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Growth and nitrogen mineralization of selected fungi and fungal-feeding nematodes on sand amended with organic matter

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Abstract

Isolates of *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum*, *Penicillium sp*., *Rhizoctonia solani*, *Stemphylium* sp., *Thielaviopsis basicola*, and *Verticillium dahliae* were cultured on potato–dextrose agar (PDA), barley–sand and alfalfa–sand substrates in petri-dish or in column microcosms. N-mineralization by fungi and fungal-feeding nematodes in combination or fungi alone was assessed. Numbers of *Aphelenchus avenae* or *Aphelenchoides composticola* supported by the fungi were measured every 7 days. Times for full colonization of the substrates by fungi ranged from 5 to 15 days. *Rhizoctonia solani* and *B. cinerea* on PDA supported the largest *A. avenae* and *A. composticola* populations, respectively. *Penicillium* sp. was a nonhost for *A. composticola* and *A. avenae. Rhizoctonia solani*, *B. cinerea*, *V. dahliae*, and *F. oxysporum* supported significantly more nematodes than the other four fungal species. The ranked order of fungi based on the amount of N mineralized in columns free of nematodes was *A. alternata* (with a rate of 0.052 μg N/g-sand per day), *Stemphylium* sp., *V. dahliae*, *T. basicola*, *B. cinerea*, *F. oxysporum*, *R. solani*, and *Penicillium* sp. (with a rate of 0.0045 μg N/g-sand per day). The presence of *A. avenae* resulted in significant increases in mineral N, compared to nematode-free columns colonized by *F. oxysporum*, *R. solani*, and *T. basicola* alone. The presence of *A. composticola* resulted in significant increases in mineral N, compared to nematode-free columns colonized by *A. alternata*, *B. cinerea*, *F. oxysporum*, and *R. solani* alone. There was more mineral N in columns in the presence of *A. composticola* than *A. avenae* in most cases.

Introduction

Fungi and bacteria are major decomposers of organic substrates in terrestrial ecosystems. Nearly 100 000 of the known fungal species are saprophages and decomposers (Agrios, 1978). There could be as many as 1.5 million species of fungi, although most of them are still undescribed (Dix and Webster, 1995; Hawksworth and Mound, 1991). Nutrient minerals from the substrates are immobilized in fungal biomass. There is considerable variability in the chemical composition of fungi (Foster, 1949), which may affect the balance between immobilization and mineralization. The balance also is often dependent on interactions with other organisms (Dighton and Boddy, 1989).

Fungal-feeding nematodes are important components of the fungal decomposition pathway. They may contribute to biological control of the causal agents of some soil-borne phytopathogenic diseases (Gupta, 1986; Ishibashi and Choi, 1991; Klink and Barker, 1968). Although there have been some investigations on the role of fungi and fungal-feeding nematodes in nutrient cycling processes in soils (Anderson et al., 1981; Chen and Ferris, 1997; Dighton, 1995; Ingham et al., 1985; Trofymow and Coleman, 1982), their effects on the fungal decomposition pathway generally have not received as much attention as those of nematode groups in bacterial decomposition channels. With renewed focus on the practice of amending soil with organic matter comes a need to investigate biological

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characteristics of soil organisms in more detail, as well as contribution of fungi and fungal-feeding nematodes to N-mineralization. In this paper, we determine (1) the growth of eight fungal species and two common fungal-feeding nematodes, *Aphelenchus avenae* Franklin, and *Aphelenchoides composticola* Bastian, on different organic substrates in a petri-dish microcosm environment; (2) N-mineralization in the presence of selected fungi in column microcosms amended with organic matter, and (3) N-mineralization in the presence of fungi and fungal-feeding nematodes in column microcosms amended with organic matter.

Methods and materials

Source and culture of fungi and nematodes

Eight fungal species included in these studies were: *Alternaria alternata* (Fr.) Keissler, *Botrytis cinerea* Pers., *Fusarium oxysporum* f. sp. lycopersici, *Penicillium* sp., *Rhizoctonia solani* Kûhn, *Stemphylium* sp., *Thielaviopsis basicola* (Berk. & Br.) Ferraris, and *Verticillium dahliae* Kleb. The potential biological and ecological roles of these fungi ranged from saprophagous to facultatively parasitic. All fungal species were isolated from soils of a long-term Sustainable Agriculture Farming System (SAFS) project at the University of California, Davis. Fungi were maintained on potato dextrose agar (PDA) at room temperature (approximately 20°C).

Fungal-feeding nematode species investigated in the study were *Aphelenchoides composticola* and *Aphelenchus avenae*. *Aphelenchoides composticola* is amphimictic and ubiquitous in temperate areas; it does not seem to feed on higher plants (Hesling, 1977). Of several fungal-feeding nematode species investigated, *A. composticola* had the widest fungal host range (Giannakis and Sanders, 1989). *Aphelenchus avenae* is parthenogenetic although males occur in samples from the SAFS site. It is present in most soils and has been reported from most parts of the world (Hooper, 1974). All nematode species were isolated from soils of the SAFS project. Voucher specimens of the nematodes are deposited in the University of California Davis Nematode Collection with the accession numbers UCDNC 3643-3644 for *A. avenae*, and UCDNC 3640-3642 for *A. composticola*. Nematodes were reared on *R. solani* on PDA at room temperature.

Petri-dish and column microcosm design

There were two components of petri-dish microcosm studies: (1) determination of growth rates of fungi on PDA, alfalfa–sand mixture, and barley–sand mixture, and (2) determination of population levels of nematodes on eight fungal species on PDA and barley–sand mixture. There were three replicates of each treatment. Plastic petri dishes (Fisher brand, 100 × 15 mm, Pittsburgh, Pennsylvania) were used for PDA cultures and glass petri dishes (Pyrex brand, 100 × 20 mm, Corning, New York) were used for amended sand cultures. For the amended sand culture, N-free acid washed sand was used as the soil medium (Ferris et al., 1998). The organic matter included leaf and stem tissue of alfalfa (*Medicago sativa*) and barley (*Hordeum vulgare*) with carbon-to-nitrogen (C:N) ratios of 11:1 and 20:1, respectively.

