INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17F–138/2018/20–3–695–700
DOI: 10.17957/IJAB/15.0563
http://www.fspublishers.org

Full Length Article

Effects of Various Interference Intensities on the Soil Bacterial Communities Diversity in the Sanjiang Plain, Northeast China

Yingnan Liu[1,2†], Xin Sui[1†], Fu Li[3], Liqiang Mu[2], Guoting Yang[2], Hongwei Ni[1*] and Shiyao Zhang[2]
[1]*Institute of Nature and Ecology, Heilongjiang Academy of Sciences, Harbin 150040, China*
[2]*Northeast Forestry University, Harbin 150070, China*
[3]*Harbin Normal University, Harbin 150080, China*
[*]For correspondence: nihongwei2000@163.com
[†]These authors have contributed equally to this article

Abstract

Large-scale agricultural land reclamation activities can trigger substantial changes in the soil bacterial community by disturbances associated with growth of crops and addition of fertilizers and pesticides. In this study, the bacterial 16S gene was sequenced on the Illumina MiSeq platform for bacterial identification and taxonomy. We investigated the (i) soil bacterial diversity and community composition in natural marsh, moderate and severe intensity of interference in wetlands, and (ii) the relationship between soil physical and chemical properties, and soil bacterial community structures in order to understand the effects of interference intensities on the marsh soil environment in the Sanjiang Plain wetland, Northeast China. The natural marsh soil contained most of 573 operational taxonomic units (OTUs) between all the three sites (n=1241), while wetland soils of moderate and severe intensity of interference had only 510 and 401 OTUs, respectively. The soil bacterial diversity and richness indices of all disturbed wetlands presented a decline at the OTU level, alpha diversity (Shannon diversity and Chao and Ace diversities). In addition, the composition of soil bacterial communities showed different trends and structure after the disturbance. There were significant variation in unclassified genera and some dominant genera (relative abundance>1% in at least one site) between natural marsh and difference in interference intensities in disturbed wetlands, including Acidobacteria, Proteobacteria, Verrucomicrobia, Actinobacteria, Chlorobi and Gemmatimonadetes. Composition of soil bacterial community was affected by Soil Moisture, pH, Soil Organic Carbon, Total Nitrogen, Available Nitrogen, Total Phosphorus, Available Phosphorus, Total Potassium, Nitrate Nitrogen, Available Potassium and Ammonium Nitrogen. This study will provide a fundamental scope to understand the bacterial community structure in wetland ecosystems and the environmental function as a predictor of bacterial community composition.

Keywords: Marshland; Bacteria; Diversity; Community structure; Miseq

Introduction

Sanjiang Plain is one of the biggest and most concentrated wetland distribution regions in northeast China and key areas of protected wetland biodiversity in the world. In the past sixty years, the typical natural wetland landscape of Sanjiang Plain has been fragmented into a farming landscape with isolated wetland patches due to rapid development of agriculture. Human activities have not only changed the water cycle patterns and decreased the groundwater tables, but also changed the soil nutrients and variability in plant community composition.

Consequently, variations in the soil microbial community structure and composition can be observed (Song *et al.,* 2011; Wang *et al.,* 2017). Over the years, this phenomenon has gradually attracted attention, and researchers are focusing on studies describing the impact of wetland cultivation (Zhou *et al.,* 2009), treatment of wetland pollution (Guo *et al.,* 2014), or wetland microbial participation in carbon and nitrogen cycles (Li *et al.,* 2013; Sui *et al.,* 2017). It is now widely recognized that soil microbes are key players in the soil ecology. Therefore, remedial methods for soil environment restoration by controlling soil microbes are being explored (Li *et al.,* 2015). Soil bacteria of abundantly distributed phyla and species are involved in decomposition of organic matter, mineralization processes, and degradation of pollutants and restoration of the ecological environment (Sura, 2015). The community composition and diversity of soil microbes can obviously indicate changes in soil nutrients and impact of environmental changes with direct effects on soil functions (Ansola *et al.,* 2014). Therefore, thorough classification and functional analysis of soil bacteria will add to the knowledge of the processes of litter decomposition, nitrogen cycles, allow us to control vegetation growth and increase decomposition of organic pesticide pollutants (Guo *et al.,* 2008; Zheng *et al.,* 2013; Xiao *et al.,* 2014).

