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Microbial rhizosphere communities in response to chlorimuron-ethyl herbicide in soils under alfafa crop

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ABSTRACT: Biolog Eco technology was used to investigate the effects of different chemical herbicide application methods, such as pre-emergence after sowing and post-emergence (stem and leaf spraying), on the characteristics of carbon source utilization by microbial communities in alfalfa rhizosphere soil. The averages of well color development (AWCD) and microbial metabolism diversity indices of post-emergence herbicide spraying on stems and leaves were significantly lower than those of pre-emergence herbicide and without herbicide treatments. Furthermore, pre-emergence after sowing herbicide treatment did not affect soil microorganisms. The principal component analysis (PCA) revealed that the microbial community diversities of the rhizosphere soils differed significantly between herbicide treatments. Carbohydrate was the carbon source type that was most sensitive to herbicide treatment changes, followed by amino acids and carboxylic acids. The herbicide application method significantly reduced the capacities of the microbial community to utilize the carbon sources, as mainly manifested in the ability to use polymers. As shown by a comprehensive analysis on the growth of alfalfa and the physiological and biochemical indices of its root system, the application of herbicide chlorimuron-ethyl reduced the alfalfa yield, and the two different application methods tested showed no significant difference. Herbicide application and different application methods showed significant impacts on alfalfa root activity and membrane permeability, suggesting that the application of herbicide damaged the membrane permeability and the activity of the alfalfa root system. Impacts of herbicide residues in the soil after stem and leaf post-emergence treatment on alfalfa growth and soil microorganisms should not be ignored.

Keywords: herbicide, biologic, soil microbial community, AWCD, PCA.

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INTRODUCTION

Alfalfa (*Medicago sativa*) is a widely cultivated legume forage and the first-choice species for many agricultural and pastoral areas (Yang, 2003; Cen et al., 2020). It has high nutritional value, good palatability, and strong adaptability. Heilongjiang province is the main production base of animal husbandry and dairy industry in China, and alfalfa has become the main source of forage for its animal husbandry.

Under large-scale mechanized production, unwanted weeds growing in the field negatively impact the yield and quality of alfalfa (Guo and Liu, 2004). The weeds compete with alfalfa for moisture and nutrients, and well-growing weeds can exert an overshadowing effect on leguminous plants, reduce their photosynthesis and growth (Xu et al., 2019). To prevent the damages caused by weeds, chemical herbicides have become an effective means of weed control. However, excessive use of chemical herbicides leads to the accumulation of chemicals in the ecosystem, polluting the environment.

Residual herbicides in the soil affect leguminous plant growth and increase the incidence of diseases by destroying the soil microbial community, jeopardizing the survival of non-target organisms, and reducing legumes-rhizobia symbiotic nitrogen fixation. Chlorimuron-ethyl is a selective pre-emergence and post-mergence herbicide, and its chemical name is ethyl 2-[(4-chloro-6-methoxypyrimidine-2-yl) carbamoyl sulfamcyl] benzoate. It is a sulfonylurea herbicide that can be absorbed by plant roots, stems and leaves, and is transported throughout the plant body, reducing the proliferation of fast-growing meristematic cells. It is mainly used for controlling broadleaf weeds in leguminous fields. Chlorimuron-ethyl blocks the biosynthesis of branched-chain amino acids and prevents cell division by blocking acetolactate synthase. In soil, Chlorimuron-ethyl is broken down through hydrolysis and biodegradation (Tao et al., 1995); however, given that it is not easily volatilized or photolyzed, its residual period in the soil is relatively long, making it particularly important to study its effects on the soil microbial ecosystem.

Soil microorganisms are important components of the soil ecosystem (Pankhurst et al., 1996). Their attributes can be sensitive indicators of soil quality and health. Quantifying the diversity of soil microbial communities can help assess the effect of soil contamination on soil productivity, and has become a major focus of research in recent years (Wardle and Parkinson, 1990; Lupwayi et al., 2004). The total amount and application area of chemical herbicide usage worldwide have exceeded the sum of fungicides and insecticides, yet only a few of the herbicides on the market are truly effective in reducing weeds.

