Miscanthus sacchariflorus exhibits sustainable yields and ameliorates soil properties but potassium stocks without any input over a 12-year period in China

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Abstract
The perennial C₄ Miscanthus spp. is used in China for bio-fuel production and its ecological functions. However, questions arise as to its economic and environmental sustainability in abandoned farmland where the costs should be very low. Little is known about its yield performance and effects on soil properties when it was harvested annually without any inputs in China. To address these questions, an experiment was implemented for 12 years on annually harvested Miscanthus sacchariflorus planted in 2006 and managed without fertilization, irrigation, or any other inputs. We determined biomass yields each year, biomass allocation, and soil properties before and after its cultivation. Biomass yields of M. sacchariflorus reached a peak value (29.67 t/ha) 3 years after cultivation and was maintained at a stable level (averaged 22.22 t/ha) during 2012–2017. Its root shoot ratio increased due to more biomass allocated below-ground with time. Long-term cultivation of M. sacchariflorus increased organic carbon contents, pH (for the absence of fertilization), microbial carbon, nitrogen and phosphorus contents, and soil carbon nitrogen ratios (0–100 cm). Soil bulk density was decreased significantly (p < .05) independent of soil depths. Annual harvest did not reduce total nitrogen and phosphorus, available nitrogen, and potassium, but total the potassium content of soil (0–100 cm). Cultivation of M. sacchariflorus increased available phosphorus contents in 40–100 cm soil and reduced that value in 20–40 cm soil. Biological nitrogen fixation provided ~218.74 kg ha⁻¹ year⁻¹ (1 m depth) nitrogen for the system offsetting nitrogen export by biomass harvest and stabilizing nitrogen levels of soil. In conclusion, M. sacchariflorus exhibited sustainable biomass yields and ameliorated soil properties but the decrease of total potassium contents after 12 years’ cultivation without any input. These conclusions could provide important information timely for the government and encourage farmers to promote large-scale utilization of M. sacchariflorus on the abandoned farmland in China.
1 | INTRODUCTION

Considerable land area will be required to meet the growing demand for bio-energy, particularly with the emergence of second generation bio-energy crops harvested for lignocellulose (Somerville, Youngs, Taylor, Davis, & Long, 2010). In the last few decades, large areas of non-suited lands for agriculture were over reclaimed into farmland due to the high pursuit of crop yields rendering many ecological problems such as non-point source pollution, soil erosion by wind, soil acidification, etc. in China (Li & Lin, 2014). Hence, the returning of this type of farmland into grassland or forestry, mandatorily required by the Chinese government for the improvement of environment, provides large areas for the bio-energy crops in China (Hao, Xu, & Zhang, 2005).

The replacement of annual row crops with perennial grass bio-energy crops is occurring globally in temperate, tropical, and semi-arid regions (Somerville et al., 2010). During the replacement process, the native energy grass species are usually recommended for the better ecological adaptation and the eliminated biological invasion risk. Miscanthus sacchariflorus (subsequently referred to as M. sacchariflorus) is a perennial rhizomatous grass with the C4 photosynthetic pathway (Lewandowski, Cliftonbrown, Scurlock, & Huisman, 2000). It is one of the most popular species in China among around 17 species of the genus Miscanthus (Lewandowski et al., 2000). It exhibits good ecological adaptability through most parts of China from the cold and dry north to the warm and wet south (Xue, Lewandowski, Wang, & Yi, 2016). Miscanthus shows great potential due to its high biomass yield, few maintenance requirements, excellent stand longevity, efficient nutrient recycling, strong resistance to various stresses, and the provisioning of several other ecosystem services (Arnould & Brancourt-Hulmel, 2015; Cadoux, Riche, Yates, & Machet, 2012; Mitchell et al., 2016). Most of the previous research work about Miscanthus was mainly studied using Miscanthus × giganteus (M × g) as material in a variety of geographical and climatic conditions (Arnould & Brancourt-Hulmel, 2015); few publications mentioned Miscanthus × giganteus (Clark et al., 2015). Compared to the fasciculate type tillers of M × g, the scattered type tillers of M. sacchariflorus could facilitate the vegetation cover on the cultivated lands through self-propagation, which indicated low economic inputs needed during the establishment process.

A huge challenge for the replacement process is the willingness of farmers to grow Miscanthus or other energy crops. The socio-economic factors lowered farmers’ willingness to grow either crop in Missouri and Iowa, and farmers with higher educational levels and smaller farm sales are more willing to grow energy crops (Gedikoglu, 2015). In China, the ultimate fate, direction, and magnitude of this promising source of energy are still unknown (Chen, Li, Guo, & Lv, 2018). Most farmers know little about Miscanthus and its utilization in bio-energy and ecological restoration due to the late start of research in this field in China. Hence, the willingness of them to grow Miscanthus is low mainly because the unknown economic benefits could be generated for them, unless with the funding of government restricted to the planting process. Though the economic benefit from the government was provided, any economic inputs for the maintaining of this system were not welcomed. Hence, the most popular model with them for Miscanthus system emerges according to the actual situation in China, which means that biomass of Miscanthus would be annually harvested and no irrigation, fertilization or any other inputs were applied. While the sustainability of biomass yields and soil quality of this system under such model remains largely unclear.

Biomass yields and its sustainability is a major concern for both the economic and environmental performance of Miscanthus. It is influenced by the growth characteristics of such plant. Biomass yields of M × g follows an establishment phase, a ceiling phase and then a phase of decline (Saletnik, Zagula, Bajcar, Czernicka, & Puchalski, 2018). Usually, the maximum productivity of M × g starts from the third year of cultivation (Saletnik et al., 2018).

