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# Effect of herbicide used with years (8 + 1) on soil enzymic activity and microbial population diversity

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**Abstract**
*Purpose* Soil microbes play important roles in plant nutrition and soil conservation, and the diversity and population of soil microbe are influenced by abiotic and biotic factors associated with different soil managements. However, the information concerning soil microbe diversity and population structure and its relation with soil fertility and enzyme activities are scarce in crop rotation under different soil management system.
*Materials and methods* This paper reports the effects of three weeding managements (herbicide (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl) acetomide, $C1_4H_{20}ClNO_2$), manual weeding, and no weeding (CK)), on soil microbial diversity, population structure, and its relationship with soil active organic matter (AOM) and pH, and the activity of soil enzymes like sucrase, catalase, and urease activities from long-term test area in red soil upland field in southeast China, which was set up since 2006. Soil samples at 0–20-cm depths were collected before (8 years) and after (8 + 1 years) weeding management in April 2014.
*Results and discussion* Soil enzymes (sucrase, catalase, and urease activity) and soil microbial populations had no significant difference ($P > 0.05$) under the three weeding treatments. Based on richness of microbial population up to 0.10%, the phyla Proteobacteria and Actinobacteria highly dominated the three soil treatments, averagely accounting for 21.76 and 21.44%. Chloroflexi was the next phylum, about accounting for 6.84%. Firmicutes, Verrucomicrobia, and Planctomycetes phylum accounted for 4.98, 4.78, and 4.23%, respectively. The percentage of Gemmatimonadetes was 2.76%, and that of Bacteroidetes was about 1.45%. Armatimonade and Nitrospira were the lowest, with 0.69 and 0.26%, respectively. Among the 20 phyla, only 5 had significant correlation with some of the soil properties. Twenty-one in 46 classes had significant correlation with some of the soil properties. Armatimonadetes and Fusobacteria had positive correlation with moisture. Acidobacteria_Gp3, Deltaproteobacteria, Chthonomonadetes, Armatimonadetes_gp4, and Euryarchaeota also were positively correlated with moisture. Negative correlation between Armatimonadetes, Chloroflexi, Chthonomonadetes, and Armatimonadetes_gp5 and AOM exists, and Armatimonadetes, Chthonomonadetes, Clostridia, Armatimonadetes, and pH were negatively correlated. Fusobacteria was positively correlated with catalase. Acidobacteria_Gp10 and Armatimonadia were positively correlated with catalase. Chthonomonadetes, Clostridia, and Armatimonadetes_gp5 were correlated with urease. Gammaproteobacteria and Flavobacteria were correlated with sucrase.
*Conclusions* For long-term herbicide experiment conducted on the Dongxiang upland site, no significant effect of

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herbicide on soil microbial community composition and enzyme activities was found. Further work is needed to relate microbial community structure and function in different herbicide systems or season sampling, even to detect herbicide effect on community structure during the growing season.

**Keywords** Herbicide · Manual weeding · Mi-seq sequencing · Soil bacterial communities · Soil enzyme

## 1 Introduction

Appropriate soil microbial community structure and diversity is of significance in maintaining the sustainability and productivity of soil ecosystem (Bell et al. 2005; Zhang et al. 2011). Soil microbes are a diverse group of microorganisms that are multifunctional and involved in many important ecosystem processes including nutrient acquisition (Smith and Read 2008), biogeochemical cycling (Bainard et al. 2013), and soil aggregation (Rillig and Mummey 2006). Reports suggest that management practices (e.g., crop rotation, application of fertilizers and herbicide) and environmental factors (e.g., pH, humidity) can influence the microbial community in the soil and thereby affect various enzyme activities in soils (Ajwaa et al. 1999; Tebrügge and Düring 1999; Giri et al. 2005; Congreves 2015).

Acetochlor (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl) acetomide) is a chloracetanilide herbicide registered for pre- and early post-emergent control of annual grasses and small-seeded broadleaved plants (Vasilakoglou et al. 2001; Zheng and Ye 2003; Foley et al. 2008). It is commonly used in the USA and countries throughout Latin America, Europe, and Africa in field, pop, seed, sweet and silage corn, sunflowers, and soybean agriculture (Foley et al. 2008; Zheng and Ye 2003). Recently, acetochlor has gained attention not only because of its widespread use but also because of its influence on ecological environment, such as the mineralization of acetochlor, its impacts on microbial communities, and its dissipation and detriment in the soil–plant system (Cai et al. 2007; Dictor et al. 2008; Xu et al. 2008; Bai et al. 2013). Peng et al. (2009) reported that acetochlor had an active influence on soil enzyme activity. While Bai et al. (2013) indicated that acetochlor did not confer a long-term impairment on viable bacterial groups, because few residual acetochlor (0.02–0.07 μg/g) could be detected in the soil 40 days after its application. Zhang et al. (2012) suggested that microbial abundance is strongly correlated with soil enzyme activities in dry desert soil. While few study has yet established the relationship between microbial communities and soil enzyme activities in crop soil, especially in long-term herbicide used soils.

