

Application of Broccoli Residues to Soil can Suppress Verticillium Wilt of Cotton by Regulating the Bacterial Community Structure of the Rhizosphere

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Abstract

[Aims] Verticillium wilt (VW) of cotton was effectively controlled by application of broccoli residues (BR) to soil. Information regarding the variation in bacterial communities in rhizosphere of cotton cultivars with different VW resistance levels under BR treatment is still lacking and finally to provide guidance for screening effective biocontrol bacteria.

[Methods] Real-time fluorescence quantitative PCR was used to determine the population of *Verticillium dahliae*, the effects of BR on the bacterial community structure in rhizosphere were determined by high-throughput sequencing technology.

[Results] Results showed that control effects for susceptible cultivar (cv. EJ-1) and resistant cultivar (cv. J863) on VW after BR treatment were 51.76% and 86.15%, the population of *V. dahliae* decreased by 18.88% and 30.27%, respectively. High-throughput sequencing showed that ACE and Chao1 indices were increased by application of BR. Actinobacteria, Proteobacteria, Bacteroidetes, Gemmatimonadetes, Acidobacteria, and Firmicutes were the most dominant phyla, and relative abundances of these bacterial taxa significantly differed between cultivars. Additionally, *Bacillus* stably increased in rhizosphere following BR treatment. Redundancy analysis (RDA) showed that relative abundances of *Bacillus*, *Lysobacter*, *Streptomyces*, *Rubrobacter*, *Gemmatimonas*, *Bryobacter* and *Nocardioides* were correlated with occurrence of VW. Field experiments demonstrated that dressing cotton seeds with *Bacillus subtilis* NCD-2 could successfully reduce occurrence of VW, and control effects for EJ-1 and J863 were 35.26% and 31.02%, respectively.

[Conclusions] The application of BR changed the bacterial community structure in cotton rhizosphere, decreased the population of *V. dahliae* in soil, and increased the abundance of beneficial microorganisms, thus significantly reducing the occurrence of VW.

Introduction

Cotton (*Gossypium hirsutum* L.) is the most important source of natural textile fibers worldwide and a significant oilseed crop (Zhang et al., 2016). Verticillium wilt (VW), caused by *Verticillium dahliae*, is a typical soil-borne disease and results in extensive economic losses. In China, losses of approximately 250–310 million US dollars have been reported for cotton annually due to *V. dahliae* (Li et al., 2015; Rehman et al., 2018). VW is particularly difficult to control due to the long-living dormant microsclerotia produced by the pathogen, which remain viable in the soil for more than two decades (Fradin and Thomma, 2006; Alstrom, 2001), as well as the inability of fungicides to contact the hyphae of *V. dahlia* after they spread inside the xylem (Klosterman et al. 2009). It is imperative to develop novel control strategies to control this devastating disease.

Previous studies have shown that soil-borne disease management has relied principally upon fumigation (Atallah et al. 2012; Johnson and Dung, 2010; Taylor et al., 2005). However, the application of chemical fumigants to the soil may be environmentally unfriendly (Uppal et al., 2008). Therefore, there is growing

interest in the search for alternatives to fumigants for disease control. Many reports have demonstrated that the use of organic soil amendments may be a potential strategy for the control of insect pests, pathogens, nematodes, and weeds (Díaz-Pérez, 2007; López-Pérez et al., 2005; Koike and Subbarao 2000); they can increase organic matter and nutrients and change the structure of the microbial community in the soil (Kavino et al., 2010; Shen et al., 2013; Akao et al., 2017). The changes in soil microbial community structure caused by organic soil amendments provide useful information on soil health and quality (Poulsen et al., 2013). In particular, the responses of soil bacterial communities to organic soil amendments are particularly important and are believed to be one of the main drivers of disease suppression (Garbeva et al., 2004). The disease-suppressive effects of certain crop residues are well documented, such as those of broccoli, buckwheat, canola, mustard, and sweet corn (Tubehleh and Stephenson 2020; Housam et al., 2018; Antoniou et al., 2017; Manuel and Celia 2017; Romanyà et al., 2019; Zhao et al., 2019a). Broccoli residues were reported to successfully decrease the incidence of VW in eggplant, potato, strawberry, sunflower and cotton by reducing microsclerotia or DNA copies of *V. dahliae* in the soil (Inderbitzin et al., 2018; Zhao et al., 2019a; Zhao et al., 2021). Broccoli residues also suppress weeds, nematodes, and pests (Koike and Subbarao 2000; Guerra et al., 2017).

As described in our previous work, VW of cotton was effectively controlled by the application of broccoli residues in greenhouse and field experiments (Zhao et al., 2019a; Wang et al., 2020). Recent evidence suggests that variability in plant genotypes or cultivars can have a significant impact on rhizosphere microbiomes, particularly bacteria (Stringlis et al., 2018; Zhang et al., 2019). Information regarding the variation in bacterial communities in rhizosphere soil that are affected by cotton cultivars that vary in resistance to VW following the application of broccoli residues is still lacking. It is unclear whether and, if so, how cultivar resistance against *V. dahliae* is related to rhizosphere bacteria.

The overall objectives of this study were therefore (i) to determine the effect of BR on the incidence of VW among different cotton cultivars, (ii) to study the differences in the bacterial composition, diversity, and community structure following the application of BR, (iii) to analyze the relationship between disease incidence and the bacterial community, and (iv) to assess the effect of the addition of exogenous *Bacillus subtilis* on VW in the field.

Materials And Methods

Field experiment site

The experimental sites were located in Quzhou County, Hebei Province. Field trials were conducted at two sites, Field A and Field B, from 2017 to 2019. The experimental sites have a long history of cotton cultivation and occurrence of VW. The plots with flat terrain, relatively uniform fertility and continuous cotton planting for more than ten years were selected as experimental fields. Soil nutrient characteristics are outlined in detail in a previous publication (Zhao et al., 2019b). Detailed information regarding the field experiment setting is described in the following statements.

Experimental setup and design

Broccoli was planted in August 2017, and the density of the broccoli plants was approximately 41 thousand plants per hectare. After harvesting the edible part of the broccoli, the remaining parts of the plants were chopped in the field with a grinder and mechanically incorporated into the soil with a rotovator in early November 2017 at a depth of 25 to 30 cm. The amount of broccoli residues amended into the soil was approximately 57 thousand kilograms per hectare. Parts of the field were not amended with broccoli residues as a blank control. The susceptible cultivar Ejing 1 (EJ-1) and resistant cultivar Ji 863 (J863) were planted in late April 2018. The experimental design included four treatments: 1) susceptible cultivar EJ-1 planted without broccoli residues (EJ-1-CK); 2) susceptible cultivar EJ-1 planted with broccoli residues (EJ-1-BR); 3) resistant cultivar J863 planted with broccoli residues (J863-BR); 4) resistant cultivar J863 planted without broccoli residues (J863-CK). The experiment had a randomized complete block design with three replicates. All plots were covered with plastic film and irrigated as necessary. For Field B, in early August 2018, broccoli planting, preparation of BR, and experimental setup and design were similar to those in Field A. After that, cotton was planted in late April 2019.