Soil nutrient levels dramatically affect the establishment (Heaton, Dohleman, Miguez, & Juvik, 2011) as well as the start and duration of the yields’ ceiling phase (Arundale et al., 2013). It has been reported that nitrogen (N), phosphorus (P) and potassium (K) all significantly (p < .05) influence the establishment and biomass yields of M × g (Haines, 2014; Shield, Barraclough, Riche, & Yates, 2014). But there seemed to be some divergences in the effects of N on M × g according to the negative or neutral influences of N on M × g's yields in short-term period (Arundale et al., 2013; Davis, David, Voigt, & Mitchell, 2014). Additionally, soil moisture as irrigation is an important factor influencing its productivity (Jans, Berndes, Heinke, Lucht, & Gerten, 2018). Beyond that, the weed and disease control cannot be ignored in Miscanthus field (Brancourt-Hulmel, Demay, Rosiash, Ferchaud, & Boizard, 2014; Song et al., 2016). Thus, the sustainability of M × g yields depends largely on the species, stand age, soil conditions, climate type and especially the agricultural practices. Under good agricultural practices, M × g

**KEYWORDS**

biological nitrogen fixation, Miscanthus sacchariflorus, soil bulk density, soil nitrogen, soil organic carbon, soil pH, soil phosphorus, soil potassium, soil properties, sustainable yield
can be harvested annually over a cultivation period of up to 20 years (Lewandowski et al., 2000), while it is sealed how would the biomass production of *M. sacchariflorus* perform when there was no irrigation, fertilization and other practices applied over long-term period.

Except for the biomass production, the sustainability in soil nutrient levels of this system is also a focus of attention. For the typical crop systems, annual harvest indicates a net removal of nutrients from the soil if no fertilizer or other soil amendments applied (Heckman et al., 2003; Wilhelm, Johnson, Hatfield, Voorhees, & Linden, 2004). And this annual removal of nutrients can be compensated for by applications of fertilizers maintaining a nutrient balance of the soil (Egli, 2008). Similarly, the annual harvest of *Miscanthus* biomass also rendered a considerable net export of soil nutrients (Masters et al., 2016; Yost, Kitchen, Sudduith, & Allphin, 2018) though it could translocate the nutrients below-ground in late autumn (Dohleman, Heaton, Arundale, & Long, 2012). Year after year, the cumulative outputs of soil nutrients might severely challenge the soil sustainability under such model.

Ecological benefits from *Miscanthus* cultivation are eagerly expected by scientists and the government due to the prominent ecological problems in China. It is verified that the cultivation of *Miscanthus* can largely prevent soil erosion no matter by wind or water, N leaching and emission (Mccalmont et al., 2017). *Miscanthus* can also increase the biodiversity and improve the landscape effects on the cultivated lands (Evers, Blanco-Canqui, Staggenborg, & Tatarko, 2013; Miriti et al., 2017). Beyond that, soil properties such as soil organic carbon (SOC) accumulation, bulk density (BD), pH and the microbial activities have also been the focus of investigations on *M × g* cultivation. Many people focused on the SOC accumulation and turnover and found that the cultivation of *M × g* could increase SOC through harvesting residues and root turnover (Harvolk, Kornatz, Otte, & Simmering, 2014; Pidlisnyuk, Stefanovska, Lewis, Erickson, & Davis, 2014). While, there was also special case that no significant change in C stocks of topsoil (0–30 cm) following 7 years commercial *Miscanthus* cultivation with three times biomass harvest during the early establishment phase and a single fertilization (Robertson et al., 2017). For BD, it has been reported that the short-term (2–3 years) cultivation of *M × g* could decrease top (<30 cm depth) soil BD (Lok, 2015). Whereas, no significant change was observed in the upper soil (0–10 cm) when a grassland was converted to *M × g*, after 5 or 20 years (Hu, Schafer, Duplay, & Kuhn, 2018). Hence, the soil benefits from cultivation of *Miscanthus* might vary with the cultivation period, land type and agricultural practices. However, little work has been carried out about the soil benefits from *Miscanthus* cultivation in China, especially under such model, rendering poor understanding about that by the government or farmers.

Summarily speaking, during the past 10 years, many reports about the biomass production and soil benefits of *Miscanthus* have been published (Clark et al., 2019; Lee, Wycislo, Guo, Lee, & Voigt, 2017; Ruf, Schmidt, Delfosse, & Emmerling, 2017; Witzel, Finger, & Reviews, 2016). However, most of them concerned case studies in Europe, US North and Midwest areas using *M × g* as material. These studies showed that the effects of *M × g* cultivation on the soil quality or properties in short or long term vary largely depending on the soil type, N addition, harvest date and other agricultural practices (Hu et al., 2018; Nebeska et al., 2018; Peyrad, Ferchaud, Mary, Gréhan, & Léonard, 2017; Ruf et al., 2017; Thompson, Deen, & Dunfield, 2018). But few reports could be referenced all over China. Thus, it is necessary and meaningful to uncover the biomass production performance and the changes in soil properties of *M. sacchariflorus* systems under such model in China.