Herbicides can directly or indirectly enter the topsoil or the cultivation layer, and can negatively impact the soil microbial flora and related soil biochemical processes (Crecchio et al., 2001; Haney et al., 2002), thus potentially damaging soil fertility and crop growth. Much of the past studies on soil microorganisms were limited to organisms that can be isolated from cultural media, however, culturable microorganisms account only for 0.1-10 % of the total microorganisms (Erb and Wagnerdöbler, 1993). Given that most soil microorganisms cannot be cultivated in lab media, traditional research methods cannot truly detect the community of soil microorganisms.

The functional diversity of soil microbial communities reflects their ecological characteristics and provides a viable, ecologically related measurement to assess microbial diversity (Zak et al., 1994). The Biolog-Eco plate method can be used to describe the characteristics of the microbial community functions and to measure the characteristics of community-level physiological profile (CLPP) in an easy, rapid and sensitive manner. It can also effectively evaluate the changes of the soil microbial community in different ecological environments (Zheng et al., 2005).

Currently, there are many reports on the effects of chemical herbicides on various organisms in the farmland (Perucci and Scarponi, 1994; Moreno et al., 2007; Weaver et al.,

2007); however, the effects of different chemical herbicide application methods, such as the pre-emergence closed weeding after sowing or the post-emergence stem leaf spraying on the soil ecological environment in the rhizosphere of alfalfa, are rarely covered. Here, the Biolog-Eco plates were used to evaluate the effects of different application methods of chemical herbicides on the functional diversities of the soil microbial community in the alfalfa rhizosphere soil, to provide a scientific basis for the risk management of herbicides, as well as offering theoretical support for the mechanization of alfalfa production.

MATERIALS AND METHODS

Site description

The study site was in the testing field of the Crop Institute of the Heilongjiang Academy of Land Reclamation Sciences in Jiamusi City, Heilongjiang Province (46° 46' N, 130° 27' E). The altitude is 95.0 m; the annual average temperature is 0.5 °C. The annual average temperature is 4 °C. The annual average rainfall is 450-550 mm, more than 59 % of the rainfall occurs between July-September. According to the USDA soil Taxonomy system (Soil Survey Staff, 2010), the soil was classified as Mollisols (Udolls). The organic nitrogen content was 0.58 g kg^{-1}; the available nitrogen content was 89.2 mg kg^{-1}, the available phosphorus content was 128.2 mg kg^{-1}, the available potassium content was 106.2 mg kg^{-1}, and its pH(H_2O) was 6.8.

Experimental design

Alfalfa (*Medicago sativa* cv. Winter star) used for the experiments was provided by the Crop Institute of the Heilongjiang Academy of Land Reclamation Sciences in Jiamusi City, Heilongjiang Province. A randomized block design was prepared with three replicates for each treatment. There were five rows in a block with a length of 5 m and the space between rows was 0.3 m (Yan, 1998). The area of the block was 7.5 m^2. Seeds were sowed with a sowing rate of 2 g row^{-1}, and a sowing depth of 2 cm. There was a serious drought at sowing, so a ditch was made for watering to ensure timely emergence. Three treatments were established: the control with no herbicide treatment (treatment I), pre-emergence herbicide spraying after sowing (treatment II), and post-emergence herbicide spraying on stems and leaves (treatment III). The herbicide tested was 20 % wettable powder of chlorimuron-ethyl provided by the Crop Institute of the Heilongjiang Academy of Land Reclamation Sciences in Jiamusi City, Heilongjiang Province. The formulation and spraying amount was 0.105 kg hm^{-2}. Alfalfa plants were harvested at the mature, and the yield was measured.

Soil sampling

The treatments were arranged in a randomized block design with three replicates (3 plots in total; plot size 5 × 5 m each). The five-point sampling method was used to collect plant samples. The topsoil layer (0.00-0.05 m) was removed, and the soil around the roots was then gently shaken off. After that, the soil attached to the root surface was gently brushed off to prepare the rhizosphere soil (Garcia et al., 2005). After removing the gravel, plant residues and other debris in the soil sample, five samples were collected from each plot and thoroughly mixed to obtain a composite sample for the replicate. The selected soil sample was then sealed in a sterile bag and brought to the lab in an ice-box. The collected soil samples were sieved with a 2 mm sieve and stored at 4 °C.

