INTRODUCTION

Plants are colonized by various microbes that are partially taken up from the surrounding environments or are derived from their parents (van Overbeek & Saikkonen, 2016). Some of these bacteria may support their host plants by biological nitrogen (N) fixation (BNF) and regulation of the plant phytohormone homeostasis (Saharan & Nehra, 2011). Furthermore, some plant-associated bacteria suppress pathogens or induce systemic resistance to maintain plant health (Eyles, Bonello, Ganley, & Mohammed, 2010; Pieterse et al., 2014). Studies in Arabidopsis, sugarcane, sorghum and many other plants have demonstrated that the bacterial communities associated with the plant are strongly influenced by the plant organ type and soil type (Lavecchia et al., 2015; Li, Voigt, & Kent, 2016; Lundberg et al., 2012; De Souza et al., 2016). A much weaker effect on the bacterial communities is imposed by different plant growth stages and the genotype (Lavecchia et al., 2015; Lundberg et al., 2012) or agricultural practice such as ploughing, while different N fertilization...
often had only minor effects on the plant-associated bacterial communities (Babin et al., 2019).

The commercial energy crop C4-fibre plant *Miscanthus × giganteus* has great potential for sustainable biomass production in temperate climate, owing to low water requirements, high nutrient efficiency and great yield per unit (Lewandowski, Clifton-Brown, Scurlock, & Huisman, 2000). Both annual and perennial field experiments have demonstrated that *M. × giganteus* grows well in temperate climate and has a relative high biomass yield without ample N fertilizer addition (Cadoux, Riche, Yates, & Machet, 2012; Heaton, Long, Voigt, Jones, & Clifton-Brown, 2004; Iqbal, Gauder, Clauepin, Graeff-Hönninger, & Lewandowski, 2015; Maughan et al., 2012). Even without any external N input by fertilization and despite perennial biomass (and N) removal, *M. × giganteus* fields maintained substantial biomass productivity (Dohleman, Heaton, Arundale, & Long, 2012; Iqbal et al., 2015). Much, but not all, of the N efficiency is explained by the perennial lifestyle and the capacity to translocate N to the rhizome at the end of the growth phase in autumn, while mobilizing stored N in spring (Beale & Long, 1997; Liu, Yang, & Ludewig, 2014).

However, multiple plants from the family Gramineae, including sugarcane and *Miscanthus*, may benefit from BNF under certain conditions. Several studies have indicated that sugarcane, a major energy crop in Brazil, largely benefits from BNF. Experimental evidence comes from the 15N-isotopic dilution technique, N-fixing bacteria isolation, inoculation experiments and *nifH* gene analysis (Baptista et al., 2014; Thaweenut, Hachisuka, Ando, Yanagisawa, & Yoneyama, 2011; Urquiaga et al., 2012). Depending on soil conditions and plant genotypes, BNF may contribute as much as 77% of total N requirement of sugarcane (Baptista et al., 2014). Similar to sugarcane, N-balance experiments and modelling studies suggest that *M. × giganteus* in the field is supported by a non-fertilizer type N source, which is likely provided by BNF (Christian, Riche, & Yates, 2008; Davis et al., 2010). Indeed, by using 15N-labelled chemical fertilizer, field experiments suggest that *M. × giganteus* could acquire 16% or more of plant total N from N fixation (Christian, Poulton, Riche, & Yates, 1997; Keymer & Kent, 2014). Estimates suggest that BNF contribute as much as 25 g N m−2 y−1 during this was omitted in the N0 plots. The long-term experiment ammonium nitrate fertilizer with nitrification inhibitor, while year in late spring to the N80 plots in form of ENTEC®), an shift the *M. × giganteus*-associated bacterial composition. To our knowledge, the present survey of underground bacterial communities of field-grown *Miscanthus* is the first high-resolution community profiling investigating the effects of compartment type and N fertilizer application on *Miscanthus* bacterial communities. Four different underground fractions, the bulk and rhizosphere soil, roots and rhizomes, were collected from *M. × giganteus* plots in southern Germany. Our results highlight compartment- and organ-specific bacterial taxonomic compositions in the underground system of *M. × giganteus*, which were moderately affected by long-term differences in N.

2 | MATERIAL AND METHODS

2.1 | Sites description and sample collection

*Miscanthus × giganteus* plots located at the University of Hohenheim (Germany) experimental station ‘Ihinger Hof’ (48.75°N, 8.92°E) were established in the year 2001 by Boehmle, Lewandowski, and Clauepin (2008). The soil is classified as a Haplic Luvisol with a silty clay texture (approximately 40% clay) and overlying loess loam. When measured in 2002, soil carbon was about 1% and total soil N was about 0.1%. The field trial was established as a split plot with different N levels (0 and 80 kg ha−1 a−1) and four replicates (180 m² each). Nitrogen fertilizer was applied every year in late spring to the N80 plots in form of ENTEC®, an ammonium nitrate fertilizer with nitrification inhibitor, while this was omitted in the N0 plots. The long-term experiment and the block design are described in detail in Iqbal et al. (2015).

Bulk soil, rhizosphere, root and rhizome (Figure 1a) were sampled in each of four replicate blocks (marked as block 1, 2, 3 and 4) from the zero nitrogen fertilizer level (N0) and moderate nitrogen fertilizer level (N80) respectively. For the total of four biological replicates per treatment (named prepoled sample), two replicates from block 1 and block 2 were mixed and pooled as a final sample, and the rest two replicates from block 3 and block 4 were mixed and pooled.
as the other final sample, finally yielding two replicates per treatment. For bacterial community analyses under both N fertilizer levels, bulk soil, rhizosphere, roots and rhizomes were sampled in the middle of July, 2015, when Miscanthus grew maximal. To reduce the interference of debris, the top 3–10 cm soil with at least 5 cm distance from the surrounding Miscanthus root was crushed and sieved through a 2 mm mesh in the field for collection of corresponding bulk soil samples. A quantity of 100 g bulk soil per treatment was used to measure the N$_{\text{min}}$ (nitrate and ammonium) by continuous-flow analysis. Loose soil was manually removed from the roots by gently shaking with sterile gloves. Soil tightly adhering to the roots was defined as a rhizosphere sample (Lavecchia et al., 2015). The bulk soil samples and rhizosphere soil samples were stored at −20°C for subsequent DNA extraction. Root samples were standardized by exclusively taking fresh lateral roots. Roots and rhizome from the top 3–10 cm in the soil for each independent block were vigorously washed with sterilized deionized water and sonicated in order to remove all soil from the root surface. The washing steps were repeated twice to avoid soil contamination in the root type samples. Subsequently, rhizome and roots were separated with sterilized scissors. The rhizome and root samples were gently dried with clean soft tissue, immediately frozen in liquid nitrogen and stored at −80°C for downstream DNA extraction.

