# EFFECT OF RECLAMATION AND RESTORATION ON SOIL MICROBIAL COMMUNITY STRUCTURES IN COLD REGION WETLAND

DING, J. N.<sup>\*</sup> – XU, N. – SHI, C. Q. – YU, S. P.

Heilongjiang Province Key Laboratory of Cold Region Wetland Ecology and Environment Research, Harbin University, Harbin 150086, China

> \*Corresponding author e-mail: ding.junnan@163.com

(Received 12<sup>th</sup> Oct 2022; accepted 6<sup>th</sup> Jan 2023)

**Abstract.** To understand the impact of reclamation and restoration on soil microbes and the corresponding soil biogeochemical processes in cold region wetland, an experiment was established that included two cultivated treatments (upland fields of soybean cultivation and paddy fields of rice cultivation), two restored treatments (with meadow and forest wetland of agricultural abandonment). Changes in soil properties and microbial communities across the different treatments were analyzed. The results showed the following: (1) The contents of SOC, TN, and TP and the Shannon diversity, Ace and Chao1 index of a wetland park nature reserve in the cold region were higher than those of the upland and paddy fields. (2) The diversity and abundance of bacterial communities in reclaimed soils was slightly different from that of restored wetland soils. Actinobacteria, Acidobacteria, Proteobacteria, Chloroflexi and Firmicutes were the predominant bacteria. (3) Some soil physicochemical properties are significantly correlated with the main bacteria in the soil, the environmental factor of AP, TP, AK, TK, TN and SOC driven by reclamation and restoration modes effect the structure microbial community. **Keywords:** *black soil, soil microbial diversity, soil properties, high throughput sequencing* 

#### Introduction

Wetland soil, as one of the important environmental factors of wetland ecosystems, is highly significant for maintaining the balance and succession of wetland ecosystems (Pei et al., 2009). A change in the original function of wetland soil will lead to changes in the types of wetland vegetation, soil biology and wetland biodiversity, and ultimately lead to a change in the function of wetland ecosystems (Angeloni et al., 2006; Mclauchlan, 2006). However, wetlands have changed dramatically in the past century. On the one hand, about half of global wetlands have been lost due to human disturbances (Zedler and Kercher, 2005). On the other hand, due to a growing awareness of the important ecological and environmental functions of wetlands, the protection and restoration of wetlands have been implemented worldwide. These extreme changes in wetlands significantly impact the cycling, retention, and release of soil nutrients and further influence global biogeochemical cycles (Gao et al., 2014; Lamers et al., 2012; Laganiere and Angers, 2020). Thus, it is important to understand the effects of wetland changes on soil biogeochemical processes.

Soil microbes play a critical role in soil biogeochemical processes. Numerous studies have confirmed that land-use changes can profoundly affect soil microbial communities (Lauber et al., 2008; He et al., 2020). Thus, soil microbial communities can serve as a sensitive indicator of soil biogeochemical changes that are related to land-use changes (Hartman et al., 2008; Zhao and Zhang, 2010). A clear understanding of changes in microbial community structures would enhance our understanding of soil

biogeochemical processes and greatly benefit land management and restoration (Morrissey et al., 2014; Zhou et al., 2017a). However, changes in the soil microbes of wetlands, caused by cultivation and agricultural abandonment, and their link to soil biogeochemical processes have rarely been studied. Many studies argue that due to changes in soil biochemical processes it is difficult to reverse the degradation of wetlands (Zhao et al., 2021). Although the plants and the hydrologic conditions could be restored to their natural states in a few years (Calhoun et al., 2014; Wang et al., 2019; Duan et al., 2020; Lin et al., 2021), the soil biogeochemical processes may still differ from those in natural wetlands, even after many years of restoration (Moreno-Mateos et al., 2012; Vogel and Conedera, 2020). Some studies included soil microbial communities as sensitive indicators of how wetlands have changed relative to their natural state, and many similarities had been found between restoration wetlands and natural wetlands, which indicated that restoration wetlands had begun to recover (Andersen et al., 2013; Card and Quideau, 2010). However, few studies have reported that microbial communities have been fully reestablished to their natural (or similar to natural) conditions, even after many years of restoration (Pellegrino et al., 2015; Deng et al., 2019). Thus, in order to provide a basis for wetland restoration and better understand the variation in soil biogeochemical processes after wetland changes, it is necessary to focus closely on the changes in soil microbial communities, as well as their relation to soil physicochemical properties.

The forest wetland in Lesser Khingan Mountains belongs to the type of inland plain wetland ecosystem, and belongs to the type of fragile ecosystem in China's ecological geographical division. Once it is damaged, it will not be easy to recover. The forest wetland ecosystem in this area is an important pillar and natural foundation to ensure the ecological balance of the Lesser Khingan Mountains, and plays a huge role in maintaining the ecological balance of Heilongjiang Province in China. Therefore, this study focuses on the impact of disturbance and restoration of forest wetland reclamation on the biochemical properties of wetland soil, soil microbial community and function, mastering its environmental impact factors, and based on the research results, the proposed protective countermeasures will help protect the ecological environment of wetland. In this study, our aims were to (i) determine if microbial composition and activity would vary in natural, restored wetlands; (ii) assess the extent of recovery for microbial composition and activity associated with wetland restoration. These results can provide a scientific theoretical basis to monitor and restore wetland soil degradation in cold regions.

