Changes in land use types affect soil bacterial communities and diversities in the Sanjiang Plain, Northeast of China


Heilongjiang Bayi Agricultural University, Daqing Heilongjiang 163319, China

*Corresponding author

E-mail: jiaofeng_1980@126.com

(Received 4th Sep 2023; accepted 30th Oct 2023)

Abstract. This study aimed to clarify the impact of land use conversion on the composition and diversity of soil bacterial communities, and to provide a reference for the selection of scientific restoration methods for degraded wetlands in the Sanjiang Plain, Northeastern of China. In 2018, Illumina MiSeq high-throughput sequencing was used to sequence amplicons of the 16S rDNA of soil bacteria in three types of land use: natural wetlands, rice fields, and restored wetlands in the Sanjiang Plain. The diversity and function of soil bacterial communities was analyzed. The results showed that wetland converted into paddy fields caused a significant decrease (P<0.05) in the Ace, Chao1 and Shannon indices of soil bacteria, and restoration of agricultural lands into wetlands significantly increased (P<0.05) these indices. The soil bacterial community structure of natural wetland, rice field and restored wetland differed significantly (P<0.05). The detected soil bacteria represented 38 phyla, 101 classes, 251 orders, 418 families, 786 genera and 1563 species. The phyla of Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Nitrospirae, Latescibacteria and Verrucomicrobia were dominant in all plots, reaching a relative abundance >1%. Wetland soil bacteria have 6 types of primary functions by PICRUST2 analysis: metabolism, environmental information processing, genetic information processing, cellular processes, human diseases, and organic systems. The soil pH, content of organic carbon and total nitrogen, and the carbon to nitrogen ratio are all factors affecting the diversity of soil bacterial communities in the investigated soils. Changes in wetland land use reduce the stability of the soil ecosystem and increases the potential ecological risk of wetland degradation.

Keywords: wetlands, land use patterns, soil bacteria, community diversity, function prediction

Introduction

Wetlands are amongst the most diverse ecosystems in nature and represent some of the most important environments for mankind. Wetlands contain abundant natural resources and have important ecological functions including regulating hydrological features, the removal of pollutants and supporting biodiversity of flora and fauna. Together with forests and oceans, they represent the three major ecosystems in the world (Zhang et al., 2017; Sui et al., 2021). In recent years, wetlands have suffered from environmental effects of changed uses, and this has become one of the hot issues of research investigating degraded ecosystems (Wang et al., 2015). Unreasonable development and utilization of wetlands have reduced remaining wetland areas, resulting in loss of biodiversity and in changes in the local wetland soils (Wang et al., 2016; Jing et al., 2017). How to coordinate the relationship between resource development and ecological protection is a key issue for maintaining the ecological balance of wetlands and promoting the sustainable development of the local economy (Yushanjiang et al., 2018).

Soil microorganisms are mainly responsible for decomposition of organic matter and are irreplaceable for maintaining the balance of an ecosystem (Xu et al., 2015). The
huge diversity of the microorganisms living in the soil shapes the overall biodiversity and has an important impact on soil ecosystem functions, as well as on the maintenance of multiple ecosystem functions including the cycling of nutrients (Dai et al., 2019). These microorganisms have a complex relationship in the overall microbial network. At a higher diversity, soil microbial communities participate in a more complex ecosystem that contributes to more ecosystem functions (Botterel et al., 2018). The in-depth study of the diversity of soil microbial communities and their response to environmental changes can assist in proper management of wetland resources and promote the healthy development of wetlands.

The Sanjiang Plain Wetland is a major food-producing area in China. A balance is required between its important role in maintaining a regional ecological sanctuary as well as ensuring national food security (Wang et al., 2011). Since the end of the 1950s, the Sanjiang Plain Wetland has undergone large-scale developments, including land reclamation, sand mining and canal construction that have changed the land use and reduced the area of the actual wetland by more than half (Yan et al., 2017). In the past 20 years, activities targeting towards protection and restoration of wetland have been achieved, and some wetland previously used as farmland has been restored. With support of state and local governments, the habitat conditions have improved year by year, but compared with the 1960s and 1970s, problems related to ecosystem degradation remain to be addressed, such as low coverage of wetland vegetation, severe soil erosion, and a general decline in biodiversity (Luo et al., 2015). The key to avoid further degradation is to protect and develop biodiversity, improve the quality of habitats, and turn the wetlands into self-sustainable entities (Jin et al., 2012).

