Quantitative assessment of the potential for soil improvement by planting Miscanthus on saline-alkaline soil and the underlying microbial mechanism

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Abstract
Phytoremediation using Miscanthus is a valuable technique for the restoration of saline-alkaline soil, but the potential is not quantified. To assess this potential, a 5 years field experiment was conducted on saline-alkaline soils with three genotypes of Miscanthus and an unplanted control. The bulk soil (0–20-cm depth) physico-chemical properties were measured annually from 2015 to 2018, while the bacterial and fungal community structures of the rhizosphere soil were investigated by high-throughput sequencing after 5 years under Miscanthus (2018). The results showed that Miscanthus cultivation on saline-alkaline soil reduced soil salinization and improved soil fertility on this site. Specifically, compared with the blank control, the five-years Miscanthus cultivation (mean of three different genotypes pooled) decreased the soil pH by 0.33 units (8.78–8.45) and electrical conductivity by 23.4 μS/cm (226.7–203.3 μS/cm), as well as increasing soil organic matter content from 18.9 to 34.9 g/kg and total potassium content from 15.2 to 18.0 g/kg. In addition, the soil improvement was more pronounced with a longer duration of growth. Miscanthus remediates saline-alkaline soils by altering the microbial community compositional abundance rather than its diversity. The improvement is due to enrichment of beneficial bacteria leading to soil desalinization (e.g., by Gemmatimonadaceae), litter decomposition (e.g., by Chitinophagaceae), plant growth promotion (e.g., by Micrococcaceae), and the reduction of harmful fungi (e.g., Pleosporales and Chitinophagaceae). Soil pH, electrical conductivity, organic matter, and total potassium were the main physico-chemical properties affecting bacterial and fungal communities. Our results quantify the soil improvement potential of Miscanthus and clarify the underlying microbial mechanisms, laying a solid foundation for further implementation of saline-alkaline soil phytoremediation using Miscanthus. In the future, more extensive multi-location trials are required to assess if these improvements are seen across a wide diversity of environments.
1 INTRODUCTION

China is facing a conflict between population increase and a decline of cropland area, and this conflict will become more serious with the accelerating urbanization (Chen, Xu, et al., 2019; Chen, Li, et al., 2019; Li et al., 2017). Because of this dilemma, the search for a viable way to increase total food production potential is an urgent task for the Chinese government. In the past few decades, attention has mainly been paid to increasing food production via breeding improvements and optimization of agricultural techniques (Wu et al., 2014; Zhang et al., 2018). However, these technology-driven improvements have now reached a bottleneck, which is difficult to breakthrough. In order to ensure food security, increasing the land area devoted to food production is an option. Marginal lands conversion is a readily available way to increase cropland area, especially in areas with lighter environmental stresses (Cao et al., 2021). This approach has been included in the national strategy of “increasing food production based on farmland management and technological application.” However, a lack of economic, sustainable, and eco-friendly conversion solutions hinders the progress in marginal land conversion (Cao et al., 2021).

Of all the marginal land types in China, saline-alkaline land is the most suitable candidate for conversion to agricultural land (Wang et al., 2011). The majority of saline-alkaline land in China is located in the humid east of the country. In this area, the main environmental problem is limited to the saline-sodic condition and the area does not face the additional drought problem found in many other marginal land types mostly locating in northwest China. Additionally, it is generally characterized as a large flat area suitable for mechanized agriculture. This is also the reason why sea rice is currently being promoted in China. As a cost-effective and environmentally beneficial technique, phytoremediation is widely recommended to convert saline-alkaline land into agricultural land by improving the soil quality (Bharti et al., 2017; Jesus et al., 2015). However, the currently available plant species (e.g., Suaeda glauca Bunge) are generally small, resulting in a long period required to achieve the expected remediation result (Cao et al., 2014; Jing et al., 2019; Rathore et al., 2017). Moreover, the current species are generally of no commercial value, leading to high phytoremediation costs. To improve the implementation of phytoremediation in saline-alkaline soil, it is necessary to screen some effective species, especially those which can simultaneously produce additional economic value.

Miscanthus is a perennial genus producing high yield and having environmentally beneficial characteristics with multi-utilization potential. It is currently an important eco-industrial crop for the bio-refinery industry (Heaton et al., 2009; Lewandowski et al., 2003; Zheng et al., 2019). Its strong tolerance to various environmental stresses such as heavy metal contamination (Barbosa et al., 2015; Sun et al., 2021), flooding (Mann et al., 2013), and drought (Shepherd et al., 2020) suggests a potential for phytoremediation use. More importantly, the large amount of biomass it produces can generate economic benefits. Miscanthus is, therefore, a promising species for phytoremediation generally and may be suitable for saline-alkaline land (Plażek et al., 2014), but there is a lack of quantitative information to confirm realizable benefits. This study is, therefore, designed to quantitatively assess the effects of long-term Miscanthus cultivation on the improvement of soil quality of saline-alkaline land.