There were two objectives in this study: (a) to evaluate the biomass production performance and changes in below-ground biomass allocation of *M. sacchariflorus* after 12 years’ cultivation under such model, (b) to evaluate the changes of soil physical and chemical properties after so many years of cultivation of *M. sacchariflorus* under such model. Our hypotheses were: (a) biomass production of *M. sacchariflorus* would perform well in the initial several years, but would decrease largely with time due to the absence of fertilization; and (b) annual harvest of *M. sacchariflorus* biomass would negatively affect the soil nutrients levels, but would improve the other soil properties. The conclusions of this study could clearly tell the government whether or not it is sustainable to maintain *M. sacchariflorus* system under such most acceptable model. Based on this work, the government could make more reasonable and feasible policies, which could largely encourage farmers to grow *Miscanthus*. Also this work could provide some information for large-scale utilization of *Miscanthus* on a similar land under similar climate type. Finally, the results of this study might benefit the research community that works on *Miscanthus*.

## 2 MATERIALS AND METHODS

### 2.1 Description of study site and management

The experimental site was located at Xiaotangshan town (40° 23′N, 116° 28′E) in the experimental base of the Beijing Academy of Agricultural and Forestry Sciences in Beijing, China (Figure 1). The soil texture was silty clay loam classified as meadow cinnamon soil according to China Soil System Classification. The clay (<2 μm), silt (2–75 μm) and sand (75–2,000 μm) fractions in the 0–15 cm topsoil layer were 17.48%, 60.10% and 22.42% (dry weight basis),
respectively. Total organic matter, available N (AN), available P (AP), available K (AK) contents and soil pH value of the top soil before the experiment started was 15.2 g/kg, 84.0 mg/kg, 16.5 mg/kg, 129.00 mg/kg and 7.62, respectively. The experimental plot was about 10 m wide and 20 m long, with flat terrain and an average elevation of 50 m, and has a warm temperate continental monsoon climate. The average annual temperature ranged from 12.1°C to 14.0°C during 2006–2017, and the annual precipitation in the growing season ranged from 318 to 733.2 mm (Figure 2). Frost-free period of experimental field was 90–200 days and ≥10°C growing degree days were 4,200°C days. The experimental field was previously crop land with wheat and maize production for at least 30 years.

In early spring of 2006, 150 kg/ha compound fertilizer (N:P:K = 20:12.5:10) was spread evenly over all this area to ensure the normal growth of *M. sacchariflorus* seedlings according to our previous experience. And the field was surface ploughed. The experimental plot was about 10 m wide and 20 m long, with flat terrain and an average elevation of 50 m, and has a warm temperate continental monsoon climate. The average annual temperature ranged from 12.1°C to 14.0°C during 2006–2017, and the annual precipitation in the growing season ranged from 318 to 733.2 mm (Figure 2). Frost-free period of experimental field was 90–200 days and ≥10°C growing degree days were 4,200°C days. The experimental field was previously crop land with wheat and maize production for at least 30 years.

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### 2.2 Plant sampling, biomass dry weight and N, P, K content determination

Plant samples were acquired from six subplots (subplots 5–10) without considering the four subplots at the corner (subplots 1–4) for the border effects on the results (Figure S1). And the same six subplots were investigated in each year. During the first 3 years after planting (year 2006–2008), *M. sacchariflorus* was characterized as a single plant. Thus, three plants were randomly selected and cut in the six subplots (not near the border of the plot). Three years after the planting, we could not separate the plants because the tillers of the adjacent plants mixed each other and evenly distributed on the ground. Hence, all the tillers in a 1 m × 1 m quadrat from each subplot were cut using a sickle. The fresh weight of the above-ground biomass from the single plant or a quadrat was determined and a
subsample from each sample was acquired and then placed in 105°C for killing (20 min) and 80°C oven for the water content in the samples. The dry weight of plants or above-ground biomass in a quadrat was calculated according to the water content and their fresh weight. Three plants or a quadrat in each subplot served as one replicate and there were six replicates.

The soil in one quadrat pit in each plot was dug manually layer by layer (20 cm for each layer down to 100 cm). All the rhizome and root in each layer were collected and cleaned using tap water. The roots were killed in 105°C for 20 min and dried in 80°C oven till constant weight. Biomass dry weight was determined using an electronic scale (Shanghai DTUO industrial Co. Ltd.). In 2019, we cut three individual tillers from each subplot each month (5.15, 6.15, 7.15, 8.15, 9.15, 10.15 and 11.15) to determine the dry weight of above-ground biomass. We also dug out the rhizome and root below each tiller to acquire the rhizosphere soil (defined as the soil 0.5 cm thickness around the root) and prepared the dry samples of the rhizome or root for determination of N contents. There were six replicates for the biomass dry weight and samples from one subplot served as one replicate.

Part of the dried above- and below-ground biomass from above procedures was crushed using a micro-mill (Tianjin TEST instrument co., LTD) for 3 min to get rough samples. Part of each rough sample was then placed in a ball crusher to make a fine powder (<0.1 mm particle size) which was used to determine total N, P and K contents. A Vario Macro CHNS instrument (Elementar Analysensysteme GmbH) was used to determine total N content (Toma et al., 2010). We used the acid digestion and colorimetric determination method to determine total P and K contents in plant materials (Cavell, 1954). During the determination process, we set two parallel samples to ensure the accuracy of the results.

