Ecological Monographs, 55(1), 1985, pp. 119–140

INTERACTIONS OF BACTERIA, FUNGI, AND THEIR NEMATODE GRAZERS: EFFECTS ON NUTRIENT CYCLING AND PLANT GROWTH[1]

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Abstract. The most common system responses attributed to microfloral grazers (protozoa, nematodes, microarthropods) in the literature are increased plant growth, increased N uptake by plants, decreased or increased bacterial populations, increased CO_2 evolution, increased N and P mineralization, and increased substrate utilization. Based on this evidence in the literature, a conceptual model was proposed in which microfloral grazers were considered as separate state variables. To help evaluate the model, the effects of microbivorous nematodes on microbial growth, nutrient cycling, plant growth, and nutrient uptake were examined with reference to activities within and outside of the rhizosphere. Blue grama grass (*Bouteloua gracilis*) was grown in gnotobiotic microcosms containing sandy loam soil low in inorganic N, with or without chitin amendments as a source of organic N. The soil was inoculated with bacteria (*Pseudomonas paucimobilis* or *P. stutzeri*) or fungus (*Fusarium oxysporum*), with half the bacterial microcosms inoculated with bacterial-feeding nematodes (*Pelodera* sp. or *Acrobeloides* sp.) and half the fungal microcosms inoculated with fungal-feeding nematodes (*Aphelenchus avenae*).

Similar results were obtained from both the unamended and the chitin-amended experiments. Bacteria, fungi, and both trophic groups of nematodes were more abundant in the rhizosphere than in nonrhizosphere soil. All treatments containing nematodes and bacteria had higher bacterial densities than similar treatments without nematodes. Plants growing in soil with bacteria and bacterial-feeding nematodes grew faster and initially took up more N than plants in soil with only bacteria, because of increased N mineralization by bacteria, NH_4^+-N excretion by nematodes, and greater initial exploitation of soil by plant roots. Addition of fungal-feeding nematodes did not increase plant growth or N uptake because these nematodes excreted less NH_4^+-N than did bacterial-feeding nematode populations and because the N mineralized by the fungus alone was sufficient for plant growth. Total shoot P was significantly greater in treatments with fungus or *Pelodera* sp. than in the sterile plant control or treatments with plants plus *Pseudomonas stutzeri* until the end of the experiment.

The additional mineralization that occurs due to the activities of microbial grazers may be significant for increasing plant growth only when mineralization by microflora alone is insufficient to meet the plants' requirements. However, while the advantage of increased N mineralization by microbial grazers may be short-term, it may occur in many ecosystems in those short periods of ideal conditions when plant growth can occur. Thus, these results support other claims in the literature that microbial grazers may perform important regulatory functions at critical times in the growth of plants.

Key words: decomposition; fungal grazers; microbial-faunal interactions; nematode nitrogen losses; nutrient cycling; plant nitrogen uptake; rhizosphere.

INTRODUCTION

In the study of the mineralization-immobilization phenomena of nutrient-cycling processes, the soil microflora have traditionally been considered responsible for the mineralization of inorganic nutrients from their immobilized forms in soil organic matter (Satchell 1974, Alexander 1977). Theoretical discussions of nutrient cycles generally represent the mineralization process as a flow from a litter or soil organic matter component to a component representing available soil nutrients (Gosz 1981, Van Cleve and Alexander 1981) or, ad-

[1] Manuscript received 27 June 1983; revised 22 February 1984; accepted 14 March 1984.

ditionally, through a soil microbe component (Melillo 1981). Rarely are the activities of faunal grazers of microflora included in the nutrient-cycling process or separated from the activities of soil microflora (Woodmansee et al. 1981, Coleman et al. 1983). Evidence is accumulating, however, that faunal grazers may be responsible for a significant portion of the mineralization previously attributed to microflora. Since mineralization is a key process in supplying nitrogen and other nutrients for plant growth in terrestrial ecosystems (Alexander 1977, Marion et al. 1981), it is important to understand the roles of all organisms involved in this process, the interactions that may occur between them, and where these interactions occur.

The present study pursues earlier observations, as reviewed by Yeates and Coleman (1982), in which some nematodes, particularly microbivorous types, were considered to have a positive effect on plant growth through enhanced nutrient mineralization. Lee and Inman (1975) concluded that a relatively small grazing component may have a significant effect on subsequent system behavior, particularly nutrient cycling. These grazing control components have been extensively reviewed for protozoa (Stout 1973, 1980), enchytraeids (Standen 1978), and soil microarthropods (Seastedt 1984).

Previous studies of biotic interactions in the rhizosphere have been more descriptive than analytical (e.g., review by Clark 1949, Head 1970, Coleman 1976). It is now important to conduct studies on the distribution and abundance of microbes and the fauna feeding on them, both inside and outside the rhizosphere region of growing plant roots. This paper reports on interactions between microflora and fauna (nematodes) in rhizosphere and nonrhizosphere areas.

The objectives of this study are twofold:

1) To propose and evaluate a conceptual model in which the levels of N and P in microfloral grazer populations are considered as separate state variables in the nutrient-cycling process, as suggested by evidence in the literature.

2) To examine the interactions between bacteria or fungi and the nematodes that feed upon them and how these relationships influence nutrient availability and plant growth.

Effects of microbial grazers on nutrient cycling

It has long been known that microbial grazers may stimulate the activities of microbial populations. Cutler and Crump (1929) reported that amoebae stimulated CO_2 production in sand with mineral amendments. It was found by de Telegdy-Kovats (1932) that decreasing the substrate C:N ratio in the presence of protozoa causes increases in CO_2 production. In sand cultures the presence of amoebae appeared to increase the rate of ammonium production in bacterial cultures growing on peptone (Meiklejohn 1930). This was confirmed by Doyle and Harding (1937), who found that most of the bacterial nitrogen ingested by the ciliate *Glaucoma* sp. was excreted as ammonia by 6 h after ingestion. A similar "regeneration" of phosphorus by paramecia was noted by Buechler and Dilleon (1974). Javornicky and Prokesova (1963) suggested that the presence of protozoa stimulates organic matter decomposition and thus may significantly influence the entire ecosystem energy flow. Mercer and Cairns (1973) reached a similar conclusion for bacterial-feeding nematodes. Johannes (1968) experimentally demonstrated that bacteria do not always directly mineralize nutrients from organic compounds and that nutrients are released at an accelerated rate when the microbial population is grazed. Barsdate et al. (1974) observed increases in gross mineralization of P in bacterial treatments grazed by protozoa as compared to those without grazers. The enhanced P turnover (using labeled phosphorus) was attributed to greater metabolic activity of bacteria in grazed systems.

Bacteria can also act as nutrient sinks in soils. With increasing additions of glucose, more inorganic phosphorus (P_i) was immobilized by bacteria, and, at the end of 160 h, there was no net mineralization of P (Elliott et al. 1979*b*). Coleman et al. (1977) found that almost all soil NH_4^+-N, and 40% of the initial P, were immobilized 21 h after inoculation of bacteria into glucose-amended sterile soils. When either amoebae or bacteriophagic nematodes were introduced to some of the microcosms, nearly all of the immobilized N was remineralized, while less than one-third of the NH_4^+-N was returned in the treatment with bacteria alone. After 24 d, significantly more P_i was remineralized in the bacteria-and-nematode treatment than in either the treatment with bacteria alone or the bacteria-and-amoebae treatment. In a similar study, more N was remineralized as NH_4^+-N in an amoebae-plus-bacteria treatment than in a treatment containing bacteria alone, a treatment with nematodes and bacteria, or one with nematodes, amoebae, and bacteria (Woods et al. 1982). In the same experiment, amoebae-and-bacteria or nematodes-and-amoebae-and-bacteria mineralized the same amount of P_i in unamended soils, while in glucose-amended soils, only the amoebae-plus-bacteria treatment remineralized significant amounts of P_i (Cole et al. 1978). Mineralization of N in chitin-amended soils has also been found to be greater in microcosms containing nematodes and bacteria than in treatments with only bacteria or with bacteria and amoebae (Gould et al. 1981, Trofymow and Coleman 1982).

Increases in the rate of substrate utilization by bacteria in nematode-grazed systems have been demonstrated by measuring substrate disappearance. Abrams and Mitchell (1980) found organic matter loss from sewage sludge to be 1.8–2.5 times greater in the presence of nematodes and bacteria than with bacteria alone. Similarly, more ^{14}C-labeled glucose was respired in

bacteria-plus-nematode treatments than with only bacteria, and soil glycol-C in the grazed systems was reduced to 40% of that in the ungrazed system in 10 d (Anderson et al. 1981*a*). In soils of cellulose- and chitin-amended microcosms, significantly more NH_4^+-N appeared and more chitin and cellulose decomposed with microbivorous nematodes present than with only bacteria (Trofymow et al. 1983).

Grazing of bacteria by nematodes has also been shown to affect the allocation of substrate C by the soil biomass. This is suggested by significantly greater CO_2 evolution in systems with bacteria and nematodes than in those with bacteria alone (Anderson and Coleman 1977, Coleman et al. 1977, 1978, Anderson et al. 1978, 1979*a*, 1981*a*, Trofymow et al. 1983).

Very little is known about the effects of fungal-feeding nematodes on nutrient cycling processes in soils. In cellulose-amended soil, N mineralization by *Fusarium oxysporum* was reduced when the fungus was grazed by the nematode *Aphelenchus avenae* (Trofymow and Coleman 1982). However, in a second experiment the nematode increased N mineralization and $^{14}CO_2$ evolution (C of cellulose origin) by *F. oxysporum* in soils amended with cellulose and chitin (Trofymow et al. 1983).

Effects of microbial grazers on microorganisms

The effects of bacterial-feeding nematodes on microbial populations in soils appears to be variable. Boucher and Chamroux (1976) found parallel growth responses between marine nematodes and bacteria in sand microcosms. A similar relationship between soil nematodes and soil bacteria was observed by Banage and Visser (1964) in bush soils of Uganda, and by Freckman and Mankau (1977) in desert soils. Santos et al. (1981), Santos and Whitford (1981), Elkins and Whitford (1982), and Whitford et al. (1982) found that when bacteriophagic nematodes were released from predation pressure by the removal of predatory mites with insecticide, decomposer bacterial populations were reduced and litter decomposition was slowed. In soil microcosms amended with glucose and nitrogen, nematodes reduced bacterial numbers to one-half or one-third of the density in the treatment without nematodes (Anderson et al. 1979*a*). Similar decreases were observed by Coleman et al. (1977), while Anderson et al. (1978) found that the reduction in bacterial numbers due to nematodes was less in microcosms that had not been amended with C and N. In contrast, Abrams and Mitchell (1980) found significantly higher bacterial densities in sewage sludge microcosms when microbivorous nematodes were present. Increased bacterial numbers with bacteriophagic nematodes were also observed by Trofymow and Coleman (1982) in microcosms amended with cellulose, chitin, or both cellulose and chitin.

The ability of mycophagous nematodes to affect growth of soil fungi has been little studied, but appears to be variable and to depend on the species of fungus and perhaps the species of nematode. Mankau and Mankau (1963) examined 18 species of fungi as food sources for *Aphelenchus avenae*. There was a range in the resulting classification from apparently poisonous species (*Pythium ultimum*) to species on which the nematodes reproduced rapidly and overgrazed the fungus, slowing or stopping hyphal growth in five of the nine species tested. Similarly, Wasilewska et al. (1975) found that inhibition of fungal growth due to consumption by *A. avenae* resulted in a 32–52% reduction in dry biomass of *Alternaria tenuis*. The response of fungi to faunal grazing may be dependent on available nutrient concentrations as well as on grazing intensity. Hanlon (1981) observed an increase in fungal respiration of as much as 100% when fungi grown in a high-nutrient regime were grazed by Collembola. However, no change in respiration was observed for grazed fungi grown in low nutrient concentrations. In addition, while moderate densities of Collembola increased fungal respiration, high densities did not, suggesting an apparent optimum grazing intensity, which increased as available nutrients increased.

