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Nitrogen mineralization by bacterial-feeding nematodes: verification and measurement

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Abstract

Bacterial feeding nematodes excrete N assimilated in excess of that required for growth. Because metabolic and developmental rates differ among nematode species, we hypothesized that their contribution to N mineralization in soil would differ. Sand-column microcosms amended with an organic substrate, bacteria, and with or without bacterial-feeding nematodes, were leached at 3-d intervals. Cumulative N, as NH_4^+ or NO_3^-, leached from columns containing nematodes was consistently greater than from columns without nematodes. Maximum N-mineralization rates for populations of rhabditid nematodes, which predominated in field soils early in the summer were at lower temperatures than those for cephalobid nematodes, which predominated later in the summer. For an organic substrate with C-to-N ratio of 11:1, rates of N mineralization among species of different body size were similar, ranging between 0.0012 and 0.0058 μg-N nematode^{-1} d^{-1}, mainly as NH_4^+. Smaller nematodes mineralized more N per unit of body weight than larger nematodes. We hypothesized that at low C-to-N ratios of the organic substrate, bacterial growth is C-limited and N-immobilization will be minimal; at high C-to-N ratios bacterial growth will be N-limited and there may be rapid immobilization of newly-mineralized N. Consequently, net N mineralization in the presence of nematodes will be lower when the organic substrate has a high C-to-N ratio. In experiments with different nematode species, net mineralization and the nematode contribution to mineralization generally decreased with increasing C-to-N ratio, consistent with the hypothesis; however, there were exceptions.

Introduction

Bacterial-feeding nematodes contribute to nitrogen mineralization by feeding on and by dispersing bacteria (Anderson et al., 1981; Bouwman et al., 1994; Freckman, 1988; Griffiths, 1994). The extent of the contribution is determined by their metabolic and behavioral attributes, by their demography and physiology, and by the relative C-to-N ratios of the nematodes and bacterial prey (Ferris et al., 1995, 1996a, 1997; Ingham et al., 1985; Venette and Ferris, 1997). The excess N assimilated during growth and egg production or to meet the C needs of respiration is excreted in mineral form (NH_4^+) (Lee and Atkinson, 1977; Wright and Newall, 1976). In recent studies we measured the mean C-to-N ratio for eight nematode species cultured on *Escherichia coli* on agar as 5.89 and the mean C-to-N ratio of five isolates of soil bacteria and *E. coli* as 4.12 (Ferris et al., 1997). From C and N budgets, we calculated that the bacterial-feeding nematode community in the top 15 cm of a field soil mineralized N at rates increasing to 1.01 μg-N g-soil^{-1} d^{-1} in the rhizosphere. Validation of the phenomenon requires measurement of N mineralization due to nematodes in soil.

When the soil food web is fueled by organic material with a low C-to-N ratio, microbial growth may be limited by availability of C but not by availability of N. In that case, N mineralized by bacteria and fungi, by nematodes and protozoa grazing on bacteria, and at each trophic link in the food web, will be available for plant uptake. When the organic material has

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a high C-to-N ratio, there will be abundant C to fuel microbial growth but availability of N will become a limiting factor. Then, N mineralized by the organisms in the food web may be immobilized by microbes and consequently rendered unavailable for plant uptake. Under such N-limited conditions, microbes compete with plants for available mineralized N (Kaye and Hart, 1997). We hypothesize that bacterial-feeding nematodes will enhance N mineralization at all C-to-N ratios of organic substrate, but that the net effect of the nematodes will be less evident at higher C-to-N ratios due to immobilization by the N-limited microbial community.

In this paper we measure N mineralization by bacterial-feeding nematodes in soil microcosms. We determine mineralization rates for nematodes of different size, life-history characteristics and taxonomic classification. We also examine the effect of the C-to-N ratios of the organic matter substrate on net mineralization due to bacterial-feeding nematodes.

Methods

Bacterial-feeding nematodes were originally isolated from populations endemic in a long-term Sustainable Agriculture Farming Systems (SAFS) project conducted on the University of California campus in Davis, California (Ferris et al., 1996b; Temple et al., 1994). The nematodes represent Maturity Index categories 1 [*Bursilla labiata*, *Cruznema tripartitum* and *Rhabditis cucumeris* (family Rhabditidae), and *Panagrolaimus detritophagus* (family Panagrolaimidae)] and 2 [*Acrobeloides bodenheimeri*, *A. buetschlii* and *Cephalobus persegnis* (family Cephalobidae)] (Bongers, 1990). All species were maintained in gnotobiotic cultures with *Escherichia coli* on nematode growth medium (NGM; Sulston and Hodgkin, 1988) as described in Ferris et al. (1995).

The soil medium used in these studies was acid-washed sand from an uncultivated area. The sand (44.5 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_2SO_4 for 48 h. The acid solution was drained and the sand was rinsed with water in the cement mixer 10 times. Moisture content of the drained sand was determined gravimetrically and 4 g air-dried ground (<0.8 mm particle size) alfalfa (*Medicago sativa*) was added per kg of dry sand. The difference between wet weight and dry weight of the sand was considered the weight of soil solution, and 1 mL of dissolved cholesterol (5 mg cholesterol/mL-ethanol) was added per liter of soil solution to provide for sterol requirements of bacterial-feeding nematodes (Sulston and Hodgkin, 1988). The sand, alfalfa and cholesterol were mixed in the cement mixer for 20 min. The mixture was autoclaved twice for 4–6 h at 24 h intervals. After cooling, the moisture level of the autoclaved sand was determined and the sand divided equally by weight into plastic buckets (19-L volume), one bucket per experimental treatment. After several repetitions of these experiments the procedure was modified by inserting an aeration tube through the sand to the bottom of the bucket.

