Received: 1 December 2020

Revised: 16 February 2021

(wileyonlinelibrary.com) DOI 10.1002/jsfa.11319

# Soil bacterial microbiota predetermines rice yield in reclaiming saline-sodic soils leached with brackish ice

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#### Abstract

BACKGROUND: Saline-sodic lands threaten the food supply and ecological security in the western Songnen Plain of northeast China, and the gypsum is commonly adopted for restoration. However, the dynamics of soil bacterial community and the correlation with crop yield during restoring processes remain poorly understood. Here, we elucidated the soil chemical properties and bacterial communities and their associations with rice yield under different flue gas desulphurization gypsum (FGDG) application rates combined with brackish ice leaching.

RESULTS: The increased application rate of FGDG generally improved soil reclamation effects, as indicated by soil chemical properties, bacterial diversity, and rice yield. Compared with fresh ice irrigation, the rice yield in brackish ice treatment increased by 15.84%, and the soil alkalinity and sodium adsorption ratio (SAR) decreased by 35.19% and 10.30%, respectively. The bacterial alpha diversity significantly correlated and predicted rice yield as early as brackish ice melt, suggesting the bacterial diversity was a sensitive indicator in predicting rice yield. Meanwhile, the bacterial communities in the control possessed a high abundance of oligotrophic Firmicutes, while eutrophic bacterial taxa (e.g. Proteobacteria) were enriched after brackish water irrigation and FGDG application. Moreover, we also established a Random Forest model and identified a bacterial consortium that explained an 80.0% variance of rice yield.

CONCLUSION: Together, our results highlight the reclaiming effect of brackish ice in the saline-sodic field and demonstrate the sensitivity and importance of the soil bacterial community in predicting crop yield, which would provide essential knowledge on the soil quality indication and bio-fertilizer development for soil reclamation. © 2021 Society of Chemical Industry.

Supporting information may be found in the online version of this article.

Keywords: saline-sodic soil; brackish ice; flue gas desulphurization gypsum; bacterial community; Random Forest

#### INTRODUCTION

Soil salinization is a global concern that threatens the food and fiber supply.<sup>1,2</sup> Saline land covers an area of about  $1 \times 10^9$  hm<sup>2</sup> worldwide.<sup>3</sup> Compared with other soil salinization types, the properties of saline-sodic soils are even worse,<sup>4,5</sup> while the related studies are scarce. Saline-sodic soil in the Songnen Plain of northeast China is one of the major saline-sodic lands across the world, which covers an area of over  $5 \times 10^6$  hm<sup>2</sup> and has progressed rapidly in recent decades.<sup>5</sup> In contrast to commonly coastal saline soils, where sodium chloride (NaCl) is the main obstacle factor, saline-sodic soil, however, is usually associated with a high amount of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and sodium hydrogen carbonate (NaHCO<sub>3</sub>).<sup>6</sup> The excessive sodium ion (Na<sup>+</sup>) in soil colloids will lead to clay dispersion and pore blockage, thus inhibits soil permeability and nutrient dispersion,<sup>7</sup> which further adversely affects the aboveground vegetation.<sup>8</sup> Sodium stress causes more severe damage to seed germination than salt stress.<sup>9</sup> For example, when rice seedlings are subjected to sodium stress, massive amounts of Na<sup>+</sup> accumulate from the root to the stem,<sup>10</sup> thus resulting in a poisoning effect on rice growth, affecting the

intracellular partial pressure of carbon dioxide (CO<sub>2</sub>),<sup>11</sup> and reducing the photosynthetic transformation rate. Since many areas are at risk of vegetation degradation and yield reduction, reclaiming the saline-sodic soils is a major task in this area, and the solutions need to be cost-effective and time-effective.

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The improvement methods of saline-sodic soil include engineering measures, physicochemical and biological measures.<sup>12</sup> Generally, chemical amendment is the most commonly adopted reclamation method of saline-sodic soils as it is more efficient and affordable.<sup>13</sup> Many studies have proved that the addition of flue gas desulphurization gypsum (FGDG) and other divalent cation containing amendments [e.g. calcium ion (Ca<sup>2+</sup>), magnesium ion (Mg<sup>2+</sup>)] can significantly decrease the excessive Na<sup>+</sup> in the soil, reducing the swelling of clay particles, and stabilizing the soil structure.<sup>14,15</sup> It has been reported that soil amended with FGDG is an effective method on reclamation of saline-sodic soils as soil electrical conductivity (EC), sodium adsorption ratio (SAR), exchangeable sodium percentage (ESP) and pH decrease in a short period.<sup>16</sup> Rice is the first alternative crop after soil reclamation since the continuous ponding water irrigation during the rice growth stage can leach the excessive Na<sup>+</sup> out of the root zone, thus improving the reclamation effect.<sup>17</sup> Furthermore, rice planting can reclaim the saline-sodic soils by inhibiting the upward salt transport and enhancing the accumulation of soil organic matter.<sup>18</sup> Studies have also included that microbial inoculant is a promising method to treat saline-sodic soils. For example, Bacillus spp., Aspergillus spp., and Alternaria spp. inoculant increased soilsaturated hydraulic conductivity.<sup>19</sup> However, the extreme level of soil salinity/sodicity might limit the survival of microbial consortia. Moreover, saline-sodic fields usually lack freshwater, therefore, a critical problem regarding the reclamation of saline-sodic soils concerns how to improve saline-sodic soil and ensure agricultural yield without impairing freshwater resources.

