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POPULATION ENERGETICS OF BACTERIAL-FEEDING NEMATODES: CARBON AND NITROGEN BUDGETS

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Summary—Bacterial-feeding nematodes participate in nitrogen mineralization in decomposition food webs to an extent determined by metabolic and behavioral attributes, by life history, and by the relative C-to-N ratios of the nematodes and their bacterial prey. The mean C-to-N ratio for eight nematode species cultured on *Escherichia coli* on agar was 5.89 (range 5.16–6.83). The mean C-to-N ratio was similar, although with greater variability among species, when nematodes were cultured in soil on a range of soil bacteria. The mean C-to-N ratio of five isolates of soil bacteria and *E. coli* was 4.12 (range 3.65–4.92). Where food was not limiting, production-to-assimilation ratios ranged from 0.58 to 0.86 and respiration-to-assimilation ratios from 0.14 to 0.42. The excess N assimilated during growth and egg production, and the excess N assimilated to meet the C needs of respiration, were similar across species at 20°C. The excess N from both sources provides an estimate of the amount of N excreted by each nematode during the life course. The weight of bacteria necessary to meet the growth and respiration costs at 20°C ranged from 0.87 μg μg-nematode^{-1} d^{-1} for *Cephalobus persegnis* to 1.99 μg μg-nematode^{-1} d^{-1} for *Bursilla labiata*, providing estimated consumption rates between 6.61×10^5 and 15.22×10^5 bacterial cells μg-nematode^{-1} d^{-1}. At a field site, we estimate that the bacterial-feeding nematode community in the top 15 cm soil mineralized N at rates increasing to 1.01 μg-N g-soil^{-1} d^{-1} in rhizosphere soil. On a monthly basis, the community contributed 0.28 kg-N ha^{-1} in April, 0.98 kg-N ha^{-1} in May and 1.38 kg-N ha^{-1} in June in bulk soil. Contributions in the rhizosphere would be considerably greater depending on the ratio of rhizosphere to bulk soil. The contribution of individual species to N mineralization in the rhizosphere varied through the first 3 months of the summer growing season as a function of their abundance and their metabolic and development rates in relation to temperature. *Rhabditis cucumeris* was the predominant contributor in April; there were similar contributions by *Acrobeloides bodenheimeri*, *B. labiata*, *Cruznema tripartitum*, and *R. cucumeris* in May; *A. bodenheimeri* and *B. labiata* were the major contributors in June. © 1997 Elsevier Science Ltd

INTRODUCTION

Bacteria and fungi are the primary decomposers of organic matter in soil food webs. Microbivorous fauna constitute the second trophic level; they are regulated by food abundance, competition for food, predatory fauna and predatory microbes (Moore and Hunt, 1988; Wardle and Yeates, 1993; Bouwman *et al.*, 1994). In decomposition pathways where bacteria are the primary decomposers, bacterial-feeding nematodes affect organic matter decomposition directly by feeding on microbes and indirectly by dispersing microbes (Bouwman *et al.*, 1994; Griffiths, 1994). Consequently, nematodes have both direct and indirect effects on mineral cycling in soil (Ingham *et al.*, 1985; Hunt *et al.*, 1987; Griffiths, 1990). The nematodes are attracted to bacterial food sources via CO_2 or exuded metabolites and possibly temperature gradients (Nicholas, 1984; Dusenbery, 1987; Freckman, 1988; Griffiths, 1994). Enhanced nutrient cycling in both the rhizosphere and bulk soil depends on consumption of bacteria by protozoa or nematodes to mineralize immobilized N (Clarholm, 1985; Griffiths *et al.*, 1991). Nematodes appear to be the primary consumers of bacteria in the rhizosphere; protozoa are equally prevalent in rhizosphere and bulk soil (Griffiths, 1990; Griffiths *et al.*, 1991; Griffiths and Caul, 1993). The abundance of bacterial-feeding nematodes may be a useful indicator of bacterial productivity in the soil (Freckman, 1988; Griffiths *et al.*, 1994).

Carbon and nitrogen budgets for the life courses of nematodes provide important estimates of the contribution of these organisms to soil fertility (e.g. Sohlenius *et al.*, 1988). Such analyses have been achieved by trophic level assignment of nematodes recovered from soil samples at various study sites. They have required measurement of nematode C-to-N ratios, assumptions of the relationship between temperature and metabolic rates, and the application of those values to all nematodes in the system. Given the complexity of soil biological systems, and the diversity of organisms represented, such assumptions provide expedience in developing C and N budgets. However, respiration and meta-

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bolic rates, and their responses to temperature, differ among nematode species from a single field (Ferris *et al.*, 1995).

C-to-N ratios of bacterial-feeding nematodes have been estimated as around 10:1 by Anderson *et al.* (1981) and as 4.6:1 by Persson (1983). The estimates of Anderson *et al.* (1981) are based on measurements of the N content of a fungal-feeding nematode, *Ditylenchus triformis* by Myers and Krusberg (1965), and on estimated C content. Those of Persson (1983) are based on measurements of C and N in an aggregate of nematode species extracted from soil. The C-to-N ratios of bacterial-feeding nematodes are purported to be higher than those of their bacterial food source (Van Veen and Paul, 1979; Hunt *et al.*, 1987) so considerable mineralization of N is associated with their metabolic activity. In consuming sufficient bacteria to provide the C necessary for their body structure and respiration, nematodes assimilate more N than necessary. The excess N is excreted mainly as ammonia which freely permeates the nematode cuticle (Rogers, 1969; Wright and Newall, 1976; Lee and Atkinson, 1977). Furthermore, perhaps 40% of the C in the food intake is respired (Marchant and Nicholas, 1974); the N consumed with that C will be in excess of structural needs and will be excreted. However, microbivorous nematodes exhibit a wide range of metabolic rates and behavioral attributes. The contribution of individual species to N cycling and soil fertility may vary considerably.

The objectives of these studies were: (1) to measure C-to-N ratios of individual species of bacterial-feeding nematodes and of their bacterial food sources; and (2) to estimate the relative importance of the individual species in N mineralization in soil. The latter goal required earlier studies on nematode metabolic rates (Ferris *et al.*, 1995) and on development and fecundity rates (Ferris *et al.*, 1996a).

