the elution solvents. The sample injection volume was set at 5 μL. MS with fowling settings of ionization mode was ESI. Nitrogen was used as the desolvation and cone gas, and argon was used as the collision gas. The source and desolvation temperatures were 150 and 350 °C, respectively. Capillary voltage was 3.5 kV. The multiple reaction

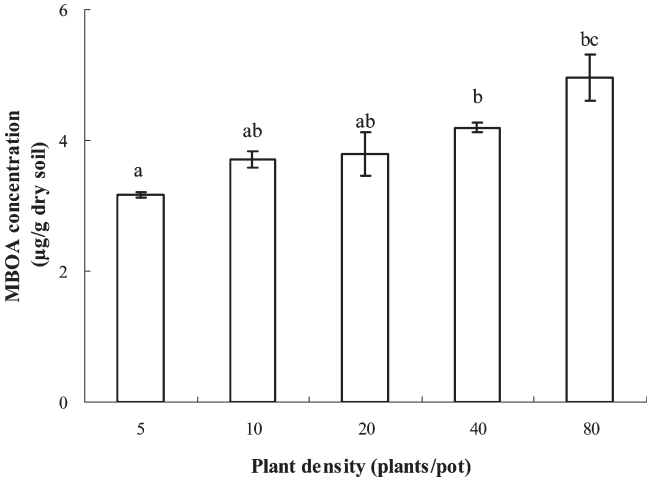

Figure 1. Soil concentration of MBOA in the rhizosphere of Jing411 seedlings with varying plant densities. Columns with the same letters are not significantly different at $p < 0.05$.

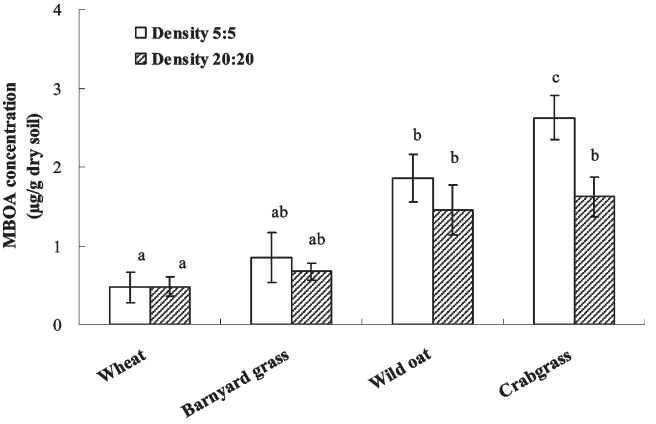

Figure 2. Response of MBOA production in the Jing411 rhizosphere associated with weeds. Columns with the same letters are not significantly different at $p < 0.05$.

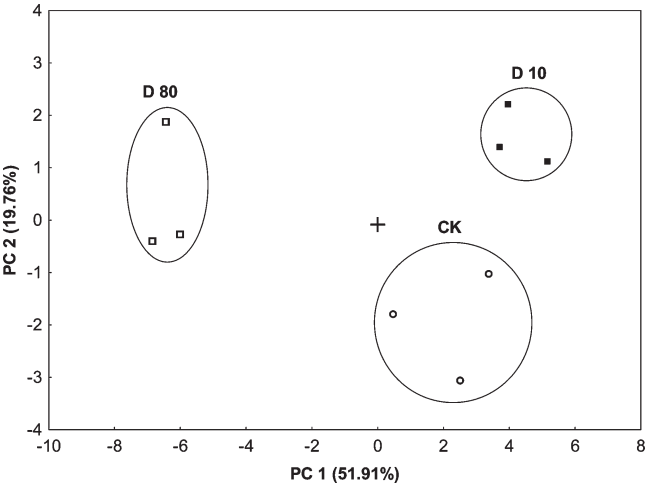

Figure 3. Plots of the soil microbial community structure in the rhizosphere of Jing411 at low (10 plants/pot, D10) and high (80 plants/pot, D80) plant densities by PCA. PC indicates a principle component, and + indicates the (0, 0) points.