2.3 Soil sampling, analysis and determination of N deposition

During the digging of the quadrat pits, the soil from each layer (0–20, 20–40, 40–60, 60–80, and 80–100 cm) was well mixed and kept in separate heaps. Five random soil samples were collected from each heap which served as one replicate of each layer. Besides, undisturbed soil was acquired using the cutting ring of a soil sampler in the middle position of each layer on the wall of the quadrat pits to determine the soil BD. The visible rocks were removed from soil samples by passing through a 2 mm soil sieve prior to pretreatment and chemical analysis. The fresh soil sample was divided into two parts. One part was air dried naturally in shade and crushed using a micro-mill (Tianjin TEST Instrument Co., LTD). The soil powder was sieved through 1 mm and stored in zip lock bags for the determination of SOC, total N (TN), total phosphorous (TP) and total potassium (TK) contents, and AN, AP and AK contents as well as the soil pH value. The other part was stored in −4°C freezer quickly for the determination of the microbial C, N and P contents within 1 week.

For each individual sample, soil gravimetric moisture content was determined by oven drying subsamples for 24 hr at 105°C. The cutting ring-acquired soil samples were oven dried till constant weight to determine soil BD. SOC and TN contents were determined using a Vario Macro CHNS instrument (Elementar Analysensysteme GmbH) as described above. Digestion and colorimetric methods were used to determine soil TP content (Bertheux, 1958). Atomic absorption method was used to determine the soil TK content (Khreish & Boltz, 1970). Alkaline KMnO4 oxidation method, Olsen’s extract method and NH4OAc extract method were used to determine AN, AP and AK contents (Dhillon & Dev, 1979). Fresh soil samples (1.0 g) were used to estimate microbial C (MC), N (MN) and P (MP) contents in each core by chloroform fumigation-K2SO4 extraction and potassium persulfate digestion methods, respectively (Li et al., 2018). Soil pH was measured using a 1:2.5 ratio of 0.01 M CaCl2 solution/soil suspension according to the professional standard of China (LY/T 1239-1999) by a Nahita pH meter, model ST 5000 (OHANUS). One subsample of the rhizosphere soil was used to determine the CFU of the non-symbiotic N-fixing bacteria (NFB) according to Emer’s methods (Emer et al., 2014). The wet/bulk deposition and the dry deposition of N were determined according to the methods described by (Xu et al., 2018). And TN amounts of deposition were the sum of the wet and dry deposition of N in this study.

2.4 Statistical analysis

The average N amounts by biological N fixation in 1 year were calculated using the following formula:

$$\text{TN}_f = \frac{\text{TN}_i(2017) + \text{TN}_i(2016) - \text{TN}_i(2012) + \text{TN}_m - \text{TN}_d}{5},$$

where \(\text{TN}_i\) indicates the TN amounts by biological N fixation in 1 m\(^3\) soil. \(\text{TN}_i(2017)\) and \(\text{TN}_i(2012)\) indicate the TN amounts of 1 m\(^3\) soil in the year 2017 and 2012, respectively. \(\text{TN}_m\) and \(\text{TN}_d\) indicate the TN amounts of the below-ground biomass in the year 2017 and 2012, respectively. \(\text{TN}_m\) indicates the TN amounts of above-ground biomass from 2013 to 2017. \(\text{TN}_d\) indicates the N amounts of wet and dry deposition from 2013 to 2017. N leakage and volatilization was not considered in the calculation.

All data in this study were organized and prepared using Microsoft Office Excel 2010. One-way ANOVA at \(p < .05\) was used for hypothesis testing and Duncan’s method was used for
comparing means through the SPSS 19.0 software program. Pearson correlation analysis was conducted using Origin 8.5. Standard deviation (SD) was provided in all figures as appropriate. All the figures in this paper were drawn using Origin 8.5.

3 | RESULTS

3.1 Production performance and below-ground biomass dry weight of *M. sacchariflorus* during the 12 year period

*M. sacchariflorus* maintained a high growth rate 12 years after establishment with annual harvesting of the above-ground biomass and with no fertilization and irrigation applied (Figure S2a). We observed that the rhizome of *M. sacchariflorus* mainly distributed in the top 30 cm of the soil and could reach up to 40 cm depth of the field (Figure S2b). Biomass yields of *M. sacchariflorus* increased gradually and reached its peak value (29.67 t/ha) 3 years after planting (Figure 3a). While, biomass yields started to show a gradual decrease after the peak value (Figure 3a). Biomass yields of *M. sacchariflorus* in 2012 decreased significantly (*p* < .05) by 31.41% compared to the peak value (Figure 3a). And then it maintained at a stable level without significant differences (*p* > .05) among the years from 2011 to 2017 and the average value during this period was 22.22 t/ha (Figure 3a). Biomass yields of *M. sacchariflorus* in 2012 decreased significantly (*p* < .05) by 31.41% compared to the peak value (Figure 3a). And then it maintained at a stable level without significant differences (*p* > .05) among the years from 2011 to 2017 and the average value during this period was 22.22 t/ha (Figure 3a). The rhizome, root and total below-ground biomass dry weight of *M. sacchariflorus* in 2017 increased significantly (*p* < .05) by 8.34, 5.52, and 7.45-fold compared with the values in 2006 (Figure 3b). The root shoot ratio of *M. sacchariflorus* in 2017 was significantly (*p* < .05) higher by 1.58-fold compared with the value in 2006 (Figure 3c).

3.2 Changes in soil physical and chemical properties after 12 year cultivation of *M. sacchariflorus*

For different soil depths (0–20, 20–40, 40–60, 60–80, and 80–100 cm), BD of soil in 2017 decreased significantly (*p* < .05) by 11.58% 10.40%, 7.37%, 8.88%, and 3.63%, respectively, compared to those values in 2006 (Figure 4). SOC contents exhibited a significant (*p* < .05) increase in 2017 by 47.28%, 32.31%, 40.65%, 113%, and 105% in soil depths of 0–20, 20–40, 40–60, 60–80, and 80–100 cm, respectively compared to the initial values (Figure 5).

