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Research Article

Effects of Herbicides on the Microbial Community and Urease Activity in the Rhizosphere Soil of Maize at Maturity Stage

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Studying the effects of herbicides on microbial community and urease activity in the rhizosphere soil of maize is helpful to clarify the mechanisms herbicides used to affect soil microbial environment. In this research, four common preemergence maize specific herbicides, nicosulfuron+atrazine (A1), alachlor+acetochlor+atrazine (A2), propisochlor+atrazine (A3), and acetochlor+atrazine (A4), were selected to use in a pot trial. A preemergence herbicide nonspecific for maize, dinitraniline (A0), was used as the positive control, whereas water instead of herbicide was considered as the negative control (CK). At the maturity stage, the microbial communities and urease activity in the 0-20 cm, 20-40 cm, and 40-60 cm rhizosphere soils of maize were analyzed. Results showed that A0 dramatically suppressed maize growth, with no grain got finally, while A1 displayed the weakest effect. The tested herbicides affected the microbial community in the 0-20 cm layer greater than in the 20-60 cm ones, with A1 displaying the greatest effect. In the 0-20 cm soil, A1 largely reduced the relative abundance of the top three dominant genera, *Prevotella*, *Barnesiella*, and *Lactobacillus* in the CK soil, by 99.0%, 98.7%, and 79.2%, and made *Pseudomonas*, *Gemmatimonas*, and *Sphingomonas* became the new dominant genera, while A2 and A3 displayed similar but slighter effects. All herbicides dramatically reduced the relative abundance of the top one dominant fungal phylum (Ascomycota) and genus (*Diatrype*) in the CK soil, from 45% to 5.2%-7.9% and 42% to 2.1%-3.2%. A0 dramatically dropped the urease activity in the 0-60 cm soils, by 30.5%-33.1%, whereas A1-A4 displayed an unsignificant effect. In conclusion, A1 is a suitable herbicide for maize. Both the bacterial community and urease activity in the 0-20 cm rhizosphere soil are suitable indices to evaluate the effects of preemergence herbicides on maize growth and soil microbial environment.

1. Introduction

Maize (*Zea mays* L.) plays a key role in keeping food security and agricultural production especially in the developing countries and is the third important cereal crop in the world [1, 2]. In China, maize has become the first grain crop with the largest planting area and total yield [3]. Weed is one of the most important factors restricting both the yield and quality of maize. It is reported that about half of the maize field faces weed damage in different levels in China [4]. The annual yield loss of maize caused by weed in China has reached to 20% [5]. Herbicides have many merits such as time-saving, economy, and high-efficiency, Therefore, the use of herbicides is still one of the most important measures to control weed in the fields of grain crops including maize [6]. However, together with the economic benefit, herbicides also bring adverse effects to the soil environment [7–9]. After entering into soils, herbicides may influence the soil environment through the processes of sorption-desorption, transformation, transporting to groundwater, or degradation [10]. Evaluating the effects of herbicides on the soil environment is crucial for the reasonable use of herbicides. The preemergence herbicide is a kind of herbicide that is sprayed directly on the soil surface before the emergence of crops and, hence,

affects soil chemical and microbial environment deeper than the postemergence ones. However, the effects of preemergence herbicides on soil microbial environment still need more researches.

Microorganisms play key roles in nutrient cycling and energy flow in soil and are important indicators for soil health, soil pollution, and ecological restoration [11, 12]. Herbicides application may inhibit, activate, or show no effects to soil microorganisms. Xu et al. reported that sterane first decreased soil bacterial diversity and abundance in maize field at 10 days but increased them at 60 days after application [13], Bezuglova et al. demonstrated that foliar application of sulfonylurea herbicide decreased the abundance of bacteria especially for the quickly growing ones on winter wheat soil [14], whereas Kepler et al. found that glyphosate did not affect the overall microbial community composition in maize or soybean grown soil [15].

Except for microorganisms, enzymes also are important evaluation indicators for soil quality as they are directly involved in the biochemical process and nutrient cycle [16]. Soil enzymes may catalyze organic substances into inorganic nutrients or assist the degradation of exogenous harmful compounds to promote plant growth [17, 18], which can then regulate the soil microbial community. Moreover, soil enzymes mainly come from soil microorganisms [19]; hence, the activity of soil enzymes is correlated with the soil microbial environment. Among soil enzymes, urease mainly participates in hydrolyzing urea into CO_2 and NH_3, regulating the soil nitrogen cycle [20]. Herbicides affect the soil urease activity had been reported. Xie et al. [21] found that applying a high dose of either bensulfuron-methyl·butachlor or quinclorac in pot paddy soil suppressed the soil urease activity. Du et al. applied mesotrione on a laboratory cultured soil and found that the soil urease was less affected than β-glucosidase [16]. Researches from Aruna Kumari et al. showed that the soil urease activity can be affected by herbicide type, concentration, and application time [2].

The rhizosphere soil is closely in contact with roots and shows a larger influence on plant growth than the nonrhizosphere soil. After entering into soil, herbicides interacted with soil microorganisms and plant roots, hence showed different effects to soil environment when compared with the nonrhizosphere soil. Studying the effects of herbicides on the microorganisms and enzyme activity in rhizosphere soil is helpful to elucidate the mechanisms of herbicides affecting both plant growth and soil environment [22–24]. However, until now, studies about the effects of herbicides on soil microorganisms and urease activity mainly focus on the nonrhizosphere soils [25–28]. Moreover, both the degradation and migration rates of herbicides in soil differ the influence degree to soil environment caused by herbicides. Effects of herbicides on soil microorganisms or enzymes in 0-20 cm soils after a short time application had been studied [13, 29, 30]. How did herbicides affect soil microbial environment in soils at different depths after a much longer time application (such as after planting a season of crop) is still unclear.