All buckets were inoculated with a nematode-free microbial slurry (20–50 mL) prepared from field soil as per Ferris et al. (1997). The buckets were loosely covered and stored at 22–24 °C. Lids were removed daily to allow air replacement, or air was blown continuously through the central aeration tube. Nematode inoculum was prepared 3–5 d later. Test nematodes were rinsed from agar cultures and the suspension poured into ten holes in the sand surface of separate buckets, one bucket for each nematode species. One bucket was not inoculated with nematodes to provide a nematode-free control.

Moisture content of the buckets was adjusted to 8% with distilled water and maintained at that level by weighing the buckets and adding water to adjust for weight loss at 2-d intervals. The buckets were incubated at 22–24 °C for 2–3 weeks to allow the nematodes to increase and disperse. Population levels were measured by removal of cores at weekly intervals. We attempted to achieve nematode population densities in the buckets between 5 and 20 nematodes/g-sand.

Soil-column microcosms were constructed from 30 cm lengths of 4 cm i.d. polyvinyl chloride pipe capped on one end. 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. When containing approximately 475 g sand, columns of these dimensions are filled to about 5 cm from the top.

Columns and stainless steel mesh screens were washed thoroughly and soaked overnight in 10% commercial bleach ($NaClO_3$ solution). Columns were rinsed with deionized water. The organic substrate necessary to provide the C-to-N ratio, and the required amount of total N (usually 37.3 mg N) for each exper-

iment was mixed into 500 g subsamples of the sand incubated in the buckets by gentle agitation in a plastic bag for 1 min. The amount and composition of organic material differed with the type of experiment; details are provided later.

The sand incubated with bacteria alone or with nematodes and bacteria was poured into the columns. Columns were tapped gently as they were filled to ensure a compact continuum without air pockets. Columns were arranged vertically, capped end down, in randomized complete blocks in a wall-mounted rack and allowed to equilibrate overnight. Each column was then leached with 100 mL of distilled deionized water to minimize initial levels of mineral N. The leachate was discarded.

At this time (day 0), and at 3-d intervals thereafter, holes in the base-cap of the columns were stoppered and 60 mL of distilled deionized water was poured into each. After 4 min the stoppers were removed and columns were allowed to drain for 2 h, during which approx. 45 mL of leachate was collected in preweighed containers. Containers were weighed to calculate the volume of leachate, capped and placed in a freezer prior to further processing. The experiments were terminated after 21 or 24 d.

N mineralization by individual species

Columns were established with 1 g of finely-ground alfalfa leaf tissue (particle size $<$0.83 mm; 3.7% N; 41.3% C) at 11:1 C-to-N ratio, to provide 37.3 mg N per column, or 74.5 μg-N/g-sand (in addition to any N remaining from the organic material used in the buckets). Four replicate columns contained bacteria and a nematode species to be tested and four control columns contained bacteria without nematodes. Three or four nematode species were tested in each trial. Eight extra columns were established for each nematode species and the nematode-free controls to allow weekly destructive sampling for determination of nematode population densities. In the destructive sampling process, the sand in each column was divided into two approx. equal parts and the nematodes in each part extracted by sugar flotation/centrifugation (Jaffee et al., 1988). The first set of destructive samples was collected after the day 0 leaching to allow assessment of the starting nematode population. Nematode population levels were assessed in all remaining columns at the end of the study. Two or more trials were conducted for several of the nematode species.

C-to-N ratio of the organic substrate

Organic substrates representing eight C-to-N ratios were prepared by combining finely-ground alfalfa leaf tissue (see previous section) with cellulose (Whatman medium fibers; W. & R. Balston, Ltd., England) so that the amount of N was constant across all combinations and was consistent with the amount used in the species comparison experiments. The cellulose had a C content of 44.4% and an N content of $<$0.1%. The materials were combined to create C-to-N ratios of 11:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1 and 45:1, with 74.5 μg-N/g-sand in each column. In early iterations of these experiments we used corn starch as the source of additional C but that restricted leaching of the columns at high C-to-N ratios. The cellulose fibers did not affect water percolation.

Columns were paired with and without the nematodes at each C-to-N level. Each treatment combination was established with three replications, requiring a total of 48 columns. Additional columns were established for determination of initial nematode population densities, measured after the day 0 leaching as in the previous experiments. Nematode populations were assessed by the sugar flotation/centrifugation (Jaffee et al., 1988) method used in the studies on N mineralization by individual species. At the final takedown, nematodes were extracted from each column. 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.

Ammonium and nitrate analyses

Two methods were used for N analysis in separate experiments during the course of these studies. The choice of method depended on availability of equipment and technical problems encountered as we changed C-to-N ratios of the organic substrate. Results for each method were qualitatively similar.

(a) Nitrogen analysis by spectrophotometry

A photometric method was used for N analysis in experiments comparing N-mineralization potential of individual species of bacterial-feeding nematodes where the C-to-N ratio of the organic material was 11:1. A 15-mL subsample was removed from each leachate sample for NH_4^+ analysis, the remainder was used for NO_3^- analysis. The NO_3^- samples were placed in a freezer. Both N–NH_4 and N–NO_3 were determined using protocols modified from Orland (1965).

Fifteen mL of 4 *M* KCl were added to each 15-mL leachate sample (providing a 2 *M* KCl solution) and the samples were shaken for 1 h to displace bound NH_4^+ (Bundy and Meisinger, 1994). Samples were treated with 300 μL of 173 m*M* $ZnSO_4 \cdot 7H_2O$ and 150 μL of 6 *M* NaOH. Samples were clarified by centrifugation at 810 *g* for 5 min. Transmittance of the supernatant was measured at 475 nm wavelength to determine background clarity. Two drops of EDTA and 1 mL of Nessler's reagent, which increases in color with NH_4^+, were added and transmittance was again measured. The reduction in transmittance due to reaction of the Nessler's reagent with the NH_4^+ in the sample was determined by subtraction. A standard curve of transmittance at known N–NH_4 concentrations was prepared and the concentration of N–NH_4 in the sample was determined from the standard curve. The concentration of N–NH_4 was expressed as μg-N/g-sand.

