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Original article

Responses of soil nematode community structure to different long-term fertilizer strategies in the soybean phase of a soybean–wheat–corn rotation

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ABSTRACT

The impact of long-term application of fertilizers in soybean fields on soil nematode community structure was studied. The long-term application model of fertilizers lasted 13 years in a soybean–wheat–corn rotation, and included three treatments: no fertilizer (NF), chemical fertilizer (urea and ammonium phosphate, CF), and pig manure combined with chemical fertilizer (MCF). The soil nematode community structures and ecological indices were determined from soil samples taken at five soybean growth stages from May to October in the soybean phase of the rotation. Fertilizer application had significant effects on abundance of plant parasites, bacterivores and fungivores ($P < 0.05$), but had no significant effects on total nematodes and omnivores-predators. Abundance of plant parasites was higher in NF than in MCF and CF, and abundance of bacterivores was highest in MCF. Fertilizer application significantly affected Plant-parasitic Nematode Maturity Index (PPI) and Nematode Channel Ratio (NCR) ecological indices ($P < 0.05$). Shannon–Weaver Index (H′) and Species Richness (SR) indices were higher in MCF than in either NF or CF. The abundances of total nematode and plant parasites showed increasing trend with soybean growth in all three treatments. This is probably due to soil environment being more suitable for soil nematode survival with more food available for plant parasites as the soybean grows. Soybean growth stage significantly affected the H′, Free Living Nematode Maturity Index (MI) and PPI. Bacterivores significantly correlated with soil nutrient status suggesting that they could be used as a potential indicator of soil fertility.

1. Introduction

Soil nematodes have been widely used as bioindicators to assess soil condition as they respond to changes in the soil environment caused by land use, agricultural management, etc. [7,13,18,27,34,40]. Soil nematodes are commonly assigned to five trophic groups basing on their feeding habits: plant parasites, bacterivores, fungivores, predators and omnivores [50]. Each nematode trophic group has the potential of reflecting a different aspect of changes in soil conditions [19]. The abundances of bacterivores and fungivores are closely related to decomposition of soil organic matter, and the ratio of numbers of these two trophic groups indirectly reflects the decomposition of organic matter and mineralization of nitrogen and carbon [17,33,52]. The numbers of bacterivores and fungivores were higher in soils treated with organic manure than in those treated with chemical fertilizer [10]. Omnivores-predators are most sensitive to environmental disturbances resulting from changes in land use, they were higher in a natural land than in an disturbed agricultural land [6,20,28]. The population of plant parasites was usually higher in agricultural fields than in natural forest or fallow fields [25,38].

Ecological indices calculated from relative populations of different soil nematode groups have been widely applied to quantify the response of the nematode community to environmental changes in soil [14,34,37,46]. The Shannon–Weaver Index (H′) is a useful measure of diversity of nematode community [12,42,52]. Nematode Channel Ratio (NCR) is a powerful index to assess the decomposition pathway of soil matter [51]. Indices of free living nematode maturity (MI) and plant-parasitic nematode maturity (PPI) represent soil nematode life-history characteristics associated

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with *r*-selection (nematode with short generation time, high fecundity and large population fluctuation) and *K*-selection (nematodes with long generation time, few offspring and generally appearing later in succession), respectively [4,33]. MI and PPI have often been used to estimate the functional responses of soil nematodes to environmental change [11,49]. MI and H′ are higher in a relatively stable, mature and natural ecosystem compared with an agricultural ecosystem [12,14,24].

Soil nematodes survive in soil and occupy all consumer trophic levels of soil food web. They have a close relationship with decomposition and mineralization of organic matter in soil, so soil nematodes should be related to some of soil physiochemical properties [15]. Plant parasites with c-p 2 had positive correlation with pH value, the ecological indices of MI and PPI had significant correlation with soil Nitrogen pool [27].

Plant nutrient supplements are required for current agriculture, and chemical fertilizer is intensively used in agricultural fields. In China, chemical fertilizer is also supplemented with manure as a common practice to improve soil fertility and soil structure. Many studies have been published on the effect of long-term fertilizer application on soil nematode community [21,43,52]. The occurrence and abundance of different trophic groups of nematodes have often been associated with crop types and soil management practices [26,32]. Soybean is the main agricultural crop in Heilongjiang province, Northeast China, but the influence of long-term fertilizer or manure supplements on soil nematode diversity in soybean fields has received little attention. The objectives of our study were to determine the effects of long-term fertilizer application on soil nematode abundance and diversity in soybean fields in Heilongjiang province, and to explore the correlation between ecological indices and soil properties.