In this study, five preemergence herbicides: four maize-specific herbicides and one nonspecific herbicide for maize were sprayed on the potted soil surface immediately after sowing maize. At the maturity stage, the effects of herbicides on the total bacterial and fungal community structures in the 0-20, 20-40, and 40-60 cm rhizosphere soils were analyzed by high-throughput sequencing technology, and the soil urease activities in the same soil layers were determined. The weight and number of maize grains were also recorded. Results from this study may help enlighten the effects of preemergence herbicides on the microbial environment in rhizosphere soil at different layers after planting a season crop and provide guides for selecting suitable herbicides for maize.

2. Materials and Methods

2.1. Materials. The maize hybrid "Bingdan 16" used in this study was provided by the Institute of Crop Science, Shanxi Academy of Agricultural Sciences. The growth period of Bingdan 16 is 120 days. The five preemergence herbicides commonly sold in market that were selected to use in this experiment (Table 1) were bought from Shanxi Taigu County Yirong Seed Industry Co., LTD, and applied based on the instructors. Dinitraniline (A0) is a nonspecific herbicide for maize, and nicosulfuron+atrazine (A1), alachlor+acetochlor +atrazine (A2), propisochlor+atrazine (A3), and acetochlor +atrazine (A4) are specific herbicides for maize.

2.2. Soil. The raw soil sample was taken from a 2 m layer from an uncultivated land in Taigu County, Shanxi Province, China. The soil was air-dried, sieved (1 mm), and evenly mixed before use. Nutrient status of the soil was as follows: 0.2 g kg^{-1} total nitrogen, 19.8 mg kg^{-1} available nitrogen, 2.9 mg kg^{-1} available phosphorus, 30.3 mg kg^{-1} available potassium, and 1.2 g kg^{-1} organic matter.

2.3. Experimental Methods. The experiment was conducted from June 3, 2014, to October 1, 2014, at the Loess Plateau Crop Research Institute, Shanxi Agricultural University, China. A special root tube device (25 cm diameter, 200 cm length) that consists of two semicylinders fixed with iron wire and steel plates was used. Soil bulk density in 0-200 cm soil layer was measured for local maize field by 20 cm interval. Based on the soil bulk density, soil mass was calculated and weighted for every 20 cm length in the tube. The soil prepared above was added to the root tube devices and compacted by every 20 cm layer. The top 0-20 cm soil was added after mixing with NPK nutrients (urea, 170 mg kg^{-1}; superphosphate, 560 mg kg^{-1}; potassium chloride, 170 mg kg^{-1}). All root tube devices were placed vertically in the grooves in a way that the soil surface inside the tubes was leveled to the outer soil surface. Five maize seeds were sown in each tube on June 3, 2014. Herbicide solutions (100 ml) were then sprayed evenly based on the dosage as mentioned in Table 1. A nonspecific herbicide for maize (A0) was sprayed as the positive control and an equal volume water instead of herbicide was considered as the negative control (CK). Each treatment was repeated three times, and all tubes were randomly placed. After emergence, seedlings were thinned to one seedling per tube and watered as needed.

2.4. Soil Sample Collection. On October 1, 2014 (at the maturity stage), the aboveground maize plants were cut and all tubes were opened. The rhizosphere soils from 0-20, 20-40,

Table 1: The tested herbicides information.

Number	Effective components and content	Actual dosage (μl tube^{-1})	Water dosage (ml pot^{-1})
A0	48% dinitraniline	14.7	100
A1	2% nicosulfuron+20% atrazine	14.7	100
A2	10% alachlor+14% acetochlor+18% atrazine	22.1	100
A3	16% propisochlor+26% atrazine	17.7	100
A4	26% acetochlor+26% atrazine	14.7	100

and 40-60 cm layer from the soil surface were collected using a banister brush from the root surface after the untight soil was removed. All soil samples were collected into seal bags and divided into two parts: one was stored in -80°C before sending to the Sangon Biological Engineering Co. Ltd. Shanghai to analyze the bacterial and fungal community structures, while the other part was used to determine the urease activity.

2.5. Measured Indices

2.5.1. Growth Indices. The grain number and weight per plant were recorded, and the thousand grains weight was calculated at the maturity stage.

2.5.2. Soil Microorganisms

(1)DNA Extraction and Gene Amplification. The collected soil samples were sent to Sangon Biotech (Shanghai) Co., Ltd., for the study of the bacterial and fungal community. Briefly, 5.0 g soil was used for DNA extraction by the PowerSoil® DNA extraction kit (MoBio, USA) following the instructions. The Qubit2.0 DNA detection kit was performed to detect the concentration and quality of the extracted DNA. The bacterial community was estimated on the V3-V4 region of the 16S rRNA gene using the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The fungal community based on the NS1-FUNG region of the 18S DNA gene was analyzed using the primers NS (5′-CCTACA CGACGCTCTTCCGATCTN GTAGTCATATGCTTGT CTC-3′) and FUNG (5′-GACTGGAGTTCCTTGGCAC CCGAGAATTCCAATTCCCCGTTACCCGTTG-3′). The purified PCR products were then sequenced using the Illumina Miseq platform.

(2)Sequencing Analysis. The obtained sequences were quality controlled through truncating the overlapped of low-quality bases in the 3′ end using the PRINSEQ-lite 0.19.5, and then, the bases in the double ends were combined to one complete sequence using FLASH v1.2.7. The obtained sequences were checked by MOTHUR (pre. cluster), and the chimeras were removed by UCHIME, and finally, the high-quality sequences were obtained. These sequences were clustered by UCLUST v1.1.579 and generated into operational taxonomic units (OTUs) at 0.97 similarity. One sequence with the highest abundance for each OTU was chosen as the representative sequence. Taxonomy was assigned using the RDP classifier and Greengenes database. The Alpha diversity indices (Shannon index, ACE index, and Alpha index) and were calculated using Mothur (http://www.mothur.org/).

