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Effect of temperature on growth and nitrogen mineralization of fungi and fungal-feeding nematodes

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Abstract

Dish and column microcosms containing alfalfa-sand medium were established to determine effect of temperature on growth and N mineralization ability of two fungi (*Rhizoctonia solani* and *Botrytis cinerea*) and two nematodes (*Aphelenchus avenae* and *Aphelenchoides composticola)*. The microcosms were incubated at 15, 20, 25 and 29 °C for 21 days. In the dish microcosms, hyphal growth rates of both fungal species increased with temperature in the range of 15–25 °C. Above that temperature range, the growth rate of *R. solani* remained almost constant while that of *B. cinerea* decrease considerably. The population growth rate of *A. avenae* increased with temperature between 15 and 29 °C on colonies of *R. solani* and *B. cinerea* in dish microcosms. The growth rate of *A. composticola* also increased in the range of 15–25 °C but decreased greatly beyond that temperature range independent of the fungal species as food source. Inorganic N (NH_4^+ + NO_3^-) was collected from each column microcosm by leaching every 3 days. In the columns containing *R. solani*, there was a significant effect of temperature on the amount of N detected in the fungus+*A avenae* or *A. composticola* but not in the fungus alone columns. The total amount of N was greatest at 29 °C for *A. avenae* and at 20 °C for *A. composticola* columns, concurrent with the population growth rates of the nematodes. In the columns containing *B. cinerea*, the effect of temperature on the amount of inorganic N was not significant in either the fungus alone or fungus+nematode columns, although the population growth rates of the both nematode species were highest at 20 °C. For *B. cinerea*, the N amount across temperatures was the same or larger for the fungus alone as for the fungus+nematode columns. In general, the contribution of fungal-feeding nematodes to N mineralization was small in any combinations of fungus and nematode species at any temperature. Similarity in C/N ratio of the fungal and nematode biomass, organic substrate C/N ratios too low for measurable increase in net mineralization by the nematodes and small reproduction of the nematodes in the column microcosms were probable contributory factors.

Introduction

Nitrogen mineralization by soil fungi and fungal-feeding nematodes has been investigated to determine the contribution of these organisms to organic matter decomposition and nutrient cycling (Anderson et al., 1981; Ingham et al., 1985; Trofymow and Coleman, 1982). The mineralization rate of a decomposition system can be affected by biological factors such as species of the fungus and nematode, and chemical factors such as C/N ratio of the organic substrates involved in the system (Chen and Ferris, 1999, 2000). The rate also can be affected by physical factors, including soil temperature and moisture, because the metabolism, growth, reproduction and activity of the organisms vary with these factors (Adams et al., 1982; Fujiie et al., 1996; Stanton and Sartori, 1990; Yeates, 1996; Young et al., 1998). Knowledge of the effects of those factors on the mineralization contribution of fungi and fungal-feeding nematodes is necessary to

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allow realistic determination of the importance of the organisms in nutrient cycling in soil.

In this paper, we report the effect of temperature on, (1) the hyphal growth of two fungi, *Rhizoctonia solani* and *Botrytis cinerea*, and the population growth of two fungal-feeding nematode species, *Aphelenchus avenae* and *Aphelenchoides composticola*; (2) N mineralization by the fungi and nematodes in column microcosms.

Materials and methods

Sources and cultures of organisms

Two fungi, *R. solani and B. cinerea,* were selected for this study because (1) they are good hosts for fungal-feeding nematodes (Mankau and Mankau, 1963), (2) they are easily cultured on sand amended with organic substrates (Chen and Ferris, 2000), and (3) optimum temperatures for their hyphal growth were different in a preliminary test. Both fungi were supplied by Mrs. Linda Bolkan, Department of Plant Pathology of the University of California at Davis. Fungi were maintained on Potato Dextrose Agar (PDA, DIFCO®) at 22–25 °C.

Both *A. avenae* and *A. composticola* feed on a variety of fungi (Giannakis and Sanders, 1989; Mankau and Mankau, 1963), but are not known to feed on higher plants (Hesling, 1977). The nematodes were isolated from field plots of the Sustainable Agriculture Farming System Project, located at the University of California, Davis, (Chen and Ferris, 1999). They were maintained at 22–25 °C on fungal colonies. *Rhizoctonia solani, B. cinerea* and *Fusarium oxysporum* cultured on PDA were used in rotation as food for the nematodes.