#### Materials and methods

#### Study area

The study area was located in the Heilongjiang Hongxing National Nature Reserve, which is located in the Lesser Khingan Mountains at Heilongjiang Province, China  $(28^{\circ}21'40''\sim128^{\circ}53'30'' \text{ N}, 48^{\circ}41'20''\sim49^{\circ}11'00'' \text{ E})$ . The total area is 11,199.5 hm<sup>2</sup>, and the wetland area is 7,669.2 hm<sup>2</sup>. The reserve belongs to the northern temperate continental monsoon climate. The annual average temperature in the reserve is -1.3 °C; the annual average precipitation is 571 mm, and the average relative humidity is 68.3%. The Heilongjiang Hongxing National Nature Reserve is rich in biology and species, with typical water, swamp, meadow wetland, shrub and forest vegetation. The zonal soil

is black soil, and the non-zonal soil includes grass, swamp soil, and peat soil. The wetland park is divided into seven types of wetlands that include rivers, flood plains, herbs, mosses, swamp meadows, shrub swamps and forest swamps. Among them, the herb and swamp meadow wetlands constitute the main wetland landscape of the wetland park, which is surrounded by farms. The vegetation of the soil sample collection site is as follows: (1) converting rice farmlands to meadow wetland (CRMW) with the primary predominant plants Deveuxia angustifolia, Carex lehmanii, Carex meyeriana, Carex appendiculata, Caltha palustris, Cyamus flavicomus, and Pedialaris langiflora, and this soil types has been restored naturally for ten years old. (2) Converting soybean farmlands to forest wetland (CSFW): the primary forest with mixed coniferous and broad-leaved forests and broad-leaved forests as the main plant types. (3) Reclaimed upland fields (soybean farmlands, SF): the main planting crop is soybean, the chosen soybean variety was Suinong 35 (Glycine max L.), which is continuously planted once a year. (4) Reclaimed paddy fields (rice farmland, RF): the main planting crop is rice, the chosen rice variety was Longjing 46 (Oryza sativa L.), which is continuously planted once a year. At the end of each April, agricultural machinery tillage is conducted once, and the soybean is sown. Farm fertilization and field management are performed according to the local practices. The crop is harvested at the end of September each year, and the land is idle from the end of October to the middle of next April.

## Sample collection

This experiment was conducted from August 20 to September 3, 2021, in the Key Laboratory of Wetland Ecology and Environmental Research in the cold regions of Harbin University (Harbin city, China) and the Heilongjiang Hongxing National Nature Reserve (Yichun city, China). According to the characteristics of land use in the nature reserve, four 100 blocks of meadow wetlands, forests and the surrounding upland and paddy fields were selected in a  $100 \times 100$  m quadrat. Five sampling points were established according to the "S" type. A 5 cm soil shovel was used to collect soil samples that were  $0 \sim 20$  cm deep at each sampling point. The mixed soil samples were combined into one repetition, and each sample was collected three times. In each quadrat, after the fresh soil sample was passed through a 2 mm sieve, the root residues were removed and placed in a sampling box. Part of the soil samples were brought back to the laboratory for storage as soon as possible at -20 °C for analysis of the bacterial microbial community structure. Another part was stored at 4 °C to determine the physical and chemical properties of the soil.

## Determination of soil properties

Soil properties were analyzed using standard soil test methods as described by the agriculture protocols for China (Lu, 2000). pH and Electric conductivity (CEC) were determined in soil: H<sub>2</sub>O (1:5; w/v soil/water) suspensions using a pH Meter (model: FE20K, Mettler Toledo, Giessen, Germany) and TDR (model: TRIME-IPH, Aozuo Inc., Beijing, China). Soil organic carbon (SOC) was determined by the dichromate oxidation process. Total nitrogen (TN) was determined by Kjeldahl digestion method. Total phosphorus (TP) was determined by Mo-Sb anti spectrophotometric method (Lu, 2000). Total potassium (TK) was determined by atomic absorption spectrophotometry. Available nitrogen (AN) was determined using the alkali-hydrolyzed diffusing method. Available

phosphorus (AP) was extracted with NH4F-HCl solution, and then determined by ultraviolet visible spectrophotometer. Available potassium (AK) was extracted with 1 mol L-1 NH4OAc, and then determined by flame absorption spectroscopy (Bao, 2005).

### DNA extraction and high-throughput assay

Total genomic DNA was extracted with an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions. The integrity of DNA was validated by agarose gel electrophoresis. The quality of the DNA samples was checked with a spectrophotometer (Nanob Drop, ND2000, Thermo Scientific, Wilmington, DE, USA) after extraction.

Genomic DNA of the soil microorganisms was extracted with an Omega E.Z.N.A DNA Kit (Omega Bio-tek, Norcross, GA, USA). The extracted genomic DNA was detected by 1% agarose gel electrophoresis. PCR was performed on a Geneamp 9700 PCR system (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The universal primers 515f (5'-gtgccagcmgcgg-3') and 907r (5'-ccgtcaattcmttragtt-3') were used to amplify the V3-V4 region of the bacterial 16S rRNA gene. The PCR products were quantified using a QuantiFluor<sup>®</sup> – ST fluorometer (Promega, Madison, WI, USA), and the samples were adjusted as needed for sequencing. Finally, they were sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for high-throughput sequencing using an Illumina HiSeq 2500 PE250 platform (San Diego, CA, USA).

### **Bioinformatics analysis**

Pairs of reads were spliced into a sequence according to the direct overlap relationship of PE (paired-end) reads. At the same time, the quality of reads and splicing effect were controlled and filtered, and correction of the sequence direction was made according to the end of the box sequence. Finally, the high-quality sequences obtained after filtering were assigned to samples according to barcodes.

Operational taxonomic unit (OTU) was defined as units with 97 percent similarity level (Stackebrandt and Goebel, 1994). OTU were used to generate rarefaction curves and Shannon-Wiener curves. The bacterial communities diversity by calculating the diversity estimator indices of Shannon-Wiener, Simpson, Chao1 and ACE using Mothur version v.1.30 (Chao and Bunge, 2002; Schloss et al., 2009).

The most abundant sequence in each OTU was clustered using Use arch at 97% sequence identity (Edgar, 2013). Representative sequences were taxonomically classified using a Bayesian classifier (Wang et al., 2007), and then the representative sequences assigned against the Silva database to gain the taxonomic information of bacterial communities (Pruesse et al., 2007; Quast et al., 2013). Bacteria were classified at the level of phylum, class, order, family, and genus.

