0971-4693/94 Euro 10.00
Allelopathy Journal 39 (2): 165-176 (2016) *International Allelopathy Foundation* 2016
Table: 1, Figs : 5

Dynamics of soil bacterial communities in Jerusalem artichoke monocropping system

X.G. Zhou[1,#], D.M. Gao[#], M.L. Zhao[2], J.H. Zhang, L. Li[2,]* and F.Z. Wu*
Department of Horticulture, Northeast Agricultural University,
Harbin, China
E. Mail: yyslili@163.com, fzwu2006@aliyun.com

(Received in revised form : October 01, 2016)

ABSTRACT

We studied the dynamics of soil microbial communities monitored in a continuously monocropped Jerusalem artichoke system, where Jerusalem artichoke was successively monocropped for 3-years in wheat field. Soil total bacterial, *Pseudomonas* and *Bacillus* spp. community structures and abundances were estimated by PCR-denaturing gradient gel electrophoresis (DGGE) and quantitative PCR, respectively. Results showed that different Jerusalem artichoke cultivation years (one, two, three years) had different total bacterial, *Pseudomonas* and *Bacillus* spp. community structures. Continuous monocropping of Jerusalem artichoke changed the soil microbial communities and total bacterial, *Pseudomonas* and *Bacillus* spp. community structures.

Key words: *Bacillus* spp., community structures, continuous monocropping, *Helianthus tuberosus* L., Jerusalem artichoke, monocropping, PCR-DGGE, *Pseudomonas* spp., quantitative PCR.

INTRODUCTION

Jerusalem artichoke (*Helianthus tuberosus* L.) is important crop, used as humans food, livestock feed after silage and source of inulin (sweetener) and ethanol production (14,27). Owing to its high tolerance to drought and salinity, it is grown in coastal and semiarid areas (18). However, its tuber yield and quality is reduced in continuously monocropped system (2) due to soil sickness (29).

Plants change the soil biology, chemistry and structure in ways that affects the subsequent plant growth and this process is called plant-soil feedback (16). Soil sickness is kind of negative plant-soil feedback in agroecosystems (11) and has been reported in several crops [rice (*Oryza sativa* L.) (15), corn (*Zea mays* L.) (11), pea (*Pisum sativum* L.) (19,23) and cucumber (*Cucumis sativus* L.) (29)]. Possible factors that contribute to this phenomenon include build-up of soil-borne pathogens, deterioration of soil physico-chemical characters, changes in nutrients availability and accumulation of toxic allelochemicals in rhizosphere (29). Recently, changes in the soil biological properties have also been suggested to contribute to soil sickness (12,23,29).

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*Correspondence author, [#]Co-first authors, [1]Postdoctoral Research Station of Biology, Northeast Agricultural University, Harbin, China, [2]Institute of Horticulture, Qinghai Academy of Agriculture and Forestry Sciences, Xining, China

Soil microorganisms are responsible for the key processes associated with soil fertility and plant health, hence, greatly influence the functioning of terrestrial ecosystems (13,26). The changes in soil microbial communities may alter the functions of community, which may have feedback effect on plant health (1). Understanding the soil microbes responses to continuous monocropping can help in determining how agricultural practices influence the soil processes mediated by microorganisms. It is known that both the structure and functions of soil microbial community differed between the diversified cropping systems (intercropping, crop rotation and cover crop) and monocropping systems (17,19,20,32). *Pseudomonas* and *Bacillus* spp. are ubiquitous bacteria in terrestrial ecosystems, and frequently found in association with plants, either as mutualists, saprophytes or pathogens. Some species of these genera also play major roles in nutrients mobilization, plant growth promotion and protection, and are important in agriculture (10,24). The *Pseudomonas* and *Bacillus* communities respond to plant species and history of land use (8,9,10). However, the dynamic changes in the soil microbial communities during the continuous cropping of crops are not well known. Information about how the continuous cropping influences the soil microbial communities may be helpful to develop practices to overcome the soil sickness problem in agriculture.

Although Jerusalem artichoke is a perennial plant but in China it is grown as an annual crop by planting the tubers. This study aimed to evaluate the responses of soil total bacteria *Pseudomonas* and *Bacillus* communities to continuous cultivation of Jerusalem artichoke.