For N-NO_3 analysis, samples were thawed by brief immersion in hot water. Following centrifugation at 810 *g*, transmittance of the supernatant was measured at 275 nm wavelength to allow correction for dissolved organic matter, and transmittance at 220 nm wavelength was determined to allow estimation of N–NO_3 from standard curves. The concentration of N–NO_3 was calculated as μg-N/g-sand in the column. The sum of the N–NH_4 and N–NO_3 provided an estimate of the total N mineralized in each column during the 3-d interval between leachings.

(b) Nitrogen analysis by diffusion-conductivity analyzer (ion electrode)

In experiments where the amount of C in the columns was increased to provide a range of C-to-N ratios, residual organic material in the samples caused the Nessler's reagent to turn dark green rather than yellow, confounding spectrophotometer readings. For those experiments we used a diffusion-conductivity analyzer (Carlson, 1978).

Frozen leachate samples were thawed by brief immersion in hot water. Each sample was shaken and 12 mL of the leachate was transferred, using a fresh pipette, into a 50-mL conical centrifuge tube containing 12 mL of 4 *M* KCl so that the sample was effectively in 2 *M* KCl solution. The centrifuge tubes were shaken for 1 h to displace bound NH_4^+ (Bundy and Meisinger, 1994). Samples were treated with 240 μL of 173 m*M* $ZnSO_4 \cdot 7H_2O$ and 120 μL of 6 *M* NaOH (Orland, 1965). Samples were clarified by centrifugation at 810 *g* for 5 min and the supernatant poured into 20-mL scintillation vials and capped. Nitrate and ammonium concentrations were determined using a diffusion-conductivity analyzer (ion electrode) analyzer by comparison with known standards. The amount of N–NH_4, and N–NO_3 in the sample was determined, and the total amount of N in the leachate sample was expressed as μg-N/g-sand in each column.

Calculation of N mineralization due to nematodes

In all experiments, the amount of N mineralized by nematodes was calculated for each 3-d interval as the difference between the average cumulative inorganic N measured in leachate from the nematode columns and that from the nematode-free control columns. Numbers of nematodes/g-sand on each leaching date were interpolated from initial, final, and intermediate (where available) nematode samples. The increase in average cumulative N during a time interval was divided by the average nematode population density during that time interval to calculate the N mineralization per nematode. By assuming a uniform life-stage distribution and average nematode weights for each stage of each species measured in previous studies, the N mineralization/μg-nematode was also calculated (Ferris et al., 1995).

Since N is a non-renewable resource being removed from the columns through leaching, the relationship between cumulative N from the nematode or control columns and time conforms to the natural growth function:

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

In this usage, the function is asymptotic with N_p, described as the Nitrogen Mineralization Potential of the system (Stanford, 1982); N_t is the cumulative amount of N mineralized by time *t*; and *k* is a regression coefficient indicating the rate of mineralization in the columns. The model transforms to the first order equation:

$$\ln(N_p - N_t) = \ln(N_p) + kt \quad (2)$$

so that $\ln(N_p)$ and *k* can be determined by linear regression. Parameter *k* is divided into two components for statistical comparison of mineralization rates in columns with and without nematodes. Rates of N mineralization differ if there is a treatment by time interaction. We include the interaction as an additional term using qualitative independent variables (Neter et al., 1990) so that Equation 2 now has the form:

$$\ln(N_p - N_t) = \ln(N_p) + k_b t + k_n t T \quad (3)$$

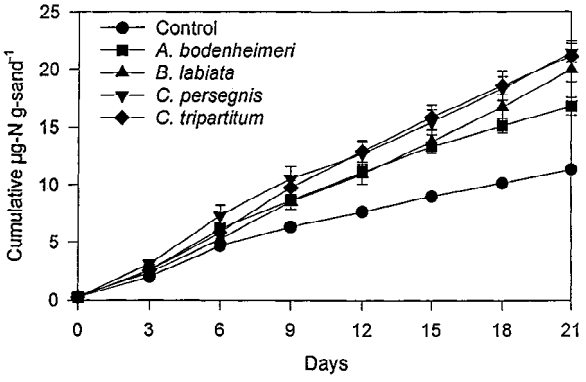

Figure 1. Cumulative N per g-sand (as NH_4^+ and NO_3^-) leached at 3-d intervals from sand columns containing bacteria and various species of bacterial-feeding nematodes or bacteria alone (control). *A. bodenheimeri* = *Acrobeloides bodenheimeri*, *B. labiata* = *Bursilla labiata*, *C. persegnis* = *Cephalobus persegnis* and *C. tripartitum* = *Cruznema tripartitum*.

where k_b is the rate of N mineralization due to microbial activity; k_n is the rate of N mineralization due to nematodes; and T is the treatment code. For columns without nematodes ($T = 0$), Equation 3 simplifies to:

$$\ln(N_p - N_t) = \ln(N_p) + k_b t. \quad (4)$$

For columns with nematodes ($T = 1$), Equation 3 becomes:

$$\ln(N_p - N_t) = \ln(N_p) + (k_b + k_n)t. \quad (5)$$

When k_n is statistically different from zero, nematodes have a significant impact on the rate of N mineralization.

Results

In all our studies the mineralized N measured in leachate from the columns was predominantly in the NH_4^+ form, consistent with the excretory products of nematodes (Lee and Atkinson, 1977; Wright and Newall, 1976). Since N–NH_4 may be transformed to N–NO_3through microbial activity, we report total N mineralized as the sum of N–NH_4 and N–NO_3.

N mineralization by individual species

These experiments were conducted six times. In the first experiment, F94, numbers of nematodes inoculated into the columns were low and population levels of two species declined during the course of the experiment. The amount of N mineralized in the nematode columns did not differ from that in the controls. In all other experiments with individual nematode species at a C-to-N ratio of 11:1, the nematode populations persisted or increased. In those cases, the exponent of the natural growth function indicated that N mineralization was significantly greater in the columns containing nematodes and bacteria (k_n + k_b) than in those containing bacteria alone (k_b) (Table 1). An example of the greater N mineralization in columns containing nematodes is provided in the data from experiment M95 (Figure 1). After the first 3 d in that experiment, cumulative N leached from nematodes columns was significantly greater than that leached from control columns.