## Statistical analysis

Differences in microbial diversity indices among samples were tested using Duncan's new multiple range test of variance performed by DPS software. The shifts in the relative abundance of the bacterial phyla were displayed by a heat map (Kolde, 2015), which was modeled with vegan package in R Core Team (2022). SPSS 22.0 software (IBM, Inc., Armonk, NY, USA) was used to perform a Pearson correlation analysis of soil properties and the diversity indices of bacterial communities. The

Pearson correlation coefficient of the top 20 abundant bacterial phyla and soil properties, as well as the top 20 abundant bacterial phyla and vegetation were calculated, and displayed on the heatmap.

### Result

### Effects of land use on soil physical and chemical properties

As shown in *Table 1*, the pH of each soil sample was neutral. The contents of SOC and TN were higher in CRMW, but lower at SF. The content of TK was highest in CRMW, and there was significant difference among RF, SF, and CSFW. The content of TP was higher in CRMW and CSFW, there was significant difference among TP values in RF and SF. The content of AN was lowest in RF and CRMW, which was significantly (P < 0.05) lower than SF and CSFW. The contents of AP and AK was higher in RF and SF than others. The value of CEC was higher in RF, followed by CRMW, but showed no difference among SF and CSFW.

Soil properties	RF	CRMW	SF	CSFW
pН	$6.71\pm0.11$	$6.33\pm0.32$	$6.43\pm0.07$	$6.42\pm0.08$
SOC (mg·kg <sup>-1</sup> )	$25.31\pm7.80$	$39.38 \pm 2.63$ *	$19.75\pm1.77$	$32.07 \pm 1.75$
TN (%)	$1.84\pm0.84$	4.87 ± 1.42 *	$3.45\pm0.45$	$2.53\pm0.85$
TP (%)	$0.05\pm0.00$	$0.48 \pm 0.13$ *	$0.03\pm0.00$	$0.22\pm0.02$
TK (%)	$1.84\pm0.11$	$3.98 \pm 0.31$ *	$1.94\pm0.09$	$2.08\pm0.47$
AN (mg·kg <sup>-1</sup> )	$116.81 \pm 14.27$	$119.31 \pm 15.29$	$149.78\pm27.57$	245.47 ± 37.65 *
AP (mg·kg <sup>-1</sup> )	$50.41 \pm 5.67$	$43.67 \pm 1.80$	70.12 ± 8.23 *	$42.38\pm2.73$
AK (mg·kg <sup>-1</sup> )	$156.51 \pm 24.71$	$649.08 \pm 27.73$ *	$150.36\pm3.39$	$182.34\pm43.81$
CEC	$12.72 \pm 4.86 *$	$9.88 \pm 1.01$	$7.53\pm2.47$	$5.53\pm2.30$

Table 1. Physical and chemical properties indices of the soil tested

The error bars on the results represent the means  $\pm$  SD (n = 3). SD, the value of \* *P* < 0.05 is marked, the same below

#### Analysis of the microbial diversity of soil bacteria

As shown in *Figure 1*, the levels of bacterial OTUs in different soil samples were significantly different. A total of 17,470 of bacterial microbial operational taxonomic unit (OTU) sequences were detected in the soil tested. The number of unique OTU sequences of bacteria in the CRMW was 1246, which accounted for 7.13% of the total OTU sequences. The number of unique OTU sequences of the soil bacteria in RF was 917, which accounted for 5.24% of the total. The number of unique OTU sequences of bacteria in the SF was 362, which accounted for 2.07% of the total OTU sequences. The number of unique OTU sequences of the soil bacteria in CSFW was 544, which accounted for 3.11% of the total. The number of OTU sequences of the wetland restoration soil bacteria was higher than those of the Upland and paddy fields soil.

#### Microbial alpha diversity

By observing the information on diversity abundance of bacterial and microbial species, the changes in alpha diversity index obtained by a statistical *t*-test under OTU level are

shown in *Figure 2*. The Shannon, Simpson, Ace and Chao1 of the diversity index of each soil sample differed. The Shannon, Ace diversity and Chao1 diversity indices of CRMW were significantly higher than CSFW, RF and SF (P < 0.05). The Simpson diversity index of SF was significantly higher than CRMW, RF, and CSFW (P < 0.05).



Figure 1. Venn diagram of soil microorganism based on the level of OTU

## Factors structuring soil bacterial community diversity

The correlation analysis showed that the soil bacterial community was not only affected by soil properties, but was also closely related to soil types. The diversity indices were positively correlated with soil nutrients, and negatively correlated with the pH value and AN. The OTU, Ace, Chao1 and Shannon indices were significantly negatively correlated with the pH value, and the Sobs index exhibited a significant positive correlation with pH value. The OTU showed a significant negatively correlated with AN. Shannon index was significantly positive correlated with TN. Sobs index was significantly negative correlated with AN, and positively correlated with pH (*Table 2*).

#### Variation of microbial community abundance

As shown in *Figure 3*, the composition of dominant bacterial species in different soil samples was similar, but the levels of abundance of bacterial microbial communities in the soil samples of land use patterns differed significantly from those of the phyla. At the taxonomic level of bacterial community in the soil samples tested, Actinobacteria, Acidobacteria, Proteobacteria, Chloroflexi and Firmicutes were the dominant bacteria and Chloroflexi were the dominant bacterial species in the farmlands (RF and SF), after farmland converting measures, the abundance of Actinobacteria and Chloroflexi were

average decreased by 28.74% and 16.78%, respectively. The abundance of Acidobacteria in RF were increased than CRMW, but the abundance changes are opposite in SF and CSFW. The change abundance of Proteobacteria contrary to Acidobacteria.