Effects of microbial grazers on plant growth

Because of high consumption and low assimilation rates, microbial grazers release a considerable amount of nutrients that may then be available as a source of nutrients for plants (Anderson et al. 1981*b*). This may increase the rate of turnover of mineral nutrients and serve a system-regulating function by increasing primary production. The response of plants to nutrient dynamics mediated by faunal grazers has been only briefly investigated. Elliott et al. (1979*a*) grew blue grama seedlings in soil with bacteria and with or without amoebae, under three levels of nitrogen fertilization. At the medium- and high-N levels, there was 50–100% more net mineralization of organic N as NH_4^+-N with amoebae than without, and at all N levels shoot-N concentrations were significantly higher with amoebae. They concluded that bacterial grazing by amoebae accelerates the mineralization of microbially immobilized nutrients, increasing the inorganic N available for uptake by the plant.

The Conceptual Model and the General Experimental Design

Based on previous work of ourselves and others, we envision the cycling of nutrients (nitrogen and phosphorus) in a soil autotroph-heterotroph system containing bacteria (non-nitrifying) and/or fungi, nematode grazers of bacteria or fungi, and plants to occur as diagrammed in Fig. 1. The state variables are represented by x_1–x_7 and include levels of N and P in each of the following: detritus, plants, inorganic forms (NH_4^+, PO_4^{3-}), fungi, bacteria, fungal-feeding nematodes, and

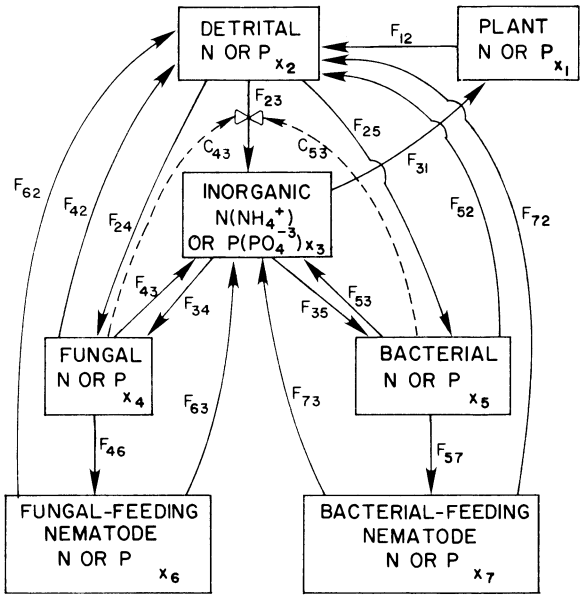

FIG. 1. Conceptual model illustrating important flows in a plant-soil system with bacteria, fungi, and their nematode grazers.

bacterial-feeding nematodes. Flows in the model are represented by F_{ij}, where i represents the state variable the flow is coming from and j represents the state variable the flow is going to. Controls on flows are represented by C_{ij}, where i is the controlling state variable and j is the state variable whose input is being controlled.

The current study examines the interactions of the biota represented in the model in order to substantiate previous observations under more rigorously controlled conditions and with a more complex assemblage of organisms, including plants. Nitrogen and phosphorus mineralization were examined in two model soil systems, one in which soil was unamended and one in which chitin was used as a representative organic-N substrate. Bacteria, fungi, and nematode grazers of these microflora were chosen from isolates to represent functional groups (Coleman 1976) operating in natural soil systems. Blue grama grass, *Bouteloua gracilis* (H.B.K.) Griffiths, was used as a test plant.

Experimental designs

The design for the unamended experiment consisted of six treatments: a sterile plant control (*p*); plant and bacteria (*pb*), plant, bacteria, and bacteriophagous nematodes (pbn_b); plant and fungus (*pf*); plant, fungus, and mycophagous nematodes (pfn_f); and plant, bacteria fungus, bacteriophagous nematodes, and mycophagous nematodes ($pbfn_bn_f$). Each treatment was replicated five times and sampled once after 40 d. Data collected included shoot and root biomass, soil bacterial and nematode numbers, soil NH_4^+-N and bicarbonate-extractable phosphorus (P_i).

The design for the amended experiment consisted of eight treatments: an uninoculated control (*uc*); a sterile plant control (*p*); bacteria and fungus (*bf*); bacteria, fungus, and mycophagous nematodes (bfn_f); plant and bacteria (*pb*); plant, bacteria, and fungus (*pbf*); plant, bacteria, and bacteriophagous nematodes (pbn_b); and plant, bacteria, fungus, and mycophagous nematodes ($pbfn_f$). Each treatment was replicated five times, with five sample dates: 7, 21, 49, 77, and 105 d after inoculation of microflora. Data collected included shoot and root biomass; rhizosphere and nonrhizosphere counts of bacteria, fungi, and nematodes; and soil NH_4^+-N, soil P_i, and shoot-N and -P concentrations.

Predictions and treatment contrasts

The treatments in the experiments outlined above were used to test the following hypotheses and predictions relating to the model in Fig. 1.

1) In nutrient-limiting soil, fungi (x_4) or bacteria (x_5) provide additional inorganic nutrients (x_3) for plant uptake (F_{31}) by mineralization of organic N and P (F_{43} and F_{53}). Thus, plants (x_1) grown in soil with microflora will grow faster and/or contain more N and P than those grown in sterile soil. (Relevant treatment contrasts: *p* vs. *pb*; *p* vs. *pf*.)

2) Mineralization by fungi (F_{43}) will be greater than that by bacteria (F_{53}). (Treatment contrasts: *pb* vs. *pf*; *pb* vs. *pbf*.)

3) Fungal-feeding nematodes (x_6) or bacterial-feeding nematodes (x_7) increase the amount of inorganic nutrients (x_3) available for plant uptake (F_{31}) by excreting microbial-immobilized N as NH_4^+-N waste (F_{63} and F_{73}) and by increasing microbial activity, which results in a positive feedback control (C_{43} and C_{53}) on decomposition (F_{23}) and further mineralization by fungi (F_{43}) and bacteria (F_{53}). Therefore, plants (x_1) will grow faster and/or contain more N and P when microbial-feeding nematodes are present than when they are not. (Treatment contrasts: *pf* vs. pfn_f; *pbf* vs. $pbfn_f$; *pb* vs. pbn_b.)

4) Bacterial-feeding nematodes will increase mineralization by bacteria (F_{53}) to a greater extent than fungal-feeding nematodes will increase mineralization by fungi (F_{43}). (Treatment contrasts: pbn_b vs. pfn_f; pbn_b vs. $pbfn_f$.)

MATERIALS AND METHODS

Isolation and culturing of organisms

All microorganisms used in these experiments were isolated from soil collected from the Pawnee National Grasslands in northeastern Colorado. The fungus used in the unamended experiment was *Mortierella* sp. isolated from soil on rose-bengal agar. The rhizosphere bacterium *Pseudomonas paucimobilis* in the unamended experiment was isolated from the roots of blue grama using the root-washing technique of Louw and Webley (1959), then cultured on nutrient agar. *Mortierella* sp. and *P. paucimobilis* were not used in the

amended experiment, because neither species adequately decomposes chitin. Chitin decomposers were isolated from Pawnee soil enriched (to 5% of soil dry mass) with purified ball-milled crustacean chitin, after a 43-d incubation (20°C) (Okafor 1966, Gould et al. 1981). *Pseudomonas stutzeri* and *Fusarium oxysporum* were chosen because they were the most chitinoclastic of the species examined and were palatable to grazers (Gould et al. 1981). In a pre-experimental study, this strain of *F. oxysporum* was found not to be pathogenic to blue grama (R. E. Ingham, *personal observation*). In the amended experiment, treatments *pb* and pbn_b were inoculated with *P. stutzeri,* while treatments *bf, bf*n_f, *pbf,* and *pbf*n_f were inoculated with *F. oxysporum* and *P. paucimobilis* to duplicate conditions in an earlier chitin-decomposition experiment so that our results would be comparable with those of another study that also included bacteria and fungi (Trofymow et al. 1983).

Aphelenchus avenae (fungal feeder) in this study was obtained from D. Freckman, University of California, Riverside, and *Pelodera* sp. and *Acrobeloides* sp. (bacterial feeders) were isolated from Pawnee soil. *Aphelenchus avenae* was cultured on plates containing 17% potato dextrose agar (in water) and a pure culture of *F. oxysporum,* while *Acrobeloides* sp. and *Pelodera* sp. were grown on agar plates containing a reference soil solution with nutrients (Herzberg et al. 1978) with a pure culture of *P. paucimobilis* or *P. stutzeri,* respectively, as a food source.

Plants were prepared in the following fashion. Seeds of blue grama, *Bouteloua gracilis* (H.B.K.) Griffiths, were surface sterilized for 15 min in 15% aqueous sodium hypochlorite containing a few drops of Tergitol as a surfactant. The seeds were then thrice rinsed in sterile de-ionized water, drained, and placed on nutrient agar for germination. After germination (4 d), the sterile seeds were planted individually into cotton-plugged test tubes containing 0.5% one-quarter strength Hoagland's agar. The plants remained in these tubes for 10 d and all contaminated seedlings were easily observed and discarded.

Microcosm design

Gnotobiotic plant microcosms were constructed from 600-mL Berzelius beakers with urethane-foam collars and with glass Petri dishes as covers (Ingham and Trofymow 1979, Trofymow et al. 1980). The original design was modified by drilling a 7–8 mm hole in the glass cover and gluing a 2.5-cm length of 5 mm diameter glass tubing into the hole with silicone sealant. To maintain sterility, the exterior end of the glass tubing was fitted with a rubber serum cap for watering the microcosm via syringe.

Treatments without plants ("nonplant microcosms") used foam-plugged 50-mL Erlenmeyer flasks as described in Coleman et al. (1977).

In the unamended experiment we used a sandy loam from the Renohill-Shingle complex (an Ustic Torriorthent) that had been sieved through a 1-mm screen, oven dried, and mixed in a twin-shell mixer for 30 min. A soil lot of 200 g was weighed into each plant microcosm and wetted to field capacity (moisture 15% of soil dry mass). After incubation for 24 h, the soil was autoclaved for 1 h. This procedure was repeated three times. Soil used in the amended experiment was a low-available-N (NH_4^+-N concentration $\approx$3 mg/g) sandy loam of the Blakeland series (found within the Truckton-Blakeland-Bresser association) (Hays et al. 1982) and was treated similarly to the unamended soil except for the following. A soil lot of 100 g was weighed into each plant microcosm, and a lot of 20 g into each nonplant microcosm. For each gram of soil, all microcosms were amended with 3.38 gm of ball-milled purified crustacean chitin (0.24 mg chitin-N) (Gould et al. 1981). An N-free Hoagland's solution was added after the first autoclaving. The soil was then thoroughly mixed before the second wetting.

Inoculation of microcosms

Treatments requiring bacteria in the unamended study received 1.25×10^5 cells/g of soil, and fungal treatments were inoculated with 1 mL of a concentrated and blended suspension of the fungus. In the amended experiment, bacterial treatments were inoculated with 1.5×10^6 cells/g of soil, and fungal treatments received a concentrated suspension of blended hyphae equalling 97.5 m/g of soil. Bacteria and fungi in both experiments were diluted with dilute mineral salts medium (MSM) before inoculation; uninoculated controls received an equal amount of sterile MSM.

Plants (three seedlings per microcosm) were added on the day of inoculation (unamended) or 7 d after inoculation (amended) of microflora, using sterile technique.

In the unamended experiment, treatments with bacterial-feeders were inoculated with *Acrobeloides* sp. (2–3 eggs, 2–3 juveniles and adults per gram of soil), while treatments containing fungal feeders were inoculated with 7.0 juvenile or adult *A. avenae* per gram of soil. All nematodes were added 10 d after addition of plants and microflora. Nematode treatments in the amended experiment received 8–10 eggs and 3–4 juveniles and adults per gram of soil (*Pelodera* sp.) or 8–10 juveniles and adults per gram of soil (*A. avenae*).