Soil sample collection and DNA extraction

Soil samples were collected at the flowering and boll-forming stages in 2018 and 2019, respectively. Within each sampling plot, three plants were randomly selected and carefully removed from the soil using a spade. The root systems of the three plants from each plot were first vigorously shaken to remove loosely adhering soil particles, and then the remaining root systems were combined as a rhizosphere sample. Soil samples were immediately preserved at 4°C for less than 48 hr. To remove plant material, the sample was sieved through a 2.0 mm sieve and stored at -80°C for subsequent DNA extraction. DNA was extracted following the instruction manual for the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) in accordance with the protocol of the manufacturer. The concentration and quality of the DNA were determined using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific Inc., Waltham, MA, USA). The extracted DNA was stored at -20°C prior to further analyses.

PCR amplification, Illumina MiSeq sequencing and DNA copies of *Verticillium dahliae*

PCR amplification of bacterial 16S rRNA targeting the V3/V4 region was conducted by using primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR protocols were used to amplify the 16S rRNA gene (Lu et al., 2018). Finally, equal amounts of PCR product from each sample were placed in individual tubes and analyzed with the Illumina MiSeq platform. Illumina MiSeq sequencing was performed at Majorbio Biopharm Technology Co., Ltd. (Shanghai, China). The DNA copies of *V. dahliae* in the different soils were studied with real-time fluorescence quantitative PCR according to the method described in our previous study (Zhao et al, 2019a).

Verification of the biocontrol function of *Bacillus subtilis* NCD-2 against VW in the field

To examine the effect of the addition of exogenous *B. subtilis* NCD-2 on cotton VW, two treatments were applied for each cultivar as described below. (1) cotton seeds were coated with *B. subtilis* (10%, w/w) at a spore concentration of 10⁹ CFU/g (representing BS), and (2) cotton seeds were not treated with *B. subtilis*

(representing CK). The experiment had a randomized complete block design with three replicates in the field. The experiment was carried out in 2019.

Disease Evaluation

VW severity on all individual plants was recorded on a scale of 0 to 4. The disease index for each plot was calculated based on a five-level categorization of cotton VW according to the percentage of plant leaves with symptoms such as chlorosis, necrosis or defoliation. 0 = healthy plants or no symptoms, 1 = diseased plants with leaf symptoms below 25%, 2 = 26–50% diseased plants with leaf symptoms and leaf margin rolled up and showing symptoms of scorch, 3 = 51–75% diseased plants with leaf symptoms and leaf margin rolled up with wilting symptoms, 4 = more than 76% diseased plants or dead with leaf symptoms. The disease incidence, disease index, and control effect were calculated using the following formula:

Disease incidence (%) = (No. of diseased plants/30) × 100

Disease index = $[100 \times \sum (\text{No. of diseased plants} \times \text{responding disease rating})] / (30 \times 4)$

Control effect (%) = $[(\text{disease index of CK} - \text{disease index of treatment}) / \text{disease index of CK}] \times 100$

Statistical Analyses

Statistically significant differences ($P < 0.05$) in disease incidence, the disease index, DNA copies of *V. dahliae*, and changes in the bacterial community composition between the control and BR treatments were evaluated with Student's t-test or one-way analysis of variance (ANOVA) using SPSS. Soil bacterial diversity indices were calculated based on resampled OTU abundance matrices in MOTHUR. Principal component analysis (PCA) was performed to explore the differences in soil bacterial community composition. Redundancy analysis (RDA) was performed to examine the relationship between disease occurrence and bacterial community composition. Analysis of similarities (ANOSIM) was performed to identify the significant differences in bacterial community structure among treatments. Data on the differences in bacterial community composition among treatments were obtained, and the relative abundances of major taxonomic groups at the phylum and genus levels were compared. Graphs were generated with Origin 8.0 software.

Results

Effects of broccoli residues on VW of different cotton cultivars

Broccoli residues had a significant impact on the disease incidence and disease index of cotton VW ($P \leq 0.05$). Compared with the blank control (no broccoli residues), the disease incidence of cultivar EJ-1 decreased by 38.76% and 53.50% and the disease index decreased by 46.47% and 57.04% in Field A and Field B, respectively. The disease incidence of cultivar J863 decreased by 100% and 63.42% and the disease index decreased by 100% and 72.30% in Field A and Field B, respectively. The average control effects for EJ-1 and J863 were 51.76% and 86.15%, respectively (Fig. 1).

Effect of broccoli residues on DNA copies of *V. dahliae* in soil

When compared with those in the blank control soils, the DNA copies of *V. dahliae* in the soils associated with the different cotton cultivars were significantly reduced by the BR treatment (Fig. 2). For EJ-1, the DNA copies of *V. dahliae* decreased by 14.31% and 23.44% in Field A and Field B, respectively. For J863, the DNA copies of *V. dahliae* decreased by 34.19% and 26.34%, respectively. The average numbers of DNA copies of *V. dahliae* for EJ-1 and J863 were decreased by 18.88% and 30.27% following the application of BR, respectively.

Alpha Diversity Of The Bacterial Community

The alpha diversity of the bacterial community was expressed by the ACE and Chao1 indices in our study (Fig. 3). In Field A, the ACE index for EJ-1 ranged from 2503 (CK) to 2667 (BR), and the Chao1 index ranged from 2501 (CK) to 2624 (BR), which were greater by 6.55% and 4.92%, respectively. The ACE index for J863 ranged from 2603 (CK) to 2652 (BR), and the Chao1 index ranged from 2585 (CK) to 2617 (BR), which were greater by 1.89% and 1.24%, respectively. In Field B, the ACE index for EJ-1 ranged from 3690 (CK) to 3751 (BR), and the Chao1 index ranged from 3466 (CK) to 3541 (BR), which were greater by 1.64% and 2.15%, respectively. The ACE index for J863 ranged from 3949 (CK) to 3972 (BR), and the Chao1 index ranged from 3520 (CK) to 3655 (BR), which were greater by 0.59% and 3.85%, respectively. These results indicate that the ACE and Chao1 indices were increased by the application of BR at the different field sites.

Bacterial Community Structure Analyses

Principal component analysis based on the OTU composition was used to study the effect of broccoli residues on the soil bacterial community structure associated with the different cotton cultivars. Figure 4 shows plots of the sites in the plane of the first two principal coordinates based on the soil bacterial communities in Field A and Field B, respectively. The results show that the bacterial community structure associated with the different cultivars was located in the same quadrant after the application of broccoli residues, while that of the blank controls of the different cultivars was located in different quadrants, which indicates that the bacterial community structure changed and tended to be the same after the application of broccoli residues. In addition, the first principal component (PC1) and the second principal component (PC2) of the bacterial community structure at the OTU level in rhizosphere soil were found to

explain 34.07% and 16.36% of all variables in Field A, and 24.83% and 21.29% of all variables in Field B, respectively. The cumulative contribution rates of variance of the two principal components reached 50.43% and 46.12%, respectively. In addition, ANOSIM indicated that the BR treatment contributed significantly to the separation of the CK treatment ($R = 0.9815$, $P = 0.001$, Field A) and ($R = 0.6481$, $P = 0.002$, Field B).

