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ORIGINAL ARTICLE

Conversion from natural wetlands to paddy field alters the composition of soil bacterial communities in Sanjiang Plain, Northeast China

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Abstract The Sanjiang Plain is the largest freshwater wetlands in Northeast China. In order to feed the growing population, about 84 % of the wetlands in this area have been converted to farmland, especially to paddy fields, since the 1950s. However, little is known about the influence of this conversion on soil microbial community composition. In this study, soil samples were collected from two natural wetlands dominated by plant species *Carex lasiocarpa* and *Deyeuxia angustifolia* and from a neighboring paddy field that was changed from wetland more than 10 years ago. The composition and diversity of bacterial communities in the soils were estimated by clone library analysis of nearly full length of 16S rDNA sequences. The results revealed that bacterial diversity was higher in paddy fields, and that the composition of bacterial communities differed among the three samples; the difference was more notable between the paddy field and two natural wetlands than between two natural wetlands. The distribution of clones into different bacterial phyla differed among soil samples, and the conversion from natural wetlands to paddy field increased the abundance of *Proteobacteria* and *Firmicutes* but decreased the abundance of *Chloroflexi*. About 63 % and 71 % of clones from two natural wetlands and 49 % of clones from the paddy field had <93 % similarity with known bacteria, suggesting that the majority of bacteria in natural wetland soils in the Sanjiang Plain are phylogenetically novel. In general, this study demonstrated that long-term conversion from natural wetlands to paddy field changes soil bacterial communities in the Sanjiang Plain.

Keywords Clone library · Land-use · 16S rRNA gene · Soil bacterial community · Wetland

Junjie Liu and Chunyu Zheng contributed equally to this work

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Introduction

The ecological functions of soils are linked to the diversity and composition of soil microorganisms (Zak et al. 2003; Gutknecht et al. 2006), which are influenced directly by changes in soil environment such as soil water content (Bossio and Scow 1998), pH (Fierer and Jackson 2006; Ge et al. 2008), soil type and field properties (Wang et al. 2009), as well as being influenced indirectly by land management procedures (Yu et al. 2011) and the plant diversity and composition growing on the soils (Garbeva et al. 2004; Carney and Matson 2006).

Wetlands are one of the most important terrestrial ecosystems, playing a crucial role in regulating the carbon cycle (Keller 2011), maintaining global water balance (McJannet et al. 2012) and protecting biodiversity (Burton and Uzarski 2009). Wetlands are ecotones between uplands and water bodies with the characteristics of a shallow water that is permanently or periodically close to the soil surface and in

which specific wetland plant communities are established (Mausbach and Parker 2001; Mitsch and Gosselink 2007). Although natural wetlands occupy only 5–8 % of the land on Earth (Mitsch and Gosselink 2007), they are regarded as the "kidney" of the Earth, playing several key biogeochemical processes such as pollutant degradation, nitrification, denitrification, methanogenesis, methanotrophy, and iron and sulfate reduction (Davidsson et al. 1997; Gutknecht et al. 2006). These biogeochemical processes are mediated mostly by microorganisms (Balasooriya et al. 2008).

The Sanjiang Plain is the largest freshwater wetland in Northeast China (NE China). It covers an area of 10.89 Mha, and about 50 % of the area was dominated by freshwater wetlands in 1950 (Zhao et al. 1999; Liu and Ma 2000). However, in order to feed the growing population over the past 50 years, approximately 84 % of the wetlands have been converted to farmland, especially to paddy fields (Liu and Ma 2000). Although several studies have evaluated the effects of this land conversion on greenhouse gas emission (Wang et al. 2002; Huang et al. 2010), soil organic carbon content (Zhang et al. 2007a), soil microbial biomass and soil respiration (Zhang et al. 2007b), and soil T4-type bacteriophages in the Sanjiang Plain (Zheng et al. 2013), no attention has been paid thus far to soil microbial community composition.

Given that land-use change is one of the dominating factors determining the microbial community structure in soils (Bossio et al. 2005; Jangid et al. 2008; Yu et al. 2011), as well as the fact that our previous study showed that T4-type bacteriophage community compositions differ between natural wetlands and paddy fields in Sanjiang Plain (Zheng et al. 2013), we hypothesized that the hosts of bacteriophages—the soil bacterial communities—might also be changed with land conversion from natural wetlands to paddy field in Sanjiang Plain. In order to test this hypothesis, we collected soil samples from two natural wetlands and a paddy field, and estimated the composition, diversity and phylogeny of soil bacterial communities in those samples by 16S rDNA clone library analysis.