Since these experiments were done at different times and different numbers of nematodes were used for each nematode species, direct comparisons cannot be made of the N-mineralization potential and k_n parameters of different species. For comparisons among species, N-mineralization rates of bacterial-feeding nematodes are expressed per individual and per unit weight of nematode (Table 2).

We used the increase in mineral N in the nematode columns relative to the control columns with the measured and interpolated nematode population levels at each leaching date, to calculate the average amount of N mineralized nematode^{-1} d^{-1}. There was often a large amount of N in both nematode and control columns on day 0 (the first leaching), presumably reflecting background N mineralized during the preliminary incubation period and not completely flushed out with the initial leaching. Consequently, N-mineralization rates per nematode were not calculated from the day 3 data points. Only the time interval over which the mineralization rate became consistent was used for the rate estimates. In the sand columns, rates of mineralization per nematode ranged from 0.0012 μg-N d^{-1} for an average individual of *A. buetschlii* to 0.0058 μg-N d^{-1} for *R. cucumeris*, with most species under 0.003 μg-N d^{-1} (Table 2B).

Since metabolic rates of nematodes vary with the size of individuals (Atkinson, 1980; Ferris et al., 1995; Klekowski et al., 1972), we calculated N-mineralization rates per unit weight of nematode from these data by assuming that each life stage was equally abundant in the population. Under that stage-distribution assumption, using average weights per individual calculated in previous research (Ferris et al., 1995), mineralization rates ranged from 0.0009 μg-N μg-nematode^{-1} d^{-1} for *C. tripartitum*

Table 1. The contribution of bacterial-feeding nematodes to N mineralization as measured in five separate experiments. Parameter k_n is the contribution of nematodes to the coefficient (k) obtained when the natural growth function $N_t = N_p(1 - e^{kt})$ is fitted to data from columns containing nematodes and bacteria or bacteria alone; the contribution of nematodes to N mineralization is least when k_n approaches zero. Nitrogen Mineralization Potential is the N_p parameter of the function for an individual nematode species in a given trial.

Genus	Species	Trial code	k_n	$p(k_n = 0.0)$	N-Min. Pot. μg-N/g-sand
Acrobeloides	*bodenheimeri*	M95	−0.0228	<0.01	24.76
Acrobeloides	*bodenheimeri*	S95	−0.0388	<0.01	22.33
Acrobeloides	*buetschlii*	S95	−0.0398	<0.01	23.36
Bursilla	*labiata*	W95	−0.0243	<0.01	22.33
Bursilla	*labiata*	M95	−0.0358	<0.01	26.45
Bursilla	*labiata*	J96	−0.0042	<0.01	8.07
Cephalobus	*persegnis*	F94	−0.1087	<0.01	4.11
Cephalobus	*persegnis*	W95	−0.0670	<0.01	25.41
Cephalobus	*persegnis*	M95	−0.0514	<0.01	26.62
Cruznema	*tripartitum*	W95	−0.0669	<0.01	24.61
Cruznema	*tripartitum*	M95	−0.0568	<0.01	27.85
Cruznema	*tripartitum*	J96	−0.0066	<0.01	8.07
Diploscapter	*coronata*	J96	−0.0275	<0.01	8.11
Panagrolaimus	*detritophagus*	S95	−0.0428	<0.01	23.08
Rhabditis	*cucumeris*	S95	−0.0309	<0.01	21.88

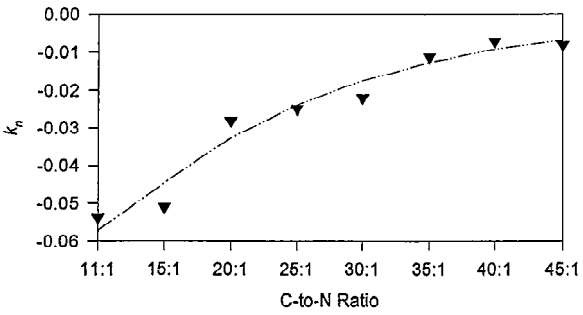

Figure 2. Rate of N-mineralization (k_n) due to nematodes across varying C-to-N ratios of the organic substrate where the amount of N is held constant. Parameter k_n is derived from the equation $\ln(N_p - N_t) = \ln(N_p) + k_b t + k_n tT$, where N_p, is the nitrogen mineralization potential of the system, N_t is the cumulative amount of N mineralized by time t, and k_b is the rate of N mineralization due to microbial activity. The contribution of nematodes to N mineralization is least when k_n approaches zero.

to 0.015 μg-N μg-nematode^{-1} d^{-1} for *A. buetschlii* (Table 2B).

C-to-N ratio of the organic substrate

The natural growth function provided an appropriate model for cumulative total N mineralization at each C-to-N ratio in the presence and absence of each nematode species. In most trials, the coefficient of the natural growth function, indicating contribution of bacteria and nematodes to N mineralization, was significantly greater than that in the nematode-free control columns at each C-to-N ratio (Table 3). Note that the contribution of nematodes to N mineralization is least when k_n approaches zero (Table 3, Figure 2).

The effect of nematodes on N mineralization in sand columns containing organic material at different C-to-N ratios can be expressed as the cumulative amount of N mineralized in the columns over the 21–27 d duration of the separate experiments (Figures 3 A–D). The relationship between nematode contribution to total N mineralization and C-to-N ratio was not consistent across experiments. The amount of N mineralized in the *A. buetschlii* experiment was greater in the presence of nematodes at all C-to-N ratios, net mineralization did not decline in the absence or presence of nematodes at higher C-to-N ratios. In fact, the nematode contribution to N mineralization was marginally greatest at the highest C-to-N ratios (Figure 3A). Reduced net mineralization at higher C-to-N ratios and the reduction of the nematode effect, consistent with the hypothesis, is clear in the *C. persegnis* (Figure 3B) and *B. labiata* (Figure 3C) experiments.