MATERIALS AND METHODS

The nematodes used were originally isolated from populations endemic in a long-term sustainable agriculture farming systems (SAFS) project conducted on the field station associated with the University of California campus in Davis, CA (Ferris *et al.*, 1996b; Temple *et al.*, 1994). Although not from the field site, *Caenorhabditis elegans* (strain N_2 var. Bristol) obtained from long-term laboratory cultures was included in all tests as a comparative standard. The nematodes are representatives of maturity index categories 1 [*Bursilla labiata*, *C. elegans*, *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).

Bacteria were also isolated from the SAFS plots, by dilution plating onto 0.1 × nutrient agar. Morphologically-unique colonies were brought into pure culture by triple-streaking on nutrient agar. Bacteria were identified by Microbe Inotech Laboratories, St Louis, MO, using BIOLOG Gram-positive and gas chromatographic fatty acid methyl esterase techniques. Identities of isolates selected for C and N analyses were *Streptomyces halstedii scabies*, *Bacillus megaterium* (GC subgroup A), *B. megaterium* (GC subgroup B), *B. polymyxa* and an unidentifiable bacterium (Red). *Escherichia coli* strain OP50, from a laboratory culture, was included in these analyses as a comparative standard. All bacteria were kept in stock cultures on nutrient agar. *Streptomyces halstedii scabies* and Red required 48–72 h to form distinguishable colonies on plates or to reach maximum turbidity in liquid media (0.1× nutrient broth). *Bacillus megaterium* (GC subgroup A), *B. megaterium* (GC subgroup B), and *E. coli* formed colonies and reached maximum turbidity in 24–48 h. *Bacillus polymyxa* did not appear to grow in liquid media even after 1 wk.

Sample preparation for C-to-N analysis

Nematodes were rinsed from the surface of NGM cultures with deionized water into 150 ml Pyrex beakers. The liquid in each beaker was repeatedly drawn up and expelled from a glass Pasteur pipette to break up the bacterial flocculant. The suspensions stood for 5 min allowing nematodes to settle and leaving bacteria in suspension. The suspended material was removed by aspiration. The mixing–aspiration procedure was conducted three times to remove much of the bacteria. The remaining liquid in each beaker, containing nematodes, was centrifuged in 15 ml conical centrifuge tubes at 750 ***g*** for 30 s. The supernatant was aspirated away. The nematodes were resuspended with sterile, deionized water in the conical tubes. The centrifugation–aspiration process was repeated twice. The third time, the resulting pellets were resuspended in 125 ml Erlenmeyer flasks in 75 ml of sterile water.

To allow expulsion of bacteria and waste products from the digestive tract, the nematode suspension in each flask was aerated for 48 h at room temperature using a bubbling stone connected to a compressed air supply. Afterwards, the nematode preparations were collected in 15 ml conical centrifuge tubes. The nematodes were spun for 2 min at 750 ***g***. The supernatant was aspirated away. The pellet was resuspended in sterile, deionized H_2O and

centrifuged a second time. Again, the supernatant was discarded, and the pellet was resuspended. The solution was centrifuged and drawn down to 1 ml and the supernatant saved. Ten microlitres of the supernatant were collected in a tin foil capsule (5 × 9 mm; Costech). Approximately 8 mg of Chromosorb (80/100 mesh; Carlo-Erba Instruments) was placed on top of the liquid and the capsule was gently sealed by folding the foil on an aluminum plate using C-free forceps. Carbon was removed from instruments and the aluminum plate by wiping them with 95% ethanol. The capsule was collapsed and placed inside another tin foil capsule. Both capsules were carefully squeezed down to a minimal size to eliminate air from the sample. Three to five replicates of the supernatant were prepared for each nematode species. Nematodes were encapsulated just as the supernatant. Three to five replicates were prepared for each species. Samples were analyzed within 3 h after preparation. The analysis was repeated on three separate occasions.

To assess the effects of bacteria within a nematode gut on our assessments of C-to-N ratios, the same procedure was followed except that nematodes were aerated in sterile water for only 24 h. To test whether the measured C-to-N ratios might be an artifact of laboratory culture conditions, representative species of the nematodes (*B. labiata*, *C. tripartitum*, *A. bodenheimeri*, and *C. persegnis*) were cultured as single species in sterilized sand amended with chopped, dry alfalfa leaves and stems (*Medicago sativa*, 2.2 g kg-dry soil^{-1}) and a nematode-free slurry of soil bacteria. The slurry was prepared by shaking 50 g of freshly-collected soil from organically-farmed SAFS plots in 500 ml sterile deionized water for 10 min. The solution was poured into 50 ml plastic centrifuge tubes and centrifuged at 810 *g* for 5 min. From a single centrifuge tube, 10 ml of the supernatant was drawn into a pipette. Care was taken to avoid disturbing the soil pellet (containing the majority of nematodes) or collecting debris floating on the water surface. The supernatant was poured through a 20 mm aperture sieve and collected in a sterile beaker. The slurry was used immediately after preparation. Nematodes were extracted from the soil using sieving and centrifugation (Jaffee *et al.*, 1988) and their identity confirmed. Nematodes were starved for 48 h, washed and encapsulated for C and N analysis as previously described.

For C and N analysis of bacteria, all isolates except *B. polymyxa* were cultured in five flasks of 100 ml 0.1× nutrient broth. Cultures were shaken at room temperature until the medium appeared turbid. To remove associated media, the bacteria were concentrated by increasing centrifugation speed to 11000 rev^{-1} min^{-1} (14,500 *g*) and allowing it to slow to zero (total centrifugation time was 10 min). The supernatant was discarded. The bacterial pellets were resuspended in saline (8.5 g NaCl l^{-1} distilled H_2O) and centrifuged, as before, a total of three times. For each isolate, after the final centrifugation, the supernatant from one tube was divided into five replicates for C-to-N analysis following the procedure used for nematodes. Because *B. polymyxa* would not grow in liquid culture, this bacterium and an *E. coli* control were cultured for 48 h on 0.1× nutrient agar. The bacteria were transferred to foil capsules covered with Chromosorb, and packed as per the nematode protocol. Five replicates were prepared for each isolate.