2.5.3. Soil Urease Activity. For each layer of soil in each pot, the collected soil samples at the maturity stage used for analyzing urease activity were divided into two even parts, and the total of six replicated soil samples were air-dried and sieved. The urease activity was analyzed by the indigo colorimetry method [19] and calculated based on the content of NH_3-N (mg g^{-1}) released from one-gram soil within the cultivated time.

2.6. Data Analysis. The Microsoft Excel 2016 software was used to analyze the data, and the Origin 9 was used to draw bar charts. The SPSS 18.0 software was used to analyze the differences between treatments or soil layers based on the least significant difference (LSD) method at the level of $P = 0.05$.

3. Results

3.1. Effects of Herbicides on the Grain Weight of Maize. Among the tested herbicides, nicosulfuron+atrazine (A1) did not affect either the early growth of maize seedlings or the grain structures during the maturity stage, whereas dinitraniline (A0) dramatically inhibited the growth of maize seedlings, and with no grain at the maturity stage (Table 2, Figure 1). The grain weight and number per plant were not significantly reduced by alachlor+acetochlor+atrazine (A2), propisochlor+atrazine (A3), or acetochlor+atrazine (A4), whereas a 27.0%-39.9% reduction in thousand-grain weight was recorded compared to the control (Table 2).

3.2. Effects of Herbicides on the Microbial Diversity in the Rhizosphere Soil of Maize. The total number of effective bacterial and fungal OTUs was 5075-6757 and 1103-1673, respectively, from different soils. Shannon index reflects the diversity degree of microorganisms, and ACE and Chao 1 indices reflect the richness of the microbial community. For bacteria, the five tested herbicides had little effect on the bacterial Shannon index in the 0-60 cm rhizosphere soil of maize. The effects of herbicides on ACE and Chao1 indices in the 0-20 cm rhizosphere soil were also weak, with only A1 changing the ACE index by more than 20% (20.9%). In the 20-40 cm rhizosphere soil, A0 and A3 reduced the ACE and Chao 1 indices, to 28.3%-40.4%, whereas increased these indices by 24.3%-55.2% in the 40-60 cm rhizosphere soil compared with the control. A5 also increased the ACE and Chao1 indices in the 40-60 cm rhizosphere soil by 46.7% and 36.8% than that of the control, respectively (Table 3).

Table 2: Effects of herbicides on the grain structures of maize.

Treatment	Grain weight (g plant^{-1})		Grain number (plant^{-1})		Thousand grain weight (g)	
	Measured value	△CK (%)	Measured value	△CK (%)	Measured value	△CK (%)
CK	67.1 ± 44.0a	—	177.0 ± 120.3ab	—	395.7 ± 41.0a	—
A0	0.0 ± 0.0b	-100.0	0.0 ± 0.0c	-100.0	0.0 ± 0.0d	-100.0
A1	41.1 ± 25.0ab	-38.7	121.7 ± 54.1b	-31.3	321.2 ± 57.5ab	-18.8
A2	58.7 ± 3.1a	-12.5	241.0 ± 4.0a	36.2	243.5 ± 8.7c	-38.5
A3	62.3 ± 25.8a	-7.1	215.3 ± 84.3ab	21.7	288.9 ± 52.6bc	-27.0
A4	43.3 ± 1.8a	-35.4	188.3 ± 40.1ab	6.4	237.8 ± 57.2c	-39.9

Notes: data in the table are X ± SD ($n = 3$), different lowercase letters in the same column indicate significant difference ($P < 0.05$) based on the LSD test.

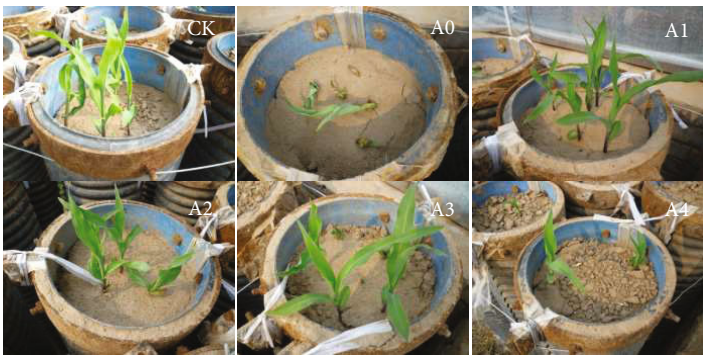

Figure 1: Effects of herbicides on the growth of maize seedlings. Note: CK, the no herbicide negative control; A0, a preemergence nonspecific herbicide for maize, dinitraniline (the active control); A1-A4: four preemergence maize specific herbicides, nicosulfuron+atrazine, alachlor +acetochlor+atrazine, propisochlor+atrazine, and acetochlor+atrazine, respectively.

Table 3: Effects of herbicides on the alpha diversity of bacteria in the rhizosphere soil of maize.

