



# The effects of nematode grazing on nitrogen mineralization during fungal decomposition of organic matter

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## Abstract

Soil fungi and nematodes isolated from a long-term Sustainable Agriculture Farming Systems (SAFS) project research site at the University of California, Davis were investigated in microcosm systems. Nitrogen-free sand in the columns was amended with ground alfalfa and cellulose, with total N held constant, to create C-to-N ratios of 11:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1 and 45:1. Nitrogen mineralization by *Aphelenchus avenae* and *Aphelenchoides composticola* feeding on *Rhizoctonia solani* and *Trichoderma* sp. was determined by measuring ammonium and nitrate concentrations in the leachate from the columns at 3-d intervals. Nematode population numbers and the fatty acid 18:2  $\omega$ 6c, a fungal biomass indicator, were monitored by destructive sampling on d 0, 7, 14 and 21. For *R. solani*, but not *Trichoderma* sp., there was significantly more N extracted from columns in the presence of either nematode species than in the absence of nematodes. Average N-mineralized nematode<sup>-1</sup> d<sup>-1</sup> was 1.8 ng for *A. avenae* and 3.3 ng for *A. composticola* when feeding on *R. solani*. As the C-to-N ratios of organic substrates increased, total mineral N decreased with *R. solani* alone, but in general remained the same in the presence of nematodes. Initial and average nematode population densities were significantly higher in columns containing *R. solani* than in those with *Trichoderma* sp. Both nematode species reduced the fungal fatty acid 18:2  $\omega$ 6c in *Trichoderma* columns on d 21. The fatty acid 18:2  $\omega$ 6c was lower in columns containing both *R. solani* and *A. composticola* on d 0 and 7 and higher on d 14 and 21 than those in the absence of nematodes. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** Nitrogen mineralization; Nematode grazing; Fungal decomposition; Effects of C-to-N ratio

## 1. Introduction

Mineralization is a key process in supplying nitrogen and other nutrients for plant growth in terrestrial ecosystems (Alexander, 1977; Marion et al., 1981). Because of high consumption and low assimilation rates, microbial grazers release considerable amounts of nutrients that are then available to plants (Anderson et al., 1981). Up to 30% of total net nitrogen mineralization has been attributed to microbial grazers (Elliott et al., 1988). Studies, confirming that N-mineralization in bacterial-dominated decomposition pathways may be enhanced by the presence of nematode grazing (Ferris et al., 1998), have suggested

benefits from managing soils to enhance the activity of these nematodes in sustainable farming systems (Ferris et al., 1996). Relatively little work, however, has been done on nematode–fungal interactions in nutrient cycling during fungal-dominated decomposition of organic matter.

The ability of nematodes to affect growth of soil fungi appears to be variable and to depend on the species of nematode (Ingham et al., 1985) and fungus (Mankau and Mankau, 1963). The fungal-feeding nematode, *Aphelenchus avenae*, containing 1.45 ng N per individual on the average, consumed 6.1 ng N from 120 ng fungal cytoplasm in 24 h (Ingham et al., 1985). Myers and Krusberg (1965) reported that total nitrogen discharged by *Ditylenchus trififormis* was 2.6 ng per nematode d<sup>-1</sup> when feeding on the fungus *Pyrenochaeta terrestris*. *A. avenae* increased N mineralization and <sup>14</sup>CO<sub>2</sub> evolution by *Fusarium oxysporum* in

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substrates amended with cellulose and chitin, but not with cellulose alone (Trofymow and Coleman, 1982; Trofymow et al., 1983).

In this investigation, we characterized and quantified the main components of a fungal decomposition pathway. The system included a range of carbon-to-nitrogen (C-to-N) ratios of the organic substrates and included fungal-nematode combinations representing good and poor hosts. The specific objectives were to (1) measure N mineralization by fungal-feeding nematodes and their fungal hosts; (2) examine the influence of different C-to-N ratios of organic substrates on N-mineralization of the fungi and fungal-feeding nematodes; and (3) monitor the dynamics of nematode populations and fungal biomass.

## 2. Methods and materials

The studies were conducted in column microcosms in the laboratory at room temperature (20–24°C). Columns were packed with N-free sand to minimize background contamination in N-mineralization measurements. The column system enabled periodic leaching for assessment of N-mineralization with minimal disturbance.

### 2.1. Preparation

#### 2.1.1. Medium

Nitrogen-free acid-washed sand was used as medium. Sand particle size (dia, % retained) consisted of 1.0 mm (0.6%), 0.5 mm (8.6%), 0.25 mm (54.2%), 0.10 mm (32.4%), and 0.05 mm (1.1%). There were 1.8% of silt and 1.3% of clay in the bulk sand. Sand (20 kg) was washed in a cement mixer for 1 min. The water was drained and the process was repeated 8–10 times until the drainage water was clear. The washed sand was placed in a plastic container and treated with 1 l of 3 M H<sub>2</sub>SO<sub>4</sub> for 48 h. The acid solution was drained and the sand was rinsed with water in a cement mixer 10 times (Ferris et al., 1998). The sand was autoclaved twice for 4–6 h at 24 h intervals. After cooling, the moisture content of the autoclaved sand was determined. The difference between wet weight and dry weight of the sand was considered the weight of soil solution.

#### 2.1.2. Organisms

Soil fungi used were *Rhizoctonia solani* and *Trichoderma* sp., provided by Dr Ariena Van Bruggen, Department of Plant Pathology, University of California, Davis. They were maintained on potato dextrose agar (PDA) culture at 20°C. Finely-ground dry barley (*Hordeum vulgare*) leaf and stem tissue (2.1% N and 41.0% C) were mixed with sand in 9-cm

glass Petri dishes at a ratio of 1-to-50 (w/w) for the purpose of fungus and nematode culture. One plug (1 cm dia) of 5-d-old PDA fungal culture was transferred to the 2% barley-sand mixture that had been autoclaved twice for 20 min. The PDA plug was removed after the fungus had colonized the barley-sand mixture. The dishes were sealed with parafilm and incubated in the dark at 20°C for 4–5 d.

Fungal-feeding nematodes, *A. avenae* Bastian, 1865 and *Aphelenchoides composticola* Franklin, 1957, were maintained in gnotobiotic cultures with *R. solani* on PDA. The two species are representatives of the Aphelenchidae and Aphelenchoididae (Order Tylenchida; Phylum Nemata), respectively. Both nematodes represent Maturity Index category 2, a measure of their reproductive potential and relative ability as colonizers of disturbed habitats (Bongers, 1990). The generation time was ca. 11 d at 20°C and 8 d at 23°C for *A. avenae* and *A. composticola*, respectively. Voucher specimens of the nematodes are deposited in the University of California Davis Nematode Collection with the following accession numbers: *A. composticola*—UCDNC 3640-3642, and *A. avenae*—UCDNC 3643-3644. All soil fungi and nematodes used were from a long-term Sustainable Agriculture Farming Systems (SAFS) project research field at University of California, Davis, CA.

#### 2.1.3. Determination of C-to-N ratio of nematodes and fungi

Fungal-feeding nematodes, cultured on PDA or 2% barley-sand mixture, were collected by incubating the cultures on Baermann funnels filled with distilled-deionized water (ddH<sub>2</sub>O) for 8 h. Three replicates were prepared for each of the nematode cultures. After centrifugation of the suspension in a 15-ml glass centrifuge tube, the supernatant was discarded, and the pellet was then resuspended in ddH<sub>2</sub>O. This process was repeated three times at 750 g for 30 s, 30 s, and 2 min. Nematodes were concentrated in the bottom of the tube. Twenty µl of nematode concentrate was packed into foil capsules (5 × 9 mm; Costech), covered with Chromosorb (80/100 mesh, Carlo-Erba Instruments).

Fungi were grown in potato dextrose broth (PDB) for 4 d on a shaker rotating at 150 rev min<sup>-1</sup> at 24°C. Fungal hyphae were harvested and washed five times using ddH<sub>2</sub>O on a vacuumed filter device. The hyphal masses were transferred and packed in foil capsules.

Nematode and fungal samples were subjected to combustion analysis in a Carlo-Erba Carbon Nitrogen Elemental Analyzer. The % C and % N were recorded for each sample and its supernatant to obtain the C-to-N ratio of nematodes or fungi (Ferris et al., 1997).

