Effects of Field-Grown Transgenic *Cry1Ah1* Poplar on the Rhizosphere Microbiome

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Abstract

Background: Poplar (*Populus*) is a genus of globally important plantation trees used widely in industrial and agricultural production. However, poplar is easily damaged by *Micromelalopha troglodyta* and *Hyphantria cunea*, resulting in a decline in poplar quality. Due to their strong insect resistance, Bt toxin-encoded *Cry* genes have been widely adopted in poplar breeding; however, potential adverse effects of *Cry1Ah1*-modified poplars on the ecological environment have raised concerns.

Results: In this study, we comprehensively analyzed the structural and functional composition of the rhizosphere microbiome in field-grown transgenic *Bt* poplar.

Conclusions: Our analysis of soil chemistry patterns revealed that soil alkaline nitrogen, soil available phosphorus, and microbial biomass nitrogen and phosphorus levels were improved, whereas microbial biomass carbon declined in *Cry1Ah1*-modified poplar rhizosphere samples. We applied metagenomic sequencing of Non-Transgenic (NT) and *Cry1Ah1*-modified poplar rhizosphere samples collected from a natural field; the predominant taxa included Proteobacteria, Acidobacteria, and Actinobacteria. We also identified microbial functional traits involved in membrane transport, amino acid metabolism, carbohydrate metabolism, and replication and repair in NT and *Cry1Ah1*-modified poplars. Together, these results demonstrate that the NT and *Cry1Ah1*-modified poplar rhizosphere microbiomes had similar diversity and structure. These differences in relative abundance were observed in a few genera but did not affect the primary genera or soil.

Background

Poplar (*Populus*) is a genus of globally important plantation trees used widely in industrial and agricultural production [1]. With the deterioration of the global environment, characterized by increasing salt, drought, pest, and disease stress, the global production of poplar is becoming challenging. One approach to address this challenge is genetic modification. The manipulation of key genes has been applied to successfully alter poplar characteristics in transgenic lines, resulting in improved traits for better growth in adverse environments [2–4].

Insecticide resistance based on *Bacillus thuringiensis* (*Bt*) has allowed the development of a variety of insect resistance proteins for use in commercial genetically modified (GM) crops [5]. *Bt* toxin-encoded *Cry* genes have been widely applied in commercial GM crops, improving plant resistance to insect pests [6, 7]. Despite the many benefits of *Bt*-modified plants, a major potential disadvantage is their effect on soil chemical properties and the structure and diversity of rhizosphere microorganisms, including bacteria and fungi [6]. Therefore, rhizosphere microorganisms associated with *Bt*-modified plants are of great interest [8–10]. Due to the complexity of field conditions and the lack of a unified approach to microorganism analysis, studies exploring the influence of *Bt*-modified plants on soil microorganisms sometimes produce conflicting results [11–14]. For example, one study showed that soil microorganisms are not adversely affected by the cultivation of *Bt*-modified cotton plants [15].

In contrast, another study suggested that exogenous gene products expressed by *Bt*-modified transgenic crops may interact with soil microorganisms and affect their activities and functions [16]. *Bt*-modified plants have been found to alter bacterial diversity population diversity compared with Non-Transgenic (NT) plants [17] and may enhance the population size of soil microbial communities [18, 19]. Compared with NT rice, *Bt*-modified rice has been found to have a short-term effect on rhizosphere microbial community function [20]. Thus, there are some differences among studies on the impact of *Bt*-modified plant varieties on the soil microbial community. Further research is needed to evaluate the safety of *Bt*-modified crop plants.

Since the commercialization of GM plants, the global planting area of GM crops has grown rapidly, and new varieties of GM plants have been emerging continually [21]. GM plants have provided great economic and environmental benefits worldwide, such as increased plant yield and reduced chemical fertilizer and pesticide application. However, the potential impacts of GM plants on the ecological environment have raised concerns [22]. Soil microorganisms are an essential part of the soil ecosystem and are involved in various biochemical processes, including organic matter accumulation and mineralization, as well as nutrient transformation and circulation [23, 24]. GM plants communicate with soil microorganisms during the growth process; therefore, research on the potential effects of GM plants on the soil microbial community is of great significance in evaluating their potential risks [25–27]. The purpose of this study was to identify the effects of *Bt*-modified poplar plantation on soil chemical properties, as well as the diversity and structure of the soil microorganism community after four years of growth under field conditions. We applied high-throughput sequencing of 16S rRNA and internal transcribed spacer 1 (ITS1) to evaluate differences in soil microorganism diversity and composition of between NT and *Cry1Ah1*-modified poplars. The results of this study contribute to our knowledge of how *Cry1Ah1*-modified poplars affect rhizosphere microbial community function and provide reference information for evaluating the safety of *Cry1Ah1*-modified poplars.

Methods

Plant material and experimental field design

Large areas of northern Jiangsu Province, China, are dedicated to poplar plantations (Fig. 1A); however, large-scale centralized poplar plantations are highly vulnerable to attacks by pests, leading to growth stagnation (Fig. 1B–D). To address this problem, the *Cry1Ah1* gene was selected to improve poplar insect resistance through breeding [2].

To study the effects of *Cry1Ah1*-modified poplars on a natural soil ecosystem, we designed a field test in Sihong, Jiangsu Province (118°68'N, 33°72'E) (Fig. 1E). Five *Cry1Ah1*-modified poplar lines were selected, and six plots were established with four replicates per clone (Fig. 1F). To isolate the influence of *Cry1Ah1*-modified poplars, we planted wild-type (WT) poplars around the experimental field (Fig. 1F). An additional 3-m-wide isolation zone was established between communities, with an area of about 676 m² (26 m × 26 m). The poplars were cultivated by cutting in March 2017 with permission from the State Forestry

Administration; the experimental field was managed conventionally without chemical fertilizers or pesticides.

After weeds and leaves were removed from the soil surface, the centre point of each transgenic plot was identified. Rhizosphere soil (10–30 cm soil depth) was excavated at 120° in a 30-cm radius around the centre point (Fig. 1G). All rhizosphere soil samples were passed through a 10-mesh sieve, mixed thoroughly, added to sterile centrifuge tubes, and then placed in a liquid nitrogen tank for transport to the laboratory.

Determination of rhizosphere soil physical and chemical indices

Chloroform fumigation was performed to obtain soil microbial biomass nitrogen (MBN), carbon (MBC), and phosphorus (MBP) [13, 28–30]. Soil samples treated with chloroform fumigation and non-chloroform fumigation were extracted with K_2SO_4 solution, and MBC, MBN, and MBP were measured using a Vario TOC cube analyzer (Elementar, Langenselbold, Germany). Briefly, fresh soil samples were dissolved in chloroform, and the mixtures were boiled for 5 min in a vacuum. Then, 0.5 mol/L K_2SO_4 was added to the mixtures, which were subjected to fumigation in the dark at 25 °C for 24 h and then filtered with a quantitative filter paper.