2.2 | DNA extraction

Rhizomes and roots were chopped into smaller sections using a sterilized razor blade in a Petri dish. Then, the root samples and rhizome samples were ground in liquid nitrogen and split into 100 mg subsamples. Total DNA was extracted using the PowerSoil DNA isolation kit (MoBio, Carlsbad, CA) from 300 mg soil sample or 100 mg plant samples following the manufacturer’s instructions, with an extending vortex mixing period for 25 min. DNA was quantified using a Thermo Scientific Nanodrop 2000c spectrophotometer. Subsequently, several replicates of DNA samples were pooled together and about 800 ng DNA per sample in 1.5 ml tubes were sent for sequencing in a package with 30 kg dry ice to the Beijing Genomics Institute (BGI, China) and arrived there within 48 hr.

2.3 | ILLUMINA MiSeq sequencing

The V4 region of the 16SrRNA gene was amplified with universal prokaryotic primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (TAATCTWTGGGVHCATCAGG) as in Caporaso et al. (2012). Library construction and 250 bp paired-end sequencing on the Illumina Miseq were done by the BGI, which included barcodes and adaptors for annealing, and quality filtering of reads.

2.4 | Operational Taxonomic Unit picking and taxonomic assignments

The raw data were demultiplexed and quality filtered to get clean data by the procedure described in Fadrosh et al. (2014). Two paired-end reads overlapping to the consensus sequence were generated with 15 bp minimal overlap and mismatch ratio of the overlapped region ≤0.1 by FLASH (Fast Length Adjustment of Short reads, v1.2.11; Magoč & Salzberg, 2011). Reads without overlaps were removed. Finally, 625,403 consensus sequences were obtained in total with on average 19.543 per sample. The average length...
was 252 bp. The consensus sequences were clustered to operational taxonomic units (OTUs) by scripts of the software USEARCH (v7.0.1090). The consensus sequences were clustered into OTUs with a 97% threshold using UPARSE. Unique OTU representative sequences were obtained with chimeras filtered out with UCHIME (v4.2.40), then all consensus sequences were mapped to each OTU representative sequence using USEARCH GLOBAL. Next, the consensus numbers of each OTU in each sample were quantified in the OTU abundance table. Finally, OTU representative sequences were taxonomically classified using Ribosomal Database Project (RDP) Classifier v.2.2, trained on the Greengenes database V201305. Several filtering steps were included: Unassigned OTUs were removed; OTUs not assigned to the target species were removed and OTUs assigned to the plant chloroplast and mitochondria were removed. The final matrix of OTU abundance in each sample was considered as meaningful for further taxonomic analysis.

2.5 | Detection of differentially enriched OTUs and taxa

Before analyses, low-abundance and rare OTUs were filtered out according to the modified procedure of Gottel et al. (2011). Only OTUs which accounted for 95% of the total relative abundance and occurred in more than three samples were considered as meaningful OTUs. Furthermore, reliable OTUs were defined by ≥5 reads in ≥3 samples (hereafter called 5 × 3 criteria). The OTU relative abundance table was calculated by dividing the absolute abundances by the total sequence counts per sample. In order to determine the bacterial diversity among different compartments, the samples at both N fertilizer levels were pooled together. Fold-change tests and false discovery rate-corrected Student’s t test were conducted to compare the N fertilizer effect on relative abundance in a given plant fraction and N condition. The enriched and depleted relative abundances of OTUs and bacterial taxa among soil–plant fractions with respect to nitrogen were determined and statistical analyses were based on fold changes (≥2). More precisely, if the mean relative abundance of one OTU in one treatment was more than twofold different compared to another treatment, this OTU was assumed to be enriched.

2.6 | Principal coordinate analysis

In order to display the differences of OTU composition in different samples, principal coordinate analysis (PCoA) was used to construct a 2-D graph. The matrix of the β-diversity distance was calculated based on bray_curtis by the QIIME software. The PCoA of OTUs used package ‘ape’ of software R (v3.1.1), based on the bray_curtis distance matrix. The effects of soil, plant organ type and N on the bacterial communities were tested using permutational multivariate analysis of variance (PerMANOVA), by using the function ‘adonis’ in package ‘vegan’ of software R (v3.1.1). Bray–Curtis parameters were used to measure the distance between different bacterial communities.

2.7 | Venn charts

Venn diagrams were chosen to visually display the number of common and unique OTUs in multisamples and were drawn by VennDiagram of software R (v3.1.1).

2.8 | Heat maps

Species heat map analysis was done based on the relative abundance in each sample. To cover a large range of relative abundances, all values were log_{10} transformed. Species with less than 1% relative abundance were pooled in the low abundance fraction. The relative abundance of species that were absent in a certain sample was substituted by the value −2.3, which corresponds to 0.005% relative abundance. Heat maps were generated using the package ‘gplots’ of software R (v3.1.1) and the distance algorithm ‘Euclidean’, with the clustering method ‘complete’.

2.9 | Diversity

The Shannon diversity index was calculated with the vegan package in R. The Tukey’s post hoc test was conducted to compare the bacterial diversity of the different soil–endosphere types.

2.10 | Taxonomy histograms and statistics

Taxonomy histograms were created with the software R (v3.1.1). The ‘low-abundance’ category contained all taxonomic groups that did not reach at least 0.5% in any one fraction. Differences at the family level were tested by the Student’s t test.

3 | RESULTS

3.1 | The soil and root organ type structure of Miscanthus × giganteus underground bacterial communities with different N fertilization

Bacterial 16S rDNA was extracted from bulk soil, rhizosphere, root and rhizome (Figure 1a) from a 14 year old Miscanthus field experiment. Plants had either not received
N for the entire period or were yearly fertilized with 80 kg N ha\(^{-1}\) a\(^{-1}\). This resulted in almost sixfold lower \(N_{\text{min}}\) levels in the N0 plots (2.88 mg/ha), compared to 16.4 mg/ha in N80 plots, respectively, with nitrate levels substantially higher than those of ammonium (Table S1). The bacterial community composition in the four different compartments at two N levels resulted in a total of eight datasets that were compared by PCoA, based on sequence abundances of the v4 conserved region of bacterial 16S rDNA. The first principle coordinate explained 41% of the variation in the dataset and separated the Miscanthus exosphere (both bulk soil and rhizosphere soil) from the root and rhizome, reflecting the distinct environmental conditions for bacterial populations (Figure 1b). Exophyte and endophyte compartments were clearly distinct (PerMANOVA: \(R^2 = 0.3859, p = 0.002\)), while the second principle coordinate that explained 18% of the variation reflected the different plant organs: lateral roots and rhizome (PerMANOVA: \(R^2 = 0.4059, p = 0.021\)). Overall, the N fertilizer effect was small, N0 and N80 data clustered closely together. Nevertheless, the relationship between fertilizer and exophytic bacterial communities (PerMANOVA: \(R^2 = 0.2045, p = 0.189\)) was less pronounced than that for endophytic bacterial communities (PerMANOVA: \(R^2 = 0.2490, p = 0.065\)). Taken together, the PCoA showed that in response to different long-term N fertilizer application, bacterial taxonomic patterns were mainly imposed by the compartment and plant organ type, but not by the distinct N availabilities.