Bacteria are the most abundant soil microorganisms, representing the widest, most complex and most diverse community relationships and functions. Small changes in the soil environment can cause significant changes in the structure and function of soil bacterial communities. At the same time, such changes can counteract the inorganic environment and be transmitted to an entire ecosystem (Sura et al., 2015). Soil bacteria can naturally protect the soil and promote the restoration of degraded ecosystems, while the characterization of their diversity and heterogeneity can describe soil fertility and predict ecological environmental risks (Hartmann et al., 2014). Changes in land use can affect the physical and chemical properties of the soil and lead to changes in the structure of soil bacterial communities, but how the changing trends of their structure and function actually affect the wetland ecosystem is still unclear (Du et al., 2019). Therefore, this study analyzed the differences in the diversity and function of wetland bacterial communities in soil collected from areas under different land use, namely natural wetland, paddy fields and restored wetlands. The study revealed the impacts of different land use patterns on the soil ecosystem of wetlands along the lower reaches of the Songhua River, with a view to formulate degraded wetland restoration methods, rational use, and scientific management of wetland resources.

Materials and methods

Study area

The study area belongs to the Sanjiang Plain. It is located in the Sanjiang Nature Reserve in China (46°56′55″ ~ 47°16″7′N, 13°22′5″ ~ 13°58′38″E). It has a temperate continental monsoon climate, an altitude of 65-81 m, an annual average rainfall of 548 mm, a multi-year average evaporation of 1,155 mm and an average frost-free period
of about 130 days (Li et al., 2023). There are 5 months on average per year with temperatures below 0°C, and the average yearly temperature is 2.1°C. The highest average temperature of 21.2°C occurs in July, and the lowest temperature occurs in January, with an average temperature of -19.4°C. The freezing and thawing period lasts from mid-October to mid-May of the following year, the ice cover period is 150 days, and the depth of the frozen layer of the wetland is 80-125 mm. The vegetation in the reserve is dominated by water plants and shrub vegetation, interspersed with artificial forests.

**Plot design and sample collection**

In June 2018, areas of natural wetland (NW), paddy fields (PF) and artificially restored wetland (RW) were selected in the Sanjiang Nature Reserve (47°44′39″N, 134°4′44″E). The selected areas had to have a slope not exceeding 5° with a consistent slope direction. A total of 9 experimental plots were set up, three for each land use type. The most abundant vegetation of NW (at an altitude of 70 m) were *Corpuscularia lehmannii* and *Deyeuxia angustifolia*. Paddy fields (altitude 71 m) contained monocultures of *Oryza sativa*, and RW (altitude 69 m) were the result of farmland that had been cultivated since 2010 but were restored to wetland in June 2015 by leveling the low-lying land of an abandoned sand quarry. These areas had been artificially replanted with *C. lehmannii* and *D. angustifolia*.

Soil samples were collected in July 2018. An area of 2 m×2 m was randomly designated in each experimental plot. A soil drill was used to sample the soil at a depth of about 0-10 cm using the 5-point sampling method. Deionized water was used to clean the soil auger between different plots. After removal of plant material and stones, the samples were put into sterile plastic ziplock bags and transported at 4°C. In the lab, 50 g soil of each sample was extracted, and the five samples taken from a single plot were combined and stored at -80 °C. These 9 samples were used for extraction of microbial DNA. The remaining 0.5 kg of the 9 soil samples were used for the determination of soil chemical properties.