*Figure 2.* Alpha diversity index of the soil bacteria. Note: the value of \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 is marked

APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH 21(2):1007-1023. http://www.aloki.hu • ISSN 1589 1623 (Print) • ISSN 1785 0037 (Online) DOI: http://dx.doi.org/10.15666/aeer/2102\_10071023 © 2023, ALÖKI Kft., Budapest, Hungary

Index	OTU	Ace	Chao 1	Shannon	Sobs
pН	-0.359 *	-0.161	-0.025	-0.008	0.331 *
SOC	0.086	0.426	0.018	0.487	-0.295
TN	0.213	0.450	0.199	0.528 **	0.182
TP	0.372	0.181	0.050	0.058	-0.454
ТК	0.513	0.259	0.043	0.278	-0.033
AN	0.325	0.469 *	0.159	0.151	-0.715 **
AP	0.136	-0.087	0.231	0.433	-0.137
AK	0.316	0.290	0.049	0.155	0.024
CEC	-0.066	0.158	-0.180	0.119	-0.404

Table 2. Correlation analysis of diversity indices and soil properties

The error bars on the results represent the means  $\pm$  SD (n = 3). SD, the value of \* *P* < 0.05, \*\* *P* < 0.01 is marked



Figure 3. Relative abundance of soil bacterial communities at the levels of phylum

The data were visualized by Circos (Krzywinski et al., 2009), the width of the bars from each phylum indicate the relative abundance of that phylum in the sample. Acidobacteria were present in the highest percentage among the four treatments and increased from 28.13% to 30.53% under SF and CSFW, but decreased from 23.23% to 19.46% under high RS and CRMW. Proteobacteria was the second most dominant phylum in the four treatments, and increased from 21.28% to 23.06% (A3) under CSFW and SF conditions, but decreased from 24.75% to 32.17% under high RS and CRMW (*Fig. 4*).

#### Correlation analysis of environmental factors

An RDA analysis revealed the correlation between the level of bacterial OTUs, community composition and varied environmental factors in the different soil samples.

The analysis indicated that there were significant differences in environmental factors among the soil samples in *Figure 5*. The interpretation of RDA1 axis, RDA2 axis and the first two axes of OTUs were 45.91%, 19.25% and 68.50%, respectively. Among them, the soil samples on the first principal component axis obviously clustered. The longer rays of pH, TN and SOC indicate that they strongly correlated with the bacterial community composition of soil samples. AP, TP, AK, SOC and TN positively correlated with the wetland soil (CRMW and CSFW). There was a positive correlation between the CEC, pH and AN with the farmland (RF and SF).



Figure 4. Distribution of microbial community for each sample at phylum level



Figure 5. RDA analysis of the soil bacterial community at the OTU level

APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH 21(2):1007-1023. http://www.aloki.hu • ISSN 1589 1623 (Print) • ISSN 1785 0037 (Online) DOI: http://dx.doi.org/10.15666/aeer/2102\_10071023 © 2023, ALÖKI Kft., Budapest, Hungary

#### Factors structuring soil bacterial community diversity

The correlation heatmap showed that the relationship between bacterial phyla and environmental factors were different. Nitrospirota, MBNT15 and Desulfobacterota showed a significant positive correlation with TP and AK, and a significant negative correlation with pH and AN. Patescubacterua demonstrated a significant negative correlation with AN and CEC, and an extremely significant positive correlation with TP and AK. WPS-2 was significantly negatively correlated with pH, and significantly positively correlated with SOC and TN. Gemmatimonadota showed an extremely significant positive correlation with TP and AK. Myxococcota was significantly negatively correlated with SOC and TN, an extremely significant positive correlation with pH. Cyanobacteria revealed a significant positive correlation with SOC and TN, and. a significant positive correlation with pH. Methylomirabilota, Acidobacteriota, Verrucomicrobiota and Chloroflexi demonstrated an extremely significant positive correlation with SOC and TN, and a significantly negative correlation with AP. Planctomycetota was significantly positive correlated with CEC (*Fig. 6*).



*Figure 6.* Correlation heatmap of top twenty phyla and soil properties. Note: X and Y axis are environmental factors and phyla. R in different colors to show, the right side of the legend is the color range of different R values. The value of \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 is marked

#### Discussion

The process of soil nutrient cycling and transformation in wetlands in cold regions is easily affected by restoration patterns and surface vegetation types (Zhang et al., 2019; Li et al., 2009). In the present study, soil microbial biomass and nutrients, such as SOC

and TN, decreased after wetland cultivation and increased after agricultural abandonment (). The results indicated that the cultivation of wetland led to soil degradation and agricultural abandonment could improve soil quality. The similar changes in soil nutrients and microbial biomass after land-use changes have been widely reported not only in wetland but also in other ecosystems such as forest and prairie (Wang et al., 2010; Lauber et al., 2008; Song et al., 2012; Guo et al., 2016). The changes in soil microbial structure in this study gave a deeper insight into soil biogeochemical processes after wetland changes occur. Relevant studies show that the basic nutrient indices, such as the soil pH, TN and TP will change correspondingly with different soil types (Zhu et al., 2019). The indicators described above are important components of soil nutrients and can directly reflect soil fertility, quality and ecosystem productivity (Zhao et al., 2008; Zhang et al., 2020). In this study, the SOC in CRMW and CSFW was higher than that of the farmland. Because the wetland in cold regions has been in the form of a swamp for a long time, the rich wetland vegetation on the soil surface and the perennial low ambient temperature can promote the process of soil humic acidification and improve the accumulation of organic carbon in the wetland soil (Wang et al., 2019). The amount of SOC in CSFW was lower than that in CRMW, which could be owing to the frequent enhancement of aerobic microbial activities because of the exchange between soil and external gas, which improves the rate of utilization of organic matter (Liu et al., 2013). The abundance of herbaceous plants on the surface of results in a biomass of litter on the surface of wetland that aids in the input of organic carbon to the soil (Wang et al., 2017). Owing to the frequent human disturbance of farmland soil, reasonable agricultural cultivation measures reduce the input of organic fertilizer and straw, which reduces the source of SOC (Xiao et al., 2003). Previous studies reported that many factors impact soil microbes, such as changes in soil texture, litter input, vegetation and moisture (Hon et al., 2018). In the present study, all of these factors changed across different treatments. However, based on the above analysis, two major factors, soil disturbance and litter input, were identified as the main drivers of soil microbial communities after wetland changes. In addition, some unexpected results were observed in this study. The AP in the restored treatments was lower than in the cultivated treatments, which indicated that the restored treatments may experience P limitation. It has been reported that soils may shift to P limitation during the first few years of soil development after wetland restoration because previous N enrichment and fertilizing before agricultural abandonment increases the rate at which P cycles, which has been reported as anthropogenic P limitation (Inglett and Inglett, 2013; Vitousek et al., 2010).