C-to-N ratio analyses and calculations

The samples were subjected to combustion analysis in a Carlo-Erba carbon–nitrogen elemental analyzer. The % C and % N were recorded for each sample and its respective supernatant. Since the sample mass was not constant in each replicate, it was necessary to adjust the C-to-N ratio as:

$$X = ((C_1 - C_2 - (1 - C_1K^{-1})10^{-1})(C_3 - C_2))^{-1}$$
$$((N_1 - N_2 - (1 - C_1K^{-1})10^{-1})(N_3 - N_2))$$

where X = the C-to-N ratio; C_1 and N_1 are the % C and N measured in one nematode replicate; C_2 and N_2 are the average background C and N measured in at least three empty tin foil capsules; C_3 and N_3 are the average % C and N of at least three supernatant replicates; and K is a constant, 248.182. The constant represents a theoretical ratio between the C content (μg) and volume (μl) of a nematode. The C content was estimated as 52% of dry wt (Persson, 1983) by calculating biomass (Andrassy, 1956) and assuming that dry wt is 20% of fresh wt (Persson *et al.*, 1980). Nematode volume was calculated using the formula for a cone, assuming that the form of a nematode can be described by two abutting cones. Since biomass and volume are both a function of the length and width of a nematode, both variables drop out of the ratio. As a result, the constant applies to all nematodes regardless of size. Therefore, C_1K^{-1} represents the "volume" of sample that is nematodes and $1 - C_1K^{-1}$ is the "volume" that is supernatant. A similar calculation was applied to the bacterial C-to-N analyses, except that we assumed that half of the analyzed volume was supernatant. We found, in both cases, that the C-to-N ratios were relatively insensitive to the adjustment and were altered by $< 1\%$.

Total C and N assimilated and mineralized during the nematode life course

We developed C and N budgets for the life course of individual species of bacterial-feeding nematodes. *Panagrolaimus detritophagus* was omitted from the calculations since life table data were not available. The step-wise procedure was:

1. We calculated the increase in length of each species during each life stage (Ferris *et al.*, 1995). Using coefficients developed to relate widths to lengths for each species, the increase in weight during each life stage was determined using the formula of Andrassy (1956) (Ferris *et al.*, 1995).
2. Total production was determined as the sum of the increase in weight during each life stage and the weight of eggs produced during the adult stage. Individual egg weight was assumed equivalent to the weight of a freshly-hatched juvenile; total egg weight was calculated from the observed egg production by females of each species (Ferris *et al.*, 1996a).
3. The amount of C necessary to achieve total production was calculated from estimated C in a nematode body and the fresh wt-to-dry wt ratio (Persson *et al.*, 1980; Persson, 1983).
4. We calculated the amount of CO_2 respired during each life stage at 20°C for each species based on the metabolic rate of individuals of average weight in that life stage (Ferris *et al.*, 1995) over the duration of each life stage at 20°C (Ferris *et al.*, 1996a). The weight of C respired was determined from the total CO_2 respired during the life course.
5. Based on our measurements of C-to-N ratios, we calculated the weight of N required for growth and egg production by the nematodes. Using the measured C-to-N ratio for the bacterial food source, we calculated the weight of N assimilated by the nematodes in acquiring the C required for total production. Excess N assimilated was the difference between the weight of N required for production and that assimilated.
6. We calculated the weight of bacterial N associated with the bacterial C necessary to provide the C respired by each nematode species over the life course. That N was assumed to be in excess of body needs.
7. The amount of N excreted by each nematode during its life course, assuming that the life course is completed, is the sum of the N assimilated in excess of growth needs and that associated with C used for respiration.

Energetics and mineralization simulations

The simulation model used for the determination of life table parameters was adapted to simulate nematode numbers, biomass and population energetics (respiration and assimilation) for each nematode species in this study. The distributed-delay population model tracks the number of individuals as they pass through developmental substages of each life stage (Schneider and Ferris, 1986; Ferris *et al.*, 1996a). The model was driven by 1993 soil temperature data from the SAFS site.

Respiration for each nematode species was calculated for the soil temperatures experienced each day based on the sizes of individuals in each substage of each life stage and the duration of each life stage (Ferris *et al.*, 1996a). The relationship $R = \Sigma n_i 24 a w_i^{0.75}$ provided the total weight of CO_2 respired in a day (R) by the n_i individuals in each of the i developmental substages of a nematode species. In the formula, w_i is the weight of an individual in the ith developmental substage, a is a temperature-specific coefficient experimentally determined for each nematode species (Ferris *et al.*, 1995) and 24 converts hourly respiration to a daily basis. The weight of C in the CO_2 respired was considered the amount of bacterial C that would need to be assimilated to meet respiration needs. All N associated with that C, as determined from the average C-to-N ratio for bacteria measured in these studies, was considered excess to nematode needs and to constitute the respiration component of N mineralization.

Growth of individuals in each life stage under the daily temperature conditions was based on the weights of individuals at the transition between life stages (Ferris *et al.*, 1996a). Growth of adult females was considered to be their increase in body size plus the weight of eggs produced d^{-1}. Total C incorporated into the nematode biomass d^{-1} was accumulated across all life stages. The amount of N incorporated into the biomass was calculated from the C-to-N ratios determined in these studies. The amount of N assimilated with the incorporated C was based on the average C-to-N ratios for bacteria measured in these studies. The excess N mineralized during growth and production was determined by subtraction of the N incorporated into nematode biomass.

Summation of excess N associated with respiration and growth provided a population-level estimate of the N mineralized d^{-1} by each nematode species. The simulation model was run with a starting uniform distribution of each life stage for each species and upper and lower thermal thresholds for each species. Lower thermal thresholds were determined from life table studies (Ferris *et al.*, 1996a) and upper thermal thresholds were determined as the temperature at which metabolic rates began to decline for each species (Ferris *et al.*, 1995). The amount of N mineralized per degree-day for an average individual in an age-distributed population of each species was simulated over the first 90 d of the growing season.

To calculate the actual amount of N associated with the numbers of bacterial-feeding nematodes at the SAFS field site, a spreadsheet was used for linear interpolation of numbers of individuals of each species in the upper 15 cm soil between sampling dates, spread 2 wk apart, during the first 90 d of the 1993 tomato growing season (April–June).