In the unamended study, microcosms were kept in a growth chamber, while for the amended experiment, plant microcosms were maintained in a 54-m^2 walk-in microbiologically "clean" room, in which conditioned air was recycled through HEPA (high efficiency particulate-free air) absolute filter (>0.3 μm) systems. Illumination (Agro-lite fluorescent lamps) was at an intensity of 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the inside of the microcosms. Nonplant microcosms were kept in an incubator at the same diurnal temperature regime as the plant microcosms (30° day/22° night). Soil moisture in

plant microcosms was maintained between 70 and 100% of field capacity by gravimetrically adding sterile de-ionized water. Nonplant microcosms did not require addition of water after the final inoculation.

During the chitin-amended experiment, *P. stutzeri* (*b*) and *P. stutzeri* plus *Pelodera* sp. (bn_b) treatments were also attempted, but *P. stutzeri* failed to survive in either of the two attempts to inoculate the bacteria into sterile soil.

Sampling of microcosms

Checks for sterility were made on sample dates by spreading ≈1 g of the mixed soil on nutrient agar. These plates were incubated for 1 wk and checked for bacterial or fungal growth.

At sampling dates, plants were carefully removed and roots with adhering rhizosphere soil (soil 2–4 mm from the root surface [Bennett and Lynch 1981, Martens 1982]) were placed in 99 mL of 0.9% NaCl. Shoots were clipped at the base of the crown, dried at 65°, and weighed. From the remaining soil, lots of ≈1 g were taken for soil moisture determination, bacterial plating, and fungal enumeration, when appropriate. Nematodes were extracted from 5 g soil in modified Baermann funnels (Anderson and Coleman 1977). Total hyphal lengths were estimated by taking 1 mL from a dilution of 1 g soil in 9 mL MSM, staining with 0.5 mL phenolic aniline blue, adding 1 mL agar, and observing the hyphal lengths in the agar film with a phase contrast microscope. Lengths of hyphae stained with FDA (fluorescein diacetate) were determined by the method of Ingham and Klein (1982) in agar film, using epifluorescent microscopy.

Rhizosphere analyses

Rhizosphere bacterial numbers were determined, after shaking roots in the dilute saline (APHA et al. 1971), with three replicate platings for each of three dilutions. From each fungal treatment, two 1-mL aliquots were removed for total hyphal and FDA-stained hyphal length analyses.

Roots were then removed, rinsed, dried at 65°, and weighed. Rhizosphere nematodes were obtained by shaking the saline-soil suspension, decanting, and centrifuging for 5 min at 1500 rpm. The centrifuged soil and nematodes were then stained (Anderson et al. 1979*b*) and counted in a gridded petri dish under a dissecting microscope (70×). The amount of rhizosphere soil was then determined gravimetrically.

Chemical analyses

The following chemical analyses were performed on nonrhizosphere or nonplant soil. Rhizosphere soil was generally in insufficient quantity for chemical analysis.

For inorganic-P, 2 g of soil was extracted with 10 mL of 0.5 mol/L $NaHCO_3$ adjusted to pH 8.5 with NaOH (Olsen et al. 1954). After shaking for 30 min and filtering the solution through a Whatman number 40 filter, the extract was analyzed for P_i using a colorimetric technique (Olsen et al. 1954).

Inorganic-N analyses (NH_4^+-N and NO_3^--N) were determined by extracting 4 g of soil with 40 mL of 2.0 mol/L KCl. The solution was shaken for 30 min and filtered through a Whatman number 1 filter. Ammonium-N concentrations were then analyzed on a Technicon Auto Analyzer I by a modification of the methods of Searcy et al. (1967) and Pym and Milham (1976). Nitrate-N levels were determined from the same KCl extract by reducing the NO_3^- to NO_2^- and measuring the NO_2^- concentration, a revision of the technique of Henriksen and Selmer-Olsen (1970).

Plant tissue N and P were determined on plant samples ground in a Wiley mill and digested with H_2SO_4-H_2O_2 (Thomas et al. 1967). Nitrogen concentration was determined colorimetrically by a salicylate assay for NH_4^+-N (Pym and Milham 1976), and an ascorbic acid reduction was used to assay for P (Murphy and Riley 1962).

Statistical analyses include ANOVA and Tukey's honestly significant difference (HSD) mean separation test (Kirk 1968). The ANOVA model used in the unamended experiment consisted of five replicates × six treatments and had 19 degrees of freedom. In the amended study, three ANOVA models were used. The first examined only treatments that contained plants (five replicates × four dates × five biological treatments; 71 df). The second model included only plant treatments inoculated with microflora (five replicates × four dates × two microbes × two grazers; 55 df), and the third model examined all treatments and analyzed nonplant soil, nonrhizosphere soil, and rhizosphere soil separately (five replicates × four dates × three soils × two grazers; 87 df). For all significant differences reported, $P < .05$.

Results

The unamended experiment

Nematode numbers.—In single culture *Acrobeloides* sp. (pbn_b) reached higher numbers per gram of soil than *Aphelenchus avenae* (pfn_f): 1058 ± 186 individuals (SE) and 646 ± 17 individuals, respectively. In concomitant culture ($pbfn_bn_f$), however, *Acrobeloides* sp. numbers per gram were significantly lower than in single culture (377 ± 79 individuals), while *A. avenae* were slightly but not significantly ($P > .05$) higher (745 ± 84 individuals).

Shoot and root biomass.—Shoot production (Table 1) was significantly higher in treatments with bacterial-feeding nematodes or with fungus than in the sterile plant control (*p*) or plant-and-bacteria treatment (*pb*). In addition, the most biologically complex treatment ($pbfn_bn_f$) had more shoot biomass than all other treatments. Root production was significantly higher in the *pf* treatment than in any other treatment.

Bacterial growth.—Occasional colonies of fungi and

TABLE 1. Biomass of plants and concentrations of P_i and NH_4^+-N in the soil after plants were grown for 40 d in unamended soil with biological assemblages ($\bar{x} \pm 1$ SE).

Assemblage*	Soil concentration (μg/g)		Biomass per plant (mg)	
	P_i†	NH_4^+-N	Shoot	Root
p	17.5 ± 0.4	33.0 ± 1.9	2.1 ± 0.1	2.0 ± 0.3
pb	16.8 ± 0.1	32.2 ± 2.1	2.6 ± 0.5	1.8 ± 0.1
pbn_b	17.5 ± 0.6	45.5 ± 1.4	6.6 ± 1.0	4.2 ± 0.3
pf	17.2 ± 0.6	48.6 ± 2.4	7.5 ± 0.9	9.6 ± 0.7
pfn_f	17.6 ± 0.5	49.1 ± 3.0	8.6 ± 1.1	4.8 ± 0.6
$pbfn_bn_f$	16.6 ± 0.7	46.4 ± 3.2	13.5 ± 2.7	4.2 ± 0.6
Q_6 value	9.7	10.2	3.0	2.9

* *p* = plant (*Bouteloua gracilis*); *pb* = plant and bacteria (*Pseudomonas paucimobilis*); pbn_b = plant, bacteria, and bacterial-feeding nematode (*Acrobeloides* sp.); *pf* = plant and fungus (*Mortierella* sp.); pfn_f = plant, fungus, and fungal-feeding nematode (*Aphelenchus avenae*); and $pbfn_bn_f$ = plant, bacteria, fungus, bacterial-feeding nematode, and fungal-feeding nematode. The Q_6 value represents the Tukey HSD ($P \leq .05$) for means from the six treatments.
† Bicarbonate-extractable phosphorus.

bacteria appeared on sterility checks for the pbn_b and pfn_f treatments, respectively. The number of bacterial cells per gram of soil increased from 1.25×10^5 at inoculation to 3.7 ± 0.6, 7.8 ± 0.4, and $5.9 \pm 0.2 \times 10^8$ in the *pb*, pbn_b, and $pbfn_bn_f$ treatments, respectively. Both treatments that included nematodes had significantly higher bacterial numbers than the treatment with only plants and bacteria, and the highest bacterial densities were in the pbn_b treatment.

Soil inorganic nutrients.—There was no significant difference in soil P_i between any pair of treatments (Table 1). All treatments with bacterial-feeding nematodes or fungi contained significantly more NH_4^+-N than the sterile plant control or plant-and-bacteria treatments (Table 1).

The chitin-amended experiment

Nematode numbers.—Nematode population densities were 11 (*A. avenae*) to 18 (*Pelodera* sp.) times greater in rhizosphere soil than in nonrhizosphere soil at the end of the experiment (Tables 2 and 3). Numbers of *Pelodera* sp. increased in the rhizosphere throughout the experiment, while the rhizosphere populations of *A. avenae* initially increased and then declined after day 49. In nonrhizosphere soil, *Pelodera* sp. reached its highest density on day 49 and then declined slightly, while *A. avenae* was most abundant on day 21 and declined considerably after day 49. Abundance of *A. avenae* in soil from the treatment without plants was generally comparable to that in nonrhizosphere soil of the treatment with plants.

Shoot and root biomass.—Shoot biomasses (Fig. 2a) in the pbn_b, *pbf*, and $pbfn_f$ treatments were higher than those for the *p* and *pb* treatments on days 21, 49, and 77 (with the exception that there was no difference between *pb* and *pbf* values on day 77). On day 105, shoot biomass in the *pb* treatment was not different from that of any other treatment containing microflora. Shoot biomass in the *p* treatment, however, remained less than in any of the other treatments throughout the experiment.

After day 21, root biomass (Fig. 2b) in all treatments with chitin decomposers was always greater than in the treatment without microflora. Among treatments with microflora, root biomass was higher in the *pb* treatment than in the *pbf* or $pbfn_f$ treatments on day 77, although it was not so on days 49 and 105.

Bacterial growth.—Within the fungal treatments, data on density of bacteria (*P. paucimobilis*) could be clas-

TABLE 2. Numbers of nematodes within the pbn_b* treatment in the rhizosphere and nonrhizosphere of chitin-amended soil (mean and 95% confidence interval).

Day	No. *Pelodera* per gram dry soil	
	Nonrhizosphere	Rhizosphere
7	13†	13†
21	30.5 ± 16.5	94.1 ± 59.0
49	63.8 ± 29.6	535.5 ± 543.9
77	44.8 ± 24.9	429.6 ± 259.3
105	49.5 ± 36.8	912.2 ± 699.0

* pbn_b = plant (*Bouteloua gracilis*), bacteria (*Pseudomonas stutzeri*), and bacterial-feeding nematode (*Pelodera* sp.).
† Values for day 7 represent inoculum level. Replicate sampling to determine confidence intervals was not attempted.

TABLE 3. Numbers of nematodes within the nonplant (bfn_f*) treatment and in rhizosphere and nonrhizosphere soil within the $pbfn_f$† treatment (mean and 95% confidence interval).

Day	No. *Aphelenchus* per gram dry soil		
	Nonplant	Nonrhizosphere	Rhizosphere
7	9‡	9‡	9‡
21	16.0 ± 6.5	61.0 ± 41.9	154.6 ± 125.0
49	29.2 ± 16.8	42.2 ± 20.6	311.5 ± 145.2
77	29.2 ± 23.1	7.2 ± 2.6	121.5 ± 59.4
105	14.4 ± 14.8	13.1 ± 9.6	142.4 ± 59.8

* bfn_f = bacteria (*Pseudomonas paucimobilis*), fungus (*Fusarium oxysoporum*), and fungal-feeding nematode (*Aphelenchus avenae*).
† $pbfn_f$ = plant (*Bouteloua gracilis*), bacteria, fungus, and fungal-feeding nematode.
‡ Values for day 7 represent inoculum level. Replicate sampling to determine confidence intervals was not attempted.