The diversity of soil microorganisms represents the stability of microbial communities and reflects the effects of soil ecological mechanisms on communities (Yang et al., 200). We compared the degree of restoration of diversity and richness of the bacterial community following different reclamation period and found that samples in CRMW and CSFW were extremely close to those of normal farmland. These results supported the findings of a previous investigation of the effects of different reclamation methods and periods on soil microorganisms (Jousset et al., 2011). Microbial community succession was found in reclaimed soils, and the ratios of dominant bacterial communities in the soil ecological system from reclaimed land successively changed. The role of the dominant bacterial community is crucial in recovering soil quality. As the most active part of wetland ecosystems, soil microorganisms can respond more sensitively to changes in land use and can be used to evaluate the quality

and health level of wetland soil. The soil microbial community structure was significantly affected by land use patterns (Fu et al., 2019). In this study, the reclamation and restoration was significantly changed the number of soil bacterial the Shannon, Ace and Chao1 index and significantly changed the abundance of bacterial community in wetland soil. The analysis of the composition of soil microbial community from the reclamation and restoration mode shows that the dominant species of soil microbial community in wetland nature reserves were Actinobacteria, Acidobacteria, Proteobacteria, Chloroflexi and Firmicutes, which can account for more than 80% of the total bacterial community in the soil samples tested. Proteobacteria is considered to be an oligotrophic bacterium, which is related to the storage of SOC and content of TN (He et al., 2011). Acidobacteria are both acidophilic and oligotrophic. Their distribution is regulated by environmental factors, such as pH, organic carbon, TN, C/N, TP, AN, ammonium N, soil humidity, soil temperature and soil respiration (Feng et al., 2016; Wang et al., 2016). They have important ecological functions, such as degrading plant residue polymers and participating in iron cycles, the light absorption of Fenna-Matthews-Olson (FMO) proteins under plant photosynthesis, and in the metabolism of single carbon compounds (Pankratov et al., 2011; Lu et al., 2010; Blöthe et al., 2008; Müh et al., 2007; Radajewski et al., 2002; Kalyuzhnaya et al., 2008). Acidobacteria, as the second largest group of soil bacteria in northern China, is much less abundant in forestland soil (Liu et al., 2015; Zhang et al., 2014). At the phylum level, different relationships between bacterial communities and the soil quality of wetlands were observed after different restoration periods (Hou et al., 2018). The wetland showed increasing P, N, K, and SOC with increasing Firmicutes and Patescibacteria, indicating a direct correlation between increased availability of the better growth of Firmicutes. The quantities of other bacterial phyla have been shown to be increased by increasing the SOC, BD, pH, and EC, which supports the findings of Nkongolo et al. (2016). Our research revealed that the distribution of soil bacterial communities was significantly influenced by the SOC, N, K, and P.

The role of the dominant bacterial community is crucial in recovering soil quality. A correlation analysis of the environmental factors showed that the composition of soil microbial communities in wetland positively correlated with soil AP, TP, AK, TK, TN and SOC. These environmental factors also restricted the relative abundance of Acidobacteriota and Chloroflexi. The relative abundance of Acidobacteriota in the reclamation wetland of Sanjiang Plain was 67.6% higher than that in the marsh wetland soil. The difference in relative abundance of Acidobacteriota significantly negatively correlated with the contents of soil water, soil carbon and N and significantly positively correlated with the soil pH, which is consistent with the results of this study (Xu et al., 2017). We speculate that samples of reclaimed wetland with stable soil ecology some beneficial bacteria; namely, Proteobacteria. Previous studies have indicated that the resilience of a microbial community will be promoted by increasing the abundance of bacteria that can be classified as copiotrophs, such as members of Proteobacteria (De Vries and Shade, 2013; Bastida et al., 2015), as well as decreasing abundance of oligotrophs, such as many members of Acidobacteria (Fierer and Jackson, 2006). This may be because higher proportions of Proteobacteria in soil communities have been associated with facilitated plant growth during remediation of restoration soils, suggesting a positive feedback relationship between available phosphorus from root growth and members of this phylum (Zhou et al., 2017b). At present, numerous studies have analyzed the effect of compound microbial agents on the quality of reclaimed soil.

For example, when continuously applying ripening biofertilizer to reclaimed soil, TP, AP, and SOC in the soil increased by 7.1%, 27.9% and 30.2%, respectively, comparing to the control group. Moreover, the number of bacteria increased by 28.2% (Xu, 2011). Further, different microbial agents have been shown to have a great influence on the quantity and structure of microorganisms in the soil. Hence, it is important to identify suitable and efficient bacterial strains to improve soil quality, which can promote the rapid restoration of soil function for the reclamation soil. Moreover, when applying bacterial fertilizer to reclaimed soil, managers should pay special attention to the influence of soil heavy metals on bacterial activity (Perilli et al., 2010).