TN content in soil depths 60–80 cm in 2017 exhibited a significant (*p* < .05) increase by 46% compared to the value in 2006 (Figure 6a). TN contents in soil depths of 0–60 and 80–100 cm in 2017 all exhibited a slight increase trend compared to the values in 2006, though the differences were not significant at *p* < .05 (Figure 6a). We found no significant differences in TP contents for each soil depth between the 2 years measured (Figure 6b). However, TK contents in all soil depths in 2017 decreased significantly (*p* < .05) by 13.54%, 13.61%, 11.28%, 6.90%, and 13.62%, respectively compared to the values in 2006 (Figure 6c). Soil C/N values in soil depths 0–20 and 80–100 cm in 2017 were both significantly (*p* < .05) higher by 18.70% and 192.29%, respectively compared to the values in 2006 (Figure S3). Whereas, there was no significant difference in the soil C/N ratios in depths 20–60 cm compared between the 2 years (Figure S3).

AN contents in all soil depths in 2017 exhibited no significant differences compared with the values in 2006 (Figure 7a). AP contents exhibited different changes in different soil depths (Figure 7b). For the surface soil (0–20 cm), there was no significant difference in AP contents between 2006 and 2017 (Figure 7b). For 20–40 cm depth, AP content decreased significantly (*p* < .05) by 36.70% in 2017 compared to the initial value (Figure 7b). On the other hand, AP contents in 40–100 cm depths all exhibited large increase in 2017 by 91.79%, 115.90%, and 135.11% compared with the values in 2006 (Figure 7b). All AK contents exhibited a slight decrease (*p* > .05) trend in soil depths 20–100 cm in 2017 compared with the values in 2006 (Figure 7c). While there was no significant difference in AK contents of the surface soil (0–20 cm) between 2006 and 2017 (Figure 7c). MC, MN, and MP contents in the surface soil (0–20 cm) were all the highest among the five soil depths in 2012 and...
2017 (Figure 8a–c). Specifically speaking, MC contents in all soil depths 0–20, 20–40, 40–60, 60–80, and 80–100 cm in 2017 were significantly \((p < .05)\) higher by 1.30, 2.33, 6.07, 3.58, and 3.36-fold, respectively compared with the values in 2012 (Figure 8a). MN contents of the soil in all these five depths in 2017 increased significantly \((p < .05)\) by 3.36, 3.62, 4.41, 2.08, and 18.24-fold, respectively compared with the values in 2012 (Figure 8b). MP contents in soil depths 0–20, 20–40, and 60–80 cm in 2017 were significantly \((p < .05)\) higher by 1.19, 2.27, and 3.55-fold, respectively compared with the values in 2012 (Figure 8c). Microbial C/N values exhibited different changes in different soil depths (Figure S4). For soil depths 0–20, 20–40, and 80–100 cm, microbial C/N values of the soil in 2017 decreased significantly \((p < .05)\) by 47.23%, 27.90%, and 77.35%, respectively compared with the values in 2012, while the values in soil depths 40–60 and 60–80 cm in 2017 increased significantly \((p < .05)\) by 30.59% and 48.60%, respectively compared with the values in 2012 (Figure S4). Soil pH value in all these five depths in 2017 increased significantly \((p < .05)\) by 2.69%, 3.95%, 4.21%, 4.09%, and 4.28%, respectively compared with the values in 2006 (Figure 9).

### 3.3 NFB dynamic in the rhizosphere soil of M. sacchariflorus and N amounts it contributed to the soil

CFU of NFB exhibited a gradual and significant \((p < .05)\) increase from May to July and the value in July was the highest among the whole year (Figure 10a). After that, CFU of NFB decreased gradually and significantly \((p < .05)\) from July to December (Figure 10a). N amounts in the above-ground biomass (reflected by N amounts in one single tiller) showed a similar variation trend with CFU of NFB (Figure 10b). Pearson correlation analysis showed significant \((p < .01)\) correlation relationship between CFU of NFB and N amounts in above-ground biomass at different time of 2019 (Figure 10c). We calculated N amounts contributed by NFB according to the formula presented in the statistical analysis part. The calculation process and details could be found in Tables S1–S4. The results showed that NFB could contribute an average of 218.74 kg N ha\(^{-1}\) year\(^{-1}\) in 1 m soil depths.

![Figure 4](image4.png) **FIGURE 4** Bulk density in different soil depths in 2006 and 2017. Data are presented as means ± SD error bars. Different small letters indicate significant differences compared with 2006 and 2017 at \(p < .05\)

![Figure 5](image5.png) **FIGURE 5** Soil organic carbon contents in different soil depths in 2006, 2012, and 2017. Data are presented as means ± SD error bars. Different small letters indicate significant differences compared with 2006 and 2017 at \(p < .05\)

![Figure 6](image6.png) **FIGURE 6** (a) Total nitrogen, (b) total phosphorus, and (c) total potassium contents in different soil depths in 2006 and 2017. Data are presented as means ± SD error bars. Different small letters indicate significant differences compared with 2006 and 2017 at \(p < .05\)
DISCUSSION