Effect of temperature on growth of organisms in dish microcosms

Fungi

Fungal growth rates were measured on sand medium (sand amended with alfalfa and cellulose). To prepare the medium, 0.89 g fibrous cellulose powder (Whatman , C/N ratio of 645:1) and 0.4 g chopped dried leaf and stem tissue of alfalfa (*Medicago sativa*, C/N ratio of 11:1), ground in a Wiley mill into 0.83 mm particle size were mixed into 20 g sand in glass petri dishes (100 × 20 mm) to result in a final C/N ratio of 35:1. The medium was autoclaved twice at 121 °C for 30 min. One piece of 10-mm diameter PDA fungal culture of either *R. solani* or *B. cinerea* was transferred onto the sand medium in each dish. After inoculation, the dishes were incubated at 15, 20, 25 and 29 °C. This range of temperatures is experienced during the cropping season in the soil at the Sustainable Agriculture Farming System Project site (Venette and Ferris, 1997). The fungal colony diameter in each dish was measured daily until the hyphae reached the edge of the dish. Hyphal growth rate (mm/day) was calculated for each fungus.

The experiments for both fungal species were conducted once simultaneously. There were five replicates for each fungal species at each temperature. Data were first subjected to two-way ANOVA, with fungal species and temperatures as factors, then to regression analysis to clarify the effects of temperature on the hyphal growth rate for each fungal species. The statistical analysis was performed using SAS® (SAS Institute Inc.).

Nematodes

Glass petri dishes containing the same type of sand medium employed in the fungal growth study were used for the nematode study. *Rhizoctonia solani* and *B. cinerea* were inoculated at different times so that the colonies covered the dishes at the same time. Nematodes maintained in *R. solani* culture were extracted under sterile conditions, and used as inoculum. Twenty individuals of either *A. avenae* or *A. composticola* were inoculated onto each fungal colony when the colony completely covered the dish. Dishes were maintained at 15, 20, 25 and 29 °C. To determine their population density 21 days after inoculation, the nematodes were extracted by placing the sand medium in each dish on Kimwipes®(Kimberly-Clark, 113 × 113 mm) supported on a wire screen on a Baermann funnel. The funnels were kept at the incubation temperature of the dishes to prevent changes in rates of development during extraction. After 24 h, the nematodes in the water suspension were counted. Population growth rates of nematodes (R) were calculated as:

$$R = Pf/Pi$$

where Pf is the number of nematodes extracted on day 21 and Pi is the number inoculated (20 per each dish).

The experiments for *A. avenae* and *A. composticola* were conducted once at different times. Four replicates were prepared for each fungal species at each temperature for each nematode species. Data

were either power or log transformed, if necessary, to improve inequality of variance before statistical analyses (Hinz and Eagles, 1976; Little and Hills, 1978). The data were subjected to two-way ANOVA, with fungal species and temperature as factors, then to regression analysis to detect population growth response to temperature for each nematode species. The statistical analyses were performed using SAS® (SAS Institute Inc.).

Effect of temperature on N mineralization by organisms in column microcosms

To determine effect of temperature, column microcosms (Chen and Ferris, 1999) were set up containing *R. solani* or *B. cinerea* with and without nematodes (*A. avenae* or *A. composticola*) at 15, 20, 25 and 29 °C. The experiments for each fungal species were conducted once at different times. Three types of columns (fungus alone, fungus+*A. avenae,* and fungus+*A. composticola)* were established in each experiment. Three replications were prepared for each type of columns at four temperatures resulting in a total of 36 columns for each of the *Rhizoctonia* and *Botrytis* experiments.

Column set up

Column microcosms were constructed from 300-mm lengths of 40-mm i.d. polyvinyl chloride pipe capped on one end as described in Ferris et al. (1998). A 6-mm dia. hole was drilled in the center of each of the caps. 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.

Alfalfa leaf and stem tissue (2.28 g) and cellulose powder (5.05 g) were mixed into 380 g N-free sand to provide C/N ratio of 35/1 for each column. The sand medium was autoclaved twice at 116 °C before packing into columns. One fully-colonized dish of fungal culture on sand medium was mixed into the sand in each column. To set up columns containing nematodes, either *A. avenae* o *r A. composticola* was inoculated together with a fungal culture. The inoculation rate of the nematodes was 50 individuals/g sand for both species. The columns were arranged in a completely randomized design in an incubation room at each temperature. At the end of the experiment (22 days after column set up), 80 g of sand was taken from each column to determine the nematode density and calculate population growth rates.

Sampling and analysis to determine N amounts in columns

Periodic leaching was used to measure the N mineralized in each column (Chen and Ferris, 2000; Ferris et al., 1998). Every 3 days for 21 days, stoppers were inserted into the column drain holes and 60 ml of distilled water was poured into the top of each column. Four minutes later, stoppers were removed, the columns were drained for 30 min and the leachates were collected. The volume of leachate from each column was recorded and 6 ml was poured into a plastic centrifuge tube. The tubes were kept in a freezer (−10 °C) until processed for N analysis. When the columns were not being leached, they were stored horizontally in boxes to minimize soil moisture gradients.

To process for N analysis, the samples in tubes were thawed in warm water. Six ml of 4 M KCl solution was added to each tube. The sample tubes were shaken for 1 h to mix the leachate and KCl solution completely before N analysis (Chen and Ferris, 2000). Both ammonium (NH_4^+) and nitrate (NO_3^-) forms of N were measured in the leachate by a diffusion conductivity analyzer (Carlson, 1978). Amount of inorganic N (NH_4^++NO_3^-) accumulated over 21 days was calculated for each column.

