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Watermelon planting is capable to restructure the soil microbiome that regulated by reductive soil disinfestation

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ABSTRACT

Reductive soil disinfestation (RSD) is an effective method to suppress many types of soil-borne diseases in various crops. However, the changes of soil-borne pathogens and microbial communities after crop cultivation in the RSD-treated soils are poorly understood. In the present study, pot experiment was conducted to evaluate the impacts of RSD treatment, with the incorporation of sugar fermentation liquor, on soil *Fusarium oxysporum* populations, microbial activities, and microbial communities at both after RSD treatment and watermelon planting. The results showed that RSD treatment could considerably inhibit the *F. oxysporum* and Fusarium wilt disease, promote the growth of watermelon, and enhance microbial activity. However, the pathogen of *F. oxysporum* resurged quickly after the watermelon cultivation. Although RSD treatment could significantly alter soil microbial communities, the watermelon cultivation also had the capacity to restructure the soil microbial communities to a similar status with control. Collectively, RSD treatment could considerably reduce soil conduciveness to Fusarium wilt and this conduciveness was quickly resurged after watermelon planting. Thus, the changes of *F. oxysporum* and soil microbial community after watermelon cultivation were likely due to the root exudates released by watermelon that had enough powerful driving force to select similar taxa by filtering the microbial seed bank.

1. Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai) widely planted and consumed around the world is a popular and important fruit. To meet the growing consumption demand worldwide, monocultures become the major cropping system for watermelon production recently. However, watermelon subjected to consecutive monoculture is susceptible to Fusarium wilt caused by the soil-borne fungus of *Fusarium oxysporum* f. sp. *niveum* that leads to the huge production and economic losses annually (Hopkins and Elmstrom, 1984; Ren et al., 2008). Once the soil has been infected by *F. oxysporum*, it is virtually impossible to remove this pathogen, with survival up to 10 years due to the strong resistant ability to environmental stress (Miguel et al., 2004)

To suppress this soil-borne disease, applying soil chemical fumigants, such as methyl bromide, has been considered as the most effective measure for a long time (Cebolla et al., 2000). However, the long-term use of chemical fumigants leads to the environmental and food quality problems. Particularly, since 2004, the use of methyl bromide has been banned under the Montreal protocol because of its ozone-depleting traits (Gamliel et al., 2000). Therefore, developing biological and environmentally friendly approaches have been urgent to prevent this disease in the last two decades. For example, Ren et al. (2008) demonstrated that intercropping with aerobic rice significantly alleviated Fusarium wilt of watermelon and altered the rhizosphere microbial communities through the production of rice root exudates. Application of bioorganic fertilizer containing the antagonistic microorganisms also effectively suppressed this disease and promoted watermelon production (Ling et al., 2010; Wu et al., 2009). Nonetheless, the suppressive efficiencies of these approaches were not consistent and stable under fluctuating environmental conditions, such as temperature, soil moisture, crop season, etc. (Wei et al., 2011).

Reductive soil disinfestation (RSD), alternatively named as

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anaerobic soil disinfestation (ASD) or biological soil disinfestation (BSD), firstly and independently developed in the Netherlands (Blok et al., 2000) and Japan (Shinmura, 2000) in 2000, has been proven to successfully control multiple soil-borne diseases in various crops by effectively suppressing their causal pathogens, such as *Ralstonia solanacearum*, *Fusarium oxysporum* f. spp. *cubense*, *spinaciae*, *lycopersici*, *Verticillium dahliae*, etc. (Huang et al., 2015a; Messiha et al., 2007; Mowlick et al., 2013a; Shinmura, 2004). Furthermore, RSD only requires a moderate temperature and short duration time with a relatively simple field operation procedure. Thus, RSD has been attracted considerable attention and widely applied as an alternative to chemical fumigations and biological control in Japan, USA, and the Netherlands (Goud et al., 2004; Momma et al., 2013; Shennan et al., 2014)

It is well known that the soil microbial community play vital roles in maintaining soil health and suppressing plant diseases (Qiu et al., 2012), whereas plants also have the capacity to drive and shape the microbiome via releasing a broad variety of chemical compounds (Haichar et al., 2008). In addition, increasing researches indicated that RSD could significantly alter soil microbial communities which were mainly dominated by microbiome conducive to non-pathogens, such as *Clostridia*, *Bacillus*, *pseudomonas*, etc (Huang et al., 2015b, 2016; Mowlick et al., 2013a). However, the responses of soil microbial communities regulated by RSD to plant cultivation are still unclear. Therefore, two hypotheses were proposed here: 1) the microbial communities in the RSD-regulated and control soils become much more different after plant growth because the communities differences are exacerbated under the influence of root exudates; 2) the microbial communities in the RSD-regulated and control soils tend to be similar after plant growth because certain root exudates are capable to select some similar specific soil microbiome. To test these hypotheses, pot experiment using homogenized soil with controlled temperature, moisture, and nutrient contents was conducted to evaluate the changes in soil microbial populations, microbial activities, and microbial communities at both after RSD and watermelon cultivation via Miseq sequencing approach coupled with quantitative PCR.