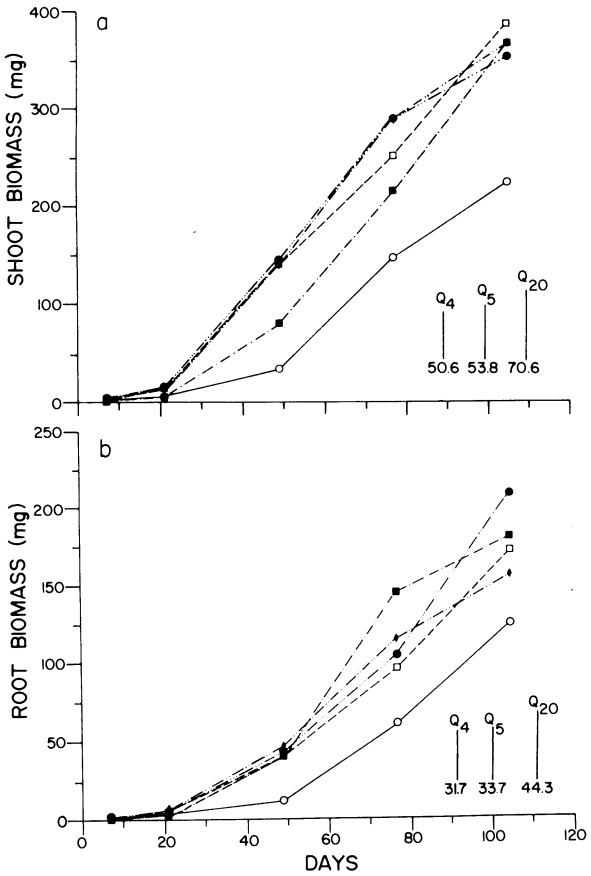

FIG. 2. Shoot biomass (a) and root biomass (b) of *Bouteloua gracilis* grown in soil microcosms with different biological treatments. The Q_4 value represents Tukey's HSD, the difference needed for significance ($P < .05$), between dates for the same treatment; Q_5 represents that for different treatments on the same date; and Q_{20} can be used to compare treatments on different dates. ○——○ Plant (*p*); ■—·—■ Plant + bacteria (*pb*); □—–—□ Plant + bacteria + fungus (*pbf*); ◆—··—◆ Plant + bacteria + bacterial-feeding nematode (pbn_b); ●—···—● Plant + bacteria + fungus + fungal-feeding nematode ($pbfn_f$).

sified in three groups: lowest in the nonplant treatments, slightly higher in nonrhizosphere soil, and highest in the rhizosphere (Fig. 3). In nearly every case, a treatment with *A. avenae* had higher bacterial numbers than the corresponding treatment without nematodes; this resulted in a significant main effect of grazing (Fig. 3). Bacterial numbers changed very little over time in all treatments except $pbfn_f$ rhizosphere soil, where bacteria increased significantly during the experiment.

Bacteria (*P. stutzeri*) in the *pb* and pbn_b treatments (Fig. 4) increased rapidly between inoculation and day 7. After this time, numbers slowly declined in the *pb* and pbn_b nonrhizosphere, while numbers increased in the rhizosphere of both treatments. Bacterial numbers in the pbn_b nonrhizosphere were significantly greater on day 21 than those in *pb* nonrhizosphere soil, but were not different later on. In contrast, rhizosphere

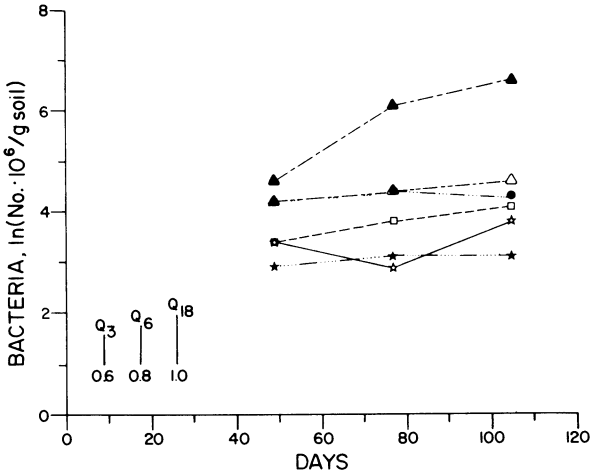

FIG. 3. Natural log (ln) of bacterial numbers in soil microcosms from treatments containing fungi. Q values are Tukey's HSD. Q_3 compares dates for the same treatment, Q_6 different treatments on the same date, and Q_{18} treatments on different dates. ★—·····—★ Bacteria + fungus (*bf*); ☆——☆ Bacteria + fungus + fungal-feeding nematode (bfn_f); □-----□ Plant + bacteria + fungus (nonrhizosphere) (*pbf*); △—--—△ Plant + bacteria + fungus (rhizosphere) (*pbf*); ●—···—● Plant + bacteria + fungus + fungal-feeding nematode (nonrhizosphere) ($pbfn_f$); ▲—-—▲ Plant + bacteria + fungus + fungal-feeding nematode (rhizosphere) ($pbfn_f$).

bacterial numbers were the same on day 21 for both the *pb* and pbn_b treatments, but after this date those in the pbn_b remained significantly greater than those in the *pb* treatment.

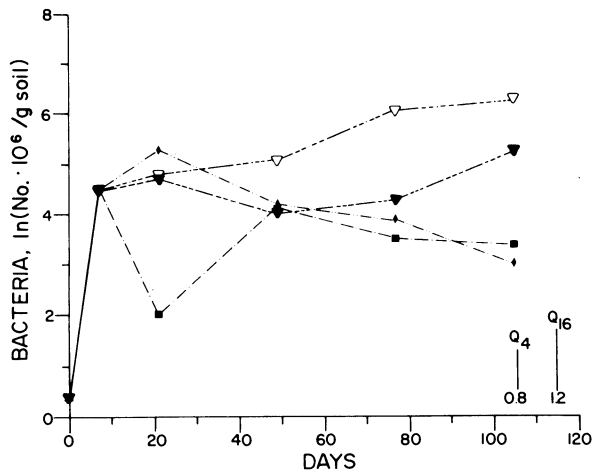

FIG. 4. Natural log (ln) of bacterial numbers in rhizosphere and nonrhizosphere soil of different treatments with bacteria but without fungi. Q values are Tukey's HSD. Q_4 compares dates for the same treatment, and treatments on the same date; Q_{16} compares treatments on different dates. ■—·—■ Plant + bacteria (nonrhizosphere) (*pb*); ▼———▼ Plant + bacteria (rhizosphere) (*pb*); ◆—··—◆ Plant + bacteria + bacterial-feeding nematode (nonrhizosphere) (pbn_b); ▽—-·-·-—▽ Plant + bacteria + bacterial-feeding nematode (rhizosphere) (pbn_b).

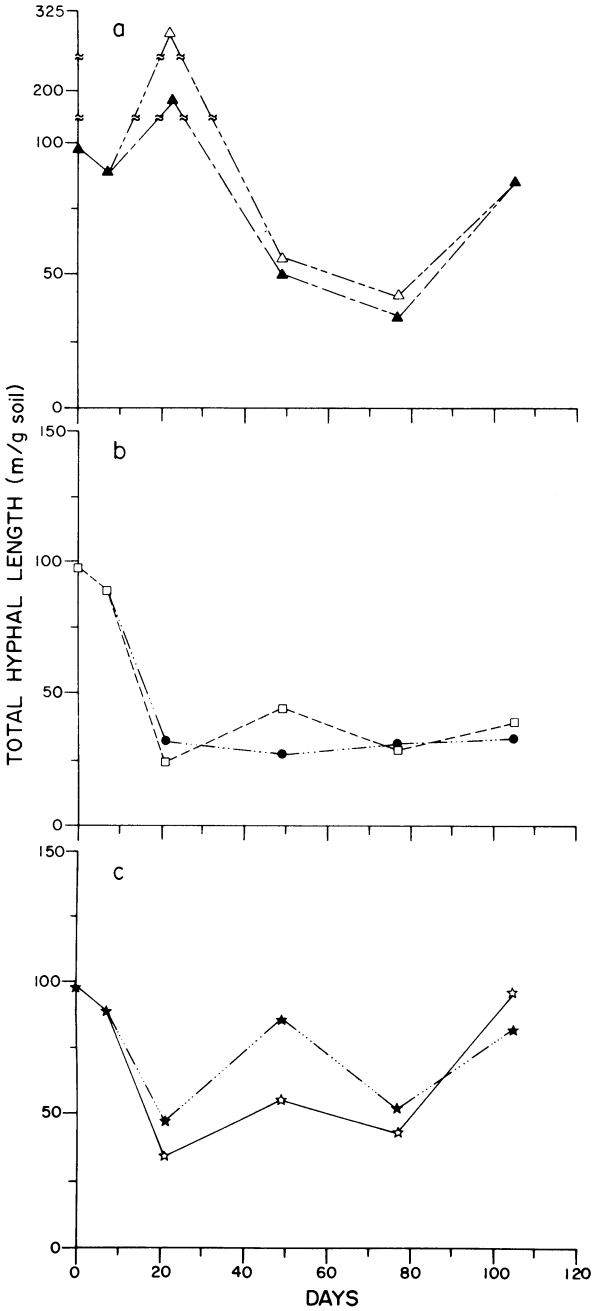

FIG. 5. Total length of fungal hyphae in (a) rhizosphere soil, (b) nonrhizosphere soil, and (c) nonplant soil, from different biological treatments in soil microcosms. Symbols as in Fig. 3.

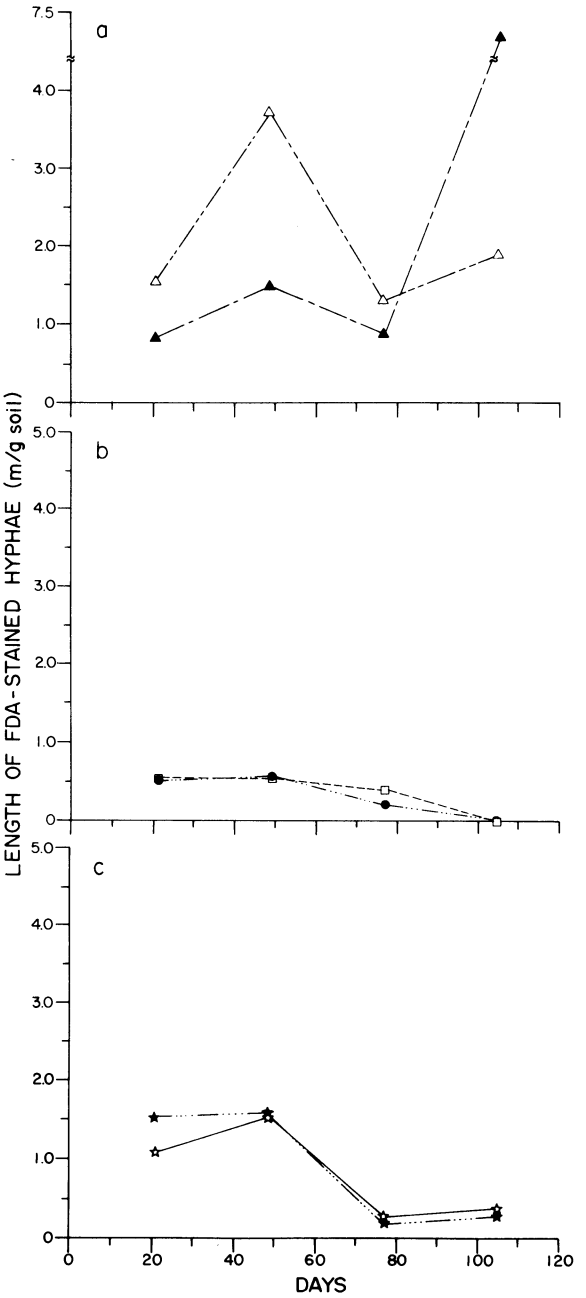

FIG. 6. Length of FDA-stained (metabolically active) hyphae in (a) rhizosphere soil, (b) nonrhizosphere soil, and (c) nonplant soil, from different biological treatments in soil microcosms. Symbols as in Fig. 3.

Bacterial densities within the rhizosphere were nearly comparable between the *pb* and *pbf* treatments and between the pbn_b and $pbfn_f$ treatments. Treatments with nematodes always contained more bacteria than treatments without nematodes.

