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# Earthworms change the abundance and community structure of nematodes and protozoa in a maize residue amended rice–wheat rotation agro-ecosystem

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ABSTRACT

The influence of earthworms on nematodes and protozoan communities was determined during the wheat phase of a six year rice–wheat rotation agro-ecosystem. Experimental plots in the rotation had five treatments, i.e. incorporation or mulching of maize residues with or without added earthworms and a control. The addition of maize residues to soil strongly affected the abundance and community structure of nematodes and protozoa in the absence of earthworms. The presence of earthworms gave significantly lower total nematode numbers at all soil depths following maize residue incorporation than the same treatment without earthworms, and also gave lower (although not significantly) total nematode numbers in the upper soil layer following maize residue mulching than the same treatment without earthworms. This was mainly due to a significant decrease in bacterial-feeding nematode numbers. Earthworms also strongly affected the distribution of the number of total nematodes and two trophic groups (bacterial and plant feeders) with soil depth. In the presence of earthworms, total protozoan and flagellate numbers significantly increased at all soil depths following both incorporation and mulching of maize residues, while numbers of amoebae increased only when maize residues were mulched. Additionally, in earthworm casts total nematode numbers (mainly bacterial and fungal feeders) were significantly higher, whereas total protozoa numbers (mainly flagellates and amoebae) were significantly lower than that in soil from 0 to 5 cm layer.

These results indicated that earthworm activity could affect the abundance and community structure of microfauna, and change their distribution between soil layers and cast material, depending on the mode of application of organic residues.



## 1. Introduction

In temperate soils earthworms, as ecosystem engineers, have a large effect on biotic and abiotic properties of the soil ecosystem. Previous studies have shown that earthworm activity promoted soil carbon and nitrogen transformations through their impact on soil microorganisms (Blair et al., 1995; Martin et al., 1992; Li et al., 2002). The influence of earthworms on the populations and activity of the soil microfauna (protozoa and nematodes) has also been studied by previous workers. Yeates (1981) had found that the total soil nematode population was reduced by earthworm activity. The presence of earthworms reduced numbers of nematodes in a peat meadow soil (Ilieva-Makulec and Makulec, 2002) and a forest soil (Räty and Huhta, 2003), so interactions between earthworms and nematodes probably show direct effects as a result of earthworm predation. Domínguez et al. (2003) found that earthworm activity strongly decreased nematode numbers (more than 50%) in fresh organic wastes because of direct grazing. The reduction of nematode populations may be associated with earthworm gut passage (Brown et al., 2000). In addition, laboratory experiments have shown that the presence of earthworms (*Lumbricus festivus* and *Lumbricus terrestris*) increased protozoan activity and biomass in bulk soil (Winding et al., 1997) and in the earthworm burrow wall (Alexei et al., 2001). Binet et al. (1998) also had found that protozoan population density was significantly greater (3–19 times) in the presence of earthworms. However, lower numbers of protozoa were discovered in *Allolobophora caliginosa* casts by Aira et al. (2003) and through the digestive tract of *L. terrestris* by Cai et al. (2002), probably due to earthworm digestion. In general, earthworms can directly or indirectly influence soil microbial and

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microfaunal population via their comminution, feeding, burrowing, casting activities and dispersal.

Microbial grazers constitute the main consumers of soil microorganisms and affect nutrient cycling in soil. Nematodes, as typical soil habitants, play a significant role in decomposition of organic matter, mineralization (Griffiths, 1990) and affect plant growth and nutrient uptake (Li and Hu, 2001). Protozoan grazing is required to enhance the mobilisation of nutrients from microbial biomass and microbial turnover (Alexei et al., 2001). Some workers considered the microbial feeding fauna as a better indicator of the activity of microbial populations than the microbial biomass itself (Sohlenius, 1990; Griffiths et al., 1992). Soil microfauna, with limited ability to move within the soil, may be affected by the comparatively long ranging movements of earthworms and appear to be sensitive to earthworm activity (Brown, 1995). Thus, feeding activities of earthworms may be able to change the distribution of microfaunal population in different soil layers, probably because of the redistribution and incorporation of soil microorganisms and organic matter. For example, Ilieva-Makulec and Makulec (2007) found earthworms were able to change the vertical distribution of nematodes in planted soil.

Many previous studies have been focused either over a short time period or laboratory simulations. The effects of earthworms on microfaunal populations ultimately need to be examined with natural food webs and under field conditions. Particularly, when organic residues were applied to agricultural soil in different ways, the effects of earthworms on microfaunal populations were rarely studied in two crops rotation agro-ecosystem. The present study is a part of a field experiment in which the influence of an engineering earthworm species on soil nutrient cycling processes and soil microorganisms is being studied in a six year rice–wheat rotation agro-ecosystem. The aim of this study is to investigate the effect of earthworms on the abundance and community structure of nematodes and protozoa in such a maize residues amended agro-ecosystem.

