

Original Research

Bacterial Communities Structure and Diversity of Soil Rhizosphere of *Brassica oleracea var.italica* with Different Reclamation Duration in Coastal Reclamation Areas of Taizhou Estuary

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Abstract

Land reclamation transforms a tidal flat from a marine ecosystem to terrestrial ecosystem, which significantly changes the soil ecosystem process, affecting soil bacterial community structure. In order to reveal the changes of soil quality after reclamation and provide a theoretical basis for the scientific regulation of the soil ecosystem, the changes of bacterial community structure in rhizosphere soil of *Brassica oleracea var.italica* collected from coastal reclamation area with different years of reclamation (12, 35, 56) in Taizhou estuary were analyzed by using the Illumina Miseq high-throughput sequencing technology. The results showed that a total of 123355 effective sequences and 7660 OTUs were obtained from nine soil samples based on high-throughput sequencing of the 16S gene. Bacterial species detected in these samples covered 23 phylum, 62 class, 148 orders, 651 gene. The abundance and diversity of bacterial communities in soil rhizosphere of *Brassica oleracea var.italica* collected from coastal reclamation areas with 35 years of reclamation was the highest among all soil samples, while that of 12 years of reclamation was the lowest among all soil samples. The dominant phyla were *Proteobacteria*, *Acidobacteria*, *Bacteroidetes* and *Actinobacteria* in all the soil samples. At the class level, *Alphaproteobacteria*, *Gammaproteobacteria*, *Betaproteobacteria*, *Actinobacteria* and *Gemmatimonadetes* were the predominant bacterial communities existed in all the soil samples. The long term land reclamation had significant effects on soil bacteria communities compositions.

Keywords: Illumina Miseq high-throughput sequencing, communities structure of soil bacterial, coastal reclamation areas, reclamation duration, *Brassica oleracea var.italica*

Introduction

Coastal mudflat soil is developed from the parent material of coastal facies through a long biological cycle and weak shower under periodic wet conditions [1]. The reclamation of coastal tidal flat wetland by human beings makes tidal flat undergo a process of transformation from marine ecosystem to terrestrial ecosystem, and at the same time makes the community structure and diversity of animals, plants and microorganisms in the ecosystem undergo fundamental changes [2-3]. Zhang Ming et al. [4] showed in the study of typical tidal flat reclamation area in Dafeng, Jiangsu that with the increase of reclamation years, the soil salt content and base salt ions gradually decreased, and the desalination process was basically completed after reclamation to about 60 years. Zhang Huan et al. [5] showed in the studies of Dafeng District of Yancheng City in Jiangsu Province, Nantong City such as the Eastern District, and the reclamation area of Cixi City in Zhejiang Province that the content of soil organic carbon gradually increased with the increase of reclamation years. Zhang Wenmin et al. [6] studied the changes of soil organic carbon and its active components in the farmland of Hangzhou Bay with different reclamation years with a long time span, and the results showed that the changes of soil organic carbon and its active components showed an increasing trend with the increase of reclamation years, but their contents tended to be stable after 40 years of reclamation. However, the research on soil bacterial community structure and diversity in different reclamation years of Taizhou Bay tidal wetland has not been reported.

The soil of the coastal reclamation area is developed on the silt deposited in the coastal mudflat. The soils of different reclamation years in the same area are all developed on the same parent soil. The different reclamation years of tidal flat reclamation land also represent the different periods of soil development in this reclamation land. Therefore, the changes of soil microbial community structure and diversity in the reclaimed land with different reclamation years on the coastal tidal wetland can provide a theoretical basis for studying the influence of long-term human participation on soil microorganisms in the tidal wetland, as well as for taking microbial measures to optimize the soil environment for crops in the saline-alkali land on the tidal flats [7].

Brassica oleracea var.italica is a Brassica vegetable in the Cruciferous family. Its origin is in Europe. *Brassica oleracea var.italica* is dark green in color and favored by consumers because of its unique taste and comprehensive nutrition [8]. In 1989, the villagers of Shangpan Town of Linhai City began to introduce and try to plant *Brassica oleracea var.italica* successfully, and then the planting area was expanded year by year. In 2003, Linhai City was awarded the title of China's "Broccoli Town" by China Quality Agricultural Product

Development Service Association. In 2011, Linhai became a national standardization demonstration area of *Brassica oleracea var.italica*, and in 2012, it became a national *Brassica oleracea var.italica* export quality and safety demonstration area. Taizhou has sufficient sunlight and abundant water and heat conditions, which are suitable for the growth of *Brassica oleracea var.italica*. At present, the planting area of *Brassica oleracea var.italica* in Taizhou is stable at 10 000 hm² all the year round, and the annual output is about 230 000 t, accounting for 25% of the total production of *Brassica oleracea var.italica* in China [9]. Taizhou is the largest and most concentrated winter and spring *Brassica oleracea var.italica* production center in China, and it is also an important international *Brassica oleracea var.italica* production base.