Table 2. Nitrogen-mineralization rates of bacterial-feeding nematodes expressed per individual and per unit weight of nematode averaged across four life stages. A. Averages are calculated across 6 to 9 individual leachings at 3-d intervals from sand columns in five separate trials. B. Nitrogen-mineralization rates of bacterial-feeding nematodes averaged across trials (Note: data for *Cephalobus persegnis* in F94 were omitted from these averages)

Genus	Species	Trial code	μg-N nematode^{-1} d^{-1}	s.d.	μg-N μg-nematode^{-1} d^{-1}	s.d.
A. Averages for each trial						
Acrobeloides	*bodenheimeri*	M95	0.0036	0.0012	0.0089	0.0028
Acrobeloides	*bodenheimeri*	S95	0.0024	0.0024	0.0173	0.0180
Acrobeloides	*buetschlii*	S95	0.0012	0.0013	0.0150	0.0162
Bursilla	*labiata*	W95	0.0025	0.0025	0.0153	0.0150
Bursilla	*labiata*	M95	0.0020	0.0008	0.0121	0.0049
Bursilla	*labiata*	J96	0.0013	0.0006	0.0076	0.0038
Cephalobus	*persegnis*	F94	0.0228	0.0118	0.1092	0.0563
Cephalobus	*persegnis*	W95	0.0026	0.0028	0.0121	0.0134
Cephalobus	*persegnis*	M95	0.0032	0.0012	0.0153	0.0057
Cephalobus	*persegnis*	J96	0.0022	0.0022	0.0103	0.0108
Cruznema	*tripartitum*	W95	0.0020	0.0010	0.0008	0.0004
Cruznema	*tripartitum*	M95	0.0026	0.0008	0.0010	0.0003
Cruznema	*tripartitum*	J96	0.0024	0.0010	0.0009	0.0004
Diploscapter	*coronata*	J96	0.0021	0.0006	0.0050	0.0014
Panagrolaimus	*detritophagus*	S95	0.0018	0.0012	0.0086	0.0058
Rhabditis	*cucumeris*	S95	0.0058	0.0040	0.0131	0.0090
B. Global Averages						
				s.e.		s.e.
Acrobeloides	*bodenheimeri*		0.0030	0.0008	0.0131	0.0059
Acrobeloides	*buetschlii*		0.0012	n.a.	0.0150	n.a.
Bursilla	*labiata*		0.0019	0.0004	0.0117	0.0027
Cephalobus	*persegnis*		0.0027	0.0003	0.0126	0.0015
Cruznema	*tripartitum*		0.0023	0.0002	0.0009	0.0001
Diploscapter	*coronata*		0.0021	n.a.	0.0050	n.a.
Panagrolaimus	*detritophagus*		0.0018	n.a.	0.0086	n.a.
Rhabditis	*cucumeris*		0.0058	n.a.	0.0131	n.a.

n.a. = not available.

The apparent in the lack of a nematode effect on N mineralization at higher C-to-N ratios in the *C. tripartitum* was associated with high nematode mortality at those levels (Figure 3D).

Since different numbers of nematodes were used for each nematode species, statistical comparisons among species cannot be made until the data are adjusted on a per individual basis. In the experiments involving C-to-N ratios of the organic substrate, the amounts of N mineralized nematode^{-1} d^{-1} were based on population levels measured at the beginning and end of each trial. Population levels were calculated for each leaching date by interpolation. Although there were differences between the nematode species tested, *A. buetschlii*, *B. labiata* or *C. persegnis*, the rates across C-to-N ratios for an individual species did not differ (Figures 4 A–C).

Discussion

During the time course of mineralization studies, the rate of N mineralization per nematode may increase during an early 'adaptation phase' (as nematodes adjust to conditions in sand columns) and decline during a later resource limitation phase. We terminated most experiments after 21 d which coincided with decreased recovery of mineral N.

Table 3. The contribution of bacterial-feeding nematodes to N mineralization at different C-to-N ratios of the organic substrate, as measured in three separate trials. Parameter k_n is the contribution of nematodes to the coefficient (k) obtained when the natural growth function $N_t = N_p(1 - e^{kt})$ is fitted to data from columns containing nematodes and bacteria or bacteria alone; the contribution of nematodes to N mineralization is least when k_n approaches zero. Nitrogen Mineralization Potential is the N_p parameter of the function for an individual nematode species at a C-to-N ratio

Genus	Species	Trial code	C-to-N ratio	k_n	$p(k_n = 0.0)$	N-Min. Pot. μg-N g-sand^{-1}
Acrobeloides	*buetschlii*	J97	11:1	−0.0253	<0.01	8.74
			15:1	−0.0257	<0.01	8.52
			20:1	−0.0378	<0.01	8.13
			25:1	−0.0181	<0.01	8.10
			30:1	−0.0499	<0.01	8.04
			35:1	−0.0143	<0.05	8.26
			40:1	−0.0747	<0.01	9.74
			45:1	−0.0532	<0.01	8.27
Bursilla	*labiata*	A97	11:1	−0.0490	<0.01	12.15
			15:1	−0.0185	<0.01	11.27
			20:1	−0.0197	<0.01	11.38
			25:1	−0.0038	n.s.	11.14
			30:1	−0.0130	<0.01	11.27
			35:1	−0.0089	<0.01	11.10
			40:1	−0.0211	<0.01	11.28
			45:1	−0.0083	<0.01	11.17
Cephalobus	*persegnis*	M96	11:1	−0.0538	<0.01	15.22
			15:1	−0.0510	<0.01	14.32
			20:1	−0.0281	<0.01	13.33
			25:1	−0.0249	<0.01	12.52
			30:1	−0.0219	<0.01	12.51
			35:1	−0.0113	<0.01	12.42
			40:1	−0.0072	<0.01	13.33
			45:1	−0.0082	<0.01	12.52
Cruznema	*tripartitum*	O96	11:1	−0.0509	<0.01	13.34
			15:1	−0.0061	<0.05	11.37
			20:1	−0.0023	n.s.	10.75
			25:1	0.0049	n.s.	10.69
			30:1	0.0031	n.s.	10.50
			45:1	−0.0031	n.s.	9.67

Are some bacterial-feeding nematode species better mineralizers than others?