2. Materials and methods

2.1. Experimental site

The study was carried out on an existing long-term rotation and fertilization experiment established in 1985 at the Hailun Experimental Station of Agricultural Ecology, Heilongjiang province, China (47°26′ N, 126°38′ E). Annual precipitation is about 500–600 mm, with about 80% occurring from May to September, cumulative temperature (≥10 °C) is about 2400–2500 degree-days (°C), and cumulative annual sunshine is 2600–2800 h. The soils in the region are typical black soil (Udic Mollisol), with 23.8 g kg^{-1} Organic C, 1.8 g kg^{-1} total N, 0.8 g kg^{-1} total P, 0.2 g kg^{-1} available N, 0.02 g kg^{-1} available P, and pH 6.1. The field had been a flat plain and with native prairie grasses before it was converted to agricultural land about 100 years ago. No fertilizer was applied to the soil in the region in the first 60 years of agricultural cropping, livestock manures were applied for the next 20 years, and finally, nitrogen fertilizer was applied in the last 20 years.

2.2. Experimental design

A randomized complete block experimental design was employed with four replicates. Each plot was 60 m^2. A 3-year rotation of soybean, wheat and corn was established; this is the main cropping system in NE China. Three fertilizer treatments were applied annually since 1994: no fertilizer (NF), chemical N and P fertilizer (CF) and pig manure combined with chemical N and P fertilizer (MCF). The amount of fertilizers applied was different for each crop. For the chemical fertilizer treatment, 32.3 kg N ha^{-1} and 35.8 kg P ha^{-1} was applied to soybean, 120 kg N ha^{-1} and 24 kg P ha^{-1} to wheat, and 150 kg N ha^{-1} and 32.8 kg P ha^{-1} to corn. The phosphorus fertilizer was in the form of diammonium phosphate and additional N fertilizer was applied as urea to corn and wheat; urea was not applied to soybean. For the manure combined with chemical fertilizer treatment, pig manure was applied at 15 Mg ha^{-1} (wet weight) for soybean and wheat, and 30 Mg ha^{-1} for corn in addition to the respective above amounts of chemical fertilizers. Pig manure contained average total N, P and K concentrations of 22.1, 4.9 and 2.4 g kg^{-1}, respectively. No chemical fertilizer or manure was applied to any of the crops in the no fertilizer (NF) treatment. The plots were plowed to a depth of 25 cm after crop was harvested, and cultivated and planted the following spring.

2.3. Soil sampling

Soil samples were collected on the soybean phase of the soybean–wheat–corn rotation in 2006. Samples were collected at five growth stages of soybean: May 6 (seeding stage), June 7 (seedling stage), July 11 (flowering stage), August 29 (poding stage) and October 3 (ripening stage). Ten soil cores, 5 cm diameter and 20 cm deep were collected from each plot with a manual soil coring tube. The soil samples were taken in the soybean row and between the soybean plants. The soil samples from each plot were combined and thoroughly mixed by hand. All soil samples were passed through a 6 mm mesh soil sieve to remove the plant roots or large stones, and placed in black plastic bags and stored in a refrigerator at 4 °C.

2.4. Nematode extraction and identification

Nematodes were extracted from 100 g wet soil by elutriation and centrifugation [10]. The nematodes were heat-killed at 60 °C and preserved in a triethanolamine formaldehyde (TAF) solution [44]. Total nematodes in each sample were counted with the aid of an anatomical lens. A sub-sample of one quarter of each nematode suspension was observed under a Motic microscope (400× and 1000×) and each nematode was identified to genus using diagnostic keys [3,22,45]. All nematodes were assigned to four trophic groups: plant parasites, fungivores, bacterivores, and omnivores-predators [50]. Nematode genera were also assigned “c-p” values of 1–5, corresponding to their positions along the colonizer-persister continuum of their life-history [4,5]. The abundances of total nematodes and each taxonomic group were adjusted to the number of soil nematodes per 100 g dry soil.

2.5. Ecological index calculation

Nematode communities were characterized by calculating specific indices that describe their structure and function:

(1) Shannon–Weaver Index $H' = -\sum p_i \ln p_i$, where p_i is the proportion of individuals in the *i*th taxon [42].
(2) Species Richness $SR = (S-1)/\ln(N)$, where S is the total number of genera and N is the total number of individuals in the community [49].
(3) Free Living Nematode Maturity Index, $MI = \sum v_i f_i$, where v_i is the c-p value of the *i*th taxon, and f_i is the frequency of the *i*th taxon [4].
(4) Plant-Parasitic Nematode Maturity Index, $PPI = \sum v_i f'_i$, where v_i is the c-p value of *i*th taxon, and f'_i is the frequency of *i*th taxon [4].
(5) Nematode Channel Ratio, $NCR = B/(B+F)$, where B and F are the numbers of bacterivores and fungivores in the total soil nematode population [31,48].