MATERIALS AND METHODS

Field Experiment: The experimental site was located in field of Mojiaquanwan village, Chengbei District, Xining, China (36°42’N, 101°45’E, Annual precipitation: 378.3 mm, mean annual temperature 2.6-5.3°C), which was successively monocropped with wheat (*Triticum aestivum* L.) for > 20 years. Wheat was grown from early March to early September and thereafter left fallow till next wheat crop.. The soil was castanozem, organic matter : 2.03%, available N (NH_4^+ and NO_3^-) : 69 mg kg^{-1}, available P : 65 mg kg^{-1}, available K : 229 mg kg^{-1}, EC (1:2.5, w/v) and pH (1:2.5, w/v), 8.12.

The field experiment was done from April 2010 to October 2012. The experiment consisted of 4- treatments viz., Y0, Y1, Y2 and Y3, which were designed to successively plant with Jerusalem artichoke for 0, 1, 2 and 3 years, respectively (Table 1). The treatments were replicated thrice in randomized block design. The experimental plot size was 120 m long and 80 m wide.

Table 1. Treatments of field experiment.

Treatments	2010	2012	2013
Y0	Wheat	Wheat	Wheat
Y1	Wheat	Wheat	JA
Y2	Wheat	JA	JA
Y3	JA	JA	JA

Crop wheat or Jerusalem artichoke (JA), was planted in each treatment from 2010 to 2012.

Jerusalem artichoke (cv. Qingyu 2) tubers were obtained from the Institute of Horticulture, Qinghai Academy of Agriculture and forestry Sciences, China. Every year, it was planted on April 5 and harvested on October 25. Row to row spacing was 60 cm and plant to plant spacing was 40 cm. Wheat was broadcasted in early March and harvested in early September. There was one crop (wheat or Jerusalem artichoke) per year. After the harvest of Jerusalem artichoke and wheat, the fields were left fallow for 5-months after these Crops. Both crops were grown with recommended cultural practices. Both diammonium hydrogen phosphate and urea were applied as basal fertilizer dose of 300 kg ha^{-1}. Crops were irrigated by flooding with groundwater and weeds were manually removed once a month in May and June.

Soil sampling and DNA extraction: Bulk soil samples were collected on November 25, 2012, one month after Jerusalem artichoke harvest. Eight soil cores (5 cm dia., 15 cm deep) were randomly collected between rows of crops from each plot to make a composite sample. Large stones and root debris were removed by sieving (2 mm), then fresh soils were transported to laboratory and stored at -70°C. Total soil DNA was extracted with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA) as per the manufacturer’s instructions.

PCR-denaturing gradient gel electrophoresis (DGGE): PCR amplification of the 16S rDNA of total bacterial community was done with primer GC-338f/518r (22). Semi-nested PCR protocols were used to amplify *Pseudomonas* and *Bacillus* 16S rDNA fragments. Primer PsF/PsR and GC-338f/518r were used for first and second round of PCR amplification of *Pseudomonas* (10), respectively; while BacF/BacR and GC-338f/518r were used for *Bacillus* (9).

For DGGE analysis, 8% (w/v) acrylamide gel with 30-70% denaturant gradient was used for total bacterial community, 6% (w/v) acrylamide gel with 45-65% denaturant gradient was used for *Pseudomonas* and *Bacillus* communities (9, 10, 22). The gel was run in a 1×TAE (Tris-acetate-EDTA) buffer for 14 h under conditions of 60°C and 80 V with a DCode universal mutation detection system (Bio-Rad Lab, LA, USA). After the electrophoresis, the gel was stained in 1:3300 (v/v) GelRed (Biotium, USA) nucleic acid staining solution for 20 min. DGGE profiles were photographed with an AlphaImager HP imaging system (Alpha Innotech Crop., CA, USA) under UV light.