Soil alkaline nitrogen was determined using the Conway method (i.e., the alkali hydrolysis diffusion method). Briefly, 10 mL of NaOH was used to dissolve air-dried soil samples set in the outer chamber of a diffusion dish, and 2 mL of boric acid (an indicator solution) was placed in the inner chamber. After incubation at 40 °C for 24 h, NH_3 in the inner chamber absorption solution was titrated with 0.005 mol/L H_2SO_4 as a standard solution. At the same time, a blank test was performed to correct reagent and titration errors.

Soil available phosphorus was identified by molybdenum–antimony colourimetry. Briefly, air-dried soil samples were mixed with 0.5 mol/L $NaHCO_3$ and activated carbon, shaken for 30 min, and filtered immediately with phosphate-free filter paper. Then, 1–5 mL of filtrate was extracted, and the absorbance value was determined. Soil pH was also measured using the glass electrode method. The soil samples were mixed with 2.5 × the volume of water, and the pH of the suspension was determined using a PP-25 Professional Meter electrode (Sartorius, Germany).

Rhizosphere soil DNA extraction and high-throughput sequencing

A total of 36 independent soil samples obtained from the root rhizosphere of the WT and five poplar varieties (A5-0, A4-6, Z1-3, A5-23, and A3-4) were selected for DNA extraction. The quality and integrity of the DNA were determined by electrophoresis on 0.8% agarose gel, and the extracted DNA samples were diluted 10-fold and stored at – 80 °C for further molecular analyses.

Using the extracted genomic DNA as a template, the V4–V5 region (515f/907r) of the 16S rRNA gene was amplified to identify the composition and diversity of the microbiological community [31, 32]. We then performed high-throughput sequencing using the Illumina Hiseq 2500 platform. The raw data were filtered using the Trimmatic software to obtain high-quality clean paired-end reads, which were then spliced using the FLASH software. The minimum overlap length was set to 10 bp, and the maximum mismatch ratio of the splicing sequence was 0.1. After filtering, influential splicing segment clean tags were obtained. All clean tags were clustered using the USEARCH software (<http://www.drive5.com/usearch/>). By default, the sequences were clustered into operational taxonomic units (OTUs) with 97% consistency. Relative abundances of less than 1% were merged at the phylum level, and unclassifiable bacterial groups were designated as other. Alpha and beta diversity analyses were performed using the Chao1 index to determine community richness and the Shannon index to determine community richness and evenness [33].

Data analyses

All data analyses were performed using the SPSS 19.0 software (IBM, USA). Differences in the physical and chemical properties, microbial community composition, and structure of rhizosphere soil between WT and *Cry1Ah1*-modified poplar varieties were evaluated using independent sample *t*-tests. The Bray–Curtis distance analysis and multivariate statistical methods, including principal coordinates analysis (PCoA), were applied to compare differences in bacterial community structure between WT and *Cry1Ah1*-modified poplars [34].

Results

Effects of *Cry1Ah1*-modified poplars on soil chemistry patterns

During the first three years of poplar establishment, the mean soil pH ranged from 7.73 to 8.23 in the upper soil (10–30 cm) (Fig. 2A). There was no significant change in soil pH between NT and *Cry1Ah1*-modified poplars by sampling date. For the five GM poplar varieties (A5-0, A4-6, Z1-3, A5-23, and A3-4), rhizosphere soil alkaline nitrogen ranged from 64.54 to 83.15 mg/kg, with similar values observed for NT poplars, and no significant difference between NT and *Cry1Ah1*-modified poplars (Fig. 2B). However, *Cry1Ah1*-modified poplar varieties had significantly lower soil available phosphorus in the field-grown stage compared with NT poplars (Fig. 2C). The rhizosphere MBC content of NT poplars ranged from 160 to 172 mg/kg and was significantly different from those of the *Cry1Ah1*-modified varieties (Fig. 2D). *Cry1Ah1*-modified poplars had significantly lower MBN and MBP content than NT poplars (Fig. 2E and F).

Total genome features of the NT and *Cry1Ah1*-modified poplar rhizosphere microbiomes

NT and *Cry1Ah1*-modified poplar rhizosphere samples were collected from representative locations where poplars had grown for at least three years to identify the constituents of the rhizosphere microbiome

community. Bacterial and fungal community sequencing was performed by amplifying the V5–V8 and ITS1 regions, respectively. Using the Illumina Hiseq/Miseq sequencer, an average of 53,567 and 68,783 16S rDNA tags and 33,750 and 87,922 ITS1 tags were generated from the rhizosphere microbiome (Supplementary Table 1). Chimaeras and short tag sequences were removed to obtain high-quality clean reads consisting of an average of 33,390 and 86,787 16S rDNA tags and 21,523 and 22,555 ITS2 tags (Supplementary Table 1). We also analyzed high-quality reads using the Qiime ver. 1.8.0 (<http://qiime.org/>) and Vsearch 2.7.1 (<https://github.com/torognes/vsearch>) software, removing chimeric and organelle sequences to produce 10,413 bacterial community sequencing OTUs and 7,732 fungal community sequencing OTUs (Supplementary Table 2).

Cry1Ah1 expression has marginal effects on rhizosphere bacteria of field-grown poplars

To determine whether Cry1Ah1 expression can affect the soil bacteria community, we investigated the taxonomic distinctiveness of poplar soil bacteria. The alpha diversity results showed no significant difference between NT and *Cry1Ah1*-modified poplars (Supplemental Fig. 1). According to the unweighted UniFrac distance (beta diversity) of NT and *Cry1Ah1*-modified poplars, we performed PCoA; the results revealed no difference in soil bacterial community composition between NT and *Cry1Ah1*-modified poplars (Fig. 3A and B). A comparison of the relative abundances of soil bacteria in NT and *Cry1Ah1*-modified poplars at the phylum, class, order, family, and genus levels was performed. At higher taxonomic levels, the root-associated microbiota of NT and *Cry1Ah1*-modified poplars had similar overall relative abundances, with Proteobacteria, Acidobacteria, and Actinobacteria as the dominant phyla. By contrast, multiple Archaea phyla, including Candidatus, Berkelbacteria, Peregrinibacteria, and Microgenomates, were found at lower relative abundances in the soil (Fig. 3C, Supplemental Table 5). At the class level, the root microbiota of NT and *Cry1Ah1*-modified poplars were similar (Supplemental Fig. 2A). In native fields, Alphaproteobacteria, Deltaproteobacteria, Betaproteobacteria, Subgroup6, and Thermoleophilia were present at similar relative abundances in NT and *Cry1Ah1*-modified poplar (Supplemental Table 6). Thus, the differences in root bacteria between NT and *Cry1Ah1*-modified poplars were significant at lower taxonomic ranks (order, family, and genus) (Supplemental Fig. 2B, C, and D)