An emerging strategy to improve saline-sodic soils is through salt leaching after melting the freezing saline water.<sup>6,20</sup> Recent laboratory studies revealed that melted saline ice involved a high concentration of electrolytes at the beginning, while the remainder of the melted water contained a lower amount of salt.<sup>6,20</sup> Soil column studies further confirmed that saline water melting increased soil moisture and effectively reduced the salt content.<sup>20</sup> However, the field study on saline-sodic soil restoration effect and the rice yield after brackish ice irrigation has not been previously investigated. We then proposed a combined method to provide an abundant of Ca<sup>2+</sup> through FGDG application and leached with brackish ice, which might achieve a win–win effect of increasing the replacement rate of Na<sup>+</sup> with favorable salts and providing water during the dry spring period.

To date, the evaluating methods of soil fertility and productivity include the physiochemical and microbial characters, as well as the plant yield and quality. The physiochemical properties of soil act as direct reflection of the reclamation effect of saline-sodic soils. However, the response of the soil physiochemical properties usually lagged behind microbial activity because of the sensitivity of the microbial community under perturbations.<sup>3,21</sup> Soil microbiota play an indispensable role in soil fertility and plant productivity and are involved in the carbon and nutrient cycling processes.<sup>22,23</sup> It was reported that soil enzyme activities could act as an indicator that reflected soil fertility in saline-sodic soils.<sup>21</sup> Moreover, the microbial activity and biomass could be recovered rapidly after the salt leaching.<sup>24</sup> Although the microbial pattern of biomass and enzyme activity have been thoroughly studied, the microbial community, especially the fine microbial details under the reclamation processes of saline-sodic soils by infiltration of brackish ice meltwater, has not been well documented and remains to be elucidated.

Here, we designed a field experiment to study the short-term effect of combined FGDG and brackish ice on soil chemical property, bacterial community, and rice yield. In this study, we aimed to (i) evaluate the effects of soil chemical properties and rice yield components under combined FGDG and brackish ice treatments, (ii) unveil the soil microbial diversity, community composition, and correlations with soil chemical properties, and (iii) find the soil chemical and microbial properties that could predict rice yield. We hypothesized that (i) the combination of FGDG and brackish ice would be more effective than freshwater ice in reclaiming soil chemical and microbial characters and improving rice yield, (ii) microbial diversity and community were among the most sensitive and important predictors of rice yield and the early microbial community was more predictable than other properties.

#### MATERIALS AND METHODS

#### Description of the study area

The field experiment was carried out at the Da'an Sodic Land Experimental Station, Chinese Academy of Sciences (45°35'58" N~45°36'28" N, 123°50'27" E~123°51'31" E), located in the western part of the Songnen Plain, northeast China. The region has a temperate zone continental monsoon climate. The spring is dry and windy, with large potential evapotranspiration, and the winter is dry and cold, with a long glaciation period (as low as -20 to -40 °C) for about 5 months. The annual mean temperature in this area is 4~5 °C and the annual mean precipitation is 300~400 mm. However, the evaporation is about 1800 mm, which is 5-6 times that of the precipitation. As a consequence, the freshwater resources are lacking, but the shallow groundwater resources are abundant.<sup>25</sup> The original vegetation type is Puccinellia distans (L.) Parl. and the soil in this area is typically a severe saline-sodic soil, and its pH, EC, Na<sup>+</sup>, and  $CO_3^{2-}$  concentrations are significantly higher than those of normal soils (Supporting Information, Table S1).

#### Experimental design and field management

The soil at the field experimental site was reclaimed in autumn of 2018, and the soil had not been cultivated until the experiment setup. Each of the experimental plots occupied  $150.5 \text{ m}^2$  (7 m long and 21.5 m wide) and a ridge (0.8 wide and 0.5 high) was set to avoid lateral seepage and overflow. The interval between each plot was 1.2 m. The six treatments were as follows and three replicates for each treatment were adopted in a randomized block design.

- CK: No FGDG amendment and no ice irrigation;
- T1: 25% GR (FGDG 7.5 t  $ha^{-1}$ ) + brackish ice irrigation;
- T2: 50% GR (FGDG 15 t  $ha^{-1}$ ) + brackish ice irrigation;
- T3: 75% GR (FGDG 22.5 t  $ha^{-1}$ ) + brackish ice irrigation;
- T4: 100% GR (FGDG 30 t  $ha^{-1}$ ) + brackish ice irrigation;
- T5: 100% GR (FGDG 30 t  $ha^{-1}$ ) + fresh ice irrigation.

The fraction gypsum requirement (GR) was calculated by the following formula<sup>26</sup>:

GR 
$$(tha^{-1}) = 0.0086FD_s \rho_b (E_{Nai} - E_{Naf}).$$

where *F* is the dimensionless Ca–Na exchange coefficient, *F* is 1.3 here since the  $E_{\text{Naf}}$  is 5 [ $E_{\text{Naf}}$  is the final exchangeable sodium in soil (in cmol<sub>c</sub> kg<sup>-1</sup>);  $E_{\text{Nai}}$  is the initial exchangeable sodium in soil (in cmol<sub>c</sub> kg<sup>-1</sup>)];  $D_{\text{s}} = 0.2$  m (depth of ameliorated soil),  $\rho_{\text{b}} = 1.5$  g cm<sup>-3</sup> (soil bulk density of ploughing layer).