Column microcosm studies consisted of (1) N-mineralization by fungi alone, and (2) N mineralization in the presence of nematodes. Polyvinylchloride tubes (31.5 cm in length, 4 cm in diam.), capped on one end with a 6-mm diam. drain hole, were used in the column experiments (Ferris et al., 1998). A disk of 60-mesh (0.24-mm aperture) stainless steel mesh was placed over the hole in the capped end to minimize sand loss. Treatments established were the eight fungal species in the presence or absence of *A. avenae* or *A. composticola*. All combinations were replicated three times to provide a total of 48 columns. The columns were maintained at room temperature. Preliminary trials indicated significant moisture difference between upper and lower region of the columns when they were arranged vertically. To improve consistency of moisture along their length, columns were arranged horizontally in boxes in a randomized complete block design, and were rotated 180° every 3 days. Every 3 days, each column was set upright for 2 h so that a leachate sample could be taken.

Glass petri dishes, columns and stainless steel mesh screens were washed thoroughly before use. All dishes and columns, complete with organic matter and sand, were autoclaved at 121°C for 30 min on two consecutive days before the designated fungal and nematode inoculation was made.

N leaching

Each column was arranged vertically and supported on a 150-ml beaker. On day 0, columns were leached with 100 ml of deionized distilled H_20 (ddH_2O) to minimize initial mineral N levels. The columns were

then leached with 60 ml of ddH_2O, and leachates were collected. Columns were leached with 60 ml of ddH_2O every 3 days thereafter. The volumes of leachates were recorded and 6 ml of each leachate subsamples was poured into a 15-ml polypropylene centrifuge tube (Corning, New York). Samples were capped and stored in a freezer before processing. After being thawed in warm water, 6 ml of 4 *M* KCl was added to each sample, and the tubes were shaken for 1 h. Serum separators (16 × 4 mm, I. B. Model, Fisher Scientific) were used to filter the sample solution in tubes before N concentration analysis. Nitrate and ammonium concentrations were determined using a diffusion-conductivity (ion electrode) analyzer (Carlson, 1978).

Mineral N released from organic matter associated with autoclaving

Petri-dishes

Three additional dishes of barley–sand substrate, without added organisms, were used to measure the amount of N released during autoclaving. They were destructively sampled after 7, 14, and 21 days for N analysis. Each dish was flooded with ddH_20. After 1 h, the liquid was poured into a vial and an equal volume of 4 *M* KCl added. The suspension was shaken for 1 h. The samples were treated with $ZnSO_4$ and NaOH, and were clarified by centrifugation at 810 × *g* for 5 min (Ferris et al., 1998).

Columns

Additional columns were employed to measure the influence of autoclaving on the amount of N released from organic materials. Three columns of alfalfa–sand substrate without added organisms were autoclaved on day 0 only and one additional column was repeatly autoclaved every 3 days. On day 0, columns were leached with 100 ml sterilized ddH_2O. Every 3 days columns were leached with 60 ml sterilized ddH_2O. Collected leachates were analyzed to determine the concentration of N.

Growth rates of fungi and population levels of fungal-feeding nematodes

The growth rates of fungi were measured on the following substrates (1) PDA, (2) sand amended with alfalfa, and (3) sand amended with barley. Dried leaf and stem tissue of alfalfa or barley (0.4 g) were ground in a Wiley mill to 0.833 mm particle size and mixed into 20 g of N-free sand in glass dishes. One piece of 1-cm diam. PDA fungal culture was transferred onto each PDA and amended-sand dish. The rates of colonization on PDA and amended sand were recorded for each of the fungi.

Fungi were inoculated at different times so that the colonies covered ca. 50% of the dishes simultaneously. Vermiform nematodes, including juveniles and adults, were inoculated when each fungal colony covered ca. 50% of the dish. Nematode population levels were monitored after 7, 14, and 21 days. The agar or amended-sand mixture containing nematodes was put on a wire-screen supporting Whatman No. 4 filter paper on a Baermann funnel (Chen and Abawi, 1996). After 24 h, the number of nematodes in the suspension was counted. Nematode reproduction rates (R) were calculated as $R = P_f/P_i$, where P_f is the final and P_i the initial nematode population level.

There were three replicates for each treatment. Data were subjected to ANOVA and other statistical analysis. The standard error (SE) of the mean was calculated to show the precision of the sample mean (Microsoft Excel, Microsoft Corporation, Redmond, WA). Duncan's multiple range test was applied for mean separations of (1) population levels of *A. avenae* and *A. composticola* on eight fungal species; and (2) the growth of eight fungal species on three substrates. Factorial analysis was used to detect if there was a significant fungus × substrate or fungus × nematode interaction (MSTAT, Michigan State University, East Lansing, MI).

N mineralization by fungi and nematodes

Fungus alone

Alfalfa leaf and stem tissue (1.0 g) and cellulose (1.2 g, fibrous cellulose powder, W. & R, Balston, Ltd., England, with a C:N ratio of 645:1) were mixed into 450 g N-free sand to provide a C:N ratio of 25:1 for each column microcosm. Based on their growth rates (Table 1), the eight fungal species were inoculated at different times so that the colonies fully covered the dishes simultaneously. A fully colonized dish of fungal culture on barley–sand (20 g) was mixed with the amended sand before the sand was packed into a column. N-mineralization was determined by leaching at 3-day intervals.

