

Responses of grassland soil nematodes and protozoa to multi-year and single-year applications of dairy manure slurry and fertilizer

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Abstract

In order to improve understanding of how long-term use of manure affects nitrogen cycling processes, the effects of multiple years of manure applications on abundance of protozoa and nematode community structure were assessed. Plots of a grass sward in the Fraser Valley of British Columbia were either left untreated or were treated with dairy manure slurry or fertilizer, each at 50 or 100 kg NH₄-N ha⁻¹, two to four times per year for six consecutive years. Nematode community structure and protozoan abundance were determined at 19 sample dates during the fourth (1997), fifth (1998) and sixth (1999) years of application. Protozoa, bacterivorous nematodes and fungivorous nematodes were consistently more abundant in soil treated with manure at both rates than in fertilized and untreated soil, indicating that microbial turnover and flux of nutrients through the soil food web was enhanced in manured soil relative to fertilized or untreated soil. The Maturity Index (MI) and the MI2-5 were both reduced by fertilization and manure, relative to the control. The MI for the manure treatment was lower than for the fertilizer treatment as a result of greater relative abundance of enrichment opportunist nematodes in manure-treated soil. Accordingly, the MI2-5 did not differ between the manure and fertilizer treatments, suggesting that with the exception of enrichment opportunists fertilizer and manure have similar effects on structural complexity of the soil food web.

Populations of micro-fauna were also assessed through 1998 and 1999 in subplots that had been treated with manure or fertilizer for four years and stopped receiving manure or fertilizer in 1998, and in subplots given manure in 1998 that had previously either been fertilized or left untreated. Protozoa and bacterivorous and fungivorous nematodes remained more abundant through 1998 and 1999 in previously manure-treated plots than in previously fertilized plots, indicating that the cumulative effects of manure application on enhancement of microbial production can be detected through at least two growing seasons after applications cease. Application of manure for one year to previously non-treated or fertilized soil raised the abundance of protozoa and bacterivorous and fungivorous nematodes to levels comparable to continuously manured soil.

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1. Introduction

Utilizing livestock manures as sources of nutrients is an important component of sustainable crop production systems. In addition to major plant nutrients such as nitrogen (N) and phosphorus (P), manures contain an array of organic compounds such as carbohydrates, fatty acids and peptides that are substrates for growth of heterotrophic soil micro-organisms (Paul and Beauchamp, 1989), and application of

manure generally increases soil microbial biomass whereas chemical fertilizer does not (Bittman et al., 2005). N immobilized in the microbial biomass is subsequently mineralized as the microbial biomass turns over (Paul and Clark, 1996). Better knowledge of factors affecting turnover of the microbial biomass would improve current understanding of nutrient fluxes in manure-treated soil.

The soil micro-fauna, i.e. protozoa and nematodes, are important consumers of the microbial biomass (Hunt et al., 1987; Forge et al., 2003) and are therefore indicative of microbial turnover and flux of nutrients through the soil food web. The abundance of microbivorous nematodes and some indices of nematode community structure have been correlated with net N mineralization or potentially

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mineralizable N across sites or fertility treatments in field studies (Hassink et al., 1993; Forge and Simard, 2000, 2001; Ferris and Matute, 2003). Opperman et al. (1993) correlated bacterivorous nematode population growth with soil nitrate levels after application of cattle manure slurry to soil. Griffiths et al. (1998) related differences between cattle and pig manure slurries, in terms of dissolved organic carbon content and potential for denitrification, to differential population growth of bacterivorous nematodes and protozoa.

A few common genera of bacterivorous nematodes have 'enrichment opportunist' ecological characteristics. The enrichment opportunists are capable of rapid population growth under conditions of high bacterial availability coupled with favourable physico-chemical conditions (Ferris et al., 1996a; Venette and Ferris, 1997), and increase in dominance under agricultural management practices that stimulate microbial production/turnover and mineralization (Ettema and Bongers, 1993; Ferris et al., 1996b). Increased relative abundance of enrichment opportunists may complement total numbers of bacterivorous nematodes as indicators of enhanced microbial turnover and flux of nutrients through the soil food web (Wasilewska, 1998; Ferris et al., 2001).

Nematode community structure can also be used as an indicator of structural complexity of the soil foodweb. Nematodes occupy several key functional groups within the soil food web and consequently nematode community structure is responsive to changes in the composition of basal food resources (bacteria and fungi) as well as soil physico-chemical conditions (Ferris et al., 2001; Neher, 2001). The nematode community Maturity Index (MI) is based on a ranking of nematode taxa from 1 to 5 according to their position on the 'colonizer–persister' (c–p) continuum of life-history strategies (Bongers, 1999). The MI has been used to describe effects of various soil fertility management practices on the structure of soil nematode communities and soil food webs (e.g. Porazinska et al., 1999; Sarathchandra et al., 2001). Nematode communities with high relative abundance of taxa with high c–p rankings (i.e. 4 and 5) have high MI values and are associated with stable soil ecosystems; relative to low-MI communities, such communities are generally represented by a greater diversity of functional guilds and are indicative of soil food webs with greater levels of connectance (Bongers, 1999; Ferris et al., 2001; Neher, 2001).

When comparing agricultural practices with differing levels of organic inputs, the strong response of enrichment opportunists can mask other underlying changes in structural complexity of the nematode community (Bongers, 1999; Ferris et al., 2001; Neher, 2001). Alternative indices, the MI2-5 and Structure Index (SI), have been proposed (Bongers, 1999; Ferris et al., 2001). Both indices involve removing the enrichment opportunists from calculations and complement the MI as measures of structural

complexity of the nematode community, and the food web it represents, when changes in organic inputs are involved (Bongers, 1999; Ferris et al., 2001).

Previous field-based studies of micro-faunal responses to manure have either assessed single-season responses to a single application of manure (Ettema and Bongers, 1993; Griffiths et al., 1994), or compared ongoing treatments at a single point in time (Dmowska and Kozłowska, 1988). The first objective of our study was to assess the effects of multiple years of manure applications on the abundance of protozoa and nematode community structure. Knowledge of the persistence of changes in micro-faunal communities, after long-term applications of manure have ceased, could provide insight into the cumulative effects of manure on soil fertility. Accordingly, our second objective was to use selected micro-faunal indicators of enrichment (abundance of protozoa, abundance of bacterivorous nematodes, relative abundance of enrichment opportunist nematodes) to determine how long microbial turnover remains elevated after manure applications cease. No data exist to indicate whether a single year of manure application can change the structure of the soil food web to a condition comparable to that observed in soil amended with manure over several years, or whether responses to application of manure depend on prior soil fertility management. Therefore, our third objective was to determine whether manure-induced increases in micro-faunal indicators of enrichment depend on soil management history, i.e., whether or not the soil was previously fertilized.