Materials and methods

Site description and soil sampling

The study sites were located in Sanjiang Mire Wetland Experimental Station (47° 35′ N, 133° 31′ E) of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. The altitude of the station is 56 m a.s.l. The average monthly temperature ranges from −21.6 °C in January to 21.5 °C in July, and the average annual temperature is 1.9 °C. The average annual precipitation is about 560 mm, with approximately 80 % occurring in the plant growing season from May to October. Two natural wetland sites inside the station, dominated by plant species *Carex lasiocarpa* and *Deyeuxia angustifolia*, and a paddy field nearby were chosen in this study. The site of the *C. lasiocarpa* wetland was approximately 40 m from the *D. angustifolia* wetland, and the paddy field site was approximately 500 m away from both wetlands. The paddy field was converted from natural wetland more than 10 years ago. The soil parent material at all three sites was Quaternary sediment, and the soils were classified as Albic Boric Luvisols with silty clay texture.

Soil samples (0–10 cm depth) were collected on 17 June 2011, corresponding to the rice mid-tillering growth stage. Approximately 1 kg soil was collected from five locations within each site and composited. The soil samples were put in polyethylene bags, placed in a container with ice and transported to the laboratory immediately. Soon after the samples arrived at the laboratory, approximately 2 g of each soil sample was placed in an autoclaved microcentrifuge tube (2 mL) and stored at −80 °C for DNA extraction, and the remaining soil samples were air-dried and used for the determination of chemical properties. Soil pH was measured using a pH meter after shaking the soil water (1:5 w/v) suspension for 30 min. The soil total carbon (TC) and total nitrogen (TN) were determined using an Elemental analyzer (VarioEL III, Elementar Analysensysteme, Hanau, Germany), and the chemical properties determined in this study are shown in Table 1.

Soil DNA extraction

DNA was extracted from 0.5 g of each of frozen soil sample with a Fast DNA SPIN Kit for Soil (Qbiogene, Carlsbad, CA) according to the manufacturer's instructions. The extracted DNA was diluted in 100 μL TE (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) buffer and stored at −20 °C until use.

Construction of 16S rRNA gene clone library

The DNAs extracted from each sampling site were subjected to PCR amplification with the bacterial universal primers 27f and 1492r (Lane 1991), and the PCR products were electrophoresed on a 1 % agarose gel with 1×TAE buffer (40 mM Tris–HCl, 40 mM acetate, 1.0 mM EDTA). The bands were excised from the gel and DNA was purified with a QIAEX II Gel Extraction Kit (QIAGEN, Crawley, UK). Purified DNA was cloned into the pMD18-T plasmid vector (TaKaRa,

Table 1 Chemical properties of soils in this study

Samples	Total C (g kg^{-1})	Total N (g kg^{-1})	pH (H_2O)
Carex lasiocarpa	40.3	3.1	5.5
Deyeuxia angustifolia	39.3	3.7	5.2
Paddy field	24.4	1.9	6.0

Dalian, China) and transformed into competent cells of *Escherichia coli* DH5α. Colonies were blue/white screened on LB agar medium supplemented with Ampicillin (100 μg mL^{-1}; Sigma, St. Louis, MO), X-gal (100 μg mL^{-1}; Amresco, Solon, OH) and IPTG (0.5 mM; Promega, Madison, WI). White clones were chosen randomly and amplified with the vector-specific primers M13-47 and RV-M, and amplicons with a length of ~1.5 kb were regarded as positive clones. The plasmid DNA from a positive clone was harvested from overnight cultured *E. coli* DH5α, and submitted to a commercial company for sequencing (BGI, Shenzhen, China). Clone libraries of three study sites were constructed in this study, and 200 positive clones from each library were sequenced.