Soil layer (cm)	Treatment	Shannon		ACE		Chao 1	
		Measured value	△CK (%)	Measured value	△CK (%)	Measured value	△CK (%)
0-20	CK	7.6	—	26109.7	—	15204.3	—
	A0	7.6	0.9	30042.1	15.1	16573.7	9.0
	A1	7.5	-1.2	20645.4	-20.9	13299.7	-12.5
	A2	7.7	2.3	25091.6	-3.9	15564.7	2.4
	A3	7.8	3.2	27539.3	5.5	16687.2	9.8
	A4	7.5	-0.2	29388.9	12.6	16735.1	10.1
20-40	CK	7.6	—	31795.4	—	17283.6	—
	A0	7.5	-1.6	21090.5	-33.7	12400.6	-28.3
	A1	7.4	-3.1	24043.1	-24.4	14098.2	-18.4
	A2	7.4	-2.3	30041.6	-5.5	16472.0	-4.7
	A3	7.4	-2.4	18942.7	-40.4	11330.8	-34.4
	A4	7.6	-0.3	33844.3	6.4	17851.1	3.3
40-60	CK	7.5	—	19817.4	—	12125.5	—
	A0	7.8	4.1	30746.0	55.2	18073.9	49.1
	A1	7.3	-3.2	21884.8	10.4	13089.6	8.0
	A2	7.6	1.5	22149.7	11.8	13397.3	10.5
	A3	7.5	0.5	25644.3	29.4	15070.1	24.3
	A4	7.5	-0.3	29077.7	46.7	16219.4	33.8

Note: CK, the no herbicide negative control; A0, a preemergence nonspecific herbicide for maize, dinitraniline (the active control); A1-A4: four preemergence maize-specific herbicides, nicosulfuron+atrazine, alachlor+acetochlor+atrazine, propisochlor+atrazine, and acetochlor+atrazine, respectively.

In contrast to bacteria, the Shannon index of fungi in the herbicides treated 0-20 cm soil was increased by 21.0%-39.9% than that of the control. In the 0-20 cm soils, the fungal ACE and Chao1 indices in the A3 and A4 treated soils were higher than other herbicides. For the 20-40 cm soil, A0 increased the fungal ACE and Chao1 indices by 32.8% and 29.8%, respectively, over that of the control, while the other four herbicides showed negligible effects. A0, A1, A3, and A4 increased the fungal ACE and Chao1 indices by 21.4%-62.1% in the 40-60 cm soil than the nonherbicide control treatment (Table 4).

3.3. Effects of Herbicides on the Composition of the Microbial Community in the Rhizosphere Soil of Maize

3.3.1. Bacterial Community. At the phylum level, herbicides affected the bacterial community composition in the 0-20 cm soil greater than in the 20-40 and 40-60 cm soils, with A1 showing the greatest effect. In the 0-20 cm soil, Bacteroidetes, Firmicutes, and Proteobacteria were the top three dominant phyla in the CK treatment. A1 decreased the relative abundance of Bacteroidetes and Firmicutes greatly, from 27.8% and 26.0% in the CK soil to 5.5% and 4.4%, whereas increased the relative abundance of Proteobacteria, from 22.3% in the CK soil to 37.7%. The relative abundance of Actinobacteria in the A1 treated 0-20 cm soil was 693.0% more than that in the control and made Actinobacteria become the second dominant phylum. The relative abundances of Gemmatimonadetes and Planctomycetes in the A1 treated 0-20 cm soil were also 423.5% and 225.0% more than that in the control. Compared with A1, A3 and A2 showed similar but weaker effects on the 0-20 cm soil bacterial community. A0 displayed the weakest effect on the 0-20 cm soil among the five tested herbicides. In the 20-40 cm and 40-60 cm soil, all herbicides showed weak effects on the bacterial community, with A1 and A0 displaying much greater effect than other herbicides, respectively (Figure 2(a)).

At the genus level, similar to the phylum level, A1 showed the greatest effect on the bacterial composition in the 0-20 cm soil and followed by A3 and A2. A1 reduced the relative abundance of *Prevotella*, *Barnesiella*, and *Lactobacillus*, the top three dominant genera in the 0-20 cm CK soil, from 11.3%, 7.5%, and 5.3% to 0.1%, 0.1%, and 1.1%, respectively. The relative abundance of *Clostridium* IV was also dropped from 2.1% in the CK soil to only 0.1%. In contrast, A1 increased the relative abundance of *Pseudomonas*, *Sphingomonas*, *Gemmatimonas*, Gp6, and *Aciditerrimonas* by 225.7%-1153.3% more when compared with no herbicide control. Similar with A1, A3 and A2 also increased the relative abundance of these above five bacterial genera by 118.2%-446.9% more when compared with the control. In the 20-40 cm and 40-60 cm soils, all herbicides displayed weak effects on the bacterial community, while also with A0 showed much greater effect than the other herbicides in the 40-60 cm soil. A0 reduced the relative abundances of *Pseudomonas* and *Barnesiella* by 27.9% and 27.4% when compared with the control, whereas increased the relative abundances of *Sphingomonas*, *Gemmatimonas*, *Subdivision3_genera_incertae_sedis*, and *Clostridium* IV, by 36.7%-230.3% than that in the no herbicide control (Figure 2(b)).

3.3.2. Fungal Community. At the phylum level, a total of seven phyla were classified. Different from those in the bacterial community, all the five tested herbicides showed similar and great effects on the fungi community in the 0-20 cm rhizosphere soil. Ascomycota was the top one dominant fungal phylum in the CK soil at 0-20 cm layer, accounting for 45.0% of the total fungal community. Herbicides reduced the relative abundance of this phylum to 5.2%-7.9%. The relative abundance of the unclassified fungal phylum (Others) was also increased greatly, from 9.6% in the no herbicide control soil to 44.0%-67.9% in the herbicide-treated soils. A1 also increased the relative abundance of Basidiomycota greatly, by 202.4% than that in the CK treatment. A1 and A2 also reduced the relative abundance of Glomeromycota, and Chytridiomycota, by 32.5%-43.7% than that in the no herbicide control. In the 20-40 cm soil, A0 and A2 increased the relative abundance of Glomeromycota but reduced the relative abundance of the unclassified phylum, with the variation being 31.9%-82.4% when compared with the control. The relative abundance of Basidiomycota in the A3-treated soil and Ascomycota in the A4-treated soil was 194.6% and 189.5%, respectively, more than that in the control treatment. In the 40-60 cm soil, A0 and A1 displayed similar effects on the fungal community, with increasing relative abundance of Glomeromycota, Chytridiomycota, and Basidiomycota, whereas reducing relative abundance of the unclassified phyla (Others), whereas A2 showed opposite effects (Figure 3(a)).