2. Materials and methods

2.1. Field plots

The field plots sampled in this study were part of a multi-faceted study of the long-term effects of applying dairy manure slurry to grassland at variable rates and frequencies over multiple years (Bittman et al., 2005; Raworth et al., 2004). The primary grass species is tall fescue (*Festuca arundinacea*) and the soil is a Monroe series, medium-textured, eluviated eutric brunisol (Luttmerding and Sprout, 1967). Beginning in 1994, 3 m × 100 m strip plots were treated with manure or fertilizer 2–4 times per year. In all, there were ten treatment combinations of application rate and frequency, randomized in four blocks. Only five of the ten long-term treatment combinations were considered in this study: (1) manure at 100 kg NH₄-N ha⁻¹; (2) manure at 50 kg NH₄-N ha⁻¹; (3) ammonium nitrate at 100 kg N ha⁻¹, (4) ammonium nitrate at 50 kg N ha⁻¹; and (5) untreated controls. The fertilizer plots received P and K according to soil tests performed each spring. Approximately 50% of total manure N is NH₄-N, so the low N manure treatment received approximately the same total N as the high N fertilizer treatment. In 1998, the fifth year of application, two subplots were marked off from the end of

each main plot to facilitate the formation of two additional experiments. One of the two subplots at the end of each of the main plots was left untreated, in order to determine the persistence of manure- and fertilizer-induced changes in micro-faunal community structure ('residual fertility'). The second subplot was treated with four doses of manure at $100 \text{ kg NH}_4\text{-N ha}^{-1}$ during 1998 in order to determine the effects of prior fertility management (i.e. fertilized-vs-untreated) on responses of micro-fauna to application of manure ('received manure in 1998'). The subplots treated with manure in 1998 were not treated with manure in 1999. The main plots continued to receive the treatments allocated to them through 1998 and 1999 ('Continuing treatments').

Samples were taken one and three weeks after one of the manure applications in 1997, two manure applications in 1998, and all four manure applications in 1999. The other five sample dates (two in 1997 and two in 1998) were not linked with particular manure application dates. At each sample date, 25 2.5-cm diam \times 30-cm deep cores were taken from each plot and combined to form a single composite sample representing each plot. The samples were refrigerated and processed within 7 days of sampling. Each soil sample was sieved (5 mm) to remove stones and root fragments, and assays initiated immediately after sieving.

2.2. Protozoa

A most probable number (MPN) procedure modified from Darbyshire et al. (1974) was used for enumerating protozoa. The assays were performed in 24-well cell culture plates and the growth medium in each well was 0.8 ml autoclaved and filtered soil extract (1:2, soil:water) amended with 0.1% glucose. The first well of each dilution series was inoculated with a 0.2 ml aliquot of 1:5 soil suspension shaken at 120 rpm for 5 min. Five replicate five-fold dilutions to 10^{-6} were prepared for each soil sample. The plates were incubated at 15–20 °C. After 14 days a 0.1 ml aliquot of suspension from each well was observed with a compound microscope at 600 \times and scored for the presence/absence of naked amoebae, flagellates and ciliates. Tables from Wooster (1994) were used for estimating the most probable number of each protozoan group from tabular data on the number of positive replicate wells at each dilution. Data were expressed as the total number of protozoa per gram of dry soil.

2.3. Nematodes

Baermann pans (16 cm diameter) were used to extract nematodes from 50 g subsamples of soil (Ingham, 1994). The nematodes were heat-killed and preserved in 4% formalin. Before initiating routine analyses, approximately 500 nematodes extracted from preliminary samples were observed at 600 \times and 1500 \times to identify the taxa distinguished during routine analyses at 200 \times . It was possible to classify most specimens to the genus level of

resolution during the routine analyses. For a few taxonomic groups (*Ditylenchus/Nothotylenchus*, *Cephalobus/Eucephalobus*, *Plectus/Anaplectus*, *Panagrellus/Panagrolaimellus*), two or more closely related genera were known to be present from the initial (600 \times and 1500 \times) observations but they were grouped together during routine analyses at 200 \times . For routine analyses, one-third of each nematode suspension was observed on a Sedgewick-Rafter slide at 200 \times magnification. The first 100 nematodes observed in each sample were identified, and the total number of nematodes in the aliquot was estimated after counting all nematodes in a defined portion of the aliquot. Nematode counts for each taxon were adjusted to the number of nematodes per 100 g dry soil.

The taxa were assigned c–p values from 1 to 5, corresponding to their positions along the colonizer–persister continuum of nematode life-history strategies (Bongers, 1999). The MI and MI2-5 were calculated from c–p values and abundances of the taxa in each sample as described by Bongers (1999) with the following modification. We included the fine-stylet Tylenchidae in calculations of the MI and MI2-5, whereas the scheme of Bongers (1999) considers the Tylenchidae as root-hair grazers and omits them from calculation of the MI and MI2-5. We acknowledge that there is uncertainty regarding their true feeding habits (Yeates et al., 1993) but field observations (Todd, 1996) and lab studies (Okada et al., 2002; Magnusson, 1983) strongly suggest that at least some of these nematodes are fungivorous. We assigned the Tylenchidae a c–p value of 3 based on our personal observations that, relative to the Aphelenchida (c–p 2), the Tylenchidae are less often abundant in disturbed soil. The percentage of bacterivorous nematodes with enrichment opportunist characteristics (%EO) was calculated as: (c–p 1 nematodes/total bacterivorous nematodes) \times 100.

The plant parasitic nematode community was dominated by root-lesion nematodes (*Pratylenchus* spp.) at relatively high population densities (> 100 nematodes per 100 g soil). Population dynamics of the *Pratylenchus* spp. under the various manure and fertilizer treatments will be the focus of a separate paper.

2.4. Estimates of microbial consumption

The food web model of Hunt et al. (1987), as shortened by Hassink et al. (1994), was used to estimate rates of microbial consumption necessary to maintain the observed populations of nematodes and protozoa. Nematode and protozoan abundance data were averaged over the multiple sample dates within each year and then converted to biomass-C. Andrassy's formula (Andrassy, 1956) and published values for the dimensions of adult specimens (Bongers, 1994) were used to make adult-based estimates of biomass for each nematode taxon. Published data on stage-specific biomass of some common genera of bacterivorous nematodes (Ferris et al., 1997) were used to adjust

the adult-based biomass estimates to mean biomass, assuming an equal distribution of life-stages in each population. Biomass was converted to biomass-C using estimated dry matter contents of 20% and C contents of 52% of dry mass. Protozoan bio-volume was estimated using a mean cell diameter of 9.5 μm (measured) and assuming a spherical shape. Protozoan bio-volume was converted to biomass-C using a conversion factor of 0.212 $\text{pg C } \mu\text{m}^{-3}$ (Griffiths and Ritz, 1988).