### 2.1.4. Inoculation

Each fungus cultured for 4–5 d on the barley–sand mixture was finely chopped and mixed into sand in buckets. The resulting barley content of the sand was 0.4% (w/w). The buckets were covered with lids to minimize contamination by airborne propagules and to reduce drying. Nematodes harvested from cultures on *R. solani* were mixed into the sand in one bucket for each fungus species at a rate of three nematodes  $g^{-1}$  sand. An aeration tube was inserted into the bottom of each bucket as the bucket was filled with sand. Aeration was provided through a 0.3  $\mu m$  glass microfiber in-line filter device (Whatman, Hepa-Vent). The moisture content of the soil in the buckets was maintained at 8–10% by weighing the buckets and adding water to adjust for weight loss at 2-d intervals. The buckets were maintained at room temperature for 2 wk to allow the nematodes to increase and disperse. Populations were counted by removal of cores on d 12. Preliminary observations showed that *R. solani* was a good host and *Trichoderma* sp. was a poor host for the two species of fungal-feeding nematodes. We achieved nematode population densities in the bucket with *R. solani* of 21 *A. avenae* and 40 *A. composticola*  $g^{-1}$  of sand as the initial population sizes for the microcosm experiments. Nematode populations in the bucket with *Trichoderma* sp. were 8 and 10 nematodes  $g^{-1}$  of sand for the two species, respectively.

### 2.1.5. Organic substrate

Alfalfa (*Medicago sativa*) and cellulose were used to furnish appropriate organic amendments to sand in experimental columns. Although organic material decomposed by fungi is often of larger dimension in the soil, we used relatively small particles to allow uniform distribution throughout each column and to reduce variability among columns. Alfalfa leaf and stem tissue with a C content of 41.3% and an N-content of 3.73% were ground in a Wiley mill (Arthur N. Thomas Co., Philadelphia, PA) to 833  $\mu m$  particle size. The cellulose (Fibrous Cellulose Powder, W. and R. Balston, Ltd., England) had a C content of 43.2% and an N-content of 0.067%. Alfalfa leaf and stem tissue (C-to-N=11:1) with or without additional cellulose (C-to-N=645:1) provided the C-to-N ratios of 11:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1 and 45:1 for each microcosm experiment (Ferris et al., 1998).

## 2.2. Column experiments

### 2.2.1. Column design and packing

Polyvinyl chloride tubes (31.5 cm length, 4 cm dia), capped on one end with a hole (6 mm dia), were used in the column experiments. A disk of 246  $\mu m$  stainless steel mesh was placed over the hole in the capped end to minimize sand loss. This system, compared to a

dish or flask microcosm, allowed periodic N-leaching and avoided destructive sampling for measurement of N-mineralization. Columns and stainless steel mesh screens were washed thoroughly. They were sterilized by autoclaving at 121°C for 20 min prior to use. Alfalfa leaf and stem tissue (1.00 g), and an appropriate amount of cellulose necessary to provide the C-to-N ratio, were mixed into 450–470 g subsamples of the sand incubated with fungus with or without nematodes by gentle and thorough mixing in a plastic bag. Columns were tapped gently as they were filled to ensure a compact continuum without air pockets. A 5-cm space was left at the top for addition of leaching water. Each treatment combination was established with three replications. A total of 48 columns was arranged horizontally in randomized complete blocks in boxes. The columns were rotated 180° every 3 d to minimize gravitational effects on soil moisture distribution. All experiments were conducted at  $20 \pm 2^\circ C$ , except for the experiment with *A. composticola* and *R. solani*, when the room temperature was  $24 \pm 2^\circ C$  during the course of experiment.

### 2.2.2. Sampling

Each column, arranged vertically with capped end down, and supported on a 150-ml glass beaker, was leached on d 0 with 100 ml of ddH<sub>2</sub>O to minimize initial amounts of mineral N. The leachate was discarded. Every 3-d thereafter, holes in the base-cap of the columns were stoppered and 60 ml of ddH<sub>2</sub>O was poured into each column. After 3–4 min the stoppers were removed. All columns were allowed to drain for 2 h and the amounts of leachate collected in the beakers were recorded. Six ml of leachate were poured into 15-ml polypropylene centrifuge tubes (Corning, New York), capped and placed in a freezer prior to further processing.

### 2.2.3. Nematode population and fungal biomass

Eight extra columns of C-to-N ratios 11:1, 20:1, 30:1 and 40:1 with fungi and/or nematodes; were established to allow weekly destructive sampling for determination of fungal biomass and nematode population densities. In the destructive sampling process, ca. 100 g sand from each column was collected in a plastic bag and stored in a freezer for biochemical analysis. An additional 50 g of sand was placed in a 0.5 cm layer on a Whatman No. 4 filter paper supported by a concave screen (6 cm dia at bottom) in a Baermann funnel (Anderson and Coleman, 1977). The sand was partially submerged in water. A 50 ml suspension was harvested from the funnel system 24 h later. Nematodes in three replicates of 1 ml of the suspension were counted on a Hawksley slide. Extraction efficiency of the method was determined by using a sand sample containing a known number of nematodes and counting the number

recovered after 24 h. The extraction efficiencies of 79.3% for *A. avenae* and 83.5% for *A. composticola* were used to adjust nematode population numbers. The initial nematode population was determined from two additional columns at the d 0 leaching. All columns were sampled at the end of the experiment for nematode population numbers. The population dynamics of fungal-feeding nematodes over time were plotted using nematode populations measured on d 0, 7, 14 and 21. Extractions were also performed on the nematode-free treatments as a check against contamination. None of the nematode-free columns was contaminated with nematodes in any of our experiments.

Phospholipid fatty acid (PLFA) analysis was used to measure fungal biomass (Bossio and Scow, 1997). Eight g (dw) soil samples were extracted in a one phase extraction mixture of 26.3% chloroform, 52.6% methanol, and 21.1% phosphate buffer (1:2:0.8 v/v/v). The supernatant, after centrifuging at 904 g, was mixed with phosphate buffer and chloroform, and allowed to separate overnight. The chloroform layer was decanted and dried under N. Solid phase extraction tubes, 6-ml 0.5 g Si sorbent cartridges (Supelco, Inc., Bellefonte, PA), were used to elute neutral and glycolipids with chloroform following acetone. Polar lipids were eluted with methanol and dried under nitrogen at 32°C. Samples were then subjected to mild alkaline methanolysis by dissolution in 1:1 MeOH:toluene and 0.2 M KOH, and heating at 37°C for 15 min. Resulting fatty acid methyl esters were extracted with hexane after adding ddH<sub>2</sub>O and acetic acid. Samples were redissolved in hexane containing 19:0 as an internal standard, and analyzed using a Hewlett Packard 6890 Gas Chromatograph with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column (J and W Scientific). Peaks were identified using standards and the automated microbial identification MIDI system (MIDI, Inc., Newark, DE). Biomass of fungus was measured as nanogram (ng) of fatty acid 18:2 ω6c extracted g<sup>-1</sup> sand. The total amount of bacterial PLFA was used to indicate biomass of bacteria (Bossio and Scow, 1997; Vestal and White 1989). It accounted for 18.3% of total PLFA on d 21.

#### 2.2.4. Soil chemistry

Frozen leachate samples were thawed by 5 min immersion in warm water. Six-ml leachate was added to an equal amount of 4 M KCl to displace bound NH<sub>4</sub><sup>+</sup> (Bundy and Meisinger, 1994) and shaken for 1 h. Serum separators (16 mm × 4, I.B. Model, Fisher Scientific) were used to filter the sample solution in tubes before testing. Nitrate and ammonium concentrations were determined using a diffusion-conductivity analyzer (Carlson, 1978). N as NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were expressed as N-NH<sub>4</sub> and N-NO<sub>3</sub>, respectively. The

total N-mineralized in each column was the sum of the N-NH<sub>4</sub> and N-NO<sub>3</sub>.

### 2.3. Assessment of nitrogen mineralization

#### 2.3.1. Ammonia and nitrate

Standard curves were developed using known concentrations of NH<sub>4</sub>Cl and KNO<sub>3</sub>. The amount of N-NH<sub>4</sub> and N-NO<sub>3</sub> in the sample was determined by comparison with the known standards. The total amount of N in the leachate sample was expressed as μg or ng N g<sup>-1</sup> sand. The precision of the sample mean was indicated by standard error (SE). The daily N-mineralization in the 3-d interval was interpolated as the amount of N measured at each sampling date divided by three. The N-mineralized nematode<sup>-1</sup> d<sup>-1</sup> was calculated by the daily N divided by the number of nematodes. Net-N is the amount of N in the column with nematodes subtracted from that without nematodes.