We next screened extremely rare OTUs from the amplicon-based and metagenomic sequences to determine the relative abundances of families; these are shown as a heatmap of community composition in Fig. 4A. Some communities contained a few dominant taxa (e.g., Rhodospirillaceae, Nitrosomonadaceae, Blastocatellaceae Subgroup4), whereas others contained many taxa of low abundance (e.g., *Hyaloperonospora arabidopsidis* and Pasteurellaceae). Weinhold et al. [35] performed a similarity percentage analysis to identify major differences in abundance within samples and found that highly abundant families contributed significantly to dissimilarities within samples. We examined family-level differences between NT and *Cry1Ah1*-modified poplars and found that Xanthobacteraceae and Rhodospirillaceae were substantially more abundant in *Cry1Ah1*-modified varieties than in NT (Z1-3, A5-23, and A4-6) (Fig. 4B). However, the relative abundances of Desulfurellaceae, Gemmatimonadaceae, Nitrosomonadaceae, and Blastocatellaceae Subgroup4 were higher in most *Cry1Ah1*-modified varieties

than in NT poplars (Fig. 4B). The quantities of *Corynebacteriaceae*, *Rhodothermaceae*, *Streptococcaceae*, *planctomycete A-2*, *Christensenellaceae*, and *Oscillochloridaceae* were low and not significantly different between NT and *Cry1Ah1*-modified poplars (Fig. 4B). Thus, the root microbiota of NT and *Cry1Ah1*-modified poplars differed among highly abundant families, but not among families of low abundance.

Cry1Ah1 expression has a negligible impact on the native root-associated fungus community

We investigated the taxonomic distinctiveness of poplar soil fungi to determine whether *Cry1Ah1* expression affected soil fungus communities. Beta diversity analysis showed no significant difference in soil fungus diversity between NT and *Cry1Ah1*-modified varieties among samples from the same location (Fig. 5A). PCoA of the Bray–Curtis distance revealed no distinct or dependent clusters among the root fungi of NT and *Cry1Ah1*-modified poplars (Fig. 5B). The dominant fungal phyla in poplar soils included Ascomycota, Basidiomycota, and Mortierellomycota (Fig. 5C); the sequence load of the six dominant phyla, represented by high sequence numbers, represented more than approximately 80% of the total sequence, whereas that of low-abundance phyla comprised less than 20% of the entire sequence (Supplemental Table 3). At the phylum level, there was no significant difference among groups of fungi with the highest or lowest abundance between NT poplars and *Cry1Ah1*-modified poplars (Supplemental Fig. 3).

Next, we examined the microbial species that were most and least abundant in poplar soils in greater depth, due to their essential roles in soil bacterial communities [36, 37]. We filtered extremely rare OTUs from the dataset to determine relative abundances at the genus level. Similar levels of relative abundance among *Tuber*, *Geopora*, *Fusarium*, *Archaeorhizomyces*, and *Acremonium* were observed between NT and *Cry1Ah1*-modified varieties. The relative lots of *Laetiporus*, *Penicillium*, *Stachybotrys*, and *Cladosporium* were low and not significantly different between NT and *Cry1Ah1*-modified types (Supplemental Fig. 4).

Functional traits of the rhizosphere soil microbiome in NT and Cry1Ah1-modified poplars

Gene function prediction in rhizosphere soil bacteria showed no significant difference in the number of gene-coding proteins between NT and *Cry1Ah1*-modified poplars, according to the Kyoto Encyclopedia of Genes and Genomes (KEGG). KEGG functional annotation analysis showed that the annotation sequences were distributed among 24 available categories, with high relative abundance among membrane transport, amino acid metabolism, carbohydrate metabolism, and replication and repair (Fig. 6). The gene abundance of the rhizosphere soil bacteria community in NT poplars was similar to that of *Cry1Ah1*-modified varieties. KEGG functional annotation of the rhizosphere soil fungi community also revealed that *Cry1Ah1* expression affected gene abundance among the rhizosphere soil fungi community (Supplemental Fig. 5).

Discussion

Biological diversity comprises community composition, structure, and function [38]. Interactions between soil microorganisms and other organisms influence nutrient cycling, which plays an essential role in soil condition, quality, and health [13, 39, 40]. The rhizosphere is a functional interface for materials exchange between plants and soil ecosystems. Plants assimilate CO₂ during photosynthesis and transport some photosynthetic products to their underground parts, promoting the growth and metabolism of soil microorganisms, which in turn transform organic nutrients in the soil into inorganic forms for absorption and utilization by plants [41]. With the recent emergence of transgenic plants, the impact of their cultivation on the structure and function of the rhizosphere microbial community has become a concern [16, 42, 43]. The structural diversity of the soil microbial community is an important index for evaluating the effects of GM on the soil ecological environment.

Changes in soil MBC, MBN, and MBP content in WT and *Cry1Ah1*-modified poplars

Soil microbial biomass is an essential parameter for the assessment of active soil nutrients and a sensitive indicator of environmental change in terrestrial ecosystems [44–46]. MBC, MBN, and MBP participate in the ecosystem cycling of carbon, nitrogen, and phosphorus [47, 48]. To date, the effects of GM plants on MBC, MBN, and MBP have not been reported. Therefore, in the present study, we examined the impact of field-cultivated GM plants on MBC, MBN, and MBP content to study the relationship between GM plant growth and the transformation of carbon, nitrogen, and phosphorus in natural soil.

As a part of active soil carbon, MBC is the driving force of soil organic matter decomposition, which is closely related to the cycling of soil elements. We found that MBC content was significantly higher in *Cry1Ah1*-modified varieties than in WT poplars. MBC content decreased in *Cry1Ah1*-modified poplars grown in the field, which may have affected the growth, metabolism, and structure of soil microorganisms. As an essential source of active soil nitrogen, MBN plays a vital role in regulating the supply of soil nitrogen [49]. MBP is the most active component of soil organic phosphorus, which governs the mineralization and fixation of soil phosphorus. MBP is an important source of available soil phosphorus, which reflects the capacity and turnover intensity of active soil phosphorus [50, 51]. Our results showed that *Cry1Ah1*-modified poplars altered soil MBN and MBP content, at least during the study period, affecting the capacity of soil microorganisms to metabolize carbon, nitrogen, and phosphorus. Plants can alter the available or bioaccessible concentrations of soil elements directly through absorption and removal or indirectly through plant residue decomposition. Total concentrations of soil elements are generally not affected by plants [52, 53]. However, we found that available soil phosphorus, MBN, and MBP content were decreased, whereas MBC content was significantly increased in *Cry1Ah1*-modified poplars. This result can be used to guide future poplar breeding. Although *Cry1Ah1* transformation can improve poplar insect resistance, transgenic poplars grown in fields may be affected by MBC, MBN, and MBP, which will eventually affect the growth of plants.