Determination of root activity and relative membrane permeability

Root activity was analyzed by the triphenyl tetrazolium chloride (TTC) method (Wang and Huang, 2015). In brief, 0.5 g fresh root was immersed in 10 mL of equally mixed solution

of 0.4 % TTC and phosphate buffer, and kept in the dark at 37 °C for 2 h. Subsequently, 2 mL of H_2SO_4 1 mol L^{-1} was added to stop the reaction with the root. The root was dried with filter paper and then extracted with ethyl acetate. The red extractant was transferred into the volumetric flask to reach 10 mL by adding ethyl acetate. The absorbance of the extract at 485 nm was measured.

$$Root\ activity = \frac{\text{amount of TTC reduction (mg)}}{\text{fresh root weight (g)} \times \text{time (h)}} \quad \text{Eq. 1}$$

The relative membrane permeability (RMP) was determined as Yang et al. (1996) described. The leaves were excised and put in test tubes containing 20 mL of deionized distilled water. The test tubes were vortexed for 5 s and the solution was assayed for initial electrical conductivity (EC_0). These tubes were kept at 4 °C for 24 h and then assayed for EC_1. The same samples containing leaves were autoclaved at 120 °C for 20 min to determine EC_2.

Percent RMP was calculated according to equation 2:

$$RMP\ (\%) = \frac{[EC_1 - EC_0]}{[EC_2 - EC_0]} \times 100 \quad \text{Eq. 2}$$

Substrate utilization patterns

Substrate utilization patterns were measured using Biolog-Eco plates (BIOLOG, Inc.). A 10^{-1} microbial suspension was prepared by suspending 10 g of fresh soil in 100 mL NaCl 0.85 % solution. The slurry was processed with a Vortex mixer for 1 min at maximum speed and centrifuged for 10 min at 500 *g*. Ten-fold serial dilutions were performed, and 150 µL of the 10^{-3} dilutions were pipetted into microplates using an 8-channel micropipetter. Microplates were incubated at 28 °C for 216 h. The color development at OD_{578} nm was read for each well at 24-h intervals. Negative values were set to zero. The average well color development (AWCD) value of the Biolog data was calculated for each sample at each time point by dividing the sum of the optical density data by 31 (number of substrates), as described by Garland (1996). The three replicate rhizospheric samples were used to prepare the three microbial suspensions for Biolog plates.

The 72-h data were used to measure the functional and species diversity of the soil microbial community, and the following parameters were calculated using the equations 3, 4, and 5:

Shannon-Wiener index $H = -\sum Pi \times \ln(Pi)$ Eq. 3

Simpson index $D = 1 - \sum (Pi)^2$ Eq. 4

McIntosh index $U = \sqrt{(\sum ni^2)}$ Eq. 5

in which: *Ni* is the relative OD in each carbon source well, and *Pi* is calculated by subtracting the control from the absorbance of each substrate and then by dividing this value by the total color change recorded for all 31 substrates.

Statistical analysis

Excel 2003 was used for statistical analysis of the data; SPSS 16.0 software was used for one-way ANOVA analysis and PCA (α-=0.05).

RESULTS

Alfalfa yield

The effects of different application methods of the chemical herbicide on alfalfa yield are shown in table 1. Compared with the control with no herbicide treatment, applying chlorimuron-ethyl significantly reduced alfalfa yield. Although the alfalfa yield of treatment III was lower than treatment II, the difference was not significant.

Physiological and biochemical indexes of alfalfa

Alfalfa root vitality was significantly reduced with the application of herbicide (Table 2). Alfalfa root vitalities of treatments II and III were 22.19 and 33.00 % lower than that of the control group, respectively. The relative permeability of the cell membrane was higher in treatments II and III; therefore, the herbicide increased the relative permeability of the root cell membrane. Interestingly, treatment III had the strongest impact on the relative permeability of the cells, where there was a 68.39 % increase compared with the control group.