#### 2.3.2. Rate of N-mineralization due to nematodes

The relationship between cumulative N and time corresponded to the natural growth function:

$$N_t = N_p(1 - e^{-kt})$$

where  $N_t$  was the cumulative amount of N-mineralized by time  $t$ ,  $N_p$  was the nitrogen mineralization potential of the system (Stanford, 1982). The regression coefficient,  $k$ , indicating the rate of mineralization, was divided into two components for statistical comparison of N-mineralization with or without nematodes. The interaction between nematode and fungus was a temporal event which affected the rate of mineralization and was included as an additional time term ( $T$ ) using qualitative independent variables (Neter et al., 1990). The following equations were used:

$$\ln(N_p - N_t) = \ln(N_p) + k_f t + k_n t T$$

$$\ln(N_p - N_t) = \ln(N_p) + k_f t \quad \text{for columns without} \\ \text{nematodes } (T = 0)$$

$$\ln(N_p - N_t) = \ln(N_p) + (k_f + k_n)t \quad \text{for columns with} \\ \text{nematodes } (T = 1)$$

where  $\ln(N_p)$  and  $k$ -coefficients can be determined by linear regression,  $k_f$  is the rate of mineralization due to fungal activity, and  $k_n$  is the rate of mineralization due to nematodes (Ferris et al., 1998). When  $k_n < 0$  or  $> 0$ , the cumulative N-curve for the columns with nematodes will appear graphically above or below of the curve for the columns without nematodes. The  $P$

Table 1  
The ratio of carbon to nitrogen (C-to-N ratio) of fungi and fungal-feeding nematodes

| Organism               | Substrate              | C-to-N ratio | SE   |
|------------------------|------------------------|--------------|------|
| <i>A. avenae</i>       | Potato dextrose agar   | 10.9:1       | 0.69 |
| <i>A. avenae</i>       | 2% barley-sand mixture | 8.5:1        | 0.06 |
| <i>A. composticola</i> | Potato dextrose agar   | 9.0:1        | 0.23 |
| <i>A. composticola</i> | 2% barley-sand mixture | 8.0:1        | 0.17 |
| <i>R. solani</i>       | Potato dextrose broth  | 8.0:1        | 0.69 |
| <i>Trichoderma</i> sp. | Potato dextrose broth  | 8.9:1        | 0.87 |

value associated with  $k_n$  will indicate whether nematodes have a significant influence on the rate of N-mineralization.

### 3. Results

#### 3.1. C-to-N ratios of fungi and nematodes

The C-to-N ratios of the fungi grown on PDB were 8.0:1 for *R. solani* and 8.9:1 for *Trichoderma* sp. The C-to-N ratios of the fungal-feeding nematode *A. avenae* grown on PDA and 2% barley-sand mixture were 10.9:1 and 8.5:1, respectively. The C-to-N ratios of the fungal-feeding nematode *A. composticola* grown on PDA and 2% barley-sand mixture were 9.0:1 and 8.0:1, respectively (Table 1).

#### 3.2. N-mineralization by fungi

At  $20 \pm 2^\circ\text{C}$ , the rate of N-mineralization in the columns containing *R. solani* or *Trichoderma* sp. ranged from 35 to 52 ng N  $\text{g}^{-1}$  sand  $\text{d}^{-1}$ ; and N accumulated after 21 d ranged from 0.738 to 1.082  $\mu\text{g}$  N  $\text{g}^{-1}$  sand. At  $24 \pm 2^\circ\text{C}$  in *R. solani* columns, the rate was 125 ng

N  $\text{g}^{-1}$  sand  $\text{d}^{-1}$ ; and a total of 2.7  $\mu\text{g}$  N  $\text{g}^{-1}$  sand had accumulated after 21 d (Table 2; Fig. 1).

#### 3.3. N-mineralization by nematodes

The rate of N-mineralization ranged from 31 to 37 ng N  $\text{g}^{-1}$  sand  $\text{d}^{-1}$  in the columns containing *A. avenae* or *A. composticola* feeding on *Trichoderma* sp., a poor fungal host of the nematodes. After d 21, 694 to 772 ng N  $\text{g}^{-1}$  sand had accumulated. (Table 2; Fig. 1).

In *R. solani* columns, the rate of N-mineralization in the presence of *A. avenae* was 74 ng N  $\text{g}^{-1}$  sand  $\text{d}^{-1}$ ; and 1.554  $\mu\text{g}$  N sand had accumulated after 21 d. The rate in the presence of *A. composticola* was 287 ng N  $\text{g}^{-1}$  sand  $\text{d}^{-1}$ ; and 6.0  $\mu\text{g}$  N  $\text{g}^{-1}$  sand had accumulated after 21 d (Table 2; Fig. 1).

Feeding of *A. avenae* and *A. composticola* on *R. solani* resulted in increases of N-mineralization of 1.8 and 3.3 ng N nematode $^{-1}$   $\text{d}^{-1}$ , respectively. Both nematode species feeding on *Trichoderma* sp. decreased N-mineralization by  $-1.4$  and  $-1.3$  ng N nematode $^{-1}$   $\text{d}^{-1}$  for *A. avenae* and *A. composticola*, respectively (Table 2). However, for *A. avenae* during the first 6 d and for *A. composticola* between d 16 and d 21, there was more N-mineralized in *Trichoderma* columns with nematodes than in those without nematodes. The highest net-N-mineralized by *A. avenae* and *A. composticola* feeding on *R. solani* occurred during d 7–9 and d 10–12, respectively. N-NH $_4$  accounted for 84–98% and 67–91% of the N extracted from the columns with or without nematodes, respectively. Less N-NO $_3$  was extracted in most of the columns containing nematodes than in nematode-free columns.

#### 3.4. Effect of C-to-N ratio of substrates

The amount of total N and N-NH $_4$  extracted from

Table 2  
Effect of nematode population numbers, nematode species, fungal hosts (F), and temperature on N-mineralization<sup>a</sup>

| Fungal host/nematode   | Nematode $\text{g}^{-1}$ sand |      | Temperature, $^\circ\text{C}$ | N (ng $\text{g}^{-1}$ sand $\text{d}^{-1}$ ) |              | Net N                                   |                                     |
|------------------------|-------------------------------|------|-------------------------------|----------------------------------------------|--------------|-----------------------------------------|-------------------------------------|
|                        | Initial                       | Mean |                               | F                                            | F + Nematode | ng $\text{g}^{-1}$ sand $\text{d}^{-1}$ | ng nematode $^{-1}$ $\text{d}^{-1}$ |
| <i>R. solani</i>       | 21                            | 22   | $20 \pm 2$                    | 35                                           | 74           | 39                                      | 1.8                                 |
| <i>A. avenae</i>       |                               |      |                               |                                              |              |                                         |                                     |
| <i>R. solani</i>       | 40                            | 48   | $24 \pm 2$                    | 125                                          | 287          | 162                                     | 3.3                                 |
| <i>A. composticola</i> |                               |      |                               |                                              |              |                                         |                                     |
| <i>Trichoderma</i> sp. | 8                             | 10   | $20 \pm 2$                    | 45                                           | 31           | -14                                     | -1.4                                |
| <i>A. avenae</i>       |                               |      |                               |                                              |              |                                         |                                     |
| <i>Trichoderma</i> sp. | 10                            | 12   | $20 \pm 2$                    | 52                                           | 37           | -15                                     | -1.3                                |
| <i>A. composticola</i> |                               |      |                               |                                              |              |                                         |                                     |

<sup>a</sup> N was the sum of N-NH $_4$  and N-NO $_3$ . Net-N was the amount of N in columns (F) with the absence of nematodes subtracted from columns (F + Nematode) with the presence of nematodes.

