International Scholars Journals

African Journal of Parasitology Research ISSN 2756-3391 Vol. 8 (8), pp. 001-007, August, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of microbial fertilizer on microbial activity and microbial community diversity in the rhizosphere of wheat growing on the Loess Plateau

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Accepted 22 May, 2021

In this study, the effects of microbial fertilizer on microbial activity and microbial community diversity in the rhizosphere of wheat were investigated in the field conditions. Dehydrogenase activity was measured, bacterial and fungal communities were characterized by BIOLOG analysis. Soil suspensions were applied to BIOLOG ECO and FF plates and average well-color development (AWCD) and richness were calculated. The results showed that significant differences were observed in dehydrogenase activity among the tested soils, arranging from 26.14 ± 3.30 to 48.13 ± 17.39 TF/g 24h. For ECO plate, AWCD of MI (0.77 ± 0.05) was higher than that of other treatments, whereas the AWCD for control (0.56 ± 0.03) was consistently the lowest (F=8.72, p=0.0067). Meanwhile, there is a significantly difference between EP (0.16 ± 0.03) and EP+MI (0.11 ± 0.03) treatments in fungal community. Richness index followed the order for ECO plate: MI > EP > EP+MI > Control, and also order for FF plate as: EP>MI=Control>EP+MI. Principal component analyses (PCA) of BIOLOG data further revealed large difference in microbial diversity (metabolic diversity) from treatments soils. The data gathered from this study can provide complementary information for agricultural projects on the Loess Plateau, Northwestern China.

Key words: BIOLOG, bacterial and fungal communities, microbial fertilizer, microbial activity, wheat.

INTRODUCTION

Loess Plateau, covering approximately 58 x 10^4 km^2, has become one of the weakest ecological environmental regions in Northwestern China (Zhang et al., 2006). The main crops are wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) in this arid and semiarid area. Many kinds of microbial fertilizers were used in agricultural practice, and the roles of microbial fertilizer in improve the growth of wheat seedlings has been recognized (Ogut et al., 2005; Arkhipova et al., 2006; Hu et al., 2010). Previous research has shown that measurements of soil microbial community parameters have often been used for soil quality evaluation (Stark et al., 2008). Unfortunately, little attention has been paid to study the effect of microbial fertilizer on microbial activity and microbial community diversity in the rhizosphere of wheat.

Dehydrogenase activity in soil is important as it indicate the potential of the soil microbial activity to support biochemical processes which are essential for the maintenance of soil fertility. It provides correlative information on the microbial composition in the soil. Nannipieri et al. (1990) also suggested that dehydrogenase activity is also an indicator for the microbial activity of the soil. Dehydrogenase activity has been proposed as potential

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indicator of soil microbial quality because of their sensitivities to various environment factors such as herbicides, slope aspect or management. However, until recently, little is known about dehydrogenase activity response of microbial fertilizer utilization in the rhizosphere soil of wheat planting on the Loess Plateau, Northwestern China. BIOLOG is based on the utilization by microbial communities on different carbon substrates, shifts in BIOLOG metabolic diversity can be related to shift in microbial community diversity. Community level-physiological profiles (CLPPs), constructed using BIOLOG plate, have shown their potential as an ecologically relevant method to characterize soil microbial community diversity of different ecosystems (Axelrood et al., 2002), different land use histories (Benizri and Amiaud, 2005), and different forest restoration approaches (Zheng et al., 2005). Recently, the BIOLOG study conducted by Zhang et al. (2010) suggested that application of ECM fungi significantly influenced bacterial functional diversity in the rhizosphere of *Pinus tabulaeformis* seedlings in the field conditions of the Loess Plateau. However, there is less information as regards the use of BIOLOG ECO and FF plate assay to assess the effect of microbial fertilizer on microbial activity and microbial community diversity in the rhizosphere of wheat growing on the Loess Plateau.

Consequently, the specific objective of this study was to detect, in the field conditions, the modifications of soil microbial activity and microbial community diversity in the rhizosphere of wheat after applying microbial fertilizer. The data gathered from this research could provide important information for reasonable utilization of microbial fertilizer in agricultural projects on the Loess Plateau, Northwestern China.