Fungal growth.—As with bacterial numbers, three different groups of responses were observed for total (live and dead) hyphal lengths (Fig. 5). Hyphal response in the rhizosphere soils was the most dynamic, increasing after inoculation, decreasing later, and then increasing again (Fig. 5a). Fungal lengths from nonrhizosphere soil of both the *pbf* and $pbfn_f$ treatments decreased rapidly after inoculation and then remained at a constant level of ≈30 m/g of soil (Fig. 5b). Fungi in the *bf* and bfn_f treatments also decreased after inoculation but returned to the inoculum density by the

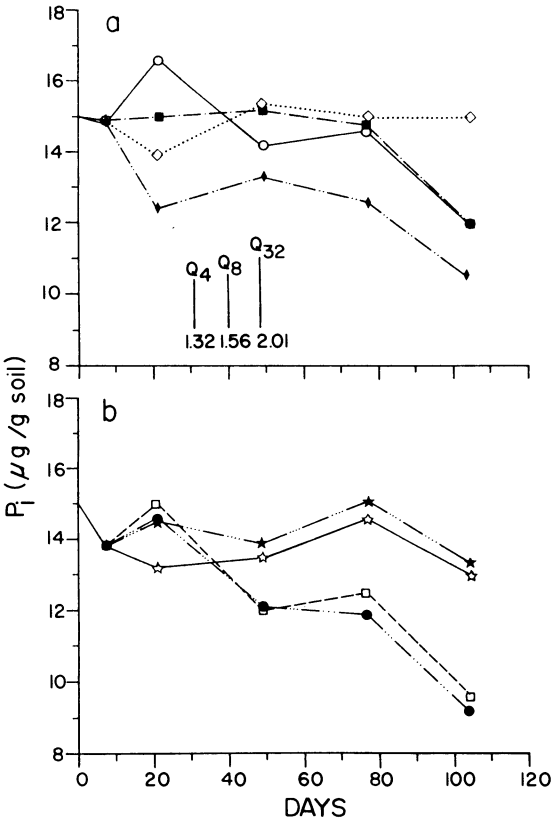

FIG. 7. Mean values of soil P_i in different treatments from soil microcosms (a) without fungi and (b) with *Fusarium oxysporum* and *Pseudomonas paucimobilis*. Q values are Tukey's HSD. Q_4 compares dates for the same treatment, Q_8 different treatments on the same date, and Q_{32} treatments on different dates. ◇---◇ Uninoculated control (*uc*); ★—·····—★ Bacteria + fungus (*bf*); ☆——☆ Bacteria + fungus + fungal-feeding nematode (bfn_f); ○——○ Plant (*p*); ■—·—■ Plant + bacteria (*pb*); □ ----- □ Plant + bacteria + fungus (*pbf*); ◆—··—◆ Plant + bacteria + bacterial-feeding nematode (pbn_b); ●—···—● Plant + bacteria + fungus + fungal-feeding nematode ($pbfn_f$).

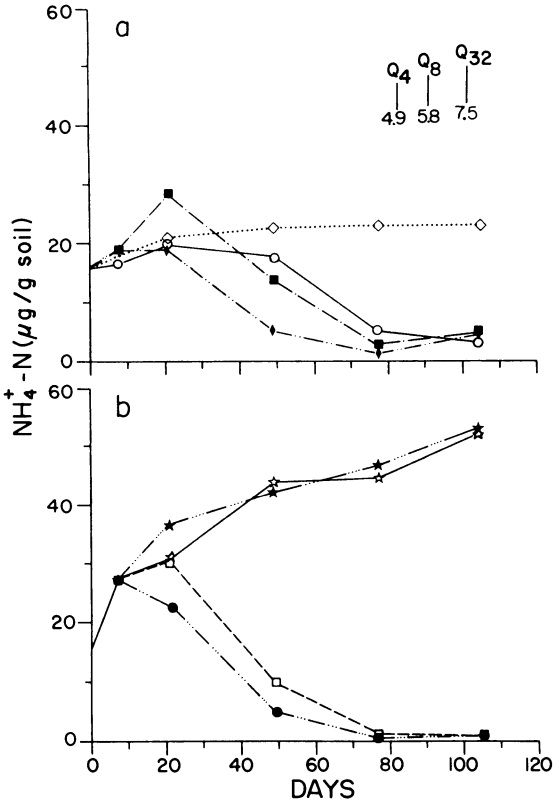

FIG. 8. Mean values of soil NH_4^+-N in different treatments from soil microcosms (a) without fungi or (b) with fungi *Fusarium oxysporum* and *Pseudomonas paucimobilis*. Q values apply to both graphs. Symbols as in Fig. 7.

end of the experiment (Fig. 5c). The amount of hyphae in grazed treatments was generally less than in ungrazed treatments.

Hyphae that stained with FDA (metabolically active hyphae; see Söderström 1977, Ingham and Klein 1982) could also be classified by treatment into three distinct groups (Fig. 6). Initially, stained hyphae in the rhizosphere were most abundant in the *pbf* treatment (Fig. 6a), in which hyphae increased significantly between days 21 and 49, then decreased to the initial level by day 77. Active hyphae in the rhizosphere of the $pbfn_f$ treatment were less abundant than in the *pbf* rhizosphere on day 21, but increased with time until they were more abundant than in the *pbf* rhizosphere on the final sample date. In the nonrhizosphere soil (Fig. 6b), both *pbf* and $pbfn_f$ treatments showed a decrease in FDA-stained hyphae over time until almost no staining could be detected. Stained hyphae in the *bf* and bfn_f treatments (Fig. 6c) were more abundant than in the nonrhizosphere soil until day 77, when stained hyphae decreased to ≈0.1 m/g of soil, a level at which they remained for the rest of the experiment.

Soil inorganic nutrients.—Extractable inorganic soil phosphorus (P_i) remained generally constant in the uninoculated control through the course of the experiment (Fig. 7a, b). Values were always less in the bfn_f treatment than in the *bf* treatment, and although the difference was never significant on any one date, the main effect of grazing was significant ($P < .01$). All plant treatments immobilized P_i, with the least immobilization in the *p* and *pb* treatments, and the most rapid initial immobilization in pbn_b. By the end of the experiment, however, the P_i values were similar for the pbn_b, *pbf*, and $pbfn_f$ treatments.

Ammonium-N increased slightly between days 9 and 21 in the uninoculated control and then remained constant for the rest of the experiment (Fig. 8a, b). Both the *bf* and bfn_f treatments showed significant net mineralization of NH_4^+-N, with no difference between the two treatments. Over time, all plant treatments immobilized significant amounts of NH_4^+-N, although

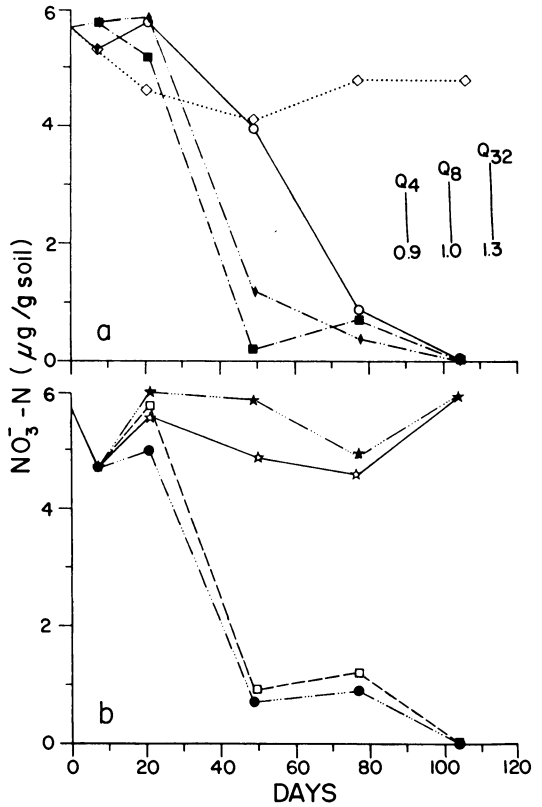

FIG. 9. Mean values of soil NO_3^--N in different treatments from soil microcosm (a) without fungi or (b) with *Fusarium oxysporum* and *Pseudomonas paucimobilis*. Q values apply to both graphs. Symbols as in Fig. 7.

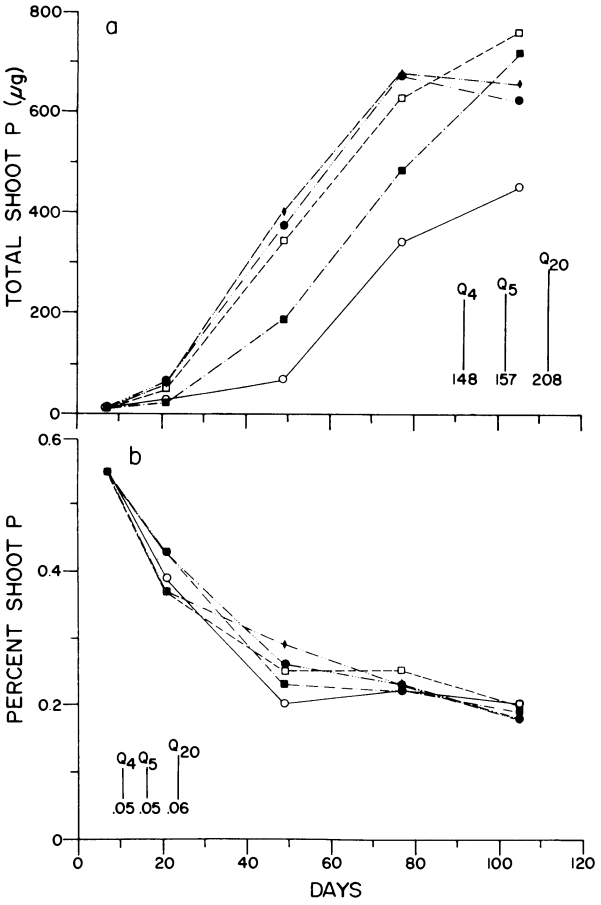

FIG. 10. Total shoot phosphorus (a) and percent shoot phosphorus (b) in *Bouteloua gracilis* grown in soil microcosms with different biological treatments. Symbols as in Fig. 2.

there was some early net mineralization in the *pb* and *pbf* treatments. The most rapid immobilization occurred in the two grazed treatments (pbn_b and $pbfn_f$).

Soil NO_3^--N levels were low, and in the uninoculated control decreased between days 0 and 49 and then increased slightly (Fig. 9a, b). Fluctuations also occurred in the *bf* and bfn_f treatments, but neither treatment differed significantly from the control or from the initial NO_3^--N value. All plant treatments immobilized all measurable NO_3^--N by the end of the experiment, with the most rapid immobilization occurring within the grazed treatments.

Shoot nitrogen and phosphorus.—Total shoot phosphorus increased throughout the experiment in all treatments except those with nematodes between days 77 and 105 (Fig. 10a). Total shoot phosphorus was significantly greater in the treatments with nematodes and/or fungi than in the plant alone or plant-plus-bacteria treatments until day 105 (except for *pb* vs. *pbf* on day 77). On the final date, the only difference among treatments was that in the *p* treatment there was less shoot phosphorus than in the treatments with microflora. However, there were only small differences in percent phosphorus in the shoots (Fig. 10b).

Total shoot N increased in all treatments between days 7 and 77, but in only the sterile plant treatment did it increase between days 77 and 105 (Fig. 11a). More N was in the shoots of the *pbf*, $pbfn_f$, and pbn_b treatments than in the *pb* or *p* treatments until day 77 (except for *pbf* vs. *pb* on day 49). The increases in total shoot N were the result of greater shoot biomass and higher N concentration in the shoots. For example, on day 21 there was 2.5% N in the *p* and *pb* treatments, while in the pbn_b, *pbf*, and $pbfn_f$ treatments the value ranged from 3.4 to 3.7% (Fig. 11b). On the final sample date there was no significant difference in total shoot N between any pair of treatments.