Fungus plus nematode

The amendment of sand, inoculation of designated fungus, and sampling and measurement of N were per-

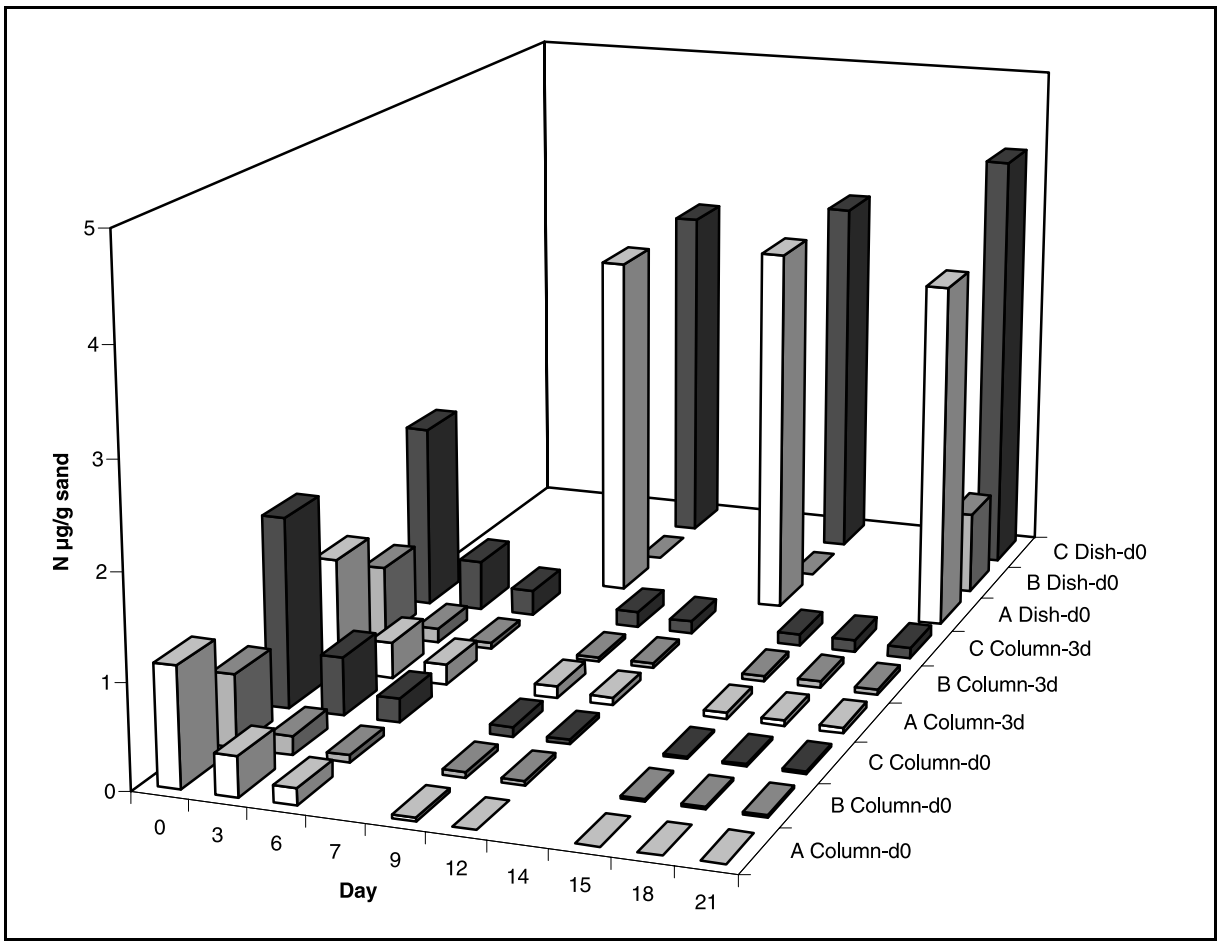

Figure 1. N release associated with autoclaving. (A) N-NH_4; (B) N-NO_3; (C) Total N. 'Dish-d0', three dishes with sand amended with barley were autoclaved on day 0, and destructively sampled every 7 days 'Column-d0', three columns with sand amended with alfalfa and cellulose were autoclaved on day 0, and leaching-sampled every 3 days. 'Column-3d', one column with sand amended with alfalfa and cellulose was autoclaved on day 0 and subsequently every 3 days, and leaching-sampled every 3 days.

formed as above. Under sterile conditions, nematodes introduced into the columns were extracted from dish cultures on an autoclaved Baermann funnel in a laminar flow hood. They consisted of juveniles and adults. The nematode suspension was pipetted onto a barley–sand dish culture for each of eight fungi. The contents of each inoculated culture dish were mixed into sand amended with alfalfa and cellulose to provide initial population density of 16 *A. avenae* and 20 *A. composticola*, including juveniles and adults, per g sand in each column.

N analysis

Total N mineralized was measured as the sum of N in the form of NH_4^+ (N-NH_4) and NO_3^- (N-NO_3). Standard curves were developed using a range of concentrations of NH_4Cl and KNO_3. The amount of N-NH_4 and N-NO_3 in the sample was determined by comparison with the standards. The total amount of N in the leachate sample was expressed as μg N/g-sand in each column. Net-N is the amount of N detected for the column in the absence of nematodes subtracted from that in the presence of nematodes. Student t-test was performed to compare mineral N extracted from the column with fungus alone and that in the presence of nematodes.