MATERIALS AND METHODS

Experimental design and sampling

The field experiment was conducted at Heng Qu farm, Mei County, Shaanxi Province, Northwestern China (107°39 E, 33°59 N). Rainfall distribution is uneven, most of which occurred between June and September, annual average precipitation is 609.5 mm, while mean annual temperature is 12.9°C. The study site has been cultivated in corn- wheat rotation for twenty years and has a basic irrigation system. The soil is classified as Lu soil. The basic physicochemical characteristics of the soil are as follows: pH (1:2.5, soil: water): 7.69, organic carbon: 8.16 $g \cdot kg^{-1}$; total nitrogen: 0.63 $g \cdot kg^{-1}$; total phosphorus: 0.34 $g \cdot kg^{-1}$, and available potassium: 82.50 $mg \cdot kg^{-1}$. The straw materials (7250 kg/ha) were added in the soil before planting establishment. The experimental design was a randomized split-plot factorial design with three randomized complete blocks. Each plot was an area of 3 x 90 m. No chemicals were applied for plant protection and the plots were weeded by hand. Microbial inoculum used in this study is *Azotobacter chroococcum* Beijerinck. The treatments were: (1) Control, (2) microbial inoculum (MI): 75 kg/ha, (3) enzyme preparations (EP) 450kg/ha, (4) microbial inoculum + enzyme preparations (MI+EP): 75 + 450 kg/ha, respectively. Enzyme preparations were obtained from Enzyme Engineering Institute of Shaanxi Academy of Sciences. Untreated winter wheat seeds cv. Xinong 889 (Northwest A and F University, China) was used in this study. The wheat seedlings protection, irrigation, and other management practices were followed during wheat growth. In the whole growth periods, no pesticides were applied. Before harvesting process, all the material used for sampling was sterilized. According to the methods conducted by Butler et al. (2003), five wheat plants with roots and soil cores were sampled using a soil corer, to a depth of 0 to 15 cm at five random spots in each plot. Wheat seedlings with root-soil systems were shaken vigorously to separate soil not tightly adhering to the roots for about 1 min. The remaining thick soils tightly attached to the root system were considered rhizosphere soil (Butler et al., 2003). Green matter, visible stones and coarse particles were removed and the composite samples were passed through a 2 mm sieve and stored at 4°C for less than 48 h until analyzed.

Microbial activity determination

According to the methods conducted by Guan et al. (1986) and little modification, soil dehydrogenase activity was determined by reducing 2, 3, 5-triphenyltetrazolium chloride (TTC). Dehydrogenase enzyme converts TTC to 2, 3, 5-triphenylformazan (TPF). The TPF formed was extracted with acetone, the extracts were filtered (Xing Hua, China) and absorption was measured at 485 nm with a spectrophotometer (UV-2100, China).

Microbial community diversity determination

BIOLOG profile was determined by using BIOLOG ECO plate for bacterial community determination and BIOLOG FF plate for fungal community determination. ECO plates (Biolog, Inc., Hayward, California, USA) where all carbon substrates are known as root exudates were used to determine culturable bacterial samples, and FF plates were employed to determine fungal community diversity (Zhang et al., 2008).The soil samples (5 g d.w.) were shaken with 45 ml of autoclave-sterilized saline solution (0.85% NaCl, w/v) for 30 min, and then brought to a final dilution of 10^{-3}. A 150 l aliquot was inoculated into each micro-plate well. All plates were placed in polyethylene bags to reduce desiccation while incubating in dark growth chambers for 240 h at 25°C. The absorbance of wells at 590_{nm} (for BIOLOG ECO plate) or at 750_{nm} (for BIOLOG FF plate) was measured using ELISA plate reader every 12 h. The raw (absorption) data were then transformed: $X\text{-}X_0$, where X was the mean of the same three wells per plate and X_0 was the mean of the control (water blanks) per plate (Classen et al., 2003). Absorbance was read at intervals and in wells that were negative or under 0.06 were set to zero. Microbial activity in each micro-plate expressed as average well-color development (AWCD), and was determined using the following formula: AWCD = $(X\text{-}X_0)$ n (n=31 or 95) functional diversity was measured as substrate Richness (R), and R is the number of oxidized carbon substrates.

Statistical analysis

Dehydrogenase activity, AWCD and Richness were statistically tested by one way analyses of variance (ANOVAs) using SAS version 8.1 software (SAS Institute Inc., Cary, NC, USA). For the BIOLOG analyses, the principle component analyses (PCA) was employed with SPSS 16.0 software for Windows. Based on examination of kinetic curves of AWCD, 72 h measurements were chosen for data analysis. Comparisons between means were carried out using Duncan's multiple range tests. Graphical work was carried out using Sigma Plot for Windows version 10.0 software packages.

