Bangladesh J. Bot. **47**(3): 689-697, 2018 (September) Special

EFFECTS OF DIFFERENT LAND USES ON COMMUNITY STRUCTURE OF SOIL ARBUSCULAR MYCORRHIZAL FUNGI IN SANJIANG WETLANDS

LIBIN YANG[1], YINGNAN LIU, XIN SUI[2*], TONG ZHANG[2], Nan Xu[1], HONGWEI NI* AND YINING WU

Institute of Nature and Ecology, Heilongjiang Academy of Sciences, Harbin, 150040, China

Keywords: Community structure, Arbuscular mycorrhizal fungi, Agricultural land, Forest, Wetlands

Abstract

The community of Arbuscular mycorrhizal fungi (AMF) was compared between the soils obtained from wetlands, rice agricultural land and artificial forest. In order to explore the influence of human activities on the soil ecology of Sanjiang wetland in China, total microbial DNA was isolated from the soil and AMF-specific amplicons were sequenced by high-throughput sequencing on an Illumina MiSeq platform. The observed variation in soil AMF community structures for the three land-use type soils was presented here for 30 OTUs with high relative abundance and for 14 AMF genera that could be recognized. The total OTU abundance and diversity was highest in wetland soil, lower in agricultural land and lowest in forest soil. Members of *Claroideoglomus*, *Funneliformis*, *Gigaspora* and *Diversispora* were commonly detected genera in all the three samples. RDA analysis showed that these dominant AMF genera correlated negatively with the acidity of the soil, with the exception of *Diversispora*, which correlated negatively with nutrient (N, P) content. This indicated that long-term farming and forestry practices have a significant impact on soil AMF community structures in wetland ecosystems.

Introduction

Wetlands account for 5 to 8% of the earth's surface area and represent the most active terrestrial ecosystems for the exchange of biomatter and energy on the earth (Lv and Liu 2008, Sui *et al.* 2016). Wetlands function as a source, sink and converter in the biogeochemical cycle (Huang *et al.* 2018). Nevertheless, these lands are also exploited by reclamation of land for agricultural utilization (Zhang *et al.* 2015, Huang *et al.* 2018). In recent years, climate change has added to the impact of human activities on wetland environments (Li *et al.* 2009). Northeast China contains vast areas of wetlands (Ping *et al.* 2011), where their exploitation is important for grain production (Huang *et al.* 2008). Reclamation of wetlands has caused multiple changes in land use patterns, which has led to a decline of water levels, changes in wetland ecosystems, a poor soil quality, and changes in soil microbial communities (Li *et al.* 2013, Zhang *et al.* 2013).

Arbuscular mycorrhizal fungi (AMF) represent an important microbial community in soil, which can form a mycorrhizal network in symbiosis with plants, improving plant nutrition and driving an environmental nutrient cycle. AMF communities play an important role for plant growth, community competition and succession, the formation of local species diversity and stabilization of an ecosystem (Jin *et al.* 2016). Cultivation of land affects the bacterial community in soil (Ping *et al.* 2011) and fungi (Sato *et al.* 2005), but research of changes in AMF communities is sparse. In particular, AMF communities in wetlands and their changes after land reclamation have not been studied so far.

*Author for corresponding: <xinsui_cool@126.com>. [1]Institute of Advanced Technology, Heilongjiang Academy of Sciences, Harbin, 150020, China. [2]College of Life Science, Heilongjiang University, Harbin, 150080, China.

This study reports changes in the community structure of soil AMF in the Sanjiang plain wetland of China after wetland was reclaimed for forestry and rice production. The relationship between soil AMF diversity and soil environmental factors was investigated, which provide a scientific basis for assessing the changes of regional ecosystems and develop more sustainable means of development, protection and utilization of wetland resources.

Materials and Methods

The study area is a part of the Honghe National Nature Reserve, located in the Sanjiang Plain wetland area in Heilongjiang Province, China (47°42'38"-47°52'00"N, 133°34'38"-133°46'29"E). The study was performed at the Inner Mongolia Wetland Ecological Field Experiment Station, Art of the Natural and Ecological Research Institute of Heilongjiang Academy of Sciences. The study area measures a total of 21, 836 hm^2 and at an altitude of 55 - 65 m, which with an average annual temperature of 1.9ºC (frost free period of about 125 days per year) and an average annual precipitation of 550 - 600 mm. The vegetation of undisturbed wetland is mainly formed by *Deyeuxia angustifolia*, drifting rafts of *Carex pseudocuraica*, and hairy Carex (*Carex lasiocapa*).