Phylogenetic affiliation of clones

The primer sequences of 27f and 1492r were removed manually from the obtained sequences. Chimeric sequences were identified by submitting the DNA sequences to GenBank at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). The closest relatives of respective clones were examined using the BLAST search program at the NCBI website against database of 16S rRNA sequences. Taxonomic affiliation was performed using the classifier tool (Wang et al. 2007) available at the Ribosomal Database Project (RDP) website (http://rdp.cme.msu.edu/). The sequences of 16S rRNA gene obtained in this study were deposited with GenBank under the accession numbers JX504885–JX505067, JX505068–JX505247 and JX505248–JX505429 for clone libraries of *C. lasiocarpa* wetland, *D. angustifolia* wetland and paddy field, respectively.

Statistical analysis of clones

The sequences obtained were aligned using ClustalX 1.81 (Thompson et al. 1997), and a neighbor-joining (NJ) tree was constructed using Molecular Evolutionary Genetic Analysis software (MEGA 4.0) with 1,000-fold bootstrap support (Tamura et al. 2007). The NJ tree then was loaded into UniFrac statistical analysis tool available at http://bmf.colorado.edu/unifrac/to compare bacterial community compositions among three clone libraries (Lozupone and Knight 2005).

Operational taxonomic units (OTUs) were determined at sequence dissimilarity of 3 % (species), 5 % (genus), and 15 % (class) levels by using mothur software (Schloss et al. 2009). A number of OTUs were used for the creation of rarefaction curve, and calculation of Shannon's diversity index (H') and Simpson's index (D) with PAST software (Hammer et al. 2001). S_{chao1} was used to estimate the true number of richness of the bacterial community based on the number of rare OTUs in the sample, where $S_{chao1}=S_{obs}+(L^2/2M)$, and S_{obs} was the total number of OTUs observed, L was the number of OTUs that were represented by only a single individual in the sample, and M was the number of OTUs represented by exactly two individuals in the sample (Chao 1984). Coverage (C) was used as a measurement of captured diversity, where C was expressed by $1-(n_1/N)$, in which n_1/N was the ratio of clones that appeared only once (n_1) to the total number of clones (N) (Chelius and Triplett 2001). Pairwise comparison of phylotype composition in clone libraries were expressed with Sorensen similarity index, $Cs=2j/(a+b)$, where j was the number of phylotypes found in both samples A and B, a was the number of phylotypes in sample A, and b was the number of phylotypes in sample B (Magurran et al. 1996). In addition, a Venn diagram was drawn based on number of OTUs among three clone libraries at 3 %, 5 % and 15 % dissimilarity by using mothur software (Schloss et al. 2009), respectively.

Results

Phylogenetic affiliation of 16S rDNA sequences

After the chimeric check, 182, 183 and 180 of high quality 16S rDNA clones were identified from clone libraries of paddy field, *C. lasiocarpa* wetland and *D. angustifolia* wetland, respectively. According to the BLAST search, 44 clones showed ≥99 % sequence similarity to their closest database entries and remaining 46, 123, and 332 clones had the similarity in the range of ≥97 %–<99 %, ≥93 %–<97 %, and <93 %, respectively (Supplementary Tables 1–3; Table 2). Thus, there were 51 clones from paddy field (28 %), 23 clones from *C. lasiocarpa* wetland (13 %) and 16 clones from *D. angustifolia* wetland (8 %) had the similarity ≥97 % with the known bacteria.

The classifier tool at the RDP website assigned 158, 101, 94, 65, 51 and 46 clones to bacterial phyla of *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria* and *Verrucomicobia*, respectively; and 6, 3, 2, 1 and 1 clones to phyla of *Planctomycetes*, *Bacteroidetes*, *Gemmatimonadetes*, *Cyanobacteria* and *Armatimonadetes*, respectively; and the remaining 16 clones were left unclassified. The distribution

Table 2 Number of clones with different ranges of highest similarity with known bacterial data in GenBank

Samples	Number of clones			
	≥99 %	≥97 %–<99 %	≥93 %–<97 %	<93 %
Paddy field	30	21	42	89
Carex lasiocarpa	7	16	45	115
Deyeuxia angustifolia	7	9	36	128
Total	44	46	123	332

of clones into the different bacterial divisions among the three clone libraries was uneven (Fig. 1). The abundance of *Proteobacteria* and *Firmicutes* in the paddy field was higher than that in the two natural wetlands. In contrast, *Chloroflexi* were remarkably abundant in two natural wetlands in comparison to the paddy field. The highest proportion of *Actinobacteria* and *Verrucomicobia* was observed in *D. angustifolia* wetland. Differences in the distribution of clones in other minor phyla were also observed among the three libraries (Fig. 1).