Taizhou City is an economically developed area in Zhejiang Province, and Taizhou Bay tidal wetland is one of the major coastal wetland distribution areas in Zhejiang Province [10]. Human activities have a severe impact on tidal wetland. After the founding of the People's Republic of China, large-scale reclamation was carried out in 1964, 1971 and 2008. In this research, the bacterial community structure and diversity of the rhizosphere soil of *Brassica oleracea var.italica* in Taizhou Bay tidal wetland during three different reclamation periods were compared and analyzed, aiming at understanding the soil microbial changes after the reclamation of coastal tidal wetland, and opening up a new way for the optimization of crop management in the reclaimed land.

Materials and Methods

Overview of the Study Area

Taizhou City is located in the central coastal area of Zhejiang Province. The length of the mainland coastline is 630.87 Km, which is 34.28% of the total length of the mainland coastline of Zhejiang Province. The continental coastline of Taizhou Bay mainly includes artificial and silt coastline, bedrock and gravel coastline, which is dominated by flat and swelling-type shoreline, and tidal shoreline is well developed. Taizhou Bay is an intersection area of offshore high water system and coastal low salt water system. Seasonal changes in the physical and chemical composition of seawater are mainly affected by Jiangsu and Zhejiang Coastal Current and Taiwan Warm Current [11]. The study area is an open sedimentation coast in Taizhou Bay and the intertidal zone is filled with silt. Tidal wetland is flat and open, with clay and silt at the bottom and thick sediment layer. Tidal soil is rich in organic matter, with soil salinity of about 0.88%~1.55%, and the parent material of soil is new shallow sea sediments [10].

The experimental site was located in the planting base of *Brassica oleracea var.italica* (28°47'N,

121°38'E) of Jiancheng Village, Shangpan Town, Linhai City, Zhejiang Province. It is in the humid subtropical monsoon climate. The annual sunshine duration is 1 785.7~3 118.6 h. The annual total solar radiation is 5 040~5 400 MJ·m⁻². The annual average temperature is 17.0°C. The maximum temperature is 37.5°C, and the minimum temperature is -6.8°C. The annual accumulated temperature $\geq 10^{\circ}\text{C}$ was 5 332.7°C. The frost-free period was 257 days. The annual rainfall was 1 300~1 400 mm, 40% of which was concentrated in the spring and plum rain seasons. Typhoon and rainstorm are prone to flood and waterlogging because of its high frequency and intensity.

The Sample Collection

After the reclamation of tidal flats in Taizhou Bay, the change of soil spatial scale represents the change of the reclamation years of tidal flats and the change of soil development years. The growing period of *Brassica oleracea var.italica* in Taizhou Bay is about 100~135 days. On March 15, 2020, the soil microorganism is more active, and the growth of *Brassica oleracea var.italica* is in the middle and late period, which is also the harvest period of *Brassica oleracea var.italica*. According to the local historical records on land use, this study chose the *Brassica oleracea var.italica* planting area concentrated in Leishikeng Village, Shangpan Town, Linhai City, as the research area. In this study, the rhizosphere soils of *Brassica oleracea var.italica* cultivated in 1964 (56 years), 1985 (35 years) and 2008 (12 years) were sampled and marked as 56 years, 35 years and 12 years respectively.

The altitude, soil type, previous crop, cultivation measures, fertilization methods and varieties of *Brassica oleracea var.italica* in each plot (3 m × 3 m) were all the same. The fertilization levels are: (1) 45% compound fertilizer (N: P₂O₅: K₂O = 15: 15: 15). The fertilizer application amount is 1 200 kg·hm⁻² (600 kg·hm⁻² was applied on September 20th and November 5th respectively); (2) The fertilizer amount of urea is 750 kg·hm⁻² (150 kg·hm⁻² applied on September 20th, 300 kg·hm⁻² applied on October 30th, 150 kg·hm⁻² applied on November 25th and 150 kg·hm⁻² applied on December 20th respectively); (3) 112.5 kg·hm⁻² potassium chloride (45 kg·hm⁻² on September 20th, 37.5 kg·hm⁻² on October 30th and 30 kg·hm⁻² on November 25th). (4) 30 kg·hm⁻² borax (15 kg·hm⁻² on September 20th and 15 kg·hm⁻² on November 25th respectively) was applied.

Each sample site was set up with 3 parallel plots, and the interval of each parallel plot was 600 m. In order to minimize the errors caused by the sampling method and environmental factors, 6 sampling points were set in an S-shape on each sample plot in this experiment, and the spacing of each sampling point was 6 m. Shaking method was used to sample the rhizosphere soil of *Brassica oleracea var.italica* [12]. The soil samples

collected from 6 sampling sites in each field were uniformly mixed into a mixed sample. The soil samples were passed through 2 mm diameter sieve. 10 g of the soil samples were air-dried at room temperature for the determination of soil physical and chemical properties. Another 10 g of the soil samples were put into sterile polyethylene sealed bags and transported at low temperature in dry ice boxes. After being transported to the laboratory, the samples were stored at -20°C for high-throughput sequencing of soil bacteria [13].