The soil of three land use types: Meadow wetland covered mostly by wetland *Deyeuxia angustifolia*, coniferous production forest and an agricultural rice field was investigated in July 2016. For each type, a plot of 20 m × 20 m was defined which was sampled at 5 points (four at the corners and one at the center). Soil samples were collected at a depth of 0 - 20 cm with a soil drill (diameter 10 cm). The soil of these five samples was mixed to reduce the influence of local heterogeneity and large plant rests, litter and small stones were removed. The soil was then grinded and divided into 2 parts, stored in the laboratory at –20ºC prior to analysis. One part was used for characterization of AMF community structure and the other part to determine soil physicochemical parameters.

The soil moisture content was determined by weight reduction after drying.The pH was determined after addition of deionized water. The content of total nitrogen (TN), available nitrogen (AN) and total organic carbon content (OC) was measured using a CN element analyzer. The content of total phosphorus (TP) was determined by the colorimetric method based on molybdenum antimony and available phosphorus (AP) was measured by the leaching rate as determined by the $NaHCO_3$ colorimetric method.

Total DNA of microorganisms in the soil was extracted by DNeasy Power Soil Kit (QiagenMOBIO 12888) starting with 0.5 gram soil. The total DNA was dissolved in 100 µl deionized water. The DNA integrity and relative concentration was visually checked by 1.0% agarose gel electrophoresis of 5µl DNA.

PCR amplification was carried out with a two-step PCR in 50 µl that contained 10 ng DNA template, 5 µl 10 × PCR buffer, 0.5 µl dNTP (10 mmol/l each), 0.5 µl Taq DNA polymerase (5 U/µl), 0.5 µl of each primer (10 µmol/l). The primers AMV4.5NF and AMDGR are specific for arbuscular mycorrhizal fungi and have been described previously by Schloss *et al.* (2011). Amplifcation was performed by initial denaturation at 95ºC for 5 min, and 25 cycles of 95ºC for 15 s, 55ºC for 30 s and 72ºC for 30 s with a final extension at 72ºC for10 min. The PCR product was separated on an 1.5 % agarose gel, excised and recovered with SanPrep column DNA Gel Recovery Kit (Berruti *et al.* 2017). The recovered product was quantified by Qubit 2.0 DNA Detection Kit (Life Technologies, USA), and adjusted to a standard relative concentration before sequencing (Magoč and Salzberg 2011).

Illumina MiSeq sequencing was performed by an external commercial sequencing company. Pairwise reads were filtered by quality control and valid sequences were obtained by the barcode and primer sequences at both ends of the sequence. Quality control (using Flash (v1.2.3)

(Schmieder and Edwards 2011)) included removal of short fragments (< 250 bp), sequences with incorrect barcode or primers and sequence with read quality control scores < 20 after assembly. Prinseq (v0.20.4) was used for sequence alignment (Hao *et al.* 2011).

Sequences classified according to dissimilarities and Operation Taxonomic Units (OTUs) were classified at a 97% similarity cut off using CROP method (Opik *et al.* 2010). Blast N was used to compare sequences to the GenBank database to exclude OTUs of the non-balloon bacteria and to sequences present in the Maarj AM database (Yang *et al.* 2017).

By using mothur software the sequences were used to determine the coverage index, and community diversity and richness was assessed by Ace, Chao1, Shannon and Simpson indices as described by Wang *et al.* (2013). Cluster analysis was performed with Mega and heatmaps were produced by R software.

A beta diversity distance matrix was constructed and an unweighted group average was calculated using Qiime software (Zhang *et al.* 2015).

Redundancy analysis (RDA) was performed based on a Principle Component Analysis as described before by Hao *et al.* (2011).

