Mechanisms of improving coastal saline-alkali soil by periphyton

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ABSTRACT

Periphyton plays an indispensable role in coastal saline-alkali land, but its function is poorly understood. Soil physical and chemical properties (pH value, salinity, soil organic matter), enzyme activity and microbial diversity (based on 16s rDNA, ITS and functional genes) were measured in periphyton formed on rice-growing coastal saline-alkali soil modified by a new type of soil conditioner. The results showed that the content of organic matter and catalase activity in periphyton were significantly higher than in the unplanted control soil. Soil pH and salinity were decreased in periphyton compared to the unplanted

control soil. Based on the relative abundance, bacterial genera *Desulfomicrobium*, *Rhodobacter*, *cyanobacterium_scsio_T−2*, *Gemmatimonas,* and *Salinarimonas* as well as fungal genus *Fusarium* were more abundant in periphyton than the unplanted control soil. In terms of functional genes, the *cbbM* and *cbbL* sequencing showed higher abundance of *Hydrogenophaga*, *Rhodovulum*, *Magnetospira*, *Leptothrix,* and *Thiohalorhabdus*, whereas the *nifH* sequencing indicated higher abundance of *Cyanobacteria* in the periphyton compared to the unplanted soil. The relative abundance and community structure of soil microorganisms were improved by periphyton, thus reducing soil salinity and pH, increasing soil organic matter and enzyme activity. This indicated that the periphyton can improve the conditions and offer a suitable environment for plant growth in coastal saline-alkali soil.

Keywords: Enzyme activity; Microbial diversity; Paddy ecosystem; Periphyton; Saline-alkali soil

1. Introduction

China has 99 million hectares of saline and alkaline land (Wang et al., 2011), and more than 80% of that resource has not been exploited yet. Soil salinization destroys the ecological balance by diminishing vital environmental properties. It also prevents the sustainable development of agriculture while causing economic losses. Therefore, soil salinization is one of the most severe issues that the humankind faces now and in the future.

It is necessary to improve the saline-alkali land resources to allow better utilization, but

this is a difficult and complex task. Planting salt-tolerant plants or halophytes is one option for using saline-alkali soils along the coast. Such planting can be facilitated by availability of salt-tolerant varieties and the knowledge of the salt tolerance mechanisms. At present, rice cultivation is considered to be an effective way to utilize saline-alkali land resources (Jesus et al., 2015; Long et al., 2016; Xu et al., 2020). Paddy soil is flooded with water during most of the rice growth period, which reduces salt accumulation at the soil surface and helps rice seedlings survive under salt stress (Lu et al., 2018).

Periphyton is a microbial film covering the surface of paddy soil (Lu et al., 2017; Su et al., 2016). It contains microorganisms such as protozoa, fungi, algae, and bacteria (Wu et al., 2010b). More specifically, periphyton is defined as a complete and independent microbial community dominated by photosynthesizing organisms; it contains algae, fungi, metazoa, bacteria, protozoa, abiotic components, detritus, and extracellular polysaccharides.

Periphyton exists in all aquatic ecosystems. The research on periphyton ecology in the modern sense has begun in the 1950s (Odum, 1956) and quickly expanded, but mainly focused on the freshwater ecosystems; in contrast, the research on the paddy ecosystem has started only recently (Larned et al., 2010). Therefore, a knowledge about composition, formation and function of the periphyton in rice paddies is scarce.

Salt-alkali stress is a vital environmental factor reducing paddy yield and affecting rice growth and development. Periphyton can absorb excess salt from soil, reducing the pH of the surface soil and providing a good environment for rice growth. In addition, it can also stabilize soil structure, provide a rich source of "food" in the soil, and provide more suitable

59growth temperature and humidity (Belnap et al., 2003). Various studies have shown that 60periphyton plays a significant role at the soil-water interface in the paddy field ecosystem. 61Therefore, periphyton can be used as an emerging biotechnology tool to improve the paddy 62ecosystem while controlling the non-point agricultural pollution in coastal saline and alkaline 63lands (Lu et al., 2018).

64 This study was aimed at determining the physical and chemical properties as well as the 65microbial diversity of various regions of periphyton, and at characterizing the composition 66and functions of paddy periphyton developing on the improved coastal saline-alkali soils.

67**2. Materials and Methods**

68*2.1. Experimental area*

69 The study field is located in the Tiaozini region, Dongtai City, coastal Jiangsu Province 70(32°50'35"N, 120°57'44"E). The area has the typical monsoon climate affected by both 71marine and continental influences, with average annual temperature of 14 ℃. The average 72annual precipitation is 1000 mm.

