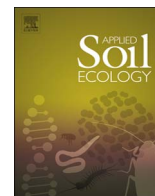




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# Bacterial community structure and functional potential of rhizosphere soils as influenced by nitrogen addition and bacterial wilt disease under continuous sesame cropping

Ruiqing Wang<sup>a,b</sup>, Yunping Xiao<sup>a</sup>, Fengjuan Lv<sup>a</sup>, Liyong Hu<sup>b,\*</sup>, Lingen Wei<sup>a,\*</sup>, Zhanqi Yuan<sup>a</sup>, Hongxin Lin<sup>a</sup>

<sup>a</sup> Soil Fertilizer and Resource Environment Institute, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, PR China

<sup>b</sup> College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

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## ABSTRACT

Effects of nitrogen addition and bacterial wilt disease on bacterial diversity, community structure and functional potential in rhizosphere soil under continuous sesame cropping were investigated by a field experiment and Illumina sequencing using two cultivars (Jinhuangma and Zhongzhi No.35) under two nitrogen levels (0-N<sub>0</sub> and 105-N<sub>1</sub> kg N ha<sup>-1</sup>) with rhizosphere soil (RS) samples collected from healthy (not infected with bacterial wilt disease) and diseased (infected with bacterial wilt disease) plants. The bacterial diversity was not affected by nitrogen addition and bacterial wilt disease ( $p > .05$ ). The relative abundance of *Ralstonia solanacearum* was 0.15 and 0.11 percentage points higher in the Diseased than in the Healthy RS samples for the two cultivars ( $p < .05$ ). N<sub>1</sub> had a decrease of 0.708 percentage points in the relative abundance of genus *Roseiflexus* as compared to N<sub>0</sub> in Jinhuangma ( $p = .031$ ). Additionally, compared to N<sub>1</sub>, N<sub>0</sub> of Zhongzhi No.35 had an increase of 0.593–2.064 percentage points in the relative abundance of five genera, such as *uncultured-f-Acidobacteriaceae\_Subgroup\_1*, *Candidatus\_Solibacter*, *Bryobacter*, *Rhizomicrobium* and *Subgroup\_2\_norank* ( $p < .05$ ). Furthermore, the Diseased RS samples of Jinhuangma were 0.655 and 0.569 percentage points higher than the Healthy RS samples in the relative abundance of *Phenylobacterium* ( $p = .031$ ) and *Pseudonocardia* ( $p = .013$ ). The diseased samples of Zhongzhi No.35 were 1.007–1.867 percentage points higher than healthy samples in the relative abundance of *Burkholderia* ( $p = .045$ ), *Massilia* ( $p = .031$ ), *Phenylobacterium* ( $p = .021$ ) and *Novosphingobium* ( $p = .005$ ), but it was just the opposite for the other six genera ( $p < .05$ ). The function potential of bacterial community varied significantly between Diseased and Healthy RS samples ( $p < .05$ ). Overall, nitrogen and bacterial wilt disease had a significant impact on the structure of bacterial communities in rhizosphere soil, and signal transduction and translation could play an important role in preserving plant health.

## 1. Introduction

Sesame is one of the five main oil-crops in China, with a planting area of  $4.291 \times 10^5$  ha, an average yield of  $1468 \text{ kg ha}^{-1}$ , and a total output of  $6.299 \times 10^5$  tons in 2014 (FAOSTAT, 2014). The cultivation zones of sesame are mainly distributed in Henan, Anhui, Hubei and Jiangxi provinces of P. R. China (Wang et al., 2016). Sesame is an oil-crop of serious continuous cropping obstacles. One of the soil-borne diseases for sesame is bacterial wilt, which is mainly developed in south China as the most serious sesame disease in the red-soil upland. Meanwhile, the farmers have formed the habit of planting sesame in continuous cropping soil, which resulted in a bacterial wilt incidence of 10%–20% in most of the plots, and even up to 50%–70% or higher in

some seriously-affected plots, thereby causing great economic losses for Jiangxi sesame industry, and restricting the sustainable development of this characteristic industry. *Ralstonia solanacearum* was reported to induce the sesame bacterial wilt as characterized through the combination of morphology and molecular biology methods by Hua et al. (2012a).