Community-level physiological profile

The average absorbance value can be used to evaluate the ability of the soil microbial community to utilize carbon, and to indicate the metabolic activity of the soil microorganisms. During the incubation period, the AWCD increased with time, and presented an S-shaped curve (Figure 1). There were significant differences in the degree of the utilization of the single carbon source by the rhizosphere soil microbial communities among the three different treatments. The activity of the soil microbial carbon metabolism of treatment II was similar to that of the control with no herbicide treatment, but it was significantly higher than that of treatment III, indicating that the rhizosphere soil microorganisms in treatment III had the lowest AWCD, the slowest exponential growth, and the weakest carbon metabolic activity.

Diversity index

The utilization of different carbon sources by the soil microbial community can be expressed by the diversity index, thus reflecting the dynamic changes of the whole community (Tian et al., 2003). After different herbicide treatments, Shannon index and Simpson

Table 1. Fresh weight of alfalfa under different treatments

Treatment	Fresh weight	
	kg plant^{-1}	kg ha^{-1}
Ⅰ	5.63 ± 0.63 a	1042.59 ± 115.75 a
Ⅱ	4.22 ± 0.08 b	781.48 ± 14.81 b
Ⅲ	4.05 ± 0.35 b	750.00 ± 64.81 b

Treatment: Ⅰ, control; Ⅱ, pre-emergence after sowing herbicide spraying; Ⅲ, post-emergence stem, and leaf herbicide spraying. Different letters indicate a significant difference at $p<0.05$.

Table 2. Root activity and relative membrane permeability in different treatments

Treatment	Root activity	Relative membrane permeability
	mg g^{-1} h^{-1}	%
Ⅰ	663.4 ± 21.5 a	30.21 ± 3.33 c
Ⅱ	516.2 ± 23.5 b	43.42 ± 4.43 b
Ⅲ	444.5 ± 34.7 c	50.87 ± 5.92 a

Treatment: Ⅰ, control; Ⅱ, pre-emergence after sowing herbicide spraying; Ⅲ, post-emergence stem, and leaf herbicide spraying. Different letters indicate a significant difference at $p<0.05$.

index showed similar patterns of change in the rhizosphere soil microbial carbon source utilization (Table 3) where there was no significant difference between treatment II and the control ($p>0.05$), but they were significantly higher than treatment III ($p<0.05$). These results suggest that treatment III significantly impacted both the richness of community species and the most common species. The McIntosh index of carbon source utilization showed no significant differences among the treatment groups, indicating that treatments II and III had little impact on microorganism community uniformity.

Principal component analysis of bacterial community

The principal component analysis (PCA) of carbon source utilization by the soil microbial community can reveal the comprehensive differences and similarities of the rhizosphere microbial community functions after different herbicide treatments. In this experiment, the culture data at 72 h were used for the PCA. The results showed that the variance contribution rates of the first three of the 31 principal components were 35.64, 17.72, and 13.00 %, respectively, and that the cumulative variance contribution rate reached 66.36 % (Figure 2), which could be used to characterize the basic profile of the rhizosphere soil microbial community after different herbicide treatments. There were significant spatial differences in the 2-dimensional system formed by the 31 principal components among the three different treatment groups (Figure 4). The projection points of the herbicide free group were obviously distributed in the positive direction of the X axis (PC1), while the projection points from treatments II and III were mainly distributed in the negative side of Y axis (PC2) and they were closed together, indicating that the soil microbes of the two treatment groups had similar carbon source utilization patterns.