73*2.2. Soil improvement*

74 "Yanpa" (ZL 201310386417.1) was used as the saline-alkali soil conditioner. The 75innovative technology in its production lies in non-metallic mineral activation and biological 76enzyme extraction and preservation, with the main raw materials being mealstone (organic 77marlstone containing abundant calcite crystals) and biological enzymes (urease, catalase,

78deoxyribonuclease, etc.). It also contains organic compounds (indoleacetic acid, carotene, 79acetylcholine, and humic acids) and wheat-rice-stone (porphyric hornblende andesite). On 80May 18, 2018, “Yanpa” was applied to soil surface. Two different treatments were set up in 81the reclamation area, each of which had three replicate 20×25 m plots. The treatment marked 82Y1 received 60 kg of "Yanpa" per plot. The treatment Y2 had 30 kg of "Yanpa" per plot. On 83June 1, 2018, rice cv. Su-xiu 867 was sowed at 187.5 kg seed/ha.

84*2.3. Sample collection*

85 Soil sampling was done on September 11, 2018 when the periphyton covered the entire 86soil surface. A complete structure of the periphyton (C) was collected with the thickness of 871-3 mm (area 10 × 10 cm) randomly in each plot. After collecting the periphyton, the surface 88soil (0-10 cm) directly under periphyton (SUC) was collected. Three replicate plots were 89sampled, with three collection points in each plot. Then, about 5 kg each of rhizosphere soil 90(HR) and non-rhizosphere soil (NHR) (close to and away from rice roots, respectively) were 91collected randomly in each plot. Soil from unplanted control (CK) was collected randomly 92from the areas around each plot where no modifier was added and no plants were present. All 93samples were packed into separate ziplock bags. In addition, about 10 g of fresh samples 94were wrapped in tin foil, snap-frozen in liquid nitrogen, and stored at -80 °C for sequencing. 95The samples used for sequencing were C and SUC in treatment Y1 and CK.

96 Soil samples for measuring enzyme activity were stored at -20 °C. The remaining 97samples were air-dried, crushed and sieved through a 0.15 mm sieve.

2.4. Analytical methods

Salinity and conductivity were measured in suspension soil:water 1:5 using a conductivity meter. Soil pH value was determined in suspension (soil:water ratio 1:5). Organic matter content was determined by potassium dichromate oxidation colorimetry. The activity of catalase in soil was determined by potassium permanganate titration (Johnson and Temple, 1964).

Soil DNA was extracted using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) following the manual. Purity and quality of the genomic DNA were checked on 0.8% agarose gels.

DNA extraction, PCR amplification and data analysis were performed at Allwegene Company (Beijing) using the standard methods (Cole, 2009; Edgar, 2017; Ji et al., 2016; Munyaka et al., 2015; Zhang et al., 2015).

The BioProject ID in NCBI Submission Portal is PRJNA563235.

2.5. Statistical analysis

Excel 2010 and IBM SPSS Statistics 20.0 were used to analyze the data in this experiment using the multi-factor correlation analysis and the single-factor ANOVA as appropriate. The correlation between physical and chemical properties was calculated by Spearman correlation coefficients using R package corrplot (v0.84) (Wei et al., 2017). The Tukey new multiple range test ($p \leq 0.05$) was used for pairwise mean comparisons.

3. Results

3.1. Soil physico-chemical properties and enzyme activity

The physical and chemical properties of periphyton were significantly different from those of the other sample types. The pH of unplanted soil (highly alkaline) was higher than the pH of periphyton (just above neutral at around 7.5) in each treatment (Fig. 1a).

As shown in Fig. 1b, the unplanted soil had the highest salt content of all the sample types. The salt content of periphyton was also very high, whereas the salinity of other sample types was significantly (about a ten-fold) lower than that of periphyton.

The soil organic matter content (SOM) of different sampling areas was shown in Fig. 1c. The soil organic matter content of periphyton in Y1 and Y2 exceeded 65 g/kg, which was significantly higher than the other sample types and about seven times that of the unplanted soil.

The catalase activity in the periphyton was significantly higher than that of the other sample types (Fig. 1d). The sample types other than periphyton did not differ significantly in catalase activity.

3.2. Soil microorganisms

3.2.1. 16s rRNA sequence analysis

Sequences were grouped as Operational Taxonomic Unit (OTU) based on similarity at >97%. As shown in Fig. 2a, 16s rRNA produced 4943 OTUs in total, and the number of

136common OTUs in CK, SUC and C was 796. As can be seen in Fig. 2b, the order of the 137Chao1 and observed_species indices was CK < C < SUC, and the order of Shannon index 138and the Faith's phylogenetic diversity pd-whole-tree was C < CK < SUC, indicating the soil 139under periphyton had the highest microbial abundance and diversity.

