Cropland-to-*Miscanthus* conversion alters soil bacterial and archaeal communities influencing N-cycle in Northern China

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**Abstract**

*Miscanthus* spp. are increasingly cultivated in cropland worldwide due to their bioenergy potential and multiple ecological services. Effects of long-term cropland-to-*Miscanthus* conversion without N fertilizer on soil microbiome and N cycling largely remain unknown. We aimed to explore the effects of *Miscanthus* conversion on soil microbiome and N cycling over a 15-year period. We analyzed diversity, composition, and abundance of bacterial and archaeal communities using 16S rRNA amplicon sequencing, and abundances of N-cycling-related genes using quantitative polymerase chain reaction of 0–10 cm soils collected from bare land, cropland, 10-year *Miscanthus × giganteus*, and 15-year *Miscanthus sacchariflorus* land in Beijing. Conversion decreased soil sand and micro-aggregate proportion, nitrate N (NiN), available phosphorus levels, conductivity, temperature, and pH, while increasing proportion of soil clay and macro-aggregate (MAA), soil organic C (SOC), available N (AN), exchangeable Mg²⁺ (EMg²⁺), and available potassium (AK) contents as well as microbial C/N. Consequently, diversity, composition, and abundance of soil bacterial community exhibited larger changes than those values of archaeal community after conversion. Soil AP, EMg²⁺, AK, and SOC were key factors in shifting microbiome from the cropland to *Miscanthus* pattern. Moreover, abundances of bacterial and archaeal communities and the N fixer gene *nifH* increased, whereas that of the bacterial ammonia monooxygenase gene decreased. The copies of other N-cycling-related genes in the two *Miscanthus* lands seemed similar to those values of cropland. The *nifH* copies negatively correlated with soil NiN and positively correlated with AN, EMg²⁺, ECa²⁺, SOC, AK, and MAA. We conclude that changes in soil microbiome pattern induced by the variation of soil properties enhance microbial N fixation potential, maintaining stable N levels and robust N cycling with lower N leakage risk after conversion. These results should inspire farmers and governments to large-scale use *Miscanthus* on marginal cropland in Northern China.
1 | INTRODUCTION

Intensive agricultural activities in marginal cropland caused by the pursuit of crop production have caused many ecological problems, including soil degradation with wind and water erosion during the past 60 years in China (Tian et al., 2010; Xu & Li, 2003). Returning marginal cropland to grassland or forests has been a profitable strategy in addressing these ecological problems (Bullock & King, 2011; Wei et al., 2018), and has drawn widespread attention from government agencies throughout China (Li et al., 2012). Recently, a new version of the “Cropland Returning Project” has been launched according to the “White Book” of the State Forestry and Grassland Administration of China. The conversion of large areas of marginal cropland provides an opportunity for Miscanthus cultivation.

Miscanthus, a perennial native grass with the C₄ photosynthetic pathway (Dufosse et al., 2014), can be considered an excellent energy crop due to its high biomass yields and quality, few maintenance requirements, excellent stand longevity, efficient nutrient recycling, and strong stress resistance, as well as having a wide range of ecological adaptations (McCalmont et al., 2017; Pidlisnyuk et al., 2014; von Cossel et al., 2019). The dense and upright stems of Miscanthus can prevent wind erosion and improve the landscape effects of cultivated land (Evers et al., 2013; Miriti et al., 2017). Its developed root system can prevent water erosion and N leakage into underground water (McCalmont et al., 2017). Moreover, Miscanthus can provide habitat for wildlife, which can obviously increase biodiversity of cultivated areas (Stewart et al., 2009). Hence, the variety of uses and multiple ecological services provided by Miscanthus make it a good candidate plant during the ecological restoration of marginal cropland in Northern China.

Soil N plays critical roles in terrestrial ecosystems because it is required for plant growth, which directly affects the stability and sustainability of terrestrial ecosystems. Changes in the soil N level after Miscanthus cultivation are of great concern by many researchers worldwide. Previous research has shown that appropriate N fertilizer application is needed to maintain long-term successive high biomass yields of Miscanthus (Arundale et al., 2014; Dufosse et al., 2014). However, it has been reported that Miscanthus has relatively low N removal rates (Alberto Oliveira et al., 2017; Masters et al., 2016) compared to grain crops (e.g., 3.7% of N removal in maize) due to its nutrients re-translocation at the end of growing stages (Heaton et al., 2009). This means long-term harvest of Miscanthus could potentially result in a soil N deficiency in cultivated land. However, many published papers indicated that Miscanthus cultivation does not reduce soil N levels, or can even increase it obviously in some cases, independent of stand age, soil depths, and management practices (Dufosse et al., 2014; Kahle et al., 1999). We previously found that long-term (12 years) successive harvest of Miscanthus sacchariflorus (Mis) without N fertilizer did not reduce 0–100 cm soil N levels compared to the initial values in cropland (Zhao et al., 2020). To better understand the mechanisms behind this phenomenon, the soil microbiome and N cycling characteristics after Miscanthus cultivation need to be studied in more depths.

The cycling of soil N, including transformations of different forms of N, is usually driven by the soil microbiome and influenced by soil properties as well as the vegetation cover types (Ma et al., 2020; Smith & Rice, 1986). The soil microbiome performs many pivotal ecological functions during the entire N cycle (Pengqiang et al., 2018; Philippot et al., 2013; Smith & Rice, 1986). Some microbial groups can fix atmospheric N₂ into ammonium N, thereby serving as N fixers (Smith & Rice, 1986). Ammonium N produced by processes of biological fixation, industrial synthesis, and the decay of organic matter can be gradually oxidized into nitrite or nitrate N (NiN), which is also mediated by some nitrifying microbial groups (Lu et al., 2017; Rosswall, 1982). In turn, the NiN can be reduced to nitrite N afterwards to nitrous oxide or N₂ by different denitrifying microbial groups (Smith & Rice, 1986). These processes directly affect soil N leakage and emission risks (Smith & Rice, 1986). All of these processes related to the soil N cycle have been systematically evaluated and studied in different ecosystems, for example, the grassland, forests, wetlands, and farmland ecosystems (Augustine & Frank, 2001; Murty et al., 2010; Yang et al., 2009). Many studies have observed that land-use change can drastically affect soil properties, N cycling characteristics, and thus reshape the soil microbiome (Battle-Aguilar et al., 2011; van Delden et al., 2016). However, a large knowledge gap exists in the current understanding of changes in the soil microbiome and N cycling after long-term conversion of cropland to Miscanthus in a temperate continental monsoon climate.