Understanding how Miscanthus cultivation can improve soil quality will help to promote its application in saline-alkaline soil remediation. Plant–microbe interactions can greatly influence plant tolerance to salt stress (Kumar & Verma, 2018). Previous studies have demonstrated that plant growth-promoting bacteria, halotolerant bacteria, and arbuscular mycorrhizal fungi can facilitate plants’ adaptation to saline stress by promoting nutrient uptake, water absorption capacity and osmolyte accumulation (Dimkpa et al., 2009; Hanin et al., 2016; Ramadoss et al., 2013). Furthermore, soil microbes are directly associated with the physicochemical characteristics of the surface soil by regulating organic matter mineralization and essential nutrient transformation to plants (Chen et al., 2020; Maarastawi et al., 2018). Previous studies involving phytoremediation of saline-alkaline soil demonstrated that salt-tolerant plants can strongly influence soil physicochemical properties (Akhter et al., 2004; Wang et al., 2020). Thus, the interaction between plants and soil microbes may help to improve soil quality. However, a knowledge gap still exists regarding the responses of the microbial community to phytoremediation of marginal land, especially saline-alkaline land. Investigation of the microbial community composition and the factors that affect it can help to better understand the underlying mechanism of soil restoration, thereby enabling the adoption of effective strategies to improve phytoremediation. The underlying microbial mechanism of saline-alkaline land phytoremediation is also, therefore, explored in this study.
2 | MATERIAL AND METHODS

2.1 | Experimental design and sampling strategies

The field experiment was established in 2014 at the Hunan Agricultural University experimental site located near the Bohai Sea, Binzhou, Shandong province, China (37°38′N, 118°07′E, 11.4 m a.s.l.). The field is within a large-area (approximately 950 ha) abandoned barren land with no human disturbance and dominated by the species of Puccinellia chinamponensis Ohwi (herbs) and Suaeda salsa (L.) Pall (i.e., the weed free bare ground). The soil (collected in May 2014 with a depth of 0–20 cm) is clay soil with a pH of 9.60, electrical conductivity of 966.0 μS/cm, total organic matter content of 10.02 g/kg, total nitrogen content of 2.00 g/kg, total phosphorus content of 0.47 g/kg, and total potassium content of 24.66 g/kg. It is classified as moderately saline-alkaline according to Zheng et al. (2019). Also, the climatic conditions including monthly average air temperature and monthly rainfall during the Miscanthus growing seasons are presented in Table S1.

A randomized complete one-factor design was adopted, including three Miscanthus hybrids (hereafter named G1 to G3) with an additional unplanted treatment CK (i.e., the original abandoned barren land). Each treatment was repeated three times in the field. The hybrids are the offspring of Miscanthus sinensis Andersson (♀) and Miscanthus lutarioriparius Liu S. L. Chen (♂), and detailed plant information is presented in Table S2, including biomass yield and field performance traits. Each genotype was planted in a 5 × 7.5 m plot in July 2014, using 300 g of rhizome per plant at a spacing of 1.0 m between plants and 1.5 m between rows. Soil tillage was carried out just before planting and the plots were irrigated once at the establishment day. To minimize weed competition, manual weeding was conducted twice, in June and September 2014. After planting, only one harvest per year (in late November to early December) was carried out and the harvested biomass was gathered stacking on edge of the field. The following soil sampling was not conducted within this biomass field storage area to keep the same effect as biomass removed from the field. No other management practices (e.g., irrigation, weeding, and pest control) were used from the 2015 growing season onwards.

From 2015 to 2018, bulk soil was collected annually during harvest for analysis of soil fertility and salinization changes. In December 2018, rhizosphere soil was collected for microbial (including fungal and bacterial) community analysis using the high-throughput sequencing method. Each composite rhizosphere and bulk soil sample was a mixture collected from five plants within a plot, randomly selected in an “S” pattern. Bulk soil samples of each plant were obtained after harvesting biomass using a hand-operated soil core sampler (with an internal diameter of 4 cm) according to the following steps: (a) four soil cores (0–20-cm depth) in four positions (north, south, east, and west) at 10 cm from the plant’s center were collected and mixed to get a composite sample; (b) another four soil cores (0–20-cm depth) in four positions at 50 cm (i.e., the middle between plants) from the plant’s center were collected and mixed by the same method; (c) samples collected at 10 and 50 cm from the plant’s center were mixed and reduced (using the quartering method) to 100 g, representing the sample of each plant. Subsamples of the five plants within a plot were mixed and reduced to 200 g, representing the sample of each plot. The rhizosphere soil samples were collected from the soil remaining attached to the roots and rhizomes after shaking plants vigorously (Chaudhary et al., 2015). Plant debris and roots in the bulk and rhizosphere soil samples were manually removed, then sieved through a 2-mm mesh. The bulk soil samples were immediately transported to the laboratory and air-dried for soil chemical analysis. Rhizosphere soil samples were divided into two subsamples. One part was air-dried in the same way as the bulk soil samples and the other part (approximately 10 g) was transported in dry ice to the laboratory, then stored in liquid nitrogen until the high-throughput sequencing was carried out.

2.2 | Soil physicochemical properties analysis

The air-dried soil samples were ground and sieved through a 100-mesh sieve before determination of soil chemical properties. The physicochemical attributes analyzed included soil electrical conductivity (EC), pH, organic matter (OM), total nitrogen (TN), total phosphorus (TP), and total potassium (TK). The analytical methods for these properties are described in detail by Zheng et al. (2019).

2.3 | DNA extraction, PCR amplification, and high-throughput sequencing of soil samples

The high-throughput sequencing analysis generally included three steps: DNA extraction, PCR amplification and Illumina MiSeq sequencing. Detailed information on each step can be found in Wu et al. (2021).