#### Conclusion

In summary, reclamation and restoration have significant impacts on soil physicochemical properties, microbial community structure diversity in a cold region wetland. These indexes can be used as indicators of wetland nutrient status in this area, and used to assess the restoration levels of wetland soil ecosystems. These findings highlight the importance of effectively managing the soil bacterial community in order to maintain a naturally functioning soil ecosystem, a collection of studies that provide detailed information would help to facilitate wetland soil management and increase our understanding of the ecological process of natural habitat restoration. In the future, China will continue to implement ecological civilization as the theme, and sustainable restoration of wetland ecological environment. The contents of SOC, TN, and TP and the Shannon diversity, Ace and Chao1 index of a wetland park nature reserve in the cold region were higher than those of the upland and paddy fields. Actinobacteria, Acidobacteria, Proteobacteria, Chloroflexi and Firmicutes were the predominant bacteria. The environmental factor of AP, TP, AK, TK, TN and SOC driven by reclamation and restoration modes affect the structure microbial community.

**Acknowledgements.** This article was supported by the Natural Science Foundation of Heilongjiang Province China (Grant No. LH2021D014).

Conflict of interests. The author confirms that this article content has no conflict of interests.

#### REFERENCES

- [1] Andersen, R., Wells, C., Macrae, M., Price, J. (2013): Nutrient mineralisation and microbial functional diversity in a restored bog approach natural conditions 10 years post restoration. Soil Biology and Biochemistry 64: 37-47.
- [2] Angeloni, N. L., Jankowski, K. J., Tuchman, N. C., Kelly, J. J. (2006): Effects of an invasive cattail species (*Typha×glauca*) on sediment nitrogen and microbial community composition in a freshwater wetland. FEMS Microbiology letters 263(1): 86-92.
- [3] Bao, S. T. (2005): Soil agrochemical analysis. China Agricultural Press.
- [4] Bastida, F., Selevsek, N., Torres, I. F., Hernández, T., García, C. (2015): Soil restoration with organic amendments: linking cellular functionality and ecosystem processes. Scientific Reports 5: 15550.
- [5] Blöthe, M., Akob, D. M., Kostka, J. E., Göschel, K., Drake, H. L., Küsel, K. (2008): pH gradient-induced heterogeneity of Fe(III)-reducing microorganisms in coal mining-associated lake sediments. Applied and Environmental Microbiology 74: 1019-1029.

- [6] Calhoun, A. J. K., Arrigoni, J., Brooks, R. P., Hunter, M. L., Richter, S. C. (2014): Creating successful vernal pools: a literature review and advice for practitioners. – Wetlands 34: 1027-1038.
- [7] Card, S. M., Quideau, S. A. (2010): Microbial community structure in restored riparian soils of the Canadian prairie pothole region. – Soil Biology and Biochemistry 42: 1463-1471.
- [8] Chao, A., Bunge, J. (2002): Estimating the number of species in a stochastic abundance model. Biometrics 58: 531-539.
- [9] De Vries, F. T., Shade, A. (2013): Control son soil microbial community stability under climate change. Frontiers in Microbiology 4: 1-16, 41-42.
- [10] Deng, J., Yin, Y., Luo, J., Zhu, W., Zhou, Y. (2019): Different revegetation types alter soil physical-chemical characteristics and fungal community in the Baishilazi Nature Reserve. – PeerJ 6: e6251.
- [11] Duan, P., Zhang, Y. C., Wang, J. G., Wang, T., Zhao, Z. Z. (2020): Functional diversity of soil microbial communities during degradation of alpine wetlands in Qinghai Tibet Plateau. Acta Agrestia Sinica 28(3): 759-767.
- [12] Edgar, R. C. (2013): UPARSE: highly accurate OTU sequences from microbial amplicon reads. – Nature Methods 10: 996-998.
- [13] Feng, H. W., Wang, D. X., Mao, L., Zhi, Y. E., Zhou, P. (2016): Influence of organic cultivation on soil microbial community in corn field. – Journal of Shanhai Jiaotong University (Agricultural Science) 34(5): 68-75.
- [14] Fierer, N., Jackson, R. B. (2006): The diversity and biogeography of soil bacterial communities. PNAS 103: 626-631.
- [15] Fu, M., He, M. M., Hu, H. Y., Ding, W. C., Zhai, M. Z., Zhang, H. Y. (2019): Responses of soil organic carbon and microbial community structure to different tillage patterns and straw returning for multiple years. – Chinese Journal of Applied Ecology 30(9): 3183-3194.
- [16] Gao, J., Zhang, X., Lei, G., Wang, G. (2014): Soil organic carbon and its fractions in relation to degradation and restoration of wetlands on the Zoige Plateau, China. – Wetlands 34: 235-241.
- [17] Guo, X., Chen, H. Y. H., Meng, M., Biswas, S. R., Ye, L., Zhang, J. (2016): Effects of land use change on the composition of soil microbial communities in a managed subtropical forest. – Forest Ecology and Management 373: 93-99.
- [18] Hartman, W. H., Richardson, C. J., Vilgalys, R., Bruland, G. L. (2008): Environmental and anthropogenic controls over bacterial communities in wetland soils. Proceedings of the National Academy of Sciences of the United States of America 105: 17842-17847.
- [19] He, Z. H., Yang, J. F., Chen, J. G., Yang, J. H. (2011): Analysis of bacterial community composition by 16SRNA clone library sampling form arctic seawater. – Transactions of Oceanology and Limnology 4: 58-67.
- [20] He, H., Miao, Y., Gan, Y., Wei, S., Tan, S., Rask, K. A., Ekelund, F. (2020): Soil bacterial community response to long-term land use conversion in Yellow River Delta. – Applied Soil Ecology 156: 103709.
- [21] Hou, H., Wang, C., Ding, Z., Zhang, S., Yang, Y., Ma, J., Chen, F., Li, J. (2018): Variation in the soil microbial community of reclaimed land over different reclamation periods. – Sustainability 10(7): 2286.
- [22] Inglett, P. W., Inglett, K. S. (2013): Biogeochemical changes during early development of restored calcareous wetland soils. – Geoderma 192: 132-141.
- [23] Jousset, A., Schmid, B., Scheu, S., Eisenhauer, N. (2011): Genotypic richness and dissimilarity opposingly affect ecosystem functioning. Ecology Letters 14: 537-545.
- [24] Kalyuzhnaya, M. G., Lidstrom, M. E., Chistoserdova, L. (2008): Real-time detection of actively metabolizing microbes by redox sensing as applied to methylotroph populations in Lake Washington. – ISME Journal 2: 696-706.