Results and Discussion

The pH of the rice field, wetland, and pine forest soil was acidic (Table 1). The organic carbon (OC) content was highest in forestland, lower in wetland and least in agricultural land, presenting a two-fold difference between agricultural and forestland soil. The total nitrogen content also differed highly, with much higher levels (2.7 times) in wetland than agricultural soil or forestland. This difference was also observed for alkaline nitrogen which was a factor of 3 higher in wetland than in agricultural land or forestland. The total phosphorus levels were also higher in wetland than in agricultural land or forestland.

Table 1. Physico-chemical characteristics of soil.

Soil type	pH	OC (g/kg)	TN (mg/kg)	AN (mg/kg) and AN/TN (%)	TP (mg/kg)	AP (mg/kg) and AP/TP (%)
Wetland	5.65	46.57	8160	613.6 (7.5)	1230	41.2 (3.3)
Forest land	5.58	56.09	3000	214.5 (7.2)	520	26.5 (5.1)
Agriculture land	5.77	27.05	3010	197.6 (6.6)	380	20.7 (5.3)

A factor of 2 compared to forestland and of 3 compared to agricultural land I of which 3~5% was present in available form (AP). In wetland the relative levels of AP were lowest (3.3% of TP) but the absolute content was still higher than in forest or agricultural land.

The total DNA isolated from the three soil types was amplified to assess the presence of AMF. To assess the coverage of the sequences, rarefaction curves were produced which revealed that the curves were flattening out but not saturated (Fig. 1A**)**. This showed that the coverage was reasonable though it may not be fully inclusive of the actual AMF community. To assess the coverage of the data further, a Coverage index was calculated, which expresses the probability of all sequence that were present and were actually detected in the dataset. With a coverage index of 0.9987, the obtained dataset for soil from forest was relatively complete, and so was that of soil from agricultural land (0.9984), while soil from the wetland plot resulted in a slightly lower index of 0.9928. In combination, these results indicate that the sequencing data can accurately reflect the real situation of the tested soil samples.

The obtained sequences were grouped into 18,490 OTUs, with 13,518 detected in wetland soil, 6436 in agricultural land and 5075 in forest soil. Thus, the vast majority of different OTUs (73%) were detected in wetland soil. A Venn diagram (Fig.1B) was created to show differences and overlaps between the three soil types. A total of 1633 AMF OTUs were detected in 3 plots. The overlap between wetland and agricultural land (2989) and between agricultural land and forestland (3099) was of the same order, and larger than the overlap between wetland and forestland (2084).

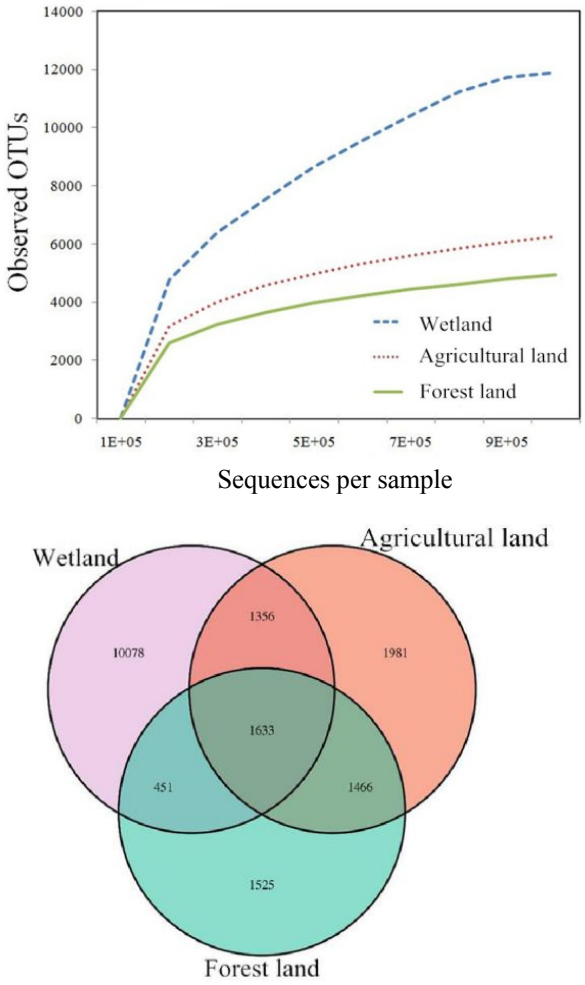

Fig. 1. Rarefation curves (A) and Venn diagram (B) of OTUs at a cutoff level of 3% in the three soil types.