N-mineralization potential was determined from the natural growth function used to describe the relationship between cumulative N from the columns and time:

$$N_t = N_{\mathrm{p}}(1 - \mathrm{e}^{kt})$$

Table 1. Colonization of selected fungi on different substrates at room temperature

Species	Substrate covered by colony (days)		
	Potato dextrose–agar	Alfalfa–sand	Barley–sand
Alternaria alternata	9 b	12 b	5 d
Botrytis cinerea	5 d	6 c	7 bcd
Fusarium oxysporum	7 c	12 b	9 ab
Penicillium sp.	7 c	6 c	6 cd
Rhizoctonia solani	5 d	5 c	5 d
Stemphylium sp.	10 ab	14 ab	8 abc
Thielaviopsis basicola	11 a	15 a	10 a
Verticillium dahliae	10 ab	14 ab	6 cd

Note: A 1-cm fungal inoculum plug was transferred to the center area of a 9.5-cm plastic or glass petri dish. There were three replicates per treatment. Means followed by the same letter in each column are not significantly different according to Duncan's multiple range test ($P = 0.05$). Factorial analysis indicated that there was a significant ($P < 0.001$) fungus × substrate interaction.

where N_p is the nitrogen mineralization potential of the microcosm system, N_t is the cumulative amount of N mineralized over time t, and k is a regression coefficient (Ferris et al., 1998; Neter et al., 1990; Stanford, 1982).

Results

Growth of fungi and fungal-feeding nematodes in petri-dish microcosms

Growth of fungi

Factorial analysis showed that there was a significant fungus × substrate interaction. Fungi required 5 – 11, 5 – 15 and 5 – 10 days to completely cover PDA, alfalfa-amended sand, and barley-amended sand, respectively, in petri-dish microcosms (Table 1). The growth rate of *R. solani* was the same on three substrates. While the shortest time required for individual fungi to cover the whole dish was 5 days for: (1) *R. solani* on any of three substrates, (2) *A. alternata* on barley–sand mixture, and (3) *B. cinerea* on PDA; the longest time required was 15 days for *T. basicola* grown on the alfalfa–sand mixture (Table 1).

Abundance and reproduction rate of nematodes on eight fungi

Factorial analysis showed that there was a significant interaction between fungal and nematode species, except for day 7 on PDA. After day 14 in most cases, fungi supported more *A. avenae* and *A. composticola* on PDA than on barley–sand mixture (Table 2). Fungi varied in their capacity for supporting nematode populations (Table 2). *Rhizoctonia solani* and *B. cinerea* were the best hosts for *A. composticola* on PDA and for both nematode species in barley–sand mixture. On PDA, *R. solani* supported the largest *A. avenae* population. *Penicillium* sp. was a nonhost for *A. composticola* and *A. avenae*. In most cases, *R. solani*, *B. cinerea*, *V. dahliae*, and *F. oxysporum* supported more individuals per PDA dish and per g of barley–sand mixture than those of the other four fungal species (Table 2).

Nematode reproduction, indicated by the ratio of the final population (P_f) and initial inoculum level (P_i), was greatest on *R. solani*, *B. cinerea*, *V. dahliae*, and *F. oxysporum* (Table 2). *Aphelenchus avenae* more often had higher reproduction rates than *A. composticola* on PDA, and *A. compoticola* more often had higher reproduction rates than *A. avenae* in barley-amended sand.

Mineral N released from organic matter in the absence of organisms

Mineral N released in petri dishes

Organisms in the petri-dish microcosms were eliminated by autoclaving on day 0. Total N released from organic material associated with autoclaving was 3.38, 3.60, and 4.21 μg N/g-sand on day 7, 14, and 21, respectively (Fig. 1). The amount of N-NH_4 in the microcosms without organisms changed little over time (average 3.46 μg N/g-sand), while N-NO_3 was only detectable on day 21 (0.81 μg N/g-sand).

Mineral N released in columns

Organisms in column microcosms were eliminated by autoclaving on day 0 only and subsequently every 3 days. The concentration of total N, N-NH_4, and N-NO_3 detected from the leachate in the columns maintained free of organisms by autoclaving was 1.9, 1.1, and 0.7 μg/g-sand on day 0 (Fig. 1). The N concentration decreased quickly over time. There was ca. 11% more N in the leachate when columns were autoclaved every 3 days than when autoclaved on day 0 only. After day 9, autoclaving every 3 days resulted in almost no change in the amount of N in the leachate (Fig. 1).

N mineralization by fungi and fungal-feeding nematodes

N mineralization by fungi

N concentration in leachate from columns with fungus

Table 2. Population of fungal-feeding nematodes supported by different fungi on potato dextrose–agar (PDA) and barley–sand mixture microcosm petri-dish systems

Fungal and Nematode Species		Population density[d]						Population development P_f/P_1[a]	
		Day 7		Day 14		Day 21			
		PDA #/dish	Barley–sand #/g-sand	PDA #/dish	Barley–sand #/g-sand	PDA #/dish	Barley–sand #/g-sand	PDA	Barley–sand
Alternaria alternata	AA[b]	3263 b	177 bc	13545 ef	244 cde	11927 def	157 fg	119	31
	AC[c]	1024 cd	73 de	7463 g	158 ef	6382 g	174 ef	64	35
Botrytis cinerea	AA	6291 a	453 a	40122 b	1164 b	36541 b	1009 c	365	202
	AC	1315 cd	103 e	36583 b	1387 a	39639 b	1450 b	396	290
Fusarium oxysporum	AA	4622 b	226 b	29415 c	425 c	24725 c	363 d	247	73
	AC	1667 cd	82 de	12874 ef	223 de	8692 fg	209 ef	87	42
Penicillium sp.	AA	3768 b	69 de	2542 h	70 ef	812 h	8 gh	8	2
	AC	99 d	21 e	3 h	0 f	0 h	0 h	0	0
Rhizoctonia solani	AA	6152 a	424 a	45499 a	1027 b	44658 a	894 c	447	179
	AC	1856 c	194 bc	35347 b	1492 a	37482 b	1662 a	375	332
Stemphylium sp.	AA	4142 b	160 c	16524 e	403 cd	10663 efg	321 de	107	64
	AC	1423 cd	54 de	23094 d	127 ef	14687 de	153 fg	147	31
Thielaviopsis basicola	AA	3725 b	164 c	14268 ef	268 cde	15642 d	153 fg	156	31
	AC	687 cd	62 de	9458 fg	134 ef	9849 fg	186 ef	99	37
Verticillium dahliae	AA	4362 b	99 d	21861 d	194 ef	21649 c	125 fgh	216	25
	AC	568 cd	57 de	13263 ef	131 ef	14881 de	201 ef	149	40
Nematode × fungus interaction[e]		n.s.	***	***	***	***	***		

[a] Nematode inoculum levels started as 100 individuals (P_i) per dish. There were three replicates per treatment. The population density level on day 21 was used as P_f.
[b] AA, *Aphelenchus avenae*.
[c] AC, *Aphelenchoides composticola*.
[d] Means followed by the same letter in each column are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[e] There was significant ($P \leq 0.001$ = ***) or not significant ($P > 0.05$ = n.s.) interaction between nematode and fungal species.