**Determination of soil physical and chemical indices**

The pH value of the soil was measured, after mixing 1 g soil with 2.5 g water, by means of a pH meter (HQ30d, HACH, U.S.). The content of soil organic carbon (SOC) and of total nitrogen (TN) were measured with an automatic carbon and nitrogen analyzer (Elementar VarioMax CN, Elementar, Germany) and from these the carbon-to-nitrogen ratio was calculated.

**Extraction of bacterial DNA and PCR amplification**

A soil DNA extraction kit (Omega Bio-tek, Norcross, GA, U.S.) was used to extract microbial DNA, and universal primers (338F/806R) were used to perform PCR amplification of the V3-V4 region of bacterial 16S rDNA. The 20 μL reaction contained 4 μL 5x Fast *Pfu* buffer cycle, 2 μL 2.5 mmol/L dNTPs, 0.8 μL 5 μmol/L of each primer, 0.4 μL TransStart Fast *pfu* DNA polymerase, 0.2 μL BSA and 10 ng template DNA. PCR amplification (ABI GeneAmp 9700) was initiated with 3 min at 95°C for denaturation followed by 27 cycles of 30 s at 95°C, 30 s at 55°C and 45 s at 72°C, followed by final amplification for 10 min at 72°C. Each sample was amplified in 3 replicates. The PCR products of the same template were mixed and checked by 2%
agarose gel electrophoresis. The AxyPrep DNA Gel Recovery Kit (Axygen) was used to recover the amplicon from a cut section of the gel. After adjustment of the DNA concentration, MiSeq libraries were constructed and sequenced using the Illumina MiSeq platform.

**Bioinformatic analysis and statistics**

The obtained DNA sequences were analyzed using QIIME (version 1.17, http://qiime.org) software on the I-Sanger biological cloud platform of the Meiji Company. The sequences were divided in operational taxonomic units (OTUs) at a similarity level of 97%. Usearch software (version 7.1) was used to remove chimeric sequences, and the RDP (ribosomal database project) classifier Bayesian algorithm was applied to perform taxonomic analysis on the OTU representative sequences, with a confidence threshold of 0.7, with reference to an alignment Database (Silva 132/16S bacteria). The diversity indices Ace, Chao1 and Shannon were calculated according to the minimum number of sample sequences using Mothur software (version v.1.30.1), and R language tools were used to produce the graphs and figures.

Principal coordinate analysis (PCoA) was used to calculate the beta diversity distance matrix, and the non-parametric factor Kruskal-Wallis rank sum test was used to detect dominant species with significant differences. The functional prediction of the soil bacteria was based on the conversion of the 16S taxonomic pedigree of the Silva database into the taxonomic pedigree of prokaryotes in the Kyoto encyclopedia of genes and genomes (KEGG) database. SPSS software (IBM SPSS Statistics22 for Windows) was used to perform Duncan multiple comparison and permutation test statistical analysis of one-way analysis of variance (ANOVA). Adonis, LefSE and RDA analysis were performed by R software with vegan and ggplot2 packages. The functions of soil bacteria were performed on the PICRUST2 (https://github.com/picrust/picrust2).

**Results**

**Soil physical and chemical properties of the different land use soil types**

The pH and the SOC and TN content varied between the three soil types, as can be seen in Table 1. Significant differences (P<0.05) were observed in the pH of the three types, with that of restored wetland being higher than that of paddy fields and natural wetland giving the lowest pH. The organic carbon and total nitrogen content were relatively high in natural wetland, while those of paddy fields were lower and lowest values were obtained with restored wetland. The nutrient index C/N was higher in NW and RW, which were both significantly higher than that of PF (Table 1), but the nutrient index C/N in NW and RW did not change significantly (Table 1).