Comparison Of Bacterial Community Composition

Among all sequences, unknown sequences were classified as “other group”. In Field A, the dominant bacterial phyla were Proteobacteria, Actinobacteria, Acidobacteria, Gemmatimonadetes, Chloroflexi, Bacteroidetes, Planctomycetes, Rokubacteria, Nitrospirae, Verrucomicrobia, Latescibacteria, Firmicutes and Patescibacteria, and these phyla accounted for more than 95% of the total sequences in each sample (Fig. S1). The changes in the relative abundances of the dominant bacterial taxa associated with the different cotton varieties after the application of broccoli residues were compared at the phylum level (Fig. 5). Notably, all dominant bacterial phyla associated with J863 increased in abundance following the application of broccoli residues, while for EJ-1, the dominant bacterial phyla were influenced to different degrees by the application of the broccoli residues. Among them, Gemmatimonadetes, Rokubacteria, Nitrospirae, Verrucomicrobia and Firmicutes increased. The most abundant group was Firmicutes, which increased by approximately 2.36 and 1.41-fold for EJ-1 and J863, respectively, when compared with the values for CK. However, Proteobacteria, Chloroflexi, Bacteroidetes, Planctomycetes, Latescibacteria and Patescibacteria decreased following the application of broccoli residues (Fig. 5A). In Field B, the dominant bacterial phyla were Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Firmicutes, Bacteroidetes, Planctomycetes, Rokubacteria, Patescibacteria, Entotheonellaeota, Nitrospirae and Verrucomicrobia, and these phyla accounted for more than 95% of the total sequences in each sample (Fig. S2). For the cultivars EJ-1 and J863, the dominant bacterial phyla were influenced to different degrees by treatment with broccoli residues. Actinobacteria, Gemmatimonadetes and Firmicutes were increased by the application of broccoli residues. The most abundant group was also Firmicutes in Field B, and the fold changes were 1.42 and 1.27 for EJ-1 and J863, respectively. Acidobacteria decreased as a result of the application of broccoli residues. In addition, Proteobacteria, Bacteroidetes, Patescibacteria, Entotheonellaeota, Nitrospirae and Verrucomicrobia decreased for EJ-1, while the opposite tendency was observed for J863 (Fig. 5B). Based on the results for the different cultivars and field sites, the relative abundances of Actinobacteria, Gemmatimonadetes and Firmicutes in the soil increased after the application of broccoli residues.

In Field A, the dominant bacterial genera (cultured and at least one group in two groups average relative abundance > 0.5%) were *Sphingomonas*, *RB41*, *MND1*, *Haliangium*, *Nitrospira*, *Lysobacter*, *Bryobacter*, *Gemmatimonas*, *Gaiella*, *Iamia*, *Pontibacter*, *Streptomyces*, *Steroidobacter*, *Ilumatobacter*, *Blastococcus*, *Dongia*, *Luedemannella*, *Rubrobacter*, *Nocardioides*, *Gemmatirosa*, *Massilia*, *Bacillus* and *Sphingobacterium* (Fig. 6A). Among them, the relative abundances of *RB41*, *Gemmatimonas*, *Pontibacter*, *Streptomyces*, *Blastococcus*, *Massilia* and *Bacillus* were increased by the application of broccoli residues

for all cultivars, while decreases in *MND1*, *Nitrospira*, *Bryobacter*, *Iamia*, *Steroidobacter*, *Ilumatobacter* and *Dongia* were observed after the application of broccoli residues.

In Field B, the dominant bacterial genera were *Arthrobacter*, *Streptomyces*, *RB41*, *Rubrobacter*, *Bacillus*, *Sphingomonas*, *Nocardioides*, *Solirubrobacter*, *MND1*, *Gaiella*, *Blastococcus*, *Steroidobacter*, *Bryobacter*, *Nitrospira*, *Marmoricola*, *Microvirga*, *Micrococcus*, *Haliangium*, *Skermanella*, *Dongia*, *Intrasporangium*, *Lysobacter*, *Gemmatimonas*, *Novosphingobium*, *Kribbella* and *Nitrolancea* (Fig. 6B). Among them, the relative abundances of *Arthrobacter*, *Bacillus*, *Microvirga*, *Intrasporangium*, *Lysobacter* and *Nitrolancea* increased, while those of *Streptomyces*, *MND1*, *Blastococcus*, *Steroidobacter*, *Nitrospira*, *Haliangium*, *Dongia* and *Kribbella* decreased. Based on the above results at the two field sites, *Bacillus* was the only genus that steadily increased after the application of broccoli residues.

Relationships between the occurrence of VW and bacterial community composition

The relationships between the occurrence of VW and bacterial community composition in Field A and Field B were studied with RDA (Fig. 7). For Field A, the RDA that was performed with the genera and disease incidence data showed that the first two RDA components could explain 52.3% of the total variation (Fig. 7A). As shown by their close grouping and by the vectors, the disease incidence of cultivar J863 was positively related to the abundant genera *Gemmatimonas*, *Pontibacter*, *RB41*, *Blastococcus* and *Massilia* after the application of broccoli residues, and it was negatively related to *Bacillus*, *Lysobacter*, and *Nitrospira*. However, the disease incidence for EJ-1 treated with BR was positively related to the abundant genera *Streptomyces*, *Rubrobacter*, *Bryobacter* and *Nocardioides*, and it was negatively related to *Gemmatimonas*, *Pontibacter*, *RB41*, *Blastococcus* and *Massilia*.

For Field B, the RDA that was performed with the genera and disease incidence data showed that the first two RDA components could explain 47% of the total variation (Fig. 7B). The disease incidence in the BR treatment for the cultivars (including J863 and EJ-1) was positively related to the abundant genera *Bacillus*, *Nocardioides*, *RB41*, *Rubrobacter*, and *Arthrobacter* and negatively related to *Streptomyces*, *Nitrospira*, *Sphingomonas*, and *Lysobacter*.

Control effect of the exogenous application of *Bacillus subtilis* NCD-2 on VW

The control effect of the application of *B. subtilis* on cotton VW was investigated in our study. As indicated in Fig. 8, the control effect of BS on the disease at the boll-forming stage for EJ-1 was 38.55%, while that for J863 was 26.73%. Additionally, further study at boll opening showed that the control effect for EJ-1 was 31.96%, while that for J863 was 35.31%. The average control effects for EJ-1 and J863 were 35.26% and 31.02%, respectively.

Discussion

The use of crop residues is an important method associated with the suppression of VW, such as those of broccoli, buckwheat, canola, mustard, and sweet corn (Subbarao et al., 1999; Wiggins and Kinkel, 2005;

Inderbitzin et al., 2018; Wheeler and Johnson, 2016; Zhao et al., 2019a; Zhao et al., 2021). In this study, resistant and susceptible cultivars of cotton VW were chosen to evaluate the effect of the application of broccoli residues on the occurrence of VW. The results further confirmed that treatment with BR could effectively reduce the incidence of VW among different cotton cultivars and decrease the population of *V. dahliae* in soil. These results are consistent with those from previous studies on disease in cauliflower, eggplant, and potato after treatment with broccoli residues (Inderbitzin et al., 2018; Ikeda et al., 2015; Davis et al., 2010; Subbarao et al., 1999). Therefore, the application of broccoli residues provides a new method and ideas for the sustainable ecological control of cotton VW. In our previous study, the main potential mechanism by which broccoli residues incorporation into the culture substrate reduced the DNA copies of *V. dahliae* and inhibited the spread of *V. dahliae* was revealed by real-time PCR and confocal microscopy methods (Wang et al., 2020; Zhao et al., 2019a). However, more in-depth research should be performed to further explore this potential mechanism, especially from the perspective of rhizosphere microbiomics. Microbiome-based research has opened a new frontier that will greatly expand our knowledge of the relationship between plant disease incidence and microbiota and offer new opportunities for developing novel approaches for biocontrol. To our knowledge, although the Illumina MiSeq high-throughput technique has been used to study the effects of cultivation or fallowing, crop rotation, and the application of common composts on the soil bacteria community composition or structure (Tian et al., 2017; Chamberlain et al., 2020; Gong et al., 2021), this detailed comparison of the soil bacteria community associated with different cotton cultivars resistant to VW after the application of broccoli residues was the first to be performed with the Illumina MiSeq method.