Table 3. Influence of *Aphelenchus avenae* (AA) and *Aphelenchoides composticola* (AC) on N-mineralization of selected fungi

	Total N μg/g-sand per day							
	A. alternata	*B. cinerea*	*F. oxysporum*	*Penicillium* sp.	*R. solani*	*Stemphylium* sp.	*T. basicola*	*V. dahliae*
F[a]	0.0694	0.0187	0.0073	0.00423	0.0069	0.0184	0.0067	0.0388
F+AA	0.0533	0.0205	0.0137	0.00416	0.0263	0.0144	0.0114	0.0387
Net-N[b]	−0.0161 n.s.[c]	0.0018 n.s.	0.0064 *	−0.00007 n.s.	0.0194*	−0.0040 n.s.	0.0047*	−0.0001 n.s.
F	0.0337	0.0061	0.0088	0.0048	0.0050	0.0525	0.0271	0.0164
F+AC	0.0720	0.0560	0.0400	0.0045	0.0150	0.0569	0.0325	0.0342
Net-N	0.0383 *	0.0499 *	0.0312 *	−0.0003 n.s.	0.0100 *	0.0044 n.s.	0.0054 n.s.	0.0178 n.s.

[a] F, Fungus; *A. alternata*, *Alternaria alternata*; *B. cinerea*, *Botrytis cinerea*; *F. oxysporum*, *Fusarium oxysporum*; *R. solani*, *Rhizoctonia solani*; *T. basicola*, *Thielaviopsis basicola*; *V. dahliae*, *Verticillium dahliae*.
[b] Net-N is the amount of mineral N in columns without nematodes subtracted from columns containing nematodes.
[c] There was significant ($P \leq 0.05$ = *) or not significant ($P > 0.05$ = n.s.) difference between mineral N in the presence of fungus alone and that of fungus plus nematode according to Student's *t*-test.

alone decreased over time (Figs. 2, 3a, 4a). After day 9, the rate of decrease in the concentration of total N was small. This general trend occurred either in the absence or in the presence of nematodes, and was described by a quadratic relationship (Fig. 2).

Under research conditions, the ranking from high to low in the amount of N mineralized by individual fungi in the absence of nematodes was *A. alternata*, *Stemphylium* sp., *V. dahliae*, *T. basicola*, *B. cinerea*, *F. oxysporum*, *R. solani*, and *Penicillium* sp. (Fig. 5). There were significant differences among the fungi. *Alternaria alternata* mineralized 0.052 μg N/g-sand per day, and ranked first and second in two experiments. It mineralized N at a rate 10 times greater than *Penicillium* sp. (0.0045 μg N/g-sand per day).