Nematode consumption of microflora

The amount of bacterial biomass carbon consumed by *Pelodera* sp. was estimated by first determining nematode respiration rates (R) according to the equation $R = 2.52W^{0.74}$, where W is nematode fresh mass in micrograms and R is O_2 consumption in nanolitres per individual per hour. (Schiemer 1982). This esti-

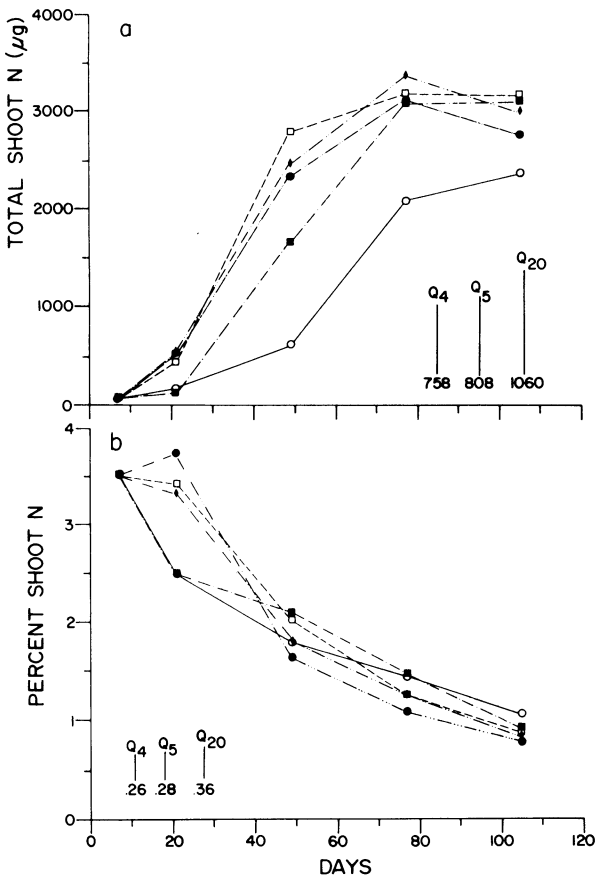

FIG. 11. Total shoot nitrogen (a) and percent shoot nitrogen (b) in *Bouteloua gracilis* grown in soil microcosms with different biological treatments. Symbols as in Fig. 2.

mation assumes a respiratory quotient (RQ) of 0.8 mol CO_2 per mol O_2 (Sohlenius 1979) and a Q_{10} of 2. The proportion of carbon mass consumed (C) and allocated towards production (Pr), respiration (R), or defecation (D) was calculated based on values for *Pelodera* sp. as follows: $C = 0.225Pr + 0.370R + 0.405D$ (Marchant and Nicholas 1972). Percent of bacterial standing crop biomass consumed was determined for each sample date assuming a dry mass of 0.44×10^{-6} μg/cell and assuming bacteria to be 50% carbon (Van Veen and Paul 1979).

Fungal biomass carbon consumed by *A. avenae* was estimated similarly using the equation for respiration rate $R = 2.75W^{0.72}$ (derived from data for *A. avenae* only; Klekowski et al. 1972) and using the values mentioned above for other parameters. Although the production and assimilation efficiencies of a fungal-feeding nematode may be different from those of a bacterial-feeding nematode, there are no reliable literature values, and the values of Marchant and Nicholas (1972) were used as the best approximation available. Amount of cytoplasm consumed was determined based on a value of 55.4% carbon for fungal cytoplasm (Hurst and Wagner 1969). Standing crop of cytoplasm was calculated by multiplying the volume of cytoplasm-filled hyphae by 0.64, since ≈64% of this hyphal volume was cytoplasm and 36% was cell wall, based on our observations of hyphae in agar films. Total cytoplasm was ≈10 times the volume of cytoplasm that stained with FDA. Cytoplasm volume was converted to dry biomass using the conversion factor 0.33 g/cm^{-3} (Van Veen and Paul 1979). Percent of total cytoplasm standing crop biomass consumed per day was determined for each sample date.

The percentage of the bacteria standing crop biomass consumed by *Pelodera* sp. ranged from 3.5 to 26.0%/d (Table 4). The percentage of the total fungal cytoplasm consumed by *A. avenae* ranged from 9.3 to 32.8%/d (Table 5).

Nitrogen losses by nematodes

The amount of N lost by *Pelodera* sp. was based on the allocation $C = Pr + R + D$ with the following assumptions. All N associated with C that is defecated or respired must also be lost, as well as half of the N associated with C that goes towards production, since the average C:N ratio of nematodes (10:1) (Anderson et al. 1983) is twice that of bacteria (5:1) (Woods et al. 1982). Nitrogen losses for *A. avenae* were determined similarly with the following assumptions. All of the N associated with C allocated to production is also allocated to production. However, since the C:N ratio of fungal cytoplasm (11:1) (Hurst and Wagner 1968) is greater than that for nematodes, this amount of N is insufficient for production of nematode biomass. Therefore, additional N must be acquired from that associated with respired C. The additional N necessary is equivalent to 6.4% of the N associated with respired C. The remaining N associated with respired C is excreted. Daily and cumulative N losses were calculated for each day of the experiment for both nematodes. The principal N loss by nematodes is in the form of ammonia (Lee and Atkinson 1977), but at times of luxury consumption of bacteria, some bacteriophagic nematodes may lose up to 50% of their metabolic N as amino-N; this phenomenon is transient (2–3 d) under normal soil conditions (Anderson et al. 1983).

Peak daily N loss from nematodes was reached on day 49 by *Pelodera* sp., when 61.4 μg/microcosm was lost, and on day 22 for *A. avenae,* when 28.0 μg/microcosm was released (Fig. 12a). Cumulative N loss per microcosm at the end of day 105 was 3671 μg for *Pelodera* sp. and 1468 μg for *A. avenae* (Fig. 12b).

DISCUSSION

Plant growth

Addition of similar biotic assemblages to sterile soil resulted in similar plant growth responses in both the unamended and chitin-amended experiments regardless of differences in soil type and species composition.

TABLE 4. Consumption of the bacterium *Pseudomonas stutzeri* by the bacterial-feeding nematode *Pelodera* sp.

Day	Nematode biomass (μg/microcosm)	Consumed bacterial biomass (μg·microcosm^{-1}·d^{-1})	Standing crop of bacterial biomass (μg/microcosm)	Percent of standing crop consumed
7	31.9	...	3916	...
21	80.6	316.0	9108	3.5
49	177.6	640.0	2992	21.4
77	109.0	427.3	2640	16.2
105	111.1	435.6	1672	26.0

TABLE 5. Consumption of cytoplasm of the fungus *Fusarium oxysporum* by the fungal-feeding nematode *Aphelenchus avenae*.

Day	*A. avenae* biomass (μg/microcosm)	Cytoplasm consumed (μg·microcosm^{-1}·d^{-1})	Standing crop total cytoplasm (μg/microcosm)	Percent of total standing crop cytoplasm consumed per day
7	18.8	...	...	...
21	173.9	726.9	2217	32.8
49	144.7	604.8	2070	29.2
77	29.2	122.0	1309	9.3
105	46.2	193.1	718	26.9

The most significant increases in available NH_4^+-N (Table 1), plant growth (Table 1, Fig. 2a), and plant-N (Fig. 11a) occurred when bacterial-feeding nematodes or fungi were present. In the unamended experiment, the rhizosphere bacterium *P. paucimobilis* did not significantly increase shoot growth over the control, whereas addition of the chitin-decomposer *P. stutzeri* (amended experiment) did result in more plant biomass with a higher N content. This resulted from additional inorganic nitrogen made available to the plant through mineralization of chitin by *P. stutzeri,* which supports prediction 1 (see The Conceptual Model: Predictions and Treatment Contrasts).

Bacterial-feeding nematodes stimulated bacterial growth in both studies. Thus, the enhanced shoot growth in the pbn_b treatments of both experiments may have been the result of nematode excretion of N, increased mineralization of N by additional bacteria, or both, as stated in prediction 3. However, in the unamended experiment, bacterial numbers in the *pb* and $pbfn_bn_f$ treatments were not significantly different, yet shoot biomass was 22% greater than in the unamended pfn_f treatment. From this evidence, we suggest that the plant growth responses were caused by the increase in N mineralization from the nematodes *Pelodera* sp. and *Acrobeloides* sp., not by the increase in bacterial numbers. In neither experiment did addition of the fungal-feeding nematode *A. avenae* significantly influence shoot growth; this did not support prediction 3 but was in agreement with prediction 4.

Bacterial populations

Bacterial densities in rhizosphere soil of the amended experiment were nearly always higher than in nonrhizosphere soil from the same treatment (Figs. 3 and 4). Similar results were reported by Starkey (1931), Darbyshire and Greaves (1973), Clarholm (1981), and Ingham and Coleman (1983). The principal mechanism responsible is the addition of soluble or labile organic matter from growing roots, which relieves energy limitations on bacteria and fungi that occur in nonrhizosphere soil (Stotzky and Norman 1963, Lynch and Panting 1980). Nonrhizosphere bacteria populations (*pbf* and $pbfn_f$) tended to be higher than in corresponding treatments without plants (*bf* and bfn_f) (Fig. 3), indicating that the influence of plant roots extended beyond the area defined as rhizosphere in this study.

Nematodes had no significant effect on bacterial numbers in nonrhizosphere soil of the amended study, where both nematode and bacterial populations were lower than in the rhizosphere (Figs. 3, 4; Tables 2, 3). However, in the rhizosphere of the amended study and

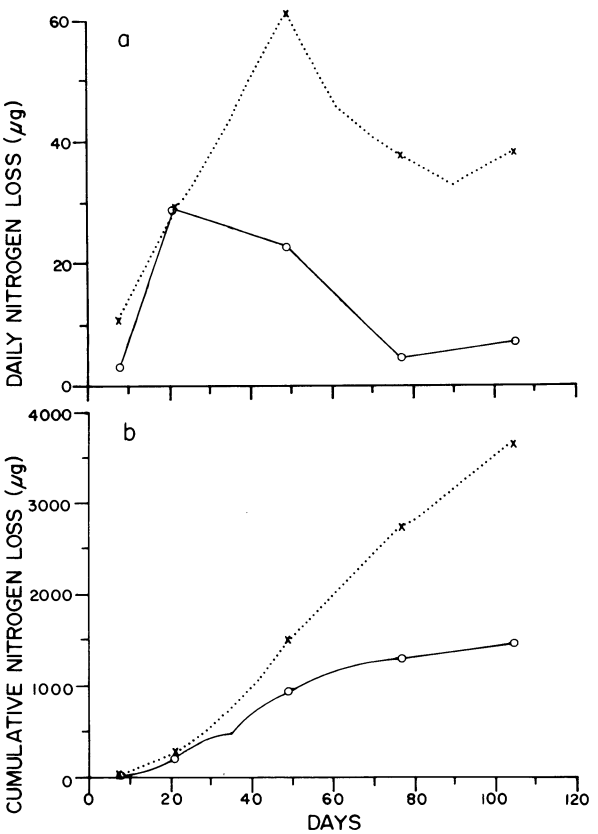

FIG. 12. Daily (a) and cumulative (b) nitrogen losses per microcosm from nematodes grown in soil microcosms with *Bouteloua gracilis*. × ····· × *Pelodera* sp.; O——O *Aphelenchus avenae.*

TABLE 6. System responses attributed to microbial grazers in soil.