2.4 | Statistical analysis

2.4.1 | Comparison of the soil improvement potentials of different Miscanthus genotypes on saline-alkaline soil

Comparisons of the soil quality indicators between genotypes were conducted using the ANOVA (analysis of variance)
procedure in SPSS 25.0 (IBM SPSS Inc., Chicago, IL, USA). For ANOVA analysis, different Miscanthus genotypes and year after planting were considered as independent variables. The mean of each trait was tested at the $p < 0.05$ level using the Duncan’s multiple range test.

Evaluation of the soil improvement potential from Miscanthus cultivation is a multi-criteria decision-making process that involves many factors. In the present study, a comprehensive soil quality index (CSQI) was established to express the soil quality, and then the soil-improving potential of three Miscanthus genotypes were compared by using CSQI. The CSQI of each genotype was defined as the average normalized values of the non-collinear related soil quality indicators, which were calculated according to Equations (1) or (2). Six indicators were finally screened and divided into a larger-is-better group including TN, TP, TK, and OM and a smaller-is-better group including EC and pH. The normalized values of the larger-is-better indicators were calculated using Equation (1), and the smaller-is-better indicators using Equation (2).

\[
y_i(k) = \frac{x_i(k) - \min x(k)}{\max x(k) - \min x(k)} \quad (1)
\]

\[
y_i(k) = \frac{\max x(k) - x_i(k)}{\max x(k) - \min x(k)} \quad (2)
\]

where $y_i(k)$ represents the normalized value of ith genotype in terms of the kth physicochemical trait (i.e., the soil quality indicator); $x_i(k)$ denotes the field-recorded value of ith genotype in terms of the kth physicochemical trait; max $x(k)$ and min $x(k)$ represent the largest and smallest value of the $x_i(k)$, respectively.

### 2.4.2 | Microbial sequencing data processing

The raw Illumina fastq sequencing reads were firstly demultiplexed, quality-filtered by Trimmomatic, and merged by FLASH using the detailed criteria described in Zhang, Riaz, et al. (2019). Microbial composition analysis was conducted on the Majorbio Cloud Platform (www.majorbio.com, assessed on October 10, 2020), which is built based on R packages. Rarefaction and Shannon index curves of the OTU number of the fungi and bacterial communities at 97% similarity were performed to check whether the sequencing data were sufficient and to characterize the bacterial and fungal richness. Non-metric multidimensional scaling (NMDS) was used to test the beta diversity among the four treatments, and significance was tested using ANOSIM analysis (Dixon, 2003). The relationship between soil microbial community structure and physicochemical properties was analyzed using the CCA (canonical correspondence analysis)/RDA (redundancy analysis) procedure based on the OTU level. All soil quality indicators pH, EC, OM, TN, TP, and TK were chosen for CCA/RDA due to their values of variance inflation factor all being less than 10 (the threshold value that represents collinearity between the tested soil physicochemical properties). The function of the fungal community of the soil samples was predicted using FUNGuild v1.0 (Nguyen et al., 2016) based on the OTU table. Twelve guilds were finally classified according to three trophic modes (pathotrophs, saprotrophs, and symbiotrophs). We considered only pathotrophs and saprotrophs due to their important roles in phytoremediation (Zhang, Yan, et al., 2019; Zhang, Sun, et al., 2019). Furthermore, only the prediction confidence levels of highly probable and probable were selected for further analysis. The FUNGuild program often assigns multiple functional guilds for a single OTU (e.g., saprotroph-plant pathogen), and such OTUs were calculated in both functional groups (Ye et al., 2021). The bacterial functional guilds were predicted based on the OTU table using FAPROTAX (Louca et al., 2016). Among all bacterial functional groups, only the relative abundances of groups that associated with nitrogen cycling and chemoheterotrophy were higher than 1% and significantly influenced soil quality. Thus, attention was focused only on the three main nitrogen cycling functional groups, diazotrophs, nitrifiers, and denitrifiers.

### 3 | RESULTS

#### 3.1 | Effect of Miscanthus cultivation on the physicochemical changes in saline-alkaline soil

Changes to physicochemical properties of the Miscanthus cultivation soils between different growing seasons (2015–2018) have an obvious trend as shown in Figure 1. Generally, soil EC (Figure 1a), pH (Figure 1b) and TK (Figure 1f) showed a significant ($p < 0.05$) decreasing trend with the duration of growth, while OM (Figure 1c), TN (Figure 1d) and TP (Figure 1e) showed a significant ($p < 0.05$) increasing trend. Soil after 5 years Miscanthus cultivation in 2018 showed on average 27.6% ($p < 0.001$) for the effect of Y, 4.6% ($p < 0.001$) and 4.7% ($p < 0.05$) lower EC (203.28 vs. 280.94 μS/cm), pH (8.45 vs. 8.86) and TK (18.07 vs. 18.97 g/kg), respectively, compared with that in the second year after establishment in 2015. Soils collected in 2018 showed on average 138.3%, 34.0%, and 21.4% higher OM (34.90 vs. 14.64 g/kg), TN (0.67 vs. 0.50 g/kg) and TP (0.85 vs. 0.70 g/kg), respectively, than in the growing season of 2015 with a significant ($p < 0.001$) effect of year (Y). Significant differences between genotypes (G) were only observed in terms of EC ($p < 0.001$), OM ($p < 0.001$), and TP ($p = 0.01$), but not pH ($p = 0.131$), TN ($p = 0.125$) and TK ($p = 0.091$). For the differences of TP between genotypes, significantly
(p = 0.010) higher contents were observed in G2, followed by G3 and G1 in all the data collection years, as indicated by the non-significant (p = 0.764) difference of G×Y interaction. However, for EC and OM, significant differences between genotypes varied in different growing seasons (p < 0.001 for the G×Y interaction). For example, in the growing season of 2015, the highest EC (312.83 μS/cm) was observed in the soil under G1 cultivation; while the lowest EC (224.83 μS/cm) was observed in the soil under G1 cultivation in 2016. Similarly, the highest OM content was observed in the G1 soil in 2015 (17.18 g/kg) and 2017 (32.96 g/kg); while the second-highest OM content was observed in the G1 soil in 2016 (26.61 g/kg) and 2018 (33.86 g/kg).