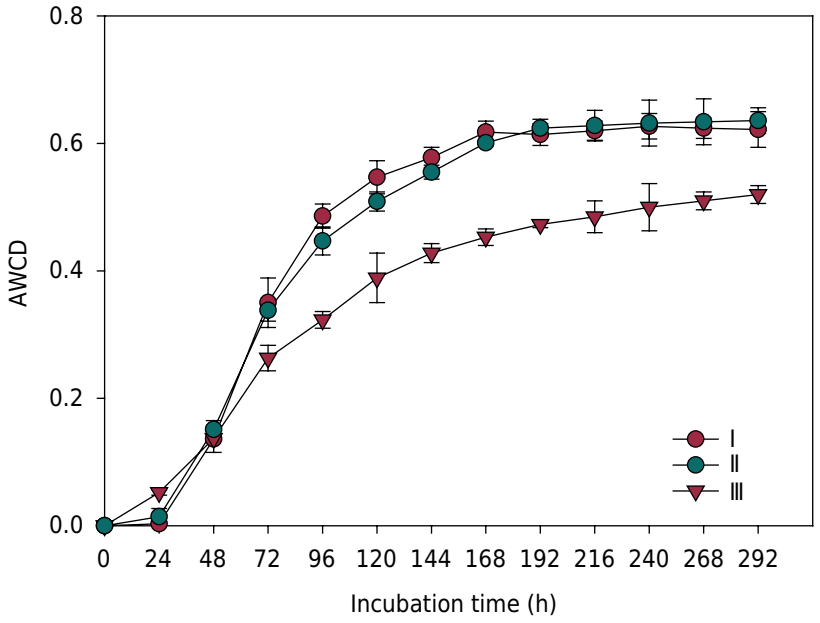

Figure 1. Average well color development (AWCD) obtained by Biolog-Eco Plate™ incubation of all treatments. Treatment: I, control; II, pre-emergence after sowing herbicide spraying; III, post-emergence stem, and leaf herbicide spraying.

Table 3. Soil microbial communities diversity indices of 72 h

Treatment	Shannon index	Simpson index	McIntosh index
I	1.42 ± 0.02 a	0.96 ± 0.00 a	2.16 ± 0.27 a
II	1.40 ± 0.04 a	0.95 ± 0.01 a	1.98 ± 0.42 a
III	1.30 ± 0.05 b	0.94 ± 0.01 b	1.72 ± 0.06 a

Treatment: I, control; II, pre-emergence after sowing herbicide spraying; III, post-emergence stem, and leaf herbicide spraying. Different letters indicate a significant difference at $p<0.05$.

As can be known from the correlation matrix of PCA, the carbon source types of the first two principal components with the eigenvector coefficient greater than 0.50 were analyzed (Table 4). There were 14 types of carbon sources that contributed significantly to PC1, among which carbohydrates accounted for 12.90 %, amino acids and carboxylic acids accounted for 9.68 % and polymers accounted for 6.45 %. Thus, the most influential carbon source of PC1 was carbohydrates, followed by amino acids, carboxylic acids and polymers. There were 12 types of carbon sources that contributed significantly to PC2, among which carbohydrates accounted for the highest proportion of 12.90 %, followed by miscellaneous and carboxylic acids, each accounting for 9.68 %. Thus, the most influential carbon source of PC2 was carbohydrates, followed by miscellaneous and carbohydrates. Ammonia/amine carbon sources did not significantly affect the principal component contribution.

Soil microbial physiological carbon metabolic fingerprint analysis

The PCA of the metabolic function of the soil microbial community reflects the overall changes of the community, but it does not reflect details about microbial metabolism.

Table 4. Loading factors of principal component of 31 carbon sources

Chemical guild	Carbon source	PC1	PC2
Amino acids	Glycyl-L-glutamic acid	0.14	-0.26
	L-threonine	0.61 *	0.17
	L-serine	0.50 *	0.49
	L-phenylalanine	0.92 **	0.12
	L-asparagine	-0.48	-0.32
	L-arginine	0.82 **	-0.29
Carboxylic acids	D-malic acid	0.60 *	0.16
	α-ketobutyric acid	0.10	0.54 *
	Itaconic Acid	0.82 **	-0.37
	γ-hydroxybutyric acid	0.73 *	0.42
	4-Hydroxy benzoic acid	0.39	0.62 *
	2-hydroxy benzoic acid	0.12	0.28
	D-galacturonic acid	0.10	-0.56 *
	D-galactonic acid γ-lactone	0.17	0.33
	D-glucosaminic acid	0.74 *	-0.22
Miscellaneous	Glucose-1-phosphate	-0.08	0.56 *
	D, L-α-glycerolohosphate	0.16	0.93 **
	Pyruvic acid methylester	0.41	0.76 *
Polymers	Tween 40℃	0.93 **	0.05
	Tween 80℃	0.26	0.82 **
	α-cyclodextrin	0.83 **	0.30
	Glycogen	0.45	0.64 *
Carbohydrates	D-cellobiose	-0.26	0.56 *
	α-D-Lactose	0.53 *	0.79 *
	β-methyl-D-glucoside	0.70 *	0.22
	D-xylose	0.82 **	0.14
	i-erythritol	0.71 *	0.23
	D-mannitol	0.09	0.71 *
	N-acetyl-D-glucosamine	0.25	0.68 *
Amines/amides	Putrescine	0.29	-0.40
	Phenylethylamine	-0.23	0.20