Quantitative PCR assay: Abundances of total bacterial, *Pseudomonas* and *Bacillus* spp. communities were estimated by quantitative PCR assays with primer sets of 338f/518r (22), PsF/PsR (10), BacF/BacR (9) as described before (5,28). Standard curves were made with a 10-fold dilution series of plasmids containing 16S rRNA genes from soil samples. Sterile water was used as a negative control to replace the template. All amplifications were done in triplicate. The specificity of the products was confirmed by melting curve analysis and agarose gel electrophoresis. The threshold cycle (*Ct*) values obtained for each sample were compared with the standard curve to determine the initial copy number of the target gene.

Statistical analysis: The DGGE profiles banding patterns were analyzed with Quantity One V4.5 (Bio-Rad Lab, LA, USA). The position and intensity of each band were determined automatically. The density value of each band was divided by the average band density of the lane in order to minimize the influence of loaded DNA concentrations among samples (31,32). Normalized data were first subjected to detrended correspondence analysis, which revealed a linear, rather than unimodal response model for the data. Thereafter, principal component analysis (PCA) was used to compare the band patterns between samples with Canoco for Windows 4.5 software (Plant Research International, Wageningen, the Netherlands). Analysis of similarities (ANOSIM) was used to test for the overall effect of treatment on microbial community structures with the Vegan package in 'R' (3). The microbial community diversity indices, including number of bands (*S*), Shannon-Wiener index (*H*) and evenness index (*E*), were calculated as described before (29). Data were analyzed following analysis of variance (ANOVA) and mean comparison between treatments was performed based on the Tukey's honestly significant difference (HSD) test at 0.05 probability level with SAS software (version 8.0).

RESULTS AND DISCUSSION

Total bacterial community structure

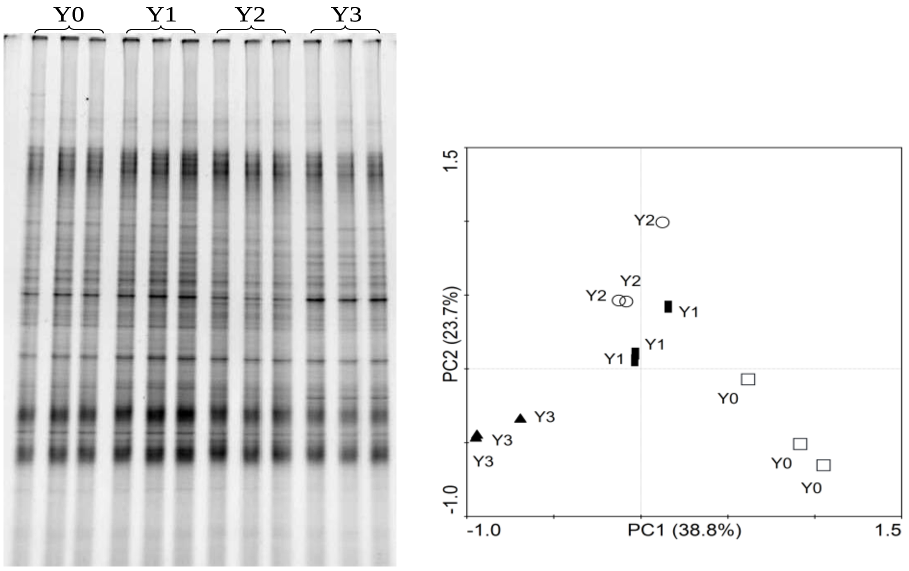
PCR-DGGE analysis showed that the banding patterns of the total bacterial community of different treatments were similar; however, the relative intensity of several bands differed among the treatments (Fig. 1a). ANOSIM analysis demonstrated that total bacterial community structure was significantly influenced by continuous monocropping of Jerusalem artichoke (R=0.914, P=0.001). PCA analysis explained 38.8% and 23.7% variation in the first two axes. On the PCA plot, Y1 and Y2 grouped together, while other treatments could be separated from each other (Fig. 1b).

Continuous monocropping of Jerusalem artichoke significantly affected the number of visible bands (*S*), Shannon-Wiener index (*H*) and Evenness (*E*) indices of the total bacterial community (P< 0.01) (Fig. 2 a, b, c). These diversity indices tended to decrease with increasing years of monocropping. The *S* and *H* indices were significantly lower in the treatment Y3 than in treatment Y0 and Y1 (P< 0.05). The *E* index was significantly lower in Y3 than in Y0 (P< 0.05). However, these indices (*S*, *H*, and *E*) were similar among Y0, Y1 and Y2 (Fig. 2 a, b, c).