**Figure 2** Operational taxonomic units (OTU) differences, composition and distribution among different fractions. (a) Numbers of shared and differentially distributed bacterial OTUs in bulk soil (S), rhizosphere (Rs), M. \(\times\) giganteus roots (Rt) and rhizomes (Rz), in N0 and N80. (b) Heat map showing the distribution of bacterial phyla in the different fractions from log\(_{10}\)-transformed relative abundances. Samples were clustered according to their Euclidean distance. (c) Histograms showing the distribution of phyla present in bulk soil, rhizosphere, roots and rhizomes. N0, no nitrogen fertilizer application; N80, 80 kg N ha\(^{-1}\) a\(^{-1}\) application.
3.2 | Bacterial taxonomic composition of soil and endosphere of *Miscanthus × giganteus* with distinct N fertilization

Using the 5 × 3 threshold (see Materials and Methods), we identified 763 OTUs defined as general units of microbial taxonomic classifications in the N0 dataset and 862 OTUs in the N80 dataset (Figure 2a). In both conditions, the OTU number reduced significantly from bulk soil to the rhizome (Figure S1). About 62% of the endosphere OTUs were also found in soil. Interestingly, both roots and rhizome showed specific OTUs (24% in roots and 20% in the rhizome respectively), which were not detected in bulk soil and rhizosphere soil. While there was substantial overlap between soil and rhizosphere compartments, the overlap between the root and rhizome communities was limited and only between 49% and 70% of the rhizome OTUs were found in the root. The rhizome was especially depleted for OTUs in the N0 dataset (only 10% overlap; Figure 2a).

To access how the different soil–plant fractions and N influenced the taxonomic distributions of the bacterial communities, their relative abundances were quantified after log10 transformation. Euclidean distance clustering of the data in a heatmap at the phylum level (Figure 2b) and at the family level (Figure S2) indicated three distinct bacterial composition patterns. In the bulk soil and rhizosphere, the major phyla were *Proteobacteria*, *Acidobacteria*, *Verrucomicrobia*, *Actinobacteria* and *Bacteriodetes*. *Proteobacteria*, *Acidobacteria*, *Actinobacteria* and *Bacteriodetes* were the main taxa identified in the root samples. The rhizome was dominated by *Proteobacteria*, *Actinobacteria* and *Bacteriodetes* (Figure 2c).

3.3 | Bacterial diversity and taxonomic distributions changed by soil and plant compartment effects

To better understand the bacterial diversity and composition from the 16s rDNA data, we applied fold-change tests to assess which OTUs were enriched or depleted in the fractions. With respect to the bulk soil, the rhizome was most substantially depleted in OTUs, with substantial overlap with the root compartment in N0 (Figure 3a). In N80, the majority of depleted OTUs overlapped between rhizosphere, root and rhizome compartments, while again a major overlap between root and rhizome was apparent (Figure 3c). This nicely indicates a gradual filtering towards the plant compartments. By contrast, the majority of enriched OTUs in both N0 and N80 were specific for each compartment: the rhizosphere, root and rhizome respectively.

**FIGURE 3** Numbers of depleted and enriched bacterial operational taxonomic units (OTUs) in the *M. × giganteus* rhizosphere, roots and rhizomes compared to the bulk soil. (a) Two-fold depleted and (b) enriched OTUs in N0; (c) Two-fold depleted and (d) enriched OTUs in N80. Rs, rhizosphere; Rt, Miscanthus root; Rz, Miscanthus rhizome; S, bulk soil.
Only very few enriched OTUs were shared, highlighting the preferences for specific environmental habitat niches (Figure 3b,d).

The relative abundance of different bacterial taxa in the soil–endosphere fractions is displayed in histograms at the phylum (Figure 4a) and family levels (Figure 4b,c). For this analysis, the N0 and N80 samples were pooled. Only Proteobacteria and Actinobacteria were enriched in the root, while Proteobacteria, Bacteriodetes and Actinobacteria were enriched in the rhizome. Conversely, the root- and rhizome-depleted phyla included Proteobacteria, Acidobacteria, Actinobacteria, Bacteriodetes, Chloroflexi and Verrucomicrobia, which resemble typical soil conditions.

**FIGURE 4** Differentiation of the Miscanthus × giganteus-associated bacterial communities. (a) Histograms showing the distributions of phyla present in the families in bulk soil, rhizosphere, root and rhizome (four left columns) and the subset of root- and rhizome-enriched (Rt↑ and Rz↑) or depleted (Rt↓ and Rz↓) phyla relative to soil (right columns). Shannon diversity (considering phyla as individuals) is given above each bar. Letters above the diversity values represent significant differences ($p < 0.05$). (b, c) Distribution of families in the phylum Actinobacteria (b) and Proteobacteria (c).
taxa, in accordance with the before-mentioned progressive filtering towards the plant. The Shannon diversity indexes of the root and rhizome fractions were 4.4 and 3.5, respectively, significantly lower than those of the soil fractions (5.7). This is consistent with the enrichment of a subset of dominant phyla in the plant.

The endosphere-enriched phyla, predominantly Proteobacteria and Actinobacteria, contain N-fixing species, which are candidates for potential Miscanthus growth promotion, especially at low N level.

The family taxonomic analysis demonstrated that the enrichment of Actinobacteria in the root and rhizome was mostly due to Streptomyces and Micromonospora in the root, as well as Mycobacteriaceae and Microbacteriaceae in the rhizome (Figure 4b). In the Proteobacteria, relatively complex differences on the family level were observed and are shown in four domain classes in histograms (Figure S3). In the root fraction, Bradyrhizobiaceae dominated the enriched families within the Alphaproteobacteria class, while Comamonadaceae dominated the enriched families in the Betaproteobacteria. Furthermore, Sinobacteraceae and Xanthomonadaceae dominated enriched families in the Gammaproteobacteria (Figure S3). Furthermore, in the rhizome fraction, a similar enrichment and depletion profile as in the root was found, except for one enriched

FIGURE 5 Operational taxonomic units (OTUs) that differentiate the N0 from the N80 microbiomes. (a) Differentially enriched and depleted bacterial OTUs in M. × giganteus bulk soil (S), rhizosphere (Rs), roots (Rt) and rhizomes (Rz) in N0 relative to N80, based on twofold change. (b) Histograms showing the distributions of enriched (N0↑) or depleted (N0↓) phyla in N0 relative to N80. (c) Distribution of enriched and depleted families in the phylum Proteobacteria.
Alphaproteobacterial family, the *Rhizobiaceae*. Compared to the bulk soil and the rhizosphere, where the main phyla remained relatively similar, the plant fractions (root and rhizome) significantly filtered against certain bacterial families and thus re-established a special subset of the bacterial communities from the soil pool.