Diverse microorganisms participate in soil nutrient transformation through complex biochemical processes. Except the culture method for the microbial population diversity studying, a culture-independent technique through the use of high-throughput sequencing have been proved to be a significant development in the field of microbial ecology (Ercolini 2013), which is yet to be applied to soil populations of soybean crop spraying with herbicide acetochlor.

In the current study, high-throughput amplicon sequencing was performed on soil DNA extracts in less than 8 + 1 years of acetochlor management, which was carried out on a red upland soil experimental site, in Dongxiang county, Jiangxi province, PRC. Several kinds of soil enzymes (catalase, sucrose, and urease) which are comparatively important and common in soils were also analyzed. We hypothesized that (1) three soil managements (herbicide, manual weeding, and no weeding) did different effects on the soil physicochemical and biological properties; (2) the long-term (8 + 1 years) use of herbicides may have great negative impact on soil enzyme activity and soil microbial community diversity.

## 2 Materials and methods

### 2.1 Sample sites

Soil samples came from a long-term location-fixed experiment site under different management modes conducted since 2006 in red soil upland field in Dongxiang County of Jiangxi Province. The experiment covered three treatments, namely, herbicide (spraying herbicide after sowing), manual weeding (hand weeding before sowing), and control (no spraying herbicide and no manual weeding, named CK). Each treatment had three replicates. The plot area was 7.2 × 4.5 $m^2$ and each plot was separated by concrete ridge. The tested crop planting system was soybean-peanut inter-year rotation. Sowing was done during the first 10-day period in April every year. The planting density was 45 × 16.67 $cm^2$ for peanut and 40 × 16.67 $cm^2$ for soybean, respectively. The tested herbicide content was 50% acetochlor EC ($C_{14}H_{20}ClNO_2$, produced by Dalian Regar Pesticides Co., Ltd), which is a selective herbicide before bud and widely used in soybeans, peanuts, and so on, and the spraying rate was 100–120 ml diluted with 40–50 l water per mu, i.e., 1500–1800 ml diluted with 600–750 l water per ha in accordance to the specifications. Except weeding methods, all other conditions including soil background and fertilizer applying modes and rates were the same for all treatments.

### 2.2 Soil sample collection

Each year, the three managements, herbicide, manual weeding, and no weeding (CK), were done in April 2014,

and soil samples were collected before and after weeding treatment at each site (herbicide, manual weeding, and CK). We named three treatments as herbicide 1, manual weeding 1, and CK 1 for the first sampling (before weeding) and as herbicide 2, manual weeding 2, and CK 2 for the second sampling (after weeding). A total of five soil cores at the 0–20-cm depths were collected according to the stratified "S-type" from each subplot by using a 7.5-cm diameter auger and bulked (well mixed) (about 2 kg per sample). Triplicate plots were sampled from each treatment, and all samples were packed in plastic bags and transported to the laboratory in a cold container. The field-moist soil samples were sieved (<2 mm) with fine roots and large debris removed. Samples were then separated into three subsamples including air-dried samples and fresh soil samples. The air-dried samples were finely ground (<150 μm) and stored at room temperature prior to analysis of soil chemical properties; some fresh soil samples were kept at 4 °C prior to the analysis of soil enzyme activity, and the other fresh soil samples were kept at −20 °C for DNA analysis.

### 2.3 Soil biochemical quality analysis

Soil biochemical quality parameters were monitored by estimating pH, soil moisture, and active organic matter (AOM). Soil pH was determined in 1:5 (*v*/*v*) soil/water extracts using a combination glass electrode and moisture by drying at 105 °C for 24 h. Soil AOMs were measured by potassium permanganate oxidation colorimetric method (Yu et al. 2005).

As we know, catalase mediates protection of living cells in the presence of activated oxygen species like $H_2O_2$ (Wang et al. 2016). The enzymes sucrase and urease are involved in the mineralization of important nutrient elements such as carbon and nitrogen. These enzymes are highly correlated with levels of biological activity in soils (Brooks et al. 2013), and thereby directly mediate the biological catabolism of soil organic and mineral components (Balota et al. 2004). The enzymatic activity of three soil enzymes were measured using the method of Lu (2000): (i) catalase, using the permanganometric method; (ii) urease, using the phenol sodium hypochlorite colorimetric method to determine the level of ammonium produced; and (iii) sucrase, using the 3,5-dinitro salicylic acid colorimetric method.

### 2.4 Soil DNA extraction and PCR sequencing

DNA from the samples were extracted using Omega Bio-tek's E.Z.N.A.® Soil DNA Kit (PRC) following the manufacturer's protocol. Multiple extractions were performed on each sample and pooled together. DNA was sequenced using the primer set 515F/806R targeting the V4 region of the 16S rRNA gene (Walters et al. 2011; Yao et al. 2014) on an Illumina MiSeq (PE250) at Beijing Genomics Institute, Shenzhen (Caporaso et al. 2012).