Before irrigation, different amounts of FGDG were evenly spread on the soil surface of each plot and then incorporated into the



0–20 cm soil layer with a rotary cultivator. In December of 2018, when the air temperature was around -20 to -30 °C, the shallow groundwater (brackish water, Table S1) in the saline-sodic soil area was extracted for the purpose of irrigation and the water was frozen in the corresponding plots (except T5 and CK treatments). Brackish water icing was conducted for four times at each plot with a 50 mm depth of water each time, which was equivalent to about 2 PV (pore volume) irrigation water. The covered brackish ice melts and infiltrates were completed in the middle of April every year. Each experimental plot was flooded with freshwater at the end of May in 2019 and kept with a ponding water depth of 3 to 5 cm. The rice was transplanted in early June. During the period of rice growth, the saline-sodic soil was flushed once by irrigation and drainage with fresh water. Rice variety (Baijing #1) and agronomy techniques were consistent among each plot.

#### Soil sampling and measurement of soil chemical properties

Soil sampling was performed before the rice was transplanted (May 14, 2019) and during the rice vegetative stage (July 14, 2019). Three randomly placed soil cores (0-20 cm) were taken from each plot, and the three soil cores were mixed to make a composite sample. Eighteen soil samples were obtained for each sampling time, with a final 36 samples in total. We divided all the soil samples into two groups: one (200 g) was air-dried and used for analysis of soil chemical properties; the other (20 g) was stored at -40 °C for DNA extraction. Soil electrical conductivity (EC<sub>1:5</sub>),  $pH_{1:5}$  and soluble salts were measured by 1:5 soil-water suspension.<sup>27</sup> Soil pH and EC were determined using a pH meter (PHSJ-3F, Shanghai Electronic Scientific Instrument Co., Ltd, Shanghai, China) and conductivity meter (DDS-120 W, Shanghai Electronic Scientific Instrument Co., Ltd), respectively. The concentration of soluble Na<sup>+</sup> was analyzed using a flame photometer (FP6410, Shanghai Electronic Scientific Instrument Co., Ltd), while  $Ca^{2+}$  and  $Mg^{2+}$  were measured through titration with ethylenediaminetetraacetic acid (EDTA).<sup>28</sup> Soluble CO<sub>3</sub><sup>2-</sup> and HCO<sub>3</sub><sup>-</sup> were analyzed through dilute acid titration. SAR and alkalinity were calculated using the following equations, where the concentrations of cations were expressed in  $mmol_c L^{-1}$  (same as milliequivalent per liter).<sup>29,30</sup>

$$SAR = \frac{[Na^{+}]}{\sqrt{\frac{[Ca^{2+} + Mg^{2+}]}{2}}} (mmol_{c}L^{-1})$$
  
Alkalinity = CO<sub>3</sub><sup>2-</sup> + HCO<sub>3</sub><sup>-</sup> (mmol\_{c}L^{-1})

#### Rice harvest and yield measurements

After naturally drying, rice grain was harvested for each plot to calculate the rice yield, thousand-grain weight (TGW), the number of filled grain per panicle (NFGP) and the number of unfilled grain per panicle (UFGP) in early October of 2019. The NFGP and UFGP were separated by ammonium sulfate solution with gravity of 1.06,<sup>31</sup> and the TGW, NFGP and UFGP were calculated by manually counting.

# High-throughput sequencing and data processing of the soil bacterial community

Soil microbial DNA was extracted from 0.4 g fresh soil using PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The extracted DNA was stored at -20 °C for downstream analysis. Highthroughput sequencing of the soil bacterial microbiota was conducted on the Illumina Novaseq PE250 platform by targeting the V4 region of 16S rRNA gene of bacteria. The primer pair 515F/806R with different barcoded sequences was used to amplify the targeted region through polymerase chain reaction (PCR). After PCR, the products were further purified and pooled in equimolar concentrations to employ the sequencing procedure on the platform.

The raw sequences of each sample were processed in QIIME 1.9.0 using USEARCH.<sup>32</sup> After truncating the primer nucleotides and the end of the reads with a nucleotide quality score < 30, the trimmed reads were merged. The merged sequences were filtered with an expected error higher than 0.5. The sequences were denoised to remove chimeras and singletons using UNOISE3.33 The denoised representative sequences were recognized as 'zero-radius' operational taxonomic units (OTUs) at 100% identity. Sequences of each sample were rarefied to 63 000 to make the OTU table for further analysis. Representative sequences of each OTU were annotated through blasting on SILVA 128 database in QIIME 1.9.0.<sup>34</sup> The microbial composition was classified by script summarize\_taxa.py. Alpha diversity indices including Observed\_species, ACE, Chao1, and PD\_whole\_tree were calculated using the alpha\_diversity.py script. Beta diversity analysis was conducted using the beta\_diversity.py script based on weighted\_unifrac distance, and non-metric multidimensional scaling (NMDS) method was used to visualize the beta diversity by script nmds. py. The raw sequences were deposited under the accession number PRJNA681295 in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA).

# Random Forest regression model and microbial network visualization

Random Forest regression model was employed to find the significant predictors of microbial alpha diversity, soil chemical properties, and microbial composition on rice yield. The prediction of rice yield by bacterial alpha diversity and soil chemical properties in two sampling stages was conducted using the *rfPermute* package on the R platform (http://cran.r-project.org/). Significant predictors and total explained variance were calculated through 1000 trees with 500 replicates. To build a Random Forest model at the OTU level, rare OTUs with a relative abundance lower than 0.05% were deleted. The samples were separated into two parts: 70% of the samples were classified as a training dataset, while the remainder of the samples were classified as a testing dataset. The importance of each OTU was evaluated and ranked, and the OTU consortia with the smallest amount of cross-validated error rate were selected as OTU predictors by the *randomForest* package.