Determination of Soil Physical and Chemical Properties

After natural drying, the ground soil was screened by 2 mm and 0.25 mm for the determination of soil physicochemical properties. The soil pH was measured with a PHS-3C pH meter. Soil available phosphorus was measured by colorimetric method. Soil available potassium was measured by flame photometry, and soil organic matter content was measured by potassium dichromate and sulfuric acid external heating method [14]. The soil conductivity was determined by a salinometer in a mixture solution with a ratio of 1:5 to water and soil. Soil total nitrogen content was determined by Kjeldahl method [15].

Extraction of Genomic DNA from Soil Bacteria

0.1g soil sample was accurately weighed. The total DNA was extracted by E.Z.N.A. Soil DNA Kit (D5625, Omega, Inc., USA). The quality of DNA was detected by 1% agarose gel electrophoresis, and the DNA was quantified by ultraviolet spectrophotometer Nano Drop ND-1000 (Nano Drop, Wilmington, DE, USA). Three of the five DNA samples were randomly mixed into three parallel samples and stored at -20°C.

PCR Amplification and High-Throughput Sequencing

Referring to the method of CAPORASO et al. [16], the V3-V4 region primers of bacterial 16S rDNA were used to amplify each sample. The upper and lower primers were 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') respectively. PCR amplification of 16S rDNA gene (V3-V4) was performed. The reaction conditions of DNA amplification were 35 cycles (98°C, 30S; 98°C, 10S. 54°C, 30S. 72°C, 45S), and extended amplification at 72°C for 10 min. The PCR product was confirmed by 2% agarose gel electrophoresis. Ultra pure water, rather than sample solution, was used throughout the DNA extraction process to rule out the possibility of a false positive PCR result as a negative control. PC products were purified by AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit(Invitrogen, USA). The amplicon pool was used for sequencing. The size and number

of amplicon libraries were evaluated on the Agilent 2100 bioanalyzer (Agilent, USA) and the Illumina (Kapa Biosciences, Woburn, MA, USA) library quantitative kit, respectively. The libraries are sorted on the Novaseq PE 250 platform [17].

Bioinformatics Analysis Process

The samples were sequenced on the Illumina Novaseq platform according to the manufacturer's recommendations and provided by LC-Bio. According to the unique barcode of the sample, the pairing end sequence is allocated to the sample, and the barcode and primer sequence introduced in the library construction are removed. FLASH was used to merge match side to read. According to fqtrim (v0.94), quality filtering is performed on the raw read data under specific filtering conditions to obtain high quality clean tags. The chimeric sequences were filtered by VSearch software (V2.3.4). DADA2 is used to demodulate, and the feature list and feature sequence are obtained. Alpha diversity is calculated by normalizing to the same random sequence. Then, according to the Silva (Release 132) classifier, the relative abundance of each sample was used to normalize the feature abundance. Alpha diversity was used to analyze the complexity of species diversity in the samples through four indicators, including Chao1, Observed species, Goods coverage, and Shannon. QIIME2 was used to calculate all these indicators in the samples. Beta diversity is calculated by QIIME2 and plotted by the R package. Blast was used for sequence alignment, and each representative sequence was annotated by SILVA database. Other images are implemented using the R package (V3.2.5) [17].

Results

Variations of Soil Physicochemical Properties

The results of soil physicochemical properties in the research area are shown in Table 1. The pH and SAL of the rhizosphere soil of *Brassica oleracea var. italica* decreased with the extension of reclamation years. The contents of soil organic matter (OM), total nitrogen (TN), available phosphorus (AP) and available potassium (AK) increased with the increase of reclamation time, and reached the maximum at 35 reclamation ages, and then showed a downward trend with the increase of reclamation time. This may be caused by the decrease of soil water content due to the dry land utilization in the late reclamation period. The contents of sand in soil particle composition decreased with the increase of reclamation time, while the contents of clay in soil particle composition increased with the increase of reclamation time.

Table 1. Variations of soil physicochemical properties with different reclamation duration.

Reclamation ages (year)	pH	AK/(mg·kg ⁻¹)	AP/(mg·kg ⁻¹)	TN/(g·kg ⁻¹)	OM/(g·kg ⁻¹)	SAL/(%)	SAND (%)	SILT (%)	CLAY (%)
12	7.9±0.030a	225.374±9.341a	74.149±3.28b	0.818±0.012b	17.824b±0.112	0.105±0.003a	1.911±0.035	2.802±0.063	95.301±5.302b
35	7.6±0.050b	231.885±3.522a	125.736±2.091a	0.976±0.031a	19.994±0.223a	0.097±0.001b	1.717±0.061	2.601±0.028	95.601±3.039b
56	7.4±0.020b	207.053±5.103b	60.131±3.512b	0.916±0.053a	15.517±0.206c	0.081±0.006c	0.798±0.015	2.009±0.019	97.302±6.012a

Values are means ±standard error (n = 3). Means followed by the same letter for a given factor are not significantly different ($P < 0.05$, LSD test)

Table 2. Variations of sequence readings and OTUs of soil bacteria of rhizosphere of *Brassica oleracea var.italica* with different reclamation duration.