## 2. Materials and methods

### 2.1. Experiment design

The study was carried out on experimental field at Nanjing Agricultural University (118°47′E and 32°03′N) which had been separated into plots (2.8 m × 1.0 m × 0.6 m) by concrete frames in 2001. The mean atmospheric temperature at the site is 16 °C and the average annual rainfall is 1106 mm. The soil (Orthic Acrisol) had a pH ($H_2O$) of 8.25 and contained 5.86 g $kg^{-1}$ soil organic C and 0.71 g $kg^{-1}$ total N. Each plot was fertilized with urea (210 kg N $ha^{-1}$), superphosphate (46 kg P $ha^{-1}$) and potassium chloride (87 kg K $ha^{-1}$) during rice and wheat cultivation every year. The field was cultivated with a rice–wheat rotation, rice being cultivated with a non-flooding method during the last 10 days of June of ever year, keeping the soil water content at about 80% of maximum water holding capacity throughout the rice season, while wheat was cultivated in the last 10 days of October every year.

*Metaphire guillelmi* was the dominant earthworm species in the experimental field and was removed from the soil in the experimental plots at establishment in 2001. *M. guillelmi* at a density of 70 g $m^{-2}$ (30 ± 2 individuals of adult earthworms) were added to the plots amended with earthworms. At the end of each cultivated season from 2001 to 2006, earthworm biomass was investigated using a sample soil (0.018 $m^3$: 0.3 m × 0.3 m × 0.2 m), and earthworm density readjusted to a density of 0 (CK) or 70 g $m^{-2}$ as appropriate. This gave the following five treatments: maize residues incorporated into soil with no earthworms added (I); maize residues incorporated into soil with earthworms added (IE); maize residues mulched on the soil surface with no earthworms added (M); maize residues mulched on the soil surface with earthworms added (ME); and control soil with no added maize residues or earthworms (CK). Maize residues (7500 kg $ha^{-1}$, chopped <2 cm) containing 7.96 g N $kg^{-1}$, 2.85 g P $kg^{-1}$, 10.67 g K $kg^{-1}$, and 65.8 C/N were applied to the appropriate plots during rice and wheat cultivation every year. There were four replicates per-treatment. For the IE treatment, soil, fertilizer and maize residues were homogenized before earthworms were added. For the ME treatment, the sequence was as follows: homogenizing soil with fertilizer, mulching with the maize residues and then adding the earthworms.

### 2.2. Sampling and analysis

Soil samples were taken after the wheat harvest on 12 June 2006. In each plot eight soil cores of an area of 2.5 $cm^2$ to depth of 20 cm were taken randomly using a steel corer. Each soil core was separated into 0–5, 5–10 and 10–20 cm soil, and then was pooled together as a composite sample. Earthworm casts were collected manually from the soil surface. These samples were kept at 4 °C prior to analysis. Earthworms were also hand-sorted from a volume of soil (0.018 $m^3$: 0.3 m × 0.3 m × 0.2 m) in all plots to investigate the earthworm biomass of each plot at the end of the wheat phase. Nematodes were extracted from 20 g fresh soil or cast soil with a modified Baermann method using trays instead of funnels (Goodfriend et al., 2000), after 48 h of extraction at room temperature, the nematode suspension was collected and the numbers of all nematodes counted under a dissecting microscope. Nematodes were heat-killed for 2 min at 60 °C and preserved in 4% formaldehyde (Griffiths et al., 2002). Subsequently, an average of 100 nematodes per sample was identified to four trophic groups (bacterial feeders, fungal feeders, plant feeders and omnivores/predators; Yeates et al., 1993). For samples in which there were fewer than 100 nematodes, all specimens were identified. Protozoa were determined by the most probable number method (Darbyshire et al., 1974). Neff's Modified Amoebae Saline (Page, 1976) with 0.2 g Nutrient Broth $l^{-1}$ was used as dilution medium (NMAS). Fresh soil samples (5 g) were suspended in 20 ml NMAS on a rotary shaker (100 rpm) for 20 min. Aliquots of the diluted suspensions were incubated in 96-well microtitre plates using twofold dilutions with four replicates each. Bacterial flora inoculated with the soil suspension served as food source for the protozoa. After 7, 14 and 21 days of incubation at 22 °C, the presence of naked amoebae, flagellates and ciliates was determined using an inverted microscope.

### 2.3. Statistical analysis

A three-way ANOVA was applied to test the effects of the presence of earthworm on microfauna with independent variables of earthworms, residue treatments and soil depth in soil with mulched (M) or incorporated (I) maize residues with (E) or without earthworms. A one-way ANOVA was applied to test the effects of residues on microfauna in different soil layers using only the treatments of CK, I and M. Differences between means were tested with LSD test ($p < 0.05$). All statistical analysis was done using the software package SPSS11.0.

## 3. Results

In those treatments with no added earthworms (CK, M and I), only a few individuals of earthworms (*M. guillelmi*) were observed at the end of each cultivated season from 2001 to 2006 (Table 1). This may be explained by contamination of earthworms during