The ACE index and Chaol index were used to estimate the total number of different species and the richness, respectively. The ACE index of wetland soil was much higher than of the other two land use types, while forestland resulted in lower values than agricultural land (Table 2). This indicated that the total number of different AMF species and their abundance was highest in soil from the wetland plot. In contrast, the Chao1 index of agricultural land was higher than the other two land use types, implying that more diverse species were typically present here.

The Shannon and Simpson indices estimated the alpha diversity and evenness of microbial communities, respectively. The Simpson index, as a measure of evenness of the data, did not vary much between the three soil types (Table 2) but the Shannon index was higher for wetland than for agricultural land and lowest for forestland soil. This suggested that the amount of information from the wetland soil AMF was the maximum with a highest level of community complexity. There are many reasons for this, including variation in plant host species and diversity (Huang *et al.* 2018), differences in available oxygen (Huang *et al.* 2018) and water (Duan and Guo 2004), soil pH (Liu *et al.* 2012) and nutrient content (Jin *et al.* 2016), which can all affect the alpha diversity and evenness of the soil microbial communities (Shi *et al.* 2017).

Table 2. Diversity indices of OTUs from each soil type at cutoff level of 3%.

Soil type	Ace index	Chao1 index	Shannon index	Simpson index
Wetland	34,071	39,170	9.9844	0.9977
Forest land	6423	6619	8.4912	0.9935
Agriculture land	8172	80,431	9.1793	0.9963

According to relative abundance of AMF types in the different soil samples, the 30 most abundant OTUs were compared by cluster analysis and a heatmap was plotted to reflect the differences in community structure of the different soil types on the level of OTU. From Fig. 2, it can be seen that the 30 relative abundant AMF OTUs fall into two major clusters and one outlier. Cluster 1 was mainly distributed in forest land plots, with 18 members. The relative abundance of

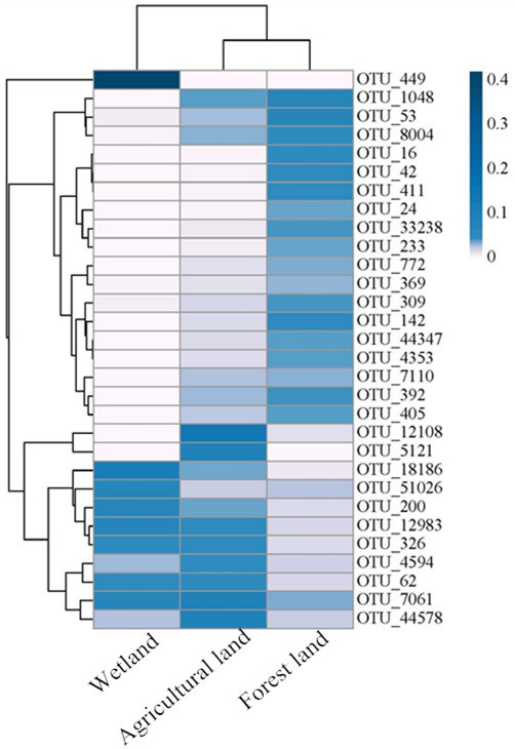

Fig. 2. Heatmap and cluster tree based on the AMF OTU sequences.

these members in forest land soil was rather even, varying between 2.18% (OTU_369) and 6.69% (OTU_53) of the data. Only three members of Cluster 1 were present at relatively high levels in agricultural land (OTU_1048, OTU_53 and OTU_8004) but members of Cluster 1 were nearly absent from wetland soil. The soil was extremely high in content of OTU_449, which made up 41.98% of the data, and forms an outlier in the cluster analysis. The OUT was absent from the other two soil types. The other abundant OTUs detected in wetland soil were members of Cluster 2, in an uneven distribution. Agricultural land contained members of both the clusters at variable abundance.

A number of the obtained sequences could be assigned to fungal genera. In total, 2 orders, 7 families and 14 genera of AMF were recognized. The 7 dominant genera in the three soil types and their relative abundance (expressed as the fraction of total recognized genera in that soil type) are presented in Table 3. Less abundant genera included *Rhizophagus*, *Scutellospora* and *Glomus*, while the genera *Pacispora*, *Entrophospora*, *Racocetra*, *Cetraspora* and *Redeckera* were detected in very low numbers (the latter was absent from wetland soil).