140 The top three bacterial phyla were *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* (Fig. 1412c). The relative abundance of *Proteobacteria* was the highest, with CK having significantly 142lower relative abundance than SUC. There was no significant difference among the three 143sample types regarding *Bacteroidetes* relative abundance. *Chloroflexi* abundance in C was 144significantly higher than CK.

145 At the genus level, the top three were *Desulfomicrobium*, *Ignavibacterium* and *Azoarcus* 146(Fig. 2d). *Desulfomicrobium* were most abundant in C (significantly higher than SUC and 147absent in CK), which was in agreement with the observations that *Desulfomicrobium* can be 148a biomarker bacterium for the periphyton (Fig. 2f and 2g). SUC had the highest relative 149abundance of *Ignavibacterium* (significantly higher than CK and C). *Azoarcus* abundance 150was significantly higher in SUC than C and CK. In addition, CK contained almost no 151*Thiobacillus*, and periphyton (C) had very low relative abundance, if any, of 152*Ignavibacterium*, *Desulfotignum*, *Thiobacillus*, *Desulfuromonas*, *Geothermobacter,* and 153*Prevotella_1*.

154 From the heatmap of 16s rRNA (Fig. 2e), it can be seen that *Rhodobacter*, *Roseivivax*, 155cyanobacterium_scsio_T−2, *SM1A02*, *Gemmatimonas,* *Salinarimonas*, and 156*Desulfomicrobium* abundances were higher in C than CK and SUC. *Ignavibacterium*

abundance was higher in SUC than CK and C. *Prevotella_1* is a pathogenic bacterium that was found in CK, but not in C and SUC.

Furthermore, LEfSe (LDA Effect Size) (Nicola et al., 2011) was used to identify the specific bacterial taxa associated with periphyton, finding 19 differentially abundant taxonomic clades with an LDA score higher than 3.0 (Fig. 2f and 2g). At the phylum level, *Cyanobacteria* was most abundant in the C, while *Parcubacteria* was most abundant in the CK.

3.2.2. ITS sequence analysis

There was no region of ITS gene amplified in the CK. According to Fig. 3a, total of 692 OTUs were produced in ITS. There were 365 common OTUs in C and SUC. All the indicators of abundance and diversity, except the Chao1 and the Shannon index, were higher in SUC than C, with the Shannon index showing a reverse difference (Fig. 3b). Hence, the fungal abundance was lower, and the fungal diversity was greater, in periphyton than the soil under it.

It was found that *Ascomycota* (phylum), *Fusarium* and *Coralloidiomyces* (genera) had the highest abundance in periphyton, and could be used as biomarkers (Fig. 3c-f). *Glomeromycota*, *Rozellomycota* (phyla) and *Periconia* (genus) had the highest abundance in the soil under periphyton. There were 30 fungal clades with statistically significant differences, including 20 biomarker fungi in C and 10 in SUC.

3.2.3. cbbL sequence analysis

Fig. 4a showed that *cbbL* sequencing generated 918 OTUs; the OTU numbers were as follows: SUC (615) > C (610) > CK (478). The number of common OTUs in CK, SUC and C was 250. On the whole, the species abundance and diversity were higher in C compared with the other samples, and were lowest in CK (Fig. 4b). Hence, the species richness and diversity were highest in periphyton, followed by the soil under periphyton, and lowest in the unplanted control.

At the phylum level, only *Proteobacteria* were found in *cbbL* sequencing. There was no significant difference among the three sample types, with the relative abundance above 97% (Fig. 4c).

At the genus level, *Cupriavidus*, *Thiobacillus* and *Thioalkalivibrio* were the top three in abundance (Fig. 4d). *Thioalkalivibrio* had the higher abundance in CK than SUC and C. *Cupriavidus* species had significantly higher abundance in SUC than C and CK. *Thiobacillus* in CK was relatively less abundant than in the other two sample types. *Hydrogenophaga* was the most abundant in C (18.9%), with very low abundances in CK (0.7%) and SUC (2.7%). Similarly, *Rhodovulum* was significantly more abundant in C than CK and SUC.

The largest differences in taxa between C and SUC, with key phylotypes identified as BmB (biomarker bacteria) at different phylogenetic levels, are shown in Fig. 4e, f. Most bacteria were significantly enriched in C, while only three clades showed abundance advantage in SUC. Specifically, *Cupriavidus* (genus), *Burkholderiaceae* (family) and *Burkholderiales* (order) were enriched in SUC. The genera *Prochlorothrix*, *Cyanothece*,

197*Geitlerinema*, *Pararhodospirillum*, and *Oscillatoria* (all belonging to *Cyanobacteria*) were 198enriched in C.