To cite this paper: Liu, Y., X. Sui, F. Li, L. Mu, G. Yang, H. Ni and S. Zhang, 2018. Effects of various interference intensities on the soil bacterial communities diversity in the Sanjiang plain, northeast China. *Int. J. Agric. Biol*., 20: 695–700

By culture-dependent methods, it is difficult to accurately reveal the community structure, function, and diversity of bacteria, because many species cannot be cultivated. Therefore, advanced methodology must be used to explore microbial species, the role of groups, and their relationship with the environment (Chi *et al.*, 2015; Sui *et al.*, 2015). Non-culturable soil bacteria can be identified by genomic analysis, and their abundance can be compared and calculated, resulting in a more reliable estimate of the components of a soil bacterial community (Singleton *et al.*, 2001).

Based on the new technology of high-throughput DNA sequencing, diversity of soil bacteria and their relative abundance and evolution can be assessed. Likewise, soil environmental factors related to soil bacterial diversity in different intensities of interference can also be determined. This study data reveal impact of human disturbance on the soil environment of wetlands in northeastern China.

Materials and Methods

Site Description and Soil Sampling

This study was carried out in the Sanjiang Plain Wetland Experimental Station (47°35'N, 133°31'E), located in the Honghe National Nature Reserve, China. The average monthly temperature of this region ranges from −21.6°C to 21.5°. The average annual precipitation (≈560 mm) usually is in May and October accounting for 80% of whole year. Three sites representing wetland, moderate interference, and severe interference wetland, were studied.

We selected two different intensities of wetland disturbance: low disturbance (*Calamagrostis angustifolia* cover of 40–50%, average height 80–90 cm, human activities interference for 1–5 years), high disturbance (*Calamagrostis angustifolia* cover of 20–30%, average height 40–50 cm, human activities interference > 15 years), and the natural *Calamagrostis angustifolia* wetland (cover of 60–70%, average height 100–110 cm) around the Honghe Nature Reserve to study the influence of disturbance on the microbial diversity (Table 1).

The top soils (0–20 cm) were selected and sampled on May 15, 2016. Approximately 1 kg soil was sampled from five locations within each site, stored in polyethylene bags in an ice-cooled container and immediately transported to the lab. After arrival in the lab, approximately 10 g of each soil sample was transferred into a sterile 2 mL Micro centrifuge tube and stored at -60°C for Ilumina-Miseq sequencing. The remaining soil samples were air-dried to determine the soil physical and chemical properties. The soil pH was measured using soil with water (1:5 w/v) for 30 min. The total organic carbon, total phosphorus and total nitrogen composition of the soil samples were tested by using an Elemental analyzer (VarioEL III, Elementar Analyses System, Hanau, Germany). Available nitrogen (AN) and available phosphorus (AP) was measured using a FLAstsr 5000 Analyzer (Foss Tecator AB Sweden Supply Company, Hoganas, Sweden). The soil physical and chemical properties of the three different disturbance intensities are summarized in Table 2.

Soil DNA Extraction and High-throughput Sequencing

DNA was extracted by using the MOBIO-12888 Power Soil DNA Isolation Kit (USA) according to the manufacturer's instructions. The extracted DNA was dissolved in 200 μL TE (10 metros–HCl, 1 mm EDTA, pH 8.0) and stored at −20°C in the fridge. The V1–V3 region were selected to amplify the bacterial 16S rRNA (Chakravorty *et al.*, 2007). The PCR products were sent to Shanghai Majorbio Biotechnology Company (Shanghai, China) to sequence.

Data Analysis

The obtained high-throughput sequences were analyzed by using QIIME Software. The bacterial community compositions were compared by using UniFrac statistical analysis software. The only effective sequences which contained both barcode and forward primers were selected to do the subsequent analysis. In order to obtain the accurate results from the bioinformatics analyses, reads having shorter lengths (<150 bp) were removed and the optimized sequence were selected to analyze subsequently. All optimized sequences were generated to operational taxonomic units (OTUs) for bioinformatic statistical analysis. Optimized sequences (lengths>350 bp) were compared with the SILVA database and then clustered by using Mothur and Chopseq software (http://www.mothur.org/wiki/Main_Page). Shannon and Chao and Coverage were represented Species richness and diversity and sequencing depth of bacteria. Alpha-diversity was tested at significance levels of 97%. The difference of OTUs in three soil samples were calculated by using Jest algorithm method and obtained the similarity index between each samples. Redundancy analysis (RDA) was conducted by R software.