**Table 1. Soil physical and chemical properties of the different land use soil types, as mean ± standard deviation (n=3)**

<table>
<thead>
<tr>
<th>Land use types</th>
<th>pH</th>
<th>SOC</th>
<th>TN</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW</td>
<td>5.60±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.25±6.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.21±0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.75±1.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RW</td>
<td>6.20±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.68±2.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.05±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.17±0.93&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PF</td>
<td>5.82±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.35±3.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.85±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.14±1.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different superscript letters indicate significant differences (P<0.05) between the land use types, as one-way ANOVA followed by Duncan test.
Sequencing analysis

The bacterial communities in the soil samples were analyzed by sequencing V3-V4 amplicons of 16S rRNA. After filtering the MiSeq high-throughput sequences to remove low-quality reads, a total of 425,505 pruned sequence reads were obtained from the 9 soil samples representing triplicates of the 3 types of land use. These represented a total number of 179,618,607 bp with an average length of 436.84 bp per read (Table 2).

The sequences were attributed to OTUs and the number of obtained OTUs were used to construct rarefaction curves (Figure 1a). As can be seen, reasonable saturation was obtained for the 9 samples, so that additional sequencing data would less likely result in the discovery of new OTU members. In total 5,096 OTUs were obtained from all sample plots combined. Figure 1b shows that 1284 OTUs (about 25.2%) were shared by all three land types. The number of OTUs unique to restored wetland was 763, accounting for 14.9% of the total (25.8% of the OTUs detected in NW). The number of OTUs unique to natural wetlands was 927 (18.2% of the total, 29.5% of all NW OTUs), and 635 OTUs were unique to the paddy soil (12.5% of the total, 20.1% of all PF OTUs).

Table 2. Sequencing data statistics

<table>
<thead>
<tr>
<th>Land use types</th>
<th>Number of reads</th>
<th>Number of base pairs</th>
<th>Average nr. of base pairs per read</th>
<th>Diversity index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ace</td>
<td>Chao1</td>
<td>Shannon</td>
<td></td>
</tr>
<tr>
<td>NW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45,820</td>
<td>19,484,596</td>
<td>432.04</td>
<td>2531 ± 180.25 b</td>
<td></td>
</tr>
<tr>
<td>46,231</td>
<td>19,896,853</td>
<td>438.10</td>
<td>2415 ± 124.30 a</td>
<td></td>
</tr>
<tr>
<td>46,125</td>
<td>19,210,843</td>
<td>437.92</td>
<td>2415 ± 124.30 a</td>
<td></td>
</tr>
<tr>
<td>RW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48,520</td>
<td>20,135,061</td>
<td>437.28</td>
<td>180.25 b 124.30 b 0.45 b</td>
<td></td>
</tr>
<tr>
<td>46,820</td>
<td>19,542,752</td>
<td>436.12</td>
<td>2998 ± 154.25 a</td>
<td></td>
</tr>
<tr>
<td>46,257</td>
<td>19,828,654</td>
<td>437.25</td>
<td>3025 ± 154.25 a</td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47,685</td>
<td>21,354,260</td>
<td>437.05</td>
<td>102.35 b 154.25 a 0.45 a</td>
<td></td>
</tr>
<tr>
<td>48,523</td>
<td>20,710,423</td>
<td>438.20</td>
<td>2156 ± 158.86 c 0.26 a</td>
<td></td>
</tr>
<tr>
<td>49,524</td>
<td>19,455,165</td>
<td>437.61</td>
<td>2196 ± 158.86 c 0.26 a</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>47,278</td>
<td>19,957,623</td>
<td>436.84</td>
<td>192.38 b 158.86 c 0.26 a</td>
</tr>
</tbody>
</table>

Different superscript letters indicate significant differences in land use types, mean ± standard deviation (n=3)

Figure 1. Rarefaction curves indicating saturation of OTUs (a), and Venn diagram of OTUs (b) for the 9 samples representing triplicates of the three different land types. Natural wetland (NW) is shown in red, restored wetland (RW) in blue and paddy fields (PF) in green.
The alpha diversity of the bacterial communities in the soil samples was assessed by calculation of Ace, Chao1 and Shannon indices. The ACE and Chao1 indices were significantly different (P<0.05) between natural wetlands, restored wetlands and paddy fields and the Shannon index of PF was lower than that of NW and RW (Table 2).