Pseudomonas spp. community structure

Visual inspection of the DGGE profiles showed that *Pseudomonas* spp. community structures differed among the treatments with respect to the number and position of bands (Fig. 3a). There were differences between the treatments, both in terms of the presence/absence of individual DGGE bands and the intensity of co-migrating DGGE bands. ANOSIM analysis showed that *Pseudomonas* spp. community structure significantly differed among the treatments (R=1, P=0.001). PCA analysis of DGGE profiles of *Pseudomonas* spp. clearly separated all treatments from each other (Fig. 3b). The PC1 and PC2 components together accounted for 72.6% of the variation.

Continuous monocropping of Jerusalem artichoke significantly affected the diversity indices (*S*, *H*, *E*) of *Pseudomonas* spp. community (P< 0.001) (Fig. 2 d, e, f). Diversity indices were significantly higher in Y1 than in Y0, but decreased in Y2 and Y3 (P< 0.05).



(a) (b)

Figure 1. PCR-DGGE profile (a) and PCA (b) analysis of total bacterial communities in the continuously monocropped Jerusalem artichoke system. Y0 : Soil from the wheat field. Y1, Y2, Y3 : First, second, third cropping of Jerusalem artichoke, respectively.

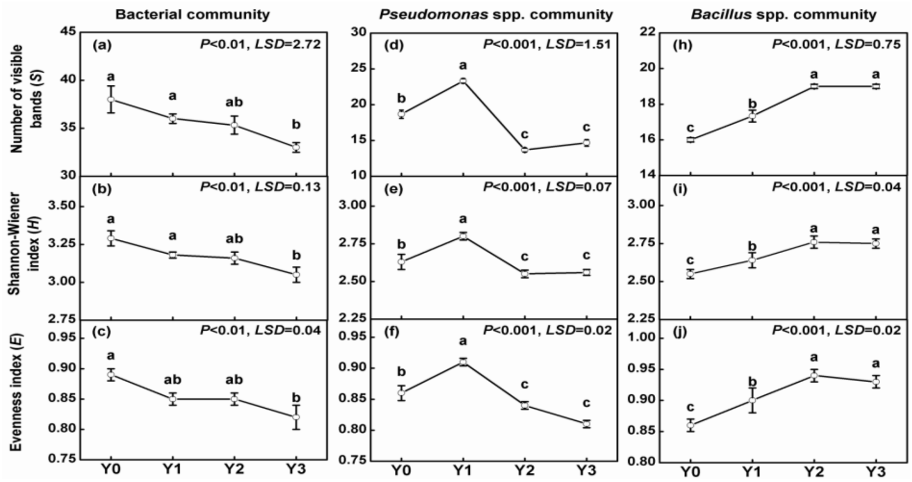
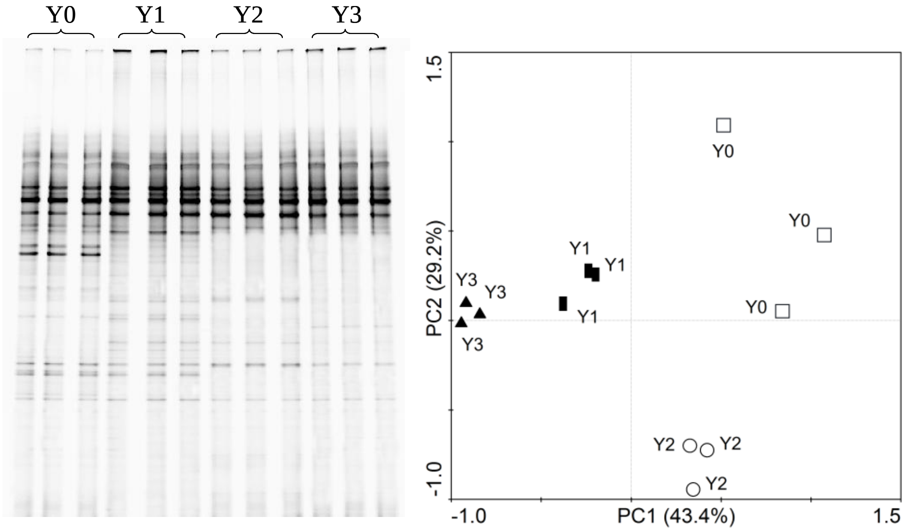