199*3.2.4. cbbM sequence analysis*

200 According to Fig. 5a, 860 OTUs were generated by *cbbM* sequencing. There was no 201*cbbM* gene amplification in CK. The C and SUC had 477 common OTUs. Chao1, 202observed_species indices and the Shannon index were higher in SUC compared to C (Fig. 2035b). Hence, the abundance as well as diversity were greater in the soil under periphyton than 204in periphyton.

205 Only *Proteobacteria* and *Euryarchaeota* were detected at the phylum level based on the 206*cbbM* sequencing (Fig. 5c), with *Proteobacteria* having the largest relative abundance (SUC 20797% and C 96%). *Euryarchaeota* were not detected in C, and the relative abundance of 208*Euryarchaeota* in SUC was 1%.

209 The top three genera were *Magnetospira*, *Thiobacillus* and *Sulfuritalea* (Fig. 5d). 210*Magnetospira* abundance was significantly higher in C than SUC [C (56%) > SUC (26%)]. 211*Thiobacillus*, *Sulfuritalea*, *Acidithiobacillus*, *Halothiobacillus,* and *Acidihalobacter* were 212more abundant in SUC than C, and they were also identified as BmB in Fig. 5e and 5f. The 213BmB in periphyton were *Streptomyces* and *Rhodanobacter*.

214*3.2.5. nifH sequence analysis*

215 According to Fig. 6a, there were 1498 OTUs produced by *nifH* sequencing, with the 216number of OTUs as follows: SUC (1194) > CK (832) > C (738). The number of common

217OTUs in CK, SUC and C was 391. Based on those values, the species abundance was higher
218in SUC than in the other sample types.

219 As can be seen from Fig. 6b, the order of Chao1 and observed_species indices was: CK
220< C < SUC, and for pd-whole-tree and Shannon indices it was: C < CK < SUC. Hence, the
221SUC had the highest bacterial species richness and diversity.

222 *Proteobacteria* had the highest abundance in CK and SUC (significantly higher than in
223C), and were also identified as BmB at the phylum level in SUC (Fig. 6c and 6f).
224*Cyanobacteria* had the highest relative abundance in C (significantly higher than in CK and
225SUC) and was also confirmed to be more abundant in C by LEfSe method. The relative
226abundance of *Firmicutes* was very low in all three sample types. Additionally, *Firmicutes*
227were biomarker bacteria in CK.

228 The three most abundant genera were *Desulfuromonas*, *Trichormus* and
229*Pseudodesulfovibrio*, and there were significant differences among the sample types (Fig.
2306d). The *Desulfuromonas* abundance was highest in CK [CK (53%) > SUC (34%) > C (4%)].
231*Trichormus* was the most abundant genus in C (25%), significantly higher than in CK (0.1%)
232and SUC (0.3%). Regarding *Pseudodesulfovibrio*, the relative abundances were SUC
233(11.3%) = C (11.0%) > CK (3.1%), with that of CK significantly lower than those of SUC
234and C.

235 A cladogram that represents the structure of the *nifH* sequencing is shown in Fig. 6e;
236with the largest differences in the taxa among the three communities displayed. The LEfSe

analysis revealed 70 discriminative features (LDA score >3, Fig. 6f). Specifically, eight genera (*Trichormus*, *Lyngbya*, *Cylindrospermum*, *Rhodopseudomonas*, *Nodosilinea*, *Cyanothece*, *Rubrivivax,* and *Pseudanabaena* were identified in C. The genera *Ectothiorhodospira*, *Zoogloea*, *Azoarcus*, *Pelodictyon*, *Desulfomicrobium*, *Pseudomonas*, *Raoultella*, *Azorhizobium*, *Methylocaldum*, *Geoalkalibacter*, *Geoalkalibacter,* and *Anaeromyxobacter* were more prevalent in SUC. The most predominant genera in CK were *Desulfuromonas*, *Pelobacter* and *Desulfurivibrio*.

Fig. S1a, c, e and Table S1 showed that there were significant differences in the microbial community structure in the three sample types. In addition, Fig. S1a indicated good reproducibility of the data among the replicate samples. Fig. S1b and d showed there was no significant difference in the microbial community structure in periphyton and the soil under periphyton.