### 3.4 Long-term nitrogen fertilizer application enriched and depleted bacterial taxa

We then applied a fold-change test to assess the enrichment and depletion of taxa with respect to the two N conditions, in both soil and plant compartments. In each fraction, a distinct subset of OTUs was compartment-specific enriched or depleted, with essentially no common overlap between these compartments (Figure 5a). Even though the N fertilization had only a minor effect on the phyla distributions between the N0 and N80 samples, we noted that several families differed in their abundance in soil–plant fractions. Interestingly, these differentially abundant species mainly belong to the phyla *Proteobacteria* and *Actinobacteria* (Figure 5b, Figure S4).

We then further classified the enriched and depleted *Proteobacteria* families, which are thought to comprise the most important bacteria with potential for N fixation and denitrification. Denitrifiers convert nitrate via nitrite to the nitrogen gases nitric oxide, nitrous oxide and dinitrogen, and thus have a direct impact on soil nitrogen availability. The differences between N0 and N80 in the *Proteobacteria* families were complex (Figure 5c). Significantly enriched and depleted families in the phylum *Proteobacteria* with respect to N and compartment are summarized in Table 1. The putative N-fixing-associated *Hyphomicrobiaceae* (up to 3.9% in roots and 2.8% in the rhizome, respectively) of the order *Alphaproteobacteria* were enriched in the N0 root and rhizome, while *Bradyrhizobiaceae* were enriched in the bulk soil (to 0.5%) and rhizome (to 7.0%) respectively. However, another putative N-fixing family, the *Rhizobiaceae* (9.9% in N80), mainly composed of the tumour-pathogen genus *Agrobacterium*, was depleted in the rhizome under N0. When considering the order *Betaproteobacteria*, only the putative denitrifier family *Comamonadaceae* (11.6% in N80) was depleted in the N0 rhizome. Furthermore, the putative N-fixing *Syntrophobacteraceae* (2.2%) and *Geobacteraceae* (2.2%) of *Deltaproteobacteria* were enriched in the N0 rhizosphere respectively. Finally, the putative plant pathogen-associated *Gammaproteobacteria* *Xanthomonadaceae* were depleted in N0 in the root (2.4% in N80) and rhizome (6.4% in N80). *Pseudomonadaceae*, which include N-fixing and denitrifying species, as well as pathogenic and protective species, were widely depleted in all fractions in N0, except for the bulk soil. At last, the *Sinobacteraceae*, putatively involved in ammonia

### Table 1 Functional microbial family abundances in the *Proteobacteria* in soil–plant fractions

| Class family       | Putative function | Average relative abundance (%) |        |        |        |        |
|--------------------|-------------------|--------------------------------|--------|--------|--------|--------|
|                    |                   |                                | Soil N0 | N80    | Root N0 | N80    | Rhizosphere N0 | N80    |
| *Alphaproteobacteria* |                   |                                |         |        |        |        |        |        |
| *Hyphomicrobiaceae*  | N-fixing          |                                | 3.9*↑  | 1.4    | 2.8*↑  | 0.2    |        |        |
| *Bradyrhizobiaceae*  | N-fixing          |                                | 0.5*↑  | 0.2    |        |        |        |        |
| *Rhodospirillaceae*  | N-fixing          |                                | 1.0*↑  | 0.4    |        |        |        |        |
| *Rhizobiaceae*       | Plant pathogen    |                                | 0.8    | 9.9*↑  |        |        |        |        |
| *Betaproteobacteria* |                   |                                | 2.5    | 11.6*↑ |        |        |        |        |
| *Comamonadaceae*     | Denitrifier        |                                |        |        |        |        |        |        |
| *Deltaproteobacteria*|                   |                                |        |        |        |        |        |        |
| *Syntrophobacteraceae*| N-fixing          |                                | 2.2*↑  | 1.0    | 0.4 *↑ | 0.2    |        |        |
| *Geobacteraceae*     | N-fixing          |                                | 2.2*↑  | 0.2    |        |        |        |        |
| *Gammaproteobacteria*|                   |                                |        |        |        |        |        |        |
| *Xanthomonadaceae*   | Plant pathogen    |                                | 0.7    | 2.4*↑  | 2.0    | 6.4*↑  |        |        |
| *Pseudomonadaceae*   | Denitrifier        |                                | 1.6    | 3.2*↑  | 0.2    | 0.6*↑  | 0.5    | 1.0*↑  |
| *Sinobacteraceae*    | AMO, N-fixing?    |                                | 2.9*↑  | 0.7    |        |        | 13.0*↑ | 0.0    |
| Summary             | N-fixing          |                                | 0.5    | 0.2    | 4.4    | 1.2    | 5.3    | 2.0    |
|                     | Denitrifier       |                                | 1.6    | 3.2    | 0.2    | 0.6    | 3.0    | 12.6   |

Note: Only families that occupied more than 0.5% and that were significantly shifted in relative abundance of the entire bacterial composition are shown. Asterisks and arrows represent significant enrichment (Student t test: *p* < 0.05).

Abbreviations: AMO, ammonia monooxygenase; N-fixing, nitrogen fixing.
oxidation, were enriched in N0 in the bulk soil (to 2.9%) and rhizome (13%).

In total, putatively N-fixing bacteria were significantly enriched among all fractions in N0 compared to the N80, 0.5% versus 0.2% in soil, 4.4% versus 1.2% in the rhizosphere, 5.3% versus 2% in the roots and 9.8% versus 0.7% in the rhizome. By contrast, putative denitrifiers were depleted in the rhizome (12.6% in N80% vs. 3% in N0).