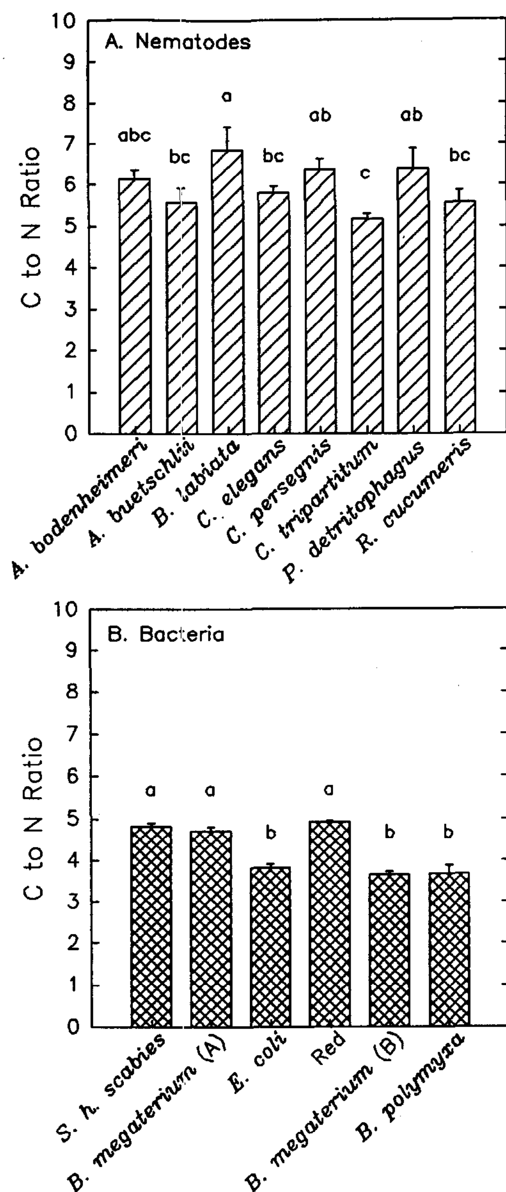

Fig. 1. C-to-N ratios. (A) Bacterial-feeding nematodes, *Acrobeloides bodenheimeri*, *A. buetschlii*, *Bursilla labiata*, *Caenorhabditis elegans*, *Cephalobus persegnis*, *Cruznema tripartitum*, *Panagrolaimus detritophagus*, *Rhabditis cucumeris*, cultured on *Escherichia coli* on agar after 48 h starvation; (B) soil bacteria isolates, *Streptomyces halstedii scabies*, *Bacillus megaterium* (GC subgroup A), unidentifiable bacterium (red), *B. megaterium* (GC subgroup B), *B. polymyxa* and *E. coli*. Values do not differ significantly when bars are marked with the same letter (α = 0.05).

Nematode numbers for each species were corrected for extraction efficiency as per earlier biomass estimates (Ferris *et al.*, 1996b). For the 90 d time interval, a degree-day schedule was calculated using the lower and upper thermal thresholds for each species. The amount of N mineralized each day by the community of bacterial-feeding nematodes at the field site was calculated by multiplying the daily abundance of each species by the number of degree-days for that species on that day and by the N mineralized individual^{-1} degree-d^{-1} from the simulation results. Four of the species observed at the field site were not included in the life table, respiration or C-to-N ratio studies. They were provided upper and lower threshold values and estimates of N mineralization per individual that were the averages of the values for the other species. N mineralization was accumulated for the days in each month to determine monthly totals for each species and the community.

Further refinement of the N-mineralization potential of field-observed population levels of bacterial-feeding nematodes was to recognize that their distribution is not uniform; densities are many times greater in the rhizosphere than in bulk soil (Griffiths, 1994). In other studies, the rhizosphere to bulk soil ratio for densities of bacterial-feeding nematodes varied from six in grass to 27 in peas after 10 wk of growth in pots; the average over four plant species and four sampling dates was nine (Griffiths, 1990). Under high nitrate conditions, the rhizosphere-to-bulk soil ratio of bacterial feeding nematodes ranged from 10 to 60 across four grass species, and from 15 to 24 under low nitrate conditions (Griffiths *et al.*, 1992). We selected a value of 15 for the rhizosphere-to-bulk soil ratio of bacterial feeding nematodes as representative of values reported in those studies. In the Griffiths (1990) experiments, the percentage of total soil considered to be in the rhizosphere was only 1.7%. We did not measure the amount of rhizosphere soil in the field-grown tomatoes, but for purposes of calculation, we assigned levels for the field area occupied by the root-zone of each plant, on the basis of above-ground canopy coverage, as 5% in mid-April, 15% in mid-May, and 25% in mid-June. We calculated the amount of soil constituting the rhizosphere as 1.7% of that root-zone soil. Based on these estimates, we linearly interpolated the proportion of rhizosphere soil present on a daily basis. Daily nematode numbers were then distributed between rhizosphere and bulk soil on a 15:1 ratio and the rate of N mineralization by nematodes in the rhizosphere and the total area was calculated on a daily and monthly basis.

RESULTS

C-to-N ratios of nematodes cultured on agar or in soil

The C-to-N ratio of all nematode species tested following culture on *E. coli* on agar, averaged across all replications and experiments, ranged from 5.16 to 6.83 with a mean value of 5.89. Most of the species were similar in C-to-N ratio, with *B. labiata* significantly higher and *C. tripartitum* significantly