- [25] Kolde, R. (2015): Pheatmap: Pretty Heatmaps. http://th.archive.ubuntu.com/cran/web/packages/pheatmap/
- [26] Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., Jones, S. J., Marra, M. A. (2009): Circos: an information aesthetic for comparative genomics. – Genome Research 19: 1639-1645.
- [27] Laganiere, J., Angers, D. A., Pare, D. (2020): Carbon accumulation in agricultural soils after afforestation: a meta-analysis. Global Change Biology 16: 439-453.
- [28] Lamers, L. P. M., van Diggelen, J. M. H., Op den Camp, H. J. M., Visser, E. J. W., Lucassen, E., Vile, M. A., Jetten, M. S. M., Smolders, A. J. P., Roelofs, J. G. M. (2012): Microbial transformations of nitrogen, sulfur, and iron dictate vegetation composition in wetlands: a review. – Frontiers in Microbiology 3: 12.
- [29] Lauber, C. L., Strickland, M. S., Bradford, M. A., Fierer, N. (2008): The influence of soil properties on the structure of bacterial and fungal communities across land use types. – Soil Biology and Biochemistry 40: 2407-2415.
- [30] Li, X., Xie, Y. H., Huang, J. S., Chen, X. S. (2009): Research progresses on the formation mechanism of vegetation distribution pattern in wetlands. – Wetland Science 7(3): 280-288.
- [31] Lin, C. Y., Li, X. L., Zhang, Y. X., Sun, H. F., Li, C. Y., Jin, L. Q., Yang, X. G., Liu, K. (2021): Responses of different degradation stages of alpine wetland on soil microbial community in the yellow river source zone. – Environmental Science 42(8): 3971-3984.
- [32] Liu, Z., Lin, Y., Lu, H., Ding, M., Tan, Y., Xu, S., Fu, S. (2013): Maintenance of a living understory enhances soil carbon sequestration in subtropical orchards. – Plos One 8(10): e76950.
- [33] Liu, J. J., Sui, Y. Y., Yu, Z. H., Shi, Y., Chu, H., Jin, J., Wang, G. (2015): Soil carbon content drives the biogeographical distribution of fungal communities in the black soil zone of northeast China. – Soil Biology and Biochemistry 83: 29-39.
- [34] Lu, R. K. (2000): Methods for Agrochemical Analysis of Soil. China Agricultural Science and Technology Press, Beijing.
- [35] Lu, S. P., Gischkat, S., Reiche, M., Akob, D. M., Hallberg, K. B., Küsel, K. (2010): Ecophysiology of Fe-cycling bacteria in acidic sediments. – Applied and Environmental Microbiology 76: 8174-8183.
- [36] Mclauchlan, K. (2006): The narure and longevity of agricultural impacts on soil carbon and nutrients: a review. Ecosystems 9: 1364-1382.
- [37] Moreno-Mateos, D., Power, M. E., Comín, F. A., Yockteng, R. (2012): Structural and functional loss in restored wetland ecosystems. Plos Biology 10: e1001247.
- [38] Morrissey, E. M., Berrier, D. J., Neubauer, S. C., Franklin, R. B. (2014): Using microbial communities and extracellular enzymes to link soil organic matter characteristics to greenhouse gas production in a tidal freshwater wetland. Biogeochemistry 117: 473-490.
- [39] Müh, F., Madjet, M. E. A., Adolphs, J., Abdurahman, A., Rabenstein, B., Ishikita, H., Renger, T. (2007): α-Helices direct excitation energy flow in the Fenna-Matthews-Olson protein. – Proceedings of the National Academy of Sciences 104: 16862-16867.
- [40] Nkongolo, K. K., Michael, P., Theriault, G., Narendrula, R., Castilloux, P., Kalubi, K. N., Bekett, P., Spiers, G. (2016): Assessing biological impacts of land reclamation in a mining region in Canada: effects of dolomitic lime applications on forest ecosystems and microbial phospholipid fatty acid signatures. – Water Air Soil Pollution 227: 1-13.
- [41] Pankratov, T. A., Ivanova, A. O., Dedysh, S. N., Liesack, W. (2011): Bacterial populations and environmental factors controlling cellulose degradation in an acidic Sphagnum peat. – Environmental Microbiology 13: 1800-1814.
- [42] Pei, X. C., Xu, Y. L., Wei, W. (2009): A review on soil microorganisms in wetland ecosystem. – Wetland Science 7(2): 181-186.
- [43] Pellegrino, E., Bosco, S., Ciccolini, V., Pistocchi, C., Sabbatini, T., Silvestri, N., Bonari, E. (2015): Agricultural abandonment in Mediterranean reclaimed peaty soils: long-term

effects on soil chemical properties, arbuscular mycorrhizas and CO2 flux. – Agriculture Ecosystems & Environment 199: 164-175.