**Differences in soil bacterial community structure and indicator genera in different land use types**

The obtained OTUs were binned according to various taxonomic levels. The total OTUs of all 9 soil samples combined represented 38 phyla, 101 classes, 251 orders, 418 families, 786 genera and 1563 species. At the phylum level (Figure 2) Proteobacteria were the most common, representing approximately 38% of all detected phyla, followed by Acidobacteria, Actinobacteria and Chloroflexi. These most common phyla were detected in the same descending order of abundance in RW and PF, although in NW Chloroflexi were more abundant than Actinobacteria. These four phyla accounted for over 80% of all detected phyla in NW, but they reached only 75% in RW. In addition, 8 other phyla reached relative abundances >1% in each plot (Figure 2). Of these, Latiscibacteria and Verrucomicrobia were more common in paddy soil, while Firmicutes were more abundant in natural wetland soil compared to the other soil types. All other detected phyla that did not reach 1% abundance were combined as ‘others’ in Figure 2.

![Figure 2. Soil bacterial phyla composition of different land use types](image)

A PCoA with a Bray-Curtis distance matrix was used to compare the similarities and differences of the soil bacterial communities at the OTU level. As shown in Figure 3, this placed the natural wetland data on the negative semi-axis, and the restored wetland and two of the three rice fields on the positive semi-axis of the PC1 axis (representing 28.53% of the data). The test of differences by ADONIS statistics confirmed that the differences in soil bacterial communities between natural wetlands, restored wetlands, and paddy fields were significant (R²=0.8651, P<0.05).
LEfSe was used to analyze the significant differences on the level of genera (LDA threshold 4.0), and a community evolutionary branch map was constructed (Figure 4). This illustrated that representatives of the genera Noviherbaspirillum (a), Aciditerrimonas (o), Skermanella (k), Pseudohongiella (e) Truepera (x), Oligoflexus (g), and Nitrosomonas (b) were the dominant genera in NW. Mesorhizobium (m), Luteolibacter (r) and Singulisphaera (s) were the dominant genera in the restored wetland and Deltaproteobacteria (h, i, j) were the dominant genera in the paddy fields.

**Functional annotation of soil bacteria in different land use types**

The annotation information of the OTUs was used to mine the KEGG primary functional metabolic pathway database. The information on abundance of each function is shown in Table 3. The soil bacterial community included 6 types of primary metabolic pathways, with the relative abundance of each functional gene in the sample ranging from high to low in the following order: metabolism, environmental information processing, organization systems, cellular processes, human diseases, genetic information processing. Among them, the genetic information processing function of the OTUs from restored wetland was significantly more frequently represented than in the other plots (P<0.05), while the other metabolic functions of the bacteria did not differ significantly.

**Relationships between bacterial alpha diversity and soil physical and chemical properties**

A correlation analysis between the alpha diversity of the soil bacteria and the soil physical and chemical properties is shown in Table 4. This showed that the Ace index significantly correlated in a positive manner with the pH and with the soil nutrient C/N
ratio ($P<0.01$ for both). The Chao1 index strongly positively correlated with pH ($P<0.01$) and negatively correlated with soil TN ($P<0.05$). The Shannon index strongly positively correlated with soil C/N ($P<0.01$).

**Figure 4.** Cladogram of all bacterial communities for the three different land use types (LDA > 4.0). The letters inside and around the cladogram represent genera as explained below the figure, with red: natural wetland, green: paddy fields and blue: restored wetland.

**Table 3.** Relative abundance information of first-level metabolic pathways of soil bacterial communities in the different land use types