The co-occurrence network among selected OTUs was conducted in Cytoscape 3.7.1 using the CoNet plug-in.<sup>35,36</sup> To build the network, each of the OTUs that appeared in less than half of samples was filtered, and the Spearman correlation values between two OTUs higher than 0.6 were selected for further analysis. We conducted the edge Score permutation with 100 iterations to avoid false-positive correlations and resultant distribution refined with 1000 bootstraps. Then, we computed the *P* value of multiple test correction at less than 0.05 based on Benjamini–Hochberg method and then merged the *P* value using a brown test.<sup>37</sup> The network was visualized in Gephi-0.9.2 and the color and size of the nodes were based on the taxonomy and degree.

#### Statistical analysis

The one-way ANOVA (analysis of variance) and Duncan test were conducted to compare the differences in rice yield, rice yield components, soil chemical properties, and bacterial alpha diversities among treatments. To meet the statistical premise of the homoscedasticity of data, the data was passed through the Levene's test based on the raw or log-transformed data before the ANOVA and Duncan tests. The correlation and significance between the soil chemical properties/bacterial alpha diversity and rice yield were calculated using the Pearson correlation coefficient at P < 0.05. Through the *vegan* package in R, permutational multivariate analysis of variance (PERMANOVA) was used to test the influence of different treatments and sampling stages on the bacterial community. Redundant analysis (RDA) was performed to correlate the bacterial community with soil chemical properties. Significantly correlated soil chemical properties with bacterial community were marked with an asterisk (P < 0.05 corresponding to '\*' and P < 0.01 corresponding to '\*\*'). The linear discriminant analysis (LDA) effect size (LEfSe) was calculated to find microbial biomarkers that significantly increased in different treatments at different levels with logarithmic LDA score > 2.0 and Kruskal-Wallis test P value <0.05 (http://huttenhower.sph. harvard.edu/galaxy).

### RESULTS

# Effects of brackish ice and FGDG application on rice yield components, economic benefits, and soil chemical properties

After the rice harvest, the total yield, TGW, NFGP, and UFGP were measured to quantitively represent the short-term reclamation effect of FGDG and brackish ice on the saline-sodic soil (Fig. 1, Supporting Information, Fig. S1). The results showed that different treatments significantly affected rice yield components, including yield, TGW, NFGP, and UFGP (P < 0.001). The rice yield improved with the increase of the FGDG application rate and became the highest in the T4 treatment (up to 3707 kg ha<sup>-1</sup>), whereas the control treatment without reclamation measures only yielded 459 kg ha<sup>-1</sup> of rice. The rice yield in the T5 treatment, in which the FGDG application rate was comparable with the T5 treatment.

Meanwhile, there was no significant difference between the control and T1 treatment, which suggested that a low amount of FGDG amendment could not reach the ameliorating effect of saline-sodic soil. The regression model between the rice yield and FGDG application rate was further constructed to figure out the replacement effect of brackish ice on reducing the FGDG application rate (Fig. 1(b)). It showed that the FGDG application rate positively correlated with the rice yield ( $R^2 = 0.863$ , P < 0.001) and that brackish ice reduced 7% of FGDG amount to reach the same yield compared with freshwater ice (Fig. 1(b)). Similarly, the TGW (ranging from 13.54 to 22.13 g) and NFGP (ranging from 13.44 to 72.56 g) of rice exhibited a similar pattern that increased with the FGDG application rate, while the UFGP of the rice was negatively correlated with the rice yield (Fig. S1). All regression curves between the T1 to T4 treatments illustrated that the values in the T5 treatment were comparable to the T3 or T4 treatments.

To evaluate the net income after the rice harvest, we conducted an economic analysis of the cost and benefit in different components of each treatment (Table 1). The cost of rice planting and FGDG application were 774.5 US\$  $ha^{-1}$  and 7.745 US\$  $t^{-1}$ , and the average price of rice on the market was 0.4647 US\$  $kg^{-1}$ . After calculation, the results showed that the net income of CK, T1 and T2 treatments was negative, thus it was not recommended to be applied for agricultural production. The treatments of T3, T4 and T5 could receive positive economic benefit, and the T4 treatment gained the highest net benefits to 715.56 US\$  $ha^{-1}$ , which was 588.39 US\$  $ha^{-1}$  and 235.43 US\$  $ha^{-1}$  higher than that in T3 and T5 treatments, respectively.

We also measured the soil chemical properties, including soil pH, EC, SAR, alkalinity, and ion contents before the rice transplant (in the middle of May) and during the vegetative stage of the rice (in the middle of July) (Figs 2 and S2). Generally, significant differences were observed in the soil chemical properties between two sampling dates, and the total salt leached across the season was 3.12 g kg<sup>-1</sup>. Soil pH, EC, SAR, and alkalinity were greatly reduced by 1.20 pH units, 62.0%, 48.5%, and 64.1% in the vegetative stage



**Figure 1.** The effect of brackish ice meltwater irrigation and flue gas desulphurization gypsum (FGDG) application on rice yield (a) and regression between FGDG application rate and rice yield (b). The mean  $\pm$  standard error of yield was presented for each treatment (n = 3) and the raw data was log-transformed to pass through the homoscedasticity test. The one-way analysis of variance (ANOVA) and Duncan tests were applied to study the differences among treatments. Different letters represent significant difference (P < 0.05) by Duncan test.