Sequence analysis was performed using Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso et al. 2010). In brief, low-quality reads were trimmed using the Qiime Suite program (Caporaso et al. 2010) and chimeras were removed with Usearch (Edgar et al. 2011). Operational taxonomic units (OTUs) were classified using 97% identity of 16S rRNA gene sequence as a cutoff, and the OTU table was generated for each sample and used for statistical analysis. Venn diagrams were used to enumerate shared or unique OTU between layers or sites. Diagrams were made using the online tool provided at the following web address: http://bioinfogp.cnb.csic.es/tools/venny/index.html (Oliveros 2007). Shannon index and Chao1 estimator were calculated at 97% sequence identity in the Ribosomal Database Project pipeline (RDP) (http://pyro.cme.msu.edu/), and beta diversity (i.e., the weighted UniFrac distances for principal coordinate analysis (PCA)) was calculated using QIIME (Caporaso et al. 2010).

### 2.5 Statistical analysis

Means were compared by Turkey's test for multiple comparison at the 0.05 level. Pearson correlation coefficients were employed to assess the linear relationship among soil microbial diversity, soil microbial population percentage, and soil chemical and biochemical properties. The datasets were subjected to principal component analysis (PCA) to classify the different weeding treatments at two sampling times. Statistical analysis was performed using SPSS 16.0 software.

## 3 Results

### 3.1 Effect of herbicide on soil physical-chemical properties

Soil pH, AOM, and moisture were measured by different sampling times under three weeding managements (Table 1). Results showed that the weeding method had no significant effect on soil pH, AOM, and moisture at each sample time ($p > 0.05$). Sample time had significant effect on soil moisture with significantly increasing at the second sampling time. AOM significantly decreased only at the herbicide site at the second sampling ($p < 0.05$) (Table 1).

Table 2 showed that catalase, urease, and sucrase activities had no significant difference for the herbicide, manual weeding, and CK treatments at the first sampling time. At the second sampling time, the soil catalase at herbicide and manual site increased significantly compared with at CK. The soil urease significantly decreased at manual weeding compared with herbicide and CK. While sucrase had no significant change at the three weeding managements.

**Table 1** Soil biochemical properties for herbicide used with 8 + 1 years

| | pH | Moisture | AOM (mg/g) |
|---|---|---|---|
| Herbicide 1 | 5.16 | 0.145 ± 0.003b | 11.980 ± 0.297a |
| Manual weeding 1 | 4.95 | 0.151 ± 0.001b | 11.324 ± 0.391abc |
| CK 1 | 5.17 | 0.146 ± 0.005b | 11.670 ± 0.265ab |
| Herbicide 2 | 4.89 | 0.178 ± 0.010a | 10.649 ± 0.353c |
| Manual weeding 2 | 4.89 | 0.176 ± 0.007a | 11.118 ± 0.030bc |
| CK 2 | 5.06 | 0.173 ± 0.003a | 11.047 ± 0.079bc |

Note: AOM presented for active organic matter; Herbicide 1, Manual weeding 1, and CK 1 presented for the first sampling (before weeding); Herbicide 2, Manual weeding 2 and CK 2 presented for the second sampling (after weeding). Data in the column are mean values ± std err (n = 3), which are compared among herbicide methods and are not different at the 5% level of significance if followed by the same letter

### 3.2 Effect of herbicide on OTUs and microbial diversity index from DNA sequencing

After removing low quality reads data from paired-end sequencing on Illumina platform (Miseq), detailed statistical data after optimization for each sample are output. The clustering under 0.97 similarity for species was called a classification OTU. A Venn diagram shows the distribution of unique or shared OTU number from soils of the three weeding managements (Fig. 1). For the first sampling time, 9700 distinct OTUs were detected, and 38% of the OTUs were common to the three weeding managements, whereas 36.9% were site-specific (distributed as follows: 16% in CK1, 14% in herbicide 1, and 6.9% in manual weeding1) (Fig. 1a). For the second sampling time, 9380 distinct OTUs were detected, and 40% of the OTUs were common to the three weeding managements, whereas 36.8% were method-specific (distributed as follows: 14% in CK2, 13% in herbicide2, and 9.8% in manual weeding2) (Fig. 1b). No matter the first sampling time or the second sampling time, CK had more specific OTUs than herbicide, and the manual weeding method had the less unique OTUs.

Illumina sequencing of 16S rRNA gene amplicons determined the microbial diversity based on α-diversity indices (i.e., observed species, chao1, Shannon, and Simpson) (Table 3). At the first sampling time, the observed species, chao1, and ace indices had the sequence of CK1 > herbicide1 > manual weeding1. At the second sampling, the observed species, chao 1, and ace indices followed the order: herbicide2 > CK2 > manual weeding2. Meanwhile, the shift trend of the Shannon index was similar at two sampling times, followed as herbicide > CK > manual weeding (Table 3). These results told us that manual weeding could reduce the microbial diversity, while herbicide had less effect on soil microbial diversity than manual weeding.

### 3.3 Effect of herbicide management on soil microbial population structure

Bacterial community structures in soils from herbicide, manual weeding, and CK sites for two sampling times were determined by Illumina sequencing (Fig. 2). Based on bacterial community richness up to 0.10%, the phyla Proteobacteria and Actinobacteria highly dominated the three soil treatments, averagely accounting for 21.76 and 21.44%. Chloroflexi was the next phylum, about accounting for 6.84%. Firmicutes, Verrucomicrobia, and Planctomycetes phyla accounted for 4.98, 4.78, and 4.23%, respectively. The percentage of Gemmatimonadetes was 2.76%, and Bacteroidetes was about 1.45%. Armatimonade and Nitrospira was the lowest with 0.69 and 0.26%, respectively. The study also presented the percentage of Crenarchaeota (0.71%) and Euryarchaeota (0.11%), which is not shown in Fig. 2.