Statistical analysis

Two-way ANOVA, regression analysis and Tukey's multiple range test were performed by SAS® procedures to determine effects of temperature and nematode inoculation on the amount of inorganic N and population growth rates of the nematodes in each fungal experiment. Data were transformed, if necessary, before the growth rate analyses, as described in the dish microcosm study. Dunnett's multiple range test was performed to detect differences in inorganic N amount between control (fungus alone) and each of the nematode columns (fungus+*A. avenae* or *A. composticola*) at each of the four temperatures.

Results

Effect of temperature on growth of organisms

Fungi

Fungal species showed main effects. *Rhizoctonia solani* grew faster than *B. cinerea* at any temperature, and the growth rate always increased with temperature

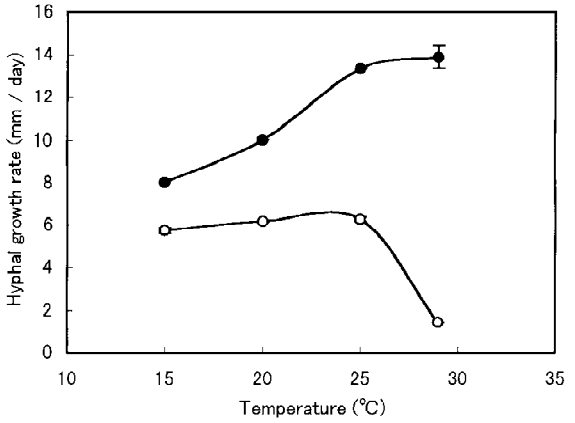

Figure 1. Hyphal growth rate (mm/day) of *Rhizoctonia solani* (solid circle) and *Botrytis cinerea* (open circle) on alfalfa-sand medium in dish microcosms at four temperatures. Vertical bars on symbols indicate standard errors. Most bars are obscured by symbols. Lines drawn in the graph show regression curves of the hyphal growth rates to temperature as follows: $y=55.93-7.73x+0.39x^2-0.01x^3$ ($r^2=0.953$) for *R. solani* and $y=72.05-10.58x+0.55x^2-0.01x^3$ ($r^2=0.993$) for *B. cinerea,* where x is temperature (°C) and y is hyphal growth rate (mm/day).

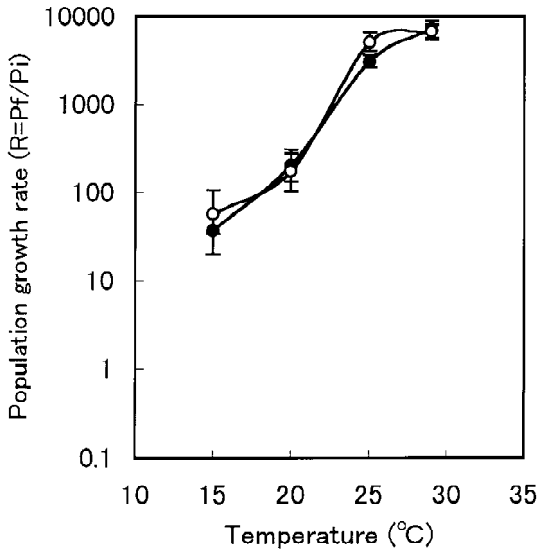

Figure 2. Population growth rate (R) of fungal-feeding nematodes, *Aphelenchus avenae* feeding *Rhizoctonia solani* (solid circle) or *Botrytis cinerea* (open circle) in dish microcosms, calculated as $R = Pf/Pi$, where Pf is the number of nematodes extracted on day 21 and Pi is the number inoculated (20 individuals/dish). Lines drawn in the graph show the regression curves of the growth rates to temperature as follows: $y=-5.14x+0.25x^2-0.004x^3+35.56$ ($r^2=0.968$) for the rate on *R. solani* and $y=-10.35x+0.50x^20.007x^3+71.03$ ($r^2=0.985$) for *B. cinerea,* where x is temperature (°C) and y is the growth rate (R) after power transformation. The rates are indicated in a logarithmic scale after detransformation. Vertical bars on symbols indicate 95% confidence range.

in the range of 15–29 °C (ANOVA, $p<0.01$). Fungal species and temperature interacted. The growth rate of *B. cinerea* slightly increased in the range of 15–25 °C, then decreased in the 25–29 °C range ($p<0.01$). The relationship between hyphal growth rate and temperature for both *R. solani* and *B.* cinerea was described by cubic regression ($p<0.01$), but the shape of the curves differed (Figure 1).