The model assumes that, for steady-state populations, the microbial biomass-C consumed is balanced by biomass-C lost to respiration, feces, natural death and predation. Therefore, annual consumption for any given group of micro-fauna can be estimated as:

Consumption ($\text{mg C kg soil}^{-1} \text{ yr}^{-1}$)

$$= [(B * D) + P] / (E_a * E_p)$$

where B = mean standing crop biomass-C (mg C kg soil^{-1}), D = natural death rate or generations/yr (yr^{-1}), P = losses to predation ($\text{mg C kg soil}^{-1} \text{ yr}^{-1}$), E_a = assimilation efficiency (1-proportion of ingested material defecated), and E_p = production efficiency (proportion of assimilated material converted to biomass) (Hunt et al., 1987; Hassink et al., 1994). With the exception of D , parameter values used in our calculations were taken directly from Hunt et al. (1987). Values of D used in our calculations were 2.5 times values used in the model of Hunt et al. (1987), which simulated conditions for a Colorado arid short-grass prairie and assumed that 40 days per year were conducive for soil biological activity. We assumed 100 days per year, as our soil accumulates approximately 1000 degree-days above 10 °C at 15 cm depth during a typical growing season (Vrain et al., 1997), corresponding to approximately 100 days at 20 °C. Soil moisture was not assumed to be limiting nematode activity at our site during the 100 days.

Our calculations started with predacious nematodes, which were treated as top predators and were assigned no losses to predation. Annual consumption by predacious nematodes was allocated to bacterivorous, fungivorous and omnivorous nematodes according to their relative biomasses. These values were then used as estimates of P in calculations of consumption for each respective group. The initial calculations, in $\text{mg C kg soil}^{-1} \text{ yr}^{-1}$, were converted to microbial biomass-N consumed $\text{kg soil}^{-1} \text{ yr}^{-1}$ using mean microbial C/N ratios of 6.8, 5.3 and 5.3 and for the control and high fertilizer and manure treatments, respectively, derived from Bittman et al. (2005). Unlike previous studies (Hunt et al., 1987; Hassink et al., 1994), we did not extend our calculations to estimate mineral N actually excreted by the various groups of micro-fauna, as these calculations require an additional layer of parameters and assumptions (e.g. biomass and resource C/N ratios) which were unnecessary for our purposes of comparing treatments with respect to fluxes of N through the microfauna.

2.5. Data analyses

All data except the estimates of microbial consumption and turnover were analyzed using a blocked split-plot-in-time analysis of variance (ANOVA) model. Treatments were considered as main-plots and the multiple sample dates within each year were considered as sub-plots. The treatment \times block mean squared error was used as the error term for testing the effect of treatment, and residual mean squared error was used as the error term for testing sample date and treatment \times date interaction effects. Separate analyses were performed on the data for each year because different sets of treatments were evaluated in each of the three years. Separate analyses were also conducted for the two sub-experiments initiated in 1998, which consisted of: (1) treatments to evaluate effects of withholding manure and fertilizer ('residual fertility'), and (2) treatments to evaluate effects of prior treatments (from 1994 to 1997) on responses to addition of manure in 1998 ('received manure in 1998'). Tukey's protected honest significant differences (HSD) calculated with $P=0.05$ was used for assessing the significance of differences between main-factor means (Snedecor and Cochran, 1980). For the main experiment, Fisher's least significant difference (LSD) was used for descriptive assessment of the number of individual sample dates with detectable differences between the high manure treatment and control. Protozoan data were log-transformed prior to final analyses to reduce heteroscedasticity. The log-transformed data are presented in tables, and back-transformed means were used in graphs.

3. Results

3.1. Effects of manure and fertilizer on community structure—continuing treatments

For protozoan abundance, the treatment \times sample date interaction was significant in 1997, and main-factor effects of treatment and sample date were significant for protozoan abundance in 1998 and 1999 (Table 1). When averaged over sample dates, mean (back-transformed) protozoan abundance in the high manure treatment was 2.7, 4.9 and 2.6 times greater than in the high fertilizer treatment (similar mineral-N inputs) in 1997, 1998 and 1999, respectively (Table 1). Average (back-transformed) protozoan abundance in the high manure treatment was likewise 1.8, 3.2 and 2.8 times greater than in the control in 1997, 1998 and 1999, respectively (Table 1). Protozoan abundance in the high fertilizer treatment did not differ from the control in any year (Table 1). Protozoan abundance in the low manure treatment was 2.3 and 1.4 times greater than in the high fertilizer treatment (similar total N inputs) in 1998 and 1999, but the difference was significant in 1998 only (Table 1). Protozoan abundance in the low manure treatment was intermediate to the control and high manure

Table 1
Main-factor means for effects of manure and fertilizer treatments on population densities of protozoa (log-*protozoa g*⁻¹ dry soil) in 1997, 1998 and 1999

	1997	1998	1999
Control	4.93 b ^a	5.05 bc	4.90 b
High mineral-N (100 kg ha ⁻¹)			
Manure	5.19 a	5.55 a	5.35 a
Fertilizer	4.76 b	4.86 c	4.93 b
Low mineral-N (50 kg ha ⁻¹)			
Manure	n.a.	5.23 b	5.09 ab
Fertilizer	n.a.	n.a.	4.93 b
ANOVA summary, F-ratios (degrees of freedom) ^b			
Treatment ^c	12.5(2)**	58.2(3)**	18.1(4)**
Date	5.3(4)**	14.6(6)**	7.63(8)**
Treatment × date	2.4(8)*	1.7(18)	0.87(32)
LSD (treatment)	0.42	0.31	0.38

Data were analyzed using split-plot ANOVA models with treatments as whole-plot and dates as sub-plot factors. Separate analyses were conducted for each year. n.a., not applicable; treatment was not sampled in that year.

^a Values within a column labeled with the same letter are not significantly different according to Tukey's HSD, $P \leq 0.05$, calculated for main-factor effects of fertility treatments ($n=20$, 28, and 36 in 1997, 1998 and 1999, respectively).

^b F-ratios and degrees of freedom (in parentheses) for principal factors in the ANOVA; values labeled with '*' and '**' are significant at $P < 0.05$ and $P < 0.01$, respectively.

^c F-test for effect of treatment = mean squared (treatment)/mean squared (treatment × block); denominator for other F-tests is mean squared error for overall ANOVA.

treatments, but was only significantly less than in the high manure treatment in 1998.

The bacterivorous, fungivorous/root-hair feeder, omnivorous + predacious, and plant-parasitic nematode trophic groups were represented by 21, 11, 14 and 3 morpho-genera,

respectively. The bacterivorous genera represented c–p rankings of 1–4; the fungivorous genera represented c–p rankings of 2–4, and the omnivorous + predacious genera represented c–p rankings of 3–5.

The treatment × date interaction and main-factor effects of treatment and sample date were significant for bacterivorous nematodes in all three years (Table 2). The interaction term apparently resulted from a greater magnitude of date-to-date changes in abundance in the manure treatment than in other treatments, rather than reversal of treatment effects through time (Fig. 1). When averaged over all sample dates, bacterivorous nematodes were approximately 4 times more abundant in the high manure treatment than in the high fertilizer treatment (similar total-N inputs), and 4.4–4.9 times more abundant than in the control (Table 2). There were no significant differences between the high fertilizer treatment and the control in any year (Table 2). The abundance of bacterivorous nematodes in the low manure treatment was approximately 3.8 and 2 times greater than in the high fertilizer treatment (similar total N inputs) in 1998 and 1999, respectively, but the difference was significant in 1998 only. Likewise, bacterivorous nematode abundance in the low manure treatment was approximately 4.1 and 2.5 times greater than in the control in 1998 and 1999, respectively, but the difference was significant in 1998 only (Table 2).

