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Effect of Different Bacterial-Feeding Nematode Species on Soil Bacterial Numbers, Activity, and Community Composition*[1]

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ABSTRACT

The effects of bacterial-feeding nematodes on bacterial number, activity, and community composition were studied through a microcosm experiment using sterilized soil inoculated with soil bacteria (soil suspension) and with bacteria and three species of bacterial-feeding nematodes (*Cephalobus persegnis*, *Protorhabditis filiformis*, and *Caenorhabditis elegans*). Catalyzed reporter deposition-fluorescence *in situ* hybridization, CO_2 evolution, and denaturing gradient gel electrophoresis (DGGE) of PCR amplified 16S rRNA gene fragments were used to investigate bacterial numbers, activity, and community composition, respectively. Our results showed that bacterial numbers and activity significantly increased in the presence of bacterial-feeding nematodes, which indicated that bacterial-feeding nematodes had a significant positive effect on soil bacteria. The different nematode species had different effects on bacterial numbers and activity. *C. persegnis* and *P. filiformis*, isolated from native soil, increased the bacterial number and activity more than *C. elegans*. The DGGE analysis results showed that dominant bacterial species significantly differed among the treatments, which suggested that bacterial-feeding nematode species modified the bacterial community composition in soil. Further gene sequence analysis results showed that the dominant bacterial species in this study were gram-negative bacteria. Given the completely same conditions except nematode species, the varied selective feeding behavior of different nematode species was the most likely reason for the altered bacterial community composition. Overall, the alteration of bacterial numbers, activity and community composition resulting from the bacterial-feeding nematodes may ultimately affect soil ecological functioning and processes.

Key Words: CARD-FISH, CO_2 evolution, DGGE, gene sequence, gram-negative bacteria

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Bacterial community structure and activity are central factors that influence terrestrial ecosystem functions (Kennedy and Gewin, 1997) and are changed by bacterivorous predators such as bacterial-feeding nematodes (Griffiths *et al.*, 1999; Rønn *et al.*, 2002; Djigal *et al.*, 2004) that graze in soils. Bacterial-feeding nematodes have been recognized as a main bacterivorous predator (Griffiths, 1994; Li and Hu, 2001) because of their greater abundance and consumption in soils. For example, Bernard (1992) and Liang *et al.* (2000) found that nematodes are the most abundant metazoans in the soil, ranging from 7.6×10^5 m^{-2} in deserts to 2.9×10^7 m^{-2} in mixed deciduous forests. Furthermore, Venette and Ferris (1998) found that an adult bacterial-feeding nematode consumes 1×10^6 cells daily. Bacterial-feeding nematodes have a huge grazing potential on soil bacteria. However, this does not mean that the number of bacteria will be substantially reduced. Bacteria respond variably to nematode grazing and conclusions regarding the changes in bacterial numbers caused by nematode grazing have been inconsistent. Researchers found that bacteria increase when predators graze on them (Abrams and Mitchell, 1980; Traunspurger *et al.*, 1997; Bardgett *et al.*, 1998), whereas others obtained the contrasting results (Coleman *et al.*, 1977; Anderson *et al.*, 1983).

Numerous studies have also reported bacterial-feeding nematodes that graze on bacteria, thereby influencing bacterial activity (usually by detecting soil respiration and enzyme activity) (Anderson and Coleman, 1977; Trofymow *et al.*, 1983; Djigal *et al.*, 2004; Fu *et al.*, 2005). Most of these studies showed increased bacterial activity in the presence of nematodes. For example, Fu *et al.* (2005) found that microcosms with nematodes produced significantly more CO_2 than those without nematodes. Although the alterations of bacterial numbers and activity in the presence of bacterial-feeding nematodes were reported by

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many previous studies, related reasons were deficiently discussed and need to be explored more deeply.