The soil bacterial community composition in the paddy field was different from those in two natural wetlands within some phylum levels. For example, within phylum *Proteobacteria*, 38, 8, 2 and 20 clones were grouped into α-, β-, γ- and δ-*Proteobacteria* in the paddy field, respectively. In contrast, 42, 1 and 2 clones from *C. lasiocarpa* wetland and 33, 1 and 8 clones from *D. angustifolia* wetland were classified to α-, β- and δ-*Proteobacteria*, respectively. Another example was phylum *Acidobacteria*, where 12 clones from the paddy field belonged to Groups 1, 6, 7, 10, 17 and 18; whereas 17 clones from the *C. lasiocarpa* wetland belonged to Groups 1, 3, 6 and 7, and 21 clones in the *D. angustifolia* wetland to Groups 1, 2, 6, 7 and 17.

Diversity of clone libraries

The diversity of bacterial communities among the three samples was calculated from 16S rDNA clone sequences at 3 % (species), 5 % (genus) and 15 % (class) dissimilarity levels. In total, 360, 321 and 148 OTUs were identified at 3 %, 5 % and 15 % dissimilarity levels, respectively (Table 3). The diversity indices at the three dissimilarity levels showed that the paddy field had the highest Shannon's diversity index and the lowest Simpson's index compared with two natural wetlands. The S_{chao1} estimator of three samples was in the order paddy field > *C. lasiocarpa* wetland > *D. angustifolia* wetland. Thus, all the diversity indices showed that the bacterial community composition in the paddy field was more diverse than that in natural wetlands (Table 3).

Table 3 Diversity indices for 16S rRNA gene libraries from a paddy field and two natural wetland soils in Sanjiang plain, NE China. *OTU* Operational taxonomic unit, *H'* Shannon's diversity index, *D* Simpson's index

	No. of clones	No. of OTUs	Coverage (%)	Diversity indices		
				H'	*D*	S_{chao1}
Species (3 % dissimilarity)						
Paddy field	200	126	48.4	4.66	0.0068	327
Carex lasiocarpa	200	120	52.5	4.58	0.0082	300
Deyeuxia angustifolia	200	114	58.9	4.58	0.0069	224
Genus (5 % dissimilarity)						
Paddy field	200	119	53.3	4.58	0.0081	318
Carex lasiocarpa	200	108	61.2	4.45	0.0101	231
Deyeuxia angustifolia	200	94	68.3	4.28	0.0129	171
Class (15 % dissimilarity)						
Paddy field	200	59	84.1	3.48	0.0557	100
Carex lasiocarpa	200	46	88.5	3.21	0.0692	94
Deyeuxia angustifolia	200	43	90.0	3.12	0.0685	61

Rarefaction curves were constructed by plotting the number of OTU against the number of clones at 3 %, 5 %, and