The soil MBC/MBN ratio can reflect soil microbial community structure. Generally, the MBC/MBN ratio is about 5:1 for bacteria, 6:1 for actinomycetes, and 10:1 for fungi [54–58]. Based on our results, the

MBC/MBN ratio for WT poplars in our study site was about 4.6 ± 0.3 , indicating that bacteria may play a dominant role in determining MBC and MBN contents. However, the MBC/MBN ratio for *Cry1Ah1*-modified poplars was about 9.2 ± 1.7 , suggesting that microbial fungi in *Cry1Ah1*-modified poplars participate widely in soil microenvironment regulation. Xu et al. [32] performed a systematic analysis of MBC, MBN, and MBP in the global terrestrial ecosystem. They reported mean values of MBC/MBN, MBC/MBP, and MBN/MBP ratios of 7.6, 42.4, and 5.6, respectively. In the present study, the MBC/MBP and MBN/MBP ratios for WT poplars were 63.2 ± 3.1 and 13.6 ± 0.9 , respectively, higher than those reported for the global terrestrial ecosystem. This discrepancy may be due to the low nitrogen and phosphorus content in the northern Jiangsu plain, resulting in lower MBN and MBP contents, and consequently lower MBC/MBP and MBN/MBP ratios. Compared with WT poplars, *Cry1Ah1*-modified poplars showed higher soil MBC/MBN, MBC/MBP, and MBN/MBP ratios; thus, *Cry1Ah1* transformation may directly affect the growth of soil microorganisms or inhibit soil microbial activity, thereby affecting the metabolism of MBC, MBN, and MBP.

Soil microbial biomass is an essential biological fertility index used to evaluate soil quality [59, 60]. Nitrogen and phosphorus deficiency generally have adverse effects on soil microbial reproduction and metabolic activity, as well as on crop growth and nutrient uptake [27, 61, 62]. The lower MBN and MBP contents in *Cry1Ah1*-modified poplars suggested that soil microorganisms were under nutrient deficiency stress due to low metabolic efficiency and more significant release of heat and CO_2 during metabolism, significantly reducing soil quality. Long-term nitrogen and phosphorus deficiency can exhaust stores of soil phosphorus, forcing the release of nitrogen and phosphorus from soil microorganisms and forming soil available nitrogen and phosphorus for absorption and use by plants. Fertilization is one approach for overcoming these adverse effects. Long-term application of inorganic fertilizers containing nitrogen and phosphorus will result in the assimilation of more nitrogen and phosphorus into microorganisms, thereby improving soil MBN and MBP. Long-term application of organic fertilizer can also speed up the decomposition of organic carbon sources by microorganisms, and more substantial assimilation rates can increase soil nutrient contents and ensure higher microbial biomass [63–66].

Effects of *Cry1Ah1* expression on native rhizosphere communities

Bt protein confers strong herbicide resistance as a dominant trait of GM crops; it has been widely used in transgenic breeding to achieve herbicide-resistant plants. Xu et al. [2] showed that field-planted *Cry1Ah1*-modified poplars had strong herbicide resistance. With increasing numbers of GM crops worldwide, the environmental and ecological impact of GM crop cultivation has raised concerns globally. Some studies have shown that GM crops have serious negative effects on biodiversity and may pose a threat to the environment [67]. Whether GM crops affect soil microbial composition, structure, and function have become a widely studied question in the fields of ecology and food safety [68]. The plant rhizosphere is a dynamic microenvironment in which many factors, such as plant species [69], soil type [70], and root location [71], affect the composition and structure of microbial communities around plant roots [72]. Therefore, to avoid the interference of these factors on the examination of soil microbial communities in

the present study, we selected poplar trees planted in a single location and collected samples simultaneously. Such a design can effectively avoid the influence of other factors on the results, focusing only on the effects of poplar type (NT or *Cry1Ah1*-modified) on the soil microbial community.

Many studies have explored the relationship between soil biodiversity and the ecological safety of transgenic plants. The high insect resistance of *Bt*-maize makes it an important transgenic crop, and it has been found not to affect soil microbial communities [73] or rhizosphere communities [6]. Field-cultivated *Bt* transgenic cotton has also been found to have no significant effect on rhizosphere communities compared with WT cotton [74]. Similarly, *Cry1Ac*-sugarcane was found to have no impact on rhizosphere microbial diversity or enzyme activity compared with NT sugarcane within a single crop season [75]. In addition, no persistent or adverse effects on the rhizosphere bacterial community population were detected between WT and *Bt*-modified rice [76]. However, Li et al. [15] reported that *Bt* transgenic rice had the potential to change bacterial community composition, but not fungal abundance or community structure. Thus, we conclude that most *Bt*-modified crops have negligible effects on soil microbial communities. In the present study, cluster heatmaps were generated from rare OTUs to show the microbial diversity and abundance of each group based on combined samples and dominant microbial genera. The results of this analysis revealed no significant difference in the structure or population of the root-associated microbial community between transgenic and NT poplars. However, the relative abundance of highly abundant bacteria, including Proteobacteria, Acidobacteria, and Actinobacteria, was affected in *Cry1Ah1*-modified poplars. Both the taxonomic diversity and structure of rhizosphere fungal communities were similar among high- and low-abundance fungi between *Cry1Ah1*-modified and NT poplars. Based on these findings, we conclude that rhizosphere microbial populations were not affected by *Cry1Ah1*-modified poplar cultivations, although some highly abundant bacteria in root-associated soil differed between NT and *Cry1Ah1*-modified poplars.