Exploring the capacity of soil microbes to utilize different carbon sources on the micro-plate through Biolog metabolic fingerprinting promotes a more comprehensive understanding of the metabolic function characteristics of the microbial community. There were relatively significant differences in the utilization intensities of soil microbes of carbon sources (Figure 3).

In the alfalfa rhizosphere soil with no herbicide treatment, the microbial utilization of carbon sources were in the order of carbohydrate > miscellaneous > polymers > amino acids > carboxylic acids > amines/amides; the order of the microbial utilization of carbon sources in the rhizosphere soil of treatment II was: carbohydrate > amino

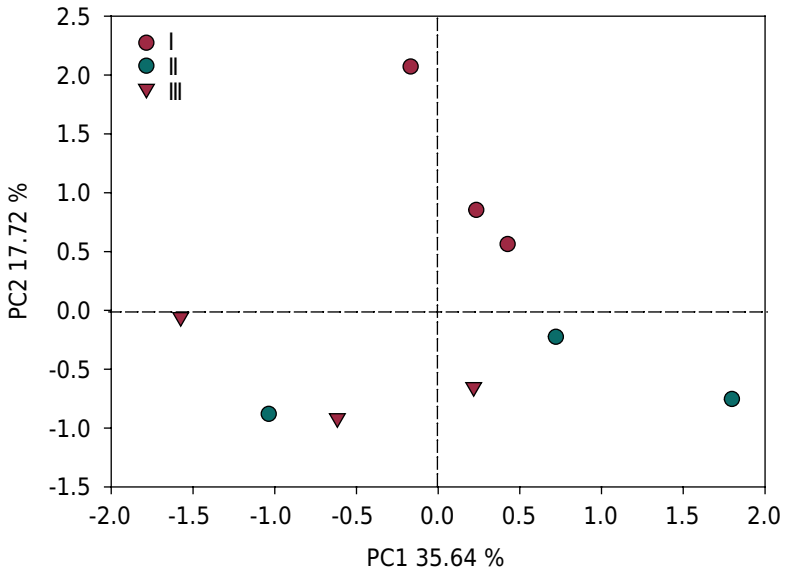

Figure 2. Principal component analysis (PCA) of the use on different carbon sources for soil microbial community. Treatment: Ⅰ, control; Ⅱ, pre-emergence after sowing herbicide spraying; Ⅲ, post-emergence stem, and leaf herbicide spraying.

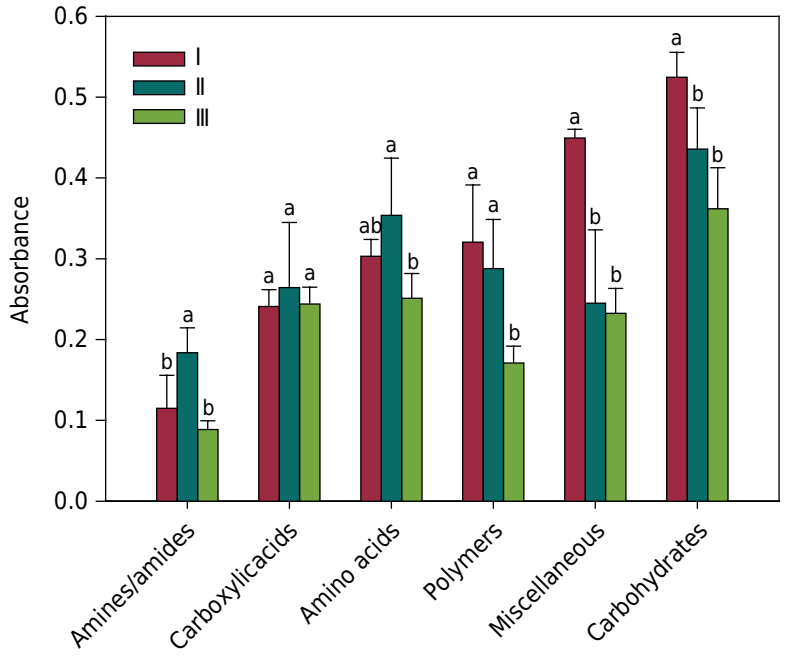

Figure 3. Relative use efficiency of soil microbial community on different carbon sources. Treatment: Ⅰ, control; Ⅱ, pre-emergence after sowing herbicide spraying; Ⅲ, post-emergence stem, and leaf herbicide spraying. Different letters indicate a significant difference at $p<0.05$.