3.3. Correlation analysis

Based on the 16s rRNA sequencing (Fig. 7a), the abundance of *Salinarimonas*, *Chloroflexi*, *Planctomycetes*, *SM1A02*, *Gemmatimonadetes* or *Proteobacteria* was negatively and significantly correlated with pH, whereas the abundance of *Desulfuromonas*, *Acidobacteria*, *Firmicutes* or *Bacteroidetes* was positively correlated with pH ($p \leq 0.01$). The *Azoarcus*, *Gemmatimonadetes*, *Proteobacteria* or *Ignavibacterium* abundance was negatively correlated with salinity ($p \leq 0.01$), whereas that of *Cyanobacteria*, *Prevotella_1* or *Bacteroidetes* was positively correlated with salinity ($p \leq 0.05$). Soil organic matter and catalase activity were positively and significantly

correlated with the abundance of *Salinarimonas*, *Cyanobacteria*, *SM1A02*, *Chloroflexi* or *Desulfomicrobium* ($p \leq 0.01$). The correlation between the *Prevotella_1* and *Gemmatimonadetes* abundance was highly significant ($p \leq 0.01$). The *Geothermobacter*, *Azoarcus* or *Thiobacillus* abundance was positively correlated with that of *Ignavibacterium* ($p \leq 0.01$). The abundance of *SM1A02* was negatively correlated with that of *Actinobacteria* and positively correlated with *Desulfomicrobium* ($p \leq 0.01$). Abundances of *Actinobacteria* and *Desulfomicrobium* were negatively correlated ($p \leq 0.01$).

Based on the *cbbL* sequencing (Fig. 7b), the abundance of *Hydrogenophaga*, *Rhodovulum*, *Sulfuricaulis* or *Nitrobacter* was negatively correlated with pH ($p<0.01$). The *Sulfurifustis*, *Rhodospirillum*, *Ectothiorhodospira* and *Thioalkalivibrio* abundance had a positive significant correlation with pH ($p<0.01$). The abundance of *Cupriavidus*, *Thiobacillus* or *Proteobacteria* was negatively correlated with salinity ($p \leq 0.01$), whereas that of *Rhodospirillum*, *Ectothiorhodospira*, *Sulfurifustis* or *Thioalkalivibrio* was positively correlated with salinity ($p<0.01$). The abundance of *Hydrogenophaga*, *Rhodovulum* or *Sulfuricaulis* was positively correlated with soil organic matter and catalase activity ($p \leq 0.05$). The correlation between *Proteobacteria* and *Thiobacillus* abundances was significantly positive ($p \leq 0.01$). The correlation between *Sulfurifustis* and *Rhodospirillum* abundances was significantly negative ($p \leq 0.01$). The abundance of *Nitrobacter* was significantly and negatively correlated with that of *Ectothiorhodospira* ($p \leq 0.01$).

Based on the *nifH* sequencing (Fig. 7c), the abundance of *Cyanobacteria*, *Lyngbya*, *Pseudodesulfovibrio* or *Trichormus* was negatively and significantly correlated with pH, but

positively and significantly correlated with soil organic matter ($p \leq 0.01$). There was a positive correlation between *Cyanobacteria*, *Lyngbya*, *Pseudodesulfovibrio* and *Trichormus* abundances ($p \leq 0.01$). The *Bradyrhizobium*, *Pelobacter* or *Desulfovibrio* abundance was positively and significantly correlated with pH ($p \leq 0.01$). Salinity was negatively and significantly correlated with the abundance of *Pseudodesulfovibrio* or *Zoogloea* ($p \leq 0.01$), but positively correlated with that of *Firmicutes*, *Desulfovibrio* or *Pelobacter* ($p \leq 0.05$). Catalase activity was positively correlated with the *Lyngbya* or *Trichormus* abundance ($p \leq 0.01$). The abundance of *Bradyrhizobium* was correlated positively with that of *Desulfuromonas* ($p \leq 0.01$). Abundances of *Pelobacter* and *Desulfovibrio* were positively and significantly correlated ($p \leq 0.01$).

4. Discussion

4.1. Effects of periphyton on pH and salinity of saline-alkaline soils

Periphyton can absorb excess salt from soil and lower the soil pH, thus keeping the paddy field ecosystem in a relatively stable state. This study (Fig. 1b) showed that the salinity of periphyton in both treatments was second only to the unplanted control soil, and significantly higher than SUC. The salinity of periphyton was 6-10 times as high as the soil underneath it. These results suggest that periphyton can absorb excess salt from soil, decreasing salinity of the underlying soil and making it more suitable for crop growth. Lu et al. (2018) showed that periphyton could get established and grow well on the salinized soil surface (electrical conductivity (EC) greater than 8 mS cm^{-1}) and greatly reduce the salinity

of surface soil (0-20 cm), which was similar to the results of the present study (Fig. 1b). Lu et al. (2018) also discovered that the presence of cyanobacteria decreased the exchangeable sodium and EC in soil, indicating that cyanobacteria may have an effect on sodium removal.