Limited information about the interactions among Miscanthus, the soil microbiome, and N cycling can be acquired from previous studies. A 5-year field experiment showed that Miscanthus × giganteus (Migi) cultivation decreased the diversity of the bacterial community while enhancing functions related to soil N and P cycling when...
compared with uncultivated land in Nanyang, China (Duan et al., 2019). Nebeská et al. (2018) observed that the G+/G− and fungal/bacterial ratios in organic and toxic metals polluted soil increased after 2 years of Miscanthus cultivation through a greenhouse pot experiment. Several studies have reported the existence of diazotrophs in bulk and rhizospheric soil of land with cultivated Miscanthus (Zhao et al., 2020), and documented their considerable contribution to N fixation gene (nifH; Li et al., 2016; Somani et al., 2018; Wewalwela et al., 2020), but lacked revelations of the responses of soil N-cycling-related genes and the microbiome for long-term Miscanthus conversion in Northern China.

The objectives of this study were (1) to evaluate effects of long-term conversion of cropland to Miscanthus (10-year Migi and 15-year Mis) on the diversity, composition, and abundance of soil bacterial and archaeal communities; and (2) to evaluate effects of the conversion on the soil N cycle in Northern China. To the best of our knowledge, this is the first study worldwide that illustrates changes in the soil microbiome and N cycling characteristics after a 15-year conversion of cropland to Miscanthus. According to previous studies and our previous findings (Zhao et al., 2020), we hypothesized that (1) long-term conversion of cropland to Miscanthus enhances N fixation by which a Miscanthus system can maintain stable soil N levels even without N fertilizer; and (2) obvious changes in the soil microbiome and N cycle occur that are caused by changes in soil properties.

2 MATERIALS AND METHODS

2.1 Study site description and management

This study site was located at the experimental base of the Beijing Research and Development Center for Grass and Environment (40°23′N, 116°28′E) at Xiaotangshan Town in Beijing, China (Figure 1). Zhao et al. (2020) described the climate type, soil texture, and some detailed information of the experiment site.

Four land-use types were employed in this experiment (Figure 1): bare land, traditional cropland (>30 years), 10-year Migi land, and 15-year Mis land. These four lands were closely located in our experimental field. Three separate subplots (4 m × 5 m) were used as three replicates in the bare land plot. Any plants were manually removed over a 15-year period. In the cropland plot (around 50 m × 120 m), we randomly selected three 4 m × 5 m subplots as replicates. Cropland was managed under traditional small-family model which is commonly used in Northern China. Rotary tillage was conducted on cropland at a depth of around 20 cm before wheat and corn sowing.

The average total amounts of N, P2O5, and K2O applied for winter wheat and summer corn in 1 year were about 480, 300, and 195 kg/hm2, respectively, according to a survey of farmers. Wheat stalks were harvested, leaving around 10 cm of stubble. The corn stalks together with the grain were harvested for silage, leaving about 10 cm of stubble. Herbicides and insecticides were applied as needed during crop production. The Migi and Mis plots were about 7 m × 16 m and 10 m × 20 m, respectively. Both of them contained more than three 4 m × 5 m subplots as replicates which could meet the requirements of the experiment. These two lands were arable beforehand because the experiment was based on the previous experimental platform. These two lands were also used for wheat and corn production before the cultivation of energy crops (Migi and Mis). Before Miscanthus planting, about 150 kg/hm2 of compound fertilizer (30 kg/hm2 N, 42.97 kg/hm2 P2O5, and 18.07 kg/hm2 K2O) was provided to ensure growth of Miscanthus based on our previous experience. A ditch (1 m wide and 1.5 m deep) was set around the Mis plot to prevent Miscanthus rhizome propagation into nearby areas (Figure 1). The subplots of the Mis and Migi plots were all isolated with cement boards (1.5 m in depth). During the first year of Miscanthus cultivation, any weeds in Miscanthus plots were artificially removed. In early November of each year, Miscanthus biomass was harvested manually using a sickle, leaving about 10 cm of stubble.

2.2 Soil sampling and determination of physical and chemical properties

Soil samples were collected on July 2, 2020 (after the last tillage) from the four land types. The height of corn seedling was around 20 cm when sampling. Before sampling, the obvious litter layer was carefully removed. We previously found that the fibrous roots (interact most with soil) of Miscanthus were the thickest in around 10 cm depth in our experimental field. Moreover, soil microbiome in the soil of 0–10 cm depth is most active. Ten 0–10 cm soil cores (3.5 cm in diameter) were randomly collected in each subplot of the four plots and combined into one composite sample as one replicate. Soil samples were sieved through a 2 mm mesh size and then homogenized thoroughly after removal of obvious stones and coarse roots. Each soil sample was divided into three subsamples in the field. One subsample was shipped back to the laboratory in insulated barrels with frozen CO2 for 16S rRNA sequence analysis. Another subsample was transported in an ice chest to a laboratory refrigerator for the determination of nitrate and nitrite N levels as well as microbial biomass (MB). The last subsample was air-dried under ambient laboratory conditions for the determination of other soil properties.
About 400 g of soil was used to analyze soil physical and chemical properties. Soil organic C (SOC), total N (TN), total phosphorus (TP), total potassium (TK), available N (AN), available phosphorus (AP), available potassium (AK), microbial C (MC), microbial N (MN), and pH were determined using methods described in Zhao et al. (2020). A WET Sensor Kit (Delta-T Devices Ltd.) was used to determine soil conductivity (SC), moisture (SM), and temperature (ST). Soil aggregate and particle size were determined using a Micotrac SDC (Kimplerstraße, Germany) according to the manufacturer’s instructions. Ammonium N and NiN concentrations in the 0.01 mol/L CaCl₂ extract were determined colorimetrically by automated flow injection analysis (Skalar Analytical B.V.) after alkaline persulfate oxidation (Cabrera & Beare, 1993). Soil exchangeable bases Mg²⁺, Na⁺, K⁺, and Ca²⁺ (referred to as EMg²⁺, ENa⁺, EK⁺, and ECa²⁺, respectively) were extracted from an ammonium chloride (0.1 mol/L)-alcohol (70%) solution (pH 8.5) and determined using atomic absorption spectrometry (SHIMADZU).