After long-term Miscanthus cultivation, the quality of the saline-alkaline soil was improved compared with the control without Miscanthus (i.e., CK in Table 1). The improved
quality was indicated by the reduced soil pH and EC, as well as increased soil OM and TK of the Miscanthus soil relative to the CK treatment. At the end of the 5th cultivation year (2018), the average (mean of three different genotypes pooled) soil pH and EC under Miscanthus cultivation was 3.8% (8.45 vs. 8.78) and 10.3% (203.28 vs. 226.67 μS/cm) lower (p < 0.05) than the CK, but 85.0% and 18.4% higher (p < 0.05) OM (34.89 vs. 18.86 g/kg) and TK (18.01 vs. 15.21 g/kg) than the CK. When the soil quality improvement potential between the three tested genotypes was compared, the trend among genotypes was not consistent for different soil quality indicators. In the growing season of 2018, the highest OM content (41.60 g/kg) was observed in the soil under G2 cultivation, followed by G1 (33.86 g/kg) and G3 (29.23 g/kg) with a significant difference (p < 0.05). However, for TK, G3 had the highest content of 18.78 g/kg, which was significantly (p < 0.05) higher than the G2 (17.75 g/kg) and G1 (17.68 g/kg) treatments. To fully compare the soil improvement potentials of different Miscanthus genotypes, a comprehensive soil quality index (CSQI) was used. Results showed that G3 had the best soil improvement potential among the three Miscanthus genotypes in this study as indicated by its highest CSQI values (range from 0.527 to 0.611) in all the four data collection years (Table 2). The soil improvement potentials of G1 and G2 varied in different years. G1 showed the worst soil improvement potential in 2015 and 2018 as suggested by its lowest CSQI values of 0.487 and 0.351, respectively; while the worst G2 soil improvement potential was observed in 2016 and 2017 with the lowest CSQI values of 0.473 and 0.550, respectively. To comprehensively evaluate the soil improvement potential of different genotypes over the years, a synthetic CSQI value was used, defined as the average CSQI value in the years 2015 to 2018. A general soil improvement potential by different Miscanthus genotypes was G3 (0.561), G2 (0.491), and G1 (0.472).

3.2 Effect of Miscanthus cultivation on the microbial structure of saline-alkaline soil

Bacterial and fungal richness indexes including Sobs, Chao1 and Shannon diversity index (expressing the α-diversity) are presented in Table S3. Good coverages, rarefaction curves showed high reliability and are representative of the sequencing results, at 97% similarity (Table S3). The above index indicated that the long-term Miscanthus cultivation on saline-alkaline soil did not have significant (p > 0.05) effects on the species richness and diversity index of both soils bacterial and fungal communities (Table S3).

To investigate the effect of Miscanthus cultivation on the microbial structure of saline-alkaline soil, β-diversity was examined using the NMDS analysis. Results showed that the Miscanthus cultivation did not significantly impact the composition of the fungal communities in the rhizosphere soil as suggested by the stress value <0.1, p > 0.05 (Figure 2). In contrast, significant (stress <0.1, p < 0.05) effects on the bacterial communities’ composition were observed. Among the four treatments, only samples of the G2 treatment and CK were visually separated according to NMDS1, while no separation could be obtained among the three Miscanthus genotypes according to NMDS1 or NMDS2. In general, differences between Miscanthus genotypes in terms of effects on microbial community structure were not obvious as both bacterial and fungal communities of three tested Miscanthus genotype samples clustered closely together (Figure 2). In addition, the relationship between Miscanthus cultivation and fungal communities (stress = 0.09, R = 0.139, p = 0.090) was less pronounced than that for bacterial communities (stress = 0.050, R = 0.2685, p = 0.002). Collectively, long-term Miscanthus cultivation on saline-alkaline soil only significantly changes the bacterial community structure and different Miscanthus genotypes tend to have the same microbial (bacterial and fungal) community structure.