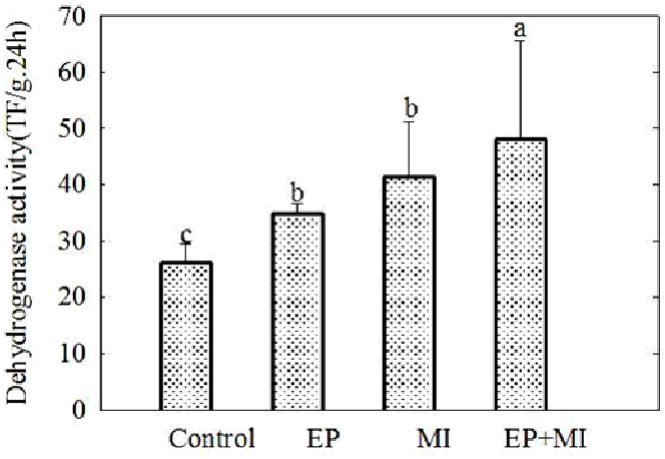

Figure 1. Dehydrogenase activities of the EP, MI, EP+MI and Control treatment soils, the data shown are the means and standard deviation. The same letter indicates no significant difference by Duncan's test. MI: Microbial inoculum, EP: Enzyme preparations, MI+EP: Microbial inoculum + Enzyme preparations, respectively.

RESULTS

Microbial activity determination

Differences of dehydrogenase activity for soil microbial compositions were significant among the treatments (Figure 1). Dehydrogenase activity ranged from 26.14 ± 3.30 TF/g 24 h for control soils to 48.13 ± 17.39 TF/g 2 4h for MI+EP soil. There were significant difference in dehydrogenase activity of soil between MI+EP soil and other treatments ($p < 0.01$), but not between EP and MI ($p > 0.05$). Dehydrogenase activity for control soil is the lowest and the differences between control and other three treatments were significant ($p < 0.01$).

Microbial community determination

The carbon substrate metabolic activities of soils from the treatments were measured by AWCD Figure 2. Soil bacterial community activity as measured by AWCD $_{(ECO)}$, continued to increase during the whole incubation and was consistently higher for MI (0.77 ± 0.05) than for other treatments, whereas the AWCD for Control (0.56 ± 0.03) was consistently the lowest ($F=8.72$, $p=0.0067$). Fungal community activity as measured by BIOLOG $_{(FF)}$, there is no a significantly difference between the treatments ($F=3.16$, $p=0.0858$) (Figure 3A). Richness index followed the order for ECO: MI>EP> EP+MI > Control (Figure 3B), and also order for FF as: EP>MI=Control>EP+MI (Figure 3B). The first and second principal component (PC1 and PC2), which explained 46.28 and 21.26% of the variance in the BIOLOG ECO data set, respectively. PCA of BIOLOG ECO profile showed a significant discrimination