Microorganism, despite its low content in the soil, occupied a dominant position for the sustainable productivity of crops, such as soil organic matter decomposition, nutrient circulation and utilization, system stability and anti-interference ability, with its quantity and activity directly affecting the soil fertility (Cardinale et al., 2006; Dorr de Quadros et al., 2012; Enwall et al., 2007; Janvier et al., 2007). Meanwhile, it could also significantly increase the diversity of soil microbial

\* Corresponding authors.

E-mail addresses: [liyonghu@mail.hzau.edu.cn](mailto:liyonghu@mail.hzau.edu.cn) (L. Hu), [lgw0021@163.com](mailto:lgw0021@163.com) (L. Wei).

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community, and played a significant role in the control of crop soil-borne disease (Miransari, 2013). Moreover, the soil microbial community structure could reflect the quality of the soil ecological environment, and the composition and species of soil microbes were simpler in the rhizosphere compared to the non-rhizosphere, primarily because of the selectivity of roots (Costa et al., 2006). The rhizosphere soil, a dynamic and complex element in the field ecological system (Yang et al., 2016), played a more important role in regulating soil fertility and protecting the healthy growth of plants through changing the composition of rhizosphere soil microbial communities, and the soil fertility directly affected the productivity of crops. Several previous studies have reported that nitrogen forms and application rate also had significant effect on the composition and diversity of soil bacterial communities (Campbell et al., 2010; Enwall et al., 2007). For instance, the use of chemical fertilizers decreased the soil microbial growth vigor (Wang et al., 2008); the diversity and richness of soil bacterial communities decreased with the increase of nitrogen fertilizer rate (Li et al., 2014); and nitrogen forms could better regulate the soil microbial composition (Giagnoni et al., 2016). However, Fierer et al. (2012) have reported that nitrogen application rate had significant effect on bacterial composition, but not on bacterial diversity.

To date, there have been several studies examining the variations of rhizosphere soil microbes from healthy and diseased plants under a continuous cropping system, mainly including crops like black spruce, cotton, tomato, notoginseng and lily (Filion et al., 2004; Li et al., 2014; Luan et al., 2015; Shang et al., 2016; Wu et al., 2016; Zhang et al., 2010). For instance, rhizosphere soil samples from the healthy cotton had the highest richness at the flowering and bolling stage, whereas the highest evenness was found in the rhizosphere soil of diseased cotton at boll opening. Meanwhile, microbes in healthy rhizosphere soils were more diverse and occupied a wider proportion in the rhizosphere soil of the healthy cotton field at the flowering stage (Zhang et al., 2010; Luan et al., 2015). Compared to those of healthy *P. notoginseng*, microbial communities in the rhizosphere soils of diseased plants showed a decrease in alpha diversity, but an increase in bacterial community dissimilarity (Wu et al., 2015). Furthermore, there were significant shifts in the microbial composition of diseased samples compared with healthy samples of greenhouse tomatoes, which had the highest bacterial diversity (Li et al., 2014).

High-throughput sequencing technologies could distinguish between the differentiation of microbial species and diversity of samples, relative abundance and evolutionary relationships under their natural environments, with no need for isolation and lab cultivation of individual species (DeLong, 2004). This technology has made possible the study of uncultivable microorganisms by genome sequencing. Meanwhile, it can provide novel and systematic information about the composition, structure, and function of microbial communities from various environments (Warnecke et al., 2007). Currently, this technology has been widely used to study rhizosphere soil microbial diversity and structure in notoginseng, potato, peanut, lettuce and soybean crops (Chen et al., 2014; Dong et al., 2016; Li et al., 2015; Li et al., 2016; Liu et al., 2014).