4 | DISCUSSION

In this study, we characterized microbiomes that were associated with M. x giganteus in different soil–endosphere compartments from a long-term field trial with two nitrogen fertilizer levels. Using amplicon sequencing, we were able to identify and classify soil and root-associated bacterial microbiomes. The PCoA analysis showed that the endophytic compartment, which comprises bacteria colonizing roots and rhizome, and the exophytic compartments, the bulk soil and the rhizosphere, were massively separated by the first principal coordinate (Figure 1b). This supports the well-accepted view that the compartment type plays a key role in determining the structure and composition of bacterial communities (Berg & Smalla, 2009; Lundberg et al., 2012). Meanwhile, bulk soil and rhizosphere samples clustered together in the PCoA plot and shared a similar Shannon diversity. The phylum and family profiles of these compartments were similar (Figures 1–4). These results are consistent with previous metagenomic analyses, which showed that the rhizosphere-associated microbiome is mainly dependent on the soil type, such as pH, soil moisture, organic matter and C/N ratio (Lundberg et al., 2012; Reinhold-Hurek, Bünger, Burbano, Sabale, & Hurek, 2015; De Souza et al., 2016). The N level had very mild effects on the bacterial communities in the soil, consistent with previous analyses (Babin et al., 2019).

Our data are overall consistent with previous microbiome studies on Miscanthus done in other soils and climates (Cope-Selby et al., 2017; Li et al., 2016), while some potentially site related differences were observed. For instance, we noted that the endophytic compartment recruited a different microbial pattern and serves as a distinct niche for only a fraction of the bacteria found in the soil and the rhizosphere. A very similar core microbiome was found for N-depleted and N-fertilized soils. Interestingly, compared to the exophytic compartment, the two phyla Proteobacteria and Actinobacteria were significantly enriched in the endophytic compartments (Figures 4 and 5). Previous work on the endophytic bacterial communities of different immunity-related Arabidopsis thaliana mutants and the endophytic fungal communities of maize under different P fertilization indicated that the endophytic microbial communities were mainly dependent on plant factors (Lebeis et al., 2015; Yu et al., 2018). The plant defence system essentially selects against detrimental microbes. We had recently observed that inoculation of Miscanthus with the endophytic bacterium Herbaspirillum frisingense GSF30T induced mild defence signalling in the plant, including the jasmonate signalling pathway and ethylene (Straub, Yang, Liu, Tsap, & Ludewig, 2013). Although an OTU representing H. frisingense was identified in the current dataset, it comprised only a very minor fraction of the exo- and endophytic bacteria, making this bacterial species unlikely having a major role in plant growth stimulation (Straub, Yang, Liu, Tsap, et al., 2013).

The plant organ type, root or rhizome, played a major further determinant role in the structure and composition of the internal bacterial communities. This was already apparent in the second principal coordinate of PCoA (Figure 1). Both roots and rhizome samples preferentially recruited Proteobacteria, Actinobacteria and Bacteroidetes, while the roots had significantly higher Shannon-diversity and harboured more Acidobacteria (Figure 2). It is now well-established that different plant organs, including roots, stalks, leaves, seeds and even flowers recruit different microbial communities in sugarcane, maize, rice, Arabidopsis and grape (Johnston-Monje, Lundberg, Lazarovits, Reis, & Raizada, 2016; Lundberg et al., 2012; Paszkowski & Gutjahr, 2013; De Souza et al., 2016; Zarraonaindia et al., 2015). Furthermore, fungal community distribution even diverged among different parts of the same maize organ, lateral and axial roots (Yu et al., 2018). Filtering of bacterial communities by the rhizosphere and further more strictly by plant organs apparently shapes these different communities (Hardoim, Overbeek, & Elsas, 2008; Reinhold-Hurek et al., 2015). The different bacterial communities between the root and the rhizome of Miscanthus are indicative of different physiological niches of these belowground compartments. The rhizome has a stem-like, but not root-like architecture and a very strong outer surface that hinders bacteria, but also nutrients and gases, such as O2 and N2, from entering the inner tissue.

Intriguingly, even though the endophytes in the M. x giganteus rhizome are expected to derive mainly from the root, there were still some OTUs predominantly identified in the rhizome. It is possible that these are directly propagated from clonal parents and are specific to the rhizome itself, as the sterile M. x giganteus is usually propagated via rhizomes. In non-sterile Miscanthus varieties, bacterial vertical transmission may be accomplished via seeds, and the endophytes of the next generation are significantly affected by these seedborne endophytes (Cankar, Kraigher, Ravnikar, & Rupnik, 2005; Hardoim, Hardoim, Overbeek, & Elsas, 2012). Also, endophytic bacteria of Miscanthus were found to form spores and other dense structures, which provided a mechanism for long-term survival and seed or rhizome transmission (Cope-Selby et al., 2017). In contrast, rhizosphere bacteria may also be derived from the plant. Using GFP-tagged bacteria, it was
found that rhizosphere bacteria may derive from plant endophytes, when injected into the maize stem. This indicates that vegetative organs are a source of additional bacteria (Johnston-Monje & Raizada, 2011).

Compared to the compartment and organ type, the effect of the N fertilizer on the composition and diversity of the bacterial community was relatively small (Figures 1–5). Nevertheless, we observed a significant N effect on the rhizosphere and the endosphere communities. This was most compelling on the family and OTU levels, especially in the rhizome. In sugarcane, with two different N fertilizer levels for 2 years, N did not change the endophytic bacterial composition, although it significantly shifted the nitrification- and denitrification-associated bacteria in the soil (Yeoh et al., 2016). Sugarcane and Miscanthus may differ in this respect, but differences may also result from the different period of distinct N supply (a difference for 14 years). In another research on Miscanthus, with two different nitrogen fertilizer levels in four different field sites for 4 years, nitrogen treatment did not change endophytic bacterial composition, but the total soil N significantly did (Li et al., 2016). The succession of the N-related bacterial communities may thus be gradual and short term of N fertilizer application may not be long enough to substantially shift the endophytic communities.

Actinobacteria were enriched in N80 in different compartments, especially in roots, showing a similar trend with the enrichment for Actinobacteria in grass roots under drought, which could depend on the ability of Actinobacteria to form spores or similar changes of plant root traits by N application and drought (more deep roots and less shallow roots; Naylor, DeGraaf, Purdom, & Coleman-Derr, 2017). The main shifted phyla between different N applications were Proteobacteria, which contain the major diazotrophic and N-cycle-associated microbe groups. Within the Proteobacteria, both enriched and depleted families were identified, with respect to the N level. Furthermore, within each compartment, differences in the diazotrophic community composition were apparent (Figure 5; Table 1). Soil diazotrophs were significantly affected by N fertilizer in rice and sorghum (Prakamhang, Minamisawa, Teamtaisong, Boonkerd, & Teaumroong, 2009; Rodrigues Coelho et al., 2008). Whether these bacteria in the root and rhizome supply substantial N to the Miscanthus, however, has to be experimentally confirmed.