At the first sampling time, Proteobacteria, Actinobacteria, Firmicutes, and Planctomycetes were the highest at CK1 (24.52, 22.13, 5.36, and 4.07%, respectively), compared to herbicide1 (19.83, 20.35, 4.64, and 3.99%, respectively) and manual weeding1 (18.19, 22.00, 4.89, and 3.50%, respectively). Chloroflexi, Gemmatimonadetes, Bacteroidete, and Nitrospira were the highest at the herbicide1 site (7.70, 2.47, 1.16, and 0.31%, respectively); Chloroflexi, Bacteroidete, and Nitrospira were lowest at the CK1 site; and Gemmatimonadetes was the lowest at manual weeding.

**Table 2** Soil enzyme activity under herbicide used with 8 + 1 years

| | Catalase mL KMnO4/g dry soil | Urease mg NH3-N/g dry soil.24 h | Sucrase mg glucose/g dry soil |
|---|---|---|---|
| Herbicide 1 | 0.153 ± 0.015ab | 1.254 ± 0.231a | 0.401 ± 0.038a |
| Manual weeding 1 | 0.147 ± 0.012ab | 0.987 ± 0.171ab | 0.504 ± 0.070a |
| CK1 | 0.150 ± 0.014ab | 1.333 ± 0.151a | 0.374 ± 0.063a |
| Herbicide 2 | 0.169 ± 0.004a | 0.858 ± 0.174ab | 0.424 ± 0.071a |
| Manual weeding 2 | 0.178 ± 0.013a | 0.692 ± 0.126b | 0.396 ± 0.044a |
| CK 2 | 0.131 ± 0.006b | 1.139 ± 0.414a | 0.346 ± 0.014a |

Note: Herbicide 1, Manual weeding 1, and CK 1 presented for the first sampling (before weeding); Herbicide 2, Manual weeding 2, and CK 2 presented for the second sampling (after weeding). Data in the column are mean values ± std err (n = 3), which are compared among herbicide methods and are not different at the 5% level of significance if followed by the same letter

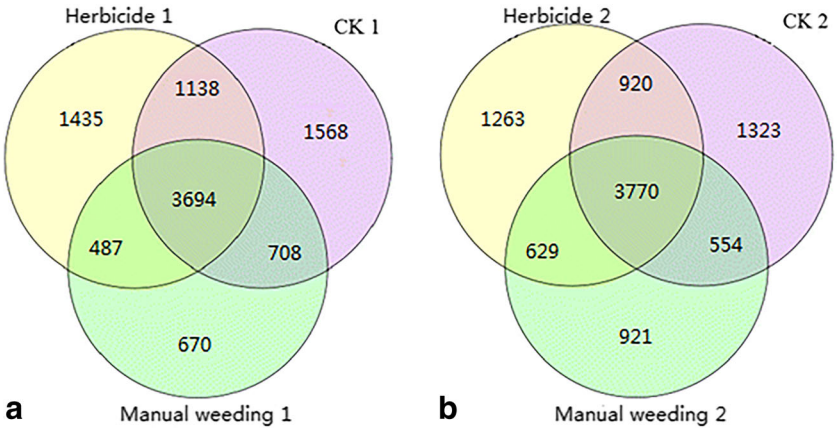


**Fig. 1** Venn diagrams of Illumina sequence data for three treatments at the first sampling (**a**) and at the second sampling (**b**). *Yellow*, *green*, and *pink* circles represent Herbicide, Manual weeding, and CK site, respectively. Values in Venn diagrams represent the number of OTUs in each site. Herbicide 1, Manual weeding 1, and CK 1 presented for the first sampling (before weeding); Herbicide 2, Manual weeding 2, and CK 2 presented for the second sampling (after weeding)

Compared to the first sampling time, the percentage of Proteobacteria, Actinobacteria, Verrucomicrobia, Firmicutes, Planctomycetes, Bacteroidetes, and Armatimonadetes increased at the herbicide2 site; only the percentage of Proteobacteria and Planctomycetes increased at the manual weeding2 site, and the content of Proteobacteria decreased at the CK2 site in the second sampling (Fig. 2).