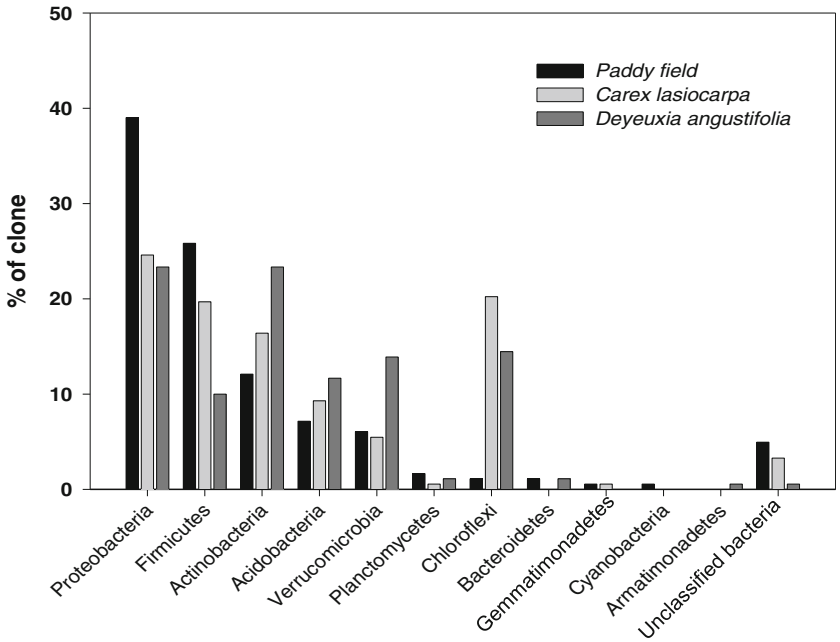

Fig. 1 Distribution of the bacterial 16S rRNA gene sequences into different phylogenetic groups in the clone libraries of the paddy field, *Carex lasiocarpa* wetland and *Deyeuxia angustifolia* wetland

15 % dissimilarity levels (Fig. 2). At 15 % dissimilarity level, all curves showed a decrease in the rate of OTU detection, indicating that clone analysis evaluated almost the full extent of taxonomic diversity at the class level, and the coverage of the clone library was 84.1 % for paddy field, 88.5 % for *C. lasiocarpa* wetland and 90.0 % for *D. angustifolia* wetland (Table 3), whereas, at the species and the genus levels, all three curves showed the increase in the number of OTU with the number of clones, indicating that the clones obtained from three clone libraries were not enough to estimate the total OTU in these clone libraries, and the coverage among these three clone libraries ranged from 48.4 % to 58.9 % at species level, and 53.3 % to 68.3 % at genus level (Table 3).

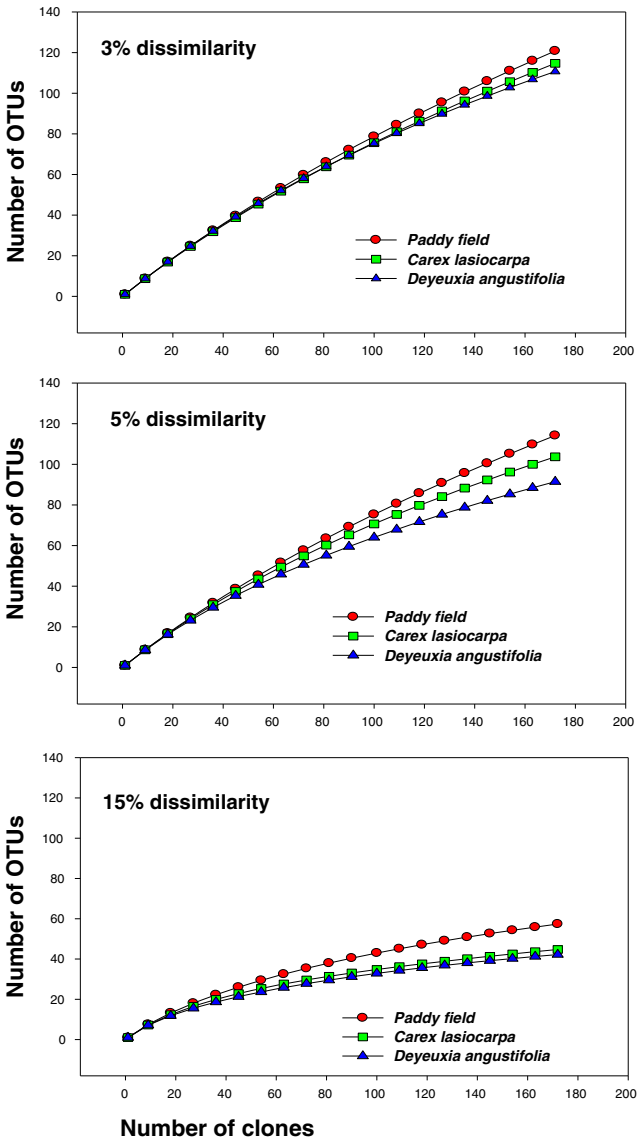

Fig. 2 Rarefaction curves for the bacterial 16S rRNA gene clone libraries from the paddy field, *Carex lasiocarpa* wetland and *Deyeuxia angustifolia* wetland. Operational taxonomic units (OTUs) were calculated based on 3 % (species), 5 % (genus) and 15 % (class) dissimilarity levels