At the genus level, similar to the phylum level, all herbicides displayed great and similar effects on the fungal community in the 0-20 cm soil, but different effects in the 20-60 cm soils. *Diatrype* was the top one dominant genus in the CK soil at 0-20 cm layer. Herbicides reduced the relative abundance of *Diatrype* from 42.0% in CK to only 2.1%-3.2%. In contrast, the relative abundance of the other genera in the herbicides applied 0-20 cm soil was increased from 18.2% in the control soil to 56.6%-73.9%. *Funneliformis* and *Powellomyces* were the third and fourth dominant fungal genus in the CK soil, accounting for 6.5% and 3.6% of the total fungal community. All herbicides reduced the relative abundance of *Funneliformis* and *Powellomyces*, with A2 and A3 showing greater reduction effects (42.9% and 55.5%, respectively) than the other three herbicides. Additionally, A1 also increased the relative abundance of *Stephanospora* greatly, from 0.1% in the control to 4.7%. In the 20-40 cm soil, A0 and A2 increased the relative abundance of *Rhizophagus*, the top one dominant genus in the CK soil, greatly, by 84.8% and 42.3% than that in the control, respectively. A0, A1, A2, and A3 reduced the relative abundance of *Diatrype*, the second dominant genus in the CK soil, with 61.3%, 67.9%, 37.6%, and 23.4% less than the control, respectively, whereas A4 increased it by 245.3% when compared with the control. In the 40-60 cm soil, A0 and A1 displayed similar effects on the fungal community, by increasing the relative abundance of *Rhizophagus*, *Funneliformis*, *Powellomyces*, and *Glomus*, whereas A2 showed opposite effects. A4 also greatly increased the relative abundance of *Diatrype* in the 20-40 cm and 40-60 cm soils, with 245.3% and 129.0% than that in the CK soil (Figure 3(b)).

Table 4: Effects of herbicides on the alpha diversity of fungi in the rhizosphere soil of maize.

Soil layer (cm)	Treatment	Shannon		ACE		Chao 1	
		Measured value	△CK (%)	Measured value	△CK (%)	Measured value	△CK (%)
0-20	CK	4.1	—	2952.8	—	2328.9	—
	A0	5.6	38.1	3190.9	8.1	2572.6	10.5
	A1	5.6	36.7	2978.8	0.9	2589.3	11.2
	A2	4.9	21.0	3043.3	3.1	2303.8	-1.1
	A3	5.7	39.9	3987.5	35.0	3026.1	29.9
	A4	5.4	31.8	3488.6	18.1	2825.7	21.3
20-40	CK	4.8	—	3373.7	—	2575.8	—
	A0	5.2	10.2	4479.9	32.8	3343.4	29.8
	A1	5.1	7.2	3495.0	3.6	2760.6	7.2
	A2	5.4	13.2	3637.7	7.8	2679.6	4.0
	A3	5.4	13.6	3261.7	-3.3	2707.8	5.1
	A4	4.8	1.1	3019.6	-10.5	2463.6	-4.4
40-60	CK	5.2	—	2584.1	—	2181.4	—
	A0	5.3	1.9	3621.1	40.1	2647.5	21.4
	A1	5.3	2.9	4189.2	62.1	2806.1	28.6
	A2	4.5	-12.5	2337.6	-9.5	1843.0	-15.5
	A3	5.4	3.7	3508.7	35.8	2670.5	22.4
	A4	5.3	1.7	3522.1	36.3	2875.7	31.8

Note: CK, the no herbicide negative control; A0, a preemergence nonspecific herbicide for maize, dinitraniline (the active control); A1-A4: four preemergence maize specific herbicides, nicosulfuron+atrazine, alachlor+acetochlor+atrazine, propisochlor+atrazine, and acetochlor+atrazine, respectively.

3.4. Effects of Herbicides on the Urease Activity in the Rhizosphere Soil of Maize. A0 significantly decreased the urease activity in the 0-60 cm layer rhizosphere soil by 30.6%-38.6% than the control, whereas A1 did not affect the urease activity in the same layer soils. A2, A3, and A5 did not significantly affect the urease activity in the 0-20 and 20-40 cm rhizosphere soils, whereas A2 and A3 increased the urease activity in the 40-60 cm rhizosphere soil, by 27.1% and 19.8% than that in the CK soils. As the layer increased, the urease activity did not change significantly in the A0, A1, and CK treatments but significantly increased in the A2- and A3-treated soils. The urease activity in the A4-treated 40-60 cm soil was also 24.2% higher than that in the 0-20 cm soil (Table 5).