Reclamation ages (year)	Raw-tags	Valid-tags	Effective (%)	OTUs
12	33274.667	29431.333	88.449	3105±10.59b
35	42810.333	37385.333	87.382	4363±12.31a
56	74666.111	59538.667	79.739	4495±11.67a

Values are means ±standard error (n = 3). Means followed by the same letter for a given factor are not significantly different (P<0.05, LSD test)

Sequencing Data Analysis

After quality control, a large number of effective sequences were obtained from the samples sequenced by Miseq at each classification level. The effective sequences obtained from soil sample with 56 years, 35 years and 12 years was 59538.667, 37385.333 and 29431.333, respectively. The total number of valid sequences obtained was 126355.333. The number of effective sequences obtained from the samples of 56 reclamation years was the highest, while the number of effective sequences obtained from the samples of 12 reclamation years was the lowest. The proportion of effective sequences in all samples was more than 79%. The sequence length between 300 and 400 accounted for 35.85% and that between 400 and 500 accounted for 60.89%. The sequence length between 300 and 500 accounted for 96.74%, which could meet the requirements of analysis.

After quality control and removal of chimeras, 97% of the effective sequences were clustered as OTUs

to obtain the abundance information of *Brassica oleracea var.italica* rhizosphere soil bacteria under different OTUs in different reclamation years. As shown in Table 2, the three soil samples produced 3,105~4,495 OTUs numbers respectively. Among them, Soil samples with 56 years of cultivation had the highest number of OTUs with 4,495 OTUs numbers, followed by soil samples with 35 years of cultivation with 4,363 OTUs numbers, and soil samples with 12 years of cultivation had the lowest number of OTUs with only 3,105.

It can be seen from the Venn figure (Fig. 1) that the total OTUs number of the rhizosphere soil of different reclamation years is 7660. The total OTUs number shared by them is 3631 accounting for 50.01% of the total. The unique OTUs number of soil sample with 56 years, 12 years and, 35 years is 624, 330 and 233, accounting for 8.15%, 4.31% and 3.04% of the total OTUs, respectively.

The rarefaction curve can reflect the sampling depth of the sample, and can evaluate whether the sequencing volume covers all the groups. As can be seen

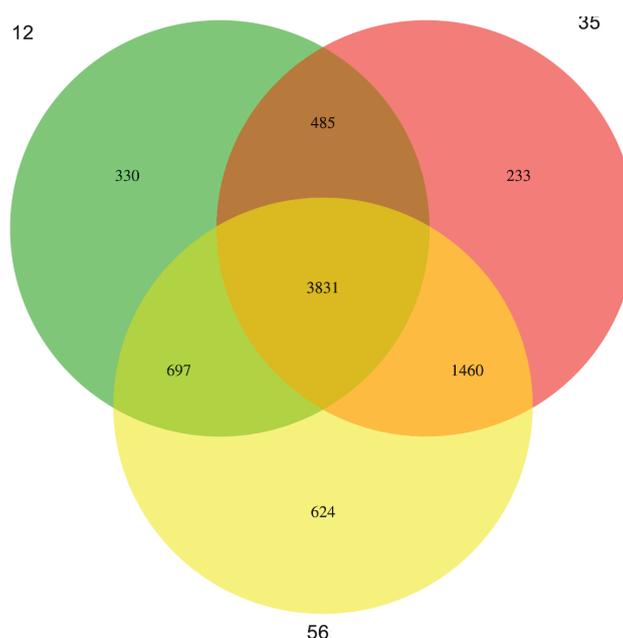


Fig. 1. Venn diagram of bacterial communities with different reclamation duration at OTU level.

12 indicated that reclamation duration has reached 12 years, while 35 indicated that reclamation duration has reached 35 years, 56 indicated that reclamation duration has reached 56 years. The same below.

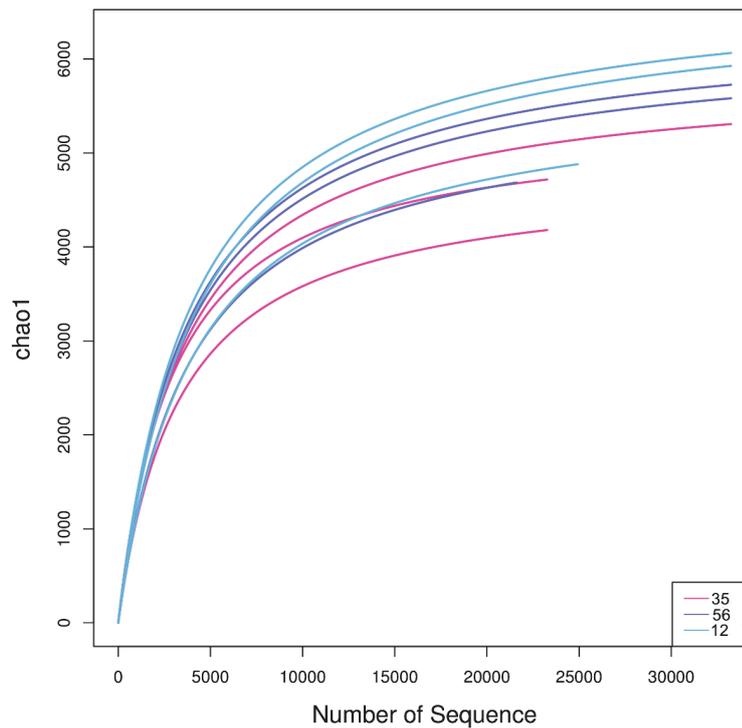


Fig. 2. Analysis of bacterial rarefaction curve.

from Fig. 2, the dilution curves of the three samples all tended to be flat, and the coverage of the libraries all exceeded 93%, which indicated that the sampling was basically reasonable and the OTUs coverage of the samples was saturated. The sequencing depth could reflect the real situation of bacterial communities in the rhizosphere soil of *Brassica oleracea var.italica* in different reclamation years of Taizhou Bay tidal flat.