monitoring (MRM) mode was used to monitor the parent and product ions. The following parameters were used for the identification of DIMBOA and MBOA, respectively. For DIMBOA: retention time, 2.03 min; parent ion, m/z 212; product ions, m/z 194 and 166; dwell time, 0.100 s; cone voltage, 20 V; and collision energy, 10 eV. For MBOA: retention time, 1.78 min; parent ion, m/z 166; product ions, m/z 110 and 95; dwell time, 0.100 s; cone voltage, 35 V; and collision energy, 20 eV. MBOA was quantified by interpolating the peak area on the LC chromatograms to a standard curve constructed by the peak area of authentic MBOA (Alfa Aesar Co., Ltd.). MBOA concentration, 1 μg/g = 2258.75 (peak area); linearity, $r^2 = 0.99$ ($y = 0.059x + 0.281$); recovery, 98%; reproducibility, 8 [relative standard deviation (RSD), $n = 5$].

PLFA Analysis. PLFA analysis was conducted by the method previously developed in the authors' laboratory (*12*). Briefly, triplicate 5 g (dry weight) sub-samples of milled and freeze-dried soil were extracted with a mixture of $CHCl_3$/MeOH/citrate butter (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 of Si, Supelco, Inc.). Samples were then subjected to mild alkaline methanolysis, and the resulting fatty acid methyl esters (FAMEs) were separated, quantified, and identified by gas chromatography−mass spectrometry (GC−MS). Identification of FAMEs was based on retention time comparisons to FAME controls (Supelco, Inc.). Quantification was carried out by calibration against standard solutions of nonadecanoate methyl ester (C19:0), which were also used as the internal standard.

A total of 36 PLFAs were identified in the soil samples. Among them, fatty acids present in proportions >0.5% were used in the analysis. The sum of 18 fatty acids was used to assess bacterial biomass (14:0, 2-OH14:0, i15:0, a15:0, 15:0, i16:0, 16:0, 10Me16:0, 16:1ω9, 16:ω7c, 2-OH16:0, i17:0, 17:0, cy17:0, 18:1ω7, 18:0, cy19:0, and 20:0). The actinobacterial biomass was indicated by the biomarker of 10Me16:0. Fungal biomass was assessed by quantifying 18:2ω6,9c and 18:1ω9. The ratio between the PLFAs i15:0, a15:0, i16:0, and i17:0 to PLFAs 16:1ω7c, cy17:0, 18:1ω7c, and cy19:0 was used as an index of Gram-negative and Gram-positive bacteria (GN/GP). Before the results were subjected to principal component analysis (PCA) and discriminant analysis (DA), they were expressed by the percentage of the total PLFA.

Data Analysis. Data were presented as the mean ± standard error (SE) from three independent experiments for each determination. We use a one-way ANOVA and Tukey's HSD tests for post-hoc analysis to compare the different effects of density and application of DIMBOA and MBOA. PCA and DA were performed using the STATISTICA software package, version 6.0 (Statsoft, Inc., Tulsa, OK). Data used in the PCA and DA plots were transformed using sample unit totals to represent the relative abundance of each PLFA (mole percentage of total PLFA).

RESULTS AND DISCUSSION

MBOA was found in the rhizosphere of six wheat cultivars tested, but its concentration varied greatly with cultivars and growth conditions. A few cultivars, such as Jing411 and Zhongbeizhong39, produced considerable amounts of MBOA in their rhizosphere (**Table 1**). Regardless of cultivars tested, there was always a higher concentration in the controlled condition than in the field situations. The MBOA concentrations in the wheat rhizosphere ranged from 0.9 to 3.7 μg/g of dry soil in greenhouse experiments but rarely exceed 0.5 μg/g of dry soil in field trials (**Table 1**). DIMBOA was not detected in the rhizosphere of all wheat cultivars tested under field situations and greenhouse conditions (**Table 1**). Although DIMBOA released from wheat roots could be detected in root exudates and agar media (*4*,*5*), it decomposed rapidly into MBOA once released into aqueous solution and soil (*3*, *25*). When wheat sprouts were incorporated into the soil, MBOA was detected as the main compound in soil (*9*), while MBOA was more resistant toward degradation in soil (*3*). Therefore, MBOA rather than DIMBOA in the wheat rhizosphere was found in this study.