Comparison of bacterial community composition between clone libraries

Shared species, genus and class among the three clone libraries are shown by Venn diagram in Fig. 3. The figure clearly indicated that 8 species, 13 genera and 19 classes were common among all three samples, while 4 species, 6 genera and 2 classes were shared between paddy field and *C. lasiocarpa* wetland, 9 species, 8 genera and 4 classes were shared between paddy field and *D. angustifolia* wetland, and 20 species, 18 genera and 6 classes were shared between two wetlands. Based on these data, pairwise comparison of community compositions of clone libraries was conducted by calculating the Sorensen similarity index, and the higher similarity indices between two natural wetlands than those between paddy field and two natural wetlands at species, genus and class levels were recognized (Table 4).

Bacterial community composition among the three clone libraries were also compared by UniFrac analysis (Table 5). The compositions of total clone sequences differed significantly among three libraries ($P<0.001$). Further UniFrac analysis at the dominant phylum levels of *Proteobacteria*, *Actinobacteria*, and *Firmicutes* showed that the *P*-test values between the paddy field and two natural wetlands were lower than those between two natural wetlands. Significant differences of bacterial member compositions among three samples were not detected at *Verrucomicobia* phylum, but were detected at *Chloroflexi* phylum. However, at *Acidobacteria* phylum, the significant difference was observed only between paddy field and *C. lasiocarpa* wetland ($P=0.001$), but not between paddy field and *D. angustifolia* wetland or between the two wetlands (Table 5).

Discussion

Genetic diversity of bacterial communities in natural wetlands and a neighboring paddy field

The diversity of bacterial communities seemed to be higher in the paddy field than in natural wetlands as calculated by the number of OTU, and the diversity indices of H', D and S_{chao1} at species, genus and class levels (Table 3), suggesting that a long-term conversion from natural wetland to paddy field increases soil bacterial diversity. This finding is consistent with the observation by Hartman et al. (2008), who stated that the diversity of bacterial communities was higher in agricultural wetlands than in natural wetlands at three locations in the North Carolina coastal plain, and they ascribed the increase of bacterial diversity in agricultural wetlands to liming or fertilization agronomic practices, which resulted in increasing soil pH. A highly positive relationship between soil pH value in the range of pH 4–7 and the diversity of soil bacterial

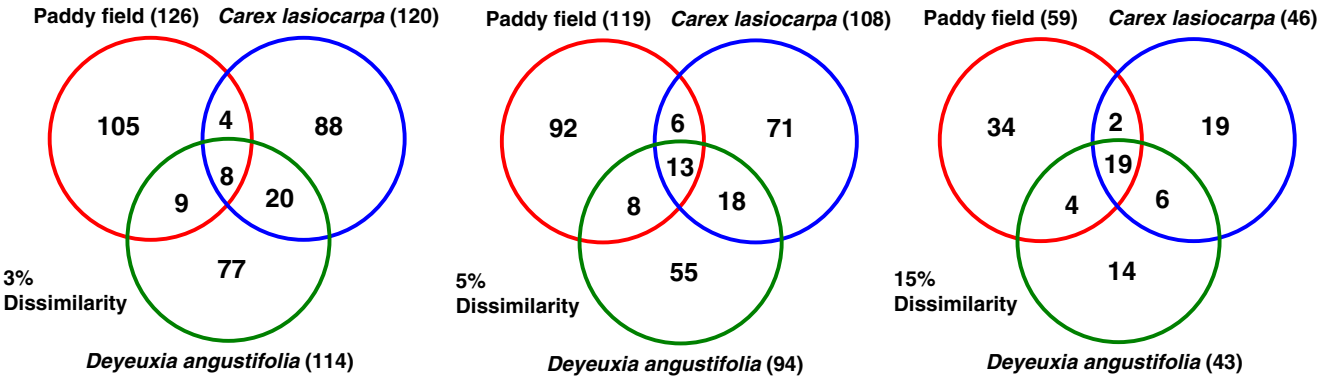

Fig. 3 Venn diagram showing shared and unique OTUs identified in 16S rRNA gene sequences obtained from clone libraries of the paddy field, *Carex lasiocarpa* wetland and *Deyeuxia angustifolia* wetland at 3 %, 5 % and 15 % dissimilarity, respectively