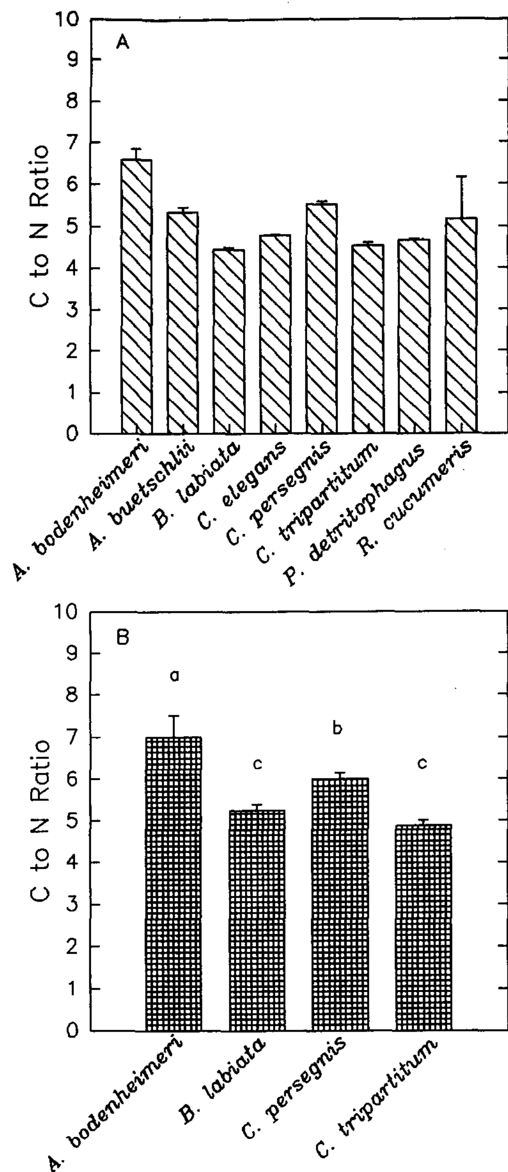

Fig. 2. C-to-N ratios. (A) Bacterial-feeding nematodes cultured on *Escherichia coli* agar after 24 h starvation; (B) bacterial-feeding nematodes cultured on soil bacteria in soil after 48 h starvation. *Acrobeloides bodenheimeri, A. buetschlii, Bursilla labiata, Caenorhabditis elegans, Cephalobus persegnis, Cruznema tripartitum, Panagrolaimus detritophagus, Rhabditis cucumeris*, cultured on *Escherichia coli* on agar after 48 h starvation. Values do not differ significantly when bars are marked with the same letter (α = 0.05), or when bars are unmarked.

lower than the others [Fig. 1(A)]. Where nematodes were only starved for 24 h to purge intestine contents before analysis, C-to-N ratios were generally lower (mean = 5.04) and more variable [Fig. 2(A)]. They ranged from 4.43 to 6.58 and did not differ between species. Minor differences did not parallel those measured after 48 h starvation [Fig. 1(A)]. Where nematodes were cultured in soil on a range of soil bacteria and then starved for 48 h before analysis, C-to-N ratios were again variable and ranged from 4.87 to 6.99 with a mean value of 5.60, similar to that measured on agar [Fig. 2(B)].

C-to-N ratios of representative soil bacteria and Escherichia coli

Bacterial C-to-N ratios ranged from 3.65 to 4.92, with three of the cultures having a significantly higher C-to-N ratio (mean = 4.82) than the other three (mean = 3.71) [Fig. 1(B)]. Since we do not know the prevalence of these bacteria in soil, recognizing that *E. coli* is not a common soil bacterium, we use the mean C-to-N ratio of all isolates tested (4.12) as an estimate of the mean C-to-N ratio of the nematode food source.

Total C and N assimilated and mineralized during the nematode life course

Carbon and nitrogen budgets for the life course of individual species of bacterial-feeding nematodes suggest that more C is used in respiration than in growth at 20°C, but when the weight of C incorporated into egg production is considered, total C for production is considerably greater than that for respiration (Table 1). Where food was not limiting, production-to-assimilation ratios ranged from 0.58 to 0.86 (Table 2). The respiration-to-assimilation ratio for C was in the 0.14–0.42 range. Two of the rhabditid nematodes in the study, *C. elegans* and *R. cucumeris*, partitioned more of the assimilated C into production than the other species, with the proportion of assimilated C used for respiration calculated as 0.16 and 0.14, respectively (Table 2). The excess N assimilated during growth and egg production and the excess N assimilated to satisfy the C needs of respiration were quite similar across species (Table 1). The excess N from both sources provides an estimate of the amount of N excreted by each nematode during the life course.

Consumption and assimilation rates of bacterial-feeding nematodes

The necessary weight of bacteria assimilated μg-nematode^{-1} d^{-1} to meet the growth and respiration costs at 20°C ranged from 0.87 μg for *C. persegnis* to 1.99 μg for *B. labiata*. Allowing for a standard defecation rate of 0.405 of all bacterial consumption (Marchant and Nicholas, 1974), required consumption rates ranged between 1.45 and 3.35 μg-bacteria μg-nematode^{-1} d^{-1}. Those consumption rates are equivalent to $6.61–15.22 \times 10^5$ bacterial cells μg-nematode^{-1} d^{-1} using the estimate of Van Veen and Paul (1979) of the weight of bacterial cells (Table 2).

Energetics and N-mineralization simulations in the rhizosphere

Based on observed field population levels and soil temperature conditions, the bacterial-feeding nematode community in the top 15 cm of soil at our

Table 1. Carbon and nitrogen utilization of species of bacterial-feeding nematodes during growth, egg production and respiration throughout the life course at 20°C

Species	Growth[a] (μg C)	Total production[b] (μg C)	Respiration[c] (μg C)	ExNP[d] (μg N)	ExNR[e] (μg N)	ExNTot[f] (μg N)
Acrobeloides bodenheimeri	0.55	1.18	0.85	0.09	0.21	0.30
Acrobeloides buetschlii	0.12	0.56	0.34	0.04	0.08	0.12
Bursilla labiata	0.15	0.35	0.25	0.03	0.06	0.09
Caenorhabditis elegans	0.32	1.17	0.22	0.08	0.05	0.13
Cephalobus persegnis	0.05	0.55	0.30	0.05	0.07	0.12
Cruznema tripartitum	1.20	2.93	1.14	0.14	0.28	0.42
Rhabditis cucumeris	0.48	2.57	0.40	0.16	0.10	0.26

[a]Total C partitioned into body mass.
[b]Total C partitioned into body mass and egg production.
[c]Total C used in respiration.
[d]Excess N assimilated with C used for production.
[e]Excess N assimilated with C used for respiration.
[f]Total excess N excreted.

Table 2. Assimilate partitioning coefficients, assimilation and consumption rates, and number of bacteria consumed at 20°C μg-body-weight^{-1} d^{-1}, averaged across all life stages, for bacterial-feeding nematodes

	Pr:As[a]	Rs:As[b]	Bacteria		
			Assimililation ($\mu g\ \mu g^{-1}\ d^{-1}$)	Consumption ($\mu g\ \mu g^{-1}\ d^{-1}$)	Consumption (cells $\mu g^{-1}\ d^{-1}$)
Acrobeloides bodenheimeri	0.58	0.42	1.04	1.75	7.95×10^5
Acrobeloides buetschlii	0.62	0.38	1.42	2.39	10.86×10^5
Bursilla labiata	0.59	0.41	1.99	3.35	15.22×10^5
Caenorhabditis elegans	0.84	0.16	1.93	3.24	14.72×10^5
Cephalobus persegnis	0.65	0.35	0.87	1.45	6.61×10^5
Cruznema tripartitum	0.72	0.28	1.11	1.86	8.45×10^5
Rhabditis cucumeris	0.86	0.14	1.58	2.65	12.04×10^5

[a]Ratio of production-to-assimilation.
[b]Ratio of respiration-to-assimilation.