Further experiments, with a MBOA-rich wheat cultivar Jing411, showed that the MBOA concentration in the wheat rhizosphere increased with an increasing plant density but the increase was much lower in the MBOA concentration than in the plant density. The significant difference in the MBOA concentration was observed up to an 8-fold increase in plant density

Table 2. Soil PLFA Concentration (nmol/g of Dry Soil) and Selected Microbial Community Characteristics in the Rhizosphere of Jing411 Seedlings with Varying Plant Densities[a]

	plant density (plants/pot)		
PLFA	0 (control)	10	80
14:0	10.868 ± 0.299 a	9.421 ± 0.496 a	13.939 ± 0.343 b
i15:0	2.435 ± 0.203 b	1.367 ± 0.098 a	2.070 ± 0.119 b
a15:0	2.591 ± 0.316 a	2.448 ± 0.282 a	3.438 ± 0.463 a
15:0	2.513 ± 0.128 a	2.630 ± 0.248 a	3.307 ± 0.463 a
16:1ω9c	18.595 ± 1.006 a	15.510 ± 0.759 a	24.664 ± 1.449 b
16:0	15.321 ± 1.193 a	19.667 ± 1.201 b	25.605 ± 2.193 c
2-OH14:0	10.283 ± 0.849 a	9.434 ± 0.804 a	10.373 ± 1.029 a
2-OH16:0	11.522 ± 0.927 a	11.039 ± 1.107 a	14.058 ± 1.105 a
i17:0	38.905 ± 4.183 a	36.595 ± 3.829 a	45.167 ± 4.022 b
17:0	22.500 ± 1.790 a	21.138 ± 1.35 a	24.519 ± 1.543 a
18:1ω7c	4.437 ± 0.637 b	2.500 ± 0.222 a	8.525 ± 0.758 c
18:0	13.490 ± 1.274 a	15.559 ± 1.280 a	12.338 ± 1.371 a
cy19:0	22.778 ± 2.427 a	24.177 ± 2.633 a	24.667 ± 1.351 a
20:0	3.372 ± 0.312 a	4.090 ± 0.337 a	3.558 ± 0.361 a
bacterial biomass	179.969 ± 10.757 a	175.576 ± 9.068 a	218.228 ± 12.395 b
18:2ω6,9c	2.166 ± 0.227 a	8.844 ± 0.933 b	18.685 ± 1.280 c
18:1ω9c	6.926 ± 0.630 a	10.597 ± 1.327 c	8.784 ± 0.801 b
fungal biomass	9.901 ± 1.154 a	19.440 ± 2.232 b	27.469 ± 3.142 c
actinobacterial biomass	29.753 ± 2.324 a	27.580 ± 2.661 a	26.272 ± 2.293 a
GN/GP	1.043 ± 0.181 a	1.044 ± 0.193 a	1.181 ± 0.163 a
fungi/bacteria	0.051 ± 0.003 a	0.111 ± 0.007 b	0.126 ± 0.012 c
total PLFA	218.813 ± 18.672 a	222.597 ± 20.665 a	271.968 ± 23.947 b

[a] Different letters indicate significant differences among plant densities at $p < 0.05$ (one-way ANOVA, followed by Tukey's HSD tests).

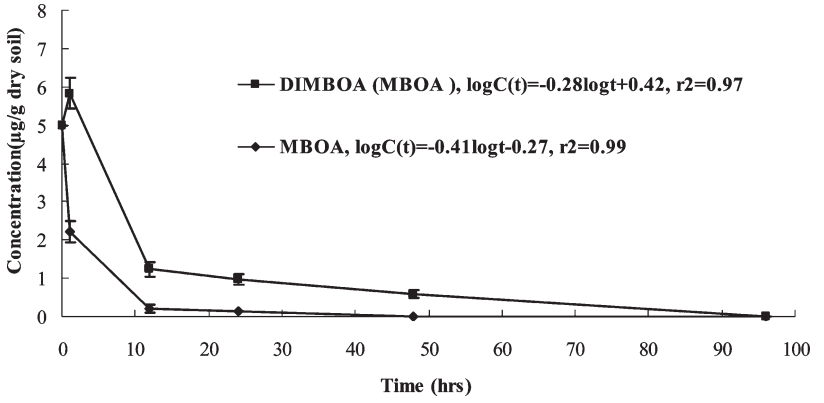

Figure 4. Soil degradation dynamics of DIMBOA and MBOA incubated with a maximal quantity (5 μg/g of dry soil) determined in the wheat rhizosphere.