There are differences in opinion on the relationships between bacterial community indices (richness and diversity) and disease incidence (Bulluck et al., 2002; Shen et al., 2014). Many studies have shown that the diversity and composition of the soil microbial community are related to the occurrence of soil-borne diseases (Gamliel et al., 2000; Bulluck et al., 2002; Zhao et al., 2021). However, Shen et al. (2014) found that there was no significant correlation between bacterial community indices and banana *Fusarium* wilt following the application of bioorganic fertilizer. In the present study, although there were no significant differences in the alpha diversity indices following the application of BR, the values of those indices increased. Moreover, the treatment with broccoli residues had a significant impact on the soil bacterial community structure, which was consistent with the results of previous studies on the changes in bacterial community structure in the rhizospheric soil of eggplant (Inderbitzin et al., 2018). In addition, the bacterial community structure associated with the different resistant cotton cultivars changed in comparison to that in CK and was located in the same quadrant after the application of broccoli residues, indicating that the bacterial community structure tended to be the same after the application of broccoli residues (Fig. 4). In terms of the bacterial community composition, the analysis at the phylum level revealed that Actinobacteria, Proteobacteria, Bacteroidetes, Gemmatimonadetes, Acidobacteria, and Firmicutes were the most common phyla, but with some changes in relative abundance. This finding roughly corresponded with those of previous articles (Inderbitzin et al., 2018; Shen et al., 2014). Inderbitzin et al. (2018) found that the five dominant phyla of soil bacteria were Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Acidobacteria. Among them, Actinobacteria and

Proteobacteria were more abundant after treatment with broccoli residues than in the control, while the opposite tendency of Bacteroidetes, Firmicutes, and Acidobacteria was observed. Our study found that the relative abundances of Actinobacteria and Proteobacteria also increased for the resistant cultivar J863, which was consistent with previous studies (Inderbitzin et al., 2018). However, there was no consistent conclusion for these two phyla for the susceptible cultivar EJ-1. The reason for this finding is still unclear and may be due to differences in the types or contents of root exudates among cotton cultivars, which can cause different microbial communities to be recruited. Moreover, the relative abundance of Firmicutes in the soil associated with different resistant cotton cultivars increased after the application of broccoli residues, which was not consistent with the findings of Inderbitzin et al. (2018).

Analysis of the dominant genera also revealed significant differences in the bacterial communities among the different treatments. Among all the cotton cultivars, the abundance of *Bacillus* was increased by treatment with the broccoli residues. RDA showed that the incidence of VW might be positively related to *MND1*, *Steroidobacter*, *Nitrospira* and *Dongia*, which belong to Proteobacteria and Nitrospirae. Moreover, we found that the *Gemmatimonas* (Gemmatimonadetes phylum) and *Streptomyces* genera (Actinobacteria phylum) might respond to the suppression of VW by treatment with broccoli residues in Field A and that *Lysobacter* (Proteobacteria phylum) might do so in Field B. Yin et al. (2013) reported that members of the *Gemmatimonas* genus were found at a relatively high frequency in the rhizosphere of healthy plants. Many articles have reported that *Streptomyces* and *Lysobacter* could be used as biological control agents against plant pathogens (Hao et al., 2019; Puopolo et al., 2015). *Bacillus*, which belongs to Firmicutes, plays an important role in the biological control of plant disease (Wang et al., 2020; Roy et al., 2018; Pandin et al., 2018). Previous studies have reported the potential of *Bacillus* sp. in the biological control of VW of cotton, potato, eggplant, sunflower, and olive (Azabou et al., 2019; Zhao et al., 2020). *Bacillus subtilis* NCD-2 was first isolated from cotton rhizosphere soil in Hebei Province and showed excellent biological control of soil-borne diseases (Li et al., 2005). In the present study, *Bacillus* significantly increased in abundance after the application of broccoli residues. Therefore, to verify the control effect of *B. subtilis* NCD-2 against VW, field experiments were executed by seed dressing with *B. subtilis*. However, the control effect of strain NCD-2 on disease was approximately 35% for different cotton cultivars. Some researchers have reported that direct applications of potentially beneficial species often result in poor disease suppression due to their low survival and colonization in soil (Saravanan et al., 2003; Lugtenberg and Kamilova, 2009). Therefore, the survival or abundance of the biocontrol inoculant *B. subtilis* in rhizosphere soil will be studied in future research.

In addition, many studies have shown that soil physical and chemical properties such as soil nutrients, pH and organic matter are important factors affecting the structure of the soil microbial community (Zhao et al., 2020; Cui et al., 2017; Zhou et al., 2015). Furthermore, soil temperature and humidity are also important environmental factors affecting soil microbial community structure (Bastida et al., 2018; Zhou et al., 2017) and need to be studied in the future.

Conclusions

In this study, the incidence of cotton VW and the population of *V. dahliae* in the rhizosphere of cotton cultivars with different verticillium wilt resistance levels were decreased by treatment with BR. High-throughput sequencing showed that bacterial diversity was increased by the application of BR. The relative abundances of *Bacillus*, *Lysobacter*, *Streptomyces*, *Rubrobacter*, *Gemmatimonas*, *Bryobacter* and *Nocardioides* were correlated with the occurrence of verticillium wilt. These results provide important information necessary for a better understanding of bacterial community structure in rhizosphere soil after treatment with BR.

Declarations

Data availability statement

The raw sequence data reported in this paper have been deposited at the National Center for Biotechnology Information (NCBI) under accession number PRJNA734729 and PRJNA734770.

Author contributions

WZ, QG, SL, and PM planned, designed the research, and experiments. WZ, PW, LD, XZ, ZS, and XL performed the experiments. WZ and PM analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