### 3.4 Correlation of soil biochemical indicators and microbial populations (at phylum and class level)

Correlation of soil microbial population data at phylum and class level, with basic soil physical and chemical characteristics data, was analyzed (Table 4). Soil AOM was positively correlated with pH and negatively correlated with the soil moisture ($P < 0.05$). Soil pH had a positive correlation with urease ($P < 0.01$). Soil moisture had a positive correlation with the Shannon index ($P < 0.05$). At phylum level, Planctomycetes showed a positive correlation with the Shannon index ($P < 0.01$). Armatimonadetes had positive correlation with soil moisture ($P < 0.01$) and a negative correlation with soil pH, AOM, and urease ($P < 0.05$). Euryarchaeota had positive correlation with moisture ($P < 0.05$). Fusobacteria was positively correlated with soil catalase ($P < 0.05$). Tenericutes had negative correlation with species ($P < 0.05$), chao ($P < 0.01$), and ace index ($P < 0.05$). At class level, 21 classes of soil microbe showed significant correlation with one or more of the 11 soil biochemical properties (Table 4). Three classes were negatively correlated with soil pH, such as Chthonomonadetes, Clostridia, and Armatimonadetes. Five (Acidobacteria_Gp3, Deltaproteobacteria, Chthonomonadetes, Armatimonadetes_gp4, and Euryarchaeota) were positively and one class (Acidobacteria_Gp13) negatively correlated with soil moisture ($P < 0.05$), respectively. Chloroflexi, Chthonomonadetes, and Armatimonadetes_gp5 were negatively correlated with AOM ($P < 0.05$). Acidobacteria_Gp10 and Armatimonadia were positively correlated with catalase. Chthonomonadetes, Clostridia, and Armatimonadetes_gp5 were negatively correlated with urease. Gammaproteobacteria and Flavobacteria were positively correlated with sucrase. Alphaproteobacteria, Planctomycetacia, Deltaproteobacteria, and Bacteroidetes were positively correlated with the Shannon index. Acidobacteria_Gp2 and Erysipelotrichia were negatively correlated with Shannon index. Deltaproteobacteria, Chthonomonadetes, Bacteroidetes, Euryarchaeota, and Acidobacteria_Gp17 were negatively correlated with Simpson index.

PCA analysis was done based on both of the soil biochemical data and sequence OTU taxa (Fig. 3). Results showed that six

**Table 3** Soil bacterial communities diversity under herbicide used with 8 + 1 years

| Sample name | Observed species | Chao 1 | Ace | Shannon | Simpson |
|---|---|---|---|---|---|
| Herbicide 1 | 8704 | 13,918 | 17,858 | 6.431 | 0.008 |
| Manual weeding 1 | 6719 | 10,971 | 13,663 | 6.282 | 0.007 |
| CK 1 | 9411 | 15,110 | 20,573 | 6.339 | 0.008 |
| Herbicide 2 | 8307 | 13,494 | 16,804 | 6.615 | 0.005 |
| Manual weeding 2 | 6799 | 9613 | 11,074 | 6.479 | 0.005 |
| CK 2 | 7863 | 11,354 | 13,897 | 6.567 | 0.005 |

Note: Herbicide 1, Manual weeding 1, and CK 1 presented for the first sampling (before weeding); Herbicide 2, Manual weeding 2, and CK 2 presented for the second sampling (after weeding)