acids > polymers > carboxylic acids > miscellaneous > amines/amides; the order of the microbial utilization of carbon sources in the rhizosphere soil of treatment III was: carbohydrate > amino acids > carboxylic acids > miscellaneous > polymers > amines/amides. On the whole, soil microorganisms had the strongest carbon source utilization capacity over carbohydrates and the weakest carbon source utilization capacity over ammonia/amine compounds. As can be seen from comparing the six major carbon source utilization capacities using different herbicide treatment methods, the ammonia/amine and carboxylic acids carbon source utilization capacities were similar among the three different herbicide treatment methods with no significant differences. Treatments II and III lowered the utilization intensities of miscellaneous and carbohydrates, and the impact of application methods was insignificant. The most significant impact of treatment methods was on the utilization intensity of polymers, and the soil microbial polymer utilization capacity in treatment II was significantly higher than that of treatment III. It demonstrated that both the application of herbicide and the application methods changed the metabolic ratio of carbon source utilization by the soil microorganisms.

DISCUSSION

In recent years, the widespread usage of herbicides has impacted non-target organisms. Many studies both at home and abroad have been reported on this subject (Slattery et al., 2001; Anderson et al., 2004). The application of herbicides has different effects on the vitality of plants. On the one hand, it affects plant growth and photosynthesis (Sajedi et al., 2013; El-Metwally et al., 2021), and on the other hand, it affects microbial survival, population size, and rhizosphere micro-ecological environment (Medo et al., 2021; Pertile et al., 2021) thereby reducing rhizosphere rhizobia survival and population size, which further reduces nitrogen uptake of plants. Bucholtz and Lavy (1979) reported that trifluralin inhibited the growth of plants, reduced the absorption of nitrogen, and caused abnormal growth and development of plants.

Another study on the effects of bentazon, metolachlor, fluchloralin, and 2,4-D on chickpea fields showed different degrees of effects on plant vitality, chlorophyll content, and plant nitrogen content and seed yield (Khan et al., 2004). In this study, we found that the application of the cholrimuron-ethyl had some effects on alfalfa yield (Table 1). Compared with the treatment with no herbicide, the application of herbicide significantly reduced alfalfa yield. Alfalfa yield in treatment III was lower than that of treatment II, but the difference was not significant. These results were in accordance with Hammok et al. (2020), which found that the spraying the post-emergence showed a significant decrease in fresh weight of plant (g) and weight of 1000 seeds (g). The decrease of yield may be that the herbicide induced oxidative stress and thus damage to and oxidation of the DNA (Demir et al., 2017). Moreover, after spraying the post-emergence may be due to the contact of the herbicide with the apical meristem zoning regions, which seems likely then that the inhibition of division and stopping of growth was observed (Singh and Srivastava, 2014). The use of herbicides and the particularly way the herbicide was applied exerted significant impacts on alfalfa root vitality and cell membrane permeability. The stem and leaf spray method decreased alfalfa root vitality and increased cell membrane permeability (Table 2), suggesting that applying herbicides can negatively affect the alfalfa root system.