### 2.3 Soil DNA extraction and quantitative PCR analyses

Microbial community genomic DNA was extracted from 0.25 g of each soil sample using a FastDNA® Spin Kit for Soil (MP Biomedicals) according to the manufacturer’s instructions. The DNA extract was checked on a 1% agarose gel, and DNA concentration and purity were determined with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific). The abundance of archaea, bacteria, denitrifiers, nitrifiers, and N fixers were determined by quantitative polymerase chain reaction (PCR) targeting bacteria and archaea (16S rRNA), nitrite reductase (nirK), nitrous oxide reductase (nosZ), ammonia monooxygenase of bacteria (AOB) and archaea (AOA), and nitrogenase (nifH) genes, respectively. A LineGene9600plus PCR thermo-cycler (Hangzhou Bior Technology Co., Ltd.) was used to finish the quantitative PCR analyses; PCR amplifications of these seven genes were performed as follows: initial denaturation at 95°C
for 5 min, followed by 40 cycles of denaturing at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 40 s, followed by a single extension at 72°C for 10 min, and ending at 4°C. The PCR mixtures contained 5× TransStart FastPfu buffer 4 μl, 2.5 mM dNTPs 2 μl, forward primer (5 μM) 0.8 μl, reverse primer (5 μM) 0.8 μl, TransStart FastPfu DNA Polymerase 0.4 μl, template DNA 10 ng, and finally ddH₂O up to 20 μl. PCRs were performed in triplicate. The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences) according to the manufacturer’s instructions and quantified using a Quantus™ Fluorometer (Promega). Serial dilutions of the known gene quantity amplified previously by PCR were used to generate the standard curves. The pair primers used in this procedure and the reaction conditions for amplification of these seven genes are listed in Table S1.

### 2.4 Illumina MiSeq sequencing and bioinformatics analyses

Amplicon sequencing targeting the V3–V4 region of the 16S rRNA gene of bacteria and V6–V8 region of archaea was performed for taxonomical profiling. Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina) according to the standard protocols of Majorbio Bio-Pharm Technology Co., Ltd. Unassembled raw amplicon data were submitted to the NCBI Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra) and can be retrieved under accession nos. PRJNA681707 (bacteria) and PRJNA681722 (archaea).

The raw 16S rRNA gene sequencing reads were demultiplexed, quality filtered by fastp version 0.20.0 (Chen et al., 2018), and merged by FLASH version 1.2.7 software (Magoc & Salzberg, 2011) with the following criteria: (1) The 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window. Any truncated reads shorter than 50 bp were discarded; reads containing ambiguous characters were also discarded. (2) Only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region was 0.2. Reads that could not be assembled were discarded. (3) Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching was done, and two nucleotide mismatches were done with primer matching. Operational taxonomic units (OTUs) with a 97% similarity cutoff (Edgar, 2013; Stackebrandt & Goebel, 1994) were clustered using UPARSE version 7.1 (Edgar, 2013), and chimeric sequences were identified and removed. The taxonomy of each representative OUT sequence was analyzed by RDP Classifier version 2.2 (Wang et al., 2007) against the 16S rRNA database using a 0.7 confidence threshold.

### 2.5 Statistical analysis

Shapiro–Wilk and Levene tests were performed to check the distribution and homoscedasticity of data prior to analysis, respectively. The high-throughput sequencing data based on 16S rRNA of bacterial and archaeal communities were prepared on a cloud platform based on a remote server. One-way ANOVA was carried out based on the least significant difference method corrected by Bonferroni correction using the “agricolae” package in R (3.5.2) software to evaluate significant differences, at p < 0.05, in soil properties, Shannon index of the bacteria and archaea, OTU index, and gene abundances among the four groups. The Euclidean method was used to determine inter-sample distances. A Wilcoxon rank-sum test corrected by the Bonferroni correction method was carried out to verify significant differences in relative abundances of different phyla between two randomly selected groups while the “bootstrap” method was used to calculate the confidence interval. Correlation analysis based on Person’s method at p < 0.05 (*), 0.01 (**) and 0.001 (***) levels was carried out using the “psych” package in R (3.5.2). Redundancy analysis (RDA) was used to determine correlations between the microbial community structure and soil properties. Initially, we conducted detrended correspondence analysis to evaluate the gradient size of the species distribution; we found that the data were linearly distributed and the length of the gradient was <3.0, which indicated that the best-fit mathematical model for the data was RDA. We used Euclidean method and Monte Carlo permutation test with 999 random permutations to finish the two-way permutational ANOVA (PERMANOVA; Anderson, 2010) to verify whether different land types had significantly different microbial communities. Figures used in this study were prepared using R (3.5.2) and origin software (2019b).

### 3 RESULTS

#### 3.1 Soil physical and chemical properties

Table 1 shows that the long-term substitution of Miscanthus of traditional crops dramatically (p < 0.05) increased soil macro-aggregate (MAA) and clay proportions but decreased soil micro-aggregate (MIA) and sand proportions (Table 1). Additionally, the variation in the magnitude of soil MAA, MIA as well as clay proportions seemed to be exacerbated (p < 0.05) with an increase in the number of years after cultivation of Miscanthus, which might also be attributed to different Miscanthus taxa (Table 1). At the end of the experiment,
soil AN, AK, EMg2+, ECa2+, and SOC contents increased in Miscanthus lands while changes of those values in Mis land were more dramatic than in Migi soils (Table 2). Miscanthus cultivation dramatically (p < 0.05) reduced NiN, ENa+, AP, SC, and ST; the variation in the magnitude of those values increased with years after cultivation of Miscanthus, when compared with those values in cropland (Table 2). Moreover, significant (p < 0.05) decreases of soil AP, pH, TP, and microbial C/N (MCN) were found in Mis land (Table 2). A high level of soil AnN was found in Mis land compared with the other three land types (Table 2). Soil chemical properties except for the TP, TK, AP, NiN, and ENa+ contents of bare land all decreased when compared with those values in the other three land types (Table 2).

**TABLE 1**  
Soil physical properties in the four land-use types

| Properties          | Units     | Bareland | Cropland | Migi       | Mis       |
|---------------------|-----------|----------|----------|------------|-----------|
| Soil aggregate MAA | %         | 4.25 ± 0.37c | 4.62 ± 0.22c | 5.61 ± 0.20b | 6.5 ± 0.35a |
| Soil aggregate MIA | %         | 95.75 ± 0.37a | 95.38 ± 0.22a | 94.39 ± 0.20b | 93.5 ± 0.35c |
| Soil particle size  |           |          |          |            |           |
| Clay (<0.002 mm)    |           | 4.17 ± 0.18c | 3.84 ± 0.15c | 5.67 ± 0.13b | 6.24 ± 0.14a |
| Silt (0.002–0.02 mm)|           | 26.78 ± 4.19a | 24.39 ± 1.74a | 26.71 ± 2.26a | 27.81 ± 1.78a |
| Sand (0.02–2 mm)    |           | 69.06 ± 2.21ab | 71.77 ± 2.58a | 67.62 ± 2.10b | 65.95 ± 1.91b |

All data are presented using mean ± standard deviation (SD). Different lower-case letters show significant differences among the four land-use types (p < 0.05). Migi and Mis indicate 10-year Miscanthus × giganteus land and 15-year Miscanthus sacchriflorus land, respectively. The same meanings are used in the following tables. Abbreviations: MAA, soil macro-aggregate; MIA, micro-aggregate.