<table>
<thead>
<tr>
<th>Land use types</th>
<th>Metabolism</th>
<th>Genetic information processing</th>
<th>Environmental information processing</th>
<th>Cellular processes</th>
<th>Human diseases</th>
<th>Organism systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW</td>
<td>0.617±</td>
<td>0.113±</td>
<td>0.166±</td>
<td>0.121±</td>
<td>0.120±</td>
<td>0.124±</td>
</tr>
<tr>
<td></td>
<td>0.011±</td>
<td>0.003b</td>
<td>0.033a</td>
<td>0.023a</td>
<td>0.020a</td>
<td>0.011a</td>
</tr>
<tr>
<td></td>
<td>0.611±</td>
<td>0.151±</td>
<td>0.169±</td>
<td>0.108±</td>
<td>0.109±</td>
<td>0.121±</td>
</tr>
<tr>
<td></td>
<td>0.006a</td>
<td>0.001a</td>
<td>0.016a</td>
<td>0.012a</td>
<td>0.014a</td>
<td>0.006a</td>
</tr>
<tr>
<td></td>
<td>0.606±</td>
<td>0.110±</td>
<td>0.199±</td>
<td>0.105±</td>
<td>0.105±</td>
<td>0.126±</td>
</tr>
<tr>
<td></td>
<td>0.002b</td>
<td>0.002b</td>
<td>0.003a</td>
<td>0.001a</td>
<td>0.001a</td>
<td>0.002a</td>
</tr>
</tbody>
</table>

Note: Different superscript letters indicate significant differences in land use types, mean ± standard deviation (n=3)
Table 4. Correlation analysis between soil bacterial alpha diversity and soil physicochemical properties

<table>
<thead>
<tr>
<th>Index</th>
<th>pH</th>
<th>SOC</th>
<th>TN</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ace</td>
<td>0.701*</td>
<td>-0.331</td>
<td>-0.618</td>
<td>0.741*</td>
</tr>
<tr>
<td>Chao1</td>
<td>0.830**</td>
<td>-0.514</td>
<td>-0.764*</td>
<td>0.590</td>
</tr>
<tr>
<td>Shannon</td>
<td>0.262</td>
<td>0.173</td>
<td>-0.155</td>
<td>0.975**</td>
</tr>
</tbody>
</table>

* indicates 0.05 level; ** indicates 0.01 level

The redundant analysis of soil bacterial community structure and the soil physical and chemical properties is shown in Figure 5. The two axes reflect the influence of soil environmental factors on the structure of the bacterial community, and the cumulative explained variation of the first and the second axis reached 56.03%. The results of the displacement test showed that soil pH ($r^2=0.842$, $P=0.023$), SOC ($r^2=0.672$, $P=0.034$), TN ($r^2=0.892$, $P=0.038$) and C/N ratio ($r^2=0.386$, $P=0.041$) represent environmental factors that all affected the differences of the bacterial communities. Moreover, the effects of pH and C/N were opposite to that of SOC.

Discussion

Diversity analysis of wetland soil bacterial communities in the different land use types

The wetland soil investigated here is affected by seasonal flooding and undergoes alternate sedimentation, which results in a soil that erodes easily under the effect of precipitation. The fixating and protective effects of wetland vegetation on the soil reaches a dynamic balance with erosion over time, ultimately resulting in a relatively stable wetland habitat (Zhou et al., 2019). When the land use locally changes, the plant cover and the soil physical and chemical properties will change accordingly, which will have an important impact on soil microbial diversity. Jia et al. (2019) compared the soil bacterial diversity of Huixian karst wetland, rice fields and dry land, and considered the
change of soil physical and chemical properties to be the main reason for the higher soil bacterial diversity index of rice fields compared to natural wetland. Xu et al. (2016) investigated the impact of cultivation of rice in wetlands in the Sanjiang Plain and found it that paddy fields increased the diversity of soil bacterial communities, based on the obtained alpha diversity index. However, our results show that wetland used as paddy fields had significantly reduced the alpha diversity of soil bacteria, compared to natural wetlands. Natural wetland soil in this region has a high nutrient content that creates a suitable habitat for soil bacteria. The higher hydrological connectivity will support growth and distribution of soil bacteria. However, after changing these lands into rice fields, the hydrological connectivity decreased. The growth and distribution of bacteria was partly inhibited, leading to a decrease in soil alpha diversity. In addition, the extensive use of chemical fertilizers and pesticides may also have had an impact on the soil bacteria.