Results

Statistics of the High-throughput Sequencing Data

The nine soil samples revealed a total of 298,234 optimized sequences. These could be attributed to 36 phyla. The total number of bases determined was 1567,500,132 and the average read length was 404.68 bp, of which reads between 401 and 500 bp (the expected size for the VI–V3 region) accounted for 99.72% of the total number of reads. The rarefaction curve was constructed through randomly sampled optimized gene sequences, as it represents the relationship between the individuals and species by selecting a random subset (Fouts *et al.*, 2012). A flat rarefaction curve indicates that sequences were sufficient, and further sequences would not produce other new OTUs.

Table 1: Community structure and function index in different interference intensity sites

Interference intensity	SDR(Sum of Dominance Ratio)	Coverage	Height	Shannon-Wiener index	Adjacent farmland reclamation time
Natural wetland	>0.8	>80%,	100-110 cm	0.1-0.2	none
Moderate interference	0.5-0.7	50-60%	70-80 cm	0.2-0.3	1-5 years
Severe interference	0.2-0.5	20-30%	40-50 cm	>0.3	> 15 years

Table 2: Physicochemical properties of soil from different interference intensity

Interference intensity	pH	Soil Organic Carbon (g/kg)	Total Nitrogen (g/kg)	Available Nitrogen (mg/kg)	Total Phosphorus (mg/kg)	Available Phosphorus (mg/kg)	Total Potassium (g/kg)	Nitrate nitrogen (mg/kg)	Available potassium (mg/kg)	Ammonium nitrogen (mg/kg)
Natural wetland	5.8±0.21	99.1±14.56	0.6±0.07	436.1±38.51	36.2±6.47	36.2±6.46	2.2±0.08	0. 7±0.17	249.7±6.47	53.4±19.78
Moderate interference	5.8±0.15	104.5±17.46	0.4±0.06	284.2±46.74	5.9±1.36	5.9±1.36	2.47±0.09	1.2±0.20	267.0±1.36	25.0±6.81
Severe interference	5.7±0.14	43.9±20.09	0.3±0.09	223.0±57.77	5.8±2.20	5.8±2.19	2.03±0.14	3.5±0.66	119.0±2.20	30.7±9.70

The rarefaction curve obtained (Fig. 1) indeed flattened out indicating that the sampling was sufficient and that diversity was sufficiently covered by the sequences.

The Distribution of Soil Bacterial Community in Different Interference Intensity Disturbance

Venn diagram (Fig. 2) illustrates the numbers of shared and exclusive OTUs of bacteria identified from different soil samples. This simple numeric visualization shows similarity and overlapping of all samples (Amato *et al.,* 2015). In total, 7448 OTUs were detected, of which 1140 (15.31%) were shared between the natural wetland, moderate-interference wetland, and severe-interference wetland (Fig. 2). Only 510 OTUs were found in moderate-interference wetland, accounting for 6.85% of the total. Natural wetland samples produced the highest number of specific OTUs (573, or 7.69%); whereas, severe-interference wetland produced 401, accounting for 5.38%. A large proportion of OTUs were shared in natural and severe-interference wetlands (25.40%), while 22.11% of OTUs were shared between natural and moderate-interference wetlands. About 17.25% were isolated from both moderate- and severe-interference wetlands. Thus, of the three sample types, there was varied distribution of OTUs from moderate- and severe-interference wetland soil.

Analysis of the Bacterial Diversity

Diversity of the bacterial OTUs detected was analyzed by means of α-diversity indices (Table 3). The results show that diversity of bacteria from natural wetland was higher than from the other sites with interference. Interference significantly decreased diversity of the soil bacterial community than in natural wetland.