- [44] Perilli, P., Mitchell, L. G., Grant, C. A., Pisante, M. (2010): Cadmium concentration in durum wheat grain (*Triticum turgidum*) as influenced by nitrogen rate, seeding date and soil type. Journal of Food Agriculture & Environment 90: 813-822.
- [45] Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., et al. (2007): SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. – Nucleic Acids Research 35: 7188-7196.
- [46] Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013): The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. – Nucleic Acids Research 41: 590-596.
- [47] R Core Team (2022): R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.Rproject.org/.12c.
- [48] Radajewski, S., Webster, G., Reay, D. S., Morris, S. A., Ineson, P., Nedwell, D. B., Murrell, J. C. (2002): Identification of active methylotroph populations in an acidic forest soil by stable-isotope probing. – Microbiology 148: 2331-2342.
- [49] Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B. (2009): Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. – Applied and Environmental Microbiology 75: 7537-7541.
- [50] Song, Y. Y., Song, C. C., Yang, G. S., Miao, Y. Q., Wang, J. Y., Guo, Y. D. (2012): Changes in labile organic carbon fractions and soil enzyme activities after marshland reclamation and restoration in the Sanjiang Plain in Northeast China. – Environmental Management 50: 418-426.
- [51] Stackebrandt, E., Goebel, B. M. (1994): Taxonomic note: a place for DNA-DNA reassociation and 16S r RNA sequence analysis in the present species definition in bacteriology. International Journal of Systematic Bacteriology 44: 846-849.
- [52] Vitousek, P. M., Porder, S., Houlton, B. Z., Chadwick, O. A. (2010): Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological Applications 20: 5-15.
- [53] Vogel, S., Conedera, M. (2020): Effects of land use-induced vegetation and topography changes on soil chemistry in the Southern Alps (Ticino, Switzerland). Plant, Soil and Environment 66(2): 73-80.
- [54] Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. (2007): Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73: 5261-5267.
- [55] Wang, L. L., Song, C. C., Song, Y. Y., Guo, Y. D., Wang, X. W., Sun, X. X. (2010): Effects of reclamation of natural wetlands to a rice paddy on dissolved carbon dynamics in the Sanjiang Plain, Northeastern China. – Ecological Engineering 36: 1417-1423.
- [56] Wang, G. H., Liu, J. J., Yu, Z. H., Wang, X. Z., Jin, J., Liu, X. B. (2016): Research progress of *Acidobacteria* ecology in soils. Biotechnology Bulletin 32(2): 14-20.
- [57] Wang, H., Yao, X., Yang, H., He, Z., Li, D. J., Ding, L. Y. (2017): Effects of different land cover types in Karst area on soil aggregates and organic carbon. Ecology and Environmental Sciences 26(9): 1506-1513.
- [58] Wang, X. Y., Zhou, C., Feng, W. X., Chen, J. L., Jiang, X. P. (2019a): Changes of soil aggregates and its organic carbon in Chinese fir plantations with different forest ages. – Journal of Soil and Water Conservation 33(5): 126-131.
- [59] Wang, Y., Ji, H., Wang, R., Guo, S. (2019b): Responses of nitrification and denitrification to nitrogen and phosphorus fertilization: does the intrinsic soil fertility matter? – Plant and Soil 440(1): 443-456.

- [60] Xiao, Y., Xie, G. D., An, K. (2003): The function and economic value of soil conservation of ecosystems in Qinghai-Tibet Plateau. – Acta Ecologica Sinica 23(11): 2367-2376.
- [61] Xu, J. (2011): Effect of biological bacterial fertilizer on the phosphorus, organic matter and microorganism in the reclamation soil in mining area. Journal of Shanxi Agriculture Science 39: 250-252.
- [62] Xu, S. Q., Zhang, B., Ma, L., Hou, A. X., Tian, L., Li, C. J. (2017): Effects of marsh cultivation and restoration on soil microbial communities in the Sanjiang Plain, Northeastern China. – European Journal of Soil Biology 82: 81-87.
- [63] Yang, H. J., Xiao, Q. M., Liu, A. Y. (2005): Soil microbial diversity and its action. Journal of Nanhua University 19: 21-31.
- [64] Zedler, J. B., Kercher, S. (2005): Wetland resources: status, trends, ecosystem services, and restore ability. Annual Review of Environment and Resources 30: 39-74.
- [65] Zhang, Y., Cong, J., Lu, H., Li, G., Qu, Y., Su, X., Li, D. (2014): Community structure and elevational diversity patterns of soil Acidobacteria. Journal of Environmental Sciences 26: 1717-1724.
- [66] Zhang, H. H., Wang, S. B., Wang, J. R., Wu, X. Y., Ma, S. L., Wu, Y. N., Li, J. B., Xu, N. (2019): Effects of different land use types on soil physicochemical properties and aggregate composition in Sanjiang Plain wetland. – Chinese Journal of Ecology 38(6): 1679-1687.
- [67] Zhang, X. Y., Zhao, R. F., Zhang, L. H., Zhao, H. L., Zhou, J. R., Zhou, Y. G., Zhao, M., Wang, Y. B. (2020): Comparative study of plant characteristics and soil properties in different ecological protected areas: a case study of middle reaches of the Heihe River. – Acta Ecologica Sinica 40(9): 3027-3039.
- [68] Zhao, Y. Q., Zhang, P. J. (2010): Ecological restoration of soils in reclaimed wetland from cultivation. Wetland Science & Management 6(3): 55-58.
- [69] Zhao, Y. K., Zhang, W. S., Wang, Y. N., Li, K. X., Jia, H. Z., Li, X. (2008): Research progress in physiology and molecular biology of plant responses to high pH. – Chinese Journal of Eco-Agriculture 16(3): 783-787.
- [70] Zhao, P. Y., Bai, X., Yan, P. M., Zhao, X. D., Wu, X. Y., Chai, B. F. (2021): Responses of soil bacterial community Structure and phenotype to soil heterogeneity in *Larix principis-rupprechtii* Forest. Scientia Silcae Sinicae 57(7): 101-109.
- [71] Zhou, X., Guo, Z., Chen, C., Jia, Z. (2017a): Soil microbial community structure and diversity are largely influenced by soil pH and nutrient quality in 78-year-old tree plantations. Biogeosciences 14(8): 2101-2111.
- [72] Zhou, Y. J., Li, J. H., Ross Friedman, C., Wang, H. F. (2017b): Variation of soil bacterial communities in a chronosequence of rubber tree (*Hevea brasiliensis*) plantations. Frontiers in Plant Science 8: 849.
- [73] Zhu, Z. X., Zhu, T. B., Yang, L., Luo, L. L., Xie, Y. C. (2019): The spatial relationship between soil alkaline-nitrogen content and environmental factors in China. Ecology and Environmental Sciences 28(11): 2199-2207.