communities has been observed by many studies (Fierer and Jackson 2006; Chu et al. 2010). However, another study by Ausec et al. (2009) observed that higher bacterial diversity in the wetland of bog soil (soil pH 4.58, organic carbon content 45.4 %) than in fen soil (soil pH 7.55, organic carbon content 16.3 %), and they concluded that the higher bacterial diversity was due to the higher soil organic carbon content. In this study, the soil pH and total carbon content (g kg^{-1}) was 6.0 and 24.4 for the paddy field, 5.5 and 40.3 for the *C. lasiocarpa* wetland, and 5.2 and 39.3 for *D. angustifolia* wetland, respectively (Table 1). Thus, it is the soil pH rather than soil organic carbon content that appears to influence the genetic diversity of bacterial communities in the wetlands of Sanjiang Plain.

Predominance of unknown bacteria in natural wetlands

It is generally understood that bacterial 16S rDNA sequences with >99 % similarity renders strain-level identification, 97 % to <99 % similarity corresponds to species-level identification, 93 % to <97 % similarity with any known bacterium falls into either new genus or new species, and <93 % similarity with any bacterium usually suggests a new genus (Han et al. 2006). According to this criterion, results from this study showed that 48.9 %, 62.8 % and 71.1 % of clones with the similarity <93 % might belong to uncharacterized genera in clone libraries of paddy field, *C. lasiocarpa* wetland and *D. angustifolia* wetland, respectively (Table 2). In contrast, about 28.0 %, 12.6 % and 8.9 % of clones in paddy field, *C. lasiocarpa* wetland and *D. angustifolia* wetland had the highest similarity ≥97 % (Table 2). These findings suggest that the natural wetland soils in the Sanjiang Plain have many unknown bacterial genera. However, after the natural wetlands were converted to paddy field, the soil environmental conditions changed, which resulted in the development of known bacterial members, since studies on microbial communities have been more intensive in paddy fields than in natural wetlands in the world (Asakawa and Kimura 2008).

Long-term conversion from natural wetlands to paddy field alters soil bacterial community composition

The bacterial community compositions were different among the three samples as shown in Fig. 1, which was also demonstrated by UniFrac analysis of all clone sequences (Table 5). The differences were more distinct between the paddy field and the natural wetlands than between two natural wetlands using both data of in-depth UniFrac analysis of clone sequences at the phylum level (Table 5) and pairwise comparison of the Sorensen similarity index between samples (Table 4). Those findings suggest that the conversion from natural wetlands to paddy field alters the soil bacterial community composition, and the compositions are more similar to

Table 4 Pairwise comparisons of phylotype compositions of 16S rRNA gene libraries from a paddy field soil and two natural wetland soils in the Sanjiang Plain, NE China

Sample	Sorensen similarity index for the libraries					
	3 % dissimilarity		5 % dissimilarity		15 % dissimilarity	
	Paddy field	*Carex lasiocarpa*	Paddy field	*Carex lasiocarpa*	Paddy field	*Carex lasiocarpa*
Carex lasiocarpa	0.0325		0.053		0.075	
Deyeuxia angustifolia	0.075	0.171	0.075	0.178	0.167	0.364

Table 5 UniFrac *P*-test values as calculated by 16S rRNA gene clone libraries at total clones and individual phylum level from a paddy field soil and two natural wetland soils in the Sanjiang Plain, NE China

Sample	Total clones		*Proteobacteria*		*Actinobacteria*		*Firmicutes*		*Acidobacteria*		*Verrucomicobia*		*Chloroflexi*	
	Pad[a]	*C. la*[a]	Pad	*C. la*	Pad	*C. la*	Pad	*C. la*	Pad	*C. la*	Pad	*C. la*	Pad	*C. la*
C. la	<0.001[b]		<0.001		<0.001		<0.001		0.01		0.25		—[c]	
D. an[a]	<0.001	<0.001	<0.001	0.01	<0.001	0.06	0.01	0.14	0.43	0.25	0.42	0.16	—	0.01

[a] Pad, *C. la*, and *D. an* represent soil samples of paddy field, wetland with plant of *Carex lasiocarpa* and *Deyeuxia angustifolia*, respectively