Composition of Soil Bacterial Community in Different Interference Intensity

Using a similarity cut-off level of 97%, the representative sequences of OTUs were combined into taxonomic groups. The results are summarized in Fig. 3. Total of 2016 OTUs included here belonged to 40 taxonomic groups, of which six taxa were most abundant (in decreasing order of abundance): Acidobacteria, Proteobacteria, Verrucomicrobia, Actinobacteria, Chlorobi, and Gemmatimonadetes. These were considered to constitute the main bacterial community and together with a significant fraction of unclassified OTUs, they represented approximately 80% of the data. There were 30 phyla with an average low relative abundance (<1%). According to the Bray Curtis similarity matrix, the similarities and differences of the communities were compared by a non-weighted method (Hong *et al.,* 2015) to construct the tree shown to the left in Fig. 3. As expected, this grouped the samples according to type of farm-use land. In case of soil similarity, the microflora structure was highest within the three natural wetlands, and this was quite different from those of moderate- interference wetland and severe-interference wetland. The abundance of each phylum was calculated and showed that there were significant differences in the dominant bacterial composition for each land type. Fig. 5 indicated differences of phylum between different soil samples. Acidobacteria, Proteobacteria, Actinobacteria, Nitrospirae, Chlorobi, and Aminicenates were significantly different between the three wetland sites (Fig. 4, $p>0.05$).

The Relationships of Bacterial Diversity and Soil Physical and Chemical Properties

At the 97% level, analysis of the composition of soil bacteria by RDA revealed that the two axes contribution rate was 47.65% (Fig. 6). The positive direction of axis I was mainly located in Severe interference, while in the negative direction of axis I located in natural wetland and moderate interference were showing that moderate interference and severe interference were the main affecting factors on axis I. of the variables tested, soil organic carbon (SOC), available potassium (AK), total nitrogen (TN), and total potassium (TK) were positively

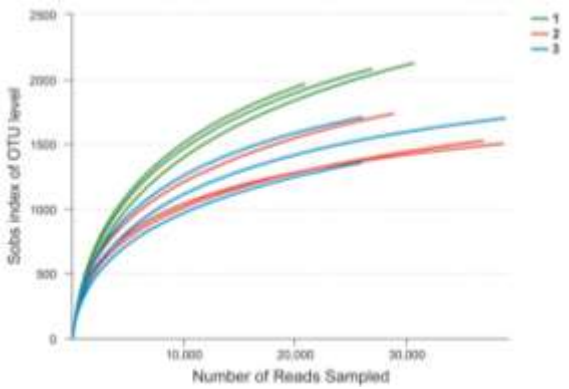

Fig. 1: Rarefaction curves showing the extent of OTU detection at the different plots

For natural wetland samples (1), moderate-interference wetland, (2) and severe-interference wetland (3).The same as below

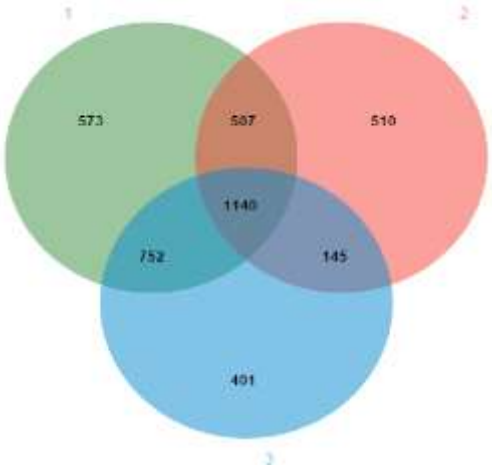

Fig. 2: Venn diagram showing the numbers of shared and exclusive OTUs of bacteria identified from the different soil samples. Note: 1 is natural wetland; 2 is moderate-interference wetland; and 3 is severe-interference wetland

correlated with moderate interference, while total phosphate (TP) were positively correlated with severe interference. These results identified that the soil nutrient elements such as pH had the most significant influence on the community structure of soil bacteria.

Discussion

As far as could be ascertained, limited research is available on soil bacterial communities to address multiple scientific questions related to the impact of disturbance on soil bacterial community (Singleton *et al.,* 2001; Liu *et al.,* 2014). In this study, we used high-throughput sequencing technology to detect the soil bacterial community in three different disturbing intensities. The results indicated that soil bacterial communities between the

Table 3: The bacterial α-diversity indices of OTUs obtained from the soil of different interference intensity

Interference intensity	Ace	Chao	Shannon	Simpson
Natural wetland	2675.75	2689.33	6.14	0.007
	2612.25	2551.70	6.24	0.006
	2777.94	2788.00	5.95	0.009
Moderate	1777.52	1815.19	5.45	0.011
	1947.64	1993.01	6.00	0.007
	2285.52	2273.54	5.68	0.010
Severe	1784.83	1788.38	5.91	0.007
	2103.62	2116.20	5.81	0.007
	2029.25	2033.86	6.02	0.007