The diversity of soil microbial community can reflect the quality of the soil environment and reveal differences in the ecological functions of microorganisms (Li et al., 2020). Changes in microbial communities are considered one of the most sensitive biological indicators of the health of the soil environment. It reflects the soil quality and even the environmental safety to a certain extent. After entering the soil, herbicides may cause structural and functional changes to the soil microbial community, thus affecting soil biochemical processes and ultimately influencing soil fertility and plant growth (Deer,

2002; Medo et al., 2021). The results of the Biolog Eco-plate AWCD and the microbial metabolic diversity indices showed no significant differences between treatment II and the control group (Figure 1 and Table 2), indicating that the herbicide did not affect soil microorganisms when applied at pre-emergence after sowing. The characteristics and mode of use of treatment II could explain the results. First, these chemical herbicides have a short light decomposition time or are rapidly degraded after rainfall; second, the herbicide is primarily applied to topsoil (Ganapathi et al., 2020). After application, the herbicide cannot immediately enter the soil, resulting in a low residual soil concentration of the herbicide, and the amount entering the rhizosphere is even less. Thus, the effects of the herbicide on the rhizosphere microbial communities are not significant. Some studies have found similar results. For example, agricultural chemicals rarely have lasting effects on the total soil microorganisms. After degradation in soil, their impact on the biological indexes that can represent the ecological effects of the soil environment is also reduced (Yao and Zhang, 2008).

Different from the effect of treatment II, treatment III significantly decreased AWCD and microbial diversity index (Figure 1 and Table 3). As shown by a comparison of different methods of applying the same chemical herbicide, treatment III lowered AWCD and the microbial metabolic diversity indexes, indicating that the microbial diversity was significantly decreased after treatment III.

The PCA showed that the functional diversities of the microbial community of the three treatment groups were significantly different. The carbon source most sensitive to the changes of herbicide application was carbohydrates, followed by amino acids and carboxylic acids. The herbicide application method significantly reduced the microbial communities' utilization of the carbon sources on the Biolog Eco-plate, as mainly manifested in the ability to use polymers.

The effect of herbicide application methods on polymer utilization intensity was the most significant, and the microbial community's ability to utilize polymers in treatment II was significantly better than that in treatment III. Therefore, the use of herbicides and the specific method by which the herbicide is applied can both cause the shift of the types of carbon source utilized by the soil microbial community, further illustrating that chlorimuron-ethyl herbicide influenced the diversity of the alfalfa rhizosphere soil microbial community.

Chemical herbicides applied on the stems and leaves can be absorbed through the foliage quickly. After diffusing through the cuticle, herbicides are transported through the phloem and enriched in the stems and roots of the plants and weeds, thus exerting their weed-killing effect (Su and Geng, 2002). Under normal circumstances, the herbicide concentration in the rhizosphere is relatively high under the post-emergence stem and leaf herbicide spraying method and its effective period in the soil is longer. This requires a longer cycle period to degrade, leading to the loss of the biochemical characteristics of the soil ecosystem required for the normal functioning of the microbial community (Imparato et al., 2016).

Compared to treatment II, the timing of the application of treatment III was different, so the applied herbicide was under the influence of environmental factors such as temperature, rainfall and sunshine (Zhang and Wang, 2002), which further results in corresponding changes in the structural and functional diversities of the soil microbial community. Studies have shown that most herbicides and pesticides can be toxic to soil microorganisms or inhibit their activities (Yao and Zhang, 2008), consistent with the results of our study. A study by Seghers et al. (2003) found that long-term use of Atrazine and Metolachlor changed the soil microbial community structure, especially the composition of the aerobic methanotrophic bacteria, but some herbicides can also increase the amount of certain microorganisms in the soil (Taiwo and Oso, 1997). In short, herbicides can change soil microbial community structure and activity.

CONCLUSION

Post-emergence stem and leaf herbicide spraying can reduce microbial community diversity and microorganisms' ability to utilize carbon sources on Biolog Eco-plates. As a result, the impact of post-emergence stem and leaf herbicide spraying on soil microorganisms must not be overlooked. When applying herbicides to a large area, the method of pre-emergence close treatment after sowing should be used because it is less expensive and has lower impact on microorganisms. However, because soil mixing after spraying can result in soil moisture loss, herbicides should be applied in the early spring or autumn prior to harvest.

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AUTHOR CONTRIBUTIONS

Conceptualization: Junnan Ding (equal).

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Formal analysis: Junnan Ding (lead) and Xin Li (supporting).

Resources: Xin Li (supporting).

Software: Xin Li (supporting).

Writing - original draft: Xin Li (supporting).

Writing - review & editing: Junnan Ding (equal) and Xin Li (supporting).

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