(a)

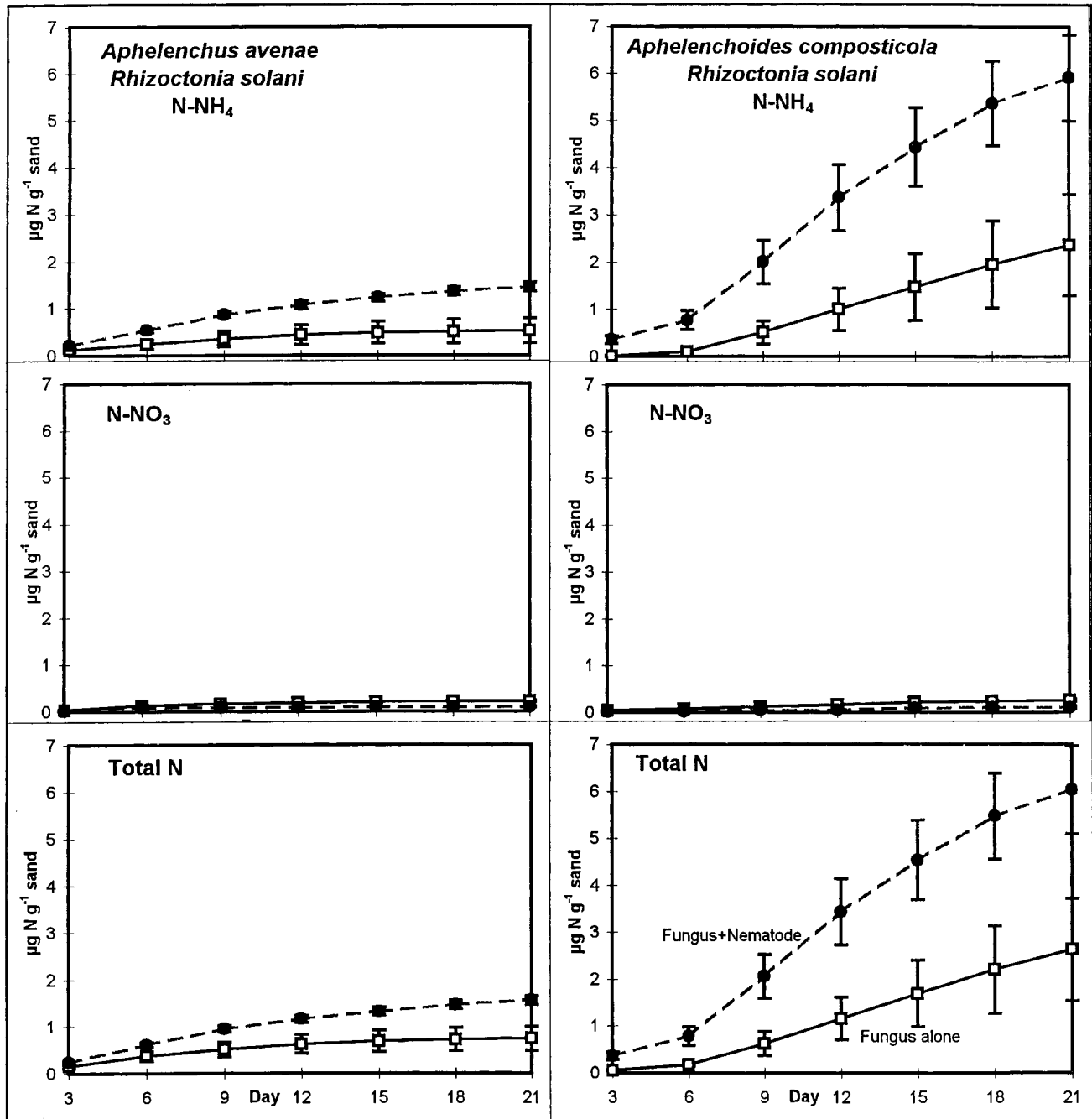


Fig. 1. Cumulative N-mineralized by fungi and fungal-feeding nematodes. (a) *A. avenae* and *A. composticola* and their fungal host, *R. solani*. (b) *A. avenae* and *A. composticola* and their fungal host, *Trichoderma* sp. Empty squares and solid lines indicate cumulative N-mineralized by fungus alone. Solid circles and dotted lines indicate cumulative N-mineralized by fungus and fungal-feeding nematode. Bars indicate SE. Some bars are obscured by symbols.

(b)

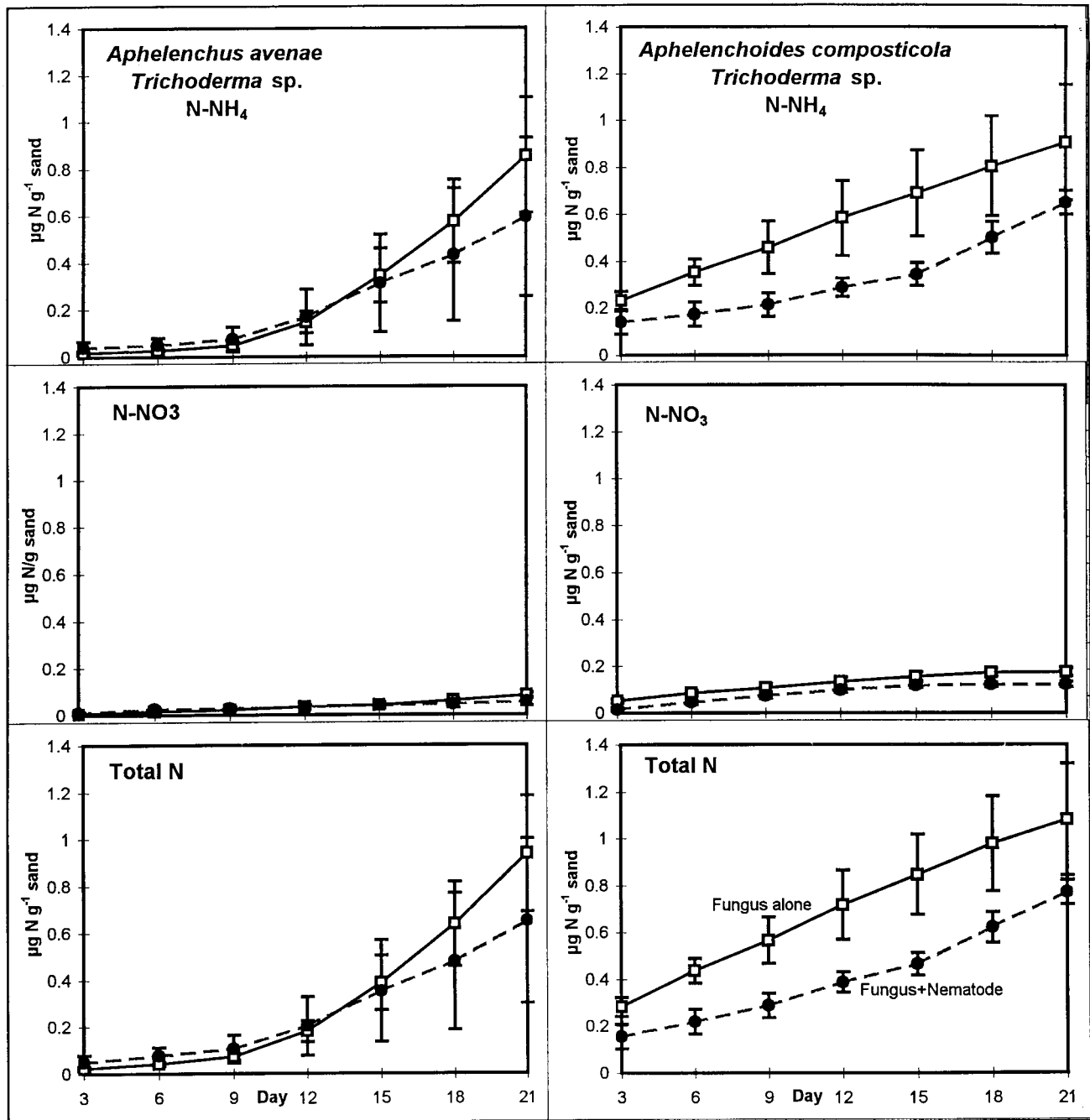


Fig. 1 (continued)

(a)

