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Novel soil fumigation strategy suppressed plant-parasitic nematodes associated with soil nematode community alterations in the field

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ABSTRACT

Banana production is severely hindered by plant-parasitic nematodes worldwide. In this study, a novel fumigation agent based on lime and ammonium bicarbonate (LAB) was evaluated as a strategy for controlling plant-parasitic nematodes by altering the soil nematode community. The results revealed that LAB fumigation altered the soil nematode community in a banana monoculture system, showing stable suppression ability ($P < 0.05$) of total nematodes and plant-parasites, especially *Rotylenchulus*, after both fumigation and harvest as well as for plant parasites in roots after harvest ($P < 0.05$). LAB fumigation significantly ($P < 0.05$) reduced the maturity index (MI); increased the ratio of plant-parasitic index and maturity index (PPI/MI), Shannon index H' and evenness index J in a short period; and improved the soil pH, NH_4^+-N, NO_3^--N and TON contents. Redundancy analysis showed that the fumigated soil (LAB) was dominated by *Acrobeles*, which was positively correlated with soil pH, while in the control (CKCM), nematode genera after the harvest was dominated by *Rotylenchulus, Helicotylenchus*, *Criconema* and *Tylenchorhynchus*, which were negatively correlated with soil pH. Variance partitioning analysis determined that LAB altered the soil nematode community by affecting soil bacteria, fungi and physicochemical properties after fumigation. The application of LAB also showed a 15% increase in banana yield compared to the control. In conclusion, the novel soil fumigation strategy based on LAB is a potentially effective strategy to suppress plant-parasitic nematode damage in the field, especially for acidic and sandy soils.

1. Introduction

Bananas are planted as a staple, important cash and major export crop in many tropical and subtropical countries. However, their production is hampered by many diseases and pests (Rahman et al., 2010), especially the widespread plant-parasitic nematodes that cause root damage leading to severe crop losses (Moens et al., 2004). More than 51 nematode genera affecting banana crop have been documented (Khan and Hasan, 2010), among those, *Pratylenchus*, *Tylenchus*, *Ditylenchus*, *Meloidogyne* and *Helicotylenchus* are the major harmful nematode pests affecting banana production worldwide (Gowen et al., 2005). Effective management for controlling banana parasitic nematodes includes biological, physical and chemical control methods (Kosma et al., 2011). However, incorporation of organic amendments into the soil or soil solarization does not always produce sufficient control efficiency (Oka et al., 2006a). Many factors, including soil type, climatic conditions and water content of the soil, can affect the effectiveness of physical treatments (Dungan et al., 2003). Abuse of pesticides can destroy the ecosystem balance and cause harm to people (Cao et al., 2002). Furthermore, biocontrol efficiency is influenced by abiotic and biotic factors, especially in soil with high disease incidence (Jiménez-Díaz et al., 2011). Therefore, it is important to explore an effective alternative to ensure healthy banana production.

Previous studies have reported that organic or inorganic nitrogen-containing materials that can release NH_4^+ have been used to suppress root-knot nematodes (Bashour et al., 2013). Ammonia has been shown

Abbreviations: CK, soil samples collected after fumigation for non-fumigation control; LAB, soil samples collected after fumigation for fumigation treatment; CKCM, soil samples collected after harvest for non-fumigation control; LABCM, soil samples collected after harvest for fumigation treatment

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to be responsible for many effects on nematodes; some plant-parasitic nematodes are very sensitive to low ammonia concentrations and die (Oka and Pivonia, 2002; Oka et al., 2006b). In our preliminary study, a novel fumigation strategy based on ammonium bicarbonate for controlling plant-parasitic nematodes through pot experiments, especially for controlling *Meloidogyne* and *Rotylenchulus* (Su et al., 2015), was explored. However, direct control efficiency regarding the novel fumigation strategy after planting banana in the field requires further study.

Nematode community structure, a measure of the abundance and diversity of soil nematode assemblages, provides insight into ecosystem resilience where larger and more diverse assemblages reflect a capacity to perform numerous ecological functions and therefore sustain soil productivity and health (Yeates, 2007). Variations of the soil nematode community after fumigation and subsequent influence after banana transplanting should warrant the same contemplation and research. Therefore, in this study, field experiments were conducted to investigate the effects of novel fumigation strategies using ammonium bicarbonate and lime on the suppression of banana parasitic nematodes, soil nematode communities and soil chemical properties. The objectives of this study were to 1) evaluate the direct efficiency of the novel fumigation strategy to control plant-parasitic nematodes in the field; 2) determine the alterations of soil nematode communities caused by fumigation and its subsequent influences on nematode communities after banana harvest and 3) explore potential correlations among banana parasitic nematode suppression and soil chemical properties.