4.1 Biomass production performance and allocation in above- and below-ground in *M. sacchariflorus*

Following field planting, *M × g* generally takes at least three growing seasons to become fully established and reach its peak yield (Lewandowski et al., 2000; Pyter, 2009). As we assumed, *M. sacchariflorus* reached its peak yield 3 years after planting and showed high biomass yields for 2 years after the peak value (Figure 3a). As a perennial rhizomatous grass, *M. sacchariflorus* needs at least 2 years to develop its rhizomes and generate daughter ramets. This is determined by the nature of such plants in the genus *Miscanthus*. After full establishment, yields of *M × g* follows a ceiling phase producing high biomass yields for more than 20 years under good climate, soils, and agricultural practices (Lewandowski et al., 2000). Without any agricultural practices, we found
that biomass yields of *M. sacchariflorus* decreased gradually after the peak value (Figure 3a). Different from our hypothesis, biomass yields of *M. sacchariflorus* exhibited significant but not large decreases during the latest years (2012–2017) compared to the peak value in 2008 (Figure 3a). During these latest years (2012–2017), biomass yields of *M. sacchariflorus* exhibited a stable level (averaged 22.22 t/ha) without much fluctuation (Figure 3a). These results indicated that *M. sacchariflorus* could maintain its biomass sustainability at a relatively low yield level under such model. It was the first time to report biomass production performance of *M. sacchariflorus* during such long period in China and could motivate the farmers to some degree to grow such energy crop on the abandoned farmland under their preferred model.

Most research work about biomass of *Miscanthus* focused on the above-ground, few mentioned the below-ground part leading to little known about its allocation into below-ground (Arnoult & Brancourt-Hulmel, 2015). In this work, below-ground biomass dry weight in 2006, 2012, and 2017 increased gradually and significantly (p < .05) with time (Figure 3b). Previous cropping history had the most persistent and largest influence on *Miscanthus* growth and production (Yost et al., 2018). It could be said that during the initial early years after *M. sacchariflorus* establishment, there might be adequate nutrients in the soil left from the wheat and corn rotation system. This could meet the demand for the nutrients of *M. sacchariflorus* ensuring the gradual increase of biomass yields during the first 3 years (Figure 3a). We speculated that the slight nutrients export during the first several years and the gradually increased stem density might be responsible for the decreased biomass yields of *M. sacchariflorus* during the period 2008–2012.

Plant root system absorbs water and nutrients from soil and plays a pivotal role in maintaining plant growth and development (Ghosh & Xu, 2014). In stress environments, plants can change the biomass ratio of below- and above-ground parts to adapt to the stress (Chang et al., 2016; Schildwacht, 1988; Xu et al., 2015). In this work, we found that the root shoot ratios of *M. sacchariflorus* increased significantly (p < .05) with time (Figure 3c) indicating an adaptive strategy of *M. sacchariflorus* to the limited nutrients levels. It has been reported that nutrients in above-ground tissues of *Miscanthus* could be transported back into root system during senescence which were used for the following year's re-growth (Beale & Long, 1997). The increased root dry weight might have helped in water and nutrient uptake and the considerable increase in rhizome dry weight (Figure 3b) signified that more nutrients could be stored in the rhizomes which could facilitate the growth and development of *M. sacchariflorus*. The expansion of the root system might contribute partly to the relative stable biomass production of *M. sacchariflorus* during the period 2011–2017 (Figure 3a). These results could enrich people's understanding in the basic agronomic characteristics that how the below-ground part of *Miscanthus* responded to such a model during the long-term period (12 years).

4.2 Effects of 12 years’ cultivation of *M. sacchariflorus* on soil BD

Aside from biomass yield, the benefits from the cultivation of *Miscanthus* for the abandoned farmlands are also expected by the government especially under the urgent background of ecological restoration by energy crops. Soil BD is an important indicator of soil compaction and mechanical resistance influencing the root elongation and growth (Goodman & Ennos, 1999). It has been reported that switchgrass (a model energy grass) managed under 0, 60, and 120 kg N/ha and two harvests per year had lower soil BD compared with no-till continuous corn after 9 years (Stewart et al., 2015). Whereas, Hu et al. (2018) found no significant (p > .05) change in BD of topsoil (0–10 cm) among 5 years’ and 20 years’ *Miscanthus* cultivation, and the original grassland. In this study, soil BD in all depths measured in 2017 decreased significantly (p < .05) compared with the values in 2006 (Figure 4) indicating the improvements in soil physical properties by the long-term cultivation of *M. sacchariflorus*. After so many years’ growth, *M. sacchariflorus* developed considerable root and rhizome biomass over these years (Figure 3b). The decomposed residues of the stubble left after biomass harvest and the more interference of the developed root or rhizome might both account for the larger decrease magnitude of topsoil (11.58% and 10.40% for 0–20 and 20–40 cm, respectively) compared with the values of deeper soil (7.37%, 8.88%, and 3.63% for 40–60, 60–80, and 80–100 cm, respectively, Figure 4). The dead and decomposed root residues could incorporate into the soil and might also have contributed to lowering the soil BD. The decreased soil BD facilitated the interpretation of any nutrient budgets (Hossain, Chen, & Zhang, 2015) and alter the gas diffusivity of soil (Blanco-Canqui & Lal, 2009) which might benefit the absorption of nutrients by *M. sacchariflorus* root system. This work expanded people's understanding in the changes in soil BD caused by *Miscanthus*’ cultivation especially in the deeper soil depths because more focus on the surface soil in previous research work. The improvement in soil physical properties through the long-term cultivation of *M. sacchariflorus* was also inspiring for the government.