N-mineralization in the presence of nematodes

In most cases, net-N in columns with *A. composticola*

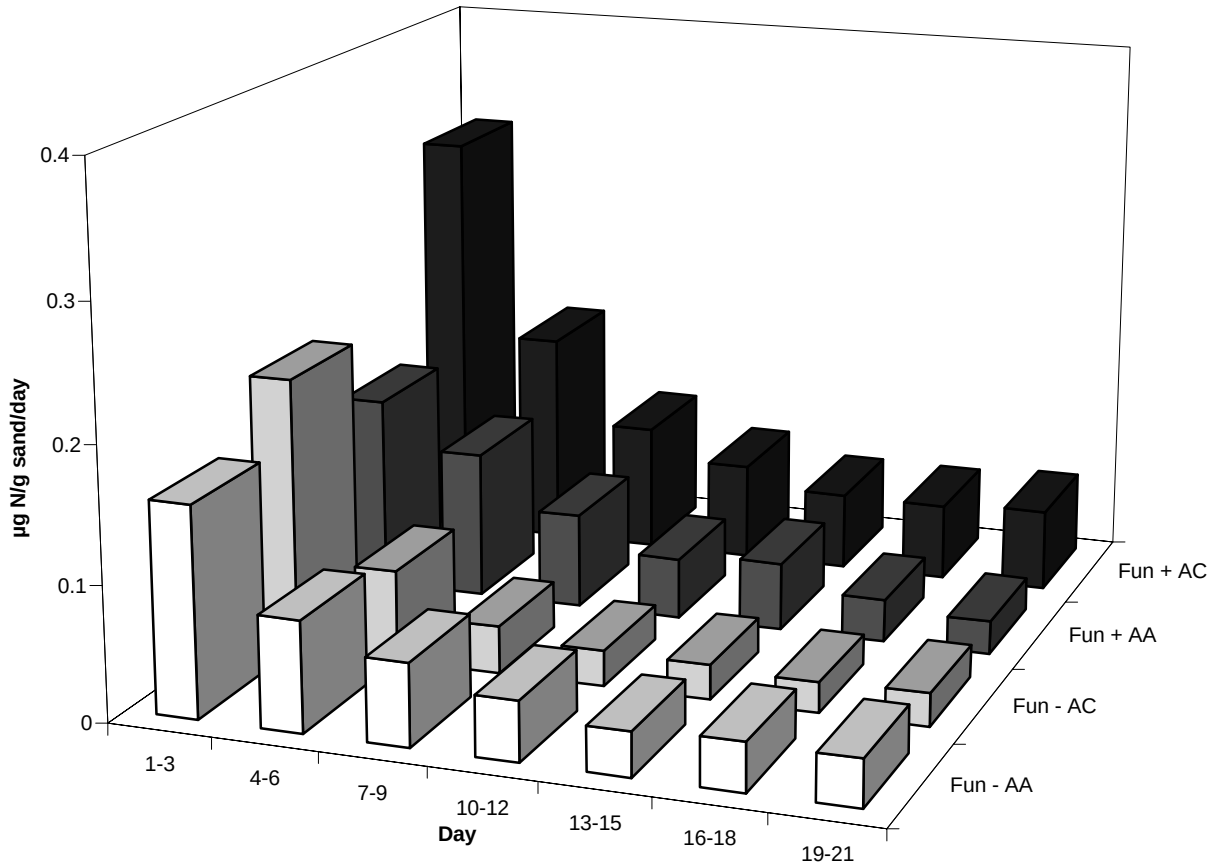

Figure 2. N dynamics in the presence of fungi and nematodes. Mineral N (Y, μg N/g-sand per day) was regressed with time (X, days). Fun-AA, columns with fungus alone in the first experiment, $Y = 0.2036 - 0.0214X + 0.0007X^2$, $r^2 = 0.957$. Fun+AA, columns with fungus and *Aphelenchus avenae*; $Y = 0.1876 - 0.0159X + 0.0004X^2$, $r^2 = 0.975$. Fun-AC, columns with fungus alone in the second experiment; $Y = 0.2698 - 0.0345X + 0.0011X^2$, $r^2 = 0.887$. Fun+AC, columns with fungus and *Aphelenchoides composticola*, $Y = 0.4188 - 0.0473$-X- $+ 0.0015X^2$, $r^2 = 0.957$.

was higher than those with *A. avenae* (Figs. 3b and 4b, Table 3). The amount of mineral N was not changed ($P \geq 0.05$) by the presence of *A. avenae* in columns with *A. alternata*, *B. cinerea*, *Penicillium* sp., *Stemphylium* sp., and *T. basicola*. The amount of mineral N was significantly ($P \leq 0.05$) increased by the presence of *A. composticola* in columns with *A. alternata*, *B. cinerea*, *F. oxysporum*, and *R. solani* (Table 3).

There were variations among individual fungi associated with nematode species for N-mineralization potential in the fungus-*A. avenae*/*A. composticola* combinations (Table 4). *Aphelenchus avenae* had the highest mineralization potential on *V. dahliae*, and *A. composticola* on *Stemphylium* sp. Lowest mineralization potentials for both nematodes were on *Penicillium* sp.

Table 4. Nitrogen mineralization potential (N_p, μg N/g-sand) of fungus–nematode combinations in column microcosms. N_p was determined from the natural growth function used to describe the relationship between cumulative N (N_t) and time, $N_t = N_p(1 - e^{kt})$, where N_t was the cumulative amount of N mineralized at time t and k was a regression coefficient.

Combination	*Aphelenchus avenae*	*Aphelenchoides composticola*
Alternaria alternata	0.878	1.002
Botrytis cinerea	0.512	0.394
Fusarium oxysporum	0.263	0.582
Penicillium sp.	0.100	0.063
Rhizoctonia solani	0.435	0.195
Stemphylium sp.	0.454	1.095
Thielaviopsis basicola	0.270	0.323
Verticillium dahliae	1.037	0.233

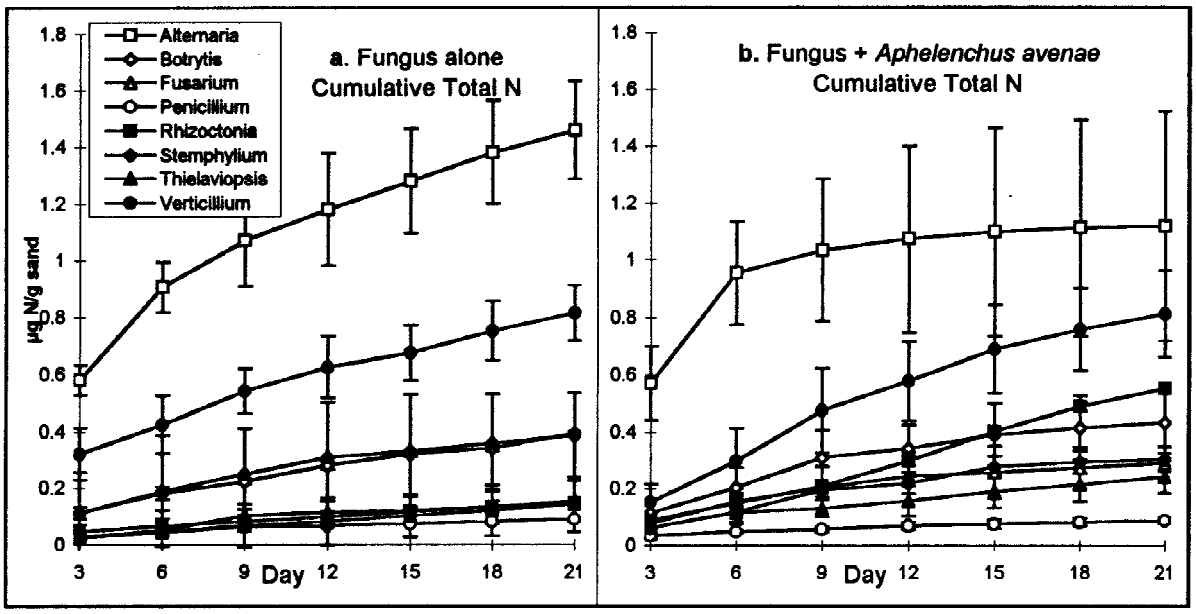

Figure 3. Cumulative total N mineralized by eight fungal species in the presence or absence of *Aphelenchus avenae.* Bars indicate standard errors. Some bars are obscured by symbols. Designation: Alternaria, *Alternaria alternata*; Botrytis, *Botrytis cinerea*; Fusarium, *Fusarium oxysporum*; Penicillium, *Penicillium* sp.; Rhizoctonia, *Rhizoctonia solani*; Stemphylium, *Stemphylium* sp.; Thielaviopsis, *Thielaviopsis basicola*; and Verticillium, *Verticillium dahliae*.