Effects of *Cry1Ah1* expression on soil nitrogen and phosphorus in the microbial community

Metagenome sequencing data allowed us to identify close relationships between bacteria and nitrogen cycling based on changes in MBN content. At the genus level, four groups of nine genera of bacteria related to nitrogen cycling were isolated from rhizosphere samples. Among these, *Bradyrhizobium*, *Rhizobium*, and *Frankia* four were identified as nitrogen-fixing bacteria; *Nitrosospira* as involved in nitrification; *Pseudomonas*, *Ralstonia*, Burkholderiales, *Bacillus*, and *Streptomyces* as denitrifying bacteria; and *Bacillus* and *Pseudomonas* as contributing to ammoniation. The relative abundances of bacteria related to nitrogen cycling decreased in the order denitrifying bacteria > nitrogen-fixing bacteria > ammonifying bacteria > nitrifying bacteria. Among these, *Bacillus* was the dominant genus, accounting for more than 30% of the total nitrogen-metabolizing bacteria, whereas Burkholderiales and *Ralstonia* accounted for smaller proportions. Our metagenome sequencing results showed that *Cry1Ah1* expression resulted in no significant changes in nitrogen cycling bacterial community structure and composition, except for the relative abundance of *Bradyrhizobium* in line Z1-3, *Nitrosospira* in line A4-6, and *Pseudomonas* in line A3-4 (Supplementary Fig. 6). *Bacillus* can transform substances into nutrients

needed by plants by dissolving phosphorus, fixing nitrogen, and resolving potassium, promoting plant growth. *Bacillus* can also affect the reproduction of some available flora, strengthening soil fertility and improving plant growth. In this study, *Bacillus* was the dominant genus of bacteria in NT and *Cry1Ah1*-modified poplars. Although the difference was not significant between NT and *Cry1Ah1*-modified poplars, *Bacillus* abundance in *Cry1Ah1*-modified poplars showed a downward trend, perhaps due to minor changes in bacteria related to nitrogen cycling, which can affect soil nitrogen metabolism and eventually lead to a decrease in rhizosphere MBN content.

Phosphorus is a nutrient element that forms an important component of nucleotides, phospholipids, and ATP; it participates in the physiological and biochemical processes of energy metabolism, carbohydrate metabolism, material transformation, and transportation in plants, thereby playing an essential role in growth and development [77, 78]. The phosphorus cycle is a typical depositional cycle in which phosphorus accumulates among soil, plants, and microorganisms [79]. Phosphorus exists in the soil in both insoluble or insoluble forms, and plants can utilize only 20% of them. Due to its low availability and utilization rate, phosphorus is a key element limiting plant growth [80]. Inorganic phosphorus forms can be absorbed and utilized by plants following transformation from organic phosphorus by microorganisms. This process depends on the population structure and quantity of phosphate-solubilizing microorganisms [81, 82]. In the present study, four groups of eight genera of bacteria connected with phosphorus cycling were isolated from the rhizosphere: *Bacillus*, *Pseudomonas*, *Pseudarthrobacter*, *Flavobacterium*, *Paenibacillus*, *Bradyrhizobium*, *Serratia*, and *Streptomyces*. All were found to be involved in organophosphorus metabolism. *Bacillus*, *Pseudarthrobacter*, and *Streptomyces* were dominant, with *Bacillus* having exceptionally high abundance. These results showed that *Cry1Ah1* expression had no significant impact on the quantity of phosphate-solubilizing microorganisms, except for the relative lot of *Pseudarthrobacter* in lines A5-25, A4-6, and A5-0 (Supplementary Fig. 6). However, soil available phosphorus and MBP content were significantly lower in *Cry1Ah1*-modified poplars than in NT poplars, possibly because they affected the activity of phosphate-solubilizing microorganisms, reducing the activity of phosphate solubilization and affecting the transformation of soil phosphorus.

Conclusion

In this study, we evaluated the ecological risk and potential impacts of herbicide-resistant poplar cultivation by comparing the effects of transgenic and NT poplar varieties on rhizosphere microbial communities. We detected no significant effect of *Cry1Ah1*-modified poplar cultivation on microbial diversity and community structure compared with that of NT poplars; however, the relative abundance of some rhizosphere microorganism genera differed among specific *Cry1Ah1*-modified poplar varieties to those of NT poplars. Our results suggest that *Cry1Ah1* expression affects MBC, MBN, and MBP contents, which are related to plant growth and development in the rhizosphere. In conclusion, we found no significant adverse impacts of *Cry1Ah1*-modified poplars on soil processes or soil organic matter stability.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The raw RNA sequencing data were deposited in the NCBI Sequence Read Archive (SRA) with the accession number PRJNA663272.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

HW and A.M. designed analyses and wrote the manuscript. ZP, CX, WS, and DL reviewed and confirmed the calculations. A.M. and QZ. supervised this project. QZ. funded this project.

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References

1. Boerjan W. Biotechnology and the domestication of forest trees. *Curr Opin Biotechnol.* 2005;16:159–66. doi:10.1016/j.copbio.2005.03.003.
2. Xu C, Wei H, Wang L, Yin T, Zhuge Q. Optimization of the cry1Ah1 Sequence Enhances the Hyper-Resistance of Transgenic Poplars to *Hyphantria cunea*. *Front Plant Sci.* 2019;10:335. doi:10.3389/fpls.2019.00335.
3. Genissel, A., Leple, J., Millet, N., et al. High tolerance against *Chrysomela tremulae* of transgenic poplar plants expressing a synthetic cry3Aa gene from *Bacillus thuringiensis* ssp *tenebrionis*. *Molecular Breeding.* 2003;11(2):103–10.

4. Yang Y, Tang R-J, Jiang C-M, Li B, Kang T, Liu H, et al. Overexpression of the PtSOS2 gene improves tolerance to salt stress in transgenic poplar plants. *Plant Biotechnol J*. 2015;13:962–73. doi:10.1111/pbi.12335.
5. Beegle CC, Yamamoto T. INVITATION PAPER (CP ALEXANDER FUND): HISTORY OF BACILLUS THURINGIENSIS BERLINER RESEARCH AND DEVELOPMENT. *Can Entomol*. 1992;124:587–616. doi:10.4039/Ent124587-4.
6. Dohrmann AB, Küting M, Jünemann S, Jaenicke S, Schlüter A, Tebbe CC. Importance of rare taxa for bacterial diversity in the rhizosphere of Bt- and conventional maize varieties. *ISME J*. 2013;7:37–49. doi:10.1038/ismej.2012.77.
7. Crecchio C, Stotzky G. Biodegradation and insecticidal activity of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound on complexes of montmorillonite–humic acids–Al hydroxypolymers. *Soil Biology and Biochemistry*. 2001;33:573–81. doi:10.1016/S0038-0717(00)00199-1.
8. Zhaolei L, Naishun B, Xueping C, Jun C, Manqiu X, Zhiping S, et al. Soil incubation studies with Cry1Ac protein indicate no adverse effect of Bt crops on soil microbial communities. *Ecotoxicol Environ Saf*. 2018;152:33–41. doi:10.1016/j.ecoenv.2017.12.054.
9. Cotta SR, Dias ACF, Marriel IE, Andreote FD, Seldin L, van Elsas JD. Different effects of transgenic maize and non-transgenic maize on nitrogen-transforming archaea and bacteria in tropical soils. *Appl Environ Microbiol*. 2014;80:6437–45. doi:10.1128/AEM.01778-14.
10. Zhaolei L, Naishun B, Jun C, Xueping C, Manqiu X, Feng W, et al. Effects of long-term cultivation of transgenic Bt rice (Kefeng-6) on soil microbial functioning and C cycling. *Sci Rep*. 2017;7:4647. doi:10.1038/s41598-017-04997-8.
11. Liu Y, Li J, Neal Stewart C, Luo Z, Xiao N. The effects of the presence of Bt-transgenic oilseed rape in wild mustard populations on the rhizosphere nematode and microbial communities. *Sci Total Environ*. 2015;530-531:263–70. doi:10.1016/j.scitotenv.2015.05.073.
12. Li, P., Li, Y., Shi, J., et al. Impact of transgenic Cry1Ac+pTI cotton on diversity and dynamics of rhizosphere bacterial community of different root environments. *Science of the Total Environment*. 2018;637:233–43.
13. Čerevková A, Miklisová D, Szoboszlay M, Tebbe CC, Cagán L. The responses of soil nematode communities to Bt maize cultivation at four field sites across Europe. *Soil Biology and Biochemistry*. 2018;119:194–202. doi:10.1016/j.soilbio.2018.01.023.
14. Shu Y, Du Y, Wang J. Presence of Cry1Ab in the Bt maize – aphid (*Rhopalosiphum maidis*) – ladybeetle (*Propylea japonica*) system has no adverse effects on insect biological parameters. *Entomol Exp Appl*. 2019;167:553–60. doi:10.1111/eea.12801.
15. Li P, Ye S, Liu H, Pan A, Ming F, Tang X. Cultivation of Drought-Tolerant and Insect-Resistant Rice Affects Soil Bacterial, but Not Fungal, Abundances and Community Structures. *Front Microbiol*. 2018;9:1390. doi:10.3389/fmicb.2018.01390.