Smaller nematodes have higher mineralization rates per unit weight, related to their higher metabolic rates. The effect of body weight (w, μg) on N-mineralization rate (R, μg-N μg-nematode^{-1} d^{-1}) was described by: R = 0.0139 – 0.0046w (r^2 = 0.65, $p < 0.05$). However, the N-mineralization rate per individual was independent of body weight (Figure 5).

We calculated N-mineralization rates from data presented in previous studies. Measured as μg-N nematode^{-1} d^{-1}, rates for *Mesodiplogaster lheritieri* are calculated as 0.0039 ± 0.0096 (Anderson et al., 1983) and 0.0089 ± 0.0075 (Anderson et al., 1979); those for *Acrobeloides* spp. as 0.0067 (Ingham et al., 1985) and 0.0031 ± 0.0027 (Anderson et al., 1979) (mean ± s.e., where available). Calculated on a unit body weight basis as μg-N μg-nematode^{-1} d^{-1}, mineralization rates for *M. lheritieri* were 0.0031 ± 0.0006 (Anderson et al., 1993) while those for *Ac-*

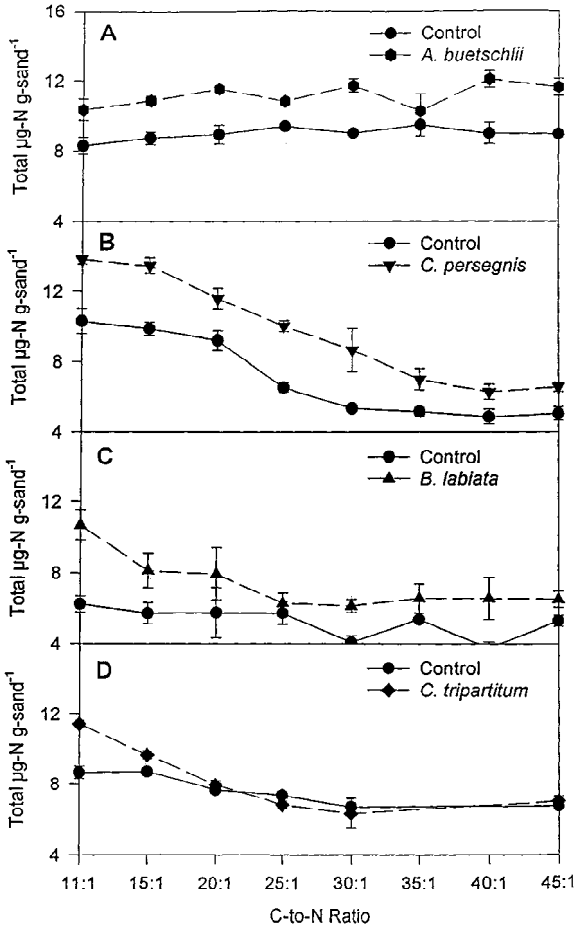

Figure 3. Total N per g-sand (as NH_4^+ and NO_3^-) in 21 d, summed across leachings at 3-d intervals from sand columns containing bacteria and various species of bacterial-feeding nematodes or bacteria alone (control), at different C-to-N ratios of the organic substrate. The amount of N is initially constant in each column. Nematode species used were: (A) *Acrobeloides buetschlii*; (B) *Cephalobus persegnis*; (C) *Bursilla labiata*; and (D) *Cruznema tripartitum*.

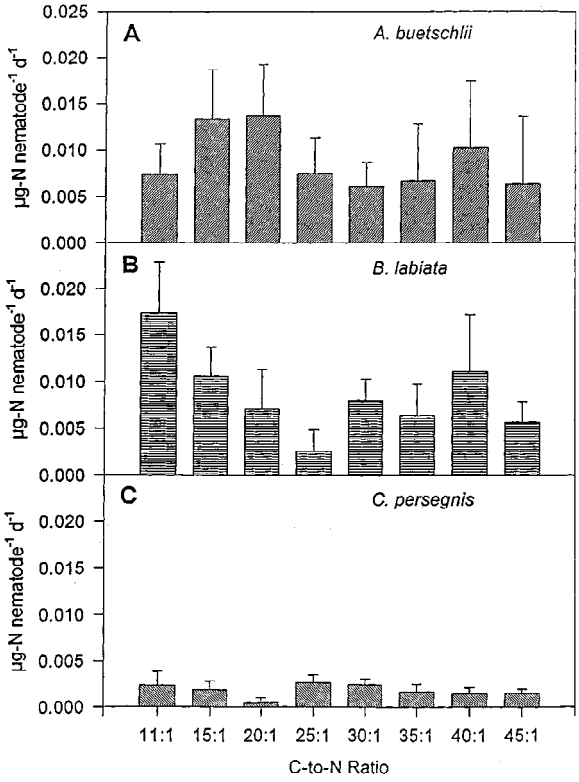

Figure 4. Calculated rate of N mineralization (μg-N nematode^{-1} d^{-1}) by bacterial-feeding nematodes in sand columns at different C-to-N ratios of the organic substrate. The amount of N is initially constant in each column. Nematode species illustrated are: (A) *Acrobeloides buetschlii*; (B) *Bursilla labiata*; and (C) *Cephalobus persegnis*.

robeloides spp. were 0.0757 ± 0.0027 (Ingham et al., 1985). These estimates are generally of the same order of magnitude as our results (Table 2A, B), although those for *Acrobeloides* spp. are higher than our measurements for either *A. bodenheimeri* or *A. buetschlii*.