Scanning electron microscopy (SEM) of periphyton found that it accumulated many crystals (such as sodium chloride), which may be due to the salt-tolerant actinomycetes in periphyton secreting extracellular polysaccharides that can chelate some toxic cations (such as sodium). This may at least partly explain the high salinity in periphyton in the present study (Fig. 1b). The presence of extracellular polysaccharides in periphyton was significant because extracellular polysaccharides can remove contaminants such as excessive salinity, heavy metals and microcystin-RR (Meylan et al., 2003; Wu et al., 2010a).

In the study presented here (Fig. 1a), the pH value was significantly lower in periphyton than in the other sample types. Lu et al. (2018) also found that the growth of cyanobacteria reduced soil pH. This might be the result of the increased leaching of soil and the release of organic acids into soil, which was consistent with the results presented here. Subhashini et al. (1981) studied the effects of applying *Calothrix braunii*, *Hapalosiphon intricatus* and *Scytonema tolypothrix ceylonica* to salt-alkali soil in the rice field. Analysis of soil samples before and after improvement showed that the pH of soil decreased from 9.2 to 8.3, and exchangeable sodium was reduced by 39%, which strengthened the notion that cyanobacteria could reduce the pH and salinity of soil, playing an ameliorating role in saline-alkali land.

Shi et al. (2017) showed that the presence of periphyton on soil surfaces with mild and severe As and Cd contamination increased soil pH from 7.59 to 8.53, depending on the

treatment. This was inconsistent with the conclusion in this study, but might have been caused by the difference in the basic properties of soil. Shi et al. (2017) used contaminated neutral soil, whereas the soil we used was alkaline and not polluted. Furthermore, periphyton may have different functions in different growth stages. We sampled periphyton when it was already in the mature stage.

4.2. Effects of periphyton on organic matter content and catalase activity in saline-alkali soil

SOM is a significant index of soil fertility and quality. High SOM can help stabilize the soil structure (Hbirkou et al., 2011; Hong et al., 2018). Catalase in soil promotes decomposition of hydrogen peroxide and decreases its toxic effects on soil organisms (Doran et al., 1994). SOM (Fig. 1c) and catalase activity (Fig. 1d) of periphyton were significantly higher than in the other sample types in the same soil improver treatment. This was because the soil improver contained mealstone, wheat-rice stone and enzymes. They can improve the soil structure, promote beneficial soil ecological cycles, accelerate nutrient conversion, and shorten the time needed to improve saline-alkali soil. Zhang et al. (2018) showed that wheat-rice stone had absorptive capacity as well as mineralization and biological activity; hence, its combination with enzymes can significantly increase the SOM in periphyton. In addition, periphyton contained abundant carbon-assimilating and nitrogen-fixing bacteria, thus effectively increasing SOM.

4.3. Microbial diversity in periphyton

So far, studies on the composition of periphyton have focused mainly on lakes, fresh water and other aquatic ecosystems, with only few studies in paddy ecosystems. Rice paddies are basically shallow water ecosystems like wetlands and ponds, but managed differently. Previous studies found that the periphyton in rice field comprised mainly algae (e.g. *Spirogyra*) and bacteria (e.g. *Cyanobacteria*) (Lu et al., 2016).

The 16s rRNA sequencing of periphyton (Fig. 2c) showed that the top three bacterial phyla were *Proteobacteria*, *Bacteroidetes* and *Chloroflexi*, which was similar to the results reported by Su et al. (2016). At the genus level (Fig. 2d and 2e), periphyton contained high abundance of *Azoarcus*, *Roseivivax*, *Cyanobacterium*, *Gemmatimonas*, *Rhodobacter,* and *Ignavibacterium*, most of which are capable of nitrogen fixation and carbon assimilation. In addition, *Gemmatimonas* can degrade complex organic macromolecules and contribute to the transformation of soil C, N and P. Hydrogen sulfide can be used by *Rhodobacter*, and some species of that genus can use macromolecular organic matter as carbon sources and electron donors for photoautotrophic growth under anaerobic conditions (Imhoff, 2015). *Ignavibacterium* belongs to the *Chloroflexi* and was found to be a marker with multiple metabolic functions in soil in the mid to later stages of rice development (Li et al., 2017). Yang et al. (2016) showed that protozoa, algae (e.g. diatoms), fungi (e.g. Candida) and bacteria such as cyanobacteria and actinomycetes were present in periphyton. This was in agreement with the sequencing results reported in the present study.

The ITS sequencing showed that *Fusarium* was the most abundant fungal genus in periphyton (Fig. 3c). This fungus may infect a variety of grain and vegetable crops, causing

root rot, stem rot, ear rot and other diseases, resulting in crop losses and severe damage to crop production worldwide. Therefore, as a complex environment with many ecological functions, periphyton can retain and potentially inactivate the harmful or unfavorable soil organisms, providing a more suitable environment for plant growth.