Fungivorous/root-feeding nematodes were overall much less abundant than bacterivorous nematodes, particularly in the manure treatments. For example, when averaged over all sample dates, bacterivorous nematodes were 3.4 times more abundant than fungivorous nematodes in the control and 7.9 times more abundant in the high manure treatment

Table 2
Main-factor means for effects of manure and fertilizer treatments on population densities (nematodes per 100 g soil) of bacterivorous, fungivorous/root-feeding, omnivorous and predacious nematode groups in 1997, 1998 and 1999

	Bacterivorous			Fungivorous/root-feeders			Omnivorous			Predacious		
	1997	1998	1999	1997	1998	1999	1997	1998	1999	1997	1998	1999
Control	287 b ^a	246 b	417 b	72 b	80 bc	128 ab	119 a	45	133 a	30	47	49
High mineral-N (100 kg ha ⁻¹)												
Manure	1366 a	1092 a	2023 a	262 a	124 a	179 ab	73 b	37	55 b	33	34	71
Fertilizer	326 b	268 b	512 b	77 b	59 c	64 b	74 b	24	58 b	29	29	32
Low mineral-N (50 kg ha ⁻¹)												
Manure	n.a.	1017 a	1031 b	n.a.	106 ab	195 a	n.a.	44	71 ab	n.a.	33	60
Fertilizer	n.a.	n.a.	371 b	n.a.	n.a.	90 ab	n.a.	n.a.	101 ab	n.a.	n.a.	60
ANOVA summary, F-ratios (degrees of freedom) ^b												
Treatment ^c	167.0(2)**	65.9(3)**	30.3(4)**	15.9(2)**	12.3(3)**	8.9(4)**	12.6(2)**	1.6(3)	5.3(4)*	0.1(2)	12.0(3)	4.5(4)*
Date	8.9(5)**	22.3(4)**	4.2(7)**	3.2(5)*	102.0(4)**	1.9(7)	18.6(5)**	6.5(4)**	3.6(7)**	2.7(5)*	10.5(4)**	1.8(7)
Trt × date	3.7(10)**	7.7(12)**	1.8(28)*	1.6(10)	7.4(12)**	0.5(28)	0.7(10)	2.6(12)*	0.4(28)	0.7(10)	2.8(12)**	0.8(28)
LSD (Trt)	379	329	748	78	44	119	34	38	68	24	24	46

Data were analyzed using split-plot ANOVA models with treatments as whole-plot and sample dates as sub-plot factors. Separate analyses were conducted for each year. n.a., not applicable; treatment was not sampled in that year.

^a Values within a column labeled with the same letter are not significantly different according to Tukey's HSD, $P \leq 0.05$, calculated for main-factor effects of fertility treatments ($n=24$, 20, and 32 in 1997, 1998 and 1999, respectively).

^b F-ratios and degrees of freedom (in parentheses) for principal factors in the ANOVA; values labeled with '*' and '**' are significant at $P < 0.05$ and $P < 0.01$, respectively.

^c F-test for effect of treatment = Mean Squared (Treatment)/Mean Squared (Treatment × Block); denominator for other F-tests is Mean Squared Error for overall ANOVA.

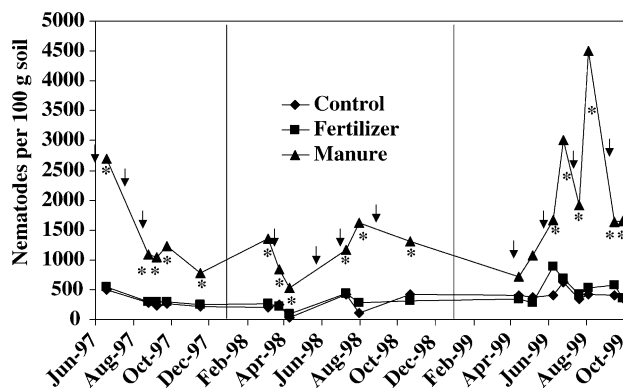


Fig. 1. Population dynamics of bacterivorous nematodes in the high manure and high fertilizer treatments and the control. For statistical analyses see Table 3. Asterisks denote single sample dates at which abundance in the manure-treated soil was significantly different from fertilized or untreated soil. Arrows mark nutrient application dates.

(Table 2). The treatment \times date interaction and main-factor effects of treatment and sample date were significant for fungivorous/root-feeding nematodes in 1998, and the main-factor effect of treatment was significant in 1997 and 1999 (Table 2). Fungivorous/root-feeding nematodes were 3.5 times more abundant in the high manure treatment than in the high fertilizer treatment (similar total-N inputs) and control in 1997; and there were no significant differences between the high fertilizer treatment and the control in any year (Table 2). In 1998 and 1999, fungivorous/root-feeding nematodes in the high manure treatment were approximately 2 times more abundant than in the high fertilizer treatment, and approximately 1.5 times more abundant than in the control, but the differences were significant in 1998 only (Table 2). Fungivorous/root-feeding nematodes were 2

and 3 times more abundant in the low manure treatment than in the high fertilizer treatment (similar mineral-N inputs) in 1998 and 1999, respectively (Table 2).

Omnivorous nematodes were less abundant than fungivorous/root-feeding nematodes in fertilizer and manure treatments but not in the controls (Table 3). Omnivorous nematodes were 1.2–2 times more abundant in the control than in the high fertilizer and manure treatments in all three years (Table 2). The main-factor effects of treatment and sample date were significant in 1997 and 1999 while the treatment \times date interaction was significant in 1998 (Table 2). For predacious nematodes, the treatment \times date interaction was significant in 1998 and the main-factor effect of treatment was significant in 1999, but the HSD did not differentiate any treatments in 1999 (Table 2).

The percentage of bacterivorous nematodes with enrichment opportunist characteristics (%EO) was affected by the treatment \times date interaction as well as the main-factor effects of treatment and sample date in 1999, whereas the main-factor effect of treatment was significant in 1997 and 1998 (Table 3). The high manure treatment had greater %EO values than the high fertilizer treatment (similar total-N inputs) and the control, which did not differ from each other (Table 3). The %EO in the low manure treatment was greater than in the high fertilizer treatment (similar mineral-N inputs) and control in 1998, but not in 1999.