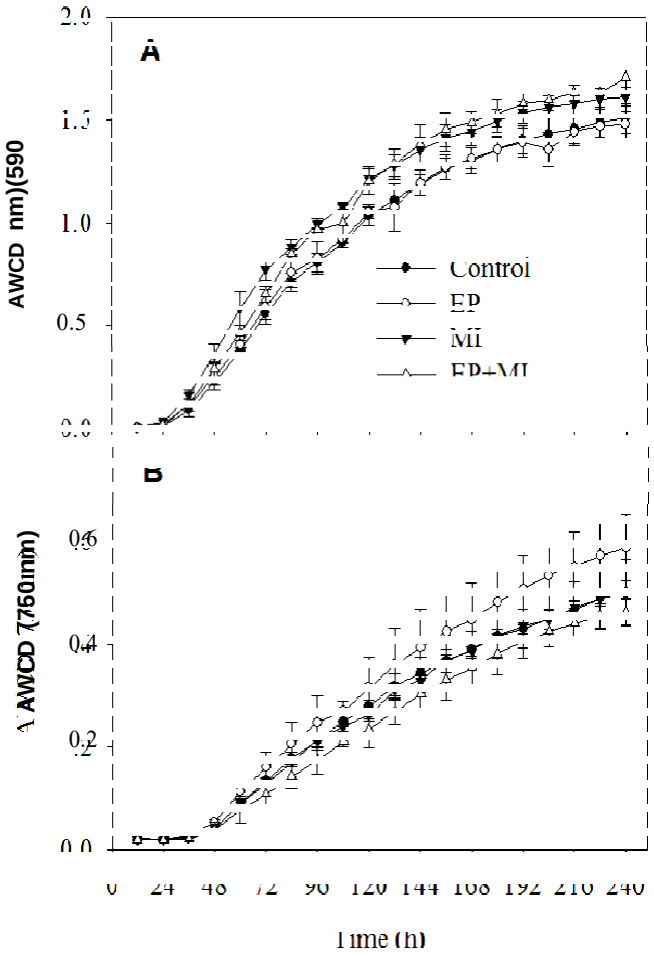

Figure 2. Average well-color development (AWCD) of metabolized carbon substrate on the BIOLOG ECO plate (A) and FF plate (B). MI: Microbial inoculum, EP: Enzyme preparations, MI+EP: Microbial inoculum + Enzyme preparations, respectively.

the soil bacterial diversity associated with different treatment soils (Figure 4A). As shown in Figure 4B, the first and second principal component (PC1 and PC2), which explained 35.17 and 18.59% of the variance in the BIOLOG FF data set, respectively. EP, MI, EP+MI and control were separated by PC1, and MI and other three treatments were separated by PC2.

DISCUSSION

Soil microbial community plays an important role in element cycling and organic matter turnover in agricultural ecosystems (Bauhus and Khanna, 1999). Understanding soil microbial community diversity is considered to be important for the sustainability of agricultural ecosystems as well as for the production of wheat. In arid and semi-arid areas, microbial activity and microbial community were modified by fresh and

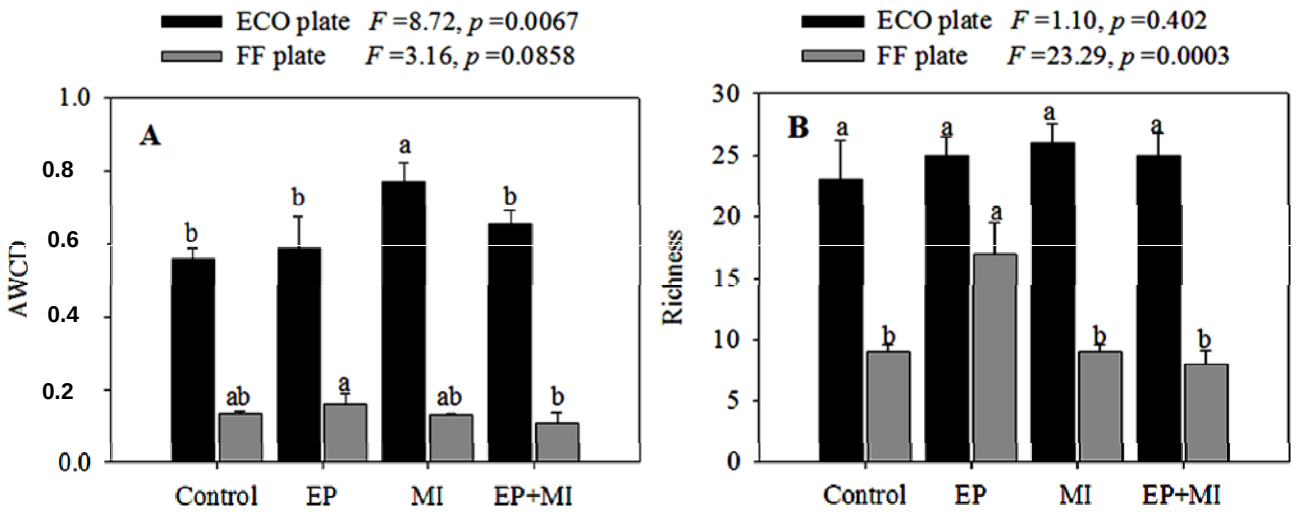

Figure 3. (A) AWCD BIOLOG ECO plate and FF plate by microbial communities in the tested soil samples. Means with different letters have significantly different values ($p < 0.05$). (B) Richness (R) of the carbon substrate utilized by microbial communities in the tested soil samples. The data shown are the means and standard deviation. The same letter indicates no significant difference by Duncan's test. MI: Microbial inoculum, EP: Enzyme preparations, MI+EP: Microbial inoculum + Enzyme preparations, respectively.

composted organic wastes (Bastida et al., 2008), different management (Acosta-Martinez et al., 2008), fertilizer management (Kanchikerimath and Singh, 2001; Chu et al., 2007; He et al., 2007), organic matter amendments (Stark et al., 2008), heavy metal contaminate (Zeng et al., 2007), and plant species (Yang et al., 2007). However, few studies focus on the effects of microbial fertilizer utilization on the microbial activity and soil microbial community diversity in the field conditions. In this work, the results shown that microbial fertilizer (MI and EP) can increase the dehydrogenase activity in the field conditions. The microbial communities were also altered by microbial fertilizers utilization. These results touch upon only individual microbial species *A. chroococcum* Beijerinck.