We attempted to further validate and to calibrate the measured rates of N mineralization in the sand-column experiments (Table 2) by calculating potential rates based on laboratory assessment of C and N requirements for growth and respiration of individual nematode species. The comparisons between measured and calculated N-mineralization rates are qualitatively consistent (Figure 6) however the mea-

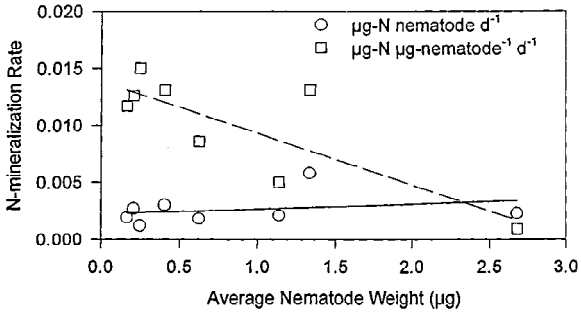

Figure 5. Relationships between N-mineralization rates (expressed as μg-N nematode^{-1} d^{-1} and μg-N μg-nematode^{-1} d^{-1}) and average body weight of different species of bacterial-feeding nematodes.

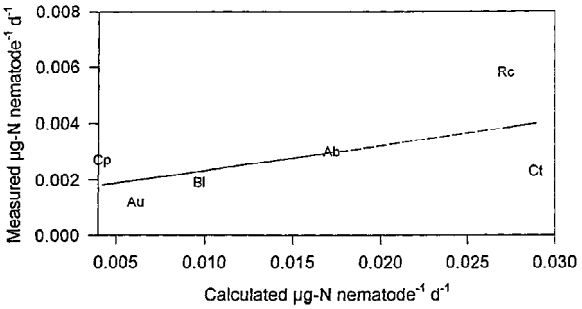

Figure 6. Relationship between rates of N mineralization measured in sand columns at ±24 °C over 21 d and rates calculated from C-to-N ratios, respiratory rates and life course parameters of the bacterial-feeding nematodes *Acrobeloides bodenheimeri* (Ab), *A. buetschlii* (Au), *Bursilla labiata* (Bl), *Cephalobus persegnis* (Cp), *Cruznema tripartitum* (Ct), and *Rhabditis cucumeris* (Rc).

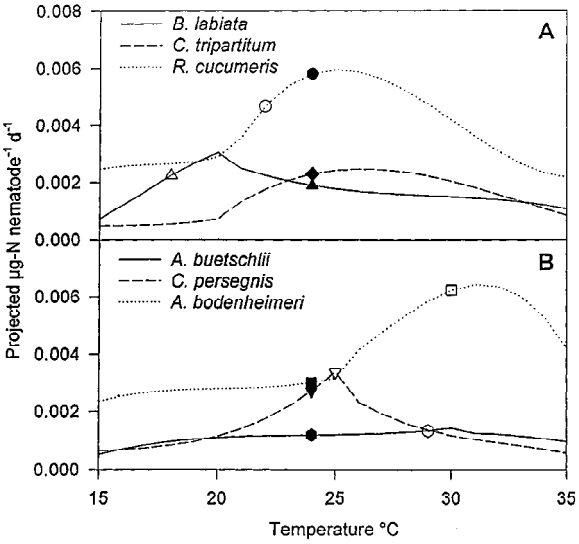

Figure 7. Projected rates of N-mineralization by bacterial-feeding nematodes (μg-N nematode^{-1} d^{-1}) based on respiratory rates across a temperature range. Optimum thermal conditions for N mineralization integrating thermal constraints on respiration (Ferris et al., 1995) and population development (Venette and Ferris, 1997) are indicated by open symbols (date were not available for *C. tripartitum*). Filled symbols indicate N-mineralization rates calculated from measurements under laboratory conditions of ±24 °C. (A) Short life course nematodes in the family Rhabditidae; (B) Longer life course nematodes in the family Cephalobidae. *A. bodenheimeri = Acrobeloides bodenheimeri, A. buetschliii = Acrobeloides buetschliii, B. labiata = Bursilla labiata, C. persegnis = Cephalobus persegnis, C. tripartitum = Cruznema tripartitum,* and *R. cucumeris = Rhabditis cucumeris*.

sured N-mineralization rates were only about 10% of those calculated from laboratory data (R^2 = 0.35). The species differed in the amount of N mineralized relative to that predicted (Figure 6). There are many potential contributors to the discrepancy between calculated and measured mineralization rates. An obvious source of difference is that measurements in the sand columns were only taken once every three days; considerable denitrification and immobilization may have occurred during that time span. Further, the calculated rates of N mineralization based on laboratory data assume unlimited resources to the organisms and optimal environmental conditions for each species, growing populations, and mortality only at the end of the natural life course.

The studies reported herein were conducted under relatively uniform laboratory temperature conditions of approximately 24 °C. We expect that N-mineralization rates will change with temperature and that the relationship with temperature will mirror that of the metabolic rates for these nematodes (Ferris et al., 1995). The respiration rate reflects the integral metabolic activity of an individual nematode. So, to adjust the N-mineralization rate to specific temperature conditions (between 15 and 35 °C), we propose to multiply the N-mineralization rate per individual (Table 2) by the relative respiration rate for an individual possessing the average weight of each life stage, expressed as a proportion of the respiration rate for that species at 24 °C (Figure 7).