Soil Bacterial Diversity

Table 3 shows the bacterial diversity analysis results of the rhizosphere soil of *Brassica oleracea var.italica* in the reclamation areas of Taizhou Bay with different reclamation years. Chao1 index represents the richness of bacterial community, and the greater the value of Chao1 index, the greater the richness of bacterial community. Shannon index is used to represent alpha diversity index of bacterial community. The higher the value of Shannon index is, the higher the diversity of

bacterial community of the sample is. As can be seen from Table 2, there were significant differences in the bacterial species richness index (Chao1 index) and diversity index (Shannon index) of the rhizosphere soil of *Brassica oleracea var.italica* in the tidal flat with different reclamation years. Soil samples of 35 years had the highest Chao 1 index and Shannon index, with values of 5551.671 and 10.339, respectively, followed by soil samples of 56 years, with Chao 1 index and Shannon index of 5433.255 and 9.838, respectively. The lowest values of Chao 1 index and Shannon index are 4687.011 and 9.768, respectively, for soil samples with the shortest reclamation years of 12 years.

Community Structure of Soil Bacteria

According to species annotation, a total of 23 phyla, 62 classes, 148 orders and 651 genera of soil bacteria were obtained from each sample. Proteobacteria (34.97%~48.16%), Acidobacteria (14.55%~21.46%),

Table 3. Variations of alpha diversity in soil bacterial communities of rhizosphere of *Brassica oleracea var.italica* with different reclamation ages.

Reclamation ages (year)	Chao1 index	Observed-species	Shannon index	Coverage
12	4687.011±14.03b	3385.111	9.768±0.053b	0.942
35	5551.671±12.51a	3636.333	10.339±0.081a	0.933
56	5433.255±11.67a	3698.031	9.838±0.076a	0.929

Values are means ±standard error (n = 3). Means followed by the same letter for a given factor are not significantly different ($P < 0.05$, LSD test)

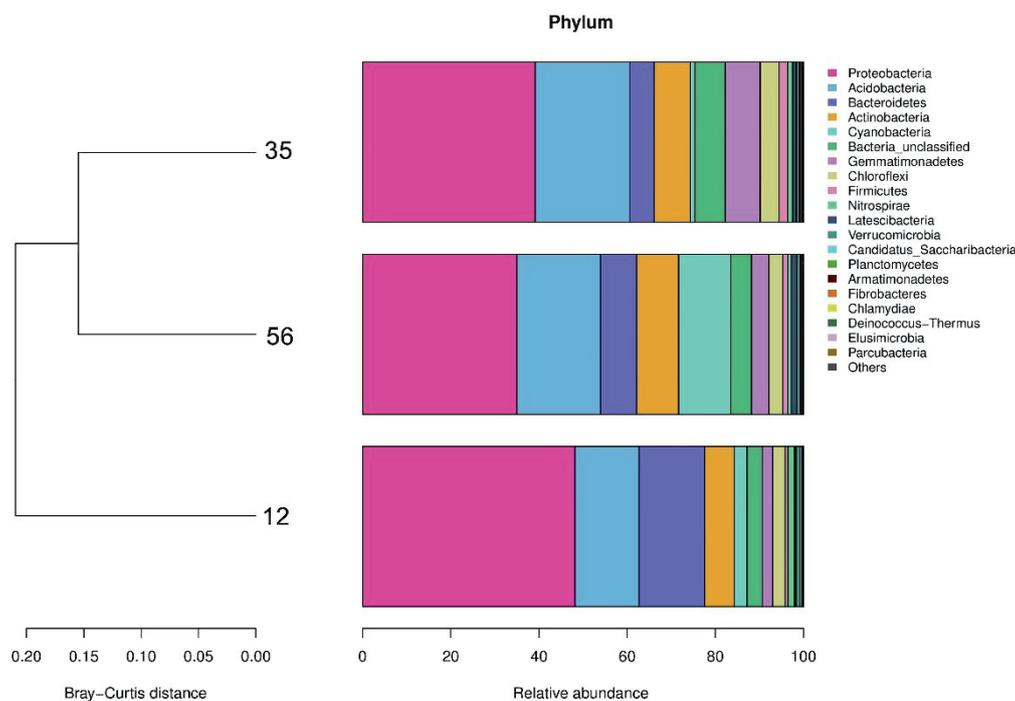


Fig. 3. The bacterial community in soil rhizosphere of *Brassica oleracea var.italica* at the phylum level.