3.3 The contribution of microbial community change to soil quality improvement

In order to quantitatively assess the microbial community’s contribution to the soil quality improvement potential, CCA/RDA was used to identify the relationship between the microbial community structure and the soil quality indicators. Results showed that the first two CCA/RDA dimensions explained 46.23% and 31.59%, respectively, for the bacterial and fungal community variations (Figure 3). As reflected by the CCA/RDA results (Figure 3), both the bacterial and fungal

| Treatment | 2015 CSQI | Order | 2016 CSQI | Order | 2017 CSQI | Order | 2018 CSQI | Order | Average CSQI | Order |
|-----------|----------|-------|----------|-------|----------|-------|----------|-------|-------------|-------|
| G1        | 0.487    | 3     | 0.497    | 2     | 0.552    | 2     | 0.351    | 3     | 0.472       | 3     |
| G2        | 0.525    | 2     | 0.473    | 3     | 0.550    | 3     | 0.414    | 2     | 0.491       | 2     |
| G3        | 0.527    | 1     | 0.611    | 1     | 0.557    | 1     | 0.548    | 1     | 0.561       | 1     |

Note: G1, G2, and G3 represent the treatments under Miscanthus by genotype G1, G2, and G3.
communities of the CK treatment were characterized by high salinization including pH and EC, while the three Miscanthus soils had high OM and TN contents. Soil physicochemical properties that could explain the variance of the fungal and bacterial community structures are shown as the CCA/RDA results in Table 3. Results indicate that the bacterial community structure positively and significantly correlates with soil pH ($p = 0.009$), EC ($p = 0.007$), OM ($p = 0.008$) and TK ($p = 0.003$), but not TN ($p = 0.852$) and TP ($p = 0.104$). The different closeness values of these significant correlations were ranked according to the correlation coefficient ($r^2$) as TK (0.7002)$>$pH (0.6484)$>$EC (0.6122)$>$OM (0.5462) (Table 3). The same four indicators were also observed to be significantly ($p$ value varied between 0.001 and 0.005) correlated with the fungal community structure. However, their closeness was not consistent with the bacterial community structure, with pH ($r^2 = 0.8297$) being the closest indicator, followed by TK (0.7891), EC (0.7014), and OM (0.6815).

In order to closely explore which kinds of microbes contribute to the soil quality improvement after Miscanthus cultivation, the variations in bacterial and fungal communities between the Miscanthus treatments and CK were analyzed at the family level. Only the top 15 families in terms of relative abundance for bacterial and fungal communities were considered (Figure 4). Non-significant differences were found in the 15 screened microbial families within the Miscanthus treatments except for the bacterial family Micrococcaceae (enriched in G3), so abundances of the same bacterial and fungal family in the three Miscanthus treatments were merged into one (averaged) group (named Miscanthus hereafter) and then compared with that in the CK treatment (Figure S1).

The proportions of three bacterial families, namely, Gemmatimonadaceae, Micrococcaceae, and Chitinophagaceae, statistically increased ($p < 0.05$) in Miscanthus treatments compared with CK (Figure 4); while proportions of
norank_o__JG30-KF-CM45 and norank_c__Actinobacteria statistically decreased ($p < 0.05$). Specifically, Gemmatimonadaceae increased from 3.1% (CK) to 4.0% (Miscanthus group); Micrococcaceae increased from 1.1% (CK) to 3.6% (Miscanthus group); and Chitinophagaceae increased from 1.0% (CK) to 2.3% (Miscanthus group). For fungal communities, only proportions of the norank_o__Pleosporales and Chaetomiaceae were significantly ($p < 0.05$) lower in Miscanthus treatment than that in CK; while no top 15 fungal families were enriched in the Miscanthus rhizosphere soil. The norank_o__Pleosporales decreased from 3.9% (CK) to 0.7% (Miscanthus group) and Chaetomiaceae decreased from 2.6% (CK) to 0.9% (Miscanthus group).

The relative abundances of bacterial and fungal functional guilds between the Miscanthus treatments and CK were analyzed in order to explore which types of microbes contribute to soil quality improvement from a functional perspective (Figure 5). For potential bacterial functional guilds involving nitrogen cycling, there was no significant effect on the relative abundance of diazotrophs (nitrogen fixation bacteria), nitrifiers and denitrifiers in soil samples with Miscanthus cultivation (Figure 5). Specifically, the highest relative abundance of diazotrophs was found in G1 (1.04%), followed by CK (1.00%), G2 (0.97%) and G3 (0.79%); the highest relative abundance of nitrifiers was found in CK (5.97%), followed by G2 (4.99%), G1 (4.81%) and G3 (4.29%); the highest relative abundance of denitrifiers was found in CK (5.97%), followed by G2 (4.99%), G1 (4.81%) and G3 (4.29%). As for fungal functional guilds, Miscanthus cultivation significantly reduced the relative abundance of pathotrophs from 22.56% in CK to 10.21%, 6.11% and 5.95% in G1, G2, and G3 treatments, respectively. The relative abundance of pathotrophs in G2 and G3 treatments were significantly higher than in G1, but no significant difference found between G2 and G3. Conversely, Miscanthus cultivation significantly increased the relative abundance of saprotrophs from 4.75% in CK to 9.24%, 9.94%, and 11.26% in G1, G2, and G3, respectively, but no significant difference found among the three Miscanthus soils.