In the bulk soil, almost no difference in the composition of the bacterial communities was apparent, although the putative N-fixing Bradyrhizobiaceae were slightly enriched in N0. Even though a number of non-legume-associated N-fixing bacteria, such as Bradyrhizobiaceae also include denitrifiers, we speculate that N fixing is more likely in N0, as denitrifiers are more likely found in the high N condition (Itakura et al., 2009; Figure 5; Table 1). In the N0 rhizosphere, two putative N-fixing bacterial families, Geobacteraceae and Syntrophobacteraceae, were enriched. These comprise candidate rhizosphere diazotrophs of Miscanthus (Bolhuis, Severin, Confurius-Guns, Wollenzien, & Stal, 2010; Holmes, Nevin, & Lovley, 2004). Pseudomonadaceae were enriched in the N80 rhizosphere, in accordance with their function in denitrification (Xun et al., 2018). The strongest nitrogen fertilizer effects were apparent in the endophytic compartments. Two putative diazotroph families, Hyphomicrobiaceae and Rhodospirillaceae, were enriched in the root to about 6% in N0, while these together only accounted for 2% in N80. Other putative diazotrophs, the Bradyrhizobiaceae, were enriched to about 7% in the rhizome, while these accounted for only 0.5% in N0. The putative diazotrophic Hyphomicrobiaceae, Rhodospirillaceae and Bradyrhizobiaceae might thus contribute nitrogen to Miscanthus, although this needs experimental confirmation. On the contrary, two putative denitrifier families, Pseudomonadaceae and Comamonadaceae, were enriched to about 12.6% in N80 (only 3.0% in N0). H. frisingense, a diazotroph previously identified in Miscanthus that promoted seedling growth (Straub, Yang, Liu, Tsap, et al., 2013), was apparently very low abundant. It therefore likely has little contribution to the N efficiency of Miscanthus (Kirchoff et al., 2001). Taken together, the yearly moderate N application enriched the relative abundance of denitrifiers, but depleted diazotrophs, compared to the non-fertilized control. The community shift to support N fixation was gradually increasing from soil to rhizome, opening the possibility that the rhizome is a niche that allows fixation from gaseous dinitrogen.

Miscanthus × giganteus is intrinsically quite resistant against many pathogens, a property that allows growing this grass without chemical protection. Interestingly, the plant pathogen-related families Xanthomonadaceae and Rhizobiaceae were more than twofold depleted in N0, compared to N80, which is in agreement with previous findings that some bacterial species are capable of suppressing plant pathogens on low fertility soils (Compant, Duffy, Nowak, Clément, & Barka, 2005; Hayat, Ali, Amara, Khalid, & Ahmed, 2010). Surprisingly, several field experiments have found an antagonistic relationship between Sinobacteraceae and Xanthomonadaceae (Ho, Lonardo, & Bodelier, 2017). Sinobacteraceae, which often express ammonia monooxygenase, tended to be enriched in N0, while the Xanthomonadaceae, mainly containing plant pathogens, were depleted in N0. As a consequence, Sinobacteraceae might contribute other plant growth-promoting functions to Miscanthus in N0.

In conclusion, we identified that three main factors shaped the M. × giganteus-associated bacterial community in the long-term experiments. The exophytic and endophytic communities substantially differed. Furthermore, the plant organ type was also important, separating the root and rhizome. Previous work on 2-year N fertilizer applications on Miscanthus and sugarcane demonstrated how the N fertilizer shifted the soil N-related bacterial community, while similar microbial communities were retained in the endophytic compartment (Li et al., 2016; Yeoh et al., 2016).
By contrast, the long-term N fertilizer difference in the field of Miscanthus allowed us to observe more N-fixing bacteria, especially in the rhizome, suggesting that pathways for gaseous nitrogen and oxygen entry into that compartment exist. By contrast, denitrification-related bacteria may be recruited by moderate or high nitrate availability (Figure 6). We also observed that the rhizome of M. × giganteus was apparently colonized by beneficial bacteria in N0. Because M. × giganteus is mainly propagated via the rhizome, it may even be beneficial to propagate this plant by choosing the rhizome from low N plots.

ACKNOWLEDGEMENTS
The authors acknowledge Prof. Dr. Iris Lewandowski for kindly providing access to the Miscanthus field as well as the University of Hohenheim Ihinger Hof station. We thank Debah Schnell for help with the DNA extraction. The work was partially supported by the China Scholarship Council.

DATA AVAILABILITY STATEMENT
Raw bacterial 16s rDNA sequencing data were deposited at the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under accession number PRJNA527123.

REFERENCES
Babin, D., Deubel, A., Jacquier, S., Sørensen, S. J., Geistlinger, J., Grosch, R., & Smalla, K. (2019). Impact of long-term agricultural management practices on soil prokaryotic communities. Soil Biology and Biochemistry, 129, 17–28. https://doi.org/10.1016/j.soilbio.2018.11.002
Baptista, R. B., de Morais, R. F., Leite, J. M., Schultz, N., Alves, B. J. R., Boddey, R. M., & Urquiaga, S. (2014). Variations in the 15N natural abundance of plant-available N with soil depth: Their influence on estimates of contributions of biological N2 fixation to sugar cane. Applied Soil Ecology, 73, 124–129. https://doi.org/10.1016/j.apsoil.2013.08.008
Beale, C. V., & Long, S. P. (1997). Seasonal dynamics of nutrient accumulation and partitioning in the perennial C4-grasses Miscanthus × giganteus and Spartina cynosuroides. Biomass & Bioenergy, 12, 419–428. https://doi.org/10.1016/S0961-9534(97)00016-0
Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology, 68, 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x
Boehmel, C., Lewandowski, I., & Claupein, W. (2008). Comparing annual and perennial energy cropping systems with different management intensities. Agricultural Systems, 96, 224–236. https://doi.org/10.1016/j.agsy.2007.08.004
Bolhuis, H., Severin, L., Confurius-Guns, V., Wollenzien, U. I., & Stal, L. J. (2010). Horizontal transfer of the nitrogen fixation gene cluster in the cyanobacterium Microcoleus chthonoplastes. The ISME Journal, 4, 121. https://doi.org/10.1038/ismej.2009.99
Cadoux, S., Riche, A. B., Yates, N. E., & Machet, J. M. (2012). Nutrient requirements of Miscanthus × giganteus: Conclusions from a review of published studies. Biomass and Bioenergy, 38, 14–22.
Cankar, K., Kraigher, H., Ravnikar, M., & Rupnik, M. (2005). Bacterial endophytes from seeds of Norway spruce (Picea abies L. Karst). FEMS Microbiology Letters, 244, 341–345.

