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Effects of conventional and reduced N inputs on nematode communities and plant yield under intensive vegetable production

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ABSTRACT

As the largest group of soil mesofauna, nematodes occupy all consumer trophic levels in soil food webs, and may serve as a proxy for soil food web structure and composition. The present study was conducted in an intensively managed, solar greenhouse vegetable-production system to investigate the effects of nitrogen management on soil nematode communities. We conducted two experimental trials. The first trial was a field survey in vegetable greenhouses with various cultivation histories $(1, 2 \text{ and } \ge 5 \text{ years})$ and open grain fields. The second trial was a series of nematode community analyses over four years from a long-term N management experiment with three treatments: NN (no nitrogen input), RN (reduced N fertilization) and CN (conventional N application). In the field survey, we found that soil total N significantly increased with planting age. After one year of cultivation, greenhouse soil had a significantly lower Shannon-Wiener diversity index (H) (1.55) and a higher abundance of root knot nematodes (RKNs) (292 nematodes per 100 g dry soil) compared to the soil in the open fields. With increasing time of cultivation, there were further decreases in H and increases in RKNs with H reaching 1.03 and RKNs 1254 after five or more years of vegetable planting. Analyses of soil nematode community in the N management experiment indicated that the abundance of RKNs significantly decreased by 55.9% to 770 per 100 g dry soil in the RN treatment compared to 1745 per 100 g dry soil in the CN treatment. The maturity index of the soil nematode community was negatively correlated with the amount of N input and soil total N. Further, tomato fruit yield was not affected by reduced N input in the RN treatment in contrast to the CN treatment. Our results clearly demonstrate that reduced N input had two benefits; (1) reducing the risk of nitrate pollution associated with excessive N input, (2) decreasing the abundance of RKNs and improving the soil nematode community for vegetable production systems.

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1. Introduction

Over the last two decades, solar greenhouse vegetables have developed rapidly in China, encompassing 2.7 million ha in 2008 (Chinese Statistical Bureau, 2009). Generally, the year round greenhouse vegetable production includes a winter–spring (WS) season and an autumn–winter (AW) season, with one month's fallow between the two growing seasons per year. Solar greenhouse vegetable cultivation is a typical intensive production model with much higher input compared to open field crop production. High nitrogen (N) fertilization in vegetable production systems is a common practice to ensure high yields of the marketable products (Agostini et al., 2010). Li et al. (2009) showed that the mean chemical fertilizer input in a vegetable greenhouse was

1600 kg N ha⁻¹ yr⁻¹ plus an estimated N input 800 kg N ha⁻¹ yr⁻¹ from organic manure. Over 3000 kg N ha⁻¹ yr⁻¹ was found in some vegetable cultivation regions in northern China (Ju et al., 2007). The high application of N fertilizer to greenhouse vegetable soils has resulted in low N use efficiency, with only 33% of applied N taken up by the vegetables in northern China (Song et al., 2009) and 18% in southern China (Min et al., 2011). This has caused substantial N leaching (Min et al., 2011), groundwater contamination (He et al., 2007; Zhu et al., 2005), and increased soil acidification (Guo et al., 2010) or salinity (Shi et al., 2009; Song et al., 2012). Deterioration of soil chemical properties may have negative effects on soil communities and overall soil health.

Nematodes play an important role in energy flow and nutrient cycling, they are the most numerous soil mesofauna, and they occupy all consumer trophic levels in soil food webs. Therefore, nematodes may serve as proxy for soil food web structure and composition (Kardol et al., 2010). Root knot nematodes (RKN) are major pests for many economically important crops, and can infect > 2000 plant species (Boina et al., 2008; Jung and Wyss, 1999)

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and reduce nitrogen uptake (Vaast et al., 1998). In addition, interactions between RKNs and other soil-borne pathogens can also contribute to crop yield losses (van der Putten et al., 2006). Growers occasionally use nematicides or fumigants to control RKNs to avoid serious yield loss. However, owing to environment and human health concerns, these chemical fumigants and nematicides have been increasingly withdrawn from use (Boina et al., 2008).

The RKN genus *Meloidogyne* is one of the most economically damaging groups of plant-parasitic nematodes, and have been spreading rapidly and causing serious economic losses in greenhouse vegetable production in China (Li et al., 2010; Wu and Shi, 2011). Generally, vegetable greenhouses with longer planting histories (time since open fields were converted to greenhouse vegetable production) experience the most serious RKN problems. In some cases we have observed significantly yield losses in newly established greenhouses on former cereal fields. However, the impact of planting age on the population density of RKNs, as well as the composition of soil nematode community, is still unknown, especially under excessive N input.

Considering the low nitrogen use efficiency and subsequent N leaching in the intensive greenhouse vegetable cultivation system, it is important to take measures to reduce nitrogen input without reducing crop yields. Under the greenhouse vegetable cultivation system, the soil ecosystem exhibited decreased microbial functional diversity (Lin et al., 2010) and increased soil acidification (Guo et al., 2010). Recently, optimized N fertilization, which aims to maintain the critical threshold of mineral N supply in the root zone soil to match crop N requirements but minimize N losses to the environment, has been implemented and nitrogen use efficiency increased (Ren et al., 2010). It is assumed that reduced of nitrogen application will gradually improve the greenhouse soil ecosystem under intensive vegetable cultivation, and may reduce the abundance of RKNs. If reduced N fertilization reduces RKN densities, N management could be used as a component of integrated RKN management in addition to growing resistant cultivars, crop rotation and chemical pesticides.