Figure 2. Numbers of visible bands (*S*), Shannon-Wiener index (*H*) and Evenness (*E*) index based on PCR-DGGE analysis of total bacterial, *Pseudomonas* and *Bacillus* spp. communities. Y0 : Soil from the wheat field. Y1, Y2, Y3 : First, second, third cropping of Jerusalem artichoke, respectively.

Bacillus spp. community structure

Most bands in the DGGE profiles of *Bacillus spp.* community were common in all treatments. However, banding patterns differed in the upper part of profile (Fig. 4a). ANOSIM analysis showed that *Bacillus* spp. community structure significantly differed among the treatments (*R*=0.926, *P*=0.001). PCA analysis showed that the PC1 and PC2 components explained the variance of 55.5% and 19.3%, respectively. On the PCA plot, Y2 and Y3 grouped together; while Y0 and Y1 could be separated from each other and from Y2 and Y3 (Fig. 4b).

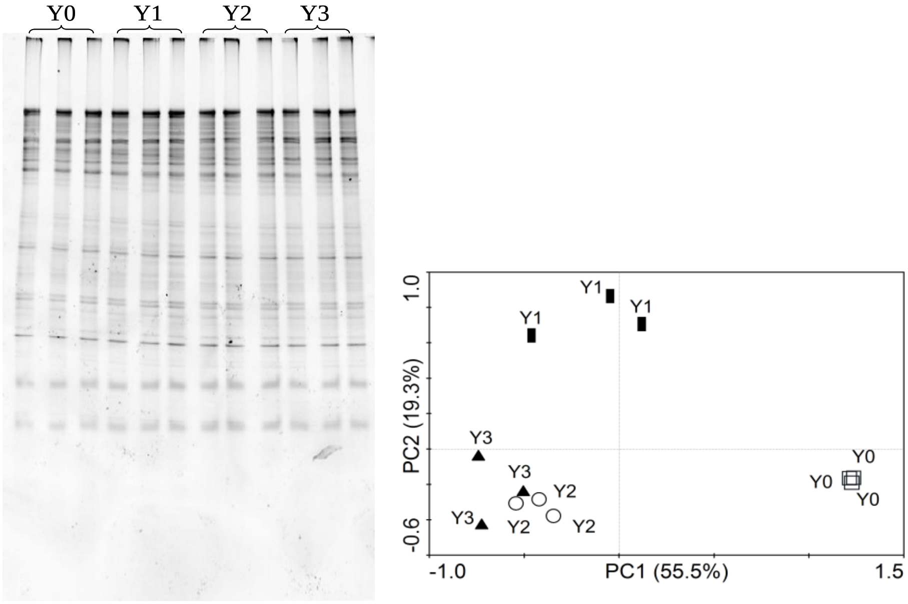


(a) (b)

Figure 3. PCR-DGGE profile (a) and PCA (b) analysis of *Pseudomonas* spp. communities in the continuously monocropped Jerusalem artichoke system. Y0 : Soil from the wheat field. Y1, Y2, Y3 : First, second, third cropping of Jerusalem artichoke, respectively.

The Continuous monocropping of Jerusalem artichoke significantly affected the diversity indices (*S*, *H*, *E*) of *Bacillus* spp. community ($P< 0.001$) (Fig. 2 h, i, j). These diversity indices were the lowest in Y0 and significantly increased in other treatments ($P< 0.05$). However, Y2 and Y3 diversity indices were similar.

The productivity and sustainability of agricultural systems depends on the functional processes carried out by soil microorganisms (26). Increasing evidences shows that the soil microbial communities are changed in response to agricultural practices, such as fertilization, irrigation, tillage, crop rotation and intercropping (1,8,10,19,28,32). The present study determined the influence of continuous monocropping on soil total bacterial *Pseudomonas* and *Bacillus* spp. communities, which may affect the soil health and productivity. Consistent with previous studies (12,23,29), our results demonstrated that continuous monocropping of Jerusalem artichoke changed these soil microbial community structures.



