European Journal of Soil Biology 71 (2015) 13–20

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Dynamics of soil nematode communities in wheat fields under different nitrogen management in Northern China Plain

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ARTICLE INFO

Article history:
Received 30 July 2015
Received in revised form
13 September 2015
Accepted 24 September 2015
Available online 2 October 2015

Keywords:
Growth stages
Nematode community
N fertilization
Winter wheat

ABSTRACT

The temporal dynamics of nematode abundance and community composition were monitored in a nitrogen (N) fertilization experiment during the main growth stages of winter wheat in Northern China Plain. A randomized complete block design was used with four levels of N fertilization (50, 100, 150 and 300 kg N ha^{-1} y^{-1} denoted as N1, N2, N3 and N4, respectively). The results showed that winter wheat aboveground biomass increased but soil pH decreased with elevating N fertilization. Total nematode abundance reached the highest values at the shooting stage of wheat, and was significantly increased by the N fertilization. On the contrary, nematode generic richness declined with increasing N fertilization. The relative abundance of bacterivores showed minor changes among different fertilization treatments whereas that of fungivores was suppressed by the N4 treatment. Plant parasites were the most abundant under the N2 treatment. The relative abundance of omnivores–predators declined with increasing N fertilization. The results indicate that trophic groups have distinctive responses to different levels of N fertilization, and N fertilization may induce an abundant but simple nematode community in the winter wheat field of North China Plain. Our findings also highlight the potential of adequate N application to reduce plant parasites and to control agriculture pests.

1. Introduction

With increasing populations and decreasing arable land areas, food security becomes a significant concern in the world, especially in the developing countries [1,2]. Wheat is regarded as the third cereal crops of the world, with more than 600 million tons produced each year (Food and Agriculture Organization of the United Nations, FAO. 2009). In China, wheat is the second largest staple crop and provides more than 20% of the total grains (National Bureau of Statistics of China). In order to increase grain yield, excessive nitrogen (N) fertilizer has often been applied [3–5], which have caused a series of problems related to production costs and environment such as eutrophication of groundwater and soil acidification [6,7].

Nematodes are ubiquitous in soil, sensitive to natural and anthropogenic disturbances, and often used as a biological indicator [8–10]. Previous studies have illustrated that inorganic N fertilizer have profound impacts on the abundance and community composition of soil nematodes [11–13]. However, the magnitude and direction of nematodes in response to N addition are still controversial, varying with the levels of N addition and among different ecosystems. In semiarid grasslands, for example, 25 and 50 kg N ha^{-1} yr^{-1} of N additions show no effects [14], but 120 kg N ha^{-1} yr^{-1} of N addition significantly suppresses soil nematode abundance [15]. By contrast, N enrichment of 300 kg N ha^{-1} yr^{-1} stimulates the population size of soil nematodes in an acid soil of subtropical China [11]. The above contradictory observations may be attributed to the balances between the positive and negative effects of N supply [16–18]. Elevated N can facilitate plant growth and indirectly benefit soil biota [12,19]. However, the direct effects of N addition such as soil acidification and ammonium toxicity may be harmful to soil nematodes [15,20,21]. The determinants of soil nematode abundance under N addition may depend on the level of N fertilization [22]. Thus, multi-level N addition experiment is needed to reveal general

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http://dx.doi.org/10.1016/j.ejsobi.2015.09.002
1164-5563/

patterns of soil nematode community under the N enrichment.

In addition to its impacts on nematode abundance, N fertilization can alter the community structure of soil nematodes due to trophic group-specific responses (i.e. bacterivores, fungivores, plant parasites, and omnivores–predators). For example, significant reductions in nematode community diversity (H′ and taxonomic richness), maturity index (MI), and the abundance of root herbivores, fungivores and omnivores–predators were found across an N enrichment gradient in a semiarid grassland ecosystem [15]. The relative abundances of fungivores (Fu%) and plant parasite (PP%) increase, whereas the relative abundances of bacterivores (Ba%) and omnivores–predators (Om%) decrease following N fertilization in a vegetable greenhouse experiment and in a hybrid napiergrass study [22,23]. In addition, the values of the basal index and channel index (CI) increase following the N fertilization compared to the control in the above mentioned experiment. Given the differential roles of functional groups in regulating biogeochemical processes [8–10], shifting community structure of soil nematodes may influence ecosystem functions under increasing N fertilization in crop fields.

In order to examine the effects of different levels of N fertilization on soil nematodes, a multi-level N fertilization experiment was established with winter wheat in Northern China Plain in 2012. The specific objectives of this study were: 1) to evaluate the responses of soil nematodes to different levels of N additions; and 2) to investigate the temporal dynamics of soil nematodes across the main growth stages of winter wheat.

