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Coupling sugarcane yield to soil nematodes: Implications from different fertilization regimes and growth stages

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ABSTRACT

Soil nematode communities provide important information for soil food web structure and function. Our study focused on the impacts of different fertilization regimes on sugarcane growth, while primarily considering their effects on soil nematode responses during different growth stages. The sugarcane yields were significantly increased in plots fertilized with functional biofertilizers (BIOs) compared with plots that received chemical fertilizers. Moreover, the BIO applications significantly ($P < 0.01$) decreased the relative abundance of plant parasite while significantly ($P < 0.01$) increased the relative abundance of beneficial nematodes: *Cephalobus, Cervidellus, Acrobeles, Caenorhabditis* in the group of bacteriores and *Ditylenchus, Tylencholaimus* in the group of fungivores. Nonmetric multidimensional scaling (NMDS) revealed that soil nematode communities were primarily separated by sampling time. A network analysis showed that BIO applications led to more simplified soil nematode community, which was possibly linked with the increased bacterivore abundance and improved soil fertility. The effects of fertilization regimes were significant ($P < 0.05$) and accounted for 3.35% of the total variation in soil nematode communities. Soil properties (e.g., pH, organic matter, total N, available K and available P) were influenced by the fertilization regimes and different sampling time, which significantly ($P < 0.05$) explained 14.53% of the soil nematode variation. In conclusion, BIOs applications might facilitate sugarcane yield by increasing the relative abundance of bacterivore while decreasing plant parasites. The changes in soil nematode communities may reflect the soil fertility and health and provide evidence for the benefits of BIO applications for sugarcane crops.

1. Introduction

The soil ecosystem supports an astounding number of microbes, microfauna, and mesofauna. As a dominant belowground component of soil microfauna (Bongers and Bongers, 1998), soil nematodes comprise a range of trophic groups that are classified according to known feeding habitats or stoma and esophageal morphology. They include bacterivores, fungivores, plant parasites and omnivores-predators. Soil nematodes occupy a versatile and important position in the soil food web structure function (Li et al., 2016a,b; Freitas et al., 2017; Šalamún et al., 2017). Accumulating evidence indicates that the soil nematode is sensitive to disturbances in the soil structure and can be used as a bioindicator of the soil's quality (Neher, 2001; Yeates, 2003; Bonkowski et al., 2009; Zhang et al., 2012; Liu et al., 2016). Therefore, monitoring their responses may help us better understand the influences of different disturbances on ecosystems. Recent reports on the responses of belowground diversities and communities to different land management practices have considerably increased and have become the focus of multiple studies (Duncan et al., 2007; Yeates, 2010; Maron et al., 2011; Bever et al., 2012; Monroy et al., 2012).

Sugarcane is a popular sugar crop and the most promising regenerative energy crop. Its cultivation has exceeded 85% of the China's sugar crop total. Sugarcane is primarily planted in soils not suitable for traditional agricultural production. However, during sugarcane cultivation, it is prone to serious soil nematode diseases, which threaten its production and cause severe economic losses worldwide (Stirling et al., 2001). Previous studies (Costa et al., 2012; Porazinska et al., 2012) have demonstrated that the contribution of the soil nematode community to ecosystem processes primarily depends on its composition and diversity. Continuous cropping may provide preferential food

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resources for specific soil nematodes, which may promote alterations in the soil nematode community structure; this may be a major cause of disease (Tom et al., 2016). The intensive use of plant protective chemicals has promoted resistant strains and severe environmental problems (Arguelles-Arias et al., 2009). Thus, an improved method that ensures healthy sugarcane crops is essential.

Applications of biofertilizers that comprise composts and functional microbes are more environment-friendly than pesticides. Multiple microbes reportedly function as potential bio-control agents that suppress soil-borne diseases to facilitate high-yield crops (Mateille et al., 2010; Xiao et al., 2013). For example, *Bacillus cereus* has been rigorously studied for its biological control features, such as its efficacies in producing antibiotics, controlling plant diseases and promoting plant growth (Halverson and Handelsman, 1991; Kevany et al., 2009). Another functional strain, *Paenibacillus polymyxa*, is a plant growth-promoting rhizobacteria (PGPR) and an effective biocontrol agent that induces plant systemic resistance and excretes antibiotics and enzymes to suppress pathogen proliferation (Raza et al., 2008; Ling et al., 2010). However, these functional microbes require suitable carriers to ensure their survival and development to a significant population of the soil microbiological community. Multiple studies (Chen et al., 2011; Huang et al., 2011; Yang et al., 2011; Xiao et al., 2013) have proved that their efficacies can be enhanced by fermenting organic matter with the functional microbes to produce biofertilizers. Applications of mineral or organic fertilizers alter soil properties (e.g., pH, soil organic matter content), which consequently exert direct or indirect influences on the nematode population through plant growth or microbial activity (Bulluck et al., 2002; Vestergård, 2004; Zhong et al., 2010). However, a few cases are reported for application of biofertilizers (that contain functional microbes) toward increasing the sugarcane yield by improving the soil nematode community in field. Soil nematode communities typically vary at different growth stages of plants due to the influences of numerous factors (Cadet et al., 2005; Zhang et al., 2015; Amosse et al., 2016 Li et al., 2016a,b; Van and Bouwmeester, 2016). Therefore, knowledge of soil nematode community dynamics in response to the different growth stages is essential for tracking soil nematode community responses over time to determine the relative impact of fertilization regimes on soil nematode communities.