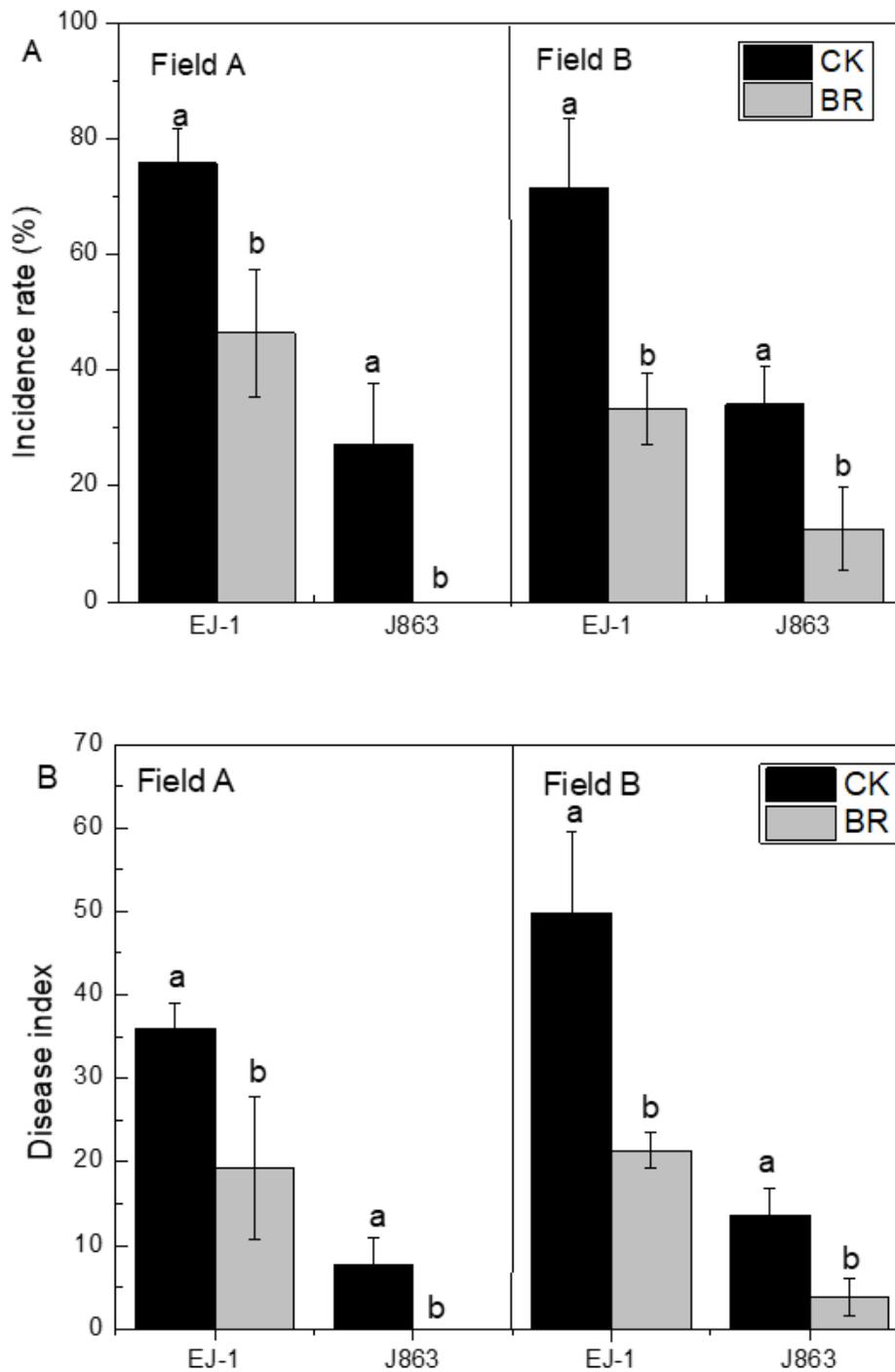


Figure 1

Comparison of the occurrence of verticillium wilt of different cotton cultivars following the application of broccoli residues