**TABLE 2**  
Soil chemical properties in the four land-use types

| Properties          | Units     | Bareland | Cropland | Migi       | Mis       |
|---------------------|-----------|----------|----------|------------|-----------|
| pH value            | –         | 7.48 ± 0.03a | 7.50 ± 0.04a | 7.41 ± 0.02a | 7.23 ± 0.04b |
| Soil orgnic C       | g/kg      | 6.64 ± 0.78b | 8.45 ± 0.63b | 10.71 ± 0.93a | 10.42 ± 0.21a |
| Total N             | %         | 0.06 ± 0.01b | 0.08 ± 0.003a | 0.09 ± 0.01a | 0.09 ± 0.01a |
| Total P             | mg/kg     | 0.10 ± 0.004ab | 0.11 ± 0.01a | 0.11 ± 0.01ab | 0.09 ± 0.004b |
| Total K             | mg/kg     | 1.99 ± 0.09a | 1.95 ± 0.08a | 2.03 ± 0.09a | 2.07 ± 0.11a |
| Available N         | mg/kg     | 46.59 ± 4.72c | 61.72 ± 3.64b | 64.23 ± 5.82ab | 77.05 ± 6.48a |
| Available P         | mg/kg     | 5.53 ± 1.81c | 17.67 ± 1.39a | 10.58 ± 0.92b | 3.95 ± 0.48c |
| Available K         | mg/kg     | 89.5 ± 14.24b | 95.83 ± 9.57b | 107.00 ± 6.26ab | 135.17 ± 10.54a |
| Ammonium N          | mg/kg     | ND        | 0.02 ± 0.003b | ND          | 0.21 ± 0.01a |
| Nitrate N           | mg/kg     | 5.39 ± 1.25b | 11.30 ± 1.24a | 2.58 ± 0.79c | 2.57 ± 0.39c |
| Microbial C         | mg/kg     | 86.65 ± 10.28b | 319.84 ± 41.68a | 311.21 ± 22.72a | 325.56 ± 27.14a |
| Microbial N         | mg/kg     | 17.07 ± 1.75b | 53.84 ± 9.53a | 48.20 ± 2.61a | 43.67 ± 4.11a |
| Soil C/N            | –         | 14.28 ± 0.93a | 13.04 ± 0.51a | 12.82 ± 0.34a | 12.94 ± 0.86a |
| Microbial C/N       | –         | 5.07 ± 0.09c | 5.97 ± 0.34b | 6.46 ± 0.29b | 7.46 ± 0.34a |
| Exchange base cation | cmol/kg   | 9.16 ± 0.46a | 9.70 ± 0.35a | 9.76 ± 0.40a | 10.07 ± 0.41a |
| quantity            |           |          |          |            |           |
| Exchangeable Mg2+   | cmol/kg   | 2.74 ± 0.13b | 2.87 ± 0.14b | 3.05 ± 0.08ab | 3.24 ± 0.12a |
| Exchangeable K+     | cmol/kg   | 5.84 ± 0.36a | 6.22 ± 0.23a | 6.00 ± 0.24a | 6.08 ± 0.34a |
| Exchangeable Na+    | cmol/kg   | 0.18 ± 0.01b | 0.22 ± 0.02a | 0.15 ± 0.01b | 0.17 ± 0.01b |
| Exchangeable Ca2+   | cmol/kg   | 0.37 ± 0.05b | 0.38 ± 0.01ab | 0.54 ± 0.12ab | 0.58 ± 0.05a |
| Soil conductivity   | mS/m      | 175.50 ± 3.50a | 179.50 ± 6.26a | 152.67 ± 9.83b | 152.67 ± 7.69b |
| Soil moisture       | %         | 31.33 ± 1.14a | 22.55 ± 2.05b | 23.45 ± 1.28b | 18.32 ± 0.55c |
| Soil temperature    | °C        | 29.20 ± 0.39a | 27.80 ± 0.20b | 26.25 ± 0.05c | 25.33 ± 0.10d |

Note: Migi and Mis indicated 10 years' Miscanthus × giganteus land and 15 years' Miscanthus sacchriflorus land, respectively. All the data was presented in a way of mean ± SD as listed in the table. Different lower-case letters showed the significant difference among the four land use types at p < 0.05.

Abbreviation: ND, not detected.
3.2 Microbial community composition, diversity, and structure

We acquired approximately $8.1 \times 10^5$ and $7.2 \times 10^5$ sequences from total 12 soil samples for bacteria and archaea, respectively, using 16S rRNA sequencing (Table S2). There were 29 phyla in the bacterial community and five phyla in the archaeal community. Actinobacteriota (35.62%, relative abundance on average), followed by Proteobacteria (24.15%), Acidobacteriota (9.44%), Planctomycetota (5.80%), Chloroflexi (4.99%), Firmicutes (4.69%), Gemmatimonadota (3.66%), Myxococcales (2.79%), Bacteroidota (1.90%), and Verrucomicrobiota (1.59%) were the most abundant phyla in the bacterial community in all soil samples and together they accounted for more than 94% of the total bacterial community (Figure 1a). For the archaeal community, Crenarchaeota followed by Thermoplasmata were the dominant phyla accounting for more than 99% of the total archaeal community (Figure 1b). Significant differences were found in relative abundances of Acidobacteriota ($p < 0.05$), Proteobacteria ($p < 0.05$), Acidobacteriota ($p < 0.01$), Planctomycetota ($p < 0.05$), Firmicutes ($p < 0.01$), Gemmatimonadota ($p < 0.01$), and Bacteroidota ($p < 0.001$) among the four land types (Figure 2). Actinobacteriota, as the most abundant phylum, was mainly composed of the genus Actinobacteria (39.81%), Vicinamibacteria (13.19%), Acidimicrobiia (10.33%), Blastocatellia (4.46%), and Acidobacteriae (3.34%), and all of these genera exhibited significant ($p < 0.05$) differences among the four land types (Figure S2). The abundance of the dominant phyla in archaeal community varied little among the four land types (data were not shown).