In this study, we applied two functional biofertilizers to sugarcane and monitored soil nematode population dynamics at four growth stages of sugarcane, namely tillering, jointing, maturity and harvest, to address the following questions: (i) Does a correlation exist between soil nematodes and sugarcane yield? (ii) How do different fertilization regimes impact the soil nematode community throughout the crop development? (iii) Which factor (e.g., sampling time, fertilization or soil properties) mainly determines the soil nematode community?

2. Materials and methods

2.1. Plant materials and fertilizers

Saccharum officinarum L. cv. YT55 sugarcane was obtained from the Guangzhou Sugarcane Industry Research Institute for this field experiment. The chemical fertilizer was supplied by the Guangzhou Sugarcane Industry Research Institute, and the N, P_2O_5, and K_2O contents were 14%, 16%, and 15%, respectively. The biofertilizers (BIO.A and BIO.B) were supplied by Xintiandi Co., Ltd (Yixing City, China), which obtained by aerobically fermenting a mixture of amino acid fertilizer and cattle manure compost (1:1, w/w) with the functional strains. The functional strains in BIO.A and BIO.B were *Paenibacillus polymyxa* SQR21 (Ling et al., 2010) and *Bacillus cereus* X5 (Xiao et al., 2013), respectively. The aerobically fermenting process maintains for 7 days at 30–40 °C and moisture at 40–45% (Zhang et al., 2013a). After that, the BIOs were air dried at room temperature until the water contents were approximately equal to 30%. BIO.A contained 44% organic matter and 4% N, and BIO.B contained 43% organic matter and 3% N. Besides, the finished densities of SQR21 and X5 in BIOs were both $\geq 5 \times 10^8$ CFU g^{-1} dry weights.

2.2. Field description and experimental design

A field experiment with different fertilization regimes was conducted at the station of the Guangzhou Sugarcane Industry Research Institute Zhanjiang Sugarcane Research Center, Zhanjiang, Guangdong Province, China, from February 2012 to January 2013. This region is characterized by a tropical and subtropical monsoon climate, with an average annual temperature range of 22.7-23.3 °C and a mean relative humidity range of 82–84% (Guangdong Meteorological service, http://www.grmc.gov.cn). The soil in this region is developed from the sediment of basalt plain and classified as Ferric Ferralsol (IUSS WG WRB, 2015), which has been planted with sugarcane for 16 years. The basic soil properties (0–20 cm depth) were 22.3 g/kg soil organic matter, 2.58 g/kg total N, 254.2 mg/kg available P, 138.5 mg/kg available K and acidity at a pH of 5.35.

The field experiment incorporated the following 3 treatments: 1) CK: 3375 kg haˆ-1 chemical fertilizer; 2) BIO.A: 1800 kg haˆ-1 BIO.A + 3375 kg haˆ-1 chemical fertilizer; 3) BIO.B: 1800 kg haˆ-1 BIO.B + 3375 kg haˆ-1 chemical fertilizer. The fertilizers were applied to soils at three different times: Feb 2012 (all BIOs and 1/6 chemical fertilizer), May 2012 (1/3 chemical fertilizer) and Jun 2012 (1/2 chemical fertilizer). Each treatment was repeated three times. Each plot contained 7 rows. The row spacing was 1.1 m and row length was 11 m, thus, the area for each plot was approximately 86.67 m^2. The field experiment was performed in a randomized block design. The sugarcane yields, sugar contents and sugar yields were evaluated on harvest day.

2.3. Soil sample collection

The soil samples for the different treatments (CK, BIO.A and BIO.B) were collected during the following growth stages: tillering stage (May 2012), jointing stage (August 2012), maturity stage (October 2012) and harvest stage (January 2013). Five soil cores (5 cm diameter) along rows at plough layer depths of 0–20 cm were cleared of gobbet and residual roots, randomly sampled and thoroughly mixed to yield one representative composite sample. Thus, each treatment group comprised three samples at every sampling stage. After being sieved through a 2.0-mm sieve and thoroughly homogenized, each sample was divided into two parts: one part was air-dried at room temperature for 7 days for the soil properties analysis, and the other part was stored at 16 °C for the follow-up soil nematode analysis.

2.4. Determination of soil physicochemical properties

Soil pH was determined with a glass electrode (soil/water = 1:5). Soil organic C (SOC) and total N (TN) were determined with an Elementar Vario EL III (Germany). Ammonium nitrogen ($NH4^+$-N) and nitrate nitrogen ($NO3^-$-N) were measured with a continuous-flow Auto Analyzer (AA3, Bran and Luebbe, Germany). Soil available phosphorus (extracted by Bray No. 1) was determined according to Bray and Kurtz (1945) and Menage and Pridmore (1973). Soil available K was extracted with ammonium acetate and measured by flame photometry (Knudsen et al., 1982).

2.5. Soil nematode isolation and identification

Nematodes were extracted from 100 g soil (fresh weight) using a modified shallow dish method (McSorley and Frederick, 2004). The extracted nematodes from each sample were randomly selected and identified to genus-level under a microscopy (Olympus BX50) at 400–1000 magnification according to their buccal and caudal characteristics (Bongers and Bongers, 1998). The identified nematodes were