Bacteroidetes (5.50%~14.68%), Actinobacteria (6.71%~8.23%), and Cyanobacteria (1.03%~11.87%) were the dominant bacteria groups in the rhizosphere soil of *Brassica oleracea var.italica* in the tidal land reclamation of Taizhou Bay. Among the above predominant flora, the relative abundance of Proteobacteria was the highest of 35 years, and the lowest of 56 years. The relative abundance of Acidobacteria was the highest of 35 years and the lowest of 12 years. The relative abundance of Bacteroidetes was the highest of 12 years and the lowest of 35 years. The relative abundance of Actinobacteria was the highest of 35 years, and the lowest of 12 years. The relative abundance of Cyanobacteria was the highest of 56 years, and the lowest of 12 years. The second most abundant species are Gemmatimonadetes (3.38%~7.93%), Chloroflexi (2.81%~4.28%), Firmicutes (0.71%~1.95%), Nitrospira (0.75%~1.42%), and the relative abundance of unclassified bacteria in the region was 3.46%~6.89%.

The heat map of microbial structure reflected the species composition and differences among different samples. As can be seen from the heat map, on the level of the phyla, three different reclamation years of *Brassica oleracea var.italica* rhizosphere bacteria community structure of 56 years and reclamation years of 35 years is similar. However, the *Brassica oleracea var.italica* rhizosphere bacteria community structure of 12 years and the other samples has large difference.

At the class level, Alphaproteobacteria was dominant in the rhizosphere soil of *Brassica oleracea var.italica* of different reclamation years in Taizhou Bay. The relative abundance of 35 years was the highest, with a value of 15.33%, while that of 56 years was the

lowest, with a value of 12.79%. The relative abundance of Gammaproteobacteria and Betaproteobacteria were slightly lower than that of Alphaproteobacteria, and the relative abundance of 12 years was 15.71% and 14.62%, respectively. The relative abundance of 35 years was the lowest, which was 8.63% and 7.20%. The relative abundance of other fungi, from high to low, is as follows: Actinobacteria (6.45%~9.17%), Acidobacteria-GP6 (5.30%~9.70%), Gemmatimonadetes (2.37% to 7.90%), Sphingobacteria (2.10% to 9.72%). The relative abundance of unclassified Bacteria at class level ranged from 3.46% to 6.89%.

Discussion

In this study, the rhizosphere soils of *Brassica oleracea var.italica* with three different reclamation years were studied. The bacterial community diversity index and richness index of 35 reclamation years were the highest, followed by the soil samples of 56 reclamation years. The bacterial community diversity index and richness index of 12 reclamation years were the lowest. The growth and reproduction of soil microorganisms in coastal reclamation areas are mainly controlled by soil salinity, pH, organic matter, nitrogen, phosphorus and other nutrients, as well as water content, enzyme activity and soil aeration. After reclamation, the soil physical and chemical properties of tidal wetland are not only affected by natural factors such as leaching, but also affected by human activities such as tillage and fertilization [18]. In the initial stage of reclamation, cotton was mainly planted in the tidal

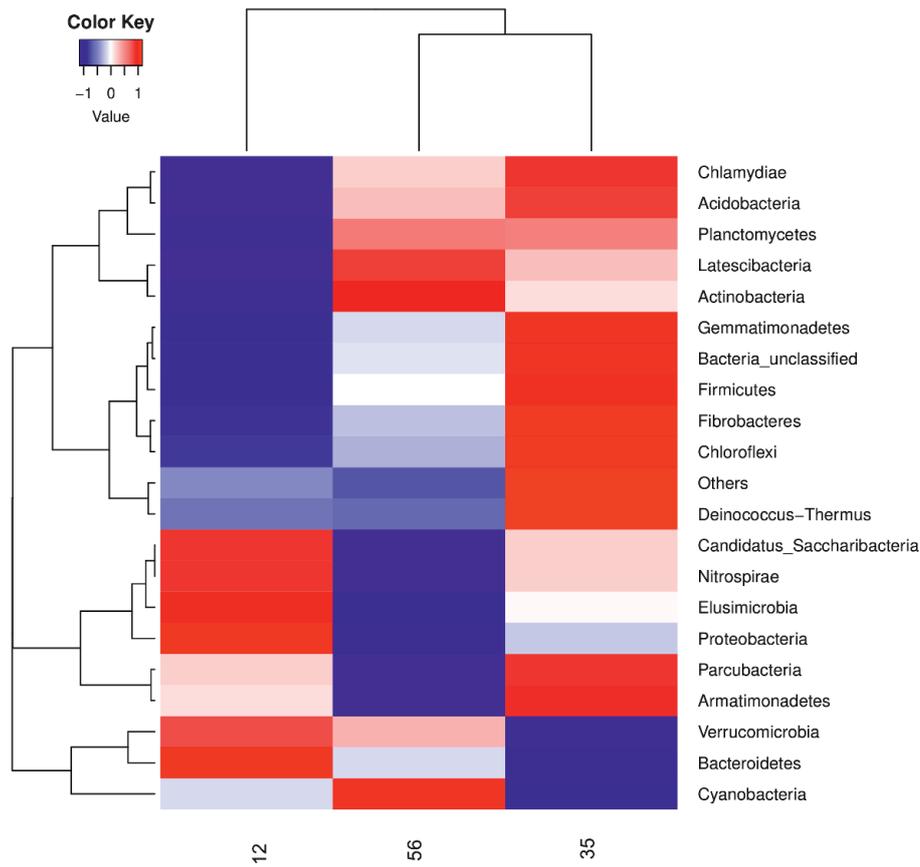


Fig. 4. Heat map showing bacterial composition in soil rhizosphere of *Brassica oleracea var.italica* at the phylum level.