(**Figure 1**). Furthermore, the MBOA concentration in the wheat rhizosphere varied with weed associations. In particular, associated weeds crabgrass and wild oat led to a great increase of the MBOA concentration in the wheat rhizosphere (**Figure 2**). Several studies have shown that the production of allelochemicals is known to respond when allelopathic crops grow in the presence of competing weeds (*26*−*28*). The present data indicated that living wheat seedlings could detect the presence of certain weeds and respond by increased MBOA in the rhizosphere even under their roots separation. The result implied that there could be soil-borne plant stress signaling interactions between wheat and associated weeds crabgrass and wild oat. However, potential signaling, awaiting detection and identification, remains obscure in this study.

There were different microbial community structures in the wheat rhizosphere. PCA scores for the PLFA extracted from different microcosms were clearly distinguished in the composition of total PLFA between plant densities of 10 or 80 plants/pot and the control (0 plants/pot), each of which occupied very different ordination space (**Figure 3**). The first principal component (PC1 = 51.91%) and second principal component (PC2 = 19.76%) together accounted for 71.67% of the variation. The PCA was conducted on changes in composition of the specific fatty acids in the rhizosphere of wheat with varying plant densities. PLFA profiling showed that signature lipid biomarkers of bacteria and fungi, except actinobacteria, were affected by wheat plant densities (**Table 2**). Total PLFA, bacterial biomass, and most bacterial indicators, such as 14:0, 16:1ω9c, i15:0, and i17:0, were increased with wheat plant density, but significant results were observed at a high density of 80 plants/pot. With the exception of fungi, a low density of 10 plants/pot did not lead to significant changes in the total PLFA and bacterial and actinobacterial biomasses when compared to the control. There was always a significant increase in the fungal biomass from the control to a high density of 80 plants/pot. Such a trend was observed in the PLFA ratio of fungi and bacteria (**Table 2**). Furthermore, there was a linear positive relationship between the MBOA level and fungi/bacteria in the wheat rhizosphere ($y = 0.023x + 0.074$; $r^2 = 0.97$). The results indicated that DIMBOA or its yielding MBOA increased fungi present in the wheat rhizosphere and, subsequently, on the microbial community structure.