[b] The values were in <0.001, 0.001–0.01, 0.01–0.05, and >0.05 indicated that two samples were highly significant, significant, marginally significant, and not significant, respectively

[c] Only a clone belong to *Chloroflexi* was observed in paddy field, the UniFrac *P*-test was not performed between the paddy field and the two natural wetlands

each other between two natural wetlands than between natural wetlands and paddy field. This finding is in accord with another report by Wang et al. (2011), who found that long-term changes in land management from subtropical wetland to paddy field shifted the soil microbial community structure as evaluated by PLFA and T-RFLP methods. That different soil microbial communities develop between natural wetlands and paddy fields is not surprising, since agronomical practices such as flooding and drainage, tillage and fertilization are involved in paddy fields, which directly or indirectly affects soil environments and thus alters soil microbial communities (Garbeva et al. 2004). It should be noted that although the two natural wetlands in this study were separated from each other by only 40 m, the dominated plant species and water level in those two sites were different. The site dominated by *C. lasiocarpa* was continuously submerged and the site dominated by *D. angustifolia* was seasonally drained (Zhao 1999). These differences led to different soil environments in the two wetlands (Song et al. 2003), and further generated the different soil microbial community compositions seen in this study as well as different T4-type bacteriophage communities (Zheng et al. 2013).

Composition of bacterial community in the wetlands of the Sanjiang Plain

There have been several studies on the bacterial composition of various kinds of wetlands around the world (reviewed by Dedysh 2011), and the majority of such research indicates that the proportion of *Acidobacteria* is larger than any other bacterial phyla in the wetlands (Hartman et al. 2008; Ausec et al. 2009; Pankratov et al. 2011). For example, Ausec et al. (2009) observed 41.6 % and 23.7 % of 16S rDNA clones were assigned to *Acidobacteria* in Slovenia bog and fen soils, respectively. The major bacterial phyla in the wetland soils of the North Carolina coastal plain were *Acidobacteria* (38.1 %), α-*Proteobacteria* (17.4 %), and *Actinobacteria* (9.7 %) with no clones belonging to *Chloroflex* (Hartman et al. 2008). Kanokratana et al. (2011) analyzed 280 clones of full-length 16S rDNA sequences from a tropical peat swamp forest soil in Thailand and found that *Acidobacteri*a (35.0 %) and *Proteobacteria* (37.9 %) dominated, and other phyla were minor members. However, in this study, about 94.4 % of total bacterial clones (515 clones) belonged to six taxonomic phyla: *Proteobacteria* (29.0 %), *Firmicutes* (18.5 %), *Actinobacteria* (17.2 %), *Chloroflexi* (11.9 %), *Acidobacteria* (9.4 %) and *Verrucomicobia* (8.4 %). The fact that the proportion of *Acidobacteria* was lower than those of *Proteobacteria*, *Firmicutes*, and *Actinobacteria* in these three soils (Fig. 1) strongly suggested that the bacterial community compositions in natural wetland or agricultural wetland (paddy field) in the Sanjiang Plain were different from those in other parts of world. The abundance of *Acidobacteria* was correlated negatively with soil carbon availability (Smit et al. 2001; Fierer et al. 2007) and soil pH (Jones et al. 2009; Rousk et al. 2010), and the pairwise correlation analysis in this study suggested that the correlation between *Acidobacteria* abundance and pH (R^2=0.94) was higher than that between *Acidobacteria* abundance and total soil carbon content (R^2=0.50). Thus it is soil pH not total soil carbon that is the major factor governing the abundance of *Acidobacteria* in the wetlands of the Sanjiang Plain.

In conclusion, this study investigated the composition and diversity of bacterial communities in two natural wetlands with different vegetations and in a neighboring paddy field in the Sanjiang Plain of NE China by clone library analysis. The conversion from natural wetlands to paddy field increased the diversity of bacterial community and altered the community composition, and the bacterial community composition was more notably different between the paddy field and two natural wetlands than between two natural wetlands. Long-term conversion from natural wetlands to paddy field increased the abundance of *Proteobacteria* and *Firmicutes* but decreased the abundance of *Chloroflexi*. In addition, a large

proportion of clones from natural wetlands had lower similarity with the known bacteria revealing that many hitherto unknown bacteria inhabit in wetlands in the Sanjiang Plain.

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