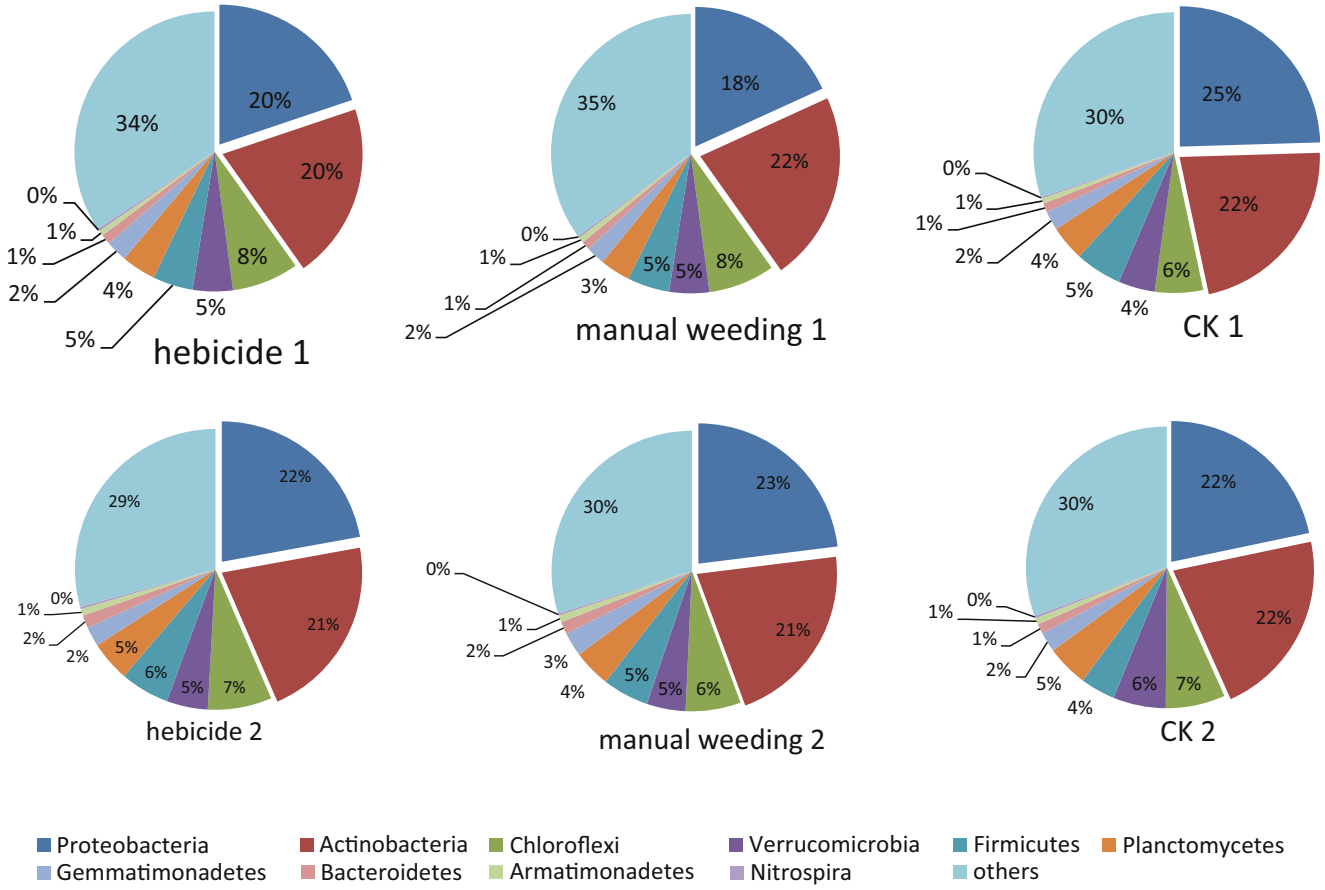


**Fig. 2** Bacterial community structures in soil from herbicide, manual weeding, and CK sites at the first and second sampling determined by Illumina sequencing. Relative abundances of bacterial 16S rRNA gene sequences affiliated phylogenetically are shown in *colors* according to explanatory note on the right side. Herbicide 1, Manual weeding 1, and CK 1 presented for the first sampling (before weeding); Herbicide 2, Manual weeding 2, and CK 2 presented for the second sampling (after weeding)

samples (3 × 2) were grouped into two clusters according to the sampling times (Fig. 3). Compared to the second sample time, the marker distance between biochemical data and OTU taxa at each weeding management were larger at the first sample time, especially at herbicide treatment. Herbicide1 and manual weeding1 had certain influence on soil biochemical properties and OTU taxa after 8 years of accumulation than control 1 (Fig. 3).

## 4 Discussion

### 4.1 Effect of herbicide management on soil properties and enzyme activity

Weeding method had no significant effect on soil pH, AOM, and moisture at each sample time, while at the second sampling time, the soil moisture increased and AOM decreased, mostly because of raining before the sampling. Enzymes play important roles in soils, such as mediating biochemical transformations involving organic residue decomposition and nutrient cycling (Burns, 1978; Zhang et al. 2012). Soil enzyme activities are often assessed to test the impact of soil management practices on soil functions in agricultural systems. Our data indicated that the herbicide did not influence the catalase, urease, and sucrase activity at the first sampling time, which demonstrated that long-term (8 years) herbicide might not influence soil biochemical properties (Tables 1 and 2). At the second time, three weeding methods decreased soil urease activity, especially manual weeding decreased soil urease activity greatly, and herbicide and manual weeding increased catalase activity while CK decreased it (Table 2). These results suggested that different weeding managements (especially in a short time) could influence soil enzyme activities (Margesin et al. 2000; Cui et al. 2014).

### 4.2 Effect of herbicide management on soil microbial population structure

Our data indicated a great diversity of bacteria in different weeding methods of red upland soil experimental site in Dongxiang county, Jiangxi province, PRC. A large proportion of the bacterial phylotypes detected were members of the Proteobacteria (subdivisions of α, β, δ, γ, and ε),