4. Discussion

4.1. Herbicides Affected Rhizosphere Soil Microbial Structure. Soil microorganisms are the main components of the agricultural microbial system, play key roles in nutrient cycling and energy flow, and are considered as indicators to reflect the effects of herbicides on the soil environment [2, 6, 16, 31]. Rhizosphere soil connected with plant roots tightly; hence, the change of rhizosphere soil environment influences plant growth stronger than the nonrhizosphere ones. Rhizosphere environment, herbicides, and microorganisms interacted with each other closely [24]. Studying the effects of herbicides on the microbial communities in rhizosphere soils contributes to reveal the effective mechanisms of herbicides on both microbial environment and plant growth. At present, the effects of herbicides on microorganisms in nonrhizosphere maize soil had been reported widely. Niewiadomska et al. found that a mixed herbicide (containing nicosulfuron or mesotrione) increased the total number of cultivable microorganisms in 0-20 cm maize soil under field condition [30]. Borowik et al. sprayed a mixture herbicide of terbuthylazine, mesotrione, and S-metolachlor to potted maize soil, and found that herbicides altered both the population and ecophysiological diversity of the cultivatable soil bacteria, actinomycetes, and fungi [19]. Xu et al. also analyzed the effects of starane on 0-15 cm maize soil on field scale that used the high-throughput sequencing technology [13]. However, few studies had been done to clarify the effects of herbicides on maize rhizosphere soil at different depths. In this present study, we tested the effects of five preemergence herbicides on maize rhizosphere soil at the maturity stage and found that the tested herbicides affected both the bacterial and fungal communities in the rhizosphere soil of maize in general, with nicosulfuron+atrazine (A1) displaying the greatest effect on bacteria, whereas dinitraniline (A0) showed the weakest effect, while all herbicides displayed similar effects on fungi diversity and community structure in the 0-20 cm soil. In our study, herbicides altered both the dominant microbial groups and abundance largely. Considering that A1 did not significantly reduce the maize grain weight but A0 application got no grain (Table 2), it indicates that the microbial community, especially the bacterial community structure in the 0-20 cm rhizosphere soil adjusted by herbicides contributes to the alleviation of the harmful effects on maize caused by herbicides.

After entering into soils, herbicides affect the growth of soil microorganisms either by directly feed soil microorganisms as

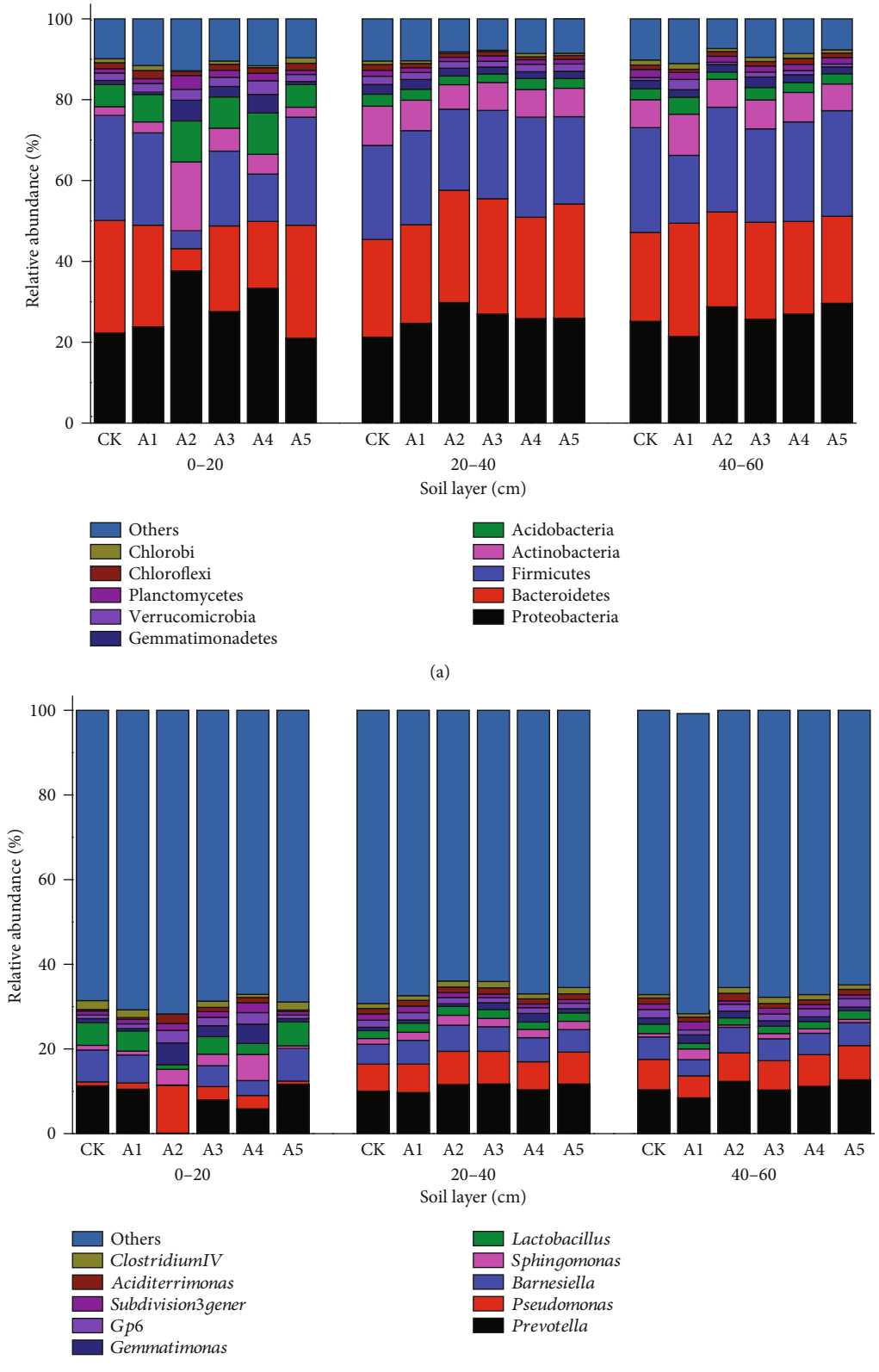

Figure 2: Relative abundance of the top ten bacteria in the phylum (a) and genus (b) level in the 0-20 cm, 20-40, and 40-60 cm rhizosphere soils. Note: CK, the no herbicide negative control; A0, a preemergence nonspecific herbicide for maize, dinitraniline (the active control); A1-A4: four preemergence maize specific herbicides, nicosulfuron+atrazine, alachlor+acetochlor+atrazine, propisochlor+atrazine, and acetochlor+atrazine, respectively.