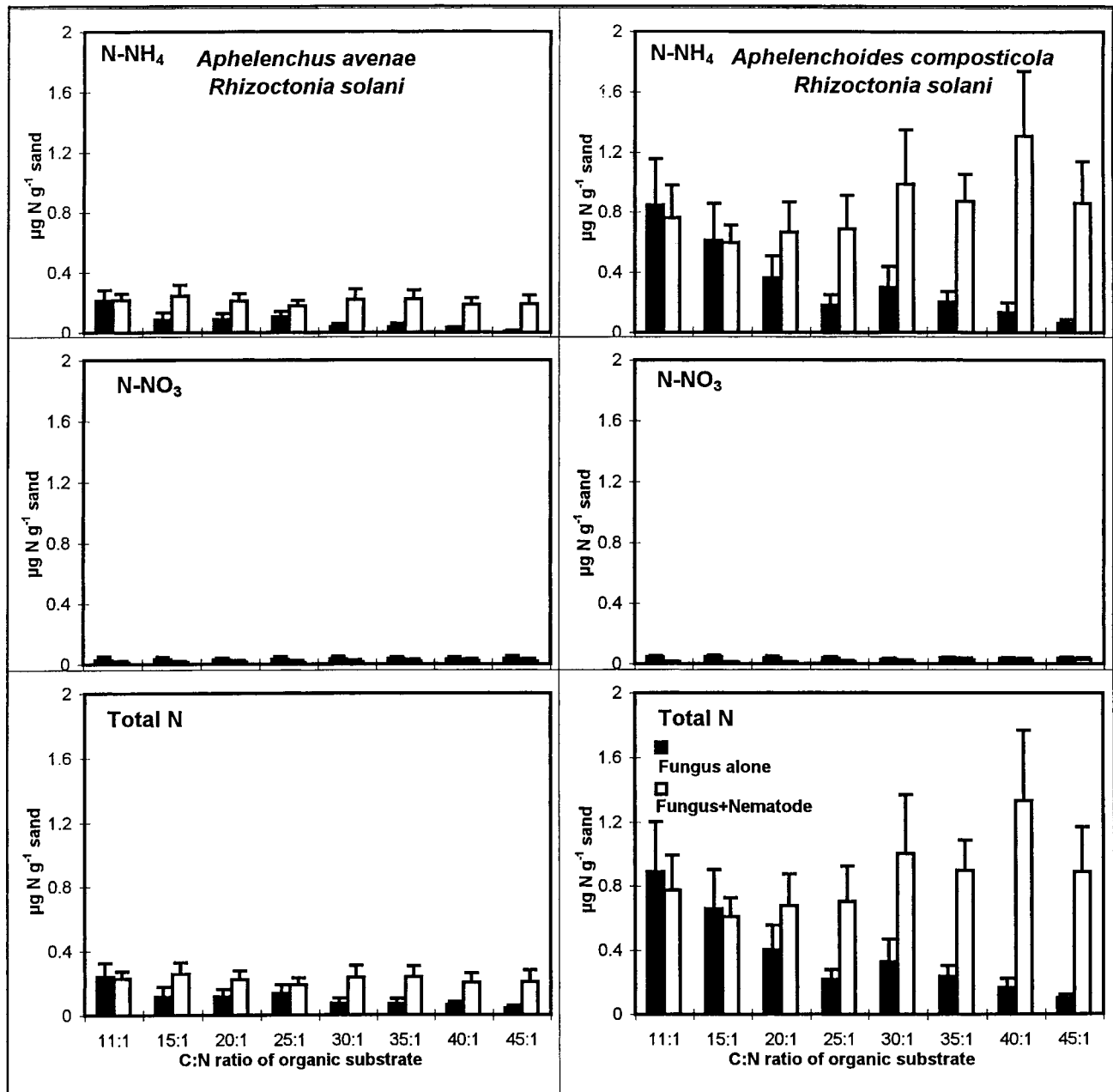


Fig. 2. Effect of C-to-N ratios of organic substrates on N-mineralization by fungi and fungal-feeding nematodes. (a) *A. avenae* and *A. composticola* and their fungal host, *R. solani*. (b) *A. avenae* and *A. composticola* and their fungal host, *Trichoderma* sp. The mean is based on N extracted over seven sampling dates. Solid columns indicate cumulative N-mineralized by fungus alone. Empty columns indicate cumulative N-mineralized by fungus and fungal-feeding nematode. Bars indicate SE. Some bars are obscured by symbols.



(b)

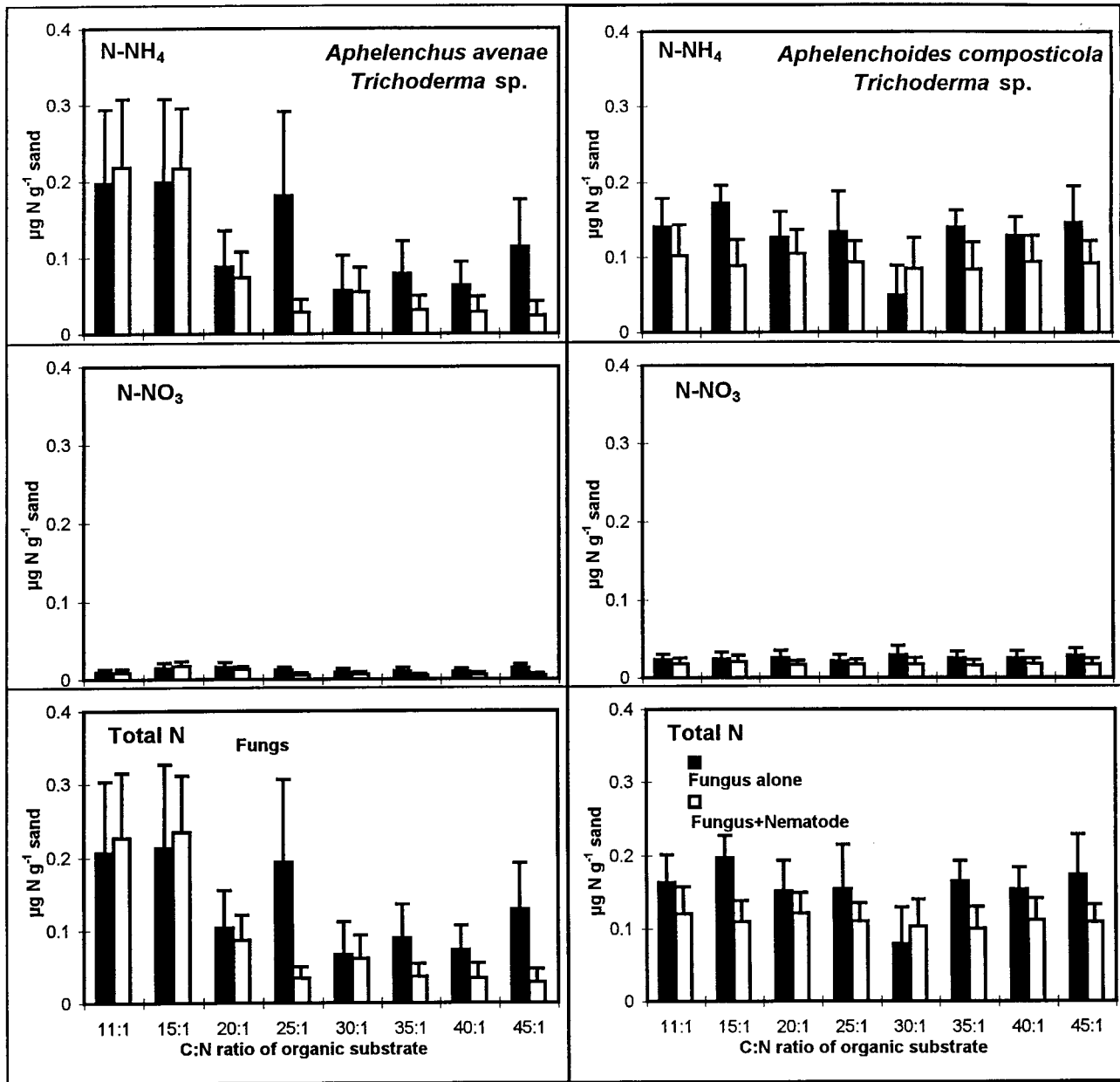


Fig. 2 (continued)