The treatment \times date interaction and main-factor effects of treatment and sample date were significant for the nematode community maturity index (MI) in 1997 and 1999, and the main-factor effect of treatment was significant in 1998 (Table 3). Both high N treatments (fertilizer and manure) had lower MI values than the control, and the high manure treatment had lower MI values than the high

Table 3
Main-factor means for effects of manure and fertilizer treatments on selected indicators of nematode community structure in 1997, 1998 and 1999

	Maturity index			Maturity index 2-5			% Enrichment opportunists		
	1997	1998	1999	1997	1998	1999	1997	1998	1999
Control	2.43 a ^a	2.54 a	2.49 a	3.10 a	2.88 a	2.78 a	38 b	32 b	28 bc
High mineral-N (100 kg ha ⁻¹)									
Manure	1.62 c	1.64 c	1.70 c	2.58 c	2.56 b	2.43 b	70 a	71 a	60 a
Fertilizer	2.13 b	2.13 b	2.11 b	2.91 b	2.53 b	2.49 b	46 b	32 b	34 bc
Low mineral-N (50 kg ha ⁻¹)									
Manure	n.a.	1.76 bc	2.03 b	n.a.	2.47 b	2.47 b	n.a.	62 a	39 b
Fertilizer	n.a.	n.a.	2.57 a	n.a.	n.a.	2.85 a	n.a.	n.a.	25 c
ANOVA summary, F-ratios (degrees of freedom) ^b									
Treatment ^c	53.4(2)**	55.8(3)**	49.6(4)**	43.6(2)**	8.2(3)**	47.9(4)**	27.3(2)**	40.5(3)**	22.2(4)**
Date	42.6(5)**	0.26(4)	3.9(7)**	1.4(5)	4.0(4)**	4.8(7)**	3.8(5)**	2.2(4)	2.6(7)*
Treatment \times date	5.1(10)**	1.1(12)	1.9(28)**	0.76(10)	0.97(12)	1.33(28)	0.6(10)	1.5(12)	1.7(28)*
HSD (treatment)	0.21	0.39	0.21	0.18	0.30	0.18	13	17	12

Data were analyzed using split-plot ANOVA models with treatments as whole-plot and dates as sub-plot factors. Separate analyses were conducted for each year. n.a., not applicable; treatment was not sampled in that year.

^a Values within a column labeled with the same letter are not significantly different according to Tukey's HSD, $P \leq 0.05$, calculated for main-factor effects of fertility treatments ($n = 24, 20$, and 32 in 1997, 1998 and 1999, respectively).

^b F-ratios and degrees of freedom (in parentheses) for principal factors in the ANOVA; values labeled with '*' and '**' are significant at $P < 0.05$ and $P < 0.01$, respectively.

^c F-test for effect of treatment = mean squared (treatment)/mean squared (treatment \times block); denominator for other F-tests is mean squared error for overall ANOVA.

fertilizer treatment (similar mineral-N inputs) in all three years (Table 3). The MI value for the low manure treatment was not significantly different from the high fertilizer treatment (similar mineral-N inputs) in either year, but was significantly greater than the high manure treatment in 1999. The MI for the low manure treatment was significantly less than for the control in all three years (Table 3). The MI value for the low fertilizer treatment did not differ from the control in 1999.

The MI2-5 was not affected by the treatment \times date interaction in any year and main-factor effects of treatment were significant in all three years (Table 3). Both high N treatments (fertilizer and manure) had lower MI2-5 values than the control in all three years, and the high manure treatment had a lower MI2-5 value than the high fertilizer treatment (similar mineral-N inputs) in 1997. MI2-5 values for the low manure treatment did not differ from either the high fertilizer treatment (similar total-N inputs) or the high manure treatment in 1998 or 1999, but was significantly less than the control in both years. The MI2-5 value for the low fertilizer treatment was greater than all other fertility treatments and did not differ from the control in 1999.

The three-year average populations of protozoa and nematodes were estimated to consume microbial biomass-C at rates of 174, 152, and 593 mg C kg soil⁻¹ yr⁻¹ in the control and high fertilizer and high manure treatments, respectively. When converted to microbial biomass-N, populations of micro-fauna in the high manure treatment were estimated to consume approximately 3 times more microbial biomass-N than populations in the fertilizer treatment and control (Fig. 2).

3.2. Temporal dynamics of faunal indicators—continuing treatments

The protozoa and bacterivorous nematodes exhibited substantial date-to-date variations in abundance (Figs. 1 and 3). Over the three years of sampling, the abundance of

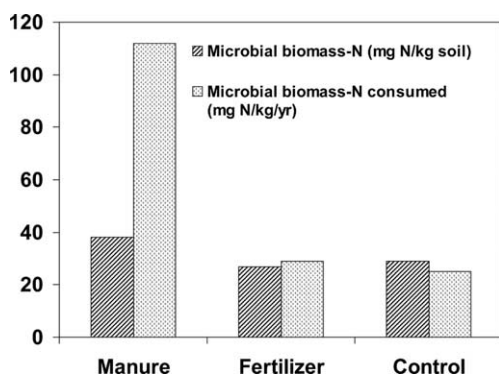


Fig. 2. Comparison of (1) microbial biomass-N (mg N kg⁻¹ soil⁻¹) and (2) rate of consumption of microbial biomass-N by nematodes and protozoa (mg N kg⁻¹ yr⁻¹) in the control and high manure and high fertilizer treatments. Presented data are averages of estimates for 1997, 1998 and 1999, and the microbial biomass N data are taken from Bittman et al. (2003).

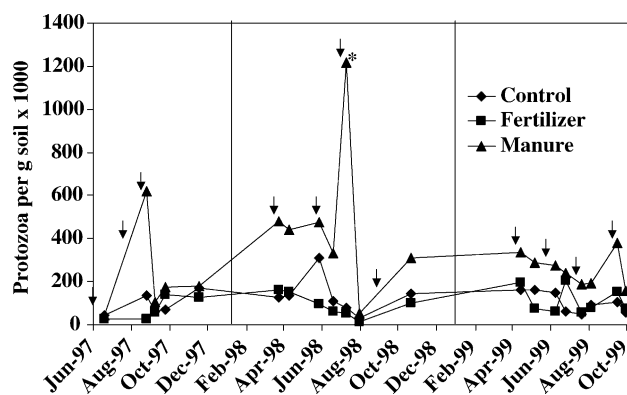


Fig. 3. Population dynamics of protozoa in the high manure and high fertilizer treatments and the control. For statistical analyses see Table 2. The asterisk denotes the single sample date at which protozoan abundance was significantly greater in manure-treated soil than in fertilized or untreated soil. Arrows mark nutrient application dates.

protozoa in the high manure treatment ranged from 4.1×10^4 to 1.2×10^6 protozoa g⁻¹ soil; this range (1.1×10^6) was 3.1 times the grand mean of 3.8×10^5 protozoa g⁻¹ soil (Fig. 3). In contrast, the ranges in protozoan abundance in the control and high fertilizer treatments were 2.7×10^5 and 1.9×10^5 protozoa g⁻¹ soil, respectively, which were 2 and 0.7 times the grand means of 1.3×10^5 and 2.6×10^5 protozoa g⁻¹ soil, respectively. The range over time in mean bacterivorous nematode abundance in the high manure treatment was 2.7 times the grand mean of 1494 nematodes per 100 g soil (Fig. 1). In contrast, the range in abundance of bacterivorous nematodes in the control and high fertilizer treatments was 1.9 and 2.2 times the grand means of 317 and 368 nematodes per 100 g soil, respectively (Fig. 1). Because the %EO is constrained to values between 0 and 100, the range over the three years in the high manure treatment (80–46) was only 52% of the grand mean of 66 (Fig. 4).