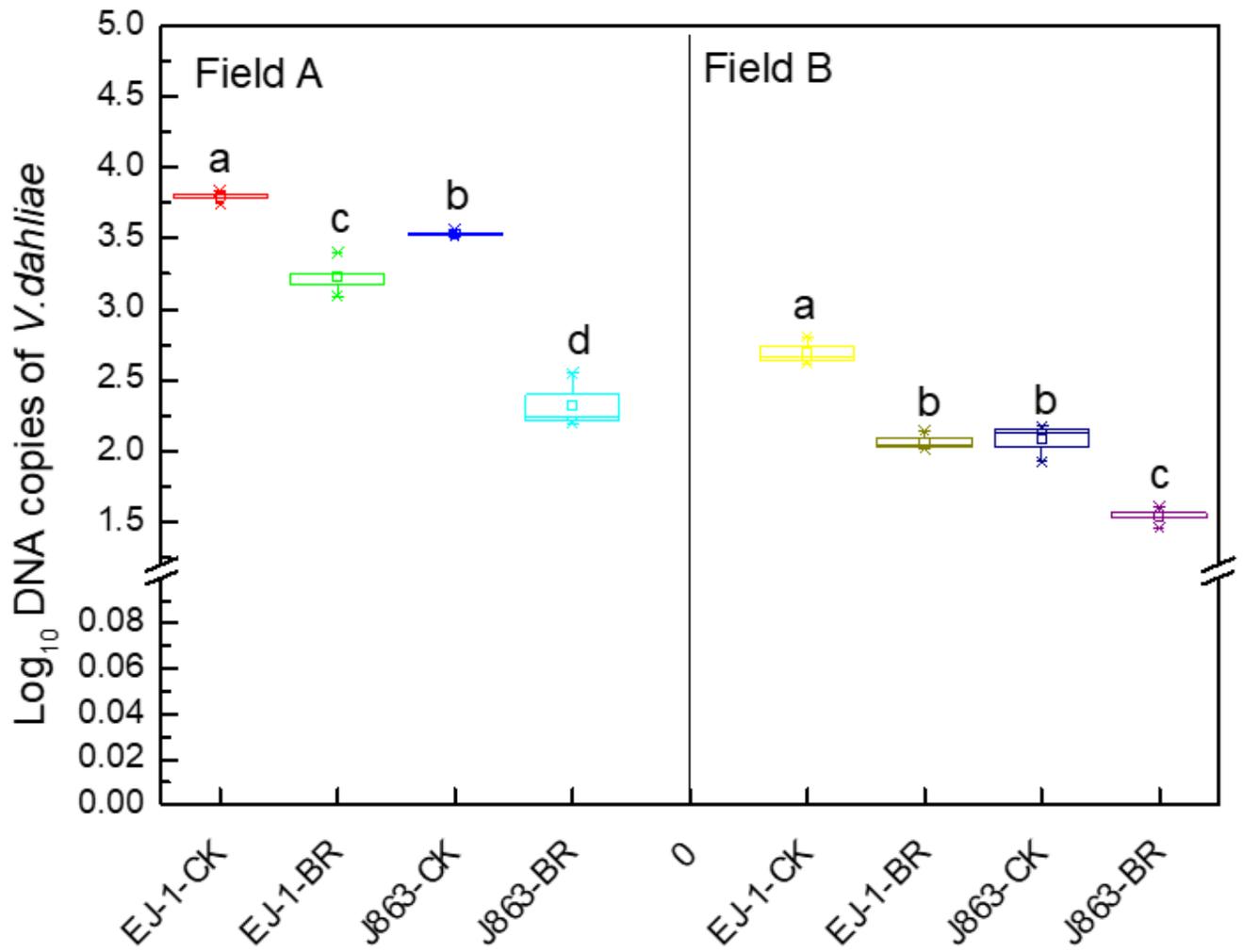


Figure 2

Quantification of *V. dahliae* in soil by real-time PCR

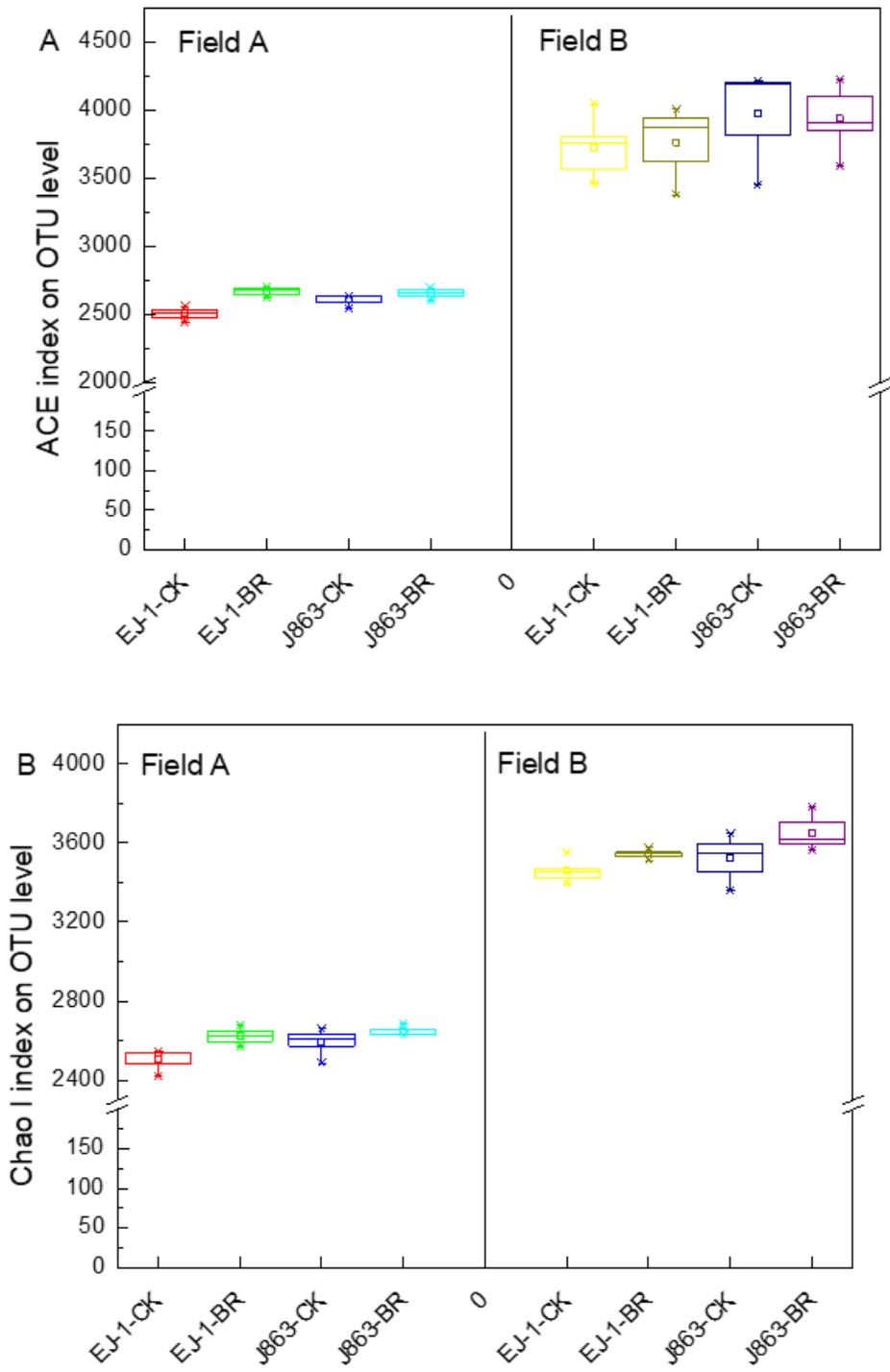


Figure 3

Effect of the BR treatment on the alpha diversity indices of the bacterial community associated with different cotton cultivars

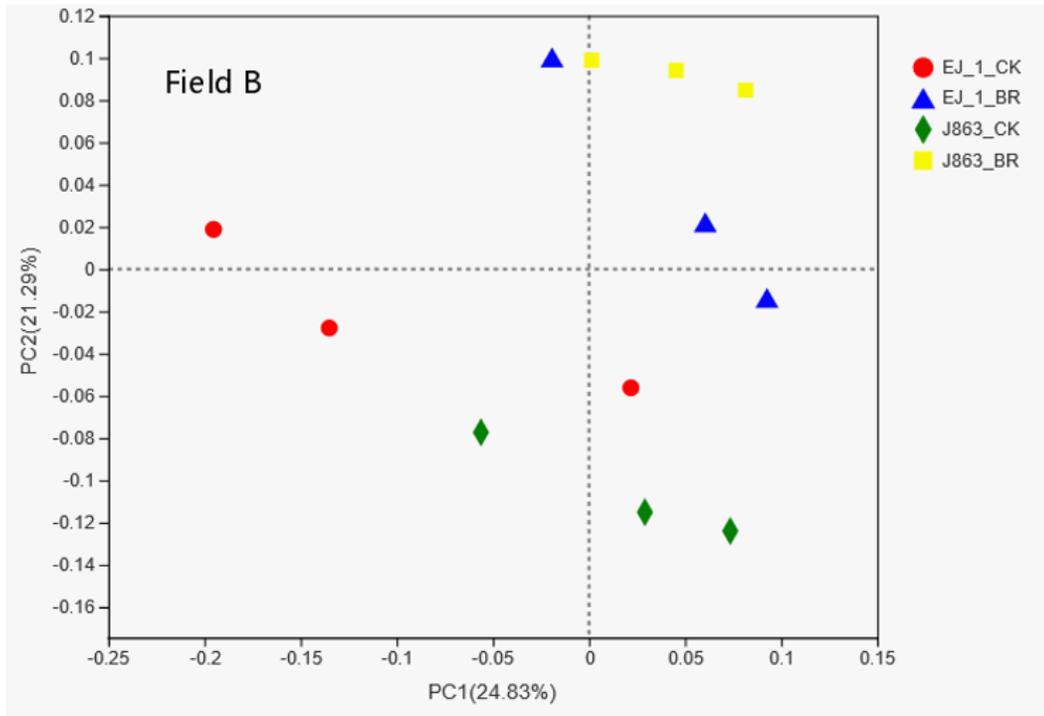
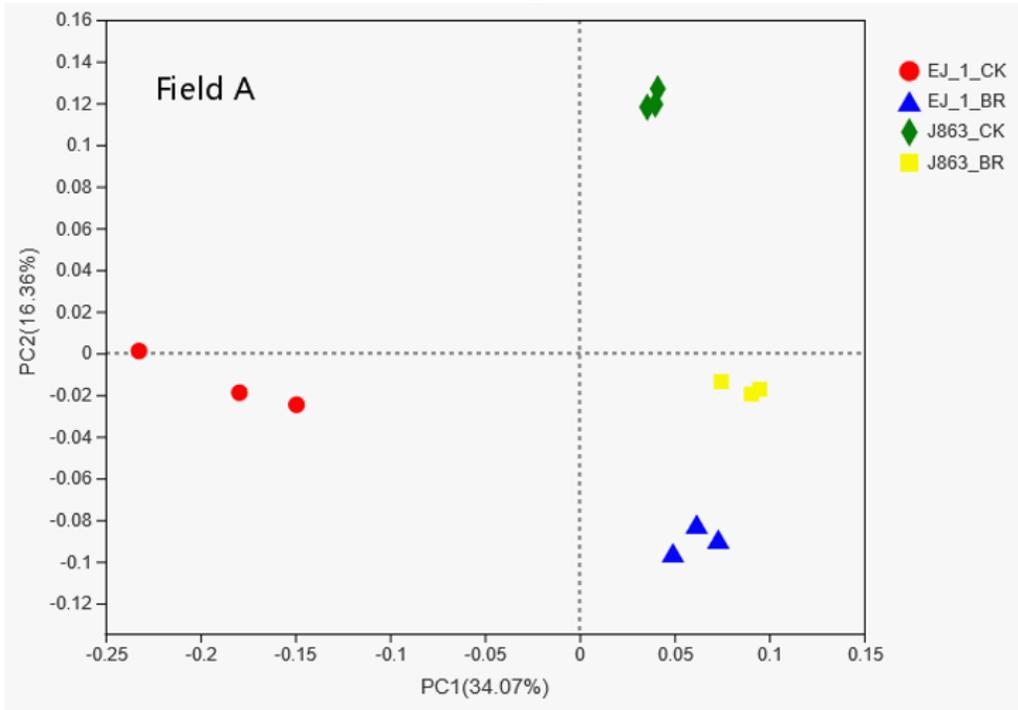