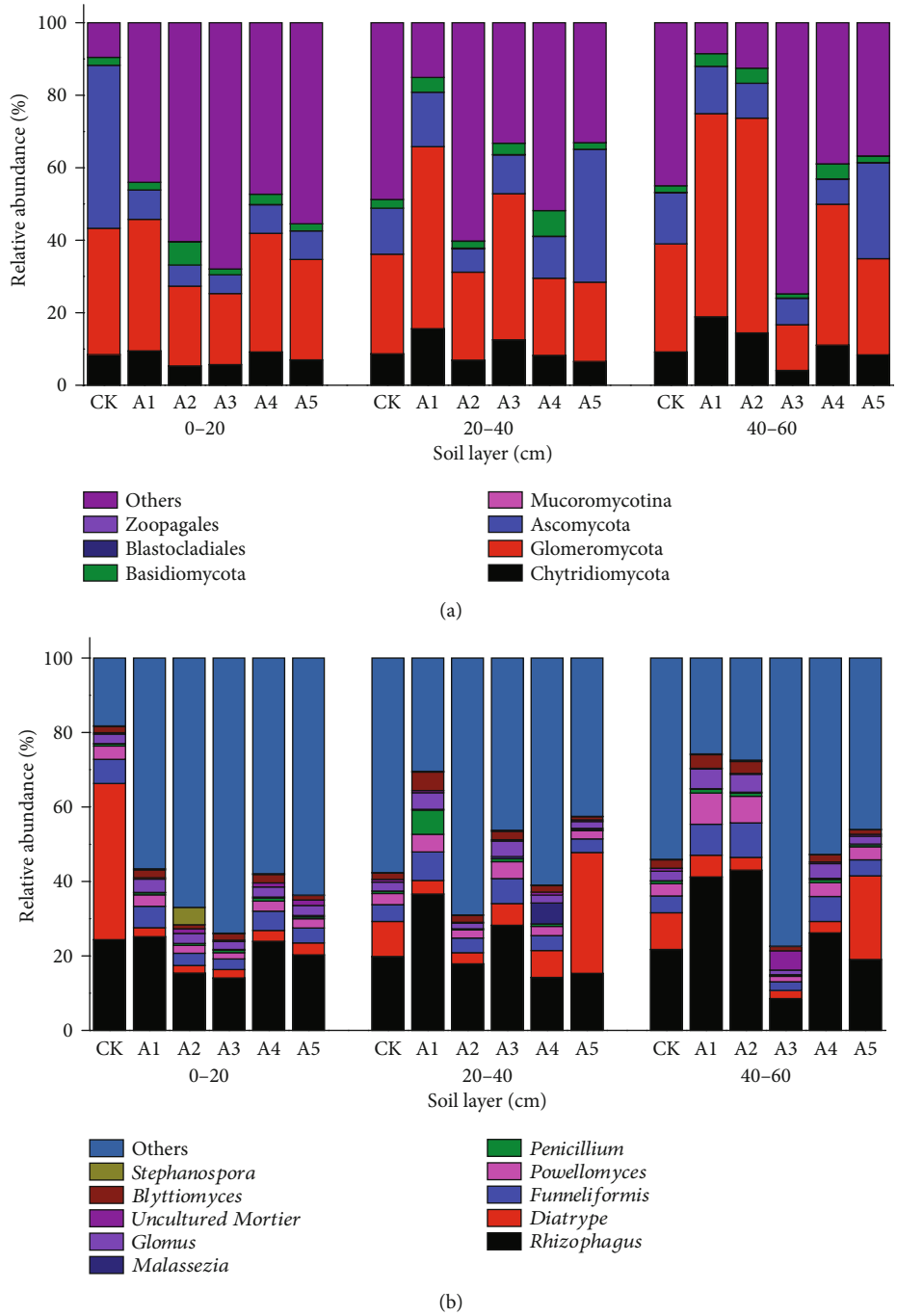

Figure 3: Relative abundance of the top ten fungi in the phylum (a) and genus (b) level in the 0-20, 20-40, and 40-60 cm rhizosphere soils. Note: CK, the no herbicide negative control; A0, a preemergence nonspecific herbicide for maize, dinitraniline (the active control); A1-A4: four preemergence maize specific herbicides, nicosulfuron+atrazine, alachlor+acetochlor+atrazine, propisochlor+atrazine, and acetochlor +atrazine, respectively.

an energy source, or indirectly by influencing the chemical environment, and then regulating the growth of soil microorganisms [32]. Zhou et al. thought that the increase of bacteria in soybean rhizosphere soil under fomesafen treatment might due to this herbicide provided more energy substances for some bacterial growth [24]. The application of sulfonylurea herbicide into winter wheat soil induced a chemical stress on soil and, then, influenced both the plants and the soil bacteria [14]. As it is well known that soil microbial community is regulated by plant root exudates, hence, herbicides may also influence soil microbial community through altering the growth of plant roots and the secretion of root exudates. This speculation still needs further researches.

Soil microorganisms may also reversely regulate the effects of herbicides on the soil environment by degrading the harmful active substances of herbicides. These herbicide-degrading microorganisms include *Bacillus* spp., *Pseudomonas* sp., and arbuscular mycorrhizal fungi [33–36]. In our research, the

Table 5: Effects of herbicides on the urease activity in the rhizosphere soil of maize.