SAFS field site mineralized N at rates increasing to 70 ng-N g-soil^{-1} d^{-1} in bulk soil (Fig. 3). On a monthly basis, the community contributed 0.28 kg-N ha^{-1} in April, 0.98 kg-N ha^{-1} in May and 1.38 kg-N ha^{-1} in June in bulk soil [Fig. 4(A)]. Using estimates of the relative distribution of nematodes in rhizosphere and bulk soil and the ratio of rhizosphere-to-bulk soil (R-to-S ratio) over time, the bacterial-feeding nematode community mineralized 0.63 μg-N g-soil^{-1} d^{-1} in rhizosphere soil by mid-May and 1.01 μg-N g-soil^{-1} d^{-1} by the end of June (Fig. 3). Estimates of the amount of N mineralized by these nematodes on a monthly basis in the total rhizosphere soil in 1 ha were 5, 40 and 90 g ha^{-1} in April, May and June, respectively [Fig. 4(A)]. *Rhabditis cucumeris* was the predominant contributor in April and May, although there were substantial contributions by other species (*A. bodenheimeri*, *B. labiata* and *C. tripartitum*) in May. *Acrobeloides bodenheimeri* and *B. labiata* were the major contributors in June [Fig. 4(B)].

DISCUSSION

Whether cultured on a single bacterial strain in Petri dishes or in soil on a range of bacteria, the C-to-N ratio of bacterial-feeding nematodes, after 48 h starvation, was between 5.16 and 6.83 (mean = 5.89) [Fig. 1(A)]. Minor differences between species had no obvious relationship to observable differences in maturity index (Bongers, 1990), metabolic activity (Ferris *et al.*, 1995), size, or life course duration (Ferris *et al.*, 1996a). The mean nematode C-to-N ratio is higher than the measurement for an aggregate of soil nematodes by Persson (1983) who also used a Carlo-Erba carbon–nitrogen elemental analyzer. However, the nematodes in that study do not appear to have been starved prior to the analysis. We obtained lower and more variable C-to-N ratios (mean = 5.04) when bacterial-feeding nematodes were starved for shorter periods, presumably reflecting the influence of residual bacteria (with a lower C-to-N ratio) in the intestine [Fig. 2(A)]. The C-to-N ratio for the nematodes was greater than that for the bacteria (mean = 4.12) [Fig. 1(B)]. As a comparative standard, our measurement of 3.88 for the C-to-N ratio

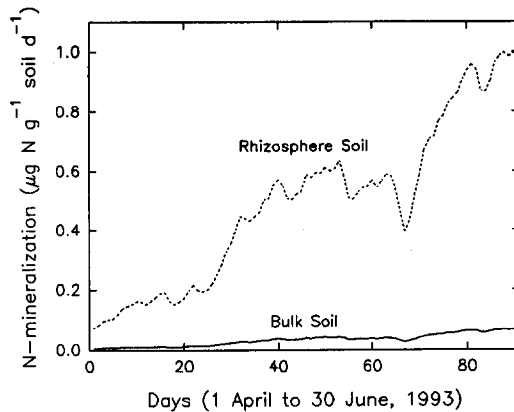

Fig. 3. Calculated daily rate of N mineralization by the community of bacterial-feeding nematodes in the upper 15 cm of soil at the SAFS field site during 1993 in bulk soil and rhizosphere soil based on estimated rhizosphere-to-bulk soil distribution coefficients.

of *E. coli* falls within the range of 3.33–4.85 reported by Luria (1960). The difference between our measurements of C-to-N ratios of bacteria and bacterial-feeding nematodes is smaller than the difference between 5:1 for bacteria and 10:1 for nematodes estimated by Anderson *et al.* (1981). In any event, the differences between C-to-N ratios of predator and prey support the hypothesis that the feeding activities of nematodes to obtain C and N for body structure and egg production, and C for respiration, should result in intake of a surfeit of N. The extra N should be excreted as ammonia (Rogers, 1969; Wright and Newall, 1976; Lee and Atkinson, 1977) and be available for uptake by plants or other organisms in the soil food web.

Between 14 and 42% of the total assimilated C was estimated to be used for respiration, in reasonable agreement with other estimates (Marchant and Nicholas, 1974) (Table 2). The rate of consumption of bacteria per unit of body weight in these studies was greater in the rhabditid nematodes than the cephalobid nematodes, and within each family it was greater in the smaller nematodes, which have higher metabolic rates (Ferris *et al.*, 1995), than in the larger nematodes. Using the measurements of Van Veen and Paul (1979) of the weights of individual bacterial cells, and nematode growth (Ferris *et al.*, 1996a), respiration (Ferris *et al.*, 1995) and defecation (Marchant and Nicholas, 1974) rates, adults of *R. cucumeris* of average size (Table 3) would consume 4.37×10^6 bacterial cells d^{-1}. We have observed that the esophageal muscles controlling the valve chamber of that species perform ca. 150 contractions min^{-1} or 216,000 contractions d^{-1} assuming incessant feeding activity. Consumption of 4.37×10^6 bacterial cells d^{-1} would require an intake of ca. 20 bacterial cells for each pulsation of the valve chamber. The consumption rate of the cephalobid nematode *C. persegnis* in contrast requires an intake of 2.54×10^5 bacterial cells d^{-1} for adults of average size (Tables 2 and 3). These rates are within ranges previously estimated for bacterial-feeding nematodes. Ingham *et al.* (1985) estimated consumption rates of 3.8 μg-bacteria μg-nematode^{-1} d^{-1} for the rhabditid nematode *Pelodera* sp., compared with our average across four rhabditid nematodes of varying size of 2.8 μg-bacteria μg-nematode^{-1} d^{-1} (Table 2). Reports of individual daily consumption rates for bacterial-feeding nematodes, calculated on a population basis, include values of 1.9×10^5 for adults of a *Pelodera* sp. [which is smaller than any of the nematodes in this study (Ingham *et al.*, 1985)] and, for populations of nematodes within the size range of those represented in this study, 3.9×10^5 for *Pelodera chitwoodi* (Mercer and Cairns, 1973) and 7.2×10^6 for *Plectus palustris* (Duncan *et al.*, 1974).