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**Table 1.** Economic analysis of different soil reclamation treatments with rice cropping (US\$  $ha^{-1}$ ). The mean  $\pm$  standard error are presented

Treatment	Cost	Gross income	Net income	
СК	774.5	213.21 ± 42.37	-561.29 ± 42.37	
T1	832.59	238.47 <u>+</u> 11.15	-594.12 <u>+</u> 11.15	
T2	890.68	414.51 <u>+</u> 22.59	-476.17 ± 22.59	
Т3	948.76	1075.93 <u>+</u> 198.43	127.17 <u>+</u> 198.43	
T4	1006.85	1722.42 <u>+</u> 172.64	715.56 <u>+</u> 172.64	
Т5	1006.85	1486.98 ± 154.82	480.13 ± 154.82	

of the rice crop than that before the rice transplanting stage, respectively. The soil pH, ranging from 9.2 to 10.0 in the middle of May, showed no significant differences between the treatments, while the soil pH value was significantly differentiated

during the vegetative stage of the rice crop and was the lowest in T4 treatment (7.9). Similarly, soil EC and SAR showed no significant difference among treatments before the rice transplant, while the T5 treatment significantly reduced soil EC and SAR during rice growth. We also observed a reduction in soil alkalinity under high FGDG application rate and found that it was the lowest in T4 treatment.

The relationship between the soil chemical properties and rice yield was evaluated to illustrate the potential effect of the chemical properties on rice yield (Figs 2(b) and S2(b)). It showed that no single chemical property was significantly correlated with the rice yield before the rice transplanting stage (P > 0.05). However, during the vegetative stage of the rice, the correlation between the chemical properties and rice yield was more significant and soil pH and alkalinity were significantly negatively correlated with rice yield (P < 0.001). The correlation between rice yield and soil ion contents, including CO<sub>3</sub><sup>2–</sup>, HCO<sub>3</sub><sup>-–</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup> and Cl<sup>-</sup>, provided similar results since the soil ion contents before the rice



**Figure 2.** Effect of brackish ice meltwater irrigation and flue gas desulphurization gypsum (FGDG) application on soil chemical properties in different sampling stages (a) and the Pearson correlation between soil chemical properties and rice yield (b). The mean  $\pm$  standard error was presented for each treatment (n = 3), and the data was passed through the homoscedasticity test by the Levene's method based on the raw or log-transformed data before the analysis of variance (ANOVA) and Duncan tests. Different letters represent significant difference (P < 0.05) by Duncan test.

transplanting stage were not significantly correlated with the rice yield, while some ions, including  $CO_3^{2-}$ ,  $HCO_3^{-}$ ,  $K^+$ , and  $Ca^{2+}$  were significantly correlated with the rice yield during the vegetative stage (P < 0.05) (Fig. S2). These results suggested that the soil chemical properties were more differentiated during the vegetative stage of rice and were closely associated with rice yield.

# Soil bacterial diversity and correlations with chemical properties under different treatments

After data processing, 2 605 984 clean reads were clustered into 10 601 OTUs at 100% identity. The soil bacterial alpha diversities, including the PD\_whole\_tree, Observed species, Chao1, and ACE indexes, were calculated. Different bacterial diversity indexes showed a similar trend that increased with increasing FGDG amendments, especially in the T4 treatment, irrespective of the difference between the sampling stages (Fig. 3). Bacterial alpha

diversity significantly increased in the vegetative stage of the rice crop, indicating that rice planting generally promoted bacterial diversity. Specifically, bacterial alpha diversity in the T5 treatment was lower than that in the T4 treatment, suggesting that brackish ice meltwater irrigation increased the bacterial diversity more than freshwater ice. We also correlated bacterial alpha diversity indexes with rice yield in separate stages (Fig. 3(b)). In contrast to the soil chemical properties, all of the bacterial alpha diversity indexes were significantly positively correlated with rice yield in the first sampling stage before rice transplant (P < 0.01), while only the PD whole tree index was significantly positively correlated with rice yield (P < 0.05). To confirm this result, we predicted the rice yield in two sampling stages through soil chemical properties and microbial alpha diversity indexes based on a Random Forest model (Fig. 3(c)). The results further validated that the microbial alpha diversity was a significant predictor and explained



**Figure 3.** Soil bacterial alpha diversity and correlation and prediction on rice yield. Soil bacterial alpha diversity changed under different treatments (a). The Pearson correlation between alpha diversity indexes and yield (b). Random Forest prediction of rice yield by soil chemical properties and bacterial alpha diversity indexes in different sampling stages (c). The mean  $\pm$  standard error was presented for each treatment (n = 3), and the data was passed through the homoscedasticity test by the Levene's method before the analysis of variance (ANOVA) and Duncan tests. Different letters represent significant difference (P < 0.05) by Duncan test. Higher %IncMSE value represents a high percentage of the explained variance of rice yield, and the colored bar denotes the significantly predicted parameters.

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**Figure 4.** Soil bacterial beta diversity in different treatments (a) and the redundant analysis (RDA) between the bacterial community, soil chemical properties, and rice yield components (b). Non-metric multidimensional scaling (NMDS) analysis was performed to visualize soil bacterial beta diversity based on weighted\_unifrac distance. Permutational multivariate analysis of variance (PERMANOVA) was applied to study the significant difference and explained variance of different treatments and sampling stages on bacterial community. The labels indicate significant levels tested by PERMANOVA and RDA (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001).