Treatment	Soil layer (cm) 0-20 Measured value (mg g^{-1})	△CK%	20-40 Measured value (mg g^{-1})	△CK %	40-60 Measured value (mg g^{-1})	△CK %
CK	132.1 ± 6.6aA	—	130.1 ± 2.4abA	—	122.2 ± 12.6bcA	—
A0	81.1 ± 35.2bA	-38.6	87.0 ± 40.9cA	-33.1	84.9 ± 35.0dA	-30.5
A1	107.3 ± 42.3abA	-18.8	109.9 ± 44.8bcA	-15.6	114.5 ± 41.6cA	-6.3
A2	135.7 ± 14.3aB	2.7	149.7 ± 4.6aA	15.0	155.4 ± 7.9aA	27.1
A3	121.5 ± 15.2aB	-8.0	143.4 ± 9.8aA	10.2	146.3 ± 16.8abA	19.8
A4	111.8 ± 14.9aB	-15.4	127.2 ± 28.4abAB	-2.2	138.8 ± 8.1abcA	13.6

Note: CK, the no herbicide negative control; A0, a preemergence nonspecific herbicide for maize, dinitraniline (the active control); A1-A4: four preemergence maize specific herbicides, nicosulfuron+atrazine, alachlor+acetochlor+atrazine, propisochlor+atrazine, and acetochlor+atrazine, respectively. Data in the table are X ± SD ($n = 6$), different lowercase letters in the same column indicate significant difference ($P < 0.05$) between treatments, and different capital letters in the same row indicate significant difference ($P < 0.05$) between layers based on the LSD test.

relative abundance of *Pseudomonas* in the A1-treated soil was far higher than that in the control soil. The harmful components in A1 were degraded into unharmful ones by soil microorganisms such as *Pseudomonas* may partly illustrate the weakest effect displayed by A1 on maize growth.

4.2. Herbicides Affected the Activity of Soil Urease. Soil enzymes take part in the biochemical process and nutrient cycling in soil, which influence soil microecology environment [37, 38]. Urease enzyme is related to the nitrogen transformation in soil and has been used to evaluate the effects of herbicides on soil biochemical environment [2, 24]. Borowik et al. demonstrated that a mixture of herbicide (containing terbuthylazine, mesotrione, and S-metolachlor) inhibited the activity of urease in 0-20 cm maize soil [19]. Results from Du et al. and Sun et al. showed that urease activity did not change much in the soil treated with mesotrione [16, 29]. In this present study, different herbicides showed different effects on urease activity in the rhizosphere soil at different layers, among which A0 significantly decreased the activity of urease in the 0-60 cm rhizosphere soil, and other herbicides did not affect urease activity largely in general, except for alachlor+acetochlor+atrazine (A2) significantly increased the urease activity in the 40-60 cm soil. A large part of soil enzymes are extracellular metabolites secreted by soil microorganisms [39]. Herbicides application altered both the structure and abundance of the soil microbial community, which affect the activity of soil enzymes. Herbicides may also affect the activity of soil enzymes by changing the cell lysis and cell membrane permeability [40, 41]. Herbicides may also act as substrates or inhibitors for the catalytic reaction of soil enzymes, which directly affect the activity of soil enzymes [24]. In addition, some researchers reported that herbicides may influence the activity of soil enzymes by regulating the growth of plant roots, altering the absorbing ability of roots to soil nutrient elements and the root activity [24]. Whether the tested herbicides displayed similar mechanisms on the activity of urease in maize rhizosphere soil still needs more studies.

In general, the effects of herbicides on soil microbial environment varied based on herbicide species and soil depths. Among the five tested herbicides, only A0 contains dinitraniline, and the other four herbicides all contain atrazine. Among the four maize-specific herbicides, only A1 contains a sulfonylurea component (nicosulfuron), whereas the other three herbicides instead with chloroacetamide ones (alachlor, acetochlor, or propisochlor). When considering soil depth, the tested herbicides affected the microbial community stronger in the 0-20 cm layer than in the 20-60 ones, whereas A0 and A1 showed consistent effects, but A2-A4 first showed insignificant effect but, then, increased the activity of soil urease with the soil depth increased. The differences in chemical characteristic, degradation, and migration rates of active components in the five tested herbicides may partly explain these inconsonant results among different soil layers. Further experiments still need to be done to confirm this speculation.

4.3. Conclusions. The use of herbicide is still a common measure to control weed damage in maize field. Herbicides affect both soil microbial environment, and the activity of soil enzymes has been reported. However, the effects of preemergence herbicides on the microbial environment of maize rhizosphere soil after applying for a relatively long time are still unclear. In this research, we applied five preemergence herbicides on maize soil and investigated the effects of herbicides on microbial community and urease activity in the rhizosphere soil of maize in different layers at the maturity stage. We found that among the five tested herbicides, nicosulfuron+atrazine (A1) largely altered the bacterial community structure especially in the 0-20 cm layers and showed insignificant effect on the activity of urease in the 0-60 cm rhizosphere soils, with other three maize-specific herbicides (A2-A4) displayed mild effects. The nonspecific herbicide of maize, A0, changed the microbial community slightly but dramatically dropped the activity of urease in the 0-60 rhizosphere soils. Considering that A1 did not affect the grain yield of maize, but A0 totally suppressed the development of maize grain, we proposed that A1 was a suitable herbicide for maize, and both the bacterial community structure and the urease activity in the rhizosphere soil at 0-20 cm layers are suitable indicators for evaluating the effects of herbicides on maize plant growth and soil microbial environment. The differences in active components may contribute to explain the differences among herbicide species and soil depths.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

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