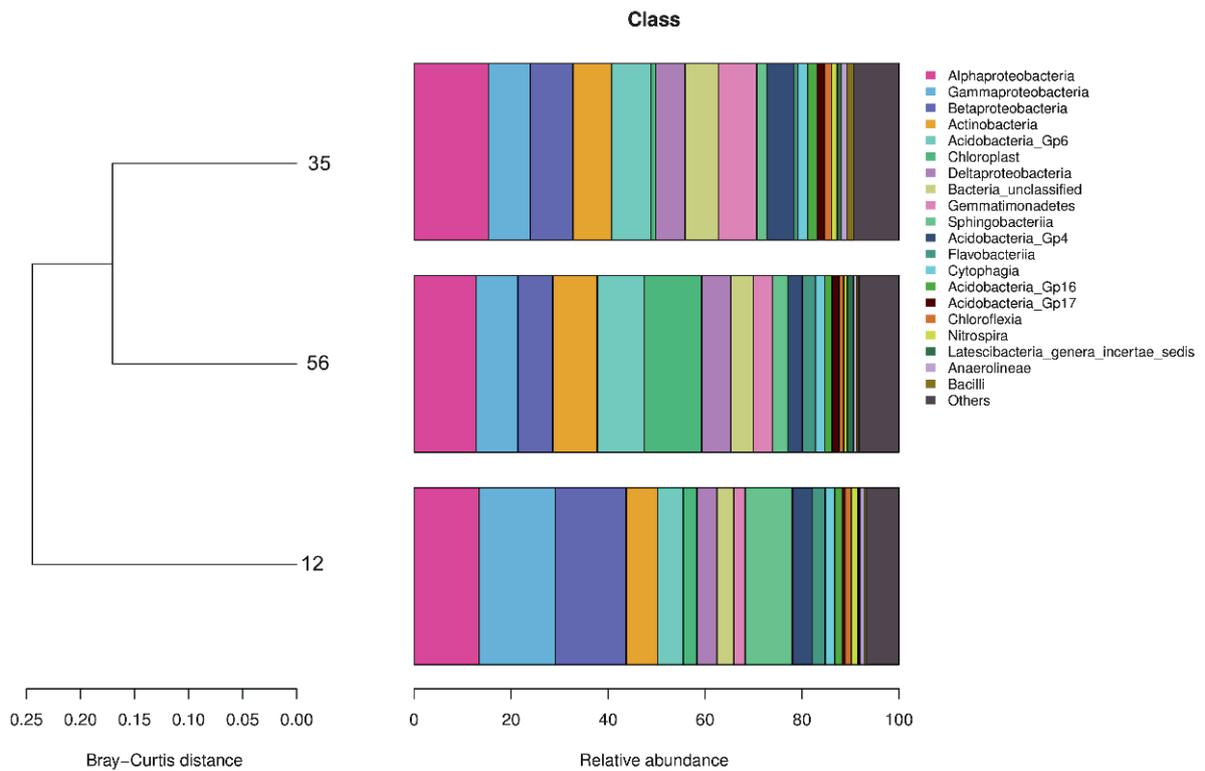


Fig. 5. The bacterial community in in soil rhizosphere of *Brassica oleracea var.italica* at the class level in this study region.

flat of the research area, and the ventilation condition of the surface soil of the tidal flat wetland was improved. Soil salt gradually reduce because of rainwater leaching [19]. With the increase of vegetation coverage, the plant residues and root exudates increase. The massive investment of chemical fertilizer and organic fertilizer, the nutrients needed for the growth and reproduction of soil bacteria are increased. Moreover, the coordination of water, air and temperature leads to a large number of soil bacteria increase. The corresponding diversity index and richness index of soil bacteria are improved. However, in the later reclamation period, *Brassica oleracea var.italica* was mainly planted on the tidal flat wetlands in the research area. Since dryland crops were planted all the time, long-term drought would lead to a decrease in soil bacterial diversity and richness. Brockett et al. [20] also showed in their research in western Canada that the decrease of soil moisture content would lead to the change of soil microbial community structure and diversity index. The results of this study may be related to the long-term dry farming land using pattern in the research area.