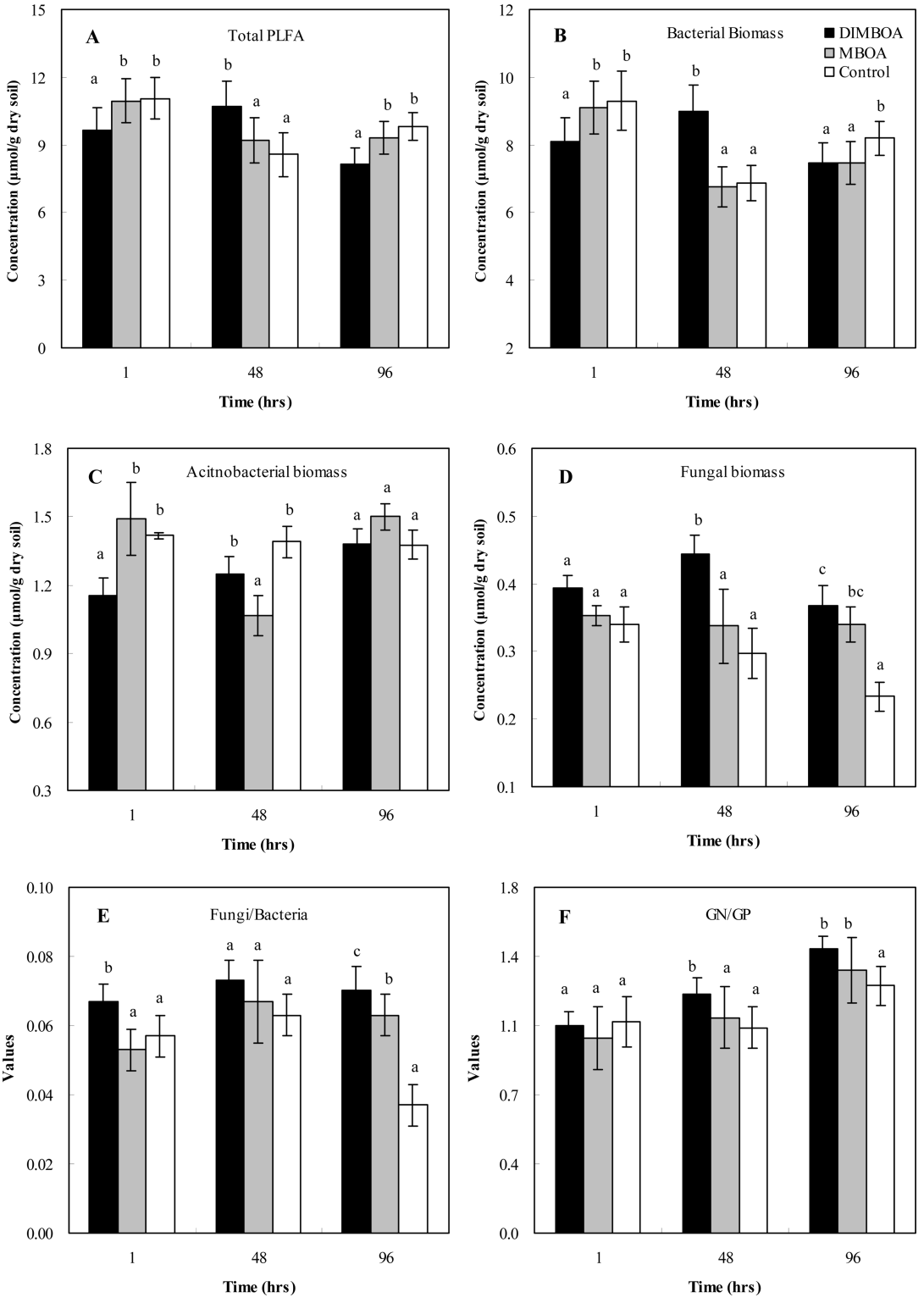

Figure 5. Total PLFA, biomasses of bacteria, actionbacteria, and fungi, and ratios of fungi to bacteria and Gram-negative and Gram-positive bacteria (GN/GP) of soil incubated with DIMBOA and MBOA in a maximal quantity (5 μg/g of dry soil) determined in the wheat rhizosphere at different incubation time intervals. Columns with the same letters are not significantly different at $p < 0.05$.

DIMBOA and MBOA released from wheat into the rhizosphere would likely contribute to the change in the soil microbial community structure. To address this, DIMBOA and MBOA at maximal concentration (5 μg/g of dry soil) determined in the wheat rhizosphere (**Figure 1**) were added to soil. Subsequently, DIMBOA was not detected in soil even at the initial incubation time (1 h). All of soil samples contained MBOA only. In the incubation with DIMBOA, yielding MBOA had a slight increase (5.82 μg/g of dry soil) after 1 h of incubation and then its amounts declined with the incubation time and were not detected after 96 h. In the incubation with MBOA, MBOA degraded rapidly and was not detected after 48 h of incubation (**Figure 4**). The results agreed with several studies that DIMBOA degraded rapidly in soil and yielding MBOA was more resistant toward degradation (*3*, *29*, *30*).