As a caveat to the above projections of N-mineralization rates across temperature conditions, we have observed that the temperature relationships of developmental rates of nematodes do not necessarily mirror those of respiration rates (Ferris et al., 1995, 1996a; Venette and Ferris, 1997). Therefore the coefficients for estimating the N-mineralization potential of species of bacterial-feeding nematodes would appropriately be applied to actual nematode counts to provide an estimate of the N-mineralization potential of the bacterial-feeding nematode community in a given soil. By calculating relative population development rates across a range of temperatures for these nematode species (using coefficients provided by Venette and Ferris, 1997) we determine the points of intersection of the relative rates of respiration and population development in relation to temperature. The points of intersection are considered the temperatures at which maximum mineralization rates will occur for the population based on the population development rates and the metabolic activity of individuals within the population. The temperatures for maximum mineralization are lower for rhabditid nematodes (Figure 7A) than for cephalobid nematodes (Figure 7B).

Clearly, individual species of bacterial-feeding nematodes differ in the rates that they mineralize N (Table 2). They probably also differ in the manner that their N-mineralization rates respond to temperature and other environmental conditions affecting metabolism and development (Figure 7). Consequently, as conditions change in the soil through space and time, individual species will differ in the magnitude of their contribution to aggregate N availability. Interestingly, the rhabditid nematodes [Maturity Index 1, Bongers (1990)] with generally short life course (Ferris et al., 1996a) and rapid response to food availability early in the growing season (Ferris et al., 1996b) have maximum population mineralization rates at temperatures experienced in the late spring and early summer (Figure 7A). The cephalobid nematodes [Maturity Index 2, Bongers (1990)] with longer life course (Ferris et al., 1996a) and slower population response to food availability during the growing season (Ferris et al., 1996b) have maximum population mineralization rates at temperatures experienced in mid to late summer (Figure 7B).

Can we improve soil fertility and nitrogen availability from organic sources?

We speculate that the effect of N mineralization by nematodes and other bacterial-grazers may be sufficient to support plant growth when the organic material source is of higher C-to-N ratio in low-input and organic farming systems. That will be of considerable importance in the usual case that the C-to-N ratio of material available for incorporation (e.g., cover crops and composted organic materials) is somewhat unpredictable. Through time after incorporation of organic material, the cumulative amount of N mineralized reaches an asymptote as the organic N resource declines (Figures. 1, 2). At all C-to-N ratios when nematode populations persisted or increased, the amount of N mineralized was increased by the presence of nematodes (Figure 3). We expected that net mineralization due to the presence of nematodes would be smaller at high C-to-N ratios than at low. That is because at high C-to-N ratios the bacterial community has abundant carbon for growth but is limited by the availability of N. Consequently, any N that becomes available through the activity of the nematodes is immediately re-immobilized by the bacteria. However, in the *A. buetschlii* experiment, N mineralization did not decrease at the highest C-to-N ratios (Figure 3A). That could happen if the bacterial community was not large enough to become limited by N availability or if shifts in microbial community structure occurred at higher C-to-N ratios. Alternatively, the cellulose fibers used to increase the C-to-N ratio may have decomposed slowly and may not have resulted in the expected microbial increase. However, since the microbial community originated from field soil we expect that it included organisms capable of decomposing cellulose, such as oligotrophic bacteria and fungi. Further, a large proportion of the C in high C-to-N ratio organic material incorporated into field soil is in the form of cellulose. We saw no evidence of residual cellulose during final nematode extractions from the columns.

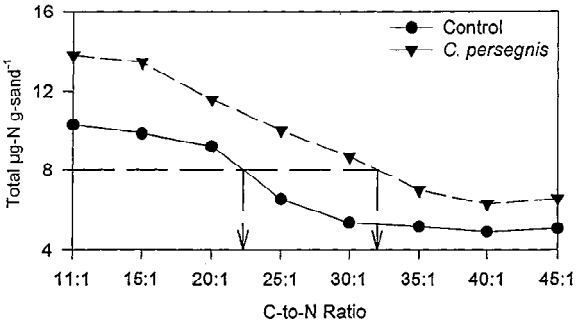

Figure 8. Theoretical enhancement of the ability of organic substrates of differing C-to-N ratio to meet N-sufficiency requirements for plant growth due to the presence and activity of a bacterial-feeding nematode population. The species *C. persegnis* = *Cephalobus persegnis* is used in this example.

In the *C. persegnis* (Figures 2, 3B) and the *B. labiata* (Figures 2, 3C) experiments, N mineralization and the nematode contribution to mineralization generally decreased with increasing C-to-N ratio, consistent with the hypothesis (Table 3). The *C. tripartitum* experiment (Figure 3D) was essentially a failure due to nematode mortality.

We continue to subscribe to the hypothesis that net-mineralization due to nematodes will decrease with increasing C-to-N ratio, even though we were only able to show it clearly in two of four experiments. We suggest that the significance of the enhanced mineralization rate at all C-to-N ratios, however, indicates that a biologically-active soil will mineralize N more rapidly than one in which numbers and activity of soil organisms are reduced. The importance of additional biological activity is illustrated by superimposing a hypothetical sufficiency level of N-availability (0.8 μg-N/g-soil) on the data for *C. persegnis* in Figure 3B to produce Figure 8. Sufficient N for plant growth can be achieved at a C-to-N ratio of no more

than 22:1 in the absence of nematodes but at up to 32:1 if nematode grazers are present.

We have demonstrated that N mineralization in soil is significantly enhanced by the activity of bacterial-feeding nematodes, as observed in previous studies (Anderson et al., 1979, 1983; Ingham et al., 1985). In our experiments, nematodes of different species mineralized between 0.0012 and 0.0058 μg-N nematode^{-1} d^{-1}, primarily in the form of NH_4^+, consistent with the known excretory products of nematodes (Lee and Atkinson, 1977; Wright and Newall, 1976). At $\pm$ 24 °C, N-mineralization rates per individual were not related to body size, although smaller nematodes mineralized more N per unit of body weight than larger nematodes. From the practical standpoint of plant nutrition and crop production, the importance of this node in the soil food web is greatest when C-to-N ratios of organic substrates fueling the web are in an intermediate range. At low C-to-N ratios primary decomposers will be C-limited and N-immobilization will be minimal; at high C-to-N ratios the decomposers will be N-limited and immobilization of N may occur rapidly.

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