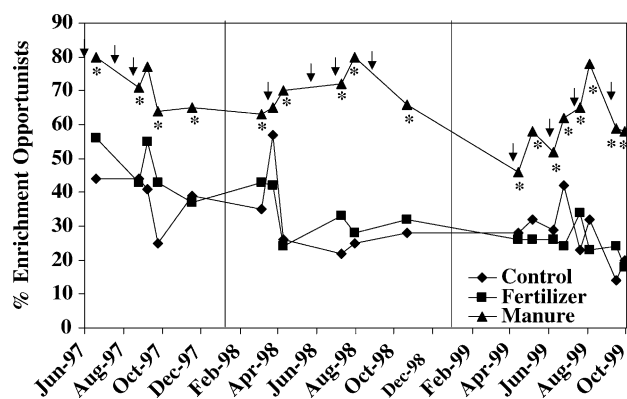


Fig. 4. Temporal dynamics of the percentage of bacterivorous nematodes with enrichment opportunist ecological characteristics (%EO) in the high manure and high fertilizer treatments and the control. For statistical analyses, see Table 4. Asterisks denote dates at which abundance in the manure-treated soil was significantly different from fertilized or untreated soil. Arrows mark nutrient application dates.

Table 4

Main-factor means for effects of prior manure and fertilizer treatments (1994–1997) on responses of selected groups of micro-fauna to cessation of treatments during 1998 and 1999: protozoa, bacterivorous and fungivorous/root-feeding nematodes, and percentage of bacterivorous nematodes with c–p rankings of 1 (% enrichment opportunists)

1994–1997 treatments	log-protozoa g ⁻¹ soil		Nematodes per 100 g soil					
			Bacterivorous		Fungivorous/root-feeding		%Enrichment opportunists	
	1998	1999	1998	1999	1998	1999	1998	1999
High manure	5.27*	5.04**	793*	994**	143*	120	41*	30
High fertilizer	4.95	4.89	387	683	51	105	31	26
ANOVA summary, F-ratios (degrees of freedom) ^a								
Treatment ^b	11.0(1)*	7.7(1)**	6.9(1)*	9.4(1)**	4.3(1)*	0.5(1)	9.0(1)**	1.3(1)
Date	9.7(6)**	4.02(8)**	5.4(4)**	1.5(7)	4.9(4)**	2.3(7)*	21.0(4)**	2.6(7)*
Treatment × date	1.3(6)	1.46(8)	1.5(4)	2.2(7)	1.6(4)	0.4(7)	2.8(4)	1.5(7)

Data were analyzed using split-plot ANOVA models with treatments as whole-plot and sample dates as sub-plot factors. Separate analyses were conducted for each year.

^a F-ratios and degrees of freedom (in parentheses) for principal factors in the ANOVA; values labeled with '*' and '**' are significant at $P < 0.05$ and $P < 0.01$, respectively.

^b F-test for effect of treatment = mean squared (treatment)/mean squared (treatment × block); denominator for other F-tests is mean squared error for the overall ANOVA.

Estimates of protozoan abundance were more variable than bacterivorous nematode abundance. Although the main-factor effect of treatment was significant in all three years, significant differences between the high manure treatment and control were detected on only one of the 19 individual sample dates (Fig. 3). In contrast, the abundance of bacterivorous nematodes (Fig. 1) and %EO (Fig. 4) discriminated the high manure treatment from the control and fertilizer treatments on 16 and 18 of the 19 sample dates, respectively. In all three years protozoan (Fig. 3) and bacterivorous nematode (Fig. 1) abundance maxima occurred during the summer, and the greatest single increase in protozoan population densities occurred between July 16 and July 30 of 1998.

3.3. Residual fertility

For protozoa and bacterivorous nematodes the date × treatment interaction was not significant in either year (Table 4). For protozoa, the main-factor effect of treatment was significant in 1998 and 1999 and population densities in previously manure-treated soil were greater than in previously fertilized soil (Table 4; Fig. 5(a)). Bacterivorous nematodes were approximately 1.5 times more abundant in the residual manure regime than in the residual fertilizer regime through 1998 and 1999 (Table 4; Fig. 5(b)). Fungivorous nematodes were 2.8 more abundant in the previous manure regime than in the previous fertilizer regime in 1998, but the difference did not persist through 1999 (Table 4). Differences between past treatments in terms of the %EO were evident in 1998, but did not persist through 1999 (Table 4; Fig. 5(c)). Differences in MI and MI2-5 between the residual manure and residual fertilizer treatments also persisted through 1998 and 1999 (data not shown).

3.4. Effects of treatment history on response to manure in 1998

Through 1998, populations of protozoa in previously fertilized and untreated soil did not differ in response to manure applications in 1998, and main-factor means for

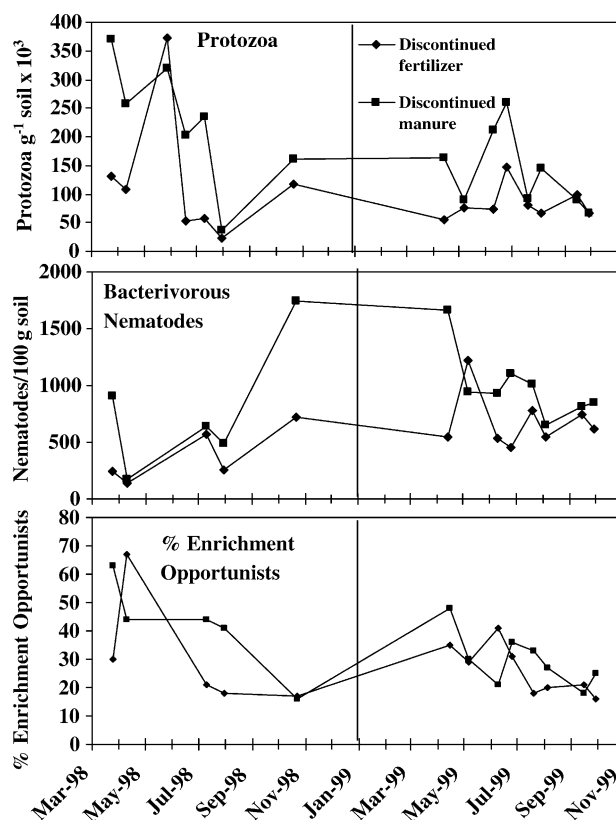


Fig. 5. Temporal dynamics of micro-faunal indicators after cessation of manure and fertilizer applications: protozoan abundance (a, top), bacterivorous nematode abundance (b, middle), and percentage of bacterivores designated as enrichment opportunists (c, bottom).