The relative abundances of the Actinobacteriota, Proteobacteria, Firmicutes, Bacteroidota, and Nitrospirata in Mis land were all significantly ($p < 0.05$) lower by 13.92%, 10.95%, 18.93%, 31.97%, and 62.18%, respectively, than those values in cropland (Figure S3a). Significant ($p < 0.05$) increases by 50.20%, 90%, 134%, 106%, and 57.14% were observed in the relative abundances of the phylum Acidobacteriota, Latescibacterota, Planctomycetota, Methylospiraita, and Desulfobacterota, respectively, after the conversion from cropland with Mis land (Figure S3a). For Migi land, only the relative abundance of Acidobacteriota was significantly ($p < 0.05$) higher by 19.38% than that value of cropland. The values of Bacteroidota and Nitrospirata in Migi land both exhibited significant ($p < 0.001$) decreases by 53.31% and 42.33%, respectively, compared with those values in cropland (Figure S3c). We detected significant ($p < 0.05$) increases of 45.64%, 216.67%, 126.67%, 94.12%, 25.83%, and 33.19% in the relative abundance of the Bacteroidota, Latescibacterota, NB1-j, Desulfobacterota, Acidobacteriota, and Myxococcales, respectively, and significant ($p < 0.05$) decreases of 15.66% and 24.47% in the relative abundances of Actinobacteriota and Firmicutes, respectively, in Mis land when compared with those values in the Migi land (Figure S3c). When compared with the bare land, the relative abundances of Bacteroidota, Verrucomicrobiota, Planctomycetota, Proteobacteria, and Actinobacteriota in the Mis and Migi lands were increased by 189% and 98%, 140% and 29%, 45.36% and 17.56%, 26.36% and 22.35%, and 2.39% and 21.41%, respectively (Figures S3b, d and Figure 4).

The Shannon index of the bacterial community based on the genus levels in Mis land was significantly ($p < 0.05$) higher than those values of the other three land types (Figure 3a). The OTU richness of cropland, Mis, and Migi lands were all significantly ($p < 0.05$) higher than that in bare land (Figure 3c). For the archaeal community, bare land had the highest OTU richness followed by Migi land ($p > 0.05$), Mis land ($p < 0.05$), and cropland ($p < 0.05$, Figure 3d).

Clustering analysis showed a sharp distinction in the β-diversity of the bacterial community among Mis land, cropland, and bare land. However, Migi land seemed to be indistinguishable to cropland (Figure S5). For the archaeal community, it was difficult to obviously differentiate Mis land, cropland, and Migi land (Figure S5). Based on Venn analysis, we found that there were 6, 4, and 16 unique OTUs in the bacterial community in Miscanthus lands, cropland, and bare land, respectively (Figure S6a). For the archaeal community, there were only five unique OTUs detected in bare land (Figure S6b). Most of the OTUs of the bacterial and archaeal communities were present in all four land types (Figure S6a).

Redundancy analysis showed that samples were grouped by different land-use types at the genus level of the bacterial community (Figure 4a). It was hard to separate Mis and Migi samples into two independent groups based on the genus level for the archaeal community while they were both obviously distinguished from cropland and bare land (Figure 4b). The first and second axes together accounted for more than 83% of the total variance in the soil bacterial community (Figure 4a) while more than 95% of the total variance in the soil archaeal community could be explained by the first and second axes (Figure 4b). Soil samples from the four land types were markedly different according to the PERMANOVA for the bacterial ($F = 14.61$, $p < 0.001$) and archaeal communities ($F = 8.55$, $p < 0.001$). Monte Carlo analysis followed by RDA showed the following: (1) a significant correlation existed between AP ($p < 0.001$) and the bacterial community structure in cropland; (2) pH ($p < 0.001$), temperature ($p < 0.001$), moisture ($p < 0.001$), NiN ($p < 0.01$), and clay proportion ($p = 0.05$) were significantly correlated with the bacterial community structure in bare land; and (3) TOC ($p < 0.001$), EMg$^{2+}$ ($p < 0.01$), and AK ($p < 0.01$) were significantly correlated with the bacterial community structure in Mis land (Figure 4a). In addition, AP was significantly correlated with the structure of the archaeal community ($p < 0.05$) in cropland while temperature ($p < 0.05$), clay proportion ($p < 0.01$), NiN ($p < 0.019$), and
moisture ($p < 0.05$) correlated with the archaeal community structure in bare land (Figure 4b). For the specific phyla of the bacterial and archaeal communities, we observed that most of the soil properties correlated significantly ($p < 0.05$) with the non-dominant phyla (Figure S7a,b). Soil pH, TP, and AP were all negatively correlated with the Acidobacteriota and soil MMA, while EMg$^{2+}$ and AK were positively correlated with the Acidobacteriota (Figure S7a).

### 3.3 Abundance of the microbial community and N-cycle-related genes determined by real-time PCR

The bacterial and archaeal communities were most abundant in Mis land, followed by cropland, Migi land, and bare land ($p < 0.05$, Figure 5a). The abundance of the ammonium oxidation gene from the bacterial community ($AOB$) in cropland was significantly ($p < 0.05$) higher than those values for the other three land types (Figure 5c). The abundances of the ammonium oxidation gene from the archaeal community ($AOA$) in cropland and Miscanthus lands were all significantly ($p < 0.05$) higher than those values in bare land. Meanwhile, the abundances of these two genes in cropland and Miscanthus lands exhibited no significant differences (Figure 5d). A similar variation trend was also detected in the abundance of the gene $nosZ$ in the four land types (Figure 5e). For the abundance of the gene $nirK$, a significant ($p < 0.05$), difference observed was between bare land and Migi land (Figure 5f). The abundances of the gene $nifH$ in the Miscanthus lands were both significantly ($p < 0.05$) higher than those values in bare land and cropland (Figure 5g).