4 | DISCUSSION

4.1 Factors contributing to the soil improvement potential by Miscanthus cultivation

The first aim of this study was to evaluate the soil improvement potential through cultivating Miscanthus on saline-alkaline soil. Our results show that Miscanthus can significantly reduce soil salinization, which is reflected by a significant lowering of soil EC and pH after 5 years of Miscanthus cultivation. Previous studies have illustrated that the secretion in plant roots of organic acids (e.g., citric and malic acid) tends to decrease soil pH (Kayama, 2001). Furthermore, better development of root systems could increase the soil porosity, which may also contribute to soil desalinization via leaching (Ado et al., 2019). Both of these mechanisms could apply to Miscanthus, due to its well-developed root and rhizome system. As a fast-growing grass, the Miscanthus plant can uptake and remove salt ions...
(e.g., Na⁺, K⁺, Mg²⁺, Ca²⁺) from saline-alkaline soils via its high-yield biomass (Zheng et al., 2019). Additionally, *Miscanthus* has a well-developed, high-density canopy that can avert the surface accumulation of salts through transpiration and thus desalinate the soil (Jing et al., 2019). This is due to the large leaf area and canopy roughness of *Miscanthus*, which can lower the soil surface temperature, thereby reducing water evaporation and surface salt accumulation.

Aside from desalinization, soil fertility improvement is another crucial aspect of the ecological restoration of saline-alkaline soil by *Miscanthus* cultivation. Soil organic matter plays a central role in maintaining soil fertility, as it carries out vital ecosystem functions, such as soil structure formation, water-holding capacity and nutrient storage (Schmidt et al., 2011). It has been widely reported that *Miscanthus* cultivation can increase soil organic carbon stock in various arable and marginal lands including degraded grassland and abandoned farmland (Chen et al., 2020; Mi et al., 2014; Zhao, Fan, et al., 2020; Zhao, Bai, et al., 2020). We also found that *Miscanthus* cultivation on saline-alkaline soil could increase soil organic matter content at a rate of 3.2 g/kg annually. A large amount of biomass, the stubble residues left on the field, and the litter returned to the soil could explain the increased organic matter content in the *Miscanthus* soil. It is reported that the annual litter lost to the soil, mainly falling leaves, could account for approximately 30% (approximately 9 dry matter t/ha) of the aboveground biomass of *Miscanthus x giganteus* (Lewandowski & Heinz, 2003). Furthermore, the extensive belowground system of *Miscanthus* can annually produce up to 80% of the biomass of the aboveground yield (Zhao, Fan, et al., 2020; Zhao, Bai, et al., 2020). Due to such a large amount of exogenous carbon input, it is understandable that the soil organic matter in the soil increases significantly. Additionally, root turnover of perennial crops and rhizodeposition might be important contributory factors to the increased soil organic matter in the present study. For example, switchgrass was found to release up to 20% of its fixed carbon via rhizodeposition, thus increasing soil organic stock (Mao et al., 2014). A study using natural ¹³C tracing found that a considerable amount of *Miscanthus*-derived C accumulated in the soil from fallen leaves, senescent rhizomes and recycling of roots (Hansen et al., 2004).
N and P are the two main macronutrients vital for plant growth, and their levels in the soil are important indicators of soil quality. *Miscanthus* is a plant with a capacity of N fixation (Li et al., 2016; Zhao, Fan, et al., 2020; Zhao, Bai, et al., 2020), especially in the condition without external N fertilization (Liu & Ludewig, 2019). It is then expected that the soil nitrogen content could increase under its cultivation. However, no significant differences were found in the TN between *Miscanthus* and non-*Miscanthus* soil. This is consistent with our result that non-significant differences in nitrogen cycling bacterial functional guilds, including diazotrophs, nitrifiers, and denitrifiers, were found between the *Miscanthus* soils and CK. In general, the N-fixing bacterial are not salt-tolerating microbes and their N fixation potential gradually decreases with the increasing salt stress level (Duro et al., 2016). This could explain why the soil TN content did not increase after *Miscanthus* cultivation in the saline-alkaline soil. Moreover, the soil TN generally decreases after plant cultivation without fertilizer input, due to the plants’ uptake and removal of nutrients from the soil. However, *Miscanthus* cultivation does not appear to take N away from the soil, as suggested by the similar soil TN content between the *Miscanthus* and CK treatments. This could result from the characteristic nutrient translocation of *Miscanthus* (Ruf et al., 2017), which reduces the amount of nutrients taken away in the harvested aboveground biomass. Similarly, no significant difference was found in TP between *Miscanthus* and non-*Miscanthus* soils. The low P demand by *Miscanthus* could be the main reason for no significant difference in TP (Cadoux et al., 2012). In addition, this could be attributed to P activation induced by *Miscanthus* (Yang et al., 2013) and P dissolution by soil microbes (Zhao, Fan, et al., 2020; Zhao, Bai, et al., 2020). Therefore, while no change in total P content was found, we speculate that the available P content could increase in *Miscanthus* cultivated soil. However, this hypothesis requires confirmation.

Based on our results, *Miscanthus* cultivation on saline-alkaline soils presents a huge potential for soil fertility improvement especially for soil organic matter accumulation, although its desalination capacity is slightly inferior compared with Jerusalem artichoke (Yue et al., 2020). For example, soil organic matter content was found to increase by a rate of 3.2 g/kg annually, which is far higher than that of Jerusalem artichoke which increases OM at a rate of 0.8 g/kg annually (Chen, Xu, et al., 2019; Chen, Li, et al., 2019). Aside from soil quality improvement, *Miscanthus* is a tall perennial grass and delivers multiple additional ecosystem services, including shelter for crops and livestock, refuge for beneficial wild animals, feedstock for biofuel production, erosion prevention, greenhouse gas mitigation, and earthworm community diversity increment (Felten & Emmerling, 2011; Littlejohn et al., 2019; Von Cossel et al., 2020). These all make it a good candidate for use in the phytoremediation of marginal land.