4.3 Effects of 12 years’ cultivation of *M. sacchariflorus* on SOC

It has been widely reported that the cultivation of *Miscanthus* can increase SOC accumulation in many climate and soil types
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(Nakajima, Yamada, Anzoua, Kubo, & Noborio, 2018; Placek et al., 2018; Zang et al., 2018; Zhu, Liang, Masters, Kantola, & DeLucia, 2018), though the amount will depend not only on crop type and management practices, but also on the former land-use history. Similar to these studies, we found that the cultivation of *M. sacchariflorus* significantly (p < .05) increased SOC stocks in 0–100 cm depths during a 12 year period in this work (Figure 5), which further verified the previous conclusions under such model in China. Though little litter returned to the field, the stubble residues left on the field could explain the increased SOC content of the surface soil (Figure 5). In addition, *M. sacchariflorus* allocated more and more carbon resource into below-ground parts (root and rhizome) with time (Figure 3b). Root turnover of energy crops also contributed partly to the increased SOC (Ferchaud, Vitte, & Mary, 2016), which might be an important factor for the increased SOC in this study. What is more, some scientists have reported that switchgrass could release up to 20% of their fixed carbon to the rhizosphere through exudation which increased the SOC stocks of the soil (Mao, Li, Smyth, Yannarell, & Mackie, 2014; Nguyen, 2003). Hence, we speculated that the rhizodeposition of *M. sacchariflorus* might be a non-negligible reason for the increased SOC stocks in this work. And the last, it is worth noting that SOC was increased through slower SOC mineralization due to the absence of soil tillage (Soussana et al., 2004) although the actual effects of soil tillage on SOC stocks is questioned (Powlson et al., 2014).

Among the environmental impacts of Miscanthus, changes in SOC stocks are of particular interest because they result in either carbon dioxide emissions or sequestration (Zang et al., 2018). SOC sequestration is an important component in the life cycle of bio-fuel production (Adler, Grosso, & Parton, 2007) and may be key in determining the greenhouse gas (GHG) reduction potential of bio-fuels relative to fossil fuels (Anderson-Teixeira, Davis, Masters, & Delucia, 2009). The increased SOC stocks of *M. sacchariflorus* field under such model in this study (Figure 3b) confirmed the potential of Miscanthus in mitigating GHG emission. Additionally, increases in SOC produce a host of other advantages including increased biomass productivity, improved water and nutrient retention, decreased runoff of both sediment and pollutants, and increased soil biodiversity (Lal, 2004). Hence, this work showed the potential of *M. sacchariflorus* in mitigating GHG emission and improving the soil quality, which met the imperious demands in understanding soil sustainability in the Miscanthus system of the Chinese government.

4.4 The contribution of NFB in maintaining the stable N level of the soil

N is one of the most important macronutrients for plant growth and plays a key role in ecosystem processes (Tamm, 1992). Previous studies have shown variable results from N applications to M × g fields (Arundale, Doehlman, Voigt, & Long, 2014; Christian, Riche, Yates, & Products, 2008; Himken, Lammel, Neukirchen, Czypionka-Krause, & Olfs, 1997). In this work, TN of soil at different depths remained at stable levels during the experimental period (Figure 6a), which indicated that the successive biomass harvest of *M. sacchariflorus* did not reduce the soil N levels though there was no fertilizer applied. This signified that the economic inputs caused by N fertilization could be eliminated or largely reduced. Soil C/N value is a quite sensitive indicator and its changes could dramatically affect the sustainability of soil quality (Sahunalu, 1990). From this work, we found that the stable N levels and increased SOC accumulation resulted in the increased soil C/N values in all five depths. We speculated that the increased but not too high C/N values (< threshold value 25) might stimulate microbe growth, reproduction and activities which could be reflected by the largely increased MC, MN and MP contents in this study (Figure 8a–c). While the effects of such increased soil C/N values on the soil C, N cycle and other soil properties, which were not exploited in this work, need to be further studied. The microbial community structure and the underlying mechanisms also need to be uncovered through the high-throughput sequencing method coupled with determination of related enzymes’ activities of soil in the next work. The non-essential N input during the long-term cultivation of *M. sacchariflorus* is quite encouraging for the farmers which signified the feasibility of their preferred management model, which also reflected the value and significance of this work.

The role of symbiotic NFB in relation with the roots of legumes is well known. Besides this, non-symbiotic NFB also play important roles in N inputs of grasslands (Abbadie, Mariotti, & Menaut, 1992), forest soils (Limmer, Drake, & Biochemistry, 1996), and farm system (Kennedy & Islam, 2001). Since its discovery in sugarcane (Baldani, Caruso, Baldani, Goi, & Döbereiner, 1997), the associative N fixation has been found and studied in many plants including Miscanthus. It has been reported that non-symbiotic NFB contributed small but measurable amounts of N for the first year M × g (Keymer & Kent, 2014). In this work, the significant correlation relationships between CFU of NFB and the N accumulation amounts in above-ground biomass (Figure 10c) indicated that NFB might play an important role in the N providing for *M. sacchariflorus*. N amounts from wet and dry deposition (Table S4) made this problem more complicated though there was an obvious decrease trend during the period 2013–2017 (Table S4) mainly due to the official management and control. The calculation (considering the changes in soil N contents, N amounts in below-ground biomass and N amounts removed with the harvested biomass, and N wet and dry deposition) results showed that NFB could contribute about 218.74 kg N ha⁻¹ year⁻¹ in 1 m soil depths.
to the fully established Miscanthus system (Tables S1–S4). This result might be not very accurate for the slight difference in N distribution in soil and the absence of N leakage and volatilization during the process of calculation. But it signified that N fixation by the non-symbiotic NFB could provide considerable amounts of N for growth and development of M. sacchariflorus offsetting the N export by biomass harvest. However, which type of the non-symbiotic NFB (including the endophytic diazotrophs, the non-symbiotic NFB in rhizosphere region or the free non-symbiotic NFB in soil) played the key role in this process still needs to be further studied. These results verified again the contribution of NFB to Miscanthus system and this was the first time to calculate the N amount provided by biological N fixation in Miscanthus system from the field scale over a 5 year period.