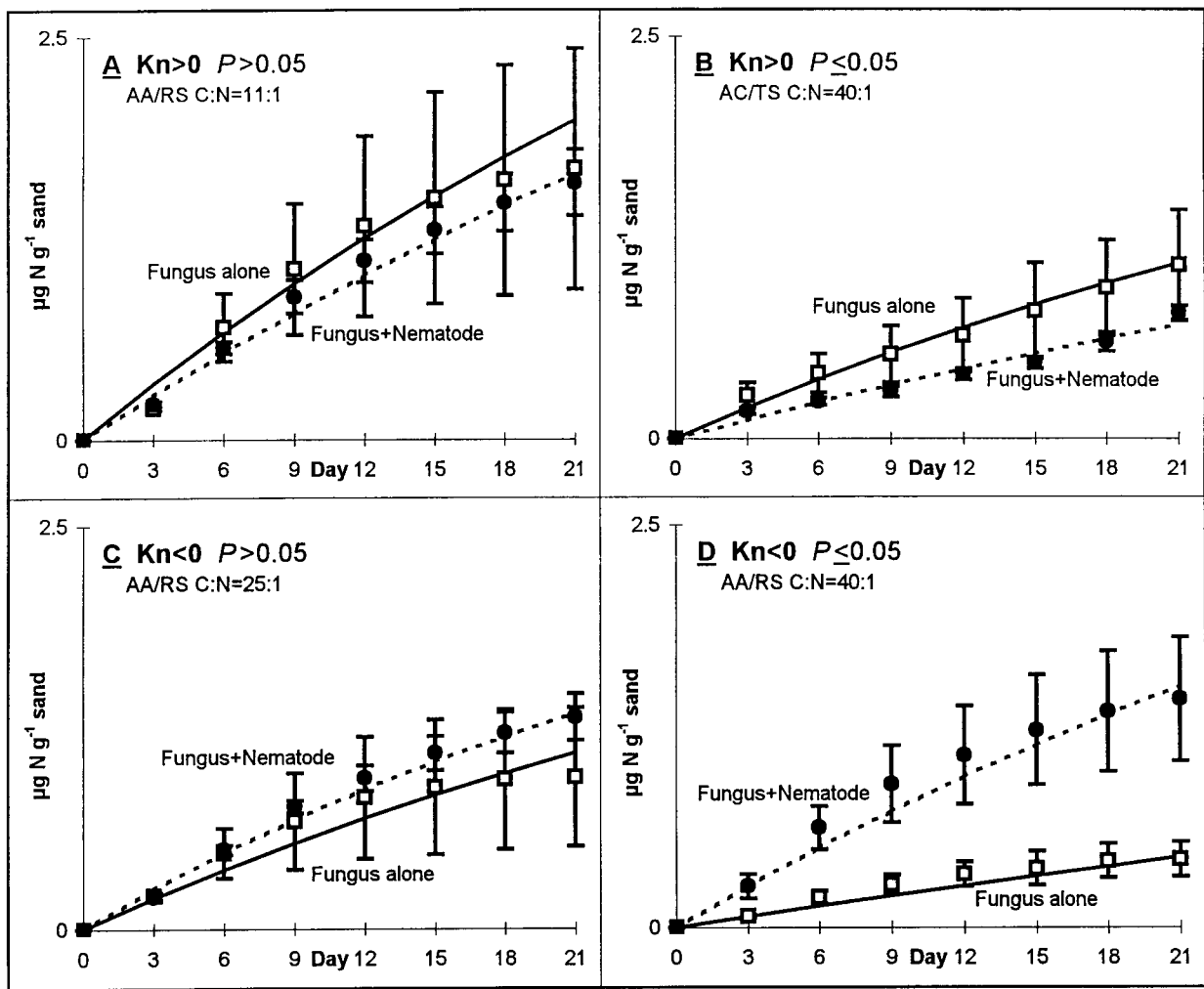


Fig. 3. N-mineralization in columns with fungus alone or fungus plus nematode.  $k_n$  is the contribution of nematodes to the coefficient ( $k$ ) of the natural growth function fitted to N-mineralized from columns with or without the presence of nematodes. A, B, C, and D represented all patterns of results, and four data sets were used in this figure to give graphic presentations. When  $k_n$  has positive and negative values, less and more N are mineralized in the columns with the presence of nematodes than in their absence, respectively. AA = *A. avenae*; AC = *A. composticola*; RS = *R. solani*; and TS = *Trichoderma* sp. Empty squares indicate observed N-mineralized by fungus alone. Solid circles indicate observed N-mineralized by fungus and nematode. Solid lines indicate cumulative N-mineralized by fungus alone. Dotted lines indicate fitted N-mineralized by fungus and nematode. Bars indicate SE. Some bars are obscured by symbols.

*R. solani* columns without nematodes decreased with increasing C-to-N ratio in both experiments (Fig. 2a). But in the columns containing fungal-feeding nematodes, the total N and N-NH<sub>4</sub> in general remained the same with increasing C-to-N ratio, except for columns containing *A. avenae* and *Trichoderma* sp. where amounts of extracted N decreased with the increase of C-to-N ratio (Fig. 2).

The amount of total N and N-NH<sub>4</sub> extracted from the columns containing *Trichoderma* sp. alone appeared to decrease in the first experiment with increasing C-to-N ratio but remained the same in the second experiment (Fig. 2b). There appeared no significant influence of C-to-N ratio of substrates on N-NO<sub>3</sub> under the research conditions (Fig. 2).

### 3.5. Nematode contribution to N-mineralization indicated by $k_n$

The fitted cumulative N, calculated by  $N_t = N_p(1 - e^{-kt})$ , corresponded to the observations at each sampling date (Fig. 3). There were three patterns of the relationship between  $k_n$  and  $k_f$  (the respective contributions of nematodes and fungi to the rate of N-mineralization):  $k_n < k_f$ ,  $k_n > k_f$ , and  $k_n = k_f$  (Fig. 3, Table 3). They are represented as four graphs: (1)  $k_n < 0$ ,  $P < 0.05$ ; (2)  $k_n < 0$ ,  $P > 0.05$ ; (3)  $k_n > 0$ ,  $P < 0.05$ ; and (4)  $k_n > 0$ ,  $P > 0.05$  (Fig. 3). In graphs A and B, there was less N-mineralized in the columns in the presence of nematodes than in their absence with the difference significant in graph B. The fitted cumu-

Table 3

Influence of fungal-feeding nematodes on N-mineralization at different C-to-N ratios of the organic substrate. Parameter  $k_n$  is the contribution of fungal-feeding nematodes to  $k$ , the coefficient of the natural growth function ( $N_t = N_p(1 - e^{-k_t t})$ ) fitting to N-mineralized from columns with or without the presence of nematodes. When  $k_n < 0$  and  $P < 0.05$ , there is significantly more nitrogen mineralized in the column with the presence of fungal-feeding nematodes than in the nematode-free columns

| Species                                        | C-to-N ratio            | $k_n$    | $P$      |
|------------------------------------------------|-------------------------|----------|----------|
| <i>A. avenae</i><br>( <i>R. solani</i> )       | 11:1                    | 0.00489  | > 0.05   |
|                                                | 15:1                    | -0.01907 | < 0.05   |
|                                                | 20:1                    | -0.01675 | < 0.05   |
|                                                | 25:1                    | -0.00528 | > 0.05   |
|                                                | 30:1                    | -0.01990 | < 0.05   |
|                                                | 35:1                    | -0.02047 | < 0.05   |
|                                                | 40:1                    | -0.01698 | < 0.05   |
|                                                | 45:1                    | -0.02512 | < 0.05   |
|                                                | <i>Trichoderma</i> sp.) | 11:1     | -0.00304 |
| 15:1                                           |                         | -0.00528 | > 0.05   |
| 20:1                                           |                         | 0.000840 | > 0.05   |
| 25:1                                           |                         | 0.013065 | < 0.05   |
| 30:1                                           |                         | -0.00111 | > 0.05   |
| 35:1                                           |                         | 0.005549 | < 0.05   |
| 40:1                                           |                         | 0.007275 | < 0.05   |
| 45:1                                           |                         | 0.014743 | < 0.05   |
| <i>A. composticola</i><br>( <i>R. solani</i> ) |                         | 11:1     | 0.000502 |
|                                                | 15:1                    | 0.000386 | > 0.05   |
|                                                | 20:1                    | -0.01091 | < 0.05   |
|                                                | 25:1                    | -0.01730 | < 0.05   |
|                                                | 30:1                    | -0.02497 | < 0.05   |
|                                                | 35:1                    | -0.01721 | < 0.05   |
|                                                | 40:1                    | -0.02149 | < 0.05   |
|                                                | 45:1                    | -0.02786 | < 0.05   |
|                                                | <i>Trichoderma</i> sp.) | 11:1     | 0.006924 |
| 15:1                                           |                         | 0.014124 | < 0.05   |
| 20:1                                           |                         | 0.013528 | < 0.05   |
| 25:1                                           |                         | 0.012022 | < 0.05   |
| 30:1                                           |                         | -0.00903 | > 0.05   |
| 35:1                                           |                         | 0.011392 | < 0.05   |
| 40:1                                           |                         | 0.010282 | < 0.05   |
| 45:1                                           |                         | 0.014902 | < 0.05   |

lative N for columns with nematodes was less than that for columns without nematodes. In graphs C and D, there was more N-mineralized in the columns in the presence of nematodes than in their absence with the difference significant in graph D. The fitted cumulative N for columns with nematodes was greater than that for those without nematodes.