ORCID
Yuan Liu https://orcid.org/0000-0002-3568-441X
Uwe Ludewig https://orcid.org/0000-0001-5456-1055
Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., ... Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal, 6, 1621. https://doi.org/10.1038/isme.2012.8

Christian, D. G., Poulton, P. R., Riche, A. B., & Yates, N. E. (1997). The recovery of 15N-labelled fertilizer applied to Miscanthus × giganteus. Biomass and Bioenergy, 12, 21–24. https://doi.org/10.1016/S0961-9534(96)00060-8

Christian, D. G., Riche, A. B., & Yates, N. E. (2008). Growth, yield and mineral content of Miscanthus × giganteus grown as a biofuel for 14 successive harvests. Industrial Crops and Products, 28, 320–327. https://doi.org/10.1016/j.indcrop.2008.02.009

Compton, S., Duffy, B., Nowak, J., Clément, C., & Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Applied and Environmental Microbiology, 71, 4951–4959. https://doi.org/10.1128/AEM.71.9.4951-4959.2005

Cope-Selby, N., Cookson, A., Squance, M., Donnison, I., Flavell, R., & Farrar, K. (2017). Endophytic bacteria in Miscanthus seed: Implications for germination, vertical inheritance of endophytes, plant evolution and breeding. GCB Bioenergy, 9, 57–77. https://doi.org/10.1111/gcbb.12364

Davis, S. C., Parton, W. J., Dohleman, F. G., Smith, C. M., Del Grosso, S., Kent, A. D., & DeLucia, E. H. (2010). Comparative biogeochemical cycles of bioenergy crops reveal nitrogen-fixation and low greenhouse gas emissions in a Miscanthus × giganteus agroecosystem. Ecosystems, 13, 144–156. https://doi.org/10.1007/s10021-009-9306-9

de Souza, R. S. C., Okura, V. K., Armanhi, J. S. L., Jorrín, B., Lozano, N., da Silva, M. J., ... Arruda, P. (2016). Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. Scientific Reports, 6, 28774. https://doi.org/10.1038/srep28774

Dohleman, F. G., Heaton, E. A., Arundale, R. A., & Long, S. P. (2012). Seasonal dynamics of above- and below-ground biomass and nitrogen partitioning in Miscanthus × giganteus and Panicum virgatum across three growing seasons. GCB Bioenergy, 4, 534–544.

Eyles, A., Bonello, P., Ganley, R., & Mohammed, C. (2010). Induced resistance to pests and pathogens in trees. New Phytologist, 185, 893–908. https://doi.org/10.1111/j.1469-8137.2009.03127.x

Fadros, D. W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R. M., & Ravel, J. (2014). An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome, 2, 6. https://doi.org/10.1186/2049-2618-2-6

Gottel, N. R., Castro, H. F., Kerley, M., Yang, Z., Pelletier, D. A., Podar, M., ... Schadt, C. W. (2011). Distinct microbial communities within the endosperm and rhizosphere of Populus deltoides roots across contrasting soil types. Applied and Environment Microbiology, 77, 5934–5944. https://doi.org/10.1128/AEM.05255-11

Hardoin, P. R., Haro, C. C., Van Overbeek, L. S., & Van Elsas, J. D. (2012). Dynamics of seed-borne rice endophytes on early plant growth stages. PLoS ONE, 7, e30438. https://doi.org/10.1371/journal.pone.0030438

Hardoin, P. R., van Overbeek, L. S., & van Elsas, J. D. (2008). Properties of bacterial endophytes and their proposed role in plant growth. Trends in Microbiology, 16, 463–471. https://doi.org/10.1016/j.tim.2008.07.008

Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: A review.

Annals of Microbiology, 60, 579–598. https://doi.org/10.1007/s13213-011-0171-7

Heaton, E. A., Long, S. P., Voigt, T. B., Jones, M. B., & Clifton-Brown, J. (2004). Miscanthus for renewable energy generation: European Union experience and projections for Illinois. Mitigation and Adaptation Strategies for Global Change, 9, 433–451. https://doi.org/10.1023/B:MITI.0000038848.94134.be

Ho, A., Di Lonoardo, D. P., & Bodelier, P. L. (2017). Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiology Ecology, 93, fix006. https://doi.org/10.1093/femsec/fix006

Holmes, D. E., Nevin, K. P., & Lovley, D. R. (2004). In situ expression of nifD in Geobacteraceae in subsurface sediments. Applied and Environmental Microbiology, 70, 7251–7259. https://doi.org/10.1128/AEM.70.12.7251-7259.2004

Iqbal, Y., Gauder, M., Clauepin, W., Graef-Hönning, S., & Lewandowski, I. (2015). Yield and quality development comparison between miscanthus and switchgrass over a period of 10 years. Energy, 89, 268–276. https://doi.org/10.1016/j.energy.2015.05.134

Itakura, M., Saeki, K., Omori, H., Yokoyama, T., Kaneko, T., Tabata, S., ... Minamisawa, K. (2009). Genomic comparison of Bradyrhizobium japonicum strains with different symbiotic nitrogen-fixing capabilities and other Bradyrhizobiaceae members. The ISME Journal, 3, 326. https://doi.org/10.1038/ismej.2008.88

Johnston-Monje, D., Lundberg, D. S., Lazarovits, G., Reis, V. M., & Raizada, M. N. (2016). Bacterial populations in juvenile maize rhizospheres originate from both seed and soil. Plant and Soil, 405, 337–355. https://doi.org/10.1007/s11104-016-2826-0

Johnston-Monje, D., & Raizada, M. N. (2011). Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology. PLoS ONE, 6, e20396. https://doi.org/10.1371/journal.pone.0020396

Keymer, D. P., & Kent, A. D. (2014). Contribution of nitrogen fixation to first year Miscanthus × giganteus. GCB Bioenergy, 6, 577–586. https://doi.org/10.1111/gcbb.12095

Kirschhoff, G., Eckert, B., Stoffels, M., Baldani, J., Reis, V., & Hartmann, A. (2001). Herbaspirillum frisingense sp. nov., a new nitrogen-fixing bacterial species that occurs in C4-fibre plants. International Journal of Systematic and Evolutionary Microbiology, 51, 157–168. https://doi.org/10.1099/00270773-51-1-157

Kirschhoff, G., Reis, V. M., Baldani, J. I., Eckert, B., Döbereiner, J., & Hartmann, A. (1997). Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in graminaceous energy plants. Plant and Soil, 194, 45–55.