**Table 4** Correlations between soil biochemical properties with soil microbial structure at phylum and class level

| | pH | Moisture | AOM | Catalase | Urease | Sucrase | Species | Chao | Ace | Shannon | Simpson |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Biochemical properties | | | | | | | | | | | |
| pH | 1 | | | | | | | | | | |
| Moisture | NS | 1 | | | | | | | | | |
| AOM | 0.814* | −0.899* | 1 | | | | | | | | |
| Catalase | NS | NS | NS | 1 | | | | | | | |
| Urease | 0.963** | NS | NS | NS | 1 | | | | | | |
| Sucrase | NS | NS | NS | NS | NS | 1 | | | | | |
| Species | NS | NS | NS | NS | NS | NS | 1 | | | | |
| Chao | NS | NS | NS | NS | NS | NS | 0.950** | 1 | | | |
| Ace | NS | NS | NS | NS | NS | NS | 0.941** | 0.993** | 1 | | |
| Shannon | NS | 0.820* | NS | NS | NS | NS | NS | NS | NS | 1 | |
| Simpson | NS | −0.959** | 0.883* | NS | NS | NS | NS | NS | NS | NS | 1 |
| Phylum level | | | | | | | | | | | |
| Planctomycetes | NS | NS | NS | NS | NS | NS | NS | NS | NS | 0.931** | NS |
| Armatimonadetes | −0.842* | 0.981** | −0.894* | NS | −0.857* | NS | NS | NS | NS | NS | −0.932** |
| Euryarchaeota | NS | 0.826* | NS | NS | NS | NS | NS | NS | NS | NS | −0.888* |
| Fusobacteria | NS | NS | NS | 0.822* | NS | NS | NS | NS | NS | NS | NS |
| Tenericutes | NS | NS | NS | NS | NS | NS | −0.864* | −0.926** | −0.887* | NS | NS |
| Class level | | | | | | | | | | | |
| Alphaproteobacteria | NS | NS | NS | NS | NS | NS | NS | NS | NS | 0.864* | NS |
| Gammaproteobacteria | NS | NS | NS | NS | NS | 0.890* | NS | NS | NS | NS | NS |
| Acidobacteria_Gp1 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Planctomycetacia | NS | NS | NS | NS | NS | NS | NS | NS | NS | 0.932** | NS |
| Acidobacteria_Gp3 | NS | 0.830* | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Deltaproteobacteria | NS | 0.922** | NS | NS | NS | NS | NS | NS | NS | 0.952** | −0.863* |
| Acidobacteria_Gp2 | NS | NS | NS | NS | NS | NS | NS | NS | NS | −0.869* | NS |
| Chloroflexi | NS | NS | −0.904* | NS | NS | NS | NS | NS | NS | NS | NS |
| Firmicutes | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Chthonomonadetes | −0.956** | 0.871* | −0.901* | NS | −0.910* | NS | NS | NS | NS | NS | −0.894* |
| Armatimonadetes_gp4 | NS | 0.832* | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Flavobacteria | NS | NS | NS | NS | NS | 0.907* | NS | NS | NS | NS | NS |
| Bacteroidetes | NS | NS | NS | NS | NS | NS | NS | NS | NS | 0.898* | −0.842* |
| Euryarchaeota | NS | 0.826* | NS | NS | NS | NS | NS | NS | NS | NS | −0.888* |
| Acidobacteria_Gp10 | NS | NS | NS | 0.858* | NS | NS | NS | NS | NS | NS | NS |
| Clostridia | −0.819* | NS | NS | NS | −0.925** | NS | NS | NS | NS | NS | NS |
| Armatimonadia | NS | NS | NS | 0.944** | NS | NS | NS | NS | NS | NS | NS |
| Armatimonadetes_gp5 | −0.937** | NS | −0.883* | NS | −0.822* | NS | NS | NS | NS | NS | NS |
| Acidobacteria_Gp13 | NS | −0.885* | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Erysipelotrichia | NS | NS | NS | NS | NS | NS | NS | NS | NS | −0.820* | NS |
| Acidobacteria_Gp17 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | −0.817* |

AOM presented for active organic matter

*$P \leq 0.05$, *$P \leq 0.01$, and *$P \leq 0.001$

Acidobacteria, Chloroflexi, Firmicutes, and Verrucomicrobia (Fig. 2). The bacterial community structures are different according to herbicide and other management (Fig. 2). For example, the private OTU number was much fewer at manual weeding site than at herbicide site (Fig. 1). Manual weeding had strong effects on soil nutrient, microbial activities, and even soil microbial populations (Zhang et al. 2006; Dong et al. 2010; Wang et al. 2013). The private OTU number was similar at herbicide and CK site, which was in accordance with many reporters. Bai et al. (2013) reported