Nematodes

Data from the *Aphelenchus* experiment were power transformed with 0.209 as a multiplier to improve inequality of variances before statistical analyses. There was a main effect of temperature on population growth rate of the nematode. The growth rates increased with temperature (ANOVA, $p<0.01$). The response patterns of the growth rate to temperature were significantly described by cubic regression curves for the nematode populations on both *R. solani* and *B. cinerea* colonies ($p<0.01$) and the shapes of the curves were almost the same with either fungal species (Figure 2). The highest growth rate was estimated at 29 °C for *R. solani* and at 27.5 °C for *B. cinerea.*

Data for the *Aphelenchoides* experiment were log transformed to improve inequality of variances. Main effects of both temperature and fungal species on population growth rates of the nematode were significant (ANOVA, $p<0.01$). The rate was always greater with *R. solani* than with *B. cinerea*; it was greatest at 23–24 °C and least at 29 °C with both fungal species. The response patterns of population growth rate to temperature were significantly described by cubic curves on both *R. solani* and *B. cinerea* colonies ($p<0.01$), and the shapes of the curves were similar on both fungal species (Figure 3).

Effect of temperature on N mineralization by organisms

Rhizoctonia *experiment*

Data for two columns with *A. composticola* at 29 °C were omitted from the statistical analyses because the columns seemed to be contaminated with bacteria. The leachate from the two columns emitted a strong odor and contained an unusually large amount of inorganic N (1.660 and 1.562 μg/g sand). In previous studies, the amount of N released from microcosms with bacteria was more than ten times larger than from microcosms with fungi (Ferris et al., 1998). Therefore, the columns that released leachate with a strong odor were considered to be contaminated with bacteria. The

Table 1. Comparison of amount of inorganic (NH_4^++NO_3^-) N between fungus alone and fungus+nematode columns at each temperature in *Rhizoctonia* and *Botrytis* experiments

Treatment[b]	Inorganic Nμg/g sand[a]							
	Rhizoctonia				*Botrytis*			
	15 °C	20 °C	25 °C	29 °C	15 °C	20 °C	25 °C	29°C
FA	0.089	0.063	0.044	0.055	0.169	0.423	0.324	0.398
FAA	0.054	0.041	0.035	0.119[d]*	0.186	0.059	0.127	0.149*
	(−0.035)[c]	(−0.022)	(−0.009)	(0.064)	(0.017)	(−0.364)	(−0.197)	(−0.249)
FAC	0.023*	0.097	0.048	0.064[e]	0.075	0.132	0.07	0.125*
	(−0.066)	(0.034)	(0.004)	(0.009)	(−0.094)	(−0.291)	(−0.254)	(−0.273)

[a] Amount of N accumulated over 21 days.
[b] FA, FAA and FAC refer to fungus alone, fungus+*Aphelenchus avenae* and fungus+*Aphelenchoides composticola*.
[c] Values in parentheses are amounts of N mineralized directly by the nematodes estimated by subtracting the N amount in the fungus alone from that in the fungus+nematode columns. Negative values mean that the N amount was larger in the fungus alone than the fungus+nematode columns.
[d] The data of only two columns were examined in the statistical test because the other one seemed to be contaminated with bacteria.
[e] The statistical analysis was not conducted because two of the three replication columns seemed to be contaminated with bacteria.
* Amount of N in the fungus+nematode columns was significantly different from that in the corresponding control (fungus alone) columns (Dunnett's multiple range test, $p<0.05$).

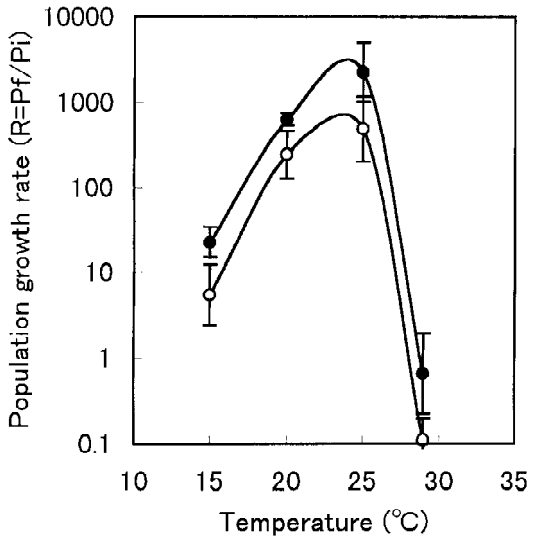

Figure 3. Population growth rate (R) of fungal-feeding nematodes, *Aphelenchoides composticola* feeding *Rhizoctonia solani* (solid circle) or *Botrytis cinerea* (open circle), calculated as $R = Pf/Pi$, where Pf is the number of nematodes extracted on day 21 and Pi is the number inoculated (20 individuals/dish). Lines drawn in the graph show the regression curves of the growth rates to temperature as follows: $y= -5.53x+0.32x^2-0.01x^3+31.14$ (r^2=0.963) for the rate on *R. solani* and $y=-6.88x+0.38x^2-0.01x^3$ (r^2=0.962) for *B. cinerea,* where x is temperature (°C) and y is the growth rate (R) after log transformation. The rates are indicated in a logarithmic scale after detransformation. Vertical bars on symbols indicate 95% confidence range.

data of one column with *A. avenae* at 29 °C were also omitted from the statistical analyses for the same reasons: the leachate from the column had a strong odor in the later part of the experimental period and the N amount was larger than that in other *A. avenae* columns (0.372 μg/g sand).