Table 5

Main-factor means for effects of prior manure and fertilizer treatments (1994–1997) on responses of selected groups of micro-fauna to the addition of manure (100 kg NH₄-N ha⁻¹) in 1998: protozoa, bacterivorous and fungivorous/root-feeding nematodes, and percentage of bacterivorous nematodes with c-p rankings of 1 (%enrichment opportunists)

1994–1997 treat- ments	log-protozoa g ⁻¹ soil		Nematodes per 100 g soil					
			Bacterivorous		Fungivorous/root-feeding		%Enrichment opportunists	
	1998	1999	1998	1999	1998	1999	1998	1999
Control	5.21 b ^a	5.03	491 b	1035	107	139	54	30 b
High mineral-N (100 kg ha ⁻¹)								
High manure	5.55 a	5.27	1092 a	1337	124	188	71	38 ab
High fertilizer	5.17 b	4.87	531 b	858	82	107	59	30 b
Low mineral-N (50 kg ha ⁻¹)								
Low manure	n.a.	5.11	n.a.	1422	n.a.	202	n.a.	44 a
Low fertilizer	n.a.	4.92	n.a.	1000	n.a.	154	n.a.	32 ab
ANOVA summary, F-ratios (degrees of freedom) ^b								
Treatment ^c	34.8(2)**	6.44(4)**	30.3(2)**	6.2(4)**	2.2(2)	4.5(4)*	7.2(2)*	13.5(4)**
Date	16.7(6)**	11.38(8)**	3.74(4)*	3.8(7)**	64.1(4)**	9.2(7)**	2.6(4)*	1.3(7)
Treatment × date	2.6(12)**	0.74(32)	1.8(8)	0.6(28)	2.9(8)*	1.2(28)	2.1(4)	0.9(28)
HSD (treatment)	0.24	0.42	378	607	60	107	18	13

Data were analyzed using split-plot ANOVA models with treatments as whole-plot and sample dates as sub-plot factors. n.a., not applicable; treatment was not sampled in that year.

^a Values within a column labeled with the same letter are not significantly different according to Tukey's HSD, $P \leq 0.05$, calculated for main-factor effects of past fertility treatments ($n=20$ and 32 in 1998 and 1999, respectively).

^b F-ratios and degrees of freedom (in parentheses) for principal factors in the ANOVA; values labeled with * and ** are significant at $P < 0.05$ and $P < 0.01$, respectively.

^c F-test for effect of treatment = mean squared (treatment)/mean squared (treatment × block); denominator for other F-tests is mean squared error for overall ANOVA.

both treatments were less than for soil that had been treated with manure continuously since 1994 (Table 5; Fig. 6(a)). Treatment, date and treatment × date interaction terms were significant in 1998; the interaction effect in 1998 appeared to be due primarily to large fluctuations in the continuously manure-treated soil rather than reversal of treatment effects through time (Fig. 6(a)). Main-factor effects of treatment and date were significant in 1999 but the HSD did not differentiate treatments (Table 5).

The responses of bacterivorous nematodes to application of manure in 1998 were affected by previous treatments. In 1998, bacterivorous nematode abundance did not differ between previously untreated and fertilized soil, although population densities in both prior treatment regimes were less than in soil treated with manure continuously from 1994 to 1998 (Table 5; Fig. 6(b)). In 1999, however, the abundance of bacterivorous nematodes in previously untreated and previously fertilized soils were no longer significantly less than in soil treated with manure continuously from 1994 to 1998 (Table 5; Fig. 6(b)). The %EO did not differ between previously fertilized and unfertilized soil in 1998 or 1999, and neither prior treatment regimes maintained lower %EO values than soil treated with manure continuously from 1994 to 1998. The %EO in soil treated with the low rate of manure (from 1994 to 1997) and then treated with the high rate of manure in 1998 was significantly greater than in previously untreated or previously fertilized soil (Table 5). The response of fungal/root-feeding nematodes to manure in 1998 was not affected by previous treatment regimes (Table 5).

4. Discussion

4.1. Effects of manure on community structure

The present data indicate that the manure-induced increase in microbial production supported substantially increased production of microbivorous protozoa and nematodes. Earthworms, which stimulate protozoan populations and microbial C fluxes (Binet et al., 1998), were also more abundant in the manure treatments at this site (Raworth et al., 2004). The increased abundance of bacterivorous protozoa and nematodes in manure-treated soil is consistent with previous laboratory and field studies (Dmowska and Kozłowska, 1988; Ettema and Bongers, 1993; Griffiths et al., 1994, 1998), whereas the increased abundance of fungivorous nematodes in manure-treated soil has not been observed previously.

An important implication of the increased abundance of microbivorous protozoa and nematodes is increased rates of microbial consumption and fluxes of N from the microbial biomass-N pool through the micro-fauna. The microbial biomass-N pool was approximately 1.4 times greater in manure-treated soil than in the control and fertilizer-treated soil (Bittman et al., 2005). However, our calculations indicate that micro-faunal consumption of microbial biomass-N in manured soil was approximately 3 times greater than in the control and fertilizer-treated soils, demonstrating that faunal analyses can reveal functional differences between soil management practices that are not evident from conventional measurements of soil microbial

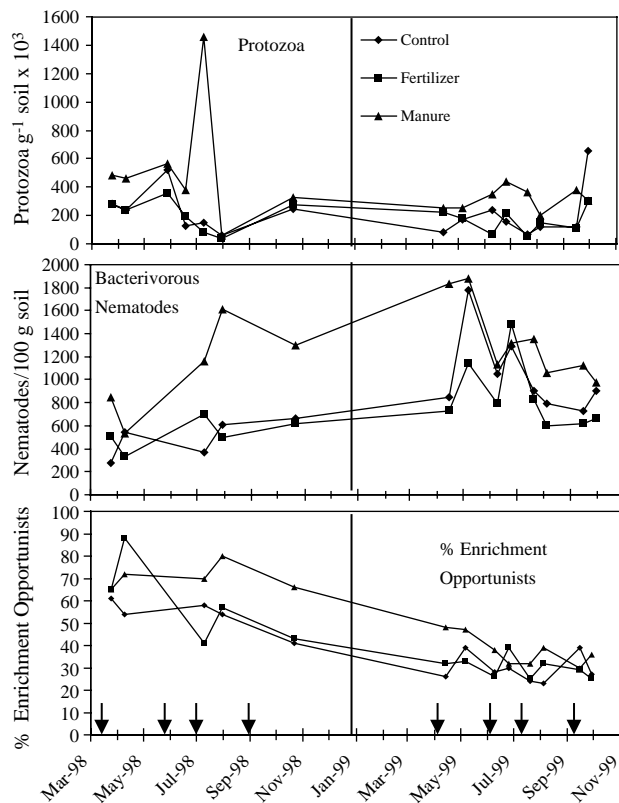


Fig. 6. Temporal dynamics of micro-faunal indicators after starting applications of manure to soil that was previously untreated or fertilized with mineral fertilizer: protozoan abundance (a, top), bacterivorous nematode abundance (b, middle), and percentage of bacterivores designated as enrichment opportunists (c, bottom). Arrows mark manure and fertilizer application dates.

biomass. The calculations used to estimate faunal consumption of the microbial biomass are based on numerous assumptions (Hunt et al., 1987; Hassink et al., 1994) and we caution against using them to make estimates of actual gross or net mineralization. The calculations are, however, a useful means of exploring the functional implications of relative differences in food web structure within a given study. Likewise, they complement results of studies correlating the abundance of bacterivorous micro-fauna with net N mineralization (e.g. Hassink et al., 1993; Opperman et al., 1993; Forge and Simard, 2000, 2001; Ferris and Matute, 2003).