41.94% of the total yield variance before the rice transplanting stage. However, the soil chemical properties in the vegetative stage of the rice crop, such as soil alkalinity, pH,  $HCO_3^-$ , and  $K^+$  contents, were significant predictors of rice yield and explained 44.25% of the total variance.

PERMANOVA test and NMDS analysis based on weighted\_unifrac distance of soil bacterial community revealed that different treatments and sampling stages co-affected the soil bacteria community (Fig. 4). In particular, the bacterial communities in the control treatment were significantly differentiated with other treatments, except T2 treatment (Table 2). Interestingly, the bacterial community of the T4 treatment showed a significant difference compared to other treatments, suggesting that the T4 treatment harbored a unique bacterial community. The bacterial communities in T5 treatment showed significant differences in the T4 treatment, whereas they showed similarities in the T2 and T3 treatments. This finding indicated that freshwater irrigation significantly changed the bacterial communities and was more comparable with treatments that reduced the gypsum amount (Table 2). Among the T1 to T3 treatments, however, there were no significant changes in these treatments, suggesting the low application amount of FGDG had a mirror impact on the bacterial community.

RDA further revealed the correlations between the bacterial community and the soil chemical properties (Fig. 4(b)). Generally,

soil pH, EC,  $CO_3^{2-}$ , and  $HCO_3^{-}$  were correlated with the soil bacterial community in all samples (P < 0.05), corresponding to the high value of these parameters before the rice transplant. Meanwhile, it showed that  $CO_3^{2-}$ ,  $Mg^{2+}$ ,  $CI^-$ , and rice yield were significantly correlated with bacterial community before the rice transplanting stage, while only pH was the significant parameter correlated with bacterial community in the rice's vegetative stage. Moreover, most of the soil chemical properties formed an

variance (PERMANOVA) test							
Treatment	СК	T1	T2	Т3	T4		
T1	**						
T2	n.s.	n.s.					
Т3	**	n.s.	n.s.				
T4	**	**	**	*			
T5	***	**	n.s.	n.s.	**		

Table 2. Pairwise comparison of the soil bacterial community

between treatments through permutational multivariate analysis of

Lack of significance was marked with n.s., and \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05.



**Figure 5.** Soil bacterial community composition and differences in different treatments of two sampling stages. Bacterial community composition at phylum level (a) and biomarkers in different treatments from phylum level to order level with logarithmic linear discriminant analysis (LDA) score > 2.0 and Kruskal–Wallis test *P*-value <0.05 based on LDA effect size (LEfSe) analysis (b).

orthogonal axis with the rice yield components, corresponding to the uncorrelated results between the parameters.

# Effects of different treatments on soil bacterial community composition

The soil bacterial community was mainly composed of Proteobacteria, Bacteroidetes, Gemmatimonadetes, Firmicutes, and Chloroflexi, corresponding to 32.8%, 20.7%, 11.3%, 10.7%, and 5.8% of total sequences at the phylum level, respectively (Fig. 5(a)). In the first sampling stage before the rice transplant, the bacterial community was more dominated by Proteobacteria and Actinobacteria than in the vegetative stage, while Chloroflexi, Planctomycetes, and Verrucomicrobia were more prevalent in the vegetative stage.

LEfSe analysis revealed microbial biomarkers of different treatments from the phylum to order levels (Fig. 5(b)). For the control treatment, phylum Firmicutes were the main biomarkers under the two sampling stages, which suggested that the Firmicutes were more abundant in saline-sodic soils without reclamation. The treatment of brackish water irrigation amended with FGDG (T1–T4) mainly increased the abundance of Actinobacteria, Gemmatimonadetes, Proteobacteria, and Chlorobi. Specifically, the bacterial order, such as Acidimicrobiales, Euzebyales, Nitriliruptorales, Solirubrobacterales, Sphaerobacterales, Deinococcales, Longimicrobiales, Phycisphaerales, and Rhizobiales were biomarkers in



**Figure 6.** Prediction of the rice yield through the bacterial community in the first sampling stage at operational taxonomic unit (OTU) level based on the Random Forest regression model (a) and co-occurrence pattern among selected OTUs by Spearman correlation ( $\rho > 0.6$ , P < 0.05) (b).

the T1 treatment. *Rhodobacterales* and *Desulfuromonadales*, which belong to Proteobacteria, increased in the T2 treatment. The T3 treatment enriched *Lactobacillales*, *Rhodocyclales*, *Puniceicoccales*, and *Chthoniobacterales*. For the T4 treatment, *Chlorobiales*, *Caulobacterales*, *Burkholderiales*, *Hydrogenophilales*, *Nitrosomonadales*, *Bdellovibrionales*, *Campylobacterales*, *Xanthomonadales*, and *Opitutales* were more prevalent at the order level. However, we did not observe any biomarkers at the phylum level in the T5 treatment, while *Flavobacteriales* and *Pseudomodales* were more abundant in the T5 treatment at the order level than other treatments.