Only interaction effects between temperature and nematode inoculation on the amount of inorganic N accumulated over 21 days were detected by ANOVA ($p<0.05$). There were significant relationships between the amount of N and temperature in the fungus+nematode columns, but not in the fungus alone columns. The relationships in the fungus+nematode columns were significantly described by a quadratic curve for *A. avenae* and by a cubic curve for *A. composticola* columns (Figure 4). In *A. avenae* columns, the amount of inorganic N decreased as temperature increased from 15 to 20 °C, but increased at temperatures higher than 20 °C. The amount of N was the greatest at 29 °C. In *A. composticola* columns, the amount of inorganic N was greatest at 20 °C. The amounts of inorganic N in most of the nematode columns were not significantly different from the corresponding control (fungus alone) columns at each temperature. However, the amount was significantly greater in fungus+*A. avenae* columns at 29 °C and significantly smaller in fungus+*A. composticola* columns at 15 °C than in the respective controls (Dunnett's multiple range test, $p<0.05$, Table 1). Nematode population growth rates in the fungus+nematode columns

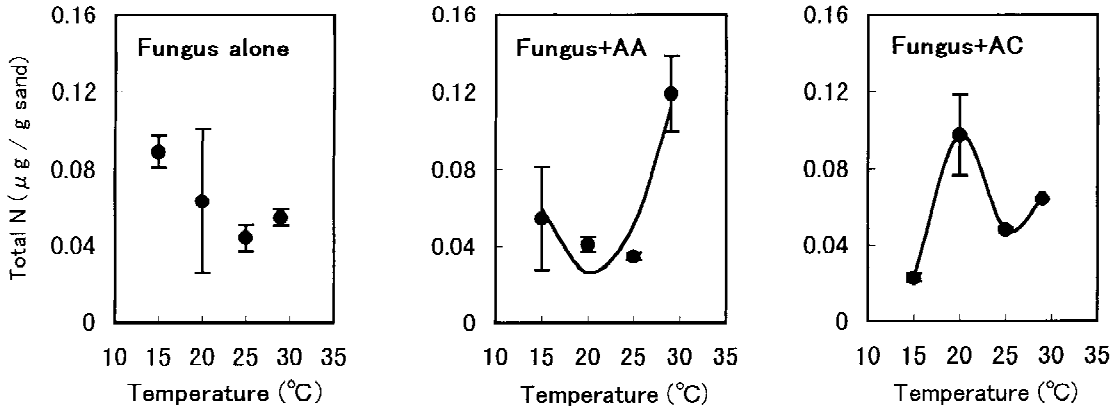

Figure 4. Influence of temperature on amounts of inorganic N (NH_4^++NO_3^-) accumulated over 21 days in *Rhizoctonia* experiment. The data of one column in fungus+AA (*Aphelenchus avenae*) and two in fungus+AC (*Aphelenchoides composticola*) treatments at 29 °C were omitted from statistical analyses due to possibilities of bacterial contaminations. Vertical bars on symbols indicate standard errors. Some of bars are obscured by symbols. Significant relationships between temperature and N amount were detected in fungus+AA (*Aphelenchus avenae*) and fungus+AC (*Aphelenchoides composticola*) treatments as follows by regression analysis ($p<0.05$): $y=-0.0454x+0.0011x^2+0.4896$ ($r^2=0.5519$) for fungus+AA and $y=0.4403x-0.0198x^2+0.0003x3$ ($r^2=0.7649$) for fungus+AC, where x is temperature (°C)and y is N amount (μg/g sand).

Table 2. Tukey's multiple comparison test for the main effect of nematode inoculation on the amount of inorganic N in *Botrytis* experiments. FA, FAA and FAC refer to fungus alone, fungus+*Aphelenchus avenae* and fungus+*Aphelenchoides composticola* treatments, respectively. The data at four temperatures were pooled for each treatment (n=12 for each). Values with the same letter were not significantly different ($p<0.05$)

Treatment	Inorganic N (average, μg/g sand)
FA	0.329 ^{a}
FAA	0.130 ^{b}
FAC	0.100 ^{b}

were power transformed with 0.191 as a multiplier before statistical analyses to improve inequality of variance. There was a main effect of temperature, and interaction effects between temperature and nematode species, on nematode population growth rates (ANOVA, $p<0.01$). The rate was highest at 29 °C in *A. avenae* columns and at 20 °C in *A. composticola* columns. The relationships between the rate and temperature were described by a straight line for *A. avenae* and by a quadratic curve for *A. composticola* (regression analysis, $p<0.01$, Figure 5)