A necessary caveat accompanies these studies. The literature abounds with estimates and assumptions in attempts to further our understanding of the functional complexities of soil biology. We have adopted the assumptions and estimates of others and made some of our own. Without such estimates we would be unable to frame our studies on C-to-N ratios of nematodes and bacteria within the larger picture of nematode population numbers, N-mineralization rates and soil fertility. As an example, we followed Ingham *et al.* (1985) in using the Marchant and Nicholas (1974) estimate of assimilation efficiency. However, Ingham *et al.* (1985) note that the estimate of defecation rate may be low since the method used by Marchant and Nicholas (1974) measured only soluble ^{14}C in the medium bathing the nematodes and would have eliminated intact bacterial cells. Those measurements yield an assimilation efficiency of 0.595, which is many times greater than the 0.08 suggested by Coleman *et al.* (1978) and used in estimates of nematode consumption of bacteria by Griffiths (1994). The validity of assumptions underlying such estimates would have

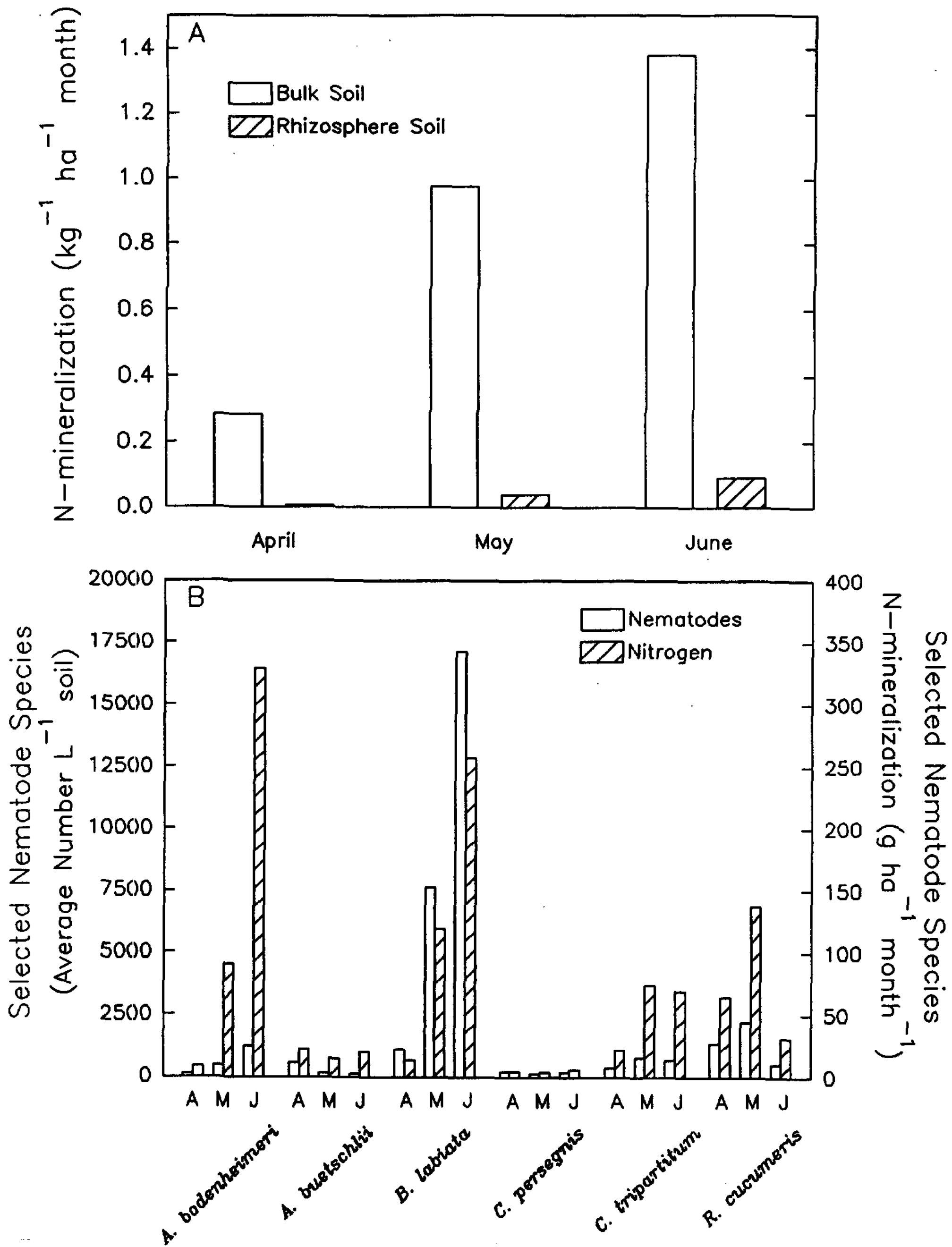

Fig. 4. Calculated contributions to N mineralization on a monthly basis by the community of bacterial-feeding nematodes in the upper 15 cm soil at the SAFS field site during 1993. (A) Rates of total N mineralization in bulk soil and rhizosphere soil based on estimated rhizosphere-to-bulk soil distribution coefficients; (B) estimated nematode abundance and monthly mineralization rates for selected species of bacterial-feeding nematodes. *Acrobeloides bodenheimeri, Acrobeloides buetschlii, Bursilla labiata, Cephalobus persegnis, Cruznema tripartitum* and *Rhabditis cucumeris*.

Table 3. Fresh weight ranges (μg) of life stages of bacterial-feeding nematodes (from Ferris *et al.*, 1995)

	<	J2	> <	J3	> <	J4	> <	A	>
Acrobeloides bodenheimeri	0.028		0.039		0.153		1.880		5.291
Acrobeloides buetschlii	0.025		0.053		0.113		0.447		1.165
Bursilla labiata	0.016		0.031		0.060		0.202		1.410
Caenorhabditis elegans	0.026		0.029		0.070		0.328		3.095
Cephalobus persegnis	0.018		0.072		0.163		0.277		0.492
Cruznema tripartitum	0.029		0.197		0.963		4.884		11.568
Panagrolaimus detritophagus	0.015		0.074		0.321		1.124		2.884
Rhabditis cucumeris	0.040		0.043		0.208		2.601		4.666

J2, J3, J4 = second, third and fourth juvenile stages.
A = adult.

a major effect on all consumption rate estimates in this study.