Grazer	System responses	Reference(s)
Systems without plants		
Acanthamoeba polyphaga (amoeba, B)*	Decreased bacterial populations Increased CO_2 evolution Increased N mineralization	Coleman et al. (1977)
Mesodiplogaster lheritieri (nematode, B)	Decreased bacterial populations Increased CO_2 evolution Increased N mineralization Increased P mineralization	
M. lheritieri	Increased CO_2 evolution	Anderson and Coleman (1978)
Unamended soil		
A. polyphaga	Decreased bacterial populations Increased CO_2 evolution Increased N mineralization Increased P mineralization	Anderson et al. (1978, 1979*a*) Cole et al. (1978) Coleman et al. (1978) Woods et al. (1982)
M. lheritieri	Decreased bacterial populations	
A. polyphaga and *M. lheritieri*	Decreased bacterial populations Increased CO_2 evolution Increased N mineralization Increased P mineralization	
C- and N-amended soil		
A. polyphaga	Decreased bacterial populations Increased CO_2 evolution Increased N mineralization Increased P mineralization	
M. lheritieri	Decreased bacterial populations Increased CO_2 evolution	
A. polyphaga and *M. lheritieri*	Decreased bacterial populations Increased CO_2 evolution	
Pelodera punctata (nematode, B)	Increased bacterial populations Increased O_2 consumption Increased substrate utilization	Abrams and Mitchell (1980)
M. lheritieri or *Acrobeloides* sp. (nematode, B)	Increased CO_2 evolution Increased substrate utilization Increased N mineralization Decreased P immobilization	Anderson et al. (1981*a*)
Pelodera sp. (nematode, B)	Increased bacterial populations Increased N mineralization	Gould et al. (1981)
Field populations of amoebae	Decreased bacterial populations	Clarholm (1981)
Rhabditis sp. (nematode, B)	Increased CO_2 evolution Increased P mineralization	Anderson et al. (1982)
M. lheritieri	Decreased bacterial populations Increased N mineralization	Anderson et al. (1983)
Pelodera sp. Cellulose-amended soil	Increased bacterial populations Increased CO_2 evolution Increased N mineralization	Trofymow and Coleman (1982) Trofymow et al. (1983)
Pelodera sp. Chitin- and cellulose-amended soil	Increased bacterial populations Increased CO_2 evolution Increased substrate utilization Increased N mineralization	
Field populations of amoebae, ciliates, and flagellates	Decreased CO_2 evolution and decreased N mineralization when densities of amoebae, ciliates, and flagellates were reduced	Coleman et al. (1984)
Field populations of bacterial-feeding nematodes	Decreased bacteria and decreased decomposition when nematodes increased following removal of predatory mites	Santos and Whitford (1981) Santos et al. (1981) Whitford et al. (1982) Elkins and Whitford (1982)
Aphelenchus avenae (nematode, F)	Decreased or killed fungal cultures	Mankau and Mankau (1963)
A. avenae	Decreased fungal biomass	Wasilewska (1975)
Oniscus asellus (collembolan, F) or *Glomeris marginata* (millipede, F)	Low and moderate densities of the microarthropods resulted in increased CO_2 evolution, but high densities re-	Hanlon and Anderson (1979)

TABLE 6. Continued.

Grazer	System responses	Reference(s)
	duced CO_2 evolution. All densities reduced fungal standing crop	
Folsomia candida (collembolan, F)	Increased CO_2 evolution at some densities and some nutrient levels	Hanlon (1981)
A. avenae	Decreased CO_2 evolution	Trofymow and Coleman (1982)
Cellulose-amended soil	Decreased N mineralization	Trofymow et al. (1983)
A. avenae		
Chitin- and cellulose-amended soil	Increased CO_2 evolution Increased N mineralization	
Systems with plants		
A. polyphaga	Increased N mineralization Increased shoot-N concentration	Elliott et al. (1979*a*)
Acrobeloides nana (nematode, B) and *A. avenae* (present in low numbers)	Decreased bacterial populations Increased loss of N in leachate during last half of experiment attributed to increased N mineralization once nematodes had achieved high population levels Slightly increased biomass of pine seedlings	Bååth et al. (1981)
Tectocepheus velatus (mite, F)	Increased total hyphae and FDA-stained active hyphae	
Field populations of amoebae, ciliates, and flagellates	Increased plant-N uptake Increased plant-N concentration	M. Clarholm (*personal communication*)
Pelodera sp. (nematode, B)	Increased bacterial populations Increased biomass of blue grama grass Increased plant-N concentration	This study
A. avenae (nematode, F)	Increased bacterial populations	

* Taxon and B = bacterial-feeder or F = fungal-feeder included in parentheses.

non-rhizosphere soil of the unamended experiment (Table 1), where the densities of nematodes were high, *Pelodera* sp., *Acrobeloides* sp., and *A. avenae* all significantly increased numbers of bacteria, relative to treatments without nematodes. In both the pbn_b and $pbfn_f$ treatments of the amended experiment, rhizosphere bacterial numbers continued to increase to the end of the experiment. These results suggest that when the microbial populations of field soils are examined without reference to the rhizosphere/nonrhizosphere separation (as is common practice), important microbial events mediated by plant roots or nematodes may be diluted and overlooked.

The mechanism by which bacterial-feeding nematodes increase the bacterial population in some studies and decrease it in others is intriguing. These inconsistencies may be nematode species-specific. While all nematode species may be capable of stimulating bacterial growth, some may also be capable of consuming the additional production before it can be observed as a net increase in numbers. For example, Anderson et al. (1983) observed a net decrease in bacterial numbers with grazing by *Mesodiplogaster lheritieri,* but they calculated that the production of nematode biomass that occurred would have required the consumption of five times as many bacteria as the number observed during the bacterial population decline. Thus, bacterial production was stimulated and the increase was then consumed by the nematodes. In four studies that recorded increased bacterial populations in the presence of a bacterial-feeding nematode, the nematode was a *Pelodera* species (the amended experiment of this study; Abrams and Mitchell 1980, Gould et al. 1981, Trofymow and Coleman 1982; study results are summarized in Table 6). All experiments in which *Mesodiplogaster lheritieri* was added as a bacterial predator resulted in net decreases in bacterial densities (Coleman et al. 1977, Anderson et al. 1979*a*; see Table 6). *Acrobeloides* sp., however, increased bacterial populations in the unamended experiment but reduced them in two other studies (Anderson et al. 1981*a*, 1983; see Table 6). The discrepancy in results may be attributable to differences in nematode density. In the current study, low nematode densities in the nonrhizosphere soil of the amended experiment did not affect bacterial numbers, while higher densities of nematodes in either the rhizosphere soil of the amended experiment or the nonrhizosphere soil of the unamended study resulted in higher bacterial populations.

There are three possible mechanisms for stimulation of bacterial population growth by bacterivorous nematodes. First, up to 60% of the bacteria passing through the gut of a nematode may be defecated alive (Smerda et al. 1971), and bacteria that survive ingestion may obtain some otherwise limiting nutrient, hormone, or growth factor while in the gut, resulting in rapid growth

after being defecated; this phenomenon has been demonstrated for phosphorus in aquatic grazing systems (Porter 1976). Second, the nematode may be important in transporting bacterial cells, either internally or externally on the cuticle, to unexploited, substrate-rich microsites that the microbe would not be able to reach as rapidly by its own form of mobility (Wasilewska et al. 1975, Gould et al. 1981, Anderson et al. 1982). Third, excretion and defecation products of nematodes may provide substrates or inorganic nutrients for bacterial growth that are locally concentrated and readily useable, and this may have a stimulatory effect. For example, nematodes defecate significant amounts of amino acids into the soil (Anderson et al. 1983), and Marchant and Nicholas (1974) noted that bacterial reproduction was stimulated by the release of waste products by *Pelodera* sp.

We believe that the first mechanism, passage of bacteria through the gut, cannot be the only mechanism involved, since *A. avenae* and *Tylenchorhynchus claytoni* (Ingham and Coleman 1983) also increased bacterial numbers even though they are incapable of ingesting bacteria and passing them through the gut. In fact, bacterial numbers in the present study were as high or higher in the presence of *A. avenae* as with *Pelodera* sp. It is likely that both the transport and excretory product mechanisms are responsible for the stimulation of bacterial growth by nematodes.

Bacterial consumption

We calculated that 3.8 μg of bacterial biomass was consumed each day for each microgram of nematode biomass (Table 4). The corresponding rate of consumption of bacterial biomass C is 1.96 $\mu g \cdot d^{-1} \cdot \mu g^{-1}$, which is greater than the rate of 0.73 $\mu g \cdot d^{-1} \cdot \mu g^{-1}$ derived from Marchant and Nicholas (1974) and based on a population of *Pelodera* sp. weighing 13.2 mg, a consumption rate of 1.277 $J \cdot h^{-1} \cdot mg^{-1}$, an energy value of 21.1 J/mg bacteria, and the assumption that 50% of bacterial dry mass is carbon. The nematode species we used was much smaller than that of Marchant and Nicholas (1974) and thus should have a higher consumption rate per unit mass. Since the average dry mass of a *Pelodera* sp. individual in this study was 0.021 μg, the average nematode consumed 1.9×10^5 bacteria/d. This is considerably lower than the rate (72×10^5 bacteria/d) reported for *Plectus parietinus* (Duncan et al. 1974) but similar to the rate for male *Pelodera chitwoodi* of 3.9×10^5 bacteria/d averaged over an 8-d life span (Mercer and Cairns 1974). However, since *P. parietinus* and *P. chitwoodi* are much larger nematodes than the *Pelodera* sp. used in this study, their individual consumption rates would be expected to be greater. The calculated consumption in this study and others underestimates the number of bacterial cells actually ingested because the value for defecation derived by Marchant and Nicholas (1974) is an underestimate. They calculated defecation from the soluble ^{14}C recovered from washes and supernatants after centrifugation. However, a large percentage of cells ingested may be defecated whole or as parts of cells (Smerda et al. 1971). These would have been removed by centrifugation and thus unaccounted for in the measurements of defecation.

Fungal growth

The rapid decline of total hyphal lengths after inoculation (Fig. 5) indicates either that the initial inoculation density was greater than the available carbon substrates could support or that inoculation of hyphae into the soil caused the destruction of some hyphae. Fungi and/or bacteria are capable of rapidly decomposing dead hyphae (Lockwood and Filonow 1981). The increase in total hyphae in the rhizosphere region after plants were added suggests that the fungus was able to utilize carbon substrates produced by plant roots.

The abundance of active hyphae (FDA stained) slowly declined after day 49 in both the nonrhizosphere and nonplant soils (Fig. 6). This was probably due to the utilization of easily metabolized substrates until only chitin and other native nonlabile compounds remained available, as also suggested by a decrease in the rate of N mineralization (Fig. 8b). There was no effect of nematodes on the abundance of active hyphae in either soil zone. As with total counts, active hyphae were more abundant in the nonplant soil than in nonrhizosphere soil. The abundance of active hyphae in the rhizosphere did not decrease with time, and significantly increased in the $pbfn_f$ treatment on the last sample date, suggesting that fungi in the rhizosphere may have been less energy-limited than in nonrhizosphere soil. Active hyphae in the treatment with *A. avenae* were significantly less abundant than in the treatment without nematodes on day 49. However, the significant increase of active hyphae in the $pbfn_f$ treatment on day 105 suggests that nematode grazing of fungi may stimulate fungal growth, as with nematode grazing of bacteria.

Fungal consumption

We calculated that 4.18 μg of fungal cytoplasm biomass, or 2.32 μg of fungal biomass C, was consumed each day for each microgram of *A. avenae* biomass (Table 5). Since the average dry mass of an individual *A. avenae* was 0.029 μg, the average nematode consumed 0.067 μg C, or 0.12 μg cytoplasm, each day. This is within the range of cytoplasm consumption of 0.01–0.17 μg/d for 1-d-old and 5-d-old *A. avenae,* respectively, as calculated from de Soza (1973). These ingestion estimates are more precise than those for bacterial-feeding nematodes, since only soluble compounds are defecated, with no need to account for whole cells or cell fragments that are not assimilated. As *A. avenae* consumption exceeded the amount of FDA-stained cytoplasm, these nematodes also must eat cy-

toplasm that is not sufficiently metabolically active to stain with FDA.

Nematode populations

In the unamended experiment, *Acrobeloides* sp. and *A. avenae* were grown both in single (pbn_b and pfn_f) and combined ($pbfn_bn_f$) cultures. Although both nematodes reached high population levels when in single culture, *Acrobeloides* sp. populations were 65% lower in the presence of *A. avenae*. Since bacterial populations were not different in the two treatments with *Acrobeloides* sp. (pbn_b and $pbfn_bn_f$) and since the two nematodes do not compete for the same food source, reduction in *Acrobeloides* sp. numbers may have been caused by interference competition with *A. avenae* for some environmental resource, such as habitable pore space.