Botrytis *experiment*

There were neither a main effect of temperature nor interaction effects between temperature and nematodes on the amount of inorganic N in leachates from *Botrytis* columns (ANOVA, $p>0.05$ and Figure 6). Only the main effect of nematode inoculation was significant in that the amount of N was significantly higher in the fungus alone columns than in the fungus+nematode columns (Tukey's multiple comparison test, $p<0.01$, Table 2). The inorganic N amount was significantly smaller in fungus+*A. avenae* or *A. composticola* than in the control (fungus alone) columns at 29 °C, but was not significantly different from the control columns at other temperatures (Dunnett's multiple range test, Table 1).

The data of nematode population growth rates were log transformed before statistical analyses to improve inequality of variance. A main effect of temperature ($p<0.01$) and interaction effects between temperature and nematode species on the population growth rate were detected by two way ANOVA ($p<0.01$). The population growth rate was highest at 20 °C for both *A. avenae* and *A. composticola*, while the rate was lowest at 15 °C for *A. avenae* and at 29 °C for *A. composticola*. The relationships between population growth rate and temperature were significantly described by a cubic curve for *A. avenae* and by a quadratic curve for *A. composticola* (Figure 5).

Discussion

Temperature is one of the important factors affecting activities of soil organisms. In these studies we ex-