Figure 4

PCA of the soil bacterial community associated with different cotton cultivars at the OTU level following the application of broccoli residues

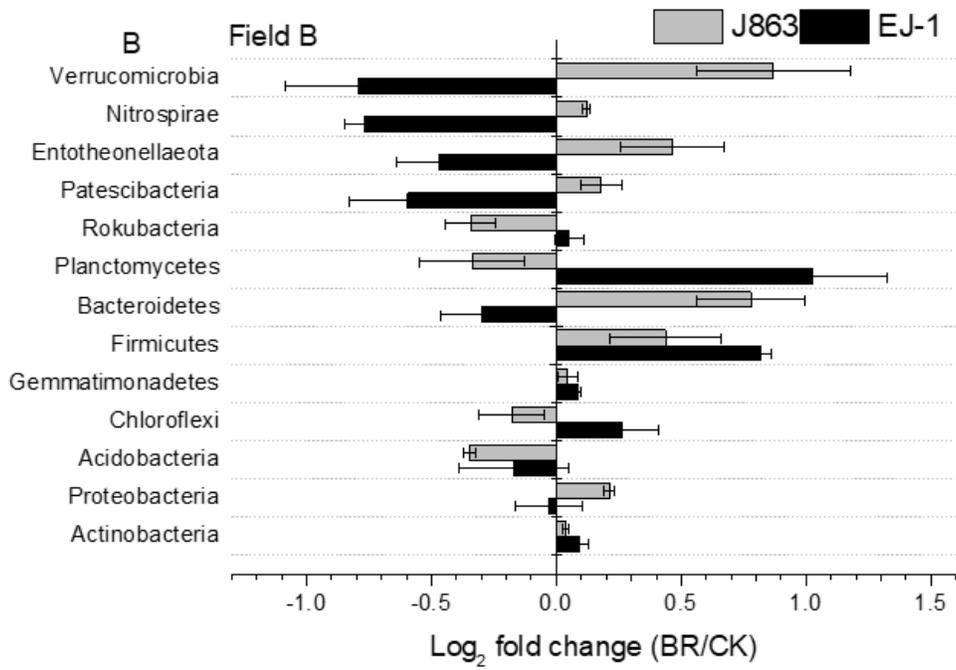
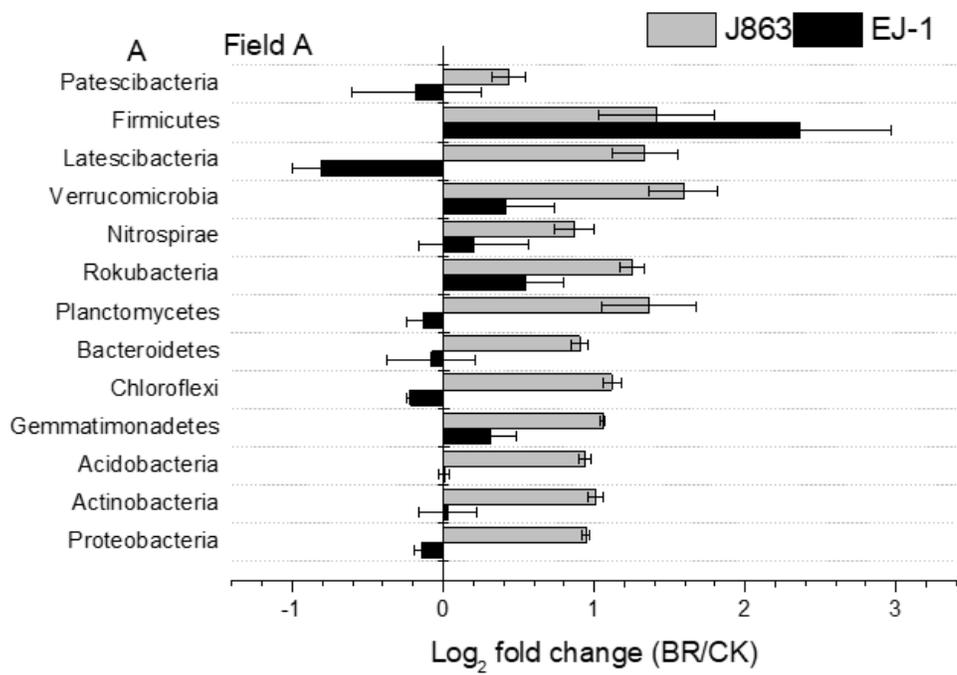


Figure 5

Comparison of the dominant bacterial taxa associated with the different cotton varieties after the application of broccoli residues at the phylum level

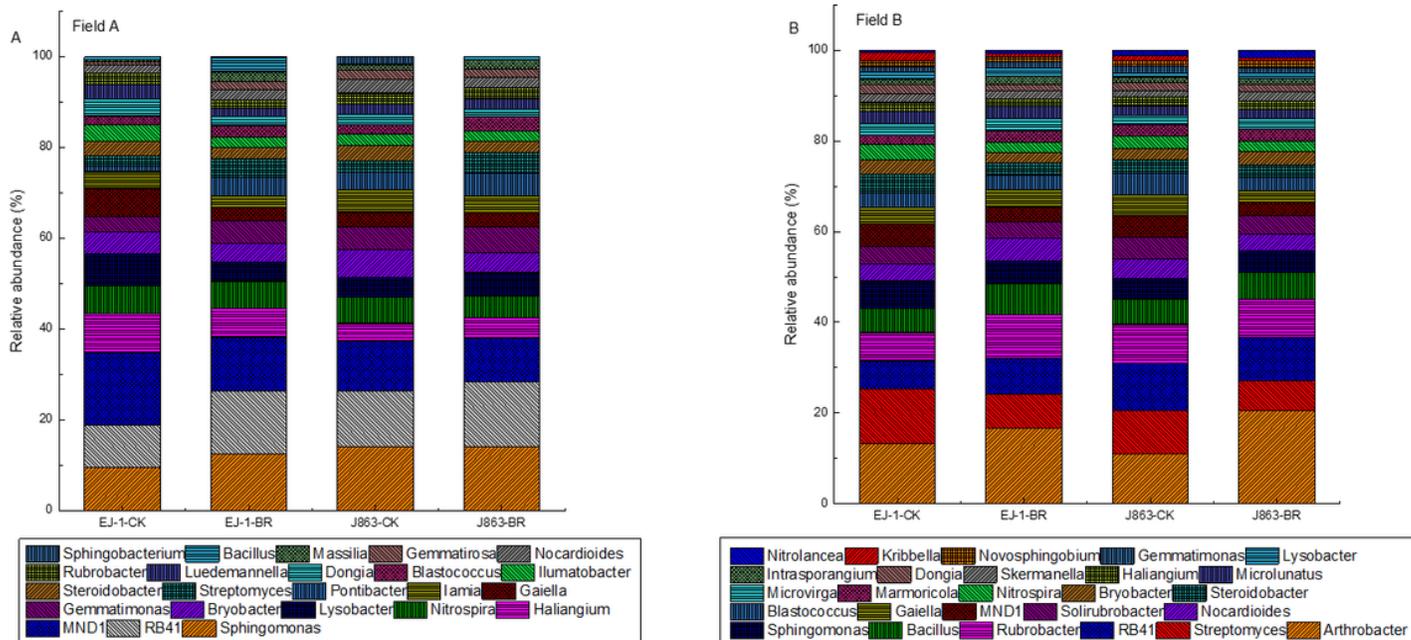


Figure 6

Changes in the relative abundances of the bacterial taxa associated with different cotton cultivars at the genus level following the application of broccoli residues