Table 3. Relative abundance of genera recognized in each soil type.

Genus	Wetland	Forest land	Agriculture land
Acaulospora		5.19%	5.82%
Claroideoglomus	12.88%	15.16%	10.62%
Dentiscutata	6.4%	7.32%	
Diversispora	11.41%	7.30%	11.94%
Funneliformis	9.01%	12.26%	11.66%
Gigaspora	10.85%	11.46%	5.86%
Scutellospora			5.88%

Based on the hierarchical clustering analysis of a beta diversity distance matrix, an unweighted group averaging algorithm was used to construct a tree to compare the similarities and differences of soil AMF genera in the three soil types (Fig. 3). Based on these data there was a similarity between the AMF community structure of AMF and agricultural land, and the difference between them and forest land was great.

A correlation between the physico-chemical properties of the three soil samples and their AMF communities was assessed by RDA (Fig. 4). The abundance of *Acaulospora*, *Scutellospora* and *Funneliformis* was positively correlated with soil organic carbon content and negatively correlated with soil pH. The contents of soil nutrients (both total and available N and P) were all grouped together in the third quadrant, and this correlated positively with the abundance of *Claroideoglomus*, *Gigaspora*, and *Glomus* but negatively with presence of *Diversispora*. The results of RDA showed that the structural characteristic of AMF community was distributed over three quadrants, which was related to differences in physical and chemical properties of the soil types. The contents of N and P, both total and available contents, decreased from wetland to forest to agricultural land soil. This corresponded to the obtained Ace, Chao1, and Shannon indices of the detected AMF OTUs. By RDA analysis it was also shown that the response of AMF communities varies, with noticeable differences between *Diversispora* sp. on the one hand, and other Glomeromycetes members on the other hand. Similar findings were reported by Bonfim *et al.* (2016) and Liu *et al.* (2012) from an Atlantic coast forest reported that soil organic matter, nitrogen and phosphorus fertilizers can significantly affect the AMF diversity results. However, those

findings are not consistent with the report by Liang (Liang *et al.* 2015) who found AFM content is negatively correlated with soil nutrient content. In addition, soil pH was also an important factor affecting the composition of soil AMF community (Jiang *et al.* 2014, Liang *et al.* 2015). In the present study, this is particularly observed for *Acaulospora*, *Scutellospora* and *Funneliformis*sp. which were negatively correlated with soil pH values.

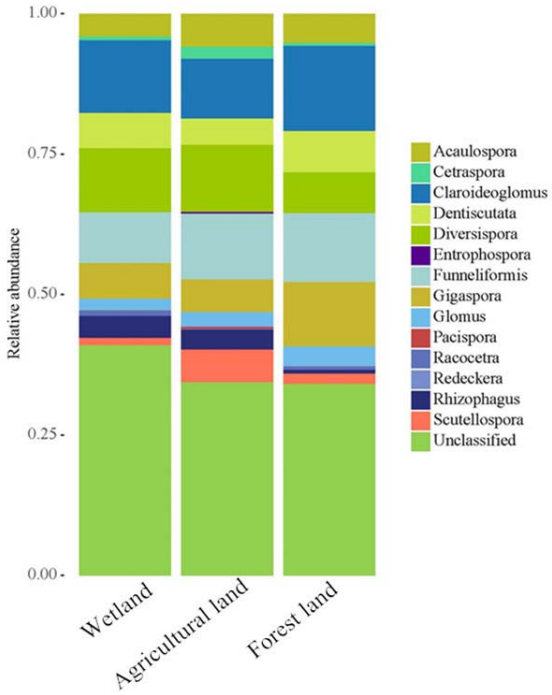

Fig. 3. Histogram based on AMF genera that could be identified from the sequences.