The abundance of 16S rRNA of the bacterial and archaeal communities as well as the genes $AOA$, $nirK$, $nifH$, and $nosZ$ were positively correlated with MC, MN, TN, SOC, EMg$^{2+}$, AN, and MCN, and negatively correlated with ST, clay proportion, and SM (Figure S8). Soil MN, TP, AP, NiN, and ENa$^+$ were positively correlated with the abundance of the $AOB$ (Figure S8). Soil pH, SC, MIA, ENa$^+$, and NiN were negatively ($p < 0.05$) correlated with the abundance of the $nifH$, whereas soil sand proportion, MAA, ECa$^{2+}$, and AK exhibited positive ($p < 0.05$) correlations with $nifH$ abundance (Figure S8).
DISCUSSION

4.1 Conversion of cropland to Miscanthus obviously affects soil properties

Little information currently available related to the effects of Miscanthus cultivation on soil mechanical properties. Kang et al. (2013) found that at a site with 2-year Miscanthus cultivation, the soil clay and silt proportions increased while the sand proportion decreased. We found that long-term conversion of cropland to Miscanthus significantly ($p < 0.05$) increased the soil clay proportion but significantly ($p < 0.05$) decreased the sand proportion (Table 1), which further supported previous research conclusions. In addition, we observed a significant ($p < 0.05$) increase in soil MAA proportion in this work. The degree of these changes in soil mechanical properties seemed to be deepened as time increased since Miscanthus cultivation, which might also be affected by different Miscanthus taxa (Table 1). Differences in distribution of roots and root exudates of grassland can help some sites to maintain relative...
high proportions of soil clay, silt, and MAA (Sollins et al., 1996; Islam & Weil, 2000), which might also be important reasons for changes in the mechanical composition of soil in the present study.

The increased accumulation of SOC caused by the effects of C sequestration by Miscanthus has been widely been shown to be independent of climate, soil type, and stand age (Alberto Oliveira et al., 2017; Dufosse et al., 2014; Guzman & Lal, 2014; Kahle et al., 1999; Mishra et al., 2013; Rehbein et al., 2015; Zhao et al., 2020), although neutral effects on SOC have also been reported in case studies (Robertson et al., 2017; Zimmermann et al., 2013). Here, long-term Miscanthus conversion significantly ($p < 0.05$) increased SOC which further demonstrated its great potential in the C sequestration. This mainly occurred because stubble residues were left on the ground, along with dead root and rhizome residues, as well as root exudates that decomposed during long-term growth of Miscanthus. Additionally, 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).

Many studies have reported that long-term cultivation of Miscanthus can increase MB (Ferrarini et al., 2017; Zhao et al., 2020) even in contaminated soil (Al Souki et al., 2017). In the present study, Miscanthus lands all had significantly ($p < 0.05$) higher MC and MN contents compared with bare land, suggesting that vegetation cover (traditional crops and Miscanthus) could stimulate microbial
reproduction and MB accumulation; this was consistent with previous studies (Shen et al., 2015; Xiao et al., 2016). The gradual increase in the ratios of microbial C and N in bare land, cropland, Migi land, and Mis land (Table 2) indicated a possible shift from a bacterial community to fungal community occurred, which needs to be explored in the future.

The low N removal rate of Miscanthus when compared with that of annual crops (Masters et al., 2016) makes it a desirable energy crop with a low need for N. However, appropriate N fertilizer should be provided to Miscanthus to ensure the long-term production of successive high biomass yields, which indicated a probable N deficiency in a Miscanthus system, especially when two harvests were completed each year (Ruf et al., 2017; Singh et al., 2015). Hence, most farmers and governments in China have misgivings and concerns that the cultivation of Miscanthus might exhaust soil N, which seriously impedes the large-scale use of Miscanthus on marginal cropland; however, many experiments have shown that Miscanthus cultivation in other countries had no or slightly positive effects on levels of soil N (Kahle et al., 1999). We previously found that 12-year-old harvested Miscanthus did not reduce soil N levels without N fertilizer in Northern China (Zhao et al., 2020). In the present work, we again found that 15 years of Miscanthus conversion resulted in slightly increased soil N levels when no N fertilizer had been applied.

In traditional cropland, heavily applied excessive N fertilizer is usually provided to compensate the soil N deficiency caused by grain harvest, which often results in much higher soil NiN contents in cropland compared to the Miscanthus lands (Table 2) and increased the N leakage risks while exacerbating soil degradation and acidification (Hu et al., 2018). Thus, long-term Miscanthus conversion without N fertilizer can provide an environmentally friendly and soil-friendly method for the restoration of degraded cropland soil. The relatively high soil AnN contents in Mis land (Table 2) observed in this study were probably caused by the degradation of stubble residues of Miscanthus and by the N fixation process of soil microbes.

Long-term repeated harvests of Miscanthus were likely to reduce soil macronutrient P levels (Alberto Oliveira et al., 2017) although this species has a relatively low P removal rate (1.8% of removal in Maize Masters et al., 2016). We previously found that 12 years of annual harvest of Miscanthus in late autumn did not reduce soil P levels in 0–100 cm soil depths in cropland, but generated different impacts on soil AP levels at different depths (Zhao et al., 2020). In the present study, the use of industrial P fertilizer resulted in much higher levels of soil AP (Table 2) in 0–10 cm soil of cropland. The exaggerated decrease in the magnitude of P content in Mis land compared to Migi land indicated non-negligible soil AP depletion after long-term Miscanthus conversion. Unlike our previous results (Zhao et al., 2020), in the present study, the TK contents in the 0–10 cm soil layer of Miscanthus lands exhibited no significant differences compared with those values of cropland and bare land (Table 2), which indicated different variations in soil TK contents, probably depending on the soil layers. The accumulation of stubble residue year after year might be an important reason for the observed stable TK and high AK levels in 0–10 cm soil, which was also reported in Hu et al. (2018).

Research has demonstrated that 2-year cultivation of Miscanthus on abandoned cropland resulted in significantly decreased soil electronic conductivity (EC) at 0–45 cm depths compared with the land without Miscanthus cultivation (Kang et al., 2013). In this work, we found that this type of conversion of land-use types dramatically decreased soil EC (Table 2), which further supported the previous studies but over a longer time scale. Soil EC reflected the amounts of total salt in soil, which were usually disrupted by irrational and excessive fertilization resulting in soil acidification and the salinization of cropland. We inferred that the absence of fertilization coupled with the salt removal effects of Miscanthus largely accounted for the significantly \( p < 0.05 \) decreased soil EC, which might effectively prevent soil salinization. It has been reported that the conversion of grass-land into Miscanthus could induce a risk of soil acidification caused by root C sequestration (Hu et al., 2018). Here, we found that Miscanthus lands and especially Mis land had the lowest pH when compared with bare land and cropland, which was quite different from our previous results. The cultivation of Miscanthus was previously found to significantly decrease the soil pH in 0–100 cm soil depths compared with the original values. The differences might have been caused by the different sampling times and sampled soil layers. The positive effects of 4–8 years of Miscanthus cultivation (Kahle et al., 1999) and the negative effects of 2 years of Miscanthus cultivation (Kahle et al., 1999) on soil EC were reported in these previous studies, exhibiting a divergence on this point. In this experiment, we observed a gradual increasing trend in the EMg\(^{2+}\) and ECa\(^{2+}\) concentrations in bare land, cropland, Migi land, and Mis land sequentially (Table 2), which implied the enrichment effects of Miscanthus cultivation on these two elements from deep soil were presumably caused by the annual incorporation of stubble and rhizome residues in a 0–10 cm depth soil. The ENa\(^{+}\) contents in Mis and Migi lands were significantly \( p < 0.05 \) lower than that in cropland (Table 2). We assumed that the application of fertilizer contributed greatly to the relatively high ENa\(^{+}\) contents in cropland soil because the fertilizer, especially organic fertilizer, was the main source of Na\(^{+}\). The trace amounts of Na\(^{+}\) (compared with the Mg\(^{2+}\) and Ca\(^{2+}\)) in Miscanthus biomass caused the enrichment effects because the volumes of stubble and rhizome residues were small and negligible.
4.2 Conversion of cropland to Miscanthus dramatically affects the soil microbiome