Lavecchia, A., Curci, M., Jingad, K., Whitman, W. B., Ricciuti, P., Pascazio, S., & Crecchio, C. (2015). Microbial 16S gene-based composition of a sorghum cropped rhizosphere soil under different fertilization managements. Biology and Fertility of Soils, 51, 661–672. https://doi.org/10.1007/s00374-015-1017-0

Lebeis, S. L., Paredes, S. H., Lundberg, D. S., Breakfield, N., Gehring, J., McDonald, M., ... Dangl, J. L. (2015). Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science, 349, 860–864. https://doi.org/10.1126/science.aaa8764

Lewandowski, I., Clifton-Brown, J. C., Scurlock, J. M. O., & Huisman, W. (2000). Miscanthus: European experience with a novel energy crop. Biomass and Bioenergy, 19, 209–227. https://doi.org/10.1016/S0961-9534(00)00032-5

Li, D., Voigt, T. B., & Kent, A. D. (2016). Plant and soil effects on bacterial communities associated with Miscanthus × giganteus.
rhizosphere and rhizomes. GCB Bioenergy, 8, 183–193. https://doi.org/10.1111/gcbb.12252

Liu, Y., Yang, H., & Ludewig, U. (2014). Dynamic element concentrations and similar proteome of the rhizome and root of Miscanthus × giganteus. Journal of Plant Biochemistry & Physiology, 2, 4. https://doi.org/10.4172/2329-9029.1000139

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., … Dangl, J. L. (2012). Defining the core Arabidopsis thaliana root microbiome. Nature, 488, 86. https://doi.org/10.1038/nature11237

Magoč, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinformatics, 27, 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Maughan, M., Bollero, G., Lee, D. K., Darmody, R., Bonos, S., Cortese, L., … Voigt, T. (2012). Miscanthus × giganteus productivity: The effects of management in different environments. GCB Bioenergy, 4, 253–265. https://doi.org/10.1111/j.1757-1707.2011.01144.x

Naylor, D., DeGraaf, S., Purdom, E., & Coleman-Derr, D. (2017). Transcriptomic and proteomic comparison of two Miscanthus genotypes: High biomass correlated with increased secondary metabolism. Plant and Soil, 338, 435–449. https://doi.org/10.1007/s11104-010-0557-1

Paszkowski, U., & Gutjahr, C. (2013). Multiple control levels of root system remodeling in arbuscular mycorrhizal symbiosis. Frontiers in Plant Science, 4, 204. https://doi.org/10.3389/fpls.2013.00204

Pieterse, C. M., Zamnioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C., & Bakker, P. A. (2014). Induced systemic resistance by beneficial microbes. Annual Review of Phytopathology, 52, 347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Prakamhang, J., Minamisawa, K., Teamtaisong, K., Boonkerd, N., & Teamroong, N. (2009). The communities of endophytic diazotrophic bacteria in cultivated rice (Oryza sativa L.). Applied Soil Ecology, 42, 141–149. https://doi.org/10.1016/j.apsoil.2009.02.008

Reinhold-Hurek, B., Bäumer, W., Burbano, C. S., Sabale, M., & Hurek, T. (2015). Roots shaping their microbiome: Global hotspots for microbial activity. Annual Review of Phytopathology, 53, 403–424. https://doi.org/10.1146/annurev-phyto-082712-102342

Rodrigues Coelho, M. R., De Vos, M., Carneiro, N. P., Marriel, I. E., Paiva, E., & Seldin, L. (2008). Diversity of nifH gene pools in the rhizosphere of two cultivars of sorghum (Sorghum bicolor) treated with contrasting levels of nitrogen fertilizer. FEMS Microbiology Letters, 279, 15–22. https://doi.org/10.1111/j.1574-6968.2007.00975.x

Saharan, B. S., & Nehra, V. (2011). Plant growth promoting rhizobacteria: A critical review. Life Sciences and Medicine Research, 21, 1–30.

Straub, D., Yang, H., Liu, Y., & Ludewig, U. (2013). Transcriptomic and proteomic comparison of two Miscanthus genotypes: High biomass correlates with investment in primary carbon assimilation and decreased secondary metabolism. Plant and Soil, 372, 151–165. https://doi.org/10.1007/s11104-013-1693-1

Straub, D., Yang, H., Liu, Y., Tsap, T., & Ludewig, U. (2013). Root ethylene signalling is involved in Miscanthus sinensis growth promotion by the bacterial endophyte Herbaspirillum frisingense GSF30T. Journal of Experimental Botany, 64, 4603–4615. https://doi.org/10.1093/jxb/ert276

Thaweenut, N., Hachisuka, Y., Ando, S., Yanagisawa, S., & Yoneyama, T. (2011). Two seasons’ study on nifH gene expression and nitrogen fixation by diazotrophic endophytes in sugarcane (Saccharum spp. hybrids): Expression of nifH genes similar to those of rhizobia. Plant and Soil, 338, 435–449. https://doi.org/10.1007/s11104-010-0557-1

Urquigaa, S., Xavier, R. P., de Morais, R. F., Batista, R. B., Schultz, N., Leite, J. M., … Boddey, R. M. (2012). Evidence from field nitrogen balance and 15N natural abundance data for the contribution of biological N2 fixation to Brazilian sugarcane varieties. Plant and Soil, 356, 5–21. https://doi.org/10.1007/s11104-011-0106-3

van Overbeek, L. S., & Saikkonen, K. (2016). Impact of bacterial–fungal interactions on the colonization of the endosphere. Trends in Plant Science, 21, 230–242. https://doi.org/10.1016/j.tplants.2016.01.003

Xun, W., Li, W., Huang, T., Ren, Y. I., Xiong, W. U., Miao, Y., … Zhang, R. (2018). Long-term agronomic practices alter the composition of asymptomatic diazotrophic bacterial community and their nitrogen fixation genes in an acidic red soil. Biology and Fertility of Soils, 54, 329–339. https://doi.org/10.1007/s00373-018-1264-y

Yeoh, Y. K., Paungfoo-Lonhienne, C., Dennis, P. G., Robinson, N., Ragan, M. A., Schmidt, S., & Hugenholtz, P. (2016). The core root microbiome of sugarcane cultivated under varying nitrogen fertilizer application. Environmental Microbiology, 18, 1338–1351. https://doi.org/10.1111/1462-2920.12925

Yu, P., Wang, C., Baldauf, J. A., Tai, H., Gutjahr, C., Hochhodinger, F., & Li, C. (2018). Root type and soil phosphate determine the taxonomic landscape of colonizing fungi and the transcriptome of field-grown maize roots. New Phytologist, 217, 1240–1253. https://doi.org/10.1111/nph.14893

Zarronaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., … Gilbert, J. A. (2015). The soil microbiome influences grapevine-associated microbiota. MBio, 6, e02527–e2614. https://doi.org/10.1128/mBio.02527-14

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Liu Y, Ludewig U. Nitrogen-dependent bacterial community shifts in root, rhizosphere and rhizospheric nutrient-efficient Miscanthus x giganteus from long-term field trials. GCB Bioenergy. 2019;11:1334–1347. https://doi.org/10.1111/gcbb.12634