The abundance of fungivorous nematodes was increased by manure even though hyphal biomass was decreased by manure and fertilizer treatments (Bittman et al., 2005). Another study has also reported apparently opposing responses of fungal biomass and the fungivorous fauna to alterations in organic matter inputs (Beare et al., 1992), suggesting that top-down forces (e.g. predation) may have more important influences on the fungal channel of the soil food web than on the bacterial channel, which appears to be influenced primarily by bottom-up forces (e.g. nutrient supply). In addition, nematophagous fungi may be inhibited by enrichment of soil with high N organic amendments

(Jaffee et al., 1994), so suppression of nematophagous fungi by manure would reduce the losses of nematodes in all trophic groups (Bouwman et al., 1994). Alternatively, a shift in the fungal community towards dominance by more palatable or nutritious species of fungi, or a greater proportion of metabolically active hyphae, may explain the greater fungivorous nematode abundance despite reduced overall fungal biomass. That changes in fungal species composition would alter populations of fungivorous nematodes is indicated by prior research demonstrating that reproduction rate of fungivorous nematodes is influenced by the species of fungi upon which they feed (Ruess and Dighton, 1996).

Fertilization did not increase bacterivorous nematode abundance, which contrasts with previous studies of nematode communities in grassland (Todd, 1996) and forest (Forge and Simard, 2001) ecosystems. In contrast to these studies, the present study involved harvesting most of the above-ground biomass 2–4 times per year, which reduces root growth and greatly limits the deposition of N-enriched detritus to the decomposer food web. Todd (1996) previously found that microbivorous nematode abundance was increased by N fertilizer in un-mowed plots but not in mowed (herbage removed) plots of tallgrass prairie in Kansas.

Relative to three-year mean population densities, there was greater sample date-to-sample date variation in populations of protozoa and bacterivorous nematodes in manure-treated soil than in non-treated soil. In addition, populations of bacterivorous nematodes varied less, relative to overall mean population densities, than populations of protozoa. The great apparent temporal variation in protozoan population densities was likely the combined result of substantial variation in actual population densities through time, as well as imprecision inherent to the most probable number (MPN) approach for quantifying protozoan population densities. These findings suggest that even though protozoa may consume more bacteria and make greater direct contributions to mineralization than bacterivorous nematodes (Hunt et al., 1987; Hassink et al., 1994), the abundance of bacterivorous nematodes may be a more stable and practical bio-indicator of soil fertility than MPN-based estimates of protozoan abundance.

Our sampling frequency was inadequate to pinpoint seasonal maxima and minima for any group of micro-fauna. However, we did observe that in all three years protozoan and bacterivorous nematode abundance maxima occurred during the summer. In addition, the greatest increase in protozoan population densities, between July 16 and July 30 of 1998, coincided with the greatest single reduction in bacterial biomass (Bittman et al., 2005).

The nematode community Maturity Index (MI) was greater for the high fertilizer treatment than for the high manure treatment in all three years. This difference was largely due to the greater abundance of enrichment opportunists in the high manure treatment. Accordingly, the MI2-5 did not indicate differences between the high

manure and high fertilizer treatments in 1998 and 1999. This pattern of results indicates that fertilizer and manure treatments had similar effects on structural complexity of the soil nematode community and differed primarily in the abundance of enrichment opportunists. The high manure and high fertilizer treatments both had lower MI and MI2-5 values than the control. These results suggest that high levels of N, regardless of source (manure or fertilizer), can reduce structural complexity of the soil nematode community and the soil food web it represents.

Omnivorous and predacious nematodes, especially those belonging to the order Dorylaimida (c–p rankings of 4 and 5), are sensitive to a variety of disturbances (Bongers, 1999; Ferris et al., 2001; Neher, 2001), including increased nutrient inputs (Ettema and Bongers, 1993; Todd, 1996). Reasons for the sensitivity of dorylaimids to nutrient inputs are not fully understood, but may include direct toxicity of high levels of ammonia or nitrate (Bongers, 1999). Our observation of reduced omnivorous nematode abundance, and reduced MI2–5 values, in high manure and fertilizer treatments is consistent with the previous observations of the sensitivity of dorylaimids to nutrient inputs (Bongers, 1999; Ettema and Bongers, 1993; Todd, 1996; Neher, 2001). However, predacious nematodes, including some dorylaimids, were more abundant in manure-treated soil than in fertilized soil, indicating that increased nutrient inputs may not necessarily have negative effects on dorylaimids representing higher-order trophic groups if the inputs are organic and therefore also enhance the availability of food resources. The predacious nematode community at our site was dominated by *Aporcelaimellus*, which may deviate from the general tendency of dorylaimids to be sensitive to increased nutrient inputs (Ettema and Bongers, 1993).

4.2. Residual fertility

Results from the residual fertility experiment implemented in 1998 indicate that the use of manure for at least four years can result in sufficient accumulation of labile organic matter to sustain elevated microbial production (Bittman et al., 2005) and populations of micro-fauna through at least two growing seasons after applications have ceased. These results indicate that manure-treated soil will continue to exhibit increased mineralization of N for up to two years after applications of manure cease. Additional research to more accurately quantify N mineralization rates during the two years after cessation of manure application would contribute to the environmentally prudent use of dairy manure slurry as a source of nutrients for forage crop production.

4.3. Effects of treatment history on response to manure

Populations of protozoa and bacterivorous and fungivorous nematodes in soil treated with manure for a single year

were still significantly smaller in the year of application (1998) than in soil treated with manure continuously through that year, and there were no significant differences between previously fertilized and previously non-treated soil. In 1999, however, populations in soil treated with manure for one year were no longer significantly different from populations in soil treated with manure continuously from 1994 to 1998. Because fertilization had selected for greater relative abundance of enrichment opportunists than in untreated soil, and because enrichment opportunists are generally capable of greater population growth in response to nutrient inputs, we had hypothesized that manure-induced growth of bacterivorous nematode populations, in terms of absolute abundance, would be greater in previously fertilized soil than in control soil. Our results were counter to this hypothesis in that the absolute abundance of bacterivorous nematodes was similar in previously fertilized and previously non-treated soil.

5. Conclusion

This research has demonstrated that in addition to increasing microbial biomass (Bittman et al., 2005), application of dairy manure slurry increases the abundance of microbivorous protozoa and nematodes that are indicative of enhanced microbial turnover and flux of nutrients through the soil food web. The manure-induced increase in microbivorous micro-fauna attained by 4 years of manure applications persisted through two growing seasons after manure applications ceased. Application of manure for one year to previously non-treated or fertilized soil raised the abundance of protozoa and bacterivorous and fungivorous nematodes to levels comparable to continuously manured soil, but the effect was not evident until the year after application. The nematode Maturity Index and the MI2-5 were both reduced by fertilization and manure. The MI for the manure treatment was lower than for the high fertilizer treatment as a result of the greater relative abundance of enrichment opportunist nematodes in manure-treated soil. Accordingly, the MI2-5 generally did not differ between the manure and fertilizer treatments, suggesting that with the exception of enrichment opportunists, manure and fertilizer have similar effects on structural complexity of the soil food web.

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