2. Materials and methods

2.1. Soil and organic material

The soil used in this experiment was collected from the Luhe Animal Science Base, Jiangsu Academy of Agriculture Sciences, Jiangsu Province, China (32°2′N, 118°8′E). The watermelon was continuously cultivated for several years in this soil and ultimately suffered from severe Fusarium wilt disease. The soil was a Tye-Fel-Stagnic Antrosols clay (IUSS Working Group WRB, 2015), with the following initial properties: pH 7.03; total organic carbon (TOC) 19.46 g kg^{-1}; total nitrogen (TN) 3.74 g kg^{-1}, and *F. oxysporum* 1.02×10^6 ITS copies·g^{-1} soil. The organic material sugar fermentation liquor (SF), a coproduct of ethanol fermentation using molasses, used in the RSD treatment was obtained from a sugar factory in Guangxi, China (22°48′, 108°22′). The TOC, TN, and easily oxidized organic carbon (EOC) contents of SF were 59.27 g kg^{-1}, 5.89 g kg^{-1}, and 58.20 g kg^{-1}, respectively.

2.2. Experimental design and watermelon cultivation

The *F. oxysporum* infected soil described above was treated by RSD before watermelon planting. Briefly, the experiment had a completely randomized design with three replications and each replicate contained 10 pots that had the following treatments, 1) CK, 4 kg soil was equipped into a pot (22 × 15 × 20 cm, Top × bottom diameter × high) without the organic matter amendment, irrigating and sealing; 2) RSD, 4 kg soil incorporated with 2% (v/w) SF was equipped into a pot, irrigated to soil saturation and sealed with plastic film. The soils in the pots were incubated for 20 days at 35 °C. During the incubation period, the soil water content in the CK treatment was maintained at 15%–18% to conform the field soil moisture condition. After the 20 days incubation, all of the soils were naturally drained, sieved (8 mm) and thoroughly mixed. The soil samples (about 30 g for each pot) were collected as three biological replicates per treatment by pooling 10 pots into one to minimize the community variation within each replicate. The collected soils were respectively stored at 4 °C and −80 °C for further analysis.

After sampling, the per kilogram soil was thoroughly incorporated with 1 g (w/w) inorganic compound fertilizer (N:P:K = 16:16:16) and then one watermelon seedling with three leaves was planted into each pot and cultivated for 90 days with the average day and night temperate at 30 °C and 15 °C, respectively. During this period, 50 mg N kg^{-1} soil of urea was applied to each pot every ten days. After 40 days growth, the plants disease index and shoot length were recorded every ten days and the plants were collected to investigate the root length and biomass at the end of cultivation according to Cao et al. (2016). The soil samples were also collected with the strategy described above for further analysis (hereby defined as CK-C and RSD-C, respectively). The plant disease index was determined by the following formula: disease index = Σ (individual disease score)/(3 × total number of plants) × 100%. Individual disease scores are as follows: 0 = healthy, 1 = less than 1/2 vascular bundle skin infected, 2 = more than 1/2 vascular bundle skin infected, 3 = leaves wilted.

2.3. Measurement of soil characteristics and total microbial activity

The TOCs in the soil and SF were measured by wet digestion with H_2SO_4–$K_2Cr_2O_7$ (Bremner and Jenkinson, 1960), and TNs were determined using semi-micro-Kjeldahl digestion (Bremner, 1960). The EOC in the SF was detected according to a previous study (Liu et al., 2016). Soil pH was measured using an S220K pH meter (Mettler-Toledo International Inc., Shanghai, China) with the ratio of 1:2.5 (soil/water, w/v). Total soil microbial activity was measured by fluorescein diacetate (FDA) hydrolysis according to Adam and Duncan (2001) and expressed as μg fluorescein released by per gram of dry weight soil per hour.

2.4. DNA extraction and real-time quantification assay

Soil DNA was extracted from 0.5 g soil using BioFast Soil Genomic Isolation kit (Bioer Technology Co., Ltd) according to the manufacturer's instructions. The extracted DNA was then quality-controlled and quantified by a Nanodrop ND-1000 spectrophotometer (Thermo, Waltman, MA, USA). All DNA extracts were subsequently stored at −20 °C for downstream molecular analyses.

Quantitative real time polymerase chain reaction (qPCR) amplifications were performed in 8-well tubes on the CFX96™ Real-Time System (Bio-Rad Laboratories Inc., Hercules, CA, USA) to enumerate the populations of bacteria, fungi, and *F. oxysporum*. The reaction mixtures and thermal conditions of qPCR were according to Liu et al. (2016). The respective primer sets of bacteria (Eub338/Eub518), fungi (ITS1-f/5.8s), and *F. oxysporum* (ITS1 F/AFR 308) were listed in Table S1. Melt curves were detected to ensure the amplification specificity after each amplification. The standard curves were generated according to Huang et al. (2015b) and the slopes of bacteria, fungi, and *F. oxysporum* were −3.003, −3.359, and −3.368, respectively.

2.5. MiSeq sequencing and raw data processing

In order to characterize the microbial communities in response to RSD treatment and watermelon cultivation, DNA extracted from all soil samples were selected for Miseq sequencing. The bacterial V4 region and fungal ITS1 region were respectively amplified using the primer sets 515F/806R and ITS1F/ITS2 (Table S1). The PCR procedures containing the reaction mixture and thermal profile were according to Zhao et al. (2014). After PCR amplification, the PCR products were purified using the SequalPrep Normalization Plate (96) Kit, pooled in equimolar

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