4.5 Effects of 12 years’ cultivation of M. sacchariflorus on soil P and K as well as pH

P and K are also two main macronutrients vital for the plants growth and development and their levels in soil are also important indicators of soil quality. Without fertilization, the annual harvest of biomass meant the net export of these two elements from soil. The annual removal of P by Miscanthus ranged from 2 to 5 kg ha\(^{-1}\) year\(^{-1}\) which is less than the removal from annual crops such as 42 kg ha\(^{-1}\) year\(^{-1}\) in corn and 58 kg ha\(^{-1}\) year\(^{-1}\) in sorghum (Propheter, Staggenborg, Wu, & Wang, 2010). In this work, we found that TP amounts removed by the biomass harvest were 6.19 and 5.51 kg ha\(^{-1}\) year\(^{-1}\) in 2012 and 2017, respectively (Table S5). This indicated that during the growth and development of M. sacchariflorus, very little P was needed. It has been reported that Miscanthus normally could activate P in the soil from fixed state (Yang, Ren, Liu, & Wang, 2013). Gerretsen and Soil (1948) also reported that plants could absorb more P from the unpasteurized soil than from the pasteurized soil due to P dissolving by soil microbe. Thus, we speculated that the root activation and microbe dissolving function both contributed to meet the low demands in P of M. sacchariflorus.

More developed root in 20–40 cm soil indicated more AP absorbed by roots, rendering less AP left in the soil compared to that in 40–100 cm soil with less root absorption and more AP left (Figure 7b). The stubble residues left on the surface soil (0–20 cm) might explain the similar AP levels in 2006 and 2017 (Figure 7b). While, the subtle changes in AP contents (Figure 7b) did not change TP stocks of the soil (Figure 6b) which indicated that long-term cultivation of M. sacchariflorus did not negatively affect the soil TP levels in this study. Different from our hypothesis, long-term cultivation of M. sacchariflorus showed no negative effects on the soil P levels which was another inspiring conclusion for the farmers and government.

The net export of soil K through biomass harvest may lead to a depletion of soil K stocks in a long-term period though Miscanthus has the properties of nutrient re-translocation (Shield et al., 2014). K removal rates of Miscanthus also vary by year, site location and species (Yost et al., 2018). In this study, total amounts of K removed from the field ranged from 85.47 to 89.47 kg ha\(^{-1}\) year\(^{-1}\) indicating a non-negligible amount of K removal (Table S5). After 12 years’ harvest, TK stocks of soil decreased significantly (\(p < .05\)) which clearly indicated the consumption of K in the soil by annual harvest of biomass (Figure 6c). However, AK contents in all five soil depths exhibited no significant (\(p > .05\)) difference compared between 2006 and 2017, which implied that the decreases in TK stocks did not influence the AK levels probably due to the large storage of K in soil. For most previous research work, people focused on the amount of K removal and the changes of K contents in top soil (0–30 cm) after a relative short-term cultivation of \(M \times g\). It still remained largely uncovered that how the deeper soil K stocks would change after the long-term cultivation of Miscanthus until this study was carried out. Meanwhile, this result could provide some suggestions for the government about the application of K element after the long-term cultivation of M. sacchariflorus which was meaningful for the soil sustainability of Miscanthus’ system.

The overuse of chemical fertilization mainly in the form of ammonium-based fertilizers severely negatively affected the soil quality and environment rendering many ecological problems such as soil acidification, soil erosion, N and P leakage, and runoff (Goulding, 2016; Martins et al., 2014). In this work, soil pH values increased significantly (\(p < .05\)) in all the five soil depths (Figure 9) which indicated that the long-term cultivation of M. sacchariflorus without fertilization could prevent the soil acidification effectively. Additionally, the developed root system, annual harvest of biomass and the absence of chemical fertilization together minimized the risk of N and P pollution to the water quality through runoff and leakage from Miscanthus system.

This work indicated that it seemed viable to manage M. sacchariflorus system under such farmer-welcomed model. The supplement of K element was not necessary but notable especially in the long-term period cultivation of M. sacchariflorus. The function of NFB in balancing N levels and mainly in the long-term period cultivation of Miscanthus system. The screening and identification of the highly efficient N fixation bacteria and the development of such bacterial fertilizer needs to be further studied in the future. The absence of chemical fertilization could avoid the environmental pollution of N and P through surface runoff and leakage. Additionally, long-term cultivation could obviously improve soil quality while providing a large quantity of biomass. Though there was some weakness in this study, the conclusions from this work were meaningful for workers on Miscanthus. They were also helpful and encouraging for the farmers and government.
and could clearly tell them the benefits and cautions of the replacement of the abandoned farmland by the energy crop *Miscanthus*. These conclusions could also provide important information for the establishment of policy by government and facilitate the popularization and application of *Miscanthus* under a similar land and climate type in China.

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SUPPORTING INFORMATION

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

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