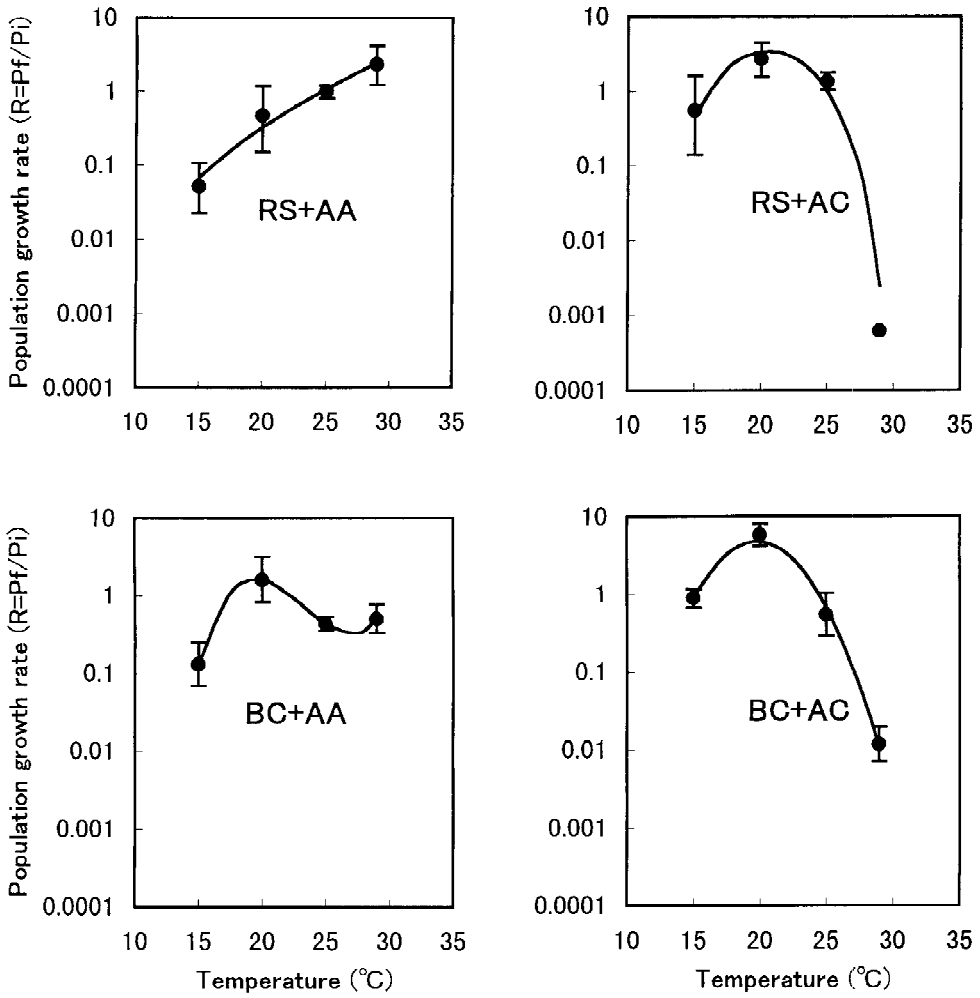

Figure 5. Influence of temperature on population growth rates ($R = Pf/Pi$) of fungal-feeding nematodes, *Aphelenchus avenae* (AA) and *Aphelenchoides composticola* (AC), in *Rhizoctonia* (RS) and *Botrytis* (BC) column experiments. Significant relationships between temperature and growth rate were detected in all of the fungus-nematode combinations by regression analysis ($p<0.05$). Lines drawn in the graphs of RS+AA and RS+AC show the regression curves of the growth rates to temperature as follows: $y=0.0418x-0.0268$ ($r^2=0.8593$) for AA and $y=0.5363x-0.0131x^2-4.2263$ ($r^2=0.8581$) for AC, where x is temperature (°C) and y is the growth rate (R) after power transformation. The line for AA is not straight due to logarithmic scale. Lines drawn in the graphs of BC+AA and BC+AC show the regression curves for the growth rate as follows: $y=5.4505x-0.2407x^2+0.0035x^3-40.13\ 6$ ($r^2=0.8476$) for AA and $y=1.2271x-0.0310x^2-11.4462$ ($r^2=0.9734$) for AC, where x is temperature (°C) and y is the growth rate (R) after log transformation. The growth rates are indicated in logarithmic scales after detransformation. Vertical bars on symbols indicate 95% confidence range.

amined effects of temperature on (1) hyphal growth rate of the soil fungi, *R. solani* and *B. cinerea*, (2) population growth rate of the nematodes, *A. avenae* and *A. composticola* feeding on the fungi, and (3) N mineralization by the fungi with or without the nematodes.

Effect of temperature on growth of organisms

Hyphal growth rates of *R. solani* and *B. cinerea* had different temperature optima. Maximum growth rate of *R. solani* occurred at higher temperature (25–29 °C) than that of *B. cinerea* (20–25 °C). These results are in concurrence with other studies (Blakeman, 1980; Sherwood, 1970).

Population growth rates of the nematodes were also affected by temperature. Our results suggest that the optimum temperature for population increase of *A. avenae* is about 28 °C or higher, which is supported by other studies (Mendis and Evans, 1983; Pillai and Taylor, 1967). Individual species of the genus

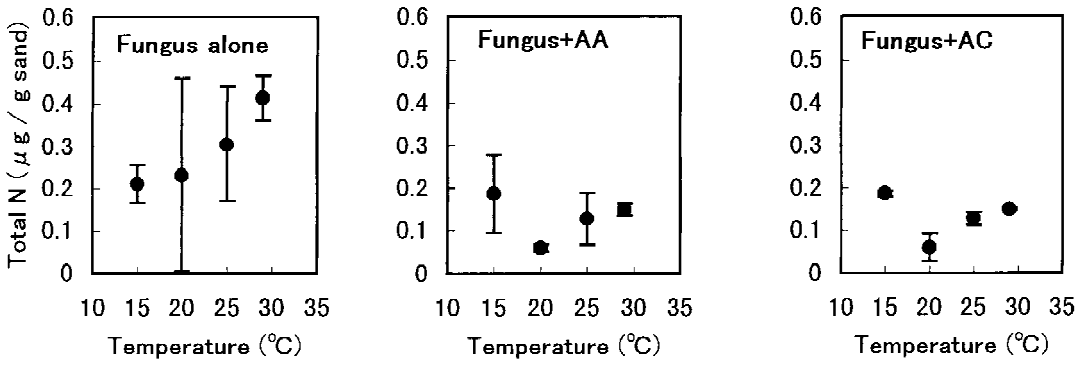

Figure 6. Influence of temperature on amounts of inorganic N (NH_4^++NO_3^-) accumulated over 21 days in *Botrytis* experiment. Vertical bars on symbols indicate standard errors. Some of bars are obscured by symbols. Significant relationships between temperature and N amount were not detected in any of the three treatments (fungus alone, fungus+AA (*Aphelenchus avenae*) and fungus+AC (*Aphelenchoides composticola*)) by regression analysis ($p>0.05$).

Aphelenchoides have different temperature optima for reproduction (Huang et al., 1972; Rössner and Nagel, 1984; Wallace, 1960; Younes, 1969). For *A. composticola,* the optimum temperature in our studies (23 °C) is slightly higher than the 20 °C reported for the strain studied by Younes (1969).

Effect of temperature on N mineralization by organisms

Mineralization by Rhizoctonia solani *with or without nematodes*

The effects of temperature on the amount of inorganic N from the fungus alone and the fungus+nematode columns differed. In the fungus alone columns, temperatures in the range of 15–29 °C did not affected the mineralization ability of the fungus. In all columns, organic substrates and fungal inoculum were dispersed uniformly, so it is possible that a hyphal network developed fully in the columns and fungal biomass quickly reached the carrying capacity at any temperature. The mineralization ability of the fungus might be closely related to the rate and extent of hyphal growth. When the fungal biomass reached the carrying capacity of the columns and the hyphal growth stopped, the mineralization may have become limited. Consequently, no difference was observed in inorganic N mineralized in the fungus alone columns across temperature.