16. Liu B, Zeng Q, Yan F, Xu H, Xu C. Effects of transgenic plants on soil microorganisms. *Plant Soil*. 2005;271:1–13. doi:10.1007/s11104-004-1610-8.
17. Singh AK, Rai GK, Singh M, Dubey SK. Bacterial Community Structure in the Rhizosphere of a Cry1Ac Bt-Brinjal Crop and Comparison to Its Non-transgenic Counterpart in the Tropical Soil. *Microb Ecol*. 2013;66:927–39. doi:10.1007/s00248-013-0287-z.
18. Velmourougane K, Sahu A. Impact of transgenic cottons expressing cry1Ac on soil biological attributes. *Plant Soil Environ*. 2013;59:108–14. doi:10.17221/616/2012-PSE.
19. Hu, H., Xie, M., Yu, Y., et al. Transgenic Bt cotton tissues have no apparent impact on soil microorganisms. *Plant Soil and Environment*. 2018;59(8):366–71.
20. Wei, M., Tan, F., Zhu, H., et al. Impact of Bt-transgenic rice (SHK601) on soil ecosystems in the rhizosphere during crop development. *Plant Soil and Environment*. 2018;58:217–23.
21. Miethling-Graff R, Dockhorn S, Tebbe CC. Release of the recombinant Cry3Bb1 protein of Bt maize MON88017 into field soil and detection of effects on the diversity of rhizosphere bacteria. *European Journal of Soil Biology*. 2010;46:41–8. doi:10.1016/j.ejsobi.2009.10.003.
22. Mina U. AN APPROACH FOR IMPACT ASSESSMENT OF TRANSGENIC PLANTS ON SOIL ECOSYSTEM. *Appl Ecol Env Res*. 2008;6:1–19. doi:10.15666/aeer/0603_001019.
23. Ali B, Sabri AN, Ljung K, Hasnain S. Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticum aestivum* L. *Lett Appl Microbiol*. 2009;48:542–7. doi:10.1111/j.1472-765X.2009.02565.x.
24. Innerebner G, Knief C, Vorholt JA. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Appl Environ Microbiol*. 2011;77:3202–10. doi:10.1128/AEM.00133-11.
25. Inceoğlu O, Salles JF, van Overbeek L, van Elsas JD. Effects of plant genotype and growth stage on the betaproteobacterial communities associated with different potato cultivars in two fields. *Appl Environ Microbiol*. 2010;76:3675–84. doi:10.1128/AEM.00040-10.
26. Debruyne, J. M., Bevard, D. A., Essington, M. E., et al. Field-grown transgenic switchgrass (*Panicum virgatum* L.) with altered lignin does not affect soil chemistry, microbiology, and carbon storage potential. *Global Change Biology Bioenergy*. 2017;9:1100–9.
27. Liu M, Sui X, Hu Y, Feng F. Microbial community structure and the relationship with soil carbon and nitrogen in an original Korean pine forest of Changbai Mountain, China. *BMC Microbiol*. 2019;19:218. doi:10.1186/s12866-019-1584-6.
28. Franzluebbers AJ, Haney RL, Hons FM, Zuberer DA. Determination of Microbial Biomass and Nitrogen Mineralization following Rewetting of Dried Soil. *Soil Science Society of America Journal*. 1996;60:1133–9. doi:10.2136/sssaj1996.03615995006000040025x.
29. Witt, C., Gaunt, J. L., Galicia, C. C., Ottow, J. C., & Neue, H. U. A rapid chloroform-fumigation extraction method for measuring soil microbial biomass carbon and nitrogen in flooded rice soils. *Biology and Fertility of Soils*. 2000;30(5):510–9.