On the basis of soil degradation dynamics of DIMBOA and MBOA, the soil microbial community was investigated after

three incubation time intervals of 1, 48, or 96 h. A comparison of PLFA patterns of the soils with DIMBOA and MBOA showed that differences in PLFA profiles between incubation times were significant. Significant differences in total PLFA (**Figure 5A**), bacterial biomass (**Figure 5B**), and actinobacterial biomass (**Figure 5C**) were observed during early incubation times (1 and 48 h), while a significant difference in fungal biomass (**Figure 5D**) was observed during late incubation times (48 and 96 h). In comparison to the controls, DIMBOA application led to a significant increase in total PLFA and bacterial biomass at 48 h but a significant decrease at 1 and 96 h. However, MBOA had no significant effect on them during early incubation times (1 and 48 h) (panels **A** and **B** of **Figure 5**). DIMBOA significantly decreased the actinobacterial biomass at 1 h, while MBOA resulted in the decrease of the actinobacterial biomass at 48 h, when compared to the controls (**Figure 5C**). Both DIMBOA and MBOA application always increased fungal biomass (**Figure 5D**). Such a similar trend was observed in the ratio of fungi and bacteria. In particular, DIMBOA and MBOA application resulted in a great increase of fungi/bacteria in 96 h (**Figure 5E**). These results agreed with the variation in biomass of fungi in soil-grown wheat seedlings with varying plant density and MBOA levels in the rhizosphere (**Table 2**). In comparison to the controls, the ratio of GN and GP (GN/GP) tended to greatly increase between the DIMBOA and MBOA treatments over the incubation time (**Figure 5F**). However, wheat seedlings did not result in a significant change in GN/GP, even at a high density of 80 plants/pot (**Table 2**).

A DA of the PLFA patterns showed that different microbial community structures were identified for DIMBOA and MBOA application and incubation times (**Figure 6**). The percentage of variance explained by DF1 was 62.81%, while the percentage of variance explained by DF2 was 23.10%. However, the PLFA pattern of the control samples was not significantly separated from all other treatments, even though the PLFA profile of DIMBOA- and MBOA-treated samples changed significantly with the incubation time (**Figure 6**).

Although the data generated in this study were not completely consistent between DIMBOA/MBOA in the wheat rhizosphere and DIMBOA/MBOA application, it is a fact that the soil microbial community structure could be affected by DIMBOA and MBOA. When released from wheat or added to soil, DIMBOA was microbially transformed to MBOA that was degraded further into other products (*11*, *29*, *30*). During the microbial transformation, DIMBOA and MBOA should exert an effect on soil microorganisms and, subsequently, a change in the microbial community structure. The effect of most allelochemicals on microbial activity is dose-dependent. A previous study showed that the microbial community structure was not affected by the addition of BOA to the soil, as investigated by analysis of signature fatty acids even at a high concentration of 30 000 nmol/g of soil (*31*). However, this study indicated that DIMBOA and it yielding MBOA at concentrations determined in the wheat rhizosphere rather than arbitrary concentrations were enough to start the effect. Furthermore, PLFA analysis clearly showed that DIMBOA and MBOA did change the soil microbial community structure even at a low concentration of 5 μg/g of dry soil. The results suggested that there were different contributions in the soil microbial community structure between benzoxazinones and their degraded benzoxazolinones, such as BOA.

An increasing number of studies have showed that DIMBOA and MBOA in wheat varieties show a correlation with fungi pathogenic resistance (*15*−*17*, *32*). It appeared from the results that DIMBOA and MBOA had a great effect on fungi present in the soil microbial community. Although this study did not clarify which soil fungi species were responsible for DIMBOA and

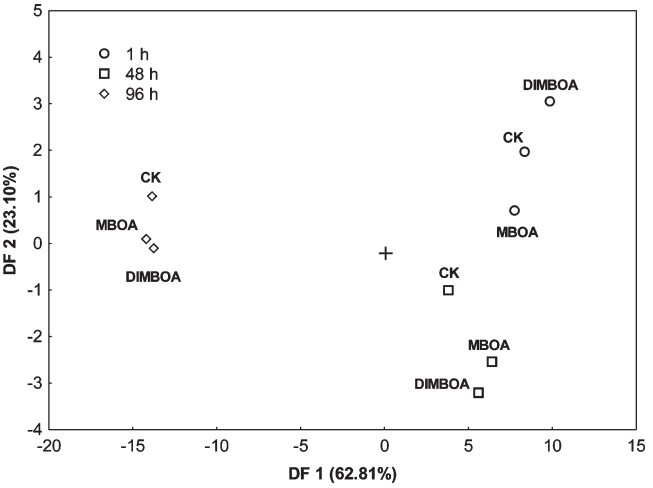

Figure 6. Plots of sample scores extracted by the DA on the PLFA of incubation times under DIMBOA and MBOA treatments and control soil (CK). + indicates the (0, 0) points.