Although, results from a limited microcosm research should be extrapolated, they are essential in assessing the positive effects that microbial fertilizer has in the agricultural soil. More filed studies must be undertaken to research the mechanisms of these effects in the future. According to results got from the present work, the following need to be discussed. Dehydrogenase activity is an important factor that for evaluation soil microbial conditions in agricultural ecosystem conditions. Dehydrogenase activity can reflect the strength of biochemical processes in soil (Liu et al., 2008). Because dehydrogenase activity is only present in viable cells, it is thought to be considered to be a good indicator of microbial activity (Nannipieri et al., 1990). Soil fertility is closely related to soil enzymatic activities (Obbard, 2001). Microbial fertilizer utilization can improve soil enzyme activities (Sun et al., 2009). The study conducted by Sun et al. (2009) shown that rhizobial inoculation can alter microbial activity (urease, acid and alkaline phosphatase activities) and microbial communities in the rhizosphere of *Medicago sativa* L. and *Elymus sibiricus* L. In this study, *A. chroococcum* Beijerinck was used. The highest dehydrogenase activity was found in EP+MI treatment, and the lowest was observed in Control. This result demonstrated that microbial fertilizer utilization could stimulate the dehydrogenase activity in the rhizosphere of wheat. The results were consisted with previous study conducted by Sun et al. (2009). The results by Yang et al. (2008) suggested that fertilization can regulate soil enzymatic activity and fertility dynamics in a cucumber field, and demonstrate soil enzymatic activity acted as a useful indicator of soil fertility dynamics. The similar study by Ge et al. (2009) demonstrated that dehydrogenase activity was higher with fertilization, especially with organic manure, than with no fertilization. However, the mechanism needs to be done in the future.

Soil microbial community can also indicate of soil quality in agricultural systems (Benintende et al., 2008). The functioning soil microbial community is necessary for soil fertility. Soil microorganisms are the most sensitive part of the soil ecosystem, determination of soil microbial community diversity have often been used for soil quality evaluation because they are very sensitive to the environmental conditions (Zornoza et al., 2007). Many previous studies have employed community level physiological profiles (CLPP) to determine soil microbial communities (Staddon et al., 1997; Shannon et al., 2002; Nautiyal et al., 2010). Nautiyal et al. (2010) investigated the functional microbial diversity were investigated in two adjacent fields located in a semi-arid dry land farming region of Maharashtra state, India using BIOLOG ECO