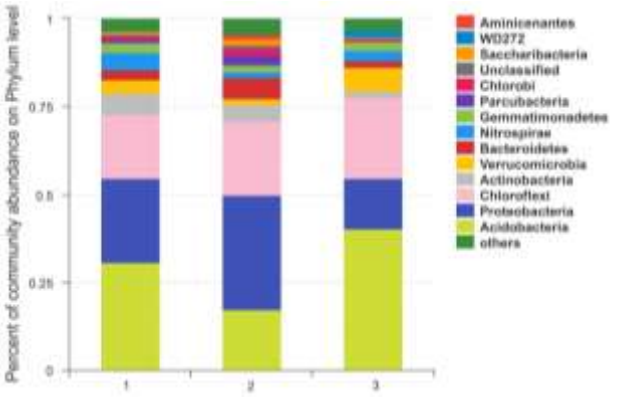

Fig. 3: Histogram of soil bacterial community in different interference intensities

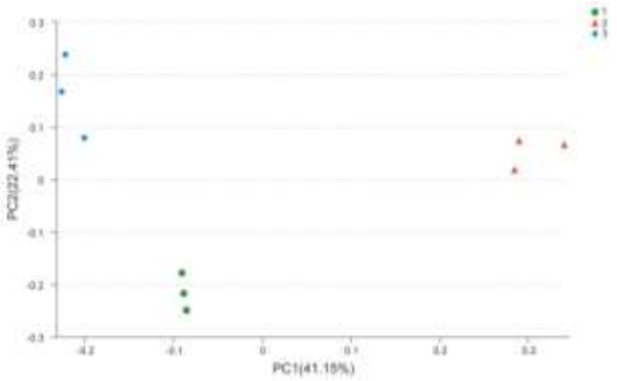

Fig. 4: PCOA of soil bacterial community in different interference intensities

different disturbance intensity differed significantly (Fig. 3). By cluster analysis, it was established that the communities thriving in soil from moderate-intensity disturbance were relatively similar to those from severe-intensity-disturbance land, despite their obviously different vegetations, indicating that vegetation type is not a good indicator for soil bacterial community structures. The observed bacterial community structure of original wetland was much different from that of the other two soil types. This suggests that by changing wetland into moderate-intensity interference, the soil properties changed considerably, consequently changing the

soil bacterial population structure (Zhang *et al.*, 2013).

Most likely, each soil micro-environment selects for specific bacterial communities (Berg and Smalla, 2009). In total, 40 phyla were identified from the soils collected from the Sanjiang Plain, with noticeable differences between disturbance intensity. Variation in bacterial composition showed the disturbing intense affected the ecological balance of the original bacterial community and changed the habitat conditions, finally led to the shifts in the dominant bacterial phyla with changed bacterial community diversity (Zhang *et al.*, 2013).

Different habitats contain specific bacterial species at high and low relative abundance (Liu *et al.*, 2015). This is also seen in our samples, for which we reported major overrepresented phyla with high abundance relative to the other land use types (Fig. 4). Such as the relative abundance of the Chlamydiae and Chloroflexi in the severe-intensity and moderate-intensity disturbed land were significantly lower than that in natural wetland, while the relative abundance of the Planctomycetes and Gemmatimonadetes in moderate-intensity disturbed soil was significantly lower than Bacteroidetes and Tenericutes in severe-intensity disturbed soil. Important determinants in the soil environment were identified as pH, SOC, and TN, which had a significant effect on soil bacterial community composition (Fig. 5). Such insights can lead to better understanding of soil bacterial communities in order to take measures to regulate balance of the soil ecosystem and promote sustainability of moderate-intensity disturbance (Cookson *et al.*, 2008).

Diversity indices provide an effective parameter to quantify the diversity of soil bacterial communities. When the alpha diversity was calculated, it was found that disturbance intensity decreased the soil bacterial diversity, in line with a study by Yu *et al*. (2011). They found that the soil bacterial diversity was higher in moderate-intensity disturbance (Shannon index 3.49–3.69) than that in the natural restoration land (3.34–3.44) (Yu *et al.*, 2011). Latitude of Helen area they analyzed is similar to that of the Sanjiang Plain, and the soil conditions are also similar; therefore, results of their study are comparable to our results. Wang *et al.* (2017), however, reported that the microbial functional diversity was highest in wetland and lowest in severe-intensity disturbed soil. This contrasts with the data presented here, suggesting that diversity of soil microbial community is dependent on different soil types. SOC, TN and pH can increase microbial diversity through improved nutrient supplies. The diversity of moderate-intensity disturbance was found to be high, probably because the soil's nutrients were externally supplied by fertilization, again indicating that nutrient conditions are important factors to affect the soil bacterial community diversity.