MBOA, DIMBOA and MBOA could affect the soil microbial community structure to their advantage through a change in fungi populations in the wheat rhizosphere. A further clarification of the interactions between DIMBOA/MBOA and soil microorganisms, particularly in soil-borne pathogenic fungi, offers many potential implications and applications in wheat disease management.

LITERATURE CITED

(1) Stochmal, A.; Kus, J.; Martyniuk, S.; Oleszek, W. Concentration of benzoxazinoids in roots of field-grown wheat (*Triticum aestivum* L.) varieties. *J. Agric. Food Chem.* **2006**, *54*, 1016−1022.

(2) Niemeyer, H. M. Hydroxamic acids derived from 2-hydroxy-2*H*-1, 4-benzoxazin-3(4*H*)-one: Key defense chemicals of cereals. *J. Agric. Food Chem.* **2009**, *57*, 1677−1696.

(3) 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.

(4) Wu, H. W.; Haig, T.; Pratley, J.; Lemerle, D.; An, M. Distribution and exudation of allelochemicals in wheat *Triticum aestivum*. *J. Chem. Ecol.* **2000**, *26*, 2141−2154.

(5) Wu, H. W.; Haig, T.; Pratley, J.; Lemerle, D.; An, M. Allelochemicals in wheat (*Triticum aestivum* L.): Production and exudation of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one. *J. Chem. Ecol.* **2001**, *27*, 1691−1700.

(6) Fomsgaard, I. S. Chemical ecology in wheat plant-pest interactions. How the use of modern techniques and a multidisciplinary approach can throw new light on a well-known phenomenon: Allelopathy. *J. Agric. Food Chem.* **2006**, *54*, 987−990.

(7) Mathiassen, S. K.; Kudsk, P.; Mogensen, B. B. Herbicidal effects of soil-incorporated wheat. *J. Agric. Food Chem.* **2006**, *54*, 1058−1063.

(8) Krogh, S. S.; Mensz, S. J. M.; Nielsen, S. T.; Mortensen, A. G.; Christophersen, C.; Fomsgaard, I. S. Fate of benzoxazinone allelochemicals in soil after incorporation of wheat and rye sprouts. *J. Agric. Food Chem.* **2006**, *54*, 1064−1074.

(9) Coja, T.; Idinger, J.; Blumel, S. Influence of the soil composition on the effects of benzoxazinoid allelochemicals on two soil nontarget organisms. *J. Agric. Food Chem.* **2006**, *54*, 1093−1098.

(10) Kato-Noguchi, H.; Macias, F. A. Inhibition of germination and α-amylase induction by 6-methoxy-2-benzoxazolinone in twelve plant species. *Biol. Plant.* **2008**, *52*, 351−354.

(11) Etzerodt, T.; Mortensen, A. G.; Fomsgaard, I. S. Transformation kinetics of 6-methoxybenzoxazolin-2-one in soil. *J. Environ. Sci. Health, Part B* **2008**, *43*, 1−7.

