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Chemical, organic and bio-fertilizer management practices effect on soil physicochemical property and antagonistic bacteria abundance of a cotton field: Implications for soil biological quality

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ABSTRACT

Cotton production in a long-term, consecutive, mono-cropping cotton field has greatly suffered from soil-borne diseases. In this study, a 4-year field experiment was conducted to investigate the effects of organic fertilizer (OF) or bio-fertilizer (BF) combined with a reduced chemical fertilizer (CF) on soil physicochemical properties, biological activities, antagonistic bacteria (*Bacillus*, *Trichoderma* and *Pseudomonas*) and pathogen(*Fusarium* and *Verticillium dahliae*). Soil physicochemical and biological properties were significantly affected by different fertilization scenarios. Soil bulk density (BD) decreased by 4.69% more with 60% CF + BF treatment than with 60% CF + OF treatment. Compared with CF treatment, 60% CF + BF treatment significantly increased soil chemical properties of total nitrogen (TN), available phosphorus (AP), available potassium (AK), soil organic carbon (SOC) and dissolved organic carbon (DOC). The resistant enzymatic activities of catalase (CAT), peroxidase (POD), polyphenoloxidase (PPO) and fluorescein diacetate (FDA) were improved by the bio-fertilizer addition. The abundances of *Bacillus*, *Trichoderma* and *Pseudomonas* followed the order of BF > OF > CF > control. The gene copy numbers of *Fusarium* and *Verticillium dahliae* were 3.18 and 2.25 cfu $\times 10^3 \cdot (g \cdot soil)^{-1}$ with CF treatment; these decreased by 47% and 32% with 80% CF + OF treatment, respectively, and they further decreased by 86% and 46% with 80% CF + BF treatment, respectively. Together, our results demonstrated that both OF and BF significantly strengthened the enzyme activities, and the antagonistic bacterial abundance but suppressed pathogens, BF was superior to OF in regulating soil microbial abundance, particularly at a high application rate, and highlighted that SOC and AP ($P < 0.05$) were positively correlated with antagonistic bacteria, whereas soil BD and pH were the dominant environment factors impacting soil pathogens. Therefore, ameliorating soil TN, AP and SOC statuses using organic or bio-fertilizer combined with chemical fertilizer is a promising approach to maintaining soil microbiota balance in a continuous, mono-cropping cotton field.

1. Introduction

As one of the indispensable soil fertility components, soil biological quality is highly affected by multiple soil physicochemical factors (Nannipieri et al., 1990; Gil-Sotresa et al., 2005). It is commonly acknowledged that organic or bio-fertilizers are overwhelmingly superior to chemical fertilizers for improving soil biological fertility. The intrinsic mechanism of organic or bio-fertilizers supersedes chemical fertilizers regarding soil biological properties regulation, but the major factors driving the functional soil microbial community shift are still unknown. Therefore, ascertaining the key physicochemical parameters driving soil microbial community structure is a prerequisite for regulating healthy soil microbiotas. Long-term application of chemical fertilizer caused a significant decrease of bacterial richness and resulted in the imbalance of soil microflora (Sun et al., 2015). Regarding the detrimental impacts of chemical fertilizer application on soil biological characteristics, a new pragmatic fertilization strategy is urgently needed.

More recently, it was reported that the application of a chemical fertilizer mixed with organic or bio-fertilizer was regarded as an

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efficient way of regulating soil microbial community structure by promoting beneficial bacteria but suppressing pathogens (Tao et al., 2015). Zhao et al. (2016) confirmed that the relative abundance of *Bacteroidetes* and *Actinobacteria* were enriched in pig manure mixed with inorganic fertilizer. Wang et al. (2013) presented that a *Bacillus* strain combined with organic fertilizer decreased the incidence rate of *Fusarium* wilt. Li et al. (2013) showed that the *Verticillium* wilt incidence rate in a cotton field significantly decreased by 42.9% in the nursery stage and by 57.1% in the transplanting stage with the application of a novel bio-fertilizer consisting of organic matter and *bacillus subtilis* strain HJ5, compared with that of the control.

A myriad of soil-borne pathogens and antagonists coexist in soils (Philippot et al., 2013). The health and equilibrium of soil microbiotas were notably influenced by soil pH, total N and available K (Zhang et al., 2015). Fierer and Jackson (2006) noted the differences of soil bacterial community diversity were predominantly explained by soil Munoz-Rojas et al. (2016) considered that the changes of soil microbial community diversity, β-glucosidase and endocellulase were primarily attributed to the variation of soil TN, SOC and pH value. Tian et al. (2015) considered that DOC must have been stronger than SOC and TN to shift soil microbial functional diversity. Hence, viewpoints of the key soil physico-chemical parameters on driving soil microbial properties changes are still controversial, and a systematic understanding of the response of the functional soil microbial populations to physico-chemical characteristic changes remains elusive.

Cotton (*Gossypium* spp.) has been widely cultivated in Xinjiang, China since the 80s. By 2013, the cotton area in Xinjiang accounted for 38.9% of the total cotton area of China, and the total yield already accounted for 53.9% of the total output of China (Ma et al., 2016). Currently, approximately one third of the arable land in Xinjiang is cotton farms. The cotton mono-cropping cultivation pattern has been extensively adopted, and the continuous cotton cropping history has even exceeded more than 30 years in some region. Unfortunately, concomitantly with the expansion of cotton replanting area, the occurrence of cotton soil-borne diseases caused by *Verticillium* and *Fusarium*, are inevitably and severely prevalent. As a result, there has been a notable reduction of cotton production and economic loss.