Although the restoration of wetlands from paddy fields has been restored in terms of vegetation and habitat, there were still significant differences between their soil nutrient conditions compared to natural wetlands, and likewise in the determined alpha diversity of the soil bacteria. Restoring wetlands showed an increase in the diversity and abundance of soil bacterial communities compared to paddy fields, which means that when the wetland habitat is restored, soil nutrients and soil water connectivity will increase, supporting growth and distribution of more diverse soil bacteria, thereby increasing the alpha diversity of the soil bacterial community.

At present, the mechanism and driving forces behind the formation and stability of soil bacterial communities is not fully understood, but the differences in soil bacterial community components can explain the impact of environmental changes on the in-situ soil bacterial community reconstruction. Environmental conditions, rather than the composition of the microbes that migrated into the soil, determine the success of reconstructing the soil microbial community structure (Xun et al., 2015). Our current study confirms that there are significant differences in the soil bacterial community structure between rice fields, restored wetlands, and natural wetlands, indicating that land use patterns have an important impact on soil bacterial communities by changing environmental factors. The study by Wang et al. (2019) described that different land use patterns has an important impact on the structure of wetland soil bacterial communities. They recorded that Proteobacteria, Actinomycota, and Acidobacteria are the dominant phyla in a wetland soil environment. Li et al. (2020) found that some members of Acidobacteria, Firmicutes and Proteobacteria play an important role in connecting soil bacterial communities. This has many similarities with the data presented here. Proteobacteria, Acidobacteria, Actinomycetes, Chloroflexus, Bacteroides, Gemmatimonadetes, and Nitrospirae were all dominant phyla in the wetland soil environment. In addition, the genera of Mesorhizobium, Luteolibacter, Singulisphaera, Noviherbaspirillum, Aciditerrimonas, Skermanella, Pseudohongiella, Truepera, Oligoflexus, Nitrosomonas can serve as indicators for wetlands of different land use types.

**Functional analysis of wetland soil bacteria in different land use types**

At present, there are few reports on the ecological functions of soil bacterial communities in wetlands. To this end, we performed PICRUST2 function prediction analysis based on the obtained partial 16S rRNA sequences. The results showed that different land use methods changed the structure of the soil bacterial community, and
the metabolic pathways of soil bacteria changed correspondingly. Wetland soil bacteria perform six functions: metabolism, environmental information processing, genetic information processing, cellular processes, human diseases, and organic systems to maintain the stability of the ecosystem. Among them, metabolic function genes account for the largest proportion of all attributed gene functions, for all three types of land use. This confirms that metabolism is the core function of soil bacteria, which is consistent with the research results of Li et al. (2018). The type of land use has a certain impact on the environmental information processing, genetic information processing, human diseases, and the functional pathways of organic systems in wetland soils. The relative abundance of these functional genes only changes significantly in a certain habitat, indicating different soils with their bacterial communities may have multiple types of primary metabolic pathways, and the primary metabolic pathways of soil bacteria have a low correlation with the composition of soil bacterial communities. This study preliminarily analyzed the differences in soil bacterial functions of different land use patterns in wetlands along the river, but PICRUST2 function prediction has its limitations. It is recommended that in-depth study of the functions of soil bacteria in wetlands along the river is combined with analysis methods such as metagenomic sequencing.

Conclusion

Land use patterns have led to significant changes in the physical and chemical properties of the soil in the Sanjiang Plain. The conversion of natural wetlands to agricultural paddy fields caused significant differences in the diversity of the bacterial communities in the soil, while restoration of agricultural land to wetlands changed that diversity again. Different land use types of habitats thus have their own distinctive dominant bacterial communities. The variation of wetland soil bacterial community is affected by soil pH, organic carbon, total nitrogen and by the carbon to nitrogen ratio. Hydrological connectivity affects the alpha diversity of the soil bacteria. The key factor to maintain a high water table level. The ecological functions of soil bacteria in various land-use types of habitats remain stable, and they are active in metabolism, environmental information processing and genetic information processing, so that degraded habitats still have a good potential for natural restoration.

REFERENCES


APPENDIX

Figure S1. The location of this research site and habitats of NW, RW and PF