The *cbbL* sequencing (Fig. 4d) showed that *Cupriavidus* had relatively high abundance in SUC. *Cupriavidus* secreted an enzyme called CupA that caused excess copper to be expelled from the bacteria. In addition, the bacterial secretions contained precious metals, such as gold (Wiesemann et al., 2013). In the present study, there were basophilic bacteria in both C and CK, which might further explain the high salt content in periphyton. The C and SUC contained genus *Rhodovulum*, marine photosynthetic bacteria that can grow well under facultative anaerobic, mildly anaerobic as well as aerobic conditions. *Hydrogenphaga* was the most abundant genus in C; it can carry out anaerobic nitrate respiration and has denitrification capacity. It can effectively use growth media containing organic acids, amino acids or peptone, but rarely uses carbohydrates (Köberl et al., 2016). *Cyanothece* was a marine nitrogen-fixing cyanobacterium that can produce high concentration of extracellular polysaccharides. Moreover, the production of extracellular polysaccharides was reported to be influenced by the concentration of salt, pH and the nitrogen source (Manivasagan et al., 2014), so it was expected that *Cyanothece* would be highly abundant in C (Fig. 4f).

In the *cbbM* sequencing, it was found that the C had abundant *Magnetospira* (more than 50%) (Fig. 5d), which can grow in the harsh environments (Bazylinski & Lefèvre, 2013).

In the *nifH* sequencing, *Cyanobacteria* were identified as an important component of

periphyton; they are large single-celled prokaryotes that perform oxygenic photosynthesis (Azim, 2009). Some early studies showed that the presence of *Cyanobacteria* in paddies increased soil fertility (Paudel et al., 2012). Therefore, it is assumed that cyanobacteria played an important role in many functions of periphyton. The strong nitrogen-fixing capacity of periphyton is not only reflected in *Cyanobacteria,* but also in other genera with nitrogen-fixing capacity, such as *Lyngbya*, *Pseudodesulfovibrio* and *Azoarcus* (Fig. 6d). Among them, *Lyngbya* can fix nitrogen under anoxic conditions. *Pseudodesulfovibrio,* existing widely in sludge that can be converted to organic fertilizer, can obtain energy from anaerobic respiration of reducible sulfide.

In terms of the number of OTUs, in all the five sequencing methods employed in the present study, the number of OTUs in SUC was higher than C and was lowest in CK. Both SUC and C were superior to CK in terms of species richness and diversity, indicating healthy microbial communities in periphyton and the topsoil under periphyton in paddy fields.

4.4. Correlation analysis

The lack of nitrogen would severely limit agricultural production. In the biosphere, nitrogen can be fixed naturally only by nitrogen-fixing microorganisms (Gaby et al., 2012). Plants influence the community composition and genetic diversity of nitrogen-fixing bacteria in the ecosystems by increasing SOM (Köberl et al., 2016). The analysis of *nifH* gene (Fig. 7c) showed that the correlation between SOM and the abundance of *Cyanobacteria*, *Lyngbya* or *Trichormus* was significant ($p \leq 0.01$). Moreover, the *Cyanobacteria* abundance (16s rRNA sequencing) was significantly correlated with catalase activity ($p \leq 0.01$) (Fig. 7a). Nitrogen-

fixing microorganisms had a strong influence on the formation and development of biological soil crusts (BSCs) that contribute significantly to the accumulation of inorganic nitrogen in soil (Pepe-Ranney et al., 2016). Periphyton has similar composition and function to BSCs. Therefore, the stimulatory effect of periphyton on biological nitrogen fixation is of vital importance in poorly fertile saline-alkali soils.

5. Conclusions

The cycling of salts and nutrients occurs at the interface between paddy water and soil, and periphyton played an important role in that cycling. In coastal saline-alkali land, periphyton could absorb excess salt, thus reducing soil salinity. In addition, the presence of periphyton also reduced the pH of topsoil and increased the organic matter content in the soil, thus promoting soil fertility and making paddy a more suitable living environment. One reason periphyton performed many functions was its abundance of cyanobacteria and other nitrogen-fixing and carbon-assimilating bacteria. These organisms provided the rice ecosystem with the compounds and nutrients it needed to grow. Moreover, periphyton accumulated harmful substances (such as excessive salt) and pathogenic bacteria. These results have showed that periphyton has a great potential in improving and managing the coastal paddy ecosystems, and its utilization prospect was immense. The periphyton formation and functioning need to be explored further.

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Authors' contributions

Y.Z. and X.H.L. conceived the study and designed the methodology; Y.J.Z. and X.M.G. collected the data; Y.Z. and T.Y.S. analyzed the data; Y.Z. and Z.R. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Notes

The authors declare no competing financial interest.