There was a significant effect of temperature on the amount of N detected in the fungus+nematode columns. The amount of N was greatest at 29 °C in *A. avenae* columns and at 20 °C in *A. composticola* columns. These results correspond to the temperatures at which greatest population growth rate of each nematode species was observed in the columns. The data for *A. avenae* columns also corresponded with the temperatures at which greatest population growth was observed in the dish microcosms. In *A. composticola* columns, population growth of the nematodes was greater at lower temperature (20 °C) than in dish microcosms (23 °C). The difference may have been due to differences in initial nematode density between the column and dish microcosms. The columns were inoculated with 50 individual nematodes/g sand and the dishes with 1 individual/g sand. In the columns at 25 °C, nematodes may have reached carrying capacity in the middle of experiment and decreased afterward, resulting in an apparent lower population growth rate at 25 °C than at 20 °C by the end of the experiment.

It is suggested that the largest growth in the nematode populations at the optimum temperatures maximized the nematode feeding on the fungal hyphae to result in release of the largest amount of the inorganic N once immobilized by the fungus.

We conclude that temperature affected N mineralization in fungus-based decomposition systems through population growth rates of fungal-feeding nematodes.

Mineralization by Botrytis cinerea *with or without nematodes*

In both fungus alone and fungus+nematode columns, temperature had no significant effect on inorganic N amount. The insignificant effect at 15–25 °C in the fungus alone columns may be explained by the small difference in hyphal growth rates in dish experiments in this temperature range (Figure 1). Interestingly,

some N was obtained at 29 °C although this temperature was too high for hyphal growth of *B. cinerea* (Figure 1). The N may have resulted from hydrolysis of dead hyphae and other organic substrates at higher temperature. In the fungus+nematode columns, the contributions of fungal-feeding nematodes to N mineralization are small, as discussed later, so there was no significant difference in the amount of N across temperatures as in the fungus alone experiments, although nematode growth rate differed depending on temperature (Figure 5).

Do fungal-feeding nematodes contribute N mineralization in fungus based decomposing system?

In these studies, the amount of N in the fungus+nematode columns was not significantly greater than that in the fungus alone columns for most combinations of fungus, nematode species and temperature. It was significantly greater than the fungus alone columns only in the combination of *R. solani*+*A. avenae* at 29 °C. Chen and Ferris (1999) also reported some larger amounts of N in fungus+nematode columns in the combination of *R. solani*+*A. avenae* or *A. composticola.* In general, the contribution of fungal-feeding nematode populations to N mineralization (N mineralized directly by nematodes estimated as the difference in N amount between microbe alone and microbe+nematode microcosms) is, however, smaller than that of bacterial feeding nematodes (Ferris et al., 1998; Ingham et al., 1985). Carbon/N ratios of the bodies of the organisms may be responsible for the small contribution. The C/N ratios of fungal-feeding nematodes and fungi are similar: 8:1 to 11:1 for nematodes and 8:1 to 10:1 for fungi (Chen and Ferris, 1999; Griffin, 1972), resulting in excretion of only small amounts of excess N associated with assimilated C used in respiration.

The small contribution of the nematodes to N mineralization might be also due to an unfavorable environment for nematode population growth in the column microcosms. In our experiments, the population growth rates of the nematodes were lower in columns than in dishes at the same temperatures across fungus-nematode combinations. Even the final nematode densities were generally lower in columns. For example, the final densities in *A. avenae* and *A. composticola* columns at 20 °C in *Rhizoctonia* experiment were 28.8 and 142.7/g sand, respectively, while in dishes the densities for the corresponding fungus-nematode combinations at 20 °C were 214.4 and 277.9/g sand. This difference might be affected by excess moisture in the column microcosms due to frequent leaching.

Carbon/N ratio of the organic substrates in the column microcosms might be another cause of the small contribution of the nematodes to N mineralization in the columns. Chen and Ferris (1999) reported that the amount of N detected in fungus alone columns became smaller when the C/N ratio of the organic substrates were higher, while it remained almost the same in fungus+nematode columns to result in larger nematode contribution at higher C/N ratio of the substrates. Perhaps more N was immobilized by fungi at higher C/N ratio in the fungus alone columns but nematodes could release the immobilized N by feeding on the fungal biomass at any C/N ratio in fungus+nematode columns. In our study, C/N ratio of the organic substrates (alfalfa and cellulose) was established at 35:1 in the hope of achieving measurable net mineralization by nematodes. Actual mineralization was, however, small in our column microcosms. We might have detected greater mineralization at C/N ratios above 35:1. However, at high C/N ratios fungal growth may be N-limited so that N mineralized by nematodes would be rapidly immobilized by fungal hyphae again. In that case, the window of time during which N remains in the mineral form would be quite small, necessitating a shorter sampling period for detection. Conditions under which contribution of fungal-feeding nematodes to N mineralization is most pronounced may be those that optimize the growth rates of both fungus and nematode.

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