### 4.2 Effect of *Miscanthus* growth duration on its soil improvement potential for saline-alkaline soils

Previous studies about the phytoremediation of saline-alkaline soils have focused on the overall level of soil quality improvement after a certain number of years of plant growth, but few have mentioned the annual soil quality indicator dynamics (Bharti et al., 2017; Chen, Xu, et al., 2019; Chen, Li, et al., 2019; Chen et al., 2020; Mi et al., 2014; Zhao, Fan, et al., 2020; Zhao, Bai, et al., 2020). In the present study, patterns of change in soil physicochemical properties under *Miscanthus* cultivation gave results from 4 consecutive years of soil quality indicator monitoring. Our results show that the soil-improving effect of *Miscanthus* cultivation was more pronounced with the ongoing years. This is the first time the pattern of soil quality change on saline-alkaline soils by phytoremediation using *Miscanthus* has been shown. *Miscanthus* is perennial and generally needs a few years (3–5 years) to achieve a ceiling yield (Jones et al., 2016; Stolarski et al., 2018; Xu et al., 2017). The lower development of the plant in the first few years results in sustained increasing biomass and canopy growth continues (Himken et al., 1997; Saletnik et al., 2018; Zhao, Fan, et al., 2020; Zhao, Bai, et al., 2020; Zheng et al., 2019). This contributes to differences in soil improvement potential between years. Normally, after 3 years of establishment, the aboveground biomass yield of *Miscanthus* reaches ceiling yield, that is, generally similar between years. However, the soil improvement potential of *Miscanthus* could still be increasing due to the continued belowground root and rhizome system growth. It has been found that the root/shoot ratio of *Miscanthus* increases with stand age, due to more biomass being allocated to belowground with time (Zhao, Fan, et al., 2020; Zhao, Bai, et al., 2020). With the additional growing years and without harvest, the belowground system of *Miscanthus* increases, suggesting the belowground system continuously inputs biomass, which may facilitate soil organic matter sequestration (Clifton-Brown et al., 2007). Additionally, the developing belowground system offers an increasing root surface for beneficial microbial growth and activity as well as for exudate secretion (Patel & Patra, 2017), which both play a vital role in the improvement process. The decomposition of litter from annually senescent leaves, rhizomes and roots, which can contribute to the cycling of carbon and nutrients in the soil, also increases as *Miscanthus* grows older (Amougou et al., 2011). For this reason, it is recommended that the soil quality improvement potential
of perennial plants should be measured after a long cultivation period. Other effects of improved soil quality were not examined in this work and need to be further studied. These include biological properties such as microbial biomass and nutrient-cycling related enzymes (C, N, and P-cycling enzymes). Additionally, although the Miscanthus soil quality was improved with more growing years, it is still unclear whether or when it will reach stability, which also points to the need for more long-term experiments.

4.3  |  Miscanthus cultivation shapes microbial community

The second objective of this study was to clarify the underlying microbial mechanisms of saline-alkaline land phytoremediation by Miscanthus. The results show that the diversity of both bacterial and fungal communities was not significantly changed by Miscanthus cultivation. This may be attributed to a highly structured and physically unperturbed rhizosphere environment, which leads to the relative stability of rhizosphere microbes. Similar results were recorded by Lovell et al. (2001), who found that the Spartina alterniflora rhizosphere microbial community did not respond dramatically to manipulated environmental variability in a salt marsh. Our results were also confirmed by a 32-year long-term fertilization practice study, which found that fungi and actinomycetes in the rhizosphere were less affected by fertilization than those in the bulk soil (Ai et al., 2015). However, Chen et al. (2020) reported that Miscanthus planting can reduce the diversity of bacteria and fungi in arable land, which is not consistent with our results. The difference may be attributable to different land types and soil properties in that study. Since the Miscanthus cultivated soil quality was improved with the greater number of growing years, it is still unclear whether or to what extent the microbial diversity will increase. A longer-term experiment is, therefore, needed to explore it.

Our results show that long-term Miscanthus planting had a significant effect on the composition of bacterial communities but did not significantly impact the composition of fungal communities in all tested genotypes. This finding is in line with the general trend that soil bacterial communities are more sensitive than fungal communities to environmental change (Chen et al., 2017; Ochoa-Hueso et al., 2018). Also, this is a possible reason why fungi were not investigated in some studies to clarify the underlying microbial mechanisms of phytoremediation (Yue et al., 2020; Zadel et al., 2020; Zhao, Fan, et al., 2020; Zhao, Bai, et al., 2020). The low quantity of fungi in soil and the insufficient database compared with the database available for bacteria could be another reason that fungal communities were less investigated in phytoremediation (Yuan et al., 2020). Based on a combination of these above-mentioned reasons and our results, we recommend that further studies should focus on the bacterial communities’ response to phytoremediation rather than the fungal communities when amplicon sequencing was used.