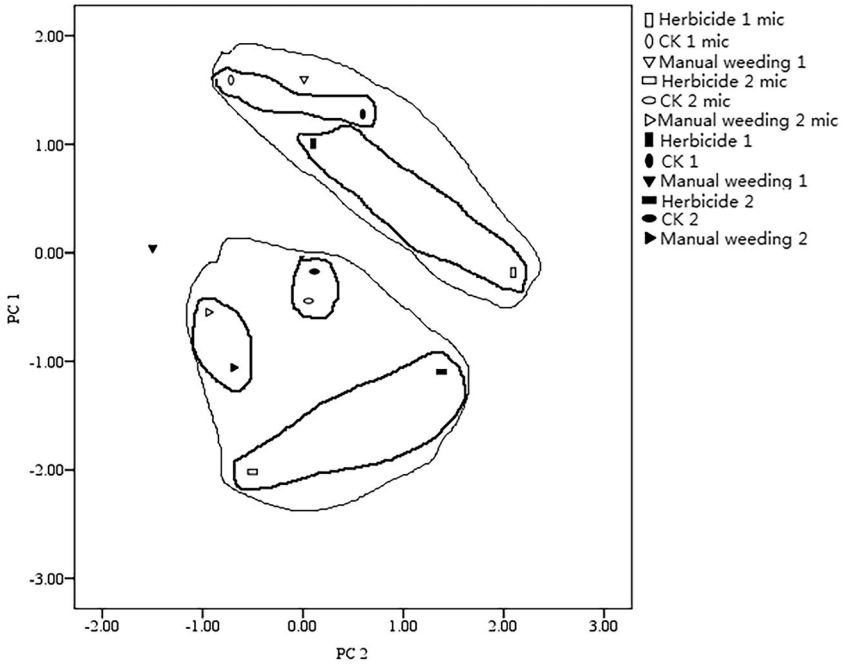


**Fig. 3** Principal component analysis (PCA) plot of Illumina sequence OTU data (*hollow*) and of soil physicochemical properties and enzyme-active data (*filled*). PCA plot was generated from the weighted UniFrac analysis of equal numbers of the bacterial sequences ($n$ = 11,409) (*hollow*). The x- and y-axes are indicated by the first and second coordinates, respectively. *Vertical rectangle*, *vertical triangles*, and *vertical ellipse* represent the Herbicide 1 and Manual weeding 1 and CK 1, respectively. *Horizontal rectangle*, *horizontal triangle*, *horizontal ellipse* represent the Herbicide 2, Manual weeding 2, and CK 2, respectively. Herbicide 1, Manual weeding 1, and CK 1 presented for the first sampling (before weeding); Herbicide 2, Manual weeding 2, and CK 2 presented for the second sampling (after weeding)

that the residual acetochlor does not significantly affect microbial populations in the soil for most cases. Although it was believed that acetochlor was toxic to bacteria in a transient way, a considerable number of bacterial species might recover soon upon the residual herbicide (Virág et al. 2007; Barriuso et al. 2010), probably owing to the available metabolites released by the herbicide across time, greater bacterial tolerance, and recovery of aboriginal resistant species of communities who take advantage of the nutrients released from killed microbes (Foley et al. 2008; Zabaloy et al. 2008; Mijangos et al. 2009).

### 4.3 Relationship of soil biochemical properties with soil microbial population structure under herbicide management

From the results of the effect of herbicide and manual weeding on soil enzyme and soil microbial populations, we found that herbicide and manual weeding had no significant influence on soil enzyme and soil microbial populations no matter at the first sampling time or at the second sampling time. At the second time, herbicide and manual weeding influence the soil catalase and urease activities in the same trend, while CK decreased both soil catalase and urease activities. Although, limited studies were reported about manual weeding influence on the soil and microbiological properties and concluded that common mechanisms regulate microbial dynamics in manual weeding systems (Petersen et al. 2002). A further explanation for the lack of influence of herbicide and manual weeding on microbial community composition in these long-term experiments may be due to continuous herbicide or manual weeding operation. Manipulation of soil organisms occurs primarily through the input of substrates and soil structure (Elliot and Coleman 1988). However, in continual manual weeding, microbial communities are bolstered by a rich spectrum of resources (Drinkwater and Snapp 2007).

In our study, the correlation of soil biochemical characteristics with microbial populations was analyzed at phylum and class level (Table 4). Among 20 phyla, only five had significant correlation with some of the soil properties. Twenty-one of the 46 classes had significant correlation with some of the soil properties (Table 4). Soil pH, moisture, and AOM may be a determinant of microbial community structure. We found that Armatimonadetes and Fusobacteria had positive correlation with moisture. Acidobacteria_Gp3, Deltaproteobacteria, Chthonomonadetes, Armatimonadetes_gp4, and Euryarchaeota also were positively correlated with soil moisture. Negative correlation between Armatimonadetes, Chloroflexi, Chthonomonadetes, and Armatimonadetes_gp5 and soil AOM and between Armatimonadetes, Chthonomonadetes, Clostridia, Armatimonadetes, and pH supports this relationship. The distribution of soil enzymes

could at least be partially determined by the species of microorganisms present (Bai et al. 2013). Based on our correlation analysis, Fusobacteria was positively correlated with catalase. Acidobacteria_Gp10 and Armatimonadia were positively correlated with catalase. Chthonomonadetes, Clostridia, and Armatimonadetes_gp5 were correlated with urease. Gammaproteobacteria and Flavobacteria were correlated with sucrase. Our study provides the first insight into the bacterial communities and soil enzyme activities in long-term herbicide and manual weeding.

## 5 Conclusions

Results of this study indicated that the impact of herbicide on soil microbe and enzyme is not universal. Catalase, urease, and sucrase activities had no significant difference among the herbicide, manual weeding, and CK treatments. Base on the microbial structure richness up to 0.10%, the phyla Proteobacteria and Actinobacteria highly dominated the three soil treatments, averagely accounting for 21.76 and 21.44%. Chloroflexi was the next phylum, about accounting for 6.84%. Firmicutes, Verrucomicrobia, and Planctomycetes phyla accounted for 4.98, 4.78, and 4.23%, respectively. The percentage of Gemmatimonadetes was 2.76%, and Bacteroidetes was about 1.45%. Armatimonade and Nitrospira were the lowest and were 0.69 and 0.26%, respectively. Proteobacteria, Actinobacteria, Firmicutes, and Planctomycetes decreased and Chloroflexi, Gemmatimonadetes, Bacteroidete, and Nitrospira increased after 8 years of using herbicide. At the next year (8 + 1), the percentage of Proteobacteria, Actinobacteria, Verrucomicrobia, Firmicutes, Planctomycetes, Bacteroidetes, and Armatimonadetes increased at herbicide site. In conclusion, for long-term herbicide experiment conducted on the Dongxiang upland site, no significant effect of herbicide on soil microbial community composition and enzyme activities was found. Further work is needed to relate microbial community structure and function in different herbicide systems or season sampling, even to detect herbicide effect on community structure during the growing season.

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