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Figure captions

Figure 1 pH (a); soil salinity (b); organic matter content (c); and catalase activity (d) in different treatments.

Note: Y1 = 60 kg/plot of "Yanpa"; Y2 = 30 kg/plot of "Yanpa". C = periphyton; SUC = soil under periphyton; HR = rhizosphere soil; NHR = non-rhizosphere soil; CK = unplanted control. The data are the means (n = 3). Different letters above the columns indicate statistically significant ($P \leq 0.05$) differences among sample types in each treatment (Y1 and Y2) separately.

Figure 2 The results of 16s rRNA sequencing. (a) Venn diagram of OTUs distribution; (b) the box plot of alpha diversity indices; (c) the relative abundance of the top ten phyla; (d) the relative abundance of the top ten genera; (e) the heatmap of top 20 OTUs at the genus level; (f) cladogram of LEfSe analysis. Each dot represents a taxon. Dots are marked for significant (LEfSe: $p<0.05$) enrichment in the control group (olive green), periphyton (red) or the soil under periphyton (blue). The size of each dot is proportional to the effect size; (g) histogram of LEfSe analysis. Taxa that reached a linear discriminant analysis score (log10) >3.0 are highlighted and labelled accordingly.

Note: C = periphyton; SUC = soil under periphyton; CK = unplanted control. The data (except in e) are means (n = 3). Different letters in a phylum or genus indicate statistically significant ($P \leq 0.05$) differences among the sample types.

Figure 3 The results of ITS sequencing. (a) Venn diagram of OTUs distribution; (b) the box

plot of alpha diversity indices; (c) the relative abundance of the specific taxa; (d) the relative abundance of the nine genera; (e) cladogram of LEfSe analysis. Each dot represents a taxon. Dots are marked for significant (LEfSe: $p<0.05$) enrichment in the periphyton (red) or the soil under periphyton (olive green). The size of each dot is proportional to the effect size; (f) histogram of LEfSe analysis. Taxa that reached a linear discriminant analysis score (log10) >3.0 are highlighted and labelled accordingly.

Note: C = periphyton; SUC = soil under periphyton. The data are means (n = 3).

Figure 4 The results of *cbbL* sequencing. (a) Venn diagram of OTUs distribution; (b) the box plot of alpha diversity indices; (c) the relative abundance of most important taxa; (d) the relative abundance of the top ten genera; (e) cladogram of LEfSe analysis. Each dot represents a taxon. Dots are marked for significant (LEfSe: $p<0.05$) enrichment in the periphyton (red) or the soil under periphyton (olive green). The size of each dot is proportional to the effect size; (f) histogram of LEfSe analysis. Taxa that reached a linear discriminant analysis score (log10) >3.0 are highlighted and labelled accordingly.

Note: C = periphyton; SUC = soil under periphyton; CK = unplanted control. The data are means (n = 3). Different letters within a phylum or genus indicate statistically significant ($P \leq 0.05$) differences among the sample types.

Figure 5 The results of *cbbM* sequencing. (a) Venn diagram of OTUs distribution; (b) the box plot of alpha diversity indices; (c) the relative abundance of most important taxa; (d) the relative abundance of the top ten genera; (e) cladogram of LEfSe analysis. Each dot represents a taxon. Dots are marked for significant (LEfSe: $p<0.05$) enrichment in the periphyton (red) or the soil under periphyton (olive green). The size of each dot is

proportional to the effect size; (f) histogram of LEfSe analysis. Taxa that reached a linear discriminant analysis score (log10) >3.0 are highlighted and labelled accordingly.

Note: C = periphyton; SUC = soil under periphyton. The data are means (n = 3).

Figure 6 The results of *nifH* sequencing. (a) Venn diagram of OTUs distribution; (b) the box plot of alpha diversity indices; (c) the relative abundance of the top three phyla; (d) the relative abundance of the top ten genera; (e) cladogram of LEfSe analysis. Each dot represents a taxon. Dots are marked for significant (LEfSe: $p<0.05$) enrichment in the control group (olive green), periphyton (red) or the soil under periphyton (blue). The size of each dot is proportional to the effect size; (f) histogram of LEfSe analysis. Taxa that reached a linear discriminant analysis score (log10) >3.0 are highlighted and labelled accordingly.

Note: C = periphyton; SUC = soil under periphyton; CK = unplanted control. The data are means (n = 3). Different letters in a phylum or a genus indicate statistically significant ($P \leq 0.05$) differences among the sample types.

Figure 7 Spearman correlation coefficient matrix of relative microbial abundance and soil properties based on sequencing (a) 16s rRNA, (b) *cbbL* and (c) *nifH*. Blue represents positive and red negative correlations. The cross indicates non-significant (at $\alpha=0.05$) p-values.