Our results also revealed that soils under different Miscanthus genotypes tend to have a similar microbial structure. This may be attributed to the three Miscanthus genotypes having the same parents and tending to harbor similar microbial communities (Li et al., 2016). However, the three Miscanthus genotypes present distinctive soil-improving potential, as reflected by a high variation of comprehensive CSQI. Furthermore, among the three Miscanthus genotypes, G3 presented the most promising soil improvement potential, as it has the highest comprehensive CSQI over the whole 4 years of study. This may be due to the change in the rhizosphere microbial composition, especially some special microbes with pronounced soil-improving function changed by Miscanthus planting. For example, among the top 15 bacterial and fungal communities, the abundance of the bacterial family Micrococccaceae in G3 was significantly higher than in G1 and G2. Micrococccaceae are salt-tolerant and can facilitate plant resistance to salt stress in saline-alkaline soils (Cordero & Zumalacárregui, 2000).

4.4  |  Relationship between microbial community composition and soil physicochemical traits

One of the most important objectives was to clarify the underlying microbial mechanisms of the soil-improving effect arising from Miscanthus cultivation. This can partly be revealed by understanding the relationship between the microbial community composition and soil physicochemical traits. Previous studies have demonstrated that soil physicochemical traits are closely related to soil microbes (Cordovez et al., 2019; Orgiazzi et al., 2012). It is well demonstrated that soil pH and EC have strong correlations with microbial communities, including bacteria and fungi, in saline-alkaline soils (Yue et al., 2020; Zhao et al., 2019), consistent with the results obtained in the present study. Our results show that both bacterial and fungal communities present a significant positive correlation with pH and EC. However, in an investigation of bacterial communities in low, medium and high salinity wetlands, Zhao, Fan, et al. (2020) and Zhao, Bai, et al. (2020) found that EC negatively affected the diversity and community composition of bacteria, which is not consistent with our results. There was a high EC (from 340 to 673 μS/cm) in their wetlands relative to our test site (below 210 μS/cm). Hence, the relationship between the microbial community and EC may differ with the salinity level. Furthermore, in the present study, the bacterial and fungal community structures showed a significant positive correlation with soil fertility, including soil organic matter content and total
potassium content. Also, the RDA/CCA results showed that the CK bacterial and fungal communities had characteristics of high salinization, while the bacterial and fungal communities of the three Miscanthus rhizosphere soils had characteristics of high soil fertility. These results together confirm that structural changes in the microbial communities induced by Miscanthus planting could be a reason for desalination and soil fertility improvement in saline-alkaline soils.

The identification of the change in the microbial abundance has clarified the contribution of soil microbes to the potential of Miscanthus for improving soil quality in saline-alkaline soils. The enrichment of beneficial bacteria in soil under Miscanthus could assist its soil improvement potential. Of the three significantly enriched bacterial families, Gemmatimonadaceae has been found to have a biomineralizing action, leading to the formation of calcium and magnesium carbonates, which might be the cause of soil desalination via Ca$^{2+}$ and Mg$^{2+}$ precipitation (Deslippe et al., 2012; Wang et al., 2014). As salt-tolerant bacteria, Micrococccaceae can facilitate plant growth due to activities such as indole-3-acetic acid (IAA) production, 1-aminoclopropane-1-carboxylate (ACC) and siderophore synthesis (Hong et al., 2016). Chitinophagaceae is characterized by high activity of β-glucosidase, which can degrade chitin and cellulose (Bailey et al., 2013), thus promoting litter decomposition and increasing soil organic matter formation. In contrast to the positive effect of enriched bacterial communities, reduction in harmful fungi is another process by which Miscanthus may assist phytoremediation of saline-alkaline soils. Of the fungal families showing a significant change in abundance, norank_o__Pleosporales is a necrotrophic group, consisting of many economically important plant pathogens (Rybak et al., 2017; Tanaka et al., 2015). Furthermore, Chaetomiaceae have been found to have a plant growth-inhibiting action (Zhang et al., 2021).

### 4.5 Phytoremediation saline-alkaline soil for agricultural land: benefits, challenges and solutions

Bioremediation of saline-alkaline soil for agricultural land by Miscanthus cultivation can alleviate the limitations of both biotic and abiotic stresses on the growth of the following crop, suggesting a yield increasing potential. The potential of lightening abiotic stresses by Miscanthus has been confirmed by the present work. For the potential of reducing biotic stresses, Miscanthus cultivation can reduce the soil contended plant pathogens, deleterious rhizosphere micro-organisms, which will prevent the declines of following crops yield caused by the diseases. Moreover, many Miscanthus-associated microbes have beneficial effects on plant growth-promoting (Liu & Ludewig, 2020). Miscanthus cultivation will enrich these beneficial microbial communities in the soil (Li et al., 2016). Such plant growth-promoting microbes might facilitate a high yield of the following crops. However, there should be a concern that converting the restored saline-alkaline land to arable land would damage the restoration achievement, due to the plowing and tillage practice used in the land conversion and following crop production. To address this concern, the perennial feed crops, for example, Lolium perenne L., are mostly recommended as the following crops to use the restored saline-alkaline land. If the annual crops are used, the conservation tillage techniques (e.g., no-tillage and reduced tillage) are mostly recommended (Busari et al., 2015). However, how long the phytoremediation process will take by Miscanthus is still uncertain and more long-term trials are needed to answer this question.

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### DATA AVAILABILITY STATEMENT

The raw sequence data of bacterial 16s rDNA and fungal ITS used in this study have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2017) in National Genomics Data Center (Nucleic Acids Res 2020), Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession number CRA004031 and are publicly accessible at https://bigd.big.ac.cn/gsa.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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