Previous studies have revealed the changes in the soil microbiome (diversity and abundance of various taxa along with community structure) caused by the alteration of land-use patterns such as the conversion from forests, and grasslands to agricultural uses (Bossio et al., 2005; Guan et al., 2018; Merloti et al., 2019). To date, no studies were found to have been conducted to illustrate the changes in the soil microbiome after the long-term (more than 10 years) conversion of cropland to Miscanthus. A pot experiment showed that the cultivation of Miscanthus sinensis obviously improved the diversity of the bacterial community in mercury polluted soil compared with that of bare soil (Zhao et al., 2019). By contrast, results from a 5-year field experiment indicated that cultivation of Miscanthus obviously decreased the diversity of the bacterial community when compared with bare land (Duan et al., 2019). In the present study, the Shannon index of the soil bacterial community (Figure 3a) indicated that the effects of long-term conversion of cropland to Miscanthus land seemed to depend on the number of years after the cultivation of Miscanthus species; the increases in that indicator were observed only in Mis land instead of both Mis and Migi lands.

It has been shown that crop cover could play a pivotal role in increasing the diversity and activity of the soil microbial community (Ashworth et al., 2017). However, crop cover had little effect on the Shannon index of the soil archaean community compared with that of the bare land (Figure 3b). Crop roots can sequester C and increase soil aggregate stability, macro-porosity, and permeability, which can increase the richness of the soil microbiome (Fernandez et al., 2016). Herein, the significantly ($p < 0.05$) higher taxa indices (OTU) of cropland, Mis land, and Migi land compared to those of bare land and the minor differences among cropland, Mis land, and Migi land (Figure 3c) indicated that vegetation cover and relative high nutrients levels compared with bare land accounted largely for the increased richness of bacterial taxa (OTU level). Fertilization levels such as N enrichment are generally considered to lead to simpler, less diverse, soil food webs (Bender et al., 2016), and declines in microbial community diversity and species richness, as shown in many experiments (Suding et al., 2005). Consistently, we observed that duration of fertilizer application rather than the vegetation cover might affect soil archaean community richness (OTU level) based on the gradual increasing trend in that indicators of cropland, Migi land, Mis land, and bare land in that order (Figure 3d) in this work. In other words, agricultural activities were most likely to negatively affect the richness of the soil archaean community. As far as we know, this study represents the first time that the changes in the diversity and composition of the whole soil bacterial and archaean communities have been demonstrated after the long-term (15 years) conversion of cropland to Miscanthus lands.

Changes in the microbial community composition in terms of the bacterial and archaean communities after the short-term (<6 years) cultivation of Miscanthus have been reported through pot and field experiments (Bourgeois et al., 2015; Mao et al., 2011; Thompson et al., 2016). Those changes lacked convergence across different numbers of years after cultivation indicating that the short-term cultivation of Miscanthus might not be sufficient to overcome pre-existing “legacies” in the composition of the soil community (Buckley & Schmidt, 2001). In the present study, the predominant phyla of the bacterial community in the four land types were similar, with few unique OTUs (Figure S6) confirming the soil “legacy effects” even after the long-term change in land uses. In addition, long-term (>10 years) cultivation of Miscanthus on what was previously cropland obviously affected the abundance of the soil bacterial community but had less of an effect on the soil archaean community at the phylum level (Figure 2; Figure S1). Moreover, the greater effects on the composition of the soil bacterial community in Mis land compared to that in the Migi land after conversion (Figure 2; Figure S1) detected in the present study imply that such effects were mostly dependent on the number of years after cultivation of Miscanthus. Beyond that, the differences in the properties of these two Miscanthus taxa (Mis and Migi), such as the different distribution of root systems (dispersive and intensive type for Mis and Migi, respectively) and yield potential, might also be important factors that should not be neglected.

Changes in the composition of the predominant soil bacterial community usually indicate variations in microbial functions for nutrient cycling in the soil. Research has shown that a great number of the members of the phylum Nitrospirae function in a closely related relationship to the oxidization of nitrite N into NiN, facilitating the absorption of N by crops in tropical and subtropical soils (Bourgeois et al., 2015; Fang et al., 2017); in addition, the phylum Bacteroidota plays a very important role in denitrification process of the soil N cycle (Lu et al., 2019). In our work, the significant ($p < 0.05$) decrease in relative abundances of the Nitrospirae and Bacteroidota in both Mis and Migi lands (Figures S3 and S4) might be attributed to the absence of N fertilizer in these two land types. Based on a previous study, the Acidobacteriota can decompose complicated carbohydrates such as cellulose and xylan in soil (Eichorst et al., 2011). The contents of complicated carbohydrates where higher in Mis and Migi lands when compared with that in cropland and was caused by stubble residues on surface of the land and the dead rhizomes in the 0–10 cm soil. This could be reflected by the increased SOC (Table 2). The SOC largely accounted for the significantly ($p < 0.05$) increased relative abundance of the Acidobacteriota in this work (Figures S3a,c.
and S4), and this effect seemed to be strengthened with the increase in the number of years since cultivation based on the higher increase rate in Mis land than that in Migi land (Figure S3). These results were in contrast to conclusions from a 5-year Miscanthus experiment, which focused on the rhizospheric soil of Miscanthus compared with that of the bare land (Duan et al., 2019). Lu et al. (2019) reported that the abundance and diversity of the Firmicutes, Actinobacteriota and Proteobacteria were closely related to the denitrification process of the soil N cycle. The dramatically \((p < 0.05)\) lower soil NiN levels in Mis land (Table 2) compared to that of cropland caused by the lack of N fertilization could explain the significant \((p < 0.05)\) decrease in the relative abundance of those three phyla (Figures S3 and S4).