Aside from affecting bacterial abundance and activity, bacterial-feeding nematodes that graze on bacteria even modify the bacterial community composition (Griffiths *et al.*, 1999; Djigal *et al.*, 2004). However, previous studies did not examine which bacterial species changed, and the possible mechanisms have been rarely reported and need more investigation. Generally, nematodes are selective and display specific preferences when grazing. In turn, different bacterial resources may be appropriate for different nematode species and affect nematode growth and fecundity. Venette and Ferris (1998) found that six different bacterial-feeding nematode species have different reproduction rates according to the ingested bacterium. Selective feeding behavior may induce competition between microbes, thereby altering the community composition and distribution in soil (Fu *et al.*, 2005; Blanc *et al.*, 2006).

Considering the complexity of environmental conditions and the limitation of research tools, observing the activity of microfauna and bacteria directly in the soil is difficult. Recently, however, catalyzed reporter deposition-fluorescent *in situ* hybridization (CARD-FISH) with horseradish peroxidase (HRP)-labeled oligonucleotide probes and tyramide signal amplification and DNA fingerprinting (such as denaturing gradient gel electrophoresis, DGGE) have been used to investigate the numbers and the community structure of bacteria, respectively. CARD-FISH, instead of conventional monolabeled FISH (oligonucleotide probes labeled with Cy3 fluorochrome), was suitable for soil microorganism analysis (Pernthaler *et al.*, 2002; Eickhorst and Tippkötter, 2008). Both the detection sensitivity and specificity of CARD-FISH were higher than those of monolabeled FISH (Haugland, 2005; Eickhorst and Tippkötter, 2008). Muyzer *et al.* (1993) and Pernthaler *et al.* (2002) successfully used CARD-FISH and DGGE to investigate the bacterial distribution and community structure.

Alterations in bacterial abundance, activity, and community composition are related to soil ecological functions because bacteria are the drivers of many biochemical reactions in soil. Considering that different nematode species likely have different effects on bacterial community composition because of their selective feeding behavior, we hypothesize that bacterial-feeding nematodes modify the bacterial community composition because of their preference for different bacterial species. Two native bacterial-feeding nematode species isolated and identified from the soil used in this study, namely, *Cephalobus persegnis* and *Protorhabditis filiformis*, and *Caenorhabditis elegans* were used to compare the effect of different bacterial-feeding nematode species on the soil bacterial number, activity, and community composition. Altered bacterial species were also examined in this study.

MATERIALS AND METHODS

Soil

The soil used in this study was a sandy soil (57.5% sand, 26.6% silt, and 15.9% clay) collected from Nantong City, Jiangsu Province, China. The soil contained 6.03 g kg^{-1} organic C, 0.70 g kg^{-1} total N, and 0.68 g kg^{-1} total P, and the pH (H_2O) was 6.85. Before use, the fresh soil was passed through a 2-mm mesh to remove stones, macrofauna, and some broken roots. A portion of the soil was used to isolate nematodes and prepare bacterial suspensions, and the remainder was sterilized by heating at 121 °C (102.9 kPa) for 30 min (Xiao *et al.*, 2010a).

Nematode isolation and incubation

Two native nematode species were isolated from the soil *via* a modified cotton wool filter method (Liang *et al.*, 2009) and identified as *Cephalobus persegnis* and *Protorhabditis filiformis*, both of which are bacterial-feeding nematodes that belong to the family Rhabditidae. A single individual of each was allowed to multiply on an agar plate with Tryptic soy broth culture containing mixed soil bacteria (soil bacterial suspension being coated onto the plates) as the food source. *C. elegans* maintained in long-term cultures in our laboratory was selected because it also belongs to the family Rhabditidae and considered a model organism. All three nematode species were reared on agar plates to generate enough for inoculation. These three nematode species had similar generation times of 4 to 5 d when incubated at 28 °C.

Soil preparation

To obtain nematode-free soil abundant with bacteria, the soil was sterilized and then treated with a bacterial suspension to increase the bacterial count. The bacterial suspension was filtered through two filter membranes with 5 μm pores to eliminate nematodes. Each pot contains 1 000 g of sterilized soil. Up to 40 mL of nematode-free inoculum of the mixed soil bacteria (about 2×10^6 g^{-1} dry soil) was inoculated into each pot.