### 3.6. Dynamics of nematode populations

The initial population and average population densities of *A. avenae* and *A. composticola* on *R. solani* were 21 and 40; and 22 and 48 nematodes  $g^{-1}$  sand,

Table 4

Effect of fungal-feeding nematodes on viable fungi. Fatty acid 18:2  $\omega$ 6c was used as a bioindicator of fungal biomass present in the soil. The fatty acid nomenclature used was as follows: total number of carbon atoms:number of double bonds, followed by the position of the double bond from the methyl end of the molecule. *Cis* geometry is indicated by the suffix c. '-Nematode' = absence of nematode; '+ Nematode' = presence of nematode. Number in the parentheses indicates SE

| Day | Organisms                                      | 18:2 $\omega$ 6c Biomass (ng $g^{-1}$ sand) |             |
|-----|------------------------------------------------|---------------------------------------------|-------------|
|     |                                                | -Nematode                                   | +Nematode   |
| 0   | <i>R. solani</i> / <i>A. composticola</i>      | 4.29 (0.62)                                 | 2.11 (0.23) |
| 7   | <i>R. solani</i> / <i>A. composticola</i>      | 17.96 (1.15)                                | 9.88 (1.28) |
| 14  | <i>R. solani</i> / <i>A. composticola</i>      | 4.33 (0.32)                                 | 4.84 (0.41) |
| 21  | <i>R. solani</i> / <i>A. composticola</i>      | 2.52 (0.51)                                 | 4.24 (0.14) |
| 21  | <i>R. solani</i> / <i>A. avenae</i>            | 4.00 (0.81)                                 | 4.17 (0.23) |
| 21  | <i>Trichoderma</i> sp./ <i>A. avenae</i>       | 4.22 (0.58)                                 | 3.11 (0.41) |
| 21  | <i>Trichoderma</i> sp./ <i>A. composticola</i> | 6.38 (0.18)                                 | 4.90 (0.33) |

respectively (Table 2; Fig. 4). The initial population and average population numbers of *A. avenae* and *A. composticola* on *Trichoderma* sp. were 8 and 10; and 10 and 12 nematodes  $g^{-1}$  sand, respectively (Table 2; Fig 4). The highest population densities usually emerged on d 7 (Fig. 4).

Fungal biomass and nematode population number for *R. solani* and *A. composticola* were plotted (Fig. 5). Significantly less fungal biomass, indicated by fatty acid 18:2  $\omega$ 6c, was extracted from the columns in the presence of nematodes than in their absence on d 0 and 7. On d 14 and 21 in columns containing both *R. solani* and *A. composticola*, the 18:2  $\omega$ 6c was higher than in those in the absence of nematodes. Both nematode species reduced the biomass of *Trichoderma* sp. on d 21 (Table 4). There was no difference between the amount of fungi in columns containing *A. avenae* feeding on *R. solani* and that in columns with *R. solani* alone on 21 d (Table 4).

## 4. Discussion

Knowledge on the composition of organic substrates, fungal host status, and fungal-feeding nematode species is useful in understanding the function of fungal-dominated decomposition pathways. In this investigation, we have measured: (1) N-mineralization by different fungal hosts and nematode species, (2) the effect of the C-to-N ratio of organic substrates, and (3) the population dynamics of fungi and fungal-feeding nematodes during the decomposition of organic matter.

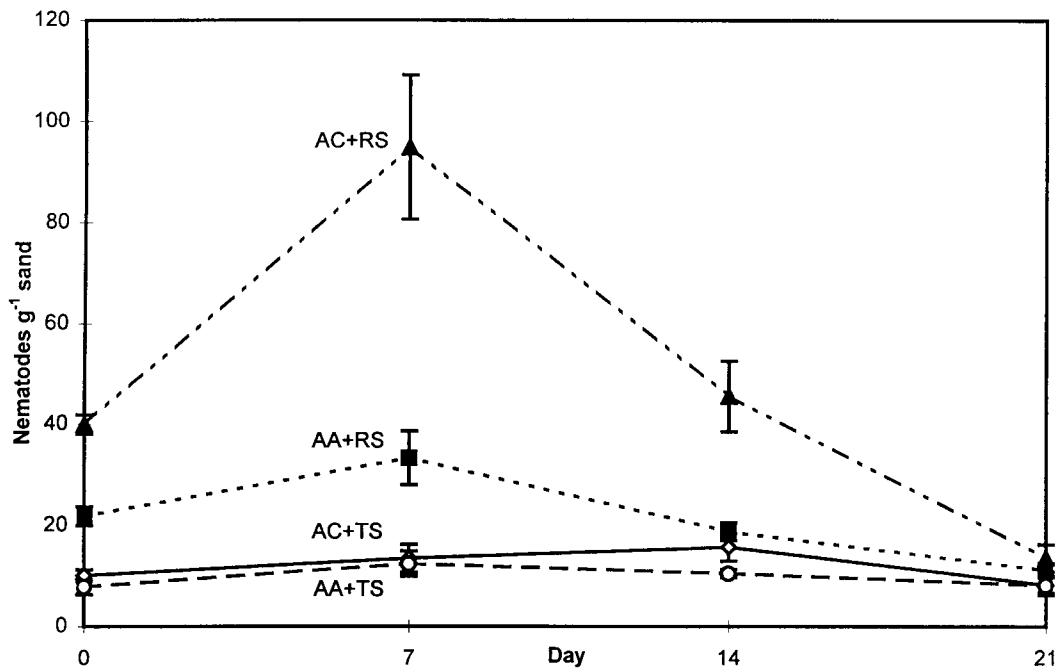


Fig. 4. The population dynamics of nematodes feeding on their fungal hosts. AA=*A. avenae*; AC=*A. composticola*; RS=*R. solani*; and TS=*Trichoderma* sp. Solid triangle=AC+RS. Solid square=AA+RS. Empty diamond=AC+TS. Empty circle=AA+TS. Bars indicate SE. Some bars are obscured by symbols.

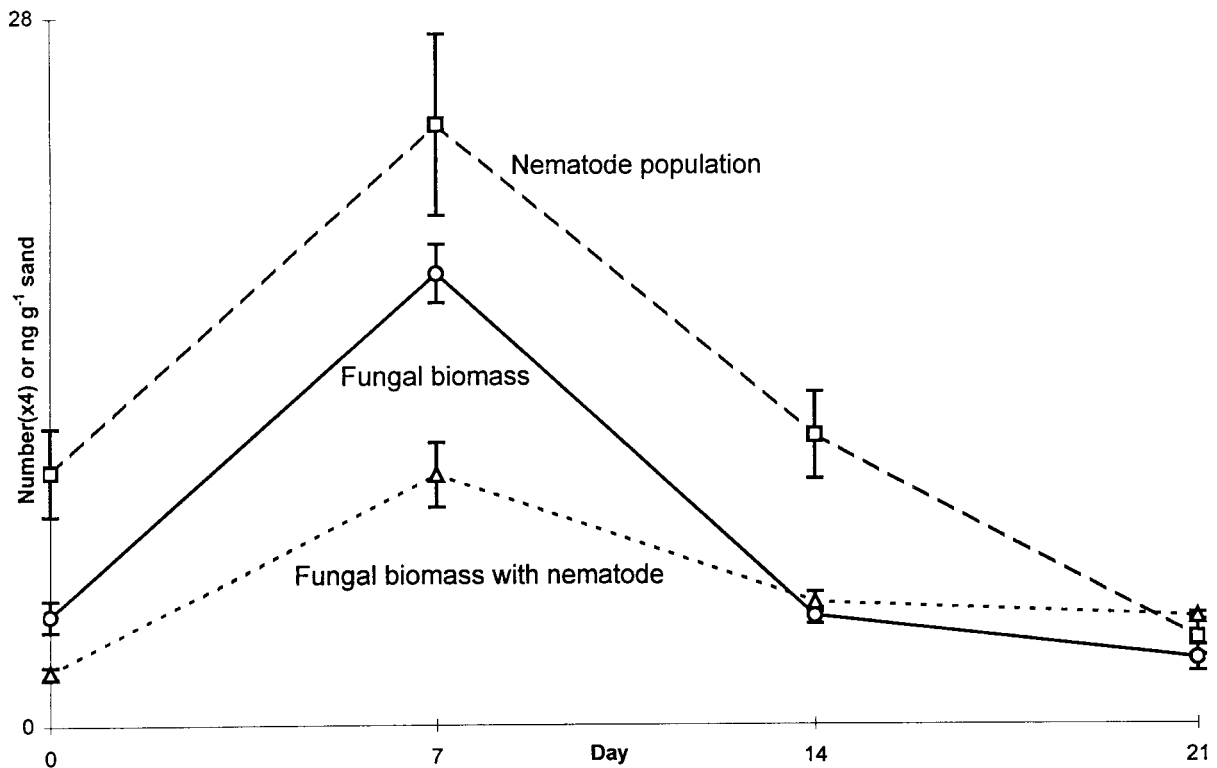


Fig. 5. Influence of fungal-feeding nematodes on fungal biomass. Fungal biomass of *R. solani* was indicated by the amount (ng) of fatty acid 18:2  $\omega$ 6c extracted on d 0, 7, 14, and 21. Nematode population of *A. composticola* is indicated by number of individuals g<sup>-1</sup> sand. Empty squares indicate nematode population densities. Empty circles indicate fungal biomass (18:2  $\omega$ 6c). Empty triangles indicate fungal biomass in the presence of nematodes. Bars indicate SE. Some bars are obscured by symbols.