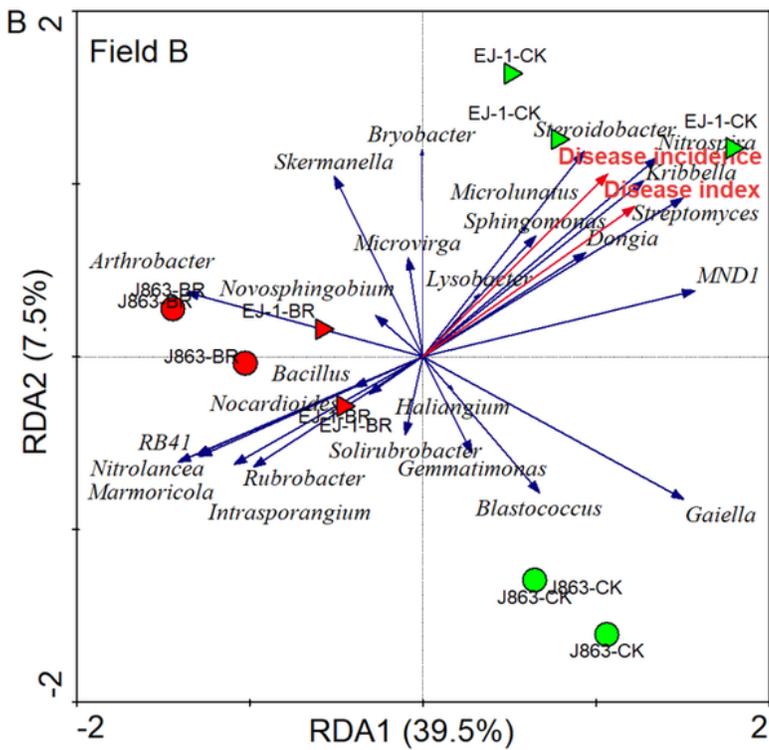
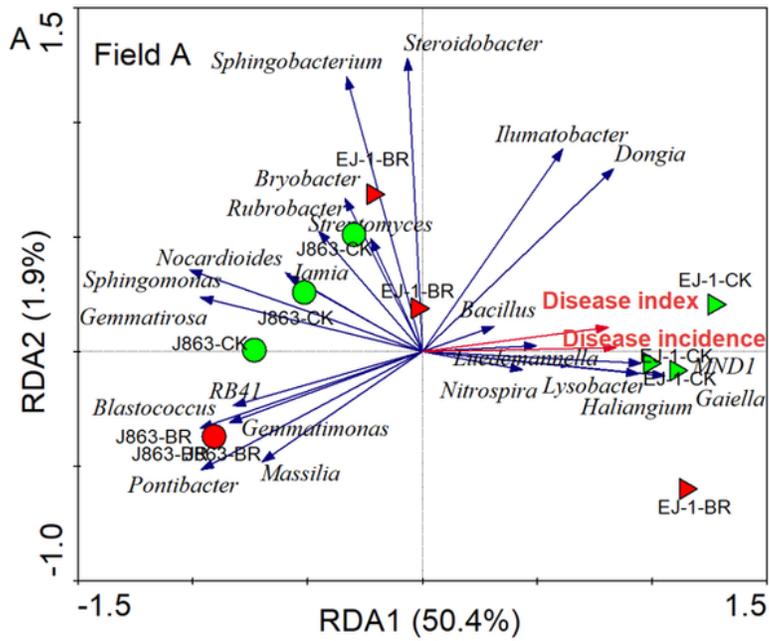


Figure 7

Redundancy analysis (RDA) of the relative abundance of genera and verticillium wilt disease occurrence for different cotton cultivars and different treatments

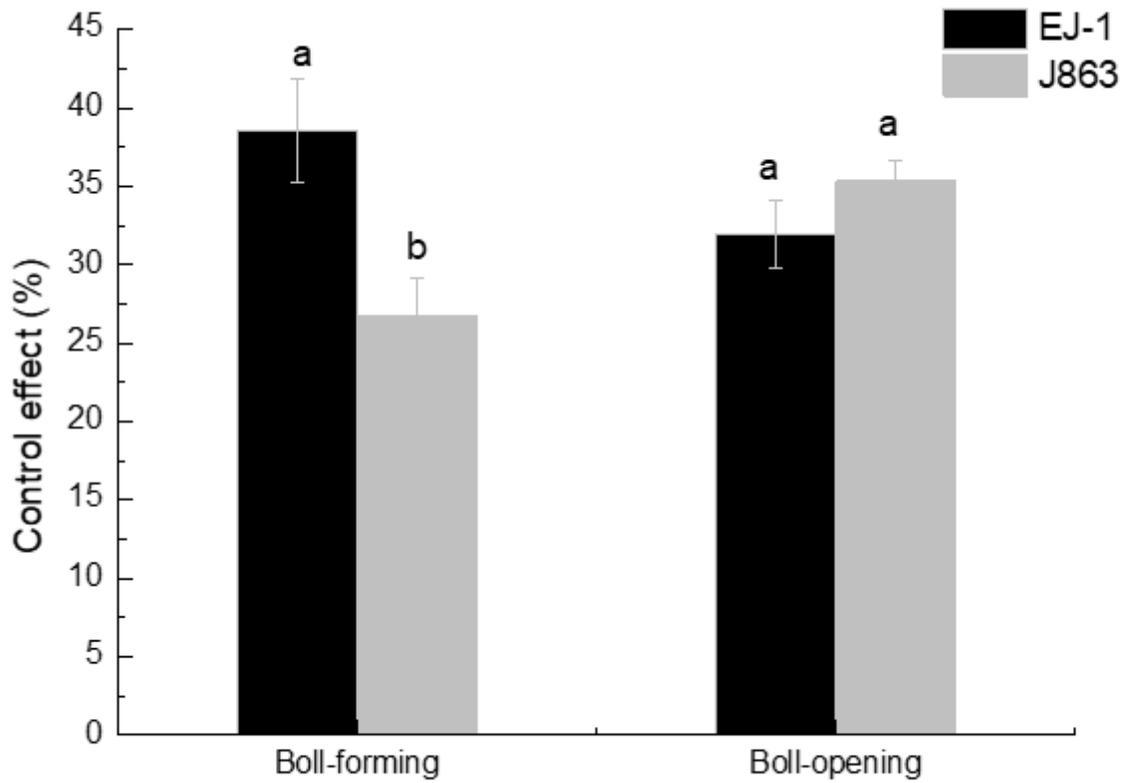


Figure 8

Control effect of *Bacillus subtilis* NCD-2 on verticillium wilt of cotton at different stages

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