The process of converting from wetland to moderate-intensity disturbance to severe-intensity disturbance can indirectly affect soil bacterial community structure by

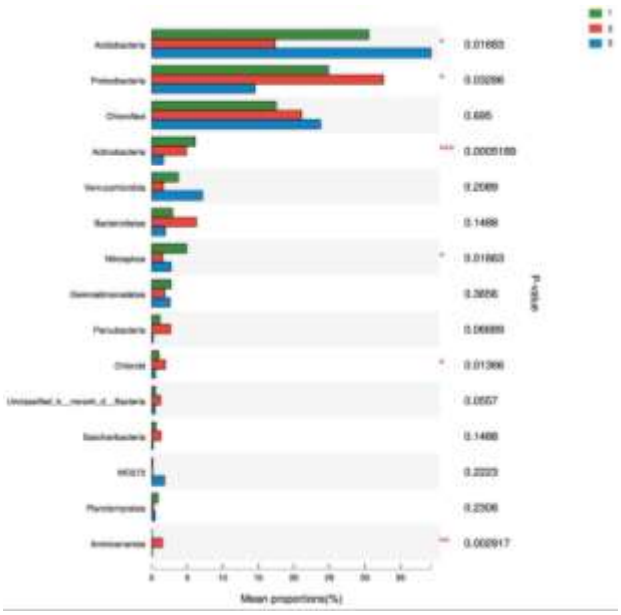

Fig. 5: The dominant phyla difference of three different land use types

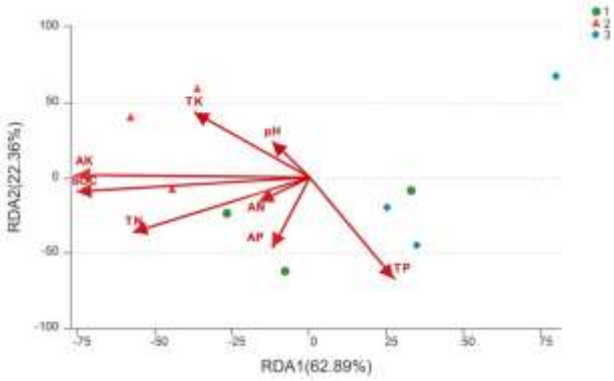

Fig. 6: RDA of the soil bacterial community and environmental factors

varying the conditions of soil hydrology and nutrient (Sheik *et al.*, 2012). Human activities can directly change the diversity of soil bacterial community, which promotes plant uptake of soil nutrients and accelerates the decomposition of soil organic matter (Staff, 2014). Changes in human disturbance resulted in changes in the physical and chemical properties, such as decreased levels of SOC, TN, AP and TP. In response to this, the soil bacterial diversity increased, showing that decrease in C and N can actually improve soil diversity. The result seems counterintuitive, because the soil organic carbon, nitrogen, available phosphor and total phosphor declined significantly from normal wetland to severe-intensity disturbed land. This phenomenon may be due to perennial accumulation of organic matter in the original swamp soil. Thus, the content

of soil nutrient is quite high, but it still cannot be used by the majority of soil bacteria in its natural form. Under new land type regimes, the original form of these soil nutrients changed, which created suitable conditions for the utilization of C and N for soil bacteria. Therefore, the content of C and N seems not the key limiting factor for soil bacterial diversity. Although we studied the soil bacterial diversity and influence environmental factors, but other factors such as soil temperature and soil oxygen content might also be important, and were not included in this study, therefore, their impact remains to be determined.

Acknowledgments

This work was executed by the National Natural Science Foundation of China (NO. 31400429, 31470019), Heilongjiang Academy of Sciences Funds For Distinguished Young Scholars and Post-doctoral Fund of Heilongjiang Province(LBH-Z14184).

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(Received 14 December 2017; Accepted 20 December 2017)