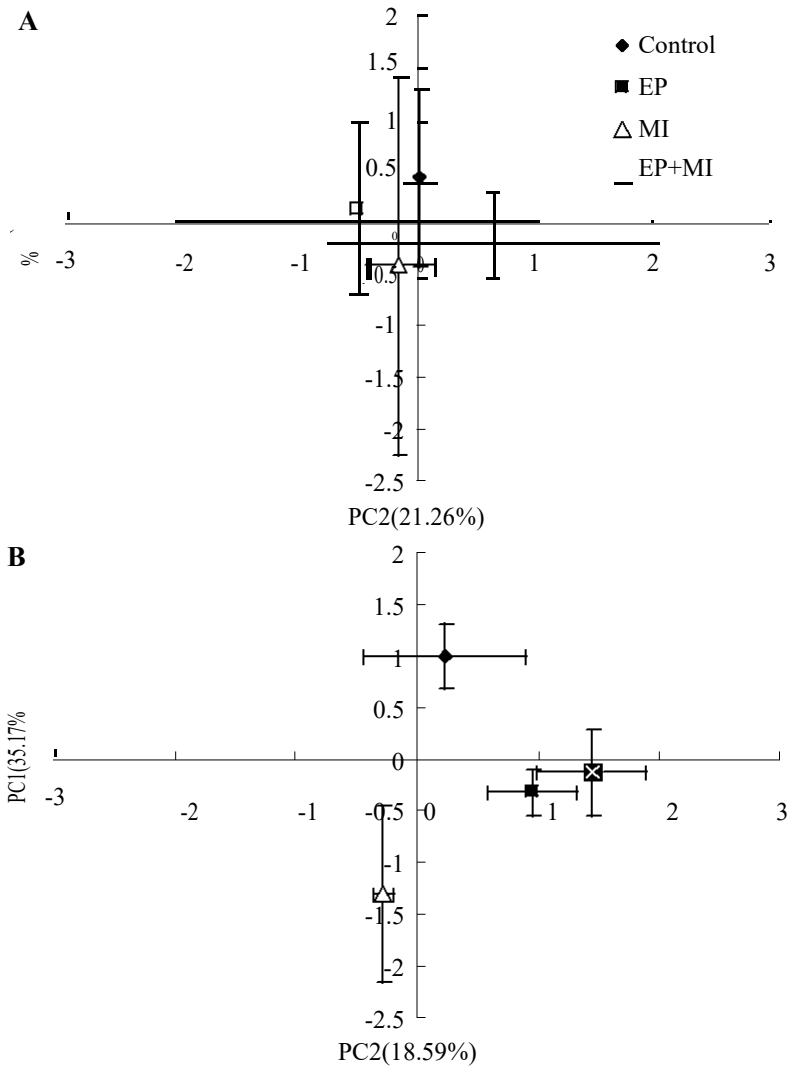

Figure 4. Principal Component analysis (PCA) of soil samples based on carbon utilization profiles of bacterial communities (A) and fungal communities (B) from the tested soils under EP, MI, EP+MI, and Control treatments. MI: Microbial inoculum, EP: Enzyme preparations, MI+EP: Microbial inoculum + Enzyme preparations, respectively. The first and second principal component (PC1 and PC2), which explained 46.28 and 21.26% of the variance in the BIOLOG ECO data set, respectively. For FF plate, the first and second principal component (PC1 and PC2), which explained 35.17 and 18.59% of variance, respectively (values are for 72 h incubation).

and GN plates. The results showed that composted cow manure utilization can enhance the soil microbial functional diversity in semi-arid filed conditions. In the present study, BIOLOG ECO and FF data attempted to determine the potential substrate utilization of microbial community. PCA of BIOLOG ECO profile showed a significant discrimination the soil bacterial diversity associated with different treatment soils. The first and second principal component (PC1 and PC2), which explained 46.28 and 21.26% of the variance in the BIOLOG ECO data set. Meanwhile, the first and second principal component (PC1 and PC2), which explained 35.17 and 18.59% of the variance in the BIOLOG FF data set. PC1 mainly separates EP, MI, EP+MI and Control. PC2 mainly separates MI and other three treatments.

Theses results suggested that microbial fertilizers can alter the bacterial and fungal community diversity in the rhizosphere of wheat in the field conditions in arid Northwestern China. Meanwhile, changes in bacterial and fungal AWCD over the microbial fertilizer addition

suggest that different organisms are active. In this study, AWCD $_{(FF)}$ is lower than AWCD $_{(ECO)}$, the results are consisted with the results of Zhang et al. (2008), similar to observations in other ecosystems, Zhang et al. (2008) also used BIOLOG ECO and FF plates to investigate the bacterial and fungal communities in a semi-arid temperate steppe in northern China. The results showed that changes in soil bacterial BIOLOG profile occur across a wide range of carbon sources utilization. In the future, some molecular methods such as Nested-PCR-DGGE, T-RFLP and LH-PCR can be used to evaluate the effects of microbial fertilization on soil microbial communities in the agricultural ecosystem in arid Northwestern China.

In conclusion, in the present work, we investigated the effect of microbial fertilizer on microbial activity and microbial community diversity in the rhizosphere of wheat growing on the Loess Plateau, Weibei, Shaanxi Province, China, and conclusions are made as follows: firstly, microbial fertilizer can increase the dehydrogenase activity in the rhizosphere of wheat cv. Xinong 889. Secondly, the results from BIOLOG ECO and FF plates shown that bacterial and fungal communities were altered in microbial fertilizer treatments compared with control. The results gathered from this work could provide essential information for reasonable utilization of microbial fertilizer in agricultural projects on the Loess Plateau, Northwestern China.

ACKNOWLEDGEMENTS

Authors thank Liu Lei and Xia Xue for their helps in experimental arrangement and soil sampling in the field conditions. We also express many thanks to the anonymous reviewers for their valuable suggestions and comments to improve this manuscript. This project was financially supported by the project of National Natural Science Foundation of China (Grant No. 40771109; Grant No. 40871119).

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