Hua et al. (2012b) have used the dilution plate method to study the rhizospheric microbial communities under a continuous sesame cropping system and reported that the abundance of *Bacteria*, *Actinomycetes*, and *Bacillus* decreased with the increase of continuous cropping years, while *Fungi*, *Fusarium oxysporum*, and *Ralstonia solanacearum* increased. To our best knowledge, there are no other reports available about the characterization of the bacterial diversity, composition and functional potential of rhizosphere soil under continuous healthy and diseased sesame cropping systems and different nitrogen rate conditions, especially by high-throughput sequencing technology. Sesame bacterial wilt often occurs under continuous cropping conditions, and is even more serious in red-soil hilly regions with a subtropical climate. High temperature and high humidity during sesame growth stage could easily induce bacterial wilt infection, which could spread from a small area to

other regions rapidly, and drug prevention and control almost had hardly any effect on sesame bacterial wilt. Meanwhile, our study team found no significant difference in the yield under a different nitrogen rate in three continuous cropping years. The objective of this study was to explore the change rules of rhizosphere soil bacterial community composition, significantly different bacterial species, and functional potential of healthy and diseased sesame plants as well as different nitrogen rate under continuous cropping conditions. Results from this study will provide theoretical and technical guidance for ecological solutions to continuous sesame cropping obstacles, and promote the sustainable development of the sesame industry.

## 2. Materials and methods

### 2.1. Description of the experiment

This experiment was conducted in Jinxian county, Jiangxi Province of P. R. China (28°37'N, 116°27'E), a main black sesame production region. This region had the humid subtropical monsoon climate and red soils, and the annual rainfall was 1046–2356 mm. The basic properties of the soil were: pH 5.10, available nitrogen 107.25 mg kg<sup>-1</sup>, available phosphorus 15.70 mg kg<sup>-1</sup>, available potassium 66.3 mg kg<sup>-1</sup>, and soluble organic matter 10.12 g kg<sup>-1</sup>.

The field experiment was conducted in a two-factor randomized block design with three replicates, and the area of each replicated plot was 12 m<sup>2</sup> (2.0 m × 6.0 m) under the same field management measures. This experiment consisted of eight treatments: 105 and 0 kg ha<sup>-1</sup> of nitrogen application rate (N<sub>1</sub> and N<sub>0</sub>) and the rhizosphere soil (RS) samples collected from plants not infected with bacterial wilt disease (Healthy RS) and plants infected with bacterial wilt disease (Diseased RS) per treatment during maturity stage. Two sesame cultivars were used as planting materials in this study: Jinhuangma (P<sub>1</sub>, the local variety) and Zhongzhi No.35 (P<sub>2</sub>, the exotic cultivar), a conventional and a hybrid sesame cultivar, which were provided by the Crop Institute of Jiangxi Academy of Agricultural Sciences and Oil Crops Research Institute, Chinese Academy of Agricultural Sciences. Twelve plots were used for the experiment under field conditions. Fertilizers used were urea for N (46.4%), single superphosphate for P<sub>2</sub>O<sub>5</sub> (12.0%), and potassium chloride for K<sub>2</sub>O (60.0%). The doses were 60 P<sub>2</sub>O<sub>5</sub> and 105 K<sub>2</sub>O (kg ha<sup>-1</sup>); 70% N, 100% P<sub>2</sub>O<sub>5</sub> and 70% K<sub>2</sub>O were applied as a basal dose before sowing; 30% N and 30% K<sub>2</sub>O were applied as a top dressing dose during the bud stage.

Sesame was cultivated in strict accordance with the standard operating procedures established by Wei et al. (2013). Sesame had been planted in the plots for three continuous cropping years including the year of the experiment. Sesame seeds were directly sown for each plot by hand on June 18th, 2016. Seedlings were thinned to 2.25 × 10<sup>5</sup> plants per hectare by hand at the 3rd double leaf stage. Under the low nitrogen condition, the bud, early flowering and maturity stages of P<sub>1</sub> started on July 17th, July 23th and Sept. 8th, and those of P<sub>2</sub> started on July 18th, July 24th and Sept. 10th, 2016, while under the high nitrogen condition, the early flowering and maturity stages of both P<sub>1</sub> and P<sub>2</sub> were delayed by 1–2 days.

### 2.2. Soil collection, yield, yield component, and incidence of sesame bacterial wilt disease

Rhizosphere soil samples were collected as reported by Hua et al. (2012b). Briefly, sesame plant was collected from plots during the maturity stage, soil was shaken off, and rhizosphere fractions were brushed for further processing. Five healthy plants and five diseased plants were selected randomly from each plot, and five RS samples were combined as a single sample. Soil samples were obtained from three replicates per treatment. A total of 24 soil samples were homogenized by passing through a 2 mm sieve and then stored at -80 °C until further processing. Meanwhile, the data were collected in terms of sesame

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