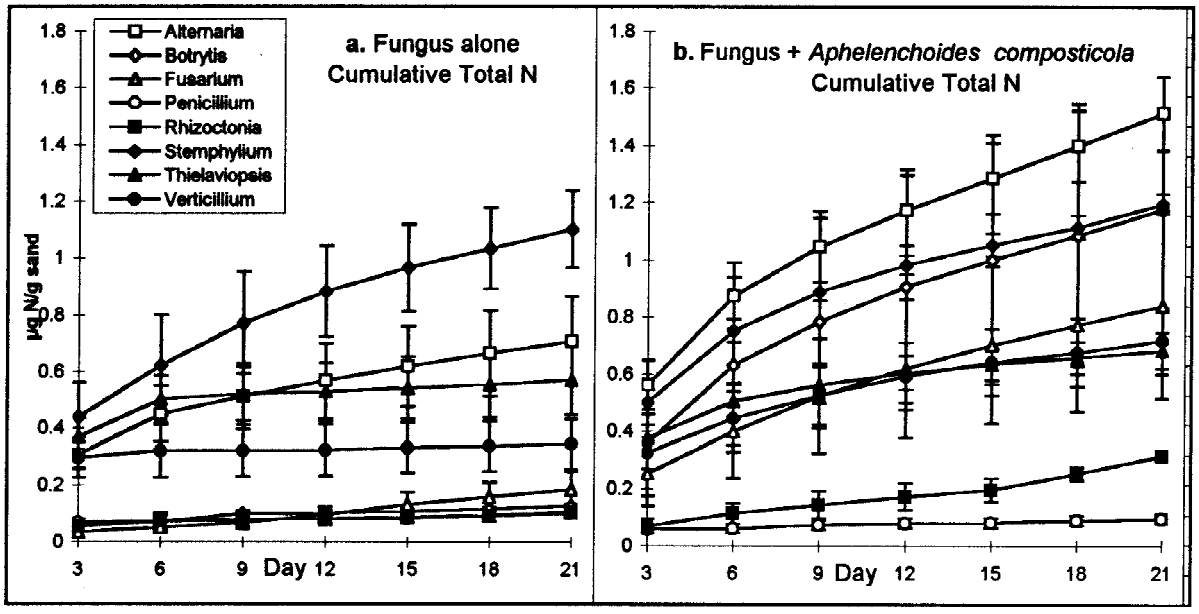

Figure 4. Cumulative total N mineralized by eight fungi in the presence or absence of *Aphelenchoides composticola.* Bars indicate standard errors. Some bars are obscured by symbols. Designation: Alternaria, *Alternaria alternata*; Botrytis, *Botrytis cinerea*; Fusarium, *Fusarium oxysporum*; Penicillium, *Penicillium* sp.; Rhizoctonia, *Rhizoctonia solani*; Stemphylium, *Stemphylium* sp.; Thielaviopsis, *Thielaviopsis basicola*; and Verticillium, *Verticillium dahliae.*

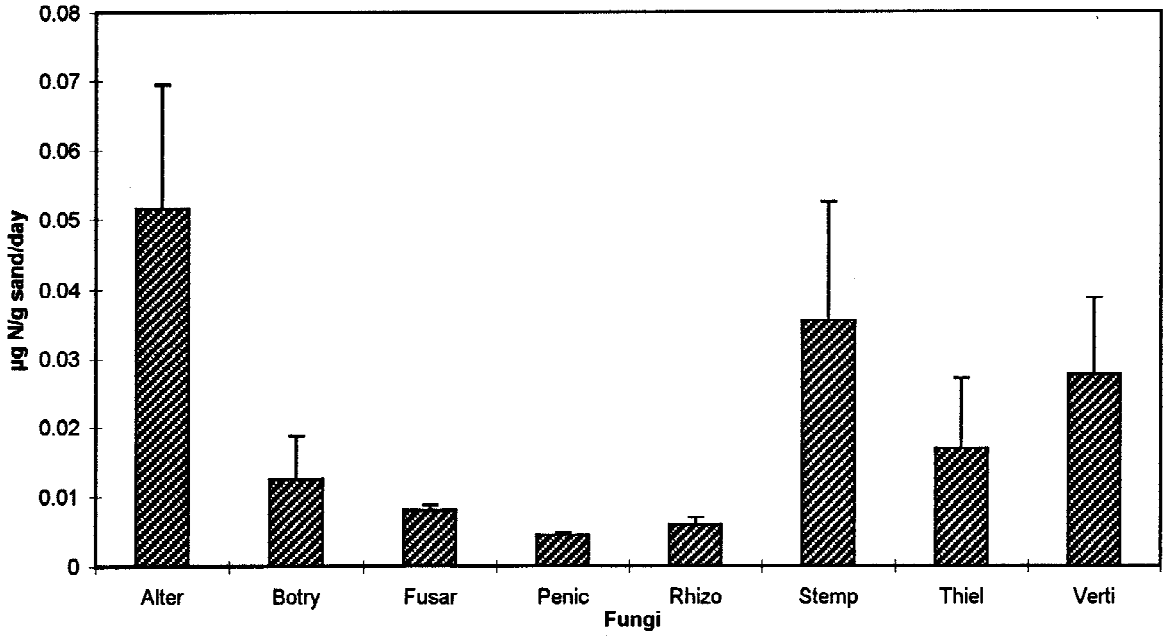

Figure 5. N-mineralization rates (μg N/g-sand per day) of selected fungi in the absence of nematodes. The total N was the combination of two experiments. Bars indicate standard errors. Designation: Alter, *Alternaria alternate*; Botry, *Botrytis cinerea*; Fusar, *Fusarium oxysporum*; Penic, *Penicillium* sp.; Rhizoc, *Rhizoctonia solani*; Stemp, *Stemphylium* sp.; Thiel, *Thielaviopsis basicola*; and Verti, *Verticillium dahliae*.