The calculated amount of N mineralized by a nematode during the life course at 20°C ranged from 0.09 to 0.42 μg, depending on the species, its size, growth rate, egg production rate, C-to-N ratio, and metabolic rates at that temperature. At that temperature, the amount of assimilated N in excess of the needs for growth was greater than that associated with C used for respiration for some species and vice versa for others (Table 1). In all cases, the amounts of excess N from each source were remarkably similar, with one never more than about two-fold the other. We recognize that the C and N budgets developed for each nematode species from these and our previous studies (Ferris *et al.*, 1995, 1996a) reflect individual growth, production and metabolic rates which assume survival for the natural duration of the life course and unlimited food supply. They do not reflect population phenomena nor do they consider sex ratios, the non-production of offspring by males, or differential survivorship among life stages.

An important area of ecological study is to determine the role of nematodes in the "health" and productivity of sustainable soils. How should soils be managed to create the sustainable condition? It would be interesting to assess the importance of each nematode species in terms of the rate of mineralization of N and the contribution to soil fertility. The extent to which an organism contributes to and participates in energy flow in an ecosystem is a function of population size and of individual and population growth rates and metabolic activities of individuals. Based on observed field populations and soil temperature conditions, calculated N mineralization nematode^{-1} degree-d^{-1} and estimated R-to-S ratios, the bacterial-feeding nematode community mineralized N at rates increasing to 1.01 μg-N g-soil^{-1} d^{-1} in rhizosphere soil (Fig. 3). On a monthly basis, we estimate that the community in the rhizosphere contributed N at rates of 5 g ha^{-1} in April, 40 g ha^{-1} in May and 90 g ha^{-1} in June. Mineralization contributions in the proportionally larger bulk soil were considerably greater on an area basis [Fig. 4(A)]. These estimates of contribution to N mineralization are quite sensitive to assumptions of the R-to-S ratio and of the relative distribution of the nematodes in rhizosphere and bulk soil; we based those assumptions on data from Griffiths (1990) and Griffiths *et al.* (1992).

The estimated amounts of N mineralized by bacterial-feeding nematodes in rhizosphere soil appear considerable and suggest a substantial contribution to plant growth. A succession of phases in the dynamics of bacterial-feeding nematodes has been documented during the decomposition of organic matter in soil. In the early period (0–32 d), growth of the nematode population occurs and there is an increase in both C and N mineralization. During the mid period (32–60 d), the nematode population stops growing but is still feeding and excreting; C mineralization is reduced and N mineralization increased. In the late period (60–180 d), mineralization of both C and N is reduced due to decline of bacteria and overgrazing by nematodes (Bouwman *et al.*, 1994). Those dynamics are in general agreement with our field observations but we find that different species tend to predominate through time (Ferris *et al.*, 1996b). Consequently, the contribution of individual species to decomposition processes and C and N mineralization is a function of their population dynamics, metabolic activity in relation to temperature and abundance [Fig. 4(B)]. The rhabditid nematodes, *B. labiata*, mineralized substantial N in May and June despite their small size due to their abundance at that time. Although numerically less abundant, the large rhabditid nematodes *C. tripartitum* and *R. cucumeris* made substantial contributions to N mineralization, the latter particularly in April and May before soil temperatures reached maximum levels. Of the cephalobid nematodes, only *A. bodenheimeri* made significant contributions to N mineralization, increasing from April to June. *Acrobeloides buetschlii* and *C. persegnis* played a relatively minor role in N cycling during the first 3 months of the tomato-growing season.

In organic and low-input farming systems, soil fertility is built through the interaction of the

microbial biomass with ambient and incorporated organic matter, mediated by soil moisture, temperature and structure (Griffiths *et al.*, 1994). In C-rich soils, N may be immobilized in the microbial biomass. The activities of bacterial-feeding nematodes and protozoa may have significant influences on the gradual release of N into the system. We recognize that not all of the mineralized N in the rhizosphere is taken up by the plant; some is re-immobilized by the microbial community; some is lost through denitrification and leaching. However, for an organic or low-input farming system to be productive and to reduce losses to the environment, N must become available at a rate that provides for optimum plant growth but that is not excessive (Powlson, 1988). Besides differing in predominance through time, bacterial-feeding nematode species may also differ in their spatial distribution. Interestingly, Griffiths *et al.* (1991) found the greatest proportion of rhabditid nematodes to be in bulk soil and of cephalobid nematodes to be in the rhizosphere. Rhabditid nematodes are maturity index 1 opportunists (Bongers, 1990); their role in bulk soil in organic farming systems may be N mineralization during decomposition of plant litter and incorporated organic matter. That would correspond with their general predominance early in the growing season (Ferris *et al.*, 1996b). It would also suggest that much of the N mineralized may be taken up by bacteria in that soil zone rather than by the plant. The cephalobid nematodes have a maturity index of 2 and are somewhat less dynamic in response to new resources than are rhabditids (Ferris *et al.*, 1996a,b). If they predominate in the rhizosphere, local activities may result in significant contribution of N availability to plant roots. Clearly this is an interesting area for future study.

Based on observed temporal dynamics of bacterial-feeding nematodes at the field level (Ferris *et al.*, 1996b) and the relationship of their growth and metabolic rates to temperature (Ferris *et al.*, 1995, 1996a) we believe there are opportunities for managing soils to enhance the activities of bacteria-feeding nematodes at key periods during the growing season. Their low abundance and contribution to N availability in the early spring (Fig. 4) may contribute to early-season N deficiency. Since metabolic rates of bacterial-feeding nematodes from the SAFS field site are extremely low at the soil temperatures experienced during the winter months (Ferris *et al.*, 1995), the opportunity to increase their abundance may be at the end of the previous growing season. At that time their food supply may be limiting (Bouwman *et al.*, 1994; Ferris *et al.*, 1996b). We postulate that microbial activity might be increased in the warm soils through about mid-October by planting a late-summer cover crop, incorporation of organic material and irrigation.

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