(a) (b)

Figure 4. PCR-DGGE profile (a) and PCA (b) analysis of *Bacillus* spp. communities in the continuously monocropped Jerusalem artichoke system.
Y0 : Soil from the wheat field. Y1, Y2, Y3 : First, second, third cropping of Jerusalem artichoke, respectively.

PCA analysis of DGGE profiles of these microbial communities showed a clear separation of Y0 and other treatments (Y1, Y2 and Y3). The treatment Y0 was successively planted with wheat for more than 20 years, while Y1, Y2 and Y3 were planted with Jerusalem artichoke. Plant species differ in their abilities to influence the soil microbial communities through varying quantities and qualities of root rhizo-depositions and plant leftovers, which are used as substrates by soil microorgasms (1,8,21). Therefore, the differences in microbial community structures and abundances observed in this study may be partly due to the changes in root rhizodepositions and accumulation of plant residues in soil.

Garbeva *et al.* (9,10) reported that maize rotation with oats, maize, barely and potato and monocropped lands differed in *Pseudomonas* spp. community structure but not in *Bacillus* spp. community structure. Our results also indicated that total bacterial, *Pseudomonas* and *Bacillus* spp. community structures differed in their responses to continuous monocropping of Jerusalem artichoke. The diversity indices of total bacterial community did not significantly change in first two years of monocropping, but significantly decreased in the third year (Y3) ($P< 0.05$). The diversity indices of *Pseudomonas* spp. community increased in Y1 and then decreased in Y2; while those of *Bacillus* spp. community tended to increase with increasing years of monocropping from

Y0 to Y2. Generally, *Pseudomonas* and *Bacillus* spp. are thought to have *r* and *k*-strategists characteristics, respectively (7). Thus, our results indicate that the soil resource conditions changed to favour more diverse *k*-strategists in this continuous monocropping system.

Total bacterial, *Pseudomonas* and *Bacillus* spp. community abundances

The abundances of total bacterial, *Pseudomonas* and *Bacillus* spp. communities are expressed as their 16S rRNA gene copies, respectively. Quantitative PCR results showed that continuous monocropping of Jerusalem artichoke significantly affected the abundances of total bacterial, *Pseudomonas* and *Bacillus* spp. communities ($P< 0.05$) (Fig. 5).

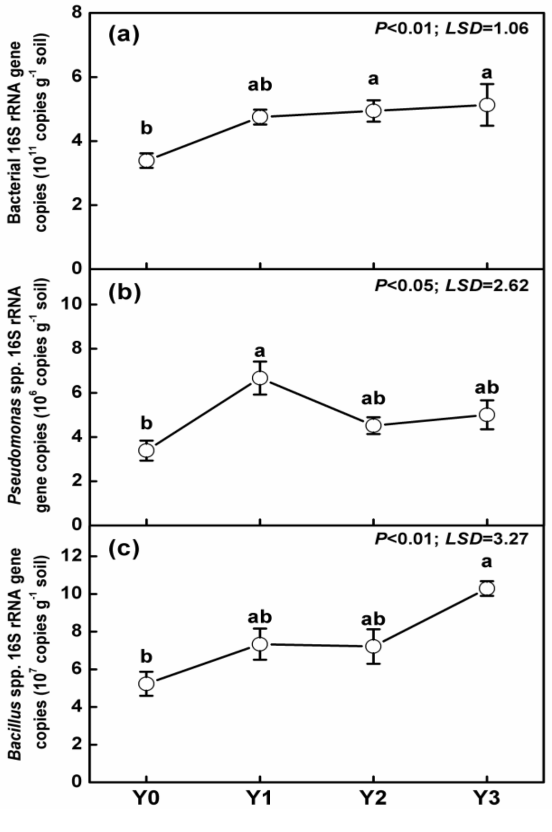


Figure 5. The abundances of total bacterial (a) *Pseudomonas* (b) and *Bacillus* spp. (c) communities in the continuously monocropped Jerusalem artichoke system. Y0 : Soil from the wheat field. Y1, Y2, Y3 : First, second, third cropping of Jerusalem artichoke, respectively. Values with different letters were significantly different between treatments ($P< 0.05$, Tukey's HSD test).