Discussion

N in soil organic matter can be released through the effects of one or more species of organisms. In this study we measured mineral N concentrations associated with colonization of organic materials by fungal species, and with the interaction between the fungi and fungal-feeding nematodes. We also assessed the growth and abundance of the fungi and fungal-feeding nematodes.

Growth and N-mineralization of fungi

Fungi generally immobilize N more effectively than bacteria when growing on complex substrates (Griffin, 1972). Rates of immobilization and mineralization are about equal when fungi colonize organic matter with C:N ratios between 15:1 and 30:1, but immobilization occurs at C:N ratios greater than 30:1 (Scarsbrook, 1965). Under field conditions, a variety of plant materials, often consisting of complex organic substances with high C:N ratios, are incorporated into soils. Such materials are decomposed, at least initially, primarily by fungi. A series of studies from our laboratory suggests that the effect on N-mineralization of nematode grazing on primary decomposers is greatest in bacterial-dominated decomposition channels when the C:N ratio of the organic materials is moderate to high (Ferris et al., 1998). When an experiment to measure effects of fungal-feeding nematode on N-mineralization became contaminated with bacteria (accounting for ca. 18% of total phospholipid fatty acids after day 21), the amount of N mineralized was greater than in the non-contaminated columns. The smallest amounts of mineral N were measured in the present study when autoclaving was used to minimize contamination by other organisms. Fungal-dominated systems in nature include: (1) forest ecosystems (Binkley et al., 1997; Dighton and Boddy, 1989; Griffiths et al., 1997); (2) agricultural soils under certain conditions, including lower pH levels (Jordan et al., 1995); (3) non-tillage agroecosystems (Hendrix et al., 1986); and 4) pasture soils (Griffiths et al., 1997).

In most cases, saprophytic or non-virulent facultative phytoparasitic fungi mineralized more N than did virulent phytoparasites. The differences probably reflect the rates of growth of the fungi on organic matter. For example, *R. solani* grew rapidly in dish microcosms. It also immobilized most of N in the column microcosms as suggested by the very small amount of mineral N present in the leachate. *Stemphylium* sp. grew slowly in the dish microcosms and may not

have immobilized N efficiently in the column microcosms, suggested by a much larger amount of mineral N present in leachate from the columns.

Abundance and N-mineralization of fungal-feeding nematodes

The C:N ratios of the body contents of fungal-feeding nematodes are similar to those of fungal mycelium (Chen and Ferris, 1999; Griffin, 1972; Ingham et al., 1985). Consequently, there is not a surfeit of N associated with fungal tissues assimilated for nematode biomass production as occurs when bacterial-feeding nematodes feed on bacteria. However, the N associated with respired C is in excess of that needed for production and is excreted. Further, fungal-feeding nematodes also deposit feces enriched with organic material into the soil, and provide reservoirs of C and N in their cells that contribute to the soil organic fraction (Ingham et al., 1985; Lee and Atkinson, 1977; Wright and Newall, 1976). Thus, nematodes provide a significant contribution to available N beyond that contained in excretory products alone.

Decomposition of complex organic matter in soil is undoubtedly dominated by rapidly growing and effective saprophytes. In this study we selected a range of fungi of varied saprophytic ability. N-mineralization was low when fungi exhibited strong saprophytic growth, probably explained by immobilization in fungal tissues. Representatives of the rapidly growing fungi were best hosts for fungal-feeding nematodes and supported greatest nematode population growth, so that the interaction resulted in the greatest additional N-mineralization.

The ability of individual fungi to support nematode populations varies. Giannakis and Sanders (1989) found that *A. composticola* had higher reproductive rates than *A. avenae* on the fungi they tested. When 18 species of fungi were tested as food sources for *A. avenae*, they ranged from apparently toxic substrates to those on which nematodes reproduced rapidly and overgrazed the fungus. In that study, both *A. avenae* and *A. composticola* were repelled by *Penicillium* sp. (Mankau and Mankau, 1963). Many important root-rotting fungi have been shown to be good hosts for *A. avenae (*Mankau and Mankau, 1962; Townshend, 1964). The effect of nematode grazing on total N-mineralized varied with the host status of a fungal species to the nematode and the growth rate of fungus and nematode. For example, *Penicillium* sp. was a non-host to both *A. avenae* and *A. composticola*; nematode grazing had no significant effects on N-mineralization by this fungus. In all seven cases where the addition of fungal-feeding nematodes significantly increased mineral N, nematode population levels and reproductive indices also were significantly higher than for other fungal/nematode combinations. For example, *A. composticola* achieved the highest population levels on *B. cinerea* and *R. solani* on barley–sand dish microcosms. In column microcosms, the amounts of mineral N in the presence of *A. composticola* were ca. nine and three times higher than *B. cinerea* alone and *R. solani* alone, respectively.

The general incidence of increase in mineral N associated with nematode grazing on primary decomposers is clear for both bacteria and fungi, particularly when organic substrates have higher C:N ratios. Management of soils in a manner conducive to (or at least not detrimental to) fungivorous and bacterivorous nematodes is likely to be beneficial to soil fertility. Relevant practices may include pre-seasonal green manure amendments, incorporation of plant residues into soils after harvest, and organic mulches during the growing season.

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