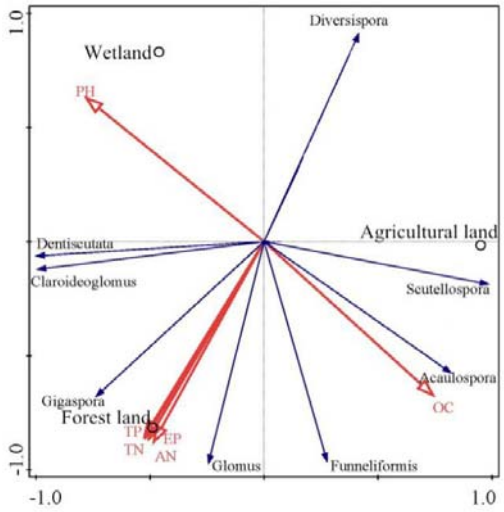

Fig. 4. RDA based on soil AMF communitiesat genus level and environmental factors of the soil.

AMF communities are important members of soil microbial flora and can collectively comprise the largest microbial biomass in soil (Jiang *et al.* 2013). The changes in land use and other human activities affect the physical and chemical properties of the soil (Jin *et al.* 2016, Liu *et al.* 2016), the activity of soil microbes and their enzymes (Huo *et al.* 2013), and the composition of the microbial community (Zhang *et al.* 2006). The results of RDA showed that the structural characteristic of AMF community was distributed over three quadrants, which was related to differences in physical and chemical properties of the soil types. By RDA analysis it was also shown that the response of AMF communities varies, with noticeable differences between *Diversispora* sp. on the one hand, and other Glomeromycetes members on the other hand. Similar findings were reported by Bonfim *et al.* (2016) and Liu *et al.* (2012) who from an Atlantic coast forest reported that soil organic matter, nitrogen and phosphorus fertilizers can significantly affect the AMF diversity results. However, those findings are not consistent with Liang (Liang *et al.* 2015) who found AFM content is negatively correlated with soil nutrient content. In addition, soil pH was also an important factor affecting the composition of soil AMF community (Jiang *et al.* 2014, Liang *et al.* 2015). In the present study, this is particularly observed for *Acaulospora*, *Scutellospora* and *Funneliformis* sp, which were negatively correlated with soil pH values.

At present, there are still many limitations in the research of AMF, with imperfect research methods, lack of database, and knowledge gaps in the clarification of the community maintenance mechanism. Only through continuous research and data collection such as presented here, this situation can be improved. In that respect the present results with assist to provide a theoretical and technical basis for further research on AMF communities.

Acknowledgements

The work was carried out with financial support by National Natural Science Foundation of China (31470019, 31600396, 31670489, 31500508,) and National Key Research Development Program (2016YFC0500405) and Institute of Basic Applied Technology, Heilongjiang Province (XKLY2018ZR01)

References

Berruti A, Desirò A, Visentin S, Zecca O and Bonfante P 2017. ITS fungal barcoding primers vs 18S AMF specific primers reveal similar AMF based diversity patterns in roots and soils of three mountain vineyards. Env Microbiol Rep. **9**(5): 658-667.

Bonfim JA, Gumiere T and Oehl F 2016. Diversity of Arbuscular Mycorrhizal Fungi in a Brazilian Atlantic Forest Toposequence. Microb Ecol. **71**(1):1-14.

Duan XN and Guo R 2004. The biomass of Phragmites australis and its influencing factors in Wuliangsuhai. Acta Phyto Sini. **28**(2):246-251.

Hao X, Jiang R and Chen T 2011. Clustering 16S rRNA for OTU prediction: a method of unsupervised Bayesian clustering. Bioinformatics **27**(5): 611-618.

Huang JY, Song CC, Song YY, Liu DY, Wan ZM and Liao YJ 2008. Influence of freshwater marsh tillage on microbial biomass and dissolved organic carbon and nitrogen. Environ. Sci. **29**(5):1380-1387.

Huang KC, Shen YY and Xu GP 2018. Effects of reclamation on soil nutrients and microbial activities in huixian karst wetland in Guilin. Environ. Sci. 4:1-17.

Huo LL, Zou YC, Guo JW and Lü XG 2013. Effect of reclamation on the vertical distribution of SOC and retention of DOC. Environ. Sci. **34**(1): 283-287.

Jiang SJ, Liu YJ, Shi GX, Pan JB and Feng HY 2014. The diversity and community assembly of arbuscular mycorrhizal fungi: a review. Chin Bull. Life Sci. **26**(2): 169-180.