#### 4.1. N-mineralization by fungi and fungal-feeding nematodes

In our study, more mineral N was measured in columns containing fungal-feeding nematodes and *R. solani* than in nematode-free columns. Less mineral N was measured in columns containing fungal-feeding nematodes and *Trichoderma* sp. than in nematode-free columns. The status of the fungi as good or poor hosts for nematodes is an important factor in N-mineralization by fungal-feeding nematodes. Although the host ranges of fungal-feeding nematodes have not been studied exhaustively, several important phytopathogenic soil fungi are good hosts of *A. avenae*, and the nematodes have been tested as biological control agents (Mankau and Mankau, 1962; Klink and Barker, 1968; Barnes et al., 1981; Gupta, 1986; Ishibashi and Choi, 1991). Some saprophytic fungi, such as *Trichoderma viride*, have been observed, on the other hand, to support meager populations of fungal-feeding nematodes (Mankau and Mankau, 1962). Besides their direct phytopathogenic effects, soil-borne plant pathogens also immobilize N so that less is available to plants (Scarsbrook, 1965). It would be interesting to examine the potential of fungal-feeding nematodes both as agents for biological control and for enhancing soil fertility. Further studies are warranted.

Fungal mycelium grown on agar cultures has been reported to contain about 5% N by dry weight and has a C-to-N ratio of about 10:1 (Griffin, 1972). In our investigation, the C-to-N ratio of the two fungi cultured on PDA ranged from 8.0:1 to 8.9:1. The C-to-N ratio of the two fungal-feeding nematodes cultured on different substrates ranged from 8.0:1 to 10.9:1. Using the estimate of 6.1 ng N consumed each day by *A. avenae* and the body content of 1.45 ng N per individual (Ingham et al., 1985), only a small amount of the N in the fungi consumed was used for production ( $N_p$ ) of nematode biomass or shed ( $N_s$ ) as cuticle and egg shell. The majority of N consumed ( $N_c$ ) was either not assimilated and released as feces ( $N_f$ ), or excreted ( $N_e$ ) according to the N balance model:  $N_c = N_p + N_s + N_e + N_f$ . This is consistent with the observation that microbial grazers have high consumption and low assimilation rates (Anderson et al., 1981), and therefore release considerable amounts of nutrients during their feeding and metabolic processes. The 1.8 and 3.3 ng N-mineralized  $d^{-1}$  individual $^{-1}$  for the two species of nematodes feeding on *R. solani* in our study were in the range of the above and other sources (De Soyza, 1973). The majority of N extracted from the leachate samples in our study was in the form of ammonia, a result that was compatible with the known excretory products of nematodes (Wright and Newall, 1976; Lee and Atkinson, 1977).

In our investigation, much more N was detected in

the columns of *R. solani* containing *A. composticola* than *A. avenae*. Since the N-effect could be attributed to a higher initial and average nematode population or higher temperature, conclusions regarding the relative importance of *A. composticola* cannot be drawn from our study. Further, in our associated field studies, *A. composticola* usually occurs in lower population numbers than *A. avenae* (Ferris et al., 1996). Due to the high contributions to mineralized N by *A. composticola* on a per capita basis, 3.3 ng N-mineralized  $d^{-1}$  individual $^{-1}$  feeding on *R. solani*, we suggest that there are research opportunities for developing field management practices to enhance population numbers of this species. Based on its greater natural abundance, *A. avenae* appears to be a potentially significant contributor to available N, particularly where decomposition pathways are fungal dominated.

#### 4.2. Effect of C-to-N ratio

C-to-N ratio in mature humus may range from 12:1 to 20:1 (Klein and Klein, 1988). The plant materials used to amend agricultural soils normally have higher C-to-N ratios, many as high as 40:1 or 50:1. Adding organic matter with a C-to-N ratio less than ca. 30:1 did not immobilize N (Gasser, 1969). As the ratio decreased, N was increasingly mineralized. During the decomposition process, the C-to-N ratio of organic matter was progressively reduced. In our study, we examined N-mineralization for C-to-N ratios between 11:1 and 45:1. The fungus *R. solani* alone mineralized less N-NH $_4$  as the C-to-N ratio of the substrate increased. However, there was no reduction of N-NH $_4$  in the presence of either nematode species as the C-to-N ratio increased. The concentration of N-NO $_3$  in leachates was low and was not affected by C-to-N ratios of the organic substrates. Deficiencies of N-NH $_4$  while decomposition is in an early stage, and by fungi with characteristics similar to those of *R. solani*, might be altered by the presence or introduction of fungal-feeding nematodes. Further studies are warranted.

Cellulose was used to make various C-to-N ratios of the organic substrates. A large part of the plant materials added to amend agricultural soils is cellulosic. Cellulose constitutes 30–60% of the chemical composition of plant-derived soil organic matter (Klein and Klein, 1988). Trofymow and Coleman (1982) amended the soil medium with cellulose and chitin. The presence of *A. avenae* resulted in more N-mineralized than *F. oxysporum* alone. In our study, the sand was amended with cellulose and alfalfa, and N-mineralization was greater in the presence of either *A. avenae* or *A. composticola* than *R. solani* alone. In general, fungi tend to immobilize N more strongly than bacteria and to mineralize it less rapidly from complex substrates (Griffin, 1972). N-mineralization and decomposition

rates of powdered cellulose and ground alfalfa used in this study were probably higher than usual due to the small particle size.

#### 4.3. Nematode and fungal dynamics

During the initial growth of fungi on organic substrates, N became immobilized by incorporation into new mycelia, or the rates of immobilization and mineralization were about equal (Scarsbrook, 1965). Analysis of the fungal fatty acid 18:2  $\omega$ 6c in our study indicated that nematodes suppressed the initial growth of *R. solani*. During this period, there was more mineral N in the nematode columns since there was more mineralization through nematode excretion, and less immobilization by the reduced fungal biomass due to nematode-feeding. The microcosm system was fungal-dominated, evidenced by the bacterial contamination accounting for ca. 18% of total PLFA on d 21.

The biochemical assessment of fungal biomass was useful in understanding the dynamics of the fungi in the soil. Fatty acids make up cell membranes of microorganisms. The fungal fatty acid 18:2  $\omega$ 6c has been used as a quantitative measurement of viable fungal biomass in soil (Vestal and White, 1989; Bossio and Scow, 1997; Mikola and Setälä, 1998). Fungal-feeding nematodes reduced the initial growth of *R. solani* in this investigation. For both nematode species, however, the amount of 18:2  $\omega$ 6c on d 21 was not lower in *R. solani* columns with nematodes present. One possible mechanism is that the response of fungi to faunal grazing might be dependent on grazing intensity (Ingham et al., 1985). As the population of both nematodes feeding on *R. solani* quickly declined, resurgence of the fungus probably occurred. When nematodes feeding on *Trichoderma* sp. maintained consistent grazing intensity, evidenced by little change in population numbers, both nematode species reduced the amount of 18:2  $\omega$ 6c in *Trichoderma* columns on d 21.

Fungal-feeding nematode populations reached their highest on d 14 in Petri dish microcosm systems (Chen and Ferris, 1997). The peaks for fungal-feeding nematode numbers and fungal biomass in the column microcosm systems frequently appeared at the early stage of the experimentation. After the peaks, nematode and fungal population numbers decreased due to food shortage, grazing, or factors other than resource availability. When fungi were heavily grazed by nematodes, N-mineralization decreased (Trofymow and Coleman, 1982). Under the research conditions in our investigation, the fungal biomass was lower later in the experiment, and so was the rate of N-mineralization.

In summary, N-mineralization in fungal decomposition pathways was affected by C-to-N ratios (11:1 to 45:1) of the substrates where the higher C-to-N ratios resulted in less N-NH<sub>4</sub> in columns with *R. solani*

alone, and no reduction of N-NH<sub>4</sub> in the presence of nematodes, compared to that at lower C-to-N ratios. It was affected by the fungal species; a good host in the presence of either *A. avenae* or *A. composticola* resulted in more mineral N than a poor host. It was also affected by fungal-feeding nematodes; more N was mineralized in columns containing nematodes and *R. solani* than in those with the fungus alone. There were differences between nematode species; more N was mineralized in the presence of *A. composticola* than *A. avenae*. Fungal-feeding nematodes, in addition to their potential as biological control agents, might also play a beneficial role in soil fertility.

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