2. Materials and methods

2.1. Field source

The field experiment was performed in "Wan Zhong" orchard (18°23′ N, 108°44′ E), Le Dong County, Hainan Province, China. The field was continuously cropped with bananas for more than ten years with a serious infestation of root-knot nematodes (over 200 individuals per 100 g dry weight soil). The area has a tropical monsoon climate. Soil was sandy with a pH of 5.6 and contained organic matter, total nitrogen, ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), available potassium, and available phosphorus contents of 7.6 g kg^{-1}, 0.4 g kg^{-1}, 10.3 mg kg^{-1}, 143.9 mg kg^{-1}, 278.2 mg kg^{-1}, and 173.6 mg kg^{-1}, respectively.

2.2. Field experiment

A two-seasonal field experiment with a completely randomized block design and three replications was performed from May 2012 to July 2014 to evaluate the nematicidal effects of a mixture of lime and ammonium bicarbonate (LAB). Quantities for LAB were developed from pot experiments in our previous study (Su et al., 2015), and the amounts of lime and ammonium bicarbonate used were 0.30 and 0.15 kg m^{-2}, respectively. The field experiment included one treatment and one control: 1) LAB treatment, soil amended with a mixture of lime and ammonium bicarbonate; and 2) control (CK), soil without any amendment. The treatment and control plots (about 90 m^2) were covered with plastic film for 15 days before applying fertilizers. The treatment and control were amended with cattle manure compost (30 t ha^{-1} soil, dry weight) and were transplanted with banana seedlings (*Musa* AAA *Cavendish*) provided by Hainan Wan Zhong Co., Ltd., China. Treatment and control were adjusted to the same amount of N, P and K by mineral fertilizer as necessary. Two-thirds of the composts and essential mineral fertilizers were applied as basic fertilizers before planting using a rotary tiller. Remaining composts and essential mineral fertilizers were applied as a top dressing during the banana bud stage. All other farm operations were managed with traditional farming methods. After the first season, plants were removed, and the soil was left to fumigate for the second season. The agronomic characteristics were measured after transplanting the seedlings for 3 months in the second field experiment. All the mature banana fruits in the second field experiment were harvested and weighed for total banana fruit yield of treatment and control plots.

2.3. Sample collection

Triplicate soil samples were collected after fumigation (three samples designed as CK and three samples designed as LAB) and after banana harvest (three samples designed as CKCM and three samples designed as LABCM, approximately 7 months after transplanting during pumping bud stage) from the two-seasonal field experiment. Briefly, five individual banana trees at least 3 m apart were selected for one sample collection, and the collected samples from each tree were mixed as a composite sample. For each tree, composite root or soil samples from 3 sites under the trunk base were collected at a depth of 20 cm. All samples were transported to the laboratory and thoroughly homogenized to determine the nematode community. Root samples were washed gently to remove soil and then macerated in a blender. Plant-parasites in roots were extracted by the Baermann funnel method (Barker et al., 1985) and calculated using an Olympus ZX10 stereomicroscope. Data were expressed as number of individuals per 100 g root to assess nematode suppression. About 100 g soil samples were chosen for nematode community analysis, and another 100 g of each soil sample after harvest of the second field experiment was sifted through a 1 mm sieve and air-dried to determine chemical properties.

2.4. Nematode community diversity and ecological indices analysis

Nematodes were counted for taxonomic identification at the trophic and family levels using keys from Yeates et al. (1998). Nematode communities were characterized using the maturity and plant-parasitic indices (MI and PPI) according to Bongers (1990). PPI/MI ratio was used to evaluate nematode communities (Bongers and Korthals, 1995). Shannon index (Shannon, 1948) was used to calculate taxonomic diversity of the nematode community and calculated as follows:

$$H' = -\Sigma pi \times \ln pi \quad (1)$$

where '*pi*' is the proportion of individuals in the *i*-th taxon.

The Pielou evenness index (Pielou, 1969) was calculated as follows:

$$J = (-\Sigma pi \times \ln pi)/\ln S \quad (2)$$

where '*pi*' is the proportion of individuals in *i*-th group in the nematode community and '*S*' is the total number of nematode genera in the community.

2.5. Assay for the quantification of total soil bacteria and fungi

Total soil bacteria and fungi were quantified using Real-Time PCR according to Fierer et al. (2005). To estimate the small-subunit rRNA gene abundances of bacteria and fungi, standard curves were generated using 10-fold serial dilutions of a plasmid containing a full-length copy of the 16S rRNA gene from *Escherichia coli* and the 18S rRNA gene from *Saccharomyces cerevisiae*. Standard and environmental DNA samples were analyzed using a CFX-96 Real-Time PCR System (BIO-RAD, USA) according to a standard procedure. Each sample was performed in three replicates, and results were expressed as log values (copy numbers g^{-1} DW soil).

2.6. Soil chemical analysis

Soil pH was determined using a glass electrode meter. Total soil nitrogen (TON) content was determined by Kjeldahl digestion. Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) contents were determined by extracting the soil with a 0.01 M $CaCl_2$ solution (1:10, w/v) and using an Auto Analyzer (AutoAnalyzer 3, Germany)