Jiang XT, Peng X, Deng GH, Sheng HF, Wang Y and Zhou HW 2013. Illumina sequencing of 16S rRNA Tag revealed spatial variations of bacterial communities in a mangrove wetland. Microb. Ecol. **66**(1): 96-104.

Jin ZJ, Zeng HH, Li Q, Cheng YP, Tang HF and Li M 2016. Comparisons of microbial numbers, biomasses and soil enzyme activities between paddy field and dryland origins in Karst Cave Wetland. Environ. Sci. **37**(1): 335-341.

Li SJ, Cai DS, Zhang HL, Shen DF, Zhao XG and Li CH 2009. Environmental changes record derived from sediment cores in huixian karst wetlands, Guilin, China. J Guangxi Norm University **27**(2): 94-100.

Li SM, Wang YQ and Liu R J 2013. Diversity of arbuscular mycorrhizal fungi in special habitats: a review. Chin J. Appl. Ecol. **24**(11): 3325-3332

Liang Y, He X, Chen C, Feng S, Liu L and Chen X 2015. Influence of plant communities and soil properties during natural vegetation restoration on arbuscular mycorrhizal fungal communities in a karst region. Ecol. Eng. **82**: 57-65.

Liu M, Zheng R, Bai SL, Yun Q, Yan W and Jia X 2016. Seasonal dynamics of arbuscular mycorrhizal fungal community in the rhizosphere of *clematis fruticosa* in the daqingshan mountains inner mongolia. Acta Botan Boreali-Occ Sini, **36**(9): 1891-1899.

Liu Y, Shi G, Mao L, Cheng G, Jiang S and Ma X 2012. Direct and indirect influences of 8 year of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. New Phytol. **194**(2): 523-535.

Lv XG and Liu XH 2008. Wetland research progresses in China-dedicated to the 50th anniversary of Northeast Institute of Geography and Agroecology, CAS. Sci.Geo. Sin. **28**(3): 301-308.

Magoč T and Salzberg SL 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics **27**(21): 2957-2963.

Opik M, Vanatoa A, Vanatoa E, Moora M, Davison J and Kalwij JM 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol. **188**(1): 223-41.

Ping-Ping HE, Zhen-Hua YU, Wang YS, Jin J, Liu JD and Li SX 2011. Seasonal changes of black soil microbial biomass C and enzyme activities as Influenced by cultivation and natural restoration treatments. Sys. Sci. & C Stu. in Agric. **27**(3): 361-365.

Sato K, Suyama Y, Saito M and Sugawara KA 2005. New primer for discrimination of arbuscular mycorrhizal fungi with polymerase chain reaction-denature gradient gel electrophoresis. Grass Sci. **51**(2):179-181.

Schloss PD, Gevers D and Westcott SL 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. Plos One **6**(12): e27310.

Schmieder R and Edwards R 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics **27**(6): 863-864.

Shi XF, Huang JJ and Shi Y 2017. On AMF diversity of pinellia ternate. J Shaanxi Univ. Chin Med. (3):75-81.

Sui X, Zhang RT, Xu N, Liu YN and Chai CR 2016. Fungal community structure of different degeneration deyeuxia angustifolia wetlands in Sanjiang Plain. Environ. Science. **37**(9):3598-3605.

Wang YT, Xin GR and Li SS 2013. An overview of the updated classification system and species diversity of arbuscular mycorrhizal fungi. Acta Ecol Sin. **33**(3): 834-843.

Yang LB, Zhu D, Cui F, Li J, Song R and University NF 2017. Study on fungal communities characteristics of different larix gmelini forest typesin cold temperate zone. JCSU Fores. & Tech. 37(12): 76-84.

Zhang JB, Song CC and Yang WY 2006. Cultivation effects on soil organic carbon dynamics in sanjiang plain, Northeast China. Scien Geog. Sin. **26**(3): 340-344.

Zhang J, Hu W, Liu YZ, Gang GE and Lan WU 2015. Response of soil microbial functional diversity to different land-use types in wetland of poyang lake, China. Acta Ecol. Sin. **35**(4): 965-971.

Zhang X, Zhang G, Chen Q and Han X 2013. Soil bacterial communities respond to climate changes in a temperate steppe. Plos. One **8**(11): e78616.

(Manuscript received on 24 July, 2018; revised on 17 September, 2018)