# Predicting rice yield by microbial communities through the Random Forest regression model

Although bacterial alpha diversity before the rice transplant and chemical properties in the vegetative stage were significantly correlated with rice yield, the explained variance of the rice yield was still low. The Random Forest regression model was then used to predict the rice yield by the soil bacterial community at the OTU level (Fig. 6). The consortia of 25 OTUs with a low cross-validation error rate in the first sampling stage were chosen to predict the rice yield with 80.0% explained variance (Fig. 6(a)). These OTUs comprised phylum Actinobacteria (two OTUs), Bacteroidetes (seven OTUs), Firmicutes (seven OTUs), and Proteobacteria (nine OTUs). Heatmap analysis showed that most of the selected OTUs belonging to the Firmicutes were more prevalent in samples with a low rice yield, whereas Bacteroidetes and Proteobacteria OTUs were more abundant in high yield samples. Interestingly, four Bacteroidetes OTUs belonging to the genus Flavobacterium (OTU4075, OTU549, OTU3418, and OTU610) were abundant in high yield samples as compared to samples with a low rice yield, suggesting that Flavobacterium played an important role in determining the rice yield. Meanwhile, the most important OTU predictor was OTU1454 (Erythrobacter) with the highest IncNodePurity value, followed by OTU612 (Hydrogenophaga), OTU610 (Flavobacterium), OTU66 (Carnobacterium), and OTU1205 (Paucimonas).

After further correlating the relative abundance of selected OTUs with the rice yield, the Spearman correlation results illustrated that most of the OTUs (16 out of 25) in the Random Forest regression model were significantly correlated with rice yield (P < 0.05, Fig. S3). Moreover, the network analysis among selected OTUs showed that most of the bacterial OTUs were involved in the network (23 out of 25 OTUs), and most connections were positive (87%) (Fig. 6(b)). Among keystone OTUs with high degrees, most of the OTUs belonged to Bacteroidetes (six out of nine OTUs with a degree over eight), indicating that Bacteroidetes OTUs played a key role in shaping the interconnected microbial consortia. A Random Forest regression model was also applied to samples collected during the vegetative stage of the rice crop and all samples from two sampling stages (Fig. S4). Although different OTUs were identified in different predicting models, the explained variances ranged from 70.5% to 71.5%, further confirming that the bacterial communities from the first sampling stage predetermined the rice yield better than chemical properties and microbial communities in the vegetative stage of the rice crop.

### DISCUSSION

### Brackish ice meltwater irrigation saved the amount of FGDG compared with freshwater ice

Due to the high content of Na<sup>+</sup> and high alkalinity, the soil particles in saline-sodic land are highly dispersed, which reduces the water infiltration rate and inhibits the root growth and nutrient availability of plants.<sup>7,8</sup> In this study, we compared the effect of irrigation by brackish ice combined with FGDG application on reclaiming heavy saline-sodic soil and rice planting in a short length of time. Our results demonstrated that the rice yield in brackish ice irrigation increased by 15.84%, and the soil alkalinity and SAR decreased by 14.30%, and 10.30%, respectively, compared with the freshwater ice irrigation treatment. It indicated that brackish ice irrigation was a promising method to reclaim the saline-sodic field and save freshwater resources. This could be attributed to the fact that gypsum with brackish ice irrigation increased the content of divalent cations (e.g. Ca<sup>2+</sup>) and the electrolyte concentration in the soil solution.<sup>15</sup> When the electrolyte concentration increases to a threshold concentration of flocculation, the fine particles combine easily to form larger aggregates,<sup>14</sup> thus improves soil permeability.<sup>38</sup> The improvement of soil infiltration performance further enhanced the leaching efficiency of excessive Na<sup>+</sup> and salts in the soil, which ameliorated the properties of saline-sodic soils. Similarly, the experiments in coastal saline soil also found that freshwater ice irrigation was less effective than brackish ice irrigation and could even result in soil alkalinization.<sup>37</sup> This is likely due to the low electrolyte concentration of freshwater ice and the meltwater will promote electrolyte losses on the soil particle surface, which, in turn, leads to clay dispersion and soil hydraulic conductivity reduction.<sup>39</sup> In addition, it should be pointed out that the rice yield is also inferior compared to brackish ice irrigation. Our results, which are based on a field experiment, provide basic evidence that brackish ice irrigation reclaims saline-sodic land and saves gypsum and freshwater compared with freshwater ice irrigation treatment, thus presents a win–win strategy to use local resources to ensure soil sustainability.

Moreover, when combined with a high amount of FGDG, brackish ice irrigation significantly reduced the salinity and alkalinity of soil, alleviated the salt and alkali stress on rice, and greatly increased the survival percentage and grain yield of rice.<sup>10</sup> In contrast, the rice yield in treatments with lower FGDG application rates (less than 15.0 t ha<sup>-1</sup>) showed no significant differences compared to the control treatment. Similarly, the soil chemical properties in treatments with a low FGDG rate also showed minor differences compared to the control treatment, which could be due to the insufficient amount of gypsum for fully replacing the excessive Na<sup>+</sup> in soil.<sup>2,16,17</sup> Thus, the unfavorable hydrophysical properties of the soil were not improved and the salt could not be washed out though brackish ice melting, which hindered the rice growth and yield.<sup>7,14,22,39</sup> All these results indicated that the rational application of gypsum was essential to reclaim the saline-sodic soils and increase rice yield.