The soil in the reclamation area is derived from silt deposited in the mudflats. Because the soil development time is short, its soil background is more uniform. In this study, the number of OTUs shared by bacteria in the rhizosphere soil of *Brassica oleracea var.italica* in three different reclamation years was 3631, accounting for 50.01% of the total. This is because the three soils with different reclamation years are all developed from the same soil parent material, and the reclamation time distance between them is not very long at present. Therefore, the proportion of the number of OTUs shared by each treatment to the total OTUs is naturally relatively high. The tidal flat soils with different reclamation years represent different stages of the soil development. The spatial change of soil development can replace the change on the time scale [20]. In Taizhou Bay, the number of OTUs endemic to the rhizosphere soil of *Brassica oleracea var.italica* after 56 years of reclamation accounted for 8.15%, the number of OTUs endemic to the rhizosphere soil of *Brassica oleracea var.italica* after 12 years of reclamation accounted for 4.31%, and the number of OTUs endemic to the rhizosphere soil of *Brassica oleracea var.italica* after 56 years of reclamation accounted for 8.15%. The number of OTUs endemic to the rhizosphere soil bacteria accounted for 3.04% of the total. It indicated that the proportion of OTUs peculiar to soil bacteria was also high in the soil with long reclamation years.

The soil in the reclamation area is derived from silt deposited in the mudflats. Due to the short time of soil development, the soil background is relatively uniform. In this study, the number of OTUs shared by bacteria in the rhizosphere soil of *Brassica oleracea var.italica* in three different reclamation years was 3831, accounting for 50.01% of the total. The three kinds of soils with different reclamation years all developed in the same soil parent material. The reclamation time is not very

long, so the proportion of common OTUs in the total OTUs of each treatment is relatively high. The tidal flat soil of different reclamation years represents the different development periods of the soil. Under natural conditions, the spatial change of soil development can replace the change on the time scale [21]. The number of bacteria specific OTUs in rhizosphere soil of *Brassica oleracea var.italica* cultivated in Taizhou Bay beach for 56 years accounted for 8.15%, 4.31% for 12 years, and 3.04% for 35 years. The proportion of OTUs with long reclamation years was the highest.

Gemmatimonadetes is salt-tolerant and even halophilic, and is abundant in soils with high salt content. The study of Zhang Huimin et al. [22] showed that the abundance of Gemmatimonadetes in the soil with high salt content was significantly higher than that in the soil with low salt content. When Gao Xiuhong et al. [23] studied the bacterial community structure in the rhizosphere soil of *Betula platyphylla* forest in Hohhot City, Inner Mongolia Autonomous Region, they found that the relative abundance of Gemmatimonadetes was 8.2%-10.20%, which was significantly higher than the reported abundance data of Gemmatimonadetes in non saline alkali soil. Yang Jing et al. [24] found that the relative abundance of Gemmatimonadetes was only 1.1%-2.6% in different mixed forests of *Dalbergia odorifera* and *Dalbergia odorifera* with low pH. Sui Xin et al. [25] found that the relative abundance of Gemmatimonadetes was only 1.0%-2.0% in the Sanjiang Plain wetland with slightly acidic soil. The tidal flat of Taizhou Bay is a typical saline alkali soil. The relative abundance of Gemmatimonadetes in the reclaimed soil of different years is 3.38%-7.93%. With the decrease of reclamation years, the relative abundance of Gemmatimonadetes in soil samples decreased gradually. The longer the reclamation time is, the stronger the degree of soil desalination and the lower the soil pH, and the lower the relative abundance of *Blastomonas* is. Therefore, this result is consistent with the conclusions obtained from the research results of the above-mentioned scholars.

Cyanobacteria is a kind of ancient prokaryotes with photosynthetic autotrophic function and has been living on earth for billions of years. Cyanobacteria are pioneer species in mudflats and other extreme environments. Li Han et al. [26] studied the diversity, community structure, abundance, and distribution of salinity gradient of cyanobacteria in salinized soil invaded by seawater from the southern bank of Laizhou Bay and the Yellow River estuary. The results showed that salinization degree was the most important environmental factor regulating the community structure and abundance of cyanobacteria in coastal tidal wetland. With the increase of salinity in coastal salinized soil, the abundance of cyanobacteria gradually decreases, and the abundance of cyanobacteria in low salt area is significantly higher than that in middle and high salt area. Li et al. [27] studied the relative abundance of cyanobacteria in Xinjiang desert soil,

and the results showed that the relative abundance of cyanobacteria decreased with the increase of salinity in soil, while the relative abundance of salt-tolerant bacteria increased. The results of this study also showed that the relative abundance of cyanobacteria in the rhizosphere soil with a long reclamation years was relatively high (10.1%), while the relative abundance of cyanobacteria in the rhizosphere soil with a short reclamation years was also relatively low (1.03%). The results of this study are consistent with previous viewpoints.

Conclusions

A total of 7660 OTUs were collected from the rhizosphere soil samples of *Brassica oleracea var. italica* from the tidal flat of Taizhou Bay with different reclamation years (12, 35, 56), belonging to 23 phyla, 62 classes, 148 orders, and 651 genera. The soil samples with 35 years of cultivation had the highest Chao1 and community diversity index, followed by the soil samples with 56 years of cultivation. The soil samples with 12 years of cultivation had the lowest value. The values of Chao1 and community diversity were significantly different among different cultivation years ($P < 0.05$). The dominant phyla of soil bacteria in the research area were Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria and Cyanobacteria. The predominant strains of soil bacteria in the study area were α -Proteobacteria, γ -Proteobacteria, β -Proteobacteria, Actinomycetes, Acidobacteria, and Blastomonas.

Conflicts of Interest

The authors declare no conflict of interest.

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