The location chosen for our investigations was Shouguang, the largest vegetable production base of China with more than 65% of the arable land under solar greenhouse production. Like other production regions, the intensified greenhouse vegetable production in the Shouguang region also has excessive nitrogen input $(2220 \text{ kg fertilizer N ha}^{-1})$ and irrigation water (1800 mm per year)applied to some crops (He et al., 2007). We hypothesized that the intensified input in vegetable greenhouse production systems would increase the abundance of RKNs and exert adverse effects on soil nematode communities whereas reduced nitrogen fertilization would reduce the abundance of RKNs and improve soil nematode community structure. The present study consisted of two parts, a field survey and an investigation of nematode communities in a long-term N management experiment. The objective of the field investigation was to evaluate the effects of time of cultivation in greenhouse systems on soil nematode communities and population densities of RKNs. In addition, soil samples from a long-term experiment initiated in 2004 for optimization of N application, were collected and analyzed during the period of 2007-2010 to address whether soil nematode communities, especially the population density of RKNs, would be affected by N management.

2. Materials and methods

2.1. Field investigation

2.1.1. Site description

The study site is located in Shouguang (36°41′–37°19′N, 118°32′–119°10′E), Shandong Province, China, a semi-humid

Table 1List of vegetables cropped in the plots investigated (greenhouses of 1-yr, 2-yr, ≥5-yr planting age and open grain fields) at three village sites (Nansunyunzi, Balizhuang, and Luojia), Shouguang, China.

Plot type	Plot no.	Site		
		Nansunyunzi	Balizhuang	Luojia
1-уг	1	Towel Gourd	Cucumber	Tomato
	2	Towel Gourd	Cucumber	Tomato
	3	Tomato	Cucumber	Tomato
2-yr	1	Towel Gourd	Cucumber	Tomato
	2	Pepper	Cucumber	Tomato
	3	Pepper	Cucumber	_a
≥5-yr	1	Towel Gourd	Cucumber	Tomato
	2	Tomato	Cucumber	Tomato
	3	Kidney bean	Cucumber	Tomato
Open grain field	1	Wheat	Wheat	Wheat
	2	Wheat	Wheat	Wheat
	3	Wheat	Wheat	Wheat

^a -, Absence.

temperate region with a mild climate and four distinct seasons. The annual mean temperature is $12.4\,^{\circ}\mathrm{C}$ with a monthly mean maximum of $26.2\,^{\circ}\mathrm{C}$ in July and a monthly mean minimum of $-3.4\,^{\circ}\mathrm{C}$ in January. The frost-free period is 195 days from mid-April until the end of October. Mean annual rainfall is 591.9 mm, which is unevenly distributed with 63% in summer and only 4.1% in winter. The soil type is a molic gleysol based on FAO-UNESCO's Soil Map of the World (21.2% clay, 44.6% sand and 34.2% silt). Unheated solar greenhouses in the region are typically constructed of clay walls and covered with polyethylene film. The material for the clay walls is collected from the soil of the greenhouse using an excavator, and so the ground surface in the greenhouses is approximately 70 cm below the outside ground level and part of the original topsoil surface is used for plant growth.

2.1.2. Soil sampling

In late April 2008 a survey was conducted at three sites (Balizhuang, Nansunyunzi and Luojia villages in Shouguang Shandong, China), located within 20 km of each other and with similar greenhouse management practices. Three categories of greenhouses were chosen in each village, namely 1, 2 and \geq 5 years after open fields were converted to greenhouse vegetable production. Three adjacent open cereal field plots in each village were also included as controls. For each type of cultivation history, three individual plots were sampled at each site. Thus, a total of 35 samples were collected (3 sites \times 3 greenhouse ages \times 3 replicates plus 3 adjacent cereal fields). Because no other representative plots were available, only two 2-year-old plots were sampled in Luojia village. The plant species cropped in the study plots and the numbers of each type of plot are listed Table 1.

Approximately 500 g of composite soil samples (five cores per sample using a 25 mm soil auger) were randomly collected at 0–25 cm depth from each plot. Each soil sample was placed in a sealed plastic bag, taken to the laboratory and stored at $4\,^{\circ}\mathrm{C}$ until analyzed. Subsamples of approximately 200 g were used for nematode extraction and 50 g for gravimetric analysis of water content.

2.1.3. Nematode sampling and identification

Nematodes were extracted using the sugar flotation and centrifugation method and nematode abundance was expressed per 100 g dry soil. Nematodes were identified to genus/family level under a stereomicroscope (100–400×). Nematode taxa were assigned to the following trophic groups: plant parasites (PP), bacterivores (BF), fungivores (FF) and omnivores–predators (OP) based on morphological characters and feeding habitat (Yeates et al.,

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