30. Cui Y, Wang X, Zhang X, Ju W, Duan C, Guo X, et al. Soil moisture mediates microbial carbon and phosphorus metabolism during vegetation succession in a semiarid region. *Soil Biology and Biochemistry*. 2020;147:107814. doi:10.1016/j.soilbio.2020.107814.
31. Dennis KL, Wang Y, Blatner NR, Wang S, Saadalla A, Trudeau E, et al. Adenomatous polyps are driven by microbe-instigated focal inflammation and are controlled by IL-10-producing T cells. *Cancer Res*. 2013;73:5905–13. doi:10.1158/0008-5472.CAN-13-1511.
32. Xu, N., Tan, G., Wang, H., et al. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *European Journal of Soil Biology*. 2016;74:1–8.
33. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol*. 2005;71:8228–35. doi:10.1128/AEM.71.12.8228-8235.2005.
34. Hong C, Si Y, Xing Y, Li Y. Illumina MiSeq sequencing investigation on the contrasting soil bacterial community structures in different iron mining areas. *Environ Sci Pollut Res Int*. 2015;22:10788–99. doi:10.1007/s11356-015-4186-3.
35. Weinhold A, Karimi Dorcheh E, Li R, Rameshkumar N, Baldwin IT. Antimicrobial peptide expression in a wild tobacco plant reveals the limits of host-microbe-manipulations in the field. *Elife* 2018. doi:10.7554/eLife.28715.
36. Huber JA, Mark Welch DB, Morrison HG, Huse SM, Neal PR, Butterfield DA, Sogin ML. Microbial population structures in the deep marine biosphere. *Science*. 2007;318:97–100. doi:10.1126/science.1146689.
37. Hua Z-S, Han Y-J, Chen L-X, Liu J, Hu M, Li S-J, et al. Ecological roles of dominant and rare prokaryotes in acid mine drainage revealed by metagenomics and metatranscriptomics. *ISME J*. 2015;9:1280–94. doi:10.1038/ismej.2014.212.
38. Clergue, B., Amiaud, B., Pervanchon, F., Lasserrejoulin, F., & Plantureux, S. Biodiversity: Function and Assessment in Agricultural Areas: A Review. *Agronomy for Sustainable Development*. 2005;25(1):1–15.
39. Zhao, J., Zhang, W., Wang, K. L., Song, T., & Du, H. Responses of the soil nematode community to management of hybrid napiergrass: The trade-off between positive and negative effects. *Applied Soil Ecology*. 2014;75:134–44.
40. Ito T, Araki M, Higashi T, Komatsuzaki M, Kaneko N, Ohta H. Responses of soil nematode community structure to soil carbon changes due to different tillage and cover crop management practices over a nine-year period in Kanto, Japan. *Applied Soil Ecology*. 2015;89:50–8. doi:10.1016/j.apsoil.2014.12.010.
41. Mahnert A, Moissl-Eichinger C, Berg G. Microbiome interplay: plants alter microbial abundance and diversity within the built environment. *Front Microbiol*. 2015;6:887. doi:10.3389/fmicb.2015.00887.
42. Garbeva P, van Elsas JD, van Veen JA. Rhizosphere microbial community and its response to plant species and soil history. *Plant Soil*. 2008;302:19–32. doi:10.1007/s11104-007-9432-0.
43. Pickett JA. The essential need for GM crops. *Nat Plants*. 2016;2:16078. doi:10.1038/nplants.2016.78.

44. Powlson DS, Prookes PC, Christensen BT. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biology and Biochemistry*. 1987;19:159–64. doi:10.1016/0038-0717(87)90076-9.
45. He ZL, Yang XE, Baligar VC, Calvert DV. Microbiological and Biochemical Indexing Systems for Assessing Quality of Acid Soils. *Advances in Agronomy*. 2003;78:89–138. doi:10.1016/S0065-2113(02)78003-6.
46. Singh JS, Gupta VK. Soil microbial biomass: A key soil driver in management of ecosystem functioning. *Sci Total Environ*. 2018;634:497–500. doi:10.1016/j.scitotenv.2018.03.373.
47. Li P, Yang Y, Han W, Fang J. Global patterns of soil microbial nitrogen and phosphorus stoichiometry in forest ecosystems. *Global Ecology and Biogeography*. 2014;23:979–87. doi:10.1111/geb.12190.
48. Chen H, Zhao X, Chen X, Lin Q, Li G. Seasonal changes of soil microbial C, N, P and associated nutrient dynamics in a semiarid grassland of north China. *Applied Soil Ecology*. 2018;128:89–97. doi:10.1016/j.apsoil.2018.04.008.
49. Xu, X., Thornton, P. E., & Post, W. M. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems: Concepts and applications. *Global Ecology and Biogeography*. 2013;22:737–49.
50. Chen G-C, He Z-L. Determination of soil microbial biomass phosphorus in acid red soils from southern China. *Biology and Fertility of Soils*. 2004;39:446–51. doi:10.1007/s00374-004-0734-6.
51. Sharma P, Rai SC, Sharma R, Sharma E. Effects of land-use change on soil microbial C, N and P in a Himalayan watershed. *Pedobiologia*. 2004;48:83–92. doi:10.1016/j.pedobi.2003.09.002.
52. Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED. A fern that hyperaccumulates arsenic. *Nature*. 2001;409:579. doi:10.1038/35054664.
53. Lasat MM. Phytoextraction of toxic metals: a review of biological mechanisms. *J Environ Qual*. 2002;31:109–20.
54. Lovell RD, Jarvis SC, Bardgett RD. Soil microbial biomass and activity in long-term grassland: Effects of management changes. *Soil Biology and Biochemistry*. 1995;27:969–75. doi:10.1016/0038-0717(94)00241-R.
55. Wardle DA. Controls of temporal variability of the soil microbial biomass: A global-scale synthesis. *Soil Biology and Biochemistry*. 1998;30:1627–37. doi:10.1016/S0038-0717(97)00201-0.
56. Wu, H., Zeng, G., Liang, J., Zhang, J., Cai, Q., Huang, L., ... & Shen, S. Changes of soil microbial biomass and bacterial community structure in Dongting Lake: Impacts of 50,000 dams of Yangtze River. *Ecological Engineering*. 2013;54(2):72–8.
57. Liu L, Wu L, Knauth S, Eickhorst T. Degradation of transgenic Bt-rice straw incorporated with two different paddy soils. *J Environ Manage*. 2019;244:415–21. doi:10.1016/j.jenvman.2019.05.075.
58. Lopes LD, Fernandes MF. Changes in microbial community structure and physiological profile in a kaolinitic tropical soil under different conservation agricultural practices. *Applied Soil Ecology*. 2020;152:103545. doi:10.1016/j.apsoil.2020.103545.