(12) Kong, C. H.; Wang, P.; Gu, Y.; Wang, M. L. The fate and impact on microorganisms of rice allelochemicals in paddy soil. *J. Agric. Food Chem.* **2008**, *56*, 5043−5049.
(13) Inderjit, S. Soil microorganisms: An important determinant of allelopathic activity. *Plant Soil* **2005**, *274*, 227−236.
(14) 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*, No. e4700.
(15) Martyniuk, S.; Stochmal, A.; Macias, F. A.; Marin, D.; Oleszek, W. Effects of some benzoxazinoids on in vitro growth of *Cephalosporium gramineum* and other fungi pathogenic to cereals and on *Cephalosporium* stripe of winter wheat. *J. Agric. Food Chem.* **2006**, *54*, 1036−1039.
(16) 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.
(17) Soltoft, M.; Jorgensen, L. N.; Svensmark, B.; Fornsgaard, I. S. Benzoxazinoid concentrations show correlation with *Fusarium* head blight resistance in Danish wheat varieties. *Biochem. Syst. Ecol.* **2008**, *36*, 245−259.
(18) Belz, R. G.; Hurle, K. Differential exudation of two benzoxazinoids—One of the determining factors for seedling allelopathy of Triticeae species. *J. Agric. Food Chem.* **2005**, *53*, 250−261.
(19) Bais, H. P.; Weir, T. L.; Perry, L. G.; Gilroy, S.; Vivanco, J. M. The role of root exudates in rhizosphere interactions with plants and other organisms. *Ann. Rev. Plant Biol.* **2006**, *57*, 233−266.
(20) 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.
(21) 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.
(22) Song, Y. N.; Zhang, F. S.; Marschner, P.; Fan, F. L.; Gao, H. M.; Bao, X.G.; Sun, J. H.; Li, L. Effect of intercropping on crop yield and chemical and microbiological properties in rhizosphere of wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and faba bean (*Vicia faba* L.). *Biol. Fertil. Soils* **2007**, *43*, 565−574.
(23) Villagrasa, M.; Guillamon, M.; Navarro, A.; Eljarrat, E.; Barcelo, D. Development of a pressurized liquid extraction−solid-phase extraction followed by liquid chromatography−electrospray ionization tandem mass spectrometry method for the quantitative determination of benzoxazolinones and their degradation products in agricultural soil. *J. Chromatogr., A* **2008**, *1179*, 190−197.
(24) Villagrasa, M.; Eljarrat, E.; Barcelo, D. Analysis of benzoxazinone derivatives in plant tissues and their degradation products in agricultural soils. *TRAC, Trends Anal. Chem.* **2009**, *28*, 1103−1114.
(25) Woodward, M. D.; Corcuera, L. J.; Helgeson, J. P.; Upper, C. D. Decomposition of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in aqueous solutions. *Plant Physiol.* **1978**, *61*, 796−802.
(26) Kong, C. H.; Li, H. B.; Hu, F.; Xu, X. H.; Wang, P. Allelochemicals released by rice roots and residues in soil. *Plant Soil* **2006**, *288*, 47−56.
(27) Kong, C. H.; Xu, X. H.; Zhou, B.; Hu, F.; Zhang, C. X.; Zhang, M. X. Two compounds from allelopathic rice accession and their inhibitory effects on weeds and fungal pathogens. *Phytochemistry* **2004**, *65*, 1123−1128.
(28) Dayan, F. E. Factors modulating the levels of the allelochemical sorgoleone in *Sorghum bicolor*. *Planta* **2006**, *224*, 339−346.
(29) Fomsgaard, I. S.; Mortensen, A. G.; Carlsen, S. C. K. Microbial transformation products of benzoxazolinone and benzoxazinone allelochemicals—A review. *Chemosphere* **2004**, *54*, 1025−1038.
(30) Etzerodt, T.; Nielsen, S. T.; Mortensen, A. G.; Christophersen, C.; Fomsgaard, I. S. Elucidating the transformation pattern of the cereal allelochemical 6-methoxy-2-benzoxazolinone (MBOA) and the trideuteriomethoxy analogue [D_3]-MBOA in soil. *J. Agric. Food Chem.* **2006**, *54*, 1075−1085.
(31) Understrup, A. G.; Ravnskov, S.; Hansen, H. C. B.; Fomsgaard, I. S. Biotransformation of 2-benzoxazolinone to 2-amino-(3*H*)-phenoxazin-3-one and 2-acetylamino-(3*H*)-phenoxazin-3-one in soil. *J. Chem. Ecol.* **2005**, *31*, 1205−1222.
(32) Zikmudova, M.; Drandarov, K.; Bigler, L. Biotransformation of 2-benzoxazolinone and 2-hydroxy-1,4-benzoxazin-3-one by endophytic fungi isolated from *Aphelandra tetragona*. *Appl. Environ. Microb.* **2002**, *68*, 4863−4870.

Received for review August 24, 2010. Revised manuscript received November 4, 2010. Accepted November 9, 2010. This work was supported by the Research Fund for the Doctoral Program of Higher Education of China (20090008110042) and the Chinese University Scientific Fund (2009TD15).