Total bacterial community abundance tended to increase with the cultivation year of Jerusalem artichoke. Total bacterial community abundance was significantly higher in treatments Y2 and Y3 than in Y0 ($P< 0.05$) (Fig. 5a). *Pseudomonas* spp. community abundance in Y1 was highest among all treatments and was significantly higher than Y0 (Fig. 5b). However, no significant difference was found among Y0, Y2 and Y3. Quantitative PCR showed *Bacillus* spp. community abundances were similar among Y0, Y1 and Y2 (Fig. 5c). Treatment Y3 had the highest *Bacillus* spp. community abundance and was significantly higher than in Y0 ($P< 0.05$). *Bacillus* spp. community abundance of Y3 was 1.97 times more than that of Y0.

Overall, quantitative PCR showed that the abundances of total bacterial, *Pseudomonas* and *Bacillus* spp. community were lower in the wheat field than in Jerusalem artichoke-cultivated soils, but did not significantly differ among the Jerusalem artichoke-cultivated soils. In continuously monocropped Jerusalem artichoke system, growth condition of Jerusalem artichoke decreased in Y3 (unpublished data). For treatment Y3, total bacterial and *Pseudomonas* spp. community structures differed from other treatments, while *Bacillus* spp. community structure was similar with Y2. Therefore, changes in the community structures of total bacterial and *Pseudomonas* spp., not *Bacillus* spp., may be related to the reduced growth of Jerusalem artichoke.

It is known that increasing the resource quantity and quality by increasing the temporal and spatial plant diversity can enhance the activity, abundance and diversity of soil microbial communities (6,25). In our experiment, treatment Y1 contained plant debris from both wheat and Jerusalem artichoke, which was more diverse than in Y0. That may explain the higher abundance of *Pseudomonas* spp. and diversities of *Pseudomonas* and *Bacillus* spp. in Y1 than in Y0. Cookson *et al.* (4) reported that more that 80% of wheat residues could be decomposed within 320 days after wheat residues are incorporated into the soil. Therefore, wheat residues may exert a larger effect on the soil microbial communities in Y1 than in Y2 and Y3.

Previous studies (12,16) suggests that allelochemicals from root exudates and decomposing plant debris can change the soil microbial communities. For example, *p*-coumaric acid from cucumber (*Cucumis sativus* L.), which is autotoxic to cucumber seedling growth, induced changes in the soil microbial communities and promoted the growth of the soil-borne pathogen of cucumber, *Fusarium oxysporum* f.sp. *cucumerinum* (30). Vidotto *et al.* (27) showed that Jerusalem artichoke had allelopathic effects on other crops viz., tomato (*Solanum lycopersicum* L.) and zucchini (*Cucurbita pepo* L.). Therefore, future studies should focus on isolation and identifying the allelochemicals involved in the soil sickness of Jerusalem artichoke cultivation and the effects of these allelochemicals on soil microbial communities.

CONCLUSIONS

We found that total bacterial, *Pseudomonas* and *Bacillus* spp. community structures changed in the continuously monocropped Jerusalem artichoke system. However, these microbial communities responded differently to continuous monocropping of Jerusalem artichoke. Compared with wheat field, Jerusalem artichoke-cultivated soils had higher microbial community abundances. Changes in the total bacterial and *Pseudomonas* community structures may be linked to the soil sickness of Jerusalem artichoke. However, the feedback effects of these changes in soil microbial communities on plant growth should be further analyzed.

ACKNOWLEDGMENTS

This work was supported by the University Nursing Programme for Young Scholars with Creative Talents in Heilongjiang Province (UNPYSCT-2015002), National Natural Science Foundation of China (41401271), China Agricultural Research System (CARS-25-08), China Postdoctoral Science Foundation (2014M551206, 2015T80320), Heilongjiang Postdoctoral Financial Assistance (LBH-Z13032, LBH-TZ06013) and 'Young Talents' Project of Northeast Agricultural University (14QC08).

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