Nitrogen fixers mainly belong to the Proteobacteria, along with the Bacteroidota, Cyanophyta, Actinobacteriota, Verrucomicrobiota, and Planctomycetota (Dixon & Kahn, 2004). Here, we compared the relative abundance of phyla involved in N fixation between Miscanthus lands and bare land to elucidate the possible bacterial communities participating in the process of N fixation. The increase in the relative abundance of the phyla Bacteroidota (189% and 98%), Verrucomicrobiota (140% and 29%), Planctomycetota (45.36% and 17.56%), Proteobacteria (26.36% and 22.35%), and Actinobacteriota (2.39% and 21.41%) in Mis and Migi lands compared to those values of the bare land (Figures S3 and S4) implied that bacterial members of these phyla, especially the Proteobacteria, might contribute largely to the compensation of soil N deficiency caused by the annual biomass harvest of Miscanthus.

A 5-year field experiment indicated that soil TN, TP, TK, SOC, and soil pH drive the variation of the microbial community structure between bare and Miscanthus lands (Duan et al., 2019). In our work, the RDA (Figure 4) indicated SOC, EMg\(^{2+}\), and AK were three major soil factors shifting the soil bacterial and archaeal community structures from the cropland to Miscanthus lands patterns. Additionally, AP was major factor in shaping the soil microbiome pattern in cropland. Moreover, variations in the soil NiN, clay proportion, ST, and SM (Tables 1 and 2) were the main driving force for changes in the structures of the bacterial and archaeal communities structure changes when cropland was converted to bare land (Figure 4).

### 4.3 Effects of the conversion of cropland to Miscanthus on soil N-cycle-related genes

The abundance of functional genes was assessed to disentangle the effects of the conversion from cropland to Miscanthus on microbial abundance and soil N cycling. Duan et al. (2019) found that 5 years of Miscanthus cultivation enhanced microbial functions related to the soil N and the phosphorus cycle. The results of quantitative PCR indicated that long-term (15 years) conversion of cropland to Mis land dramatically \((p < 0.05)\) increased soil bacterial and archaeal abundance (Figure 5), whereas it had little effect on the Migi land (Figure 5). This was probably caused by the differences in years after cultivation and species properties between these two Miscanthus taxa. The lowest values which were found on bare land (Figure 5) implied that vegetation cover strongly impacted the soil microbiome (Kim et al., 2020).

Abundances of the AOB and AOA genes can be used to reflect the status of the process of soil ammonia oxidation while the abundances of the genes nosZ and nirK were used to mirror the intensity of the process of soil denitrification. The gene nifH was the predominant gene related to the microbial N fixation process (Kuyper et al., 2018). In the present study, we observed that the soil of cropland had a much higher abundance of AOB genes (Figure 5c), which was mainly caused by the application of excessive N fertilizer in cropland. This was also the main reason for the much higher soil NiN levels in cropland (Table 2), dramatically increasing the risk of N population into the surrounding environment. The abundance of the genes AOA exhibited no significant differences among cropland, Migi land, and Mis land (Figure 5d), which indicated that the bacterial community performed greater functions during the soil ammonia oxidation process than the archaeal community. The nosZ gene is responsible for the final step of the soil denitrification process that converts N\(_2O\) to N\(_2\); the lower contents of the mineral forms of N (i.e., NH\(_4^+\) and NO\(_3^-\)) could favor the growth of denitrifiers (reflected by the increase in nosZ abundance). However, the nosZ abundance in cropland was slightly higher than those of the Migi and Mis lands in our work (Figure 5e). The reduction of NO\(_2\) to NO, an intermediate step in the soil denitrification process, is mainly driven by the gene nirK (Henry et al., 2004). The increase in its abundance might be a great potential source of N\(_2O\) (Lammel et al., 2015) which is a greenhouse gas that is 300-fold more harmful than CO\(_2\) (Stocker et al., 2013). In the present study, few differences were found in nirK abundance among cropland, Migi land, and Mis land (Figure 5f), which indicated that the long-term conversion of cropland to Miscanthus lands might not reduce the risks of N\(_2O\) emissions. This conclusion seemed to be different from some of the previous research conclusions illustrating the negative effects on N\(_2O\) emissions caused by the cultivation of Miscanthus (Drewer et al., 2012). As we hypothesized, the abundance of the gene nifH increased significantly \((p < 0.05)\) after such conversion (Figure 5g), which provided strong evidence for the enhanced function of microbial N fixation caused by the cultivation of Miscanthus especially when there was no N fertilizer applied. Hence, we can conclude here that N generated by microbial N fixation instead of the application of industrial N fertilizer compensated for N export with biomass harvest, helping to maintain
stable soil N levels and robust N cycling in Miscanthus systems. When compared with bare land, all the marked genes in the other three land types determined in this study were significantly ($p < 0.05$) higher (Figure 5), which indicated that the lands without any fertilization and vegetation cover could dramatically weaken soil N cycling.

Rates of microbial N fixation usually decline with an increase in N fertilizer application, which has been proven in other systems (Fan et al., 2019). In our work, nifH copies were negatively affected by soil NiN levels (Figure S8), which provides further evidence for the priming effects of N starvation on the function of soil microbial N fixation. The positive correlations between the SOC and almost all of the marked genes (Figure S8) implied that soil C stocks were a critical factor influencing microbial activities and N cycling. The positive correlation between soil EMg$^{2+}$ levels and most of the marked genes (Figure S8) indicates significance and necessity of further exploration of the enrichment effects of Miscanthus on micronutrient elements in future research.

As we hypothesized, the substitution of traditional crops by Miscanthus without the application of fertilizer causes dramatic changes in soil microbial communities, and stimulates soil microbial N fixation, mediating robust soil N cycling with a lower risk of NO$_3$ leakage. Although there were some weaknesses in this work, such as a case study in Beijing area, and soil depths limited to 0–10 cm, this study can provide insight into the understanding of interactions among Miscanthus, soil properties, and the soil microbiome. Second, these results can help to dispel people's misgivings of Miscanthus depletion effects on soil nutrients of the cultivated land. Third, this study can provide some suggestions for government agencies or farmers during large-scale use of Miscanthus as a part of ecological restoration efforts for marginal cropland in Northern China.

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**SUPPORTING INFORMATION**

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

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