# Bacterial diversity was an earlier determinant of rice yield than soil chemical properties

Soil physicochemical properties and microbial activity play a decisive role in plant growth and crop yield.<sup>3,40</sup> We observed that different treatments significantly changed the chemical properties in the soil, especially in the T4 or T5 treatments. Similarly, the soil bacterial alpha diversity also showed an improvement and reached the highest amount in the T4 treatment, and the alpha diversity in the T5 treatment decreased as compared to the T4 treatment, indicating the advantage of brackish ice irrigation on bio-diversity improvement. Recent studies have demonstrated that soil microbial diversity is a key factor in sustainable agriculture through driving nutrient cycling processes and maintaining soil health.<sup>12,23</sup> The increase in soil bacterial diversity in different treatments during short-term reclamation reflected soil quality improvement and high rice yield.<sup>24</sup>

Correlation analysis revealed that soil alkalinity, pH,  $CO_3^{2-}$ ,  $HCO_3^{-}$ ,  $K^+$ , and  $Ca^{2+}$  were significantly correlated with rice yield in the vegetative stage of the rice crop. It is not surprising that soil chemical properties during the vegetative stage of the rice crop interact with rice plants since soil supplies essential nutrients, whereas the sampling stage before rice transplanting has no direct interaction with plants. However, the soil bacterial alpha diversity in both sampling stages was positively correlated with rice yield, especially before the rice transplanting stage, which thus indicated that the bacterial alpha diversity was an early predictor of rice yield. Soil microbial diversity is affected by soil properties, soil water, gas, and heat conditions, as well as inorganic nutrient and soil organic matter content.<sup>41-43</sup> Similar results based on the Random Forest regression model further confirmed our

results in the correlation analysis. Bacteria are relatively small and play a major role in nutrient cycling, while they are also sensitive indicators of environmental change, such as soil temperature and pH.<sup>41</sup> The strong correlation and prediction capacity of the bacterial diversity of rice yields suggested that microbial diversity could also be used as an indicator of the degree of reclamation of saline-sodic soils. It was reported that the microbial community responded quickly to salinity, and that the change of land use significantly altered the microbial community.<sup>21,24</sup> The increase of bacterial diversity in different treatments might accelerate nutrient turnover and supply during the following rice planting season, thereby enhancing rice yield.<sup>23</sup>

# Bacterial community changed under different treatments and precisely predicted rice yield

Brackish ice meltwater irrigation and FGDG application significantly altered the bacterial community, especially in the T4 treatment. Specifically, bacterial phylum Proteobacteria was less abundant in the control treatment than in other treatments, while it was a significant biomarker in T4 treatment, suggesting Proteobacteria was the indicator of the severity of soil salinity-sodicity. The Proteobacteria is commonly recognized as the eutrophic microorganism with an R-type growth strategy and is abundant in nutrient-rich soils.<sup>44</sup> However, the bacterial phylum Firmicutes are regarded as oligotrophic microorganisms that flourish in adverse environments.<sup>44</sup> The enrichment of phylum Firmicutes in the control treatment reflects the poor soil quality without reclamation. In addition, some plant beneficial bacteria were also enriched in different treatments. For example, bacterial order, including Burkholderiales and Nitrosomonadales, were biomarkers in the T4 treatment. The Nitrosomonadales are an ammoniaoxidizing bacteria (AOB) that enhances the nitrification process in soil.<sup>45</sup> Meanwhile, the bacterial species of Burkholderiales are well-known nitrogen-fixing bacteria, and also exhibit the antipathogenic abilities.<sup>46</sup> Members of the bacterial order *Rhizobiales* enriched in the T1 treatment are important symbiotic, nitrogenfixing bacteria that supply inorganic nitrogen to plants.<sup>46,47</sup> Additionally, the enriched bacterial orders Flavobacteriales and Pseudomodales in the T5 treatment are common plant growth-regulating bacteria that can improve plant performance.48,49 These results suggested that the different treatments, especially the treatments with high rice yield, recruited several different plant-beneficial bacteria that might promote the nutrient supply and health of rice seedlinas.

We selected specific bacterial consortia that predicted the rice yield with 80.0% of the variance explained, illustrating the importance of key species in the maintenance of soil functions. Similarly, it was reported that the microbial community predicted the plant health, yield, and developmental stage with high accuracy.<sup>50,51</sup> OTUs belonging to Firmicutes were clustered in low yield samples to predict rice yield, while Proteobacteria and Bacteroidetes OTUs were abundant in high yield samples. Of these OTUs, the Proteobacteria and Bacteroidetes were more closely interconnected, suggesting the high potential interaction among them. The intensified network association reflected more facilitation of the potential physiological metabolism,<sup>52</sup> which might be closely related to rice growth and development.

### CONCLUSIONS

In this study, we characterized soil chemical properties and bacterial communities under different reclamation treatments in a

saline-sodic field. Brackish ice performed better than freshwater ice as it both increased rice yield and saved freshwater resources. Bacterial community in the T4 treatment showed significant differences compared to other treatments and enriched the abundance of eutrophic Proteobacteria. The soil bacterial community responded earlier than the chemical properties, while it also predicted rice yield with a high accuracy, especially through the selected microbial consortia using the Random Forest algorithm. These observations provide a basic knowledge of field reclamation of saline-sodic soils from the chemical and microbial aspects. Our results suggest that the microbial community is an important early indicator of crop yield and future studies should focus on developing a more accurate yield forecast system and effective bio-fertilizers for sustainable agriculture.

### ACKNOWLEDGEMENTS

This research was supported by the National Key Research and Development Program of China (2016YFC0501200), the National Natural Science Foundation of China (Nos 41971066 and 41771250), the National Science and Technology Basic Work of China (2015FY110500), the Key Laboratory Foundation of Mollisols Agroecology (2020ZKHT-03), the Science-technology Development Initiative of Jilin Province (20200402005NC) and the CAS Key Technology Talent Program.

### **CONFLICT OF INTERESTS**

The authors declare no conflict of interest.

### SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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