59. Yao, He, Wilson, Campbell. Microbial Biomass and Community Structure in a Sequence of Soils with Increasing Fertility and Changing Land Use. *Microb Ecol.* 2000;40:223–37. doi:10.1007/s002480000053.
60. Heuck C, Weig A, Spohn M. Soil microbial biomass C:N:P stoichiometry and microbial use of organic phosphorus. *Soil Biology and Biochemistry.* 2015;85:119–29. doi:10.1016/j.soilbio.2015.02.029.
61. Zheng, S., Hu, J., Chen, K., Yao, J., Yu, Z., & Lin, X. Soil microbial activity measured by microcalorimetry in response to long-term fertilization regimes and available phosphorous on heat evolution. *Soil Biology & Biochemistry.* 2009;41(10):2094–9.
62. Liu L, Zhang T, Gilliam FS, Gundersen P, Zhang W, Chen H, Mo J. Interactive effects of nitrogen and phosphorus on soil microbial communities in a tropical forest. *PLoS ONE.* 2013;8:e61188. doi:10.1371/journal.pone.0061188.
63. Goyal S, Chander K, Mundra MC, Kapoor KK. Influence of inorganic fertilizers and organic amendments on soil organic matter and soil microbial properties under tropical conditions. *Biology and Fertility of Soils.* 1999;29:196–200. doi:10.1007/s003740050544.
64. Peacock AD, Ringelberg DB. Soil microbial community responses to dairy manure or ammonium nitrate applications. Oxford, UK: Pergamon Press; 2001.
65. Böhme L, Langer U, Böhme F. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. *Agriculture, Ecosystems & Environment.* 2005;109:141–52. doi:10.1016/j.agee.2005.01.017.
66. Malhotra H, Vandana, Sharma S, Pandey R. Phosphorus Nutrition: Plant Growth in Response to Deficiency and Excess. In: Hasanuzzaman M, Fujita M, Oku H, Nahar K, Hawrylak-Nowak B, editors. *Plant Nutrients and Abiotic Stress Tolerance.* Singapore: Springer Singapore; 2018. p. 171–190. doi:10.1007/978-981-10-9044-8_7.
67. Angle JS. Release of transgenic plants: biodiversity and population-level considerations. *Molecular Ecology.* 2008;3(1):45–50.
68. Dunfield KE, Germida JJ. Impact of genetically modified crops on soil- and plant-associated microbial communities. *J Environ Qual.* 2004;33:806–15. doi:10.2134/jeq2004.0806.
69. Dohrmann AB, Tebbe CC. Effect of elevated tropospheric ozone on the structure of bacterial communities inhabiting the rhizosphere of herbaceous plants native to Germany. *Appl Environ Microbiol.* 2005;71:7750–8. doi:10.1128/AEM.71.12.7750-7758.2005.
70. Buee, M., De Boer, W. F., Martin, F., Van Overbeek, L. S., & Jurkevitch, E. The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. *Plant and Soil.* 2009;321(1):189–212.
71. Watt M, Hugenholtz P, White R, Vinall K. Numbers and locations of native bacteria on field-grown wheat roots quantified by fluorescence in situ hybridization (FISH). *Environ Microbiol.* 2006;8:871–84. doi:10.1111/j.1462-2920.2005.00973.x.
72. Yang CH, Crowley DE. Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. *Appl Environ Microbiol.* 2000;66:345–51. doi:10.1128/aem.66.1.345–

351.2000.

73. Oliveira AP, Pampulha ME, Bennett JP. A two-year field study with transgenic *Bacillus thuringiensis* maize: effects on soil microorganisms. *Sci Total Environ.* 2008;405:351–7. doi:10.1016/j.scitotenv.2008.05.046.
74. Zhang, Y., Xie, M., Wu, G., Peng, D., & Yu, W. A 3-year field investigation of impacts of Monsanto's transgenic Bt-cotton NC 33B on rhizosphere microbial communities in northern China. *Applied Soil Ecology.* 2015;89:18–24.
75. Zhou, D., Xu, L., Gao, S., Guo, J., Luo, J., You, Q., & Que, Y. Cry1Ac transgenic sugarcane does not affect the diversity of microbial communities and has no significant effect on enzyme activities in rhizosphere soil within one crop season. *Front Plant Sci.* 2016;7:265.
76. Wu WX, Liu W, Lu HH, Chen YX, Medha D, Janice T. Use of ¹³C labeling to assess carbon partitioning in transgenic and nontransgenic (parental) rice and their rhizosphere soil microbial communities. *FEMS Microbiol Ecol.* 2009;67:93–102. doi:10.1111/j.1574-6941.2008.00599.x.
77. Sun Y, Cui X, Liu F. Effect of irrigation regimes and phosphorus rates on water and phosphorus use efficiencies in potato. *Scientia Horticulturae.* 2015;190:64–9. doi:10.1016/j.scienta.2015.04.017.
78. Hasanuzzaman M, Fujita M, Oku H, Nahar K, Hawrylak-Nowak B, editors. *Plant Nutrients and Abiotic Stress Tolerance.* Singapore: Springer Singapore; 2018.
79. Stutter MI, Shand CA, George TS, Blackwell MSA, Dixon L, Bol R, et al. Land use and soil factors affecting accumulation of phosphorus species in temperate soils. *Geoderma.* 2015;257-258:29–39. doi:10.1016/j.geoderma.2015.03.020.
80. Schachtman, Reid, Ayling. Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiol.* 1998;116:447–53. doi:10.1104/pp.116.2.447.
81. Oliveira CA. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome; 2009.
82. Krey T, Vassilev N, Baum C, Eichler-Löbermann B. Effects of long-term phosphorus application and plant-growth promoting rhizobacteria on maize phosphorus nutrition under field conditions. *European Journal of Soil Biology.* 2013;55:124–30. doi:10.1016/j.ejsobi.2012.12.007.

Figures

Figure 2

Evaluation of rhizosphere soil physical and chemical properties in NT and Cry1Ah1-modified poplar varieties. Analysis of rhizosphere soil pH (A), soil alkaline nitrogen (B), soil available phosphorus (C), microbial biomass carbon (D), microbial biomass nitrogen (E), and microbial biomass phosphorus (F) in NT and Cry1Ah1-modified poplar varieties. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc comparison. * $P < 0.05$.

Figure 3

Overall composition of rhizosphere bacterial communities in field-grown NT and Cry1Ah1-modified poplar varieties. (A) Beta diversity analysis of rhizosphere soil bacteria community in terms of the unweighted UniFrac distance. (B) Principal coordinates analysis (PCoA) based on the unweighted UniFrac distance between rhizosphere soil of NT and Cry1Ah1-modified varieties in each location using 16S data. (C) Phylum-level taxonomic analysis of microbial distribution in rhizosphere samples of the NT and Cry1Ah1-modified types based on 16S amplicon and metagenomic data.

Figure 4

Relative abundances of bacteria at the family level between NT and Cry1Ah1-modified varieties. (A) Rare operational taxonomic unit (OTU) data were used to visualize the distribution of all 19 families in a heatmap of relative abundances. (B) Significance tests of high and low relative abundance of bacteria between NT and Cry1Ah1-modified varieties. Data were analyzed using one-way ANOVA and Tukey’s post hoc comparison. *P < 0.05.

Figure 5

Overall composition of rhizosphere fungal communities in field-grown NT and Cry1Ah1-modified varieties. (A) Alpha diversity comparison of rhizosphere soil from the NT and Cry1Ah1-modified varieties based on the Shannon index using internal transcribed spacer 1 (ITS1) data. (B) PCoA based on the

unweighted UniFrac distance between rhizosphere soil from the NT and Cry1Ah1-modified varieties for each location using ITS1 data. (C) Relative sequence abundance of fungal phyla in rhizosphere soil from the NT and Cry1Ah1-modified varieties.

Figure 6

Characterization of poplar rhizosphere microbiome function. Kyoto Encyclopedia of Genes and Genomes level 2 pathway functional comparison of rhizosphere soil bacteria from the NT and Cry1Ah1-modified varieties.

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