In the amended study, both *Pelodera* sp. and *Aphelenchus avenae* strongly preferred the rhizosphere (Tables 2 and 3). Although rhizosphere soil was never more than 5% by mass of the total soil in the microcosm, at times 30–40% of the total population of either species was recovered from the rhizosphere. Root-feeding nematodes are commonly known to predominate next to roots (Ingham and Coleman 1983), but this is the first quantitative report that bacterial-feeding and fungal-feeding nematodes also prefer this habitat. Andrew and Nicholas (1976) observed that living *Pseudomonas* spp. attracted bacterial-feeding nematodes, but dead bacteria did not. Similarly, fungi also produce substances that attract nematodes (Balan et al. 1976, Jansson and Nordbring-Hertz 1979). Therefore, the majority of the population is likely to spend most of the time in the rhizosphere, where microbial densities are highest. Bacterial-feeding protozoa have also been observed in greater numbers in rhizosphere soil (Darbyshire and Greaves 1973, Clarholm 1981).

Soil inorganic N and P

In the short-term unamended study, there was net N immobilization in the *p* and *pb* treatments, as the plants took up the limited available N while little or no N was mineralized (Table 1). In the treatments with fungi and/or bacterial-feeding nematodes, however, there was more N mineralized than the plants could take up, even though there was greater and more rapid plant growth in these more biologically complex treatments.

In the amended study, the bfn_f and $pbfn_f$ treatments consistently immobilized more P_i ($P < .01$) than the *bf* and *pbf* treatments (Fig. 7a, b). The increased bacterial populations in the treatment with nematodes most likely immobilized much of the P, with a small portion also being immobilized into nematode biomass. Similarly, the pbn_b treatment immobilized significantly more P_i than the *pb* treatment. While most of the additional immobilization was due to the more rapidly growing plants in the pbn_b treatment, some of the P_i was also taken up by the larger bacterial population in the rhizosphere of the pbn_b treatment. More P_i was immobilized in the pbn_b, *pbf*, and $pbfn_f$ treatments than in the plant or plant-plus-bacteria treatments because of the larger plant biomass attained in treatments with bacterial-feeding nematodes or fungi.

Before the addition of plants (days 0–7), all inoculated treatments mineralized N (Fig. 8a, b), with the fungal treatments mineralizing much more than the treatments with bacteria since *Fusarium oxysporum* is a better chitin decomposer than *Pseudomonas stutzeri* (Gould et al. 1981). The greatest net mineralization of N was in the *bf* and bfn_f treatments. The mineralization rate in these two treatments was initially rapid and then slower after day 21. Therefore, the initial mineralization of N could have been from simple native organic N compounds (proteins, amino acids, etc.), followed by a slower mineralization of N from the added chitin. Between day 7 and day 21 there was net mineralization of N in the *pbf* treatment but net immobilization in the $pbfn_f$ treatment (Fig. 8b). On day 21 there was no difference between these two treatments in shoot, root, or fungal biomass (Figs. 2a, b, 5), so the net immobilization of N in the $pbfn_f$ treatment must have been due to increased uptake of NH_4^+-N by larger bacterial populations in this treatment and incorporation into nematode biomass. After day 21, immobilization of N was rapid in both *pbf* and $pbfn_f$ treatments as plants grew rapidly. Most of the increase in soil NH_4^+-N in the *pb* treatment on day 21 probably resulted from the marked decline in bacterial populations, but the excess was rapidly immobilized again as bacterial populations increased after day 21. The greater immobilization in the pbn_b treatment was due to a larger bacterial population and more rapidly growing plants, which were already significantly larger than those in the *pb* treatment on day 21. The faster initial growth rate of the plants in the treatment with *Pelodera* sp. probably resulted from the greater availability of N as the large population of nematodes grazing on the bacteria excreted NH_4^+-N, which allowed for rapid uptake by the plants (Anderson et al. 1983). In all plant treatments, nearly all available inorganic N was immobilized by the end of the experiment (Figs. 8, 9), suggesting that the plants remained N-limited throughout the experiment and that the mineralization rate of chitin did not exceed the plant's ability to take up N.

Nitrogen release by nematodes

While appreciable N was released by nematodes in the pbn_b treatment, the amount was insufficient to explain all the early increase in plant N as compared with plants from the *pb* treatment. For example, on day 21, 647 μg of N were found in plants from the pbn_b treatment, while *pb* plants contained 175 μg N, a difference of 472 μg. By this time only 269.5 μg N had been released by nematodes. Thus, another mechanism must

be proposed for the additional N uptake by the plant. Several factors should be considered. First, the bacterial populations on day 21 were 25 times greater in the pbn_b treatment than in the *pb* treatment (Fig. 4), so chitin decomposition by bacteria was probably much greater in the treatment with nematodes. Second, since both bacteria and nematodes were concentrated in the rhizosphere, much of the N mineralized by either bacteria or nematodes was probably in close proximity to the roots, available for immediate uptake. This more advantageous N regime was probably responsible for the more rapid root growth early in the pbn_b treatment. The greater root biomass was then able to exploit a larger soil volume and reach soil inorganic N not available to roots in the *pb* treatment. This was primarily responsible for the differences between these treatments in NH_4^+-N and NO_3^--N levels, which were noted in the first half of the experiment (Figs. 8a, 9a). Therefore, it seems likely that *Pelodera* sp. had an important positive feedback effect early in the experiment.

Root biomass between the two treatments was no longer different by day 49, yet plants in the pbn_b treatment contained 813 μg more N than those in the *pb* treatment. By this time nematodes had excreted 1491 μg N per microcosm, which may have accounted for much of the additional plant N, although some of the nematode-excreted N was undoubtedly taken up by bacteria.

During the entire experiment, *A. avenae* in the $pbfn_f$ treatment lost 1468 μg of N per microcosm, appreciably less than the 3671 μg excreted by *Pelodera* sp. The fungus alone mineralized 3750 μg of N per microcosm in the nonplant (*bf*) treatment; thus, the amount of N lost by *A. avenae* did not significantly improve the N regime of the plant. Hence, the fungus treatments with and without *A. avenae* did not differ in either shoot biomass or total shoot N.

Evaluation of the Conceptual Model

The conceptual model introduced in Fig. 1 was generally supported by the results of this study and was useful in interpreting the results observed. The model proposes that microbial grazers influence the strength of feedback controls on substrate utilization by fungi (C_{43}) and bacteria (C_{53}). Our results support this, as do those of Anderson et al. (1981*a*), Abrams and Mitchell (1980), and Trofymow et al. (1983) (Table 6). Fungal feeders have been shown to increase (Hanlon 1981) or decrease (Trofymow and Coleman 1982) system respiration. System responses are likely to be dependent on grazing intensity as affected by initial population sizes (Coleman et al. 1984).

The ecological importance of grazer influence on the rate of substrate utilization C_{53} is not only that disappearance of litter would occur at a faster rate with nematodes but also that potentially more nutrients would be mineralized. The availability of these nutrients for plant uptake depends on the amount of organic matter C available for microfloral growth and hence on nutrient immobilization. By comparing the *pb* and pbn_b treatments, relative estimates of mineralization can be made, however. The higher concentration and total amount of N in the shoot tissue in the pbn_b treatment suggest that those plants were drawing from a larger pool of available N than those in the *pb* treatment. By the end of the experiment (105 d), however, there was no difference in plant or soil N between the *pb* and pbn_b treatments, indicating that the presence of nematodes may be of only short-term advantage to the plant. This concept was also proposed by Anderson et al. (1981*b*), who hypothesized that microbial grazers accelerate the rates of substrate utilization and nutrient mineralization but not necessarily the final amounts of substrate decomposed or nutrients released. However, in many ecosystems, particularly those in arid and semiarid climates, ideal conditions for plant growth occur only for short periods. Thus, a short-term advantage may be quite significant. Some of the additional N mineralized in the pbn_b treatment was immobilized into microbial biomass as bacterial populations in the rhizosphere of the pbn_b treatment increased. Thus, in the latter part of the experiment the bacteria may have been outcompeting the plant for the NH_4^+-N mineralized by the nematode. A similar increase in nitrogen concentration in plant shoot tissue in the presence of a protozoan (amoebal) bacterial grazer was also reported by Elliott et al. (1979*a*) and M. Clarholm (*personal communication*) (Table 6).

Our prediction that plants grow faster in the presence of microfloral grazers than in their absence was supported for bacterial-feeding nematodes but not for fungal-feeding nematodes. There was no evidence in this experiment (Fig. 8b) that the system with fungal-feeding nematodes mineralized more N than the one without grazers (*bf* and bfn_f treatments). Even if more N had been mineralized with *A. avenae,* however, there still may have been no plant growth response, because the amount of N mineralized by the fungus alone may have been optimal. An increase in plant growth with the addition of microfloral grazers probably occurs only in a nutrient-limited environment. Elliott et al. (1979*a*) found that addition of bacterial-grazing amoebae resulted in a smaller increase in plant-N uptake when initial NH_4^+-N levels were high (15% increase) than when they were at a medium level (132% increase). Because of greater chitin-N mineralization by *F. oxysporum* than by *P. stutzeri* (Gould et al. 1981), plants in the *pbf* treatment had more N available than those in the *pb* treatment. Thus, plant growth and amount of shoot N in the *pbf* treatment were greater than in the *pb* treatment and equal to values in the treatment with bacterial grazers.

While Bååth et al. (1981) observed that Scots pine seedlings were 17% larger in treatments with microbial-

feeding nematodes than in a like treatment without nematodes, this mass difference was not significant and there was no difference in seedling N concentration between the two treatments. However, the pine seedlings had not been inoculated with mycorrhizal fungi and thus were probably more limited by phosphorus than by nitrogen, since seedlings did not respond to nitrogen additions in a similar treatment. Had the seedlings been responsive to additional available N, there may well have been a growth response in the treatment with nematodes. Higher concentrations of N in the leachate from the nematode treatment during the last half of the experiment indicated that the microbial-feeding nematodes caused a higher rate of N mineralization than in the comparable treatment without nematodes. These results show, however, that plants may not respond to the activities of microbivorous fauna under all conditions. We suggest that the additional mineralization from microfloral grazers may be of significant importance to plant growth only when mineralization of a growth-limiting nutrient by microflora alone is insufficient to meet the plant's demand for that element under current growing conditions. The importance of this contribution by microbial grazers may vary from ecosystem to ecosystem, from microsite to microsite, and even from time to time within the same system. However, the effects of microfloral grazers on microflora growth are probably important in all systems at all times that these biota are active.

Influence of microbivorous fauna in ecosystems

An objective of this study was to illustrate that microbivorous soil nematodes have a potentially important role in ecosystems. However, these nematodes are only one of several regulators of ecosystem nutrient cycling and primary production (Anderson et al. 1981*b*, Coleman et al. 1983, Seastedt 1984). Although the biological processes observed in microcosms represent phenomena that may also occur in the field, in a native soil these processes may be mediated by other physical and biological interactions. For instance, while it is suggested here that nematodes regulate primary production, Santos et al. (1981) and Whitford et al. (1982) have noted that predatory mites may regulate nematodes in arid and semiarid ecosystems.

Understanding ecosystem functioning must be an integrative process that considers all biological processes as they interact with one another. Gnotobiotic microcosms are a tool that can be used to examine these interactions with greater resolution than can currently be achieved in the field. We suggest that in the future, use of a "functional group" approach (Coleman 1976) to build complex biotic assemblages for the combined study of soil microflora, microfloral grazers, plant nutrient uptake, and nutrient cycling will yield considerable new information about ecosystem functioning.

Acknowledgments

Cindy Cambardella, Janet Frey, and Rachel Hays provided valuable technical assistance. We also thank E. T. Elliott for critical editorial comments during the preparation of the manuscript. This research was supported by National Science Foundation (NSF) grants DEB 78-11201 and DEB 80-04193 to Colorado State University; manuscript preparation was also supported by NSF grant DEB 80-22380 to Colorado State University.

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