In the present study, a 4-year field trial was carried out in drip-irrigated conditions. The objectives were to: (i) Investigate the influences of common organic fertilizer/bio-fertilizer mixed with chemical fertilizer on soil physicochemical properties and defensive enzymatic activities; (ii) Compare the effect of different fertilizer applications on soil pathogens and antagonistic bacteria community abundances in cotton fields; (iii) Explore the driving factors in altering soil microbial community abundances in cotton field.

2. Materials and methods

2.1. Study site

The field experiment was performed at the agricultural experimental station of Shihezi University (latitude 44°23′ N, longitude 85°41′ E), situated in the Xinjiang province in the northwest of China. The average daily minimum and maximum temperatures are 2 °C and 32 °C, respectively, during the cotton growing period from April to October. The accumulated temperature above 10 °C is 3463.5 °C, and the frost-free period lasts 170 days. The annual average precipitation is 213 mm, and the evaporation of the region is 1342 mm. The soil type is *Calcaric Fluvisol* (World Reference Base for Soil Resources, WRS). The experiment site had already experienced four consecutive years of fertilization during 2011–2014. The basic soil properties of the plow layer (0–20 cm) were as follows: TN = 0.95 g kg $^{-1}$; TP = 0.3 g kg $^{-1}$; AP = 23.4 mg kg $^{-1}$; AK = 136 mg kg $^{-1}$; and SOC = 13.5 g kg $^{-1}$.

2.2. Field experiment design

The field experiment was a completely randomized block design. The cotton (*Gossypium hirsutum* L.) variety was Xinluzao No. 46 (traditional variety). Six treatments were (1) T1 (CK): no fertilizer added (control); (2) T2 (CF): NPK chemical fertilizer (300 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1}); (3) T3 (80% CF + OF): 80% CF plus 3000 kg ha^{-1} of common organic fertilizer; (4) T4 (60% CF + OF): 60% CF plus 6000 kg ha^{-1} of common organic fertilizer; (5) T5 (80% CF + BF): 80% CF plus 3000 kg ha^{-1} of bio-fertilizer; and (6) T6 (60% CF + BF): 60% CF plus 6000 kg ha^{-1} of bio-fertilizer. Each treatment was replicated three times, and the plot area was 90 m^2 (20.0 m × 4.5 m).

The basics of fertilizer characterization were described in a previous study (Tao et al., 2015). In brief, common organic fertilizer (organic matter 24.8%, total N 1.7%; i.e., 3000 kg ha^{-1} = 51 kg of N, etc.). Bio-fertilizer (organic matter 29.7%, total N 4.4%, pH 7.6; i.e., 3000 kg ha^{-1} = 132 kg of N, etc.) contains approximately 0.5×10^9 CFU g^{-1} bacteria (*B.subtilis* HJ5 and *B. subtilis* DF14), the two strains, *B. subtilis* HJ5 and *B. subtilis* DF14, were previously isolated from the rhizosphere soil of healthy cotton roots in a field severely affected by Verticillium wilt in Dafeng, Jiangsu province, China (Lang et al., 2012). The two strains have been registered in the China General Microbiological Culture Collection Center with the assigned accession numbers CGMCC no. 3301 and CGMCC no. 3302, respectively. And along with various small molecular peptides; chicken manure is the main medium (developed by Nanjing Agricultural University and supplied by Jiangsu Xintiandi Biology Fertilizer Engineering Cented CO., LTD., China).

The common organic fertilizer or bio-fertilizer was spread evenly on the surface of the soil in each plot and was then thoroughly mixed with the top 0–20 cm of the soil by rotary cultivator, fifteen days prior to cotton sowing in the spring. chemical fertilizer was applied in eight applications by fertigation during the cotton growth season: 10/June, 24/June, 5/June, 14/July, 24/July, 2/August, 12/August and 22/August. The chemical fertilizer consisted of a blend of urea (N 46%), potassium ammonium phosphate (N 5%; P_2O_5 24%) and potassium phosphate monobasic (P_2O_5 52%; K_2O 31.8%).

2.3. Soil sampling

Soil samples were collected randomly from the 0–20 cm soil layer using a 5 cm diameter auger at the bolling (15/August) and harvest stages (15/September) in 2014. Fresh samples were taken from each plot (50 random soil cores per plot) and were mixed thoroughly as one composite sample for further study. After the soil samples were collected, they were preserved in a portable storage box and transported to the lab immediately. All soil samples were passed through a 1 mm sieve, and each sample was separated into two parts. One subsample was used for the measurement of enzymatic activity and physicochemical properties, whereas the other subsample was immediately stored at −80 °C for later DNA extraction or was stored in a 4 °C fridge used for culture-dependent experiments.

2.4. Soil physicochemical analysis

Soil pH and electrical conductivity (EC) were determined using an IQ150/pH metre (Spectrum® Technologies Inc., Aurora, IL, USA) at a 1:5 soil/water ratio. Water holding capacity (WHC) was performed using the double loop gravimetric method. Total N (TN), total P (TP), cation exchange capacity (CEC), available P (AP),