2. Materials and methods

2.1. Study site and experimental design

A multi-level N addition experiment was setup at the Experimental Station of Global Change Ecology located in Jinming Campus of Henan University (34°49′16.84″N, 114°17′56.76″E), Kaifeng, Henan, China in 2012. The region is part of the Yellow-Huai-Hai River Drainage Basin where winter wheat is planted as a major crop [24,25]. Long-term (1951–2012) mean annual precipitation in the local area is 625 mm, with the majority occurring from April to October. Mean annual temperature is 14.36 °C, with monthly mean temperature ranging from −0.16 °C in January to 27.1 °C in July. The soil is sandy loam (FAO classification system) which is conducive to more evaporation, combined with the shallow groundwater, slow surface runoff and high salinity, make the soil pH (8.66) is strongly alkaline. The soil properties in the surface layer at the beginning of experiment were: 11.04 g kg^{-1} in soil organic C, 0.47 g kg^{-1} in total N, and 1.35 g cm^3 in bulk density.

A randomized complete block design was used in this study. Sixteen plots (3 × 3 m^2) were established with four treatments, N1, 50 kg N ha^{-1} y^{-1}; N2, 100 kg N ha^{-1} y^{-1}; N3, 150 kg N ha^{-1} y^{-1}; and N4, 300 kg N ha^{-1} y^{-1}, and each treatment had 4 replications. The N2 treatment was set according to the typical amount of N applied by local farmers. Corresponding amounts of urea with N concentration of 46.67% were incorporated into soil as base fertilizer (50%) before the wheat seeds were sowed on Oct. 17, 2013 and top-dressing (50%) on Mar. 4, 2014. One hundred and fifty kg phosphorus (P) ha^{-1} y^{-1} of P fertilizer (calcium superphosphate containing 21.8% P_2O_5) was applied to each plot as base fertilizer application.

2.2. Soil sampling

Soil samples were collected at 4 growth stages of winter wheat: March 5 (reviving stage), April 1 (shooting stage), April 20 (heading stage), and May 20 (maturity stage). Four cores (0–10 cm depth) were randomly taken in each plot with a 5 cm-diameter soil auger, and mixed carefully to obtain a composite sample. After removing stones, larger roots and macro-arthropods, soil was divided into two aliquots. One aliquot was used for the analysis of soil physicochemical properties and the other one was used to extract and identify soil nematodes.

2.3. Soil property and winter wheat

Soil water content was measured gravimetrically. Soil pH was determined using the glass electrode (Sartorius PB-10) in 1: 2.5 soil and distilled water. The aboveground biomass of one 1 × 1 m^2 quadrat was clipped in each plot on Jun. 3, 2014, and dried in oven at 65 °C to a constant weight. Root biomass was sampled at 20 cm depth by taking four soil cores with a soil auger (5 cm in diameter). After cleaning and drying, the constant weight was used to calculate root biomass. Across the whole experiment period, height of winter wheat was measured every 5 days. The difference in height at the beginning and end of each growth stage was used to calculate the relative growth rate (RGR) of winter wheat [26].

2.4. Nematode extraction and identification

Nematode population was extracted from 50 g soil using a modified Baermann method [27]. After extracting for 48 h, nematodes were killed at 60 °C, and then preserved in 4% formaldehyde. After counting the total number, 100 individuals were identified to genus level under an optical microscope (Eclipse E200, Nikon Corporation). All nematodes were assigned to four trophic groups: bacterivores (Ba), fungivores (Fu), plant parasites (PP) and omnivores–predators (Om) [28]. Nematode genera were also assigned"*c–p*"values of 1–5 [29]. The total abundance was adjusted to the number of soil nematodes per 100 g dry soil.

2.5. Nematode community diversity and ecological indices

The following diversity and ecological indices were calculated to describe nematode community structure and function.

1. Shannon–Wiener Index: $H' = -\sum p_i \ln p_i$, [30];
2. Simpson dominance index, $\lambda = \sum p_i^2$, where *pi* is the proportion of individuals in the *i*-th taxon [31];
3. Maturity index: $MI = \sum v_i \times f_i$, where v_i is *c–p* value of taxon in according to their *r* or *k* characteristics, f_i is the frequency of taxon *i* in the plot [32];
4. Channel index $CI = 100 \times [0.8\ Fu_2/(3.2\ Ba_1 + 0.8\ Fu_2)]$ [33];
5. Enrichment index: $EI = 100 \times \sum k_e n_e / (\sum k_b n_b + \sum k_e n_e)$ [33];
6. Structure index: $SI = 100 \times \sum k_s n_s / (\sum k_b n_b + \sum k_s n_s)$ [33], where n_b is the abundance of individuals and k_b is the weight in guilds Ba_1 and Fu_2, which represents the basal characteristics of soil food web; k_s is similar weight assigned to Ba_3–Ba_5, Fu_3–Fu_5, Om_3–Om_5, and n_s is the abundance of above mentioned guilds. Ba_x, Fu_x, Om_x (where x = 1–5) represent *c–p* values and feeding groups [34].

2.6. Statistical analysis

Nematode abundance and generic richness were logarithmically transformed ($Y' = \ln[Y + 1]$) to meet the condition of normality. All data were analysed using two-way ANOVAs (treatment and date). Duncan's multiple comparison tests were used if the effects were significant. Simple linear regression was used to examine the relationships of nematode abundance and generic richness with soil water content, soil pH, root biomass, and aboveground biomass