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Uncoupling of carbon and nitrogen mineralization: role of microbivorous nematodes ☆

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Abstract

Microfaunal grazing of soil microorganisms affects nutrient mineralization rates. However, the accessibility of microbial food resources to microfauna depends on matric potential because microfauna require water to move. Laboratory incubations of undisturbed pairs of soil cores were conducted to evaluate temporal changes in the relationships among C and N mineralization, abundance and distribution of nematode trophic groups, and matric potential. Cores were collected in May, August, and November 1997, and March 1998 from an old field.

The general relationship between C and N mineralization for all data points did not hold among sampling periods. Differences in this relationship may have been a result of microbivorous grazing. Nematode abundance did not decrease as matric potential decreased, suggesting microbivorous grazers were not merely excluded from their food resources, but survived in isolated water-filled pores as soil dried. We suggest that at −50 kPa nematodes and their microbial food resources are enclosed within spatially isolated water pockets and this entrapment leads to increased microbivorous grazing and microbial activity per unit biomass (qCO_2). Only at −50 kPa was there a strong linear relationship between qCO_2 and microbivorous nematode density. There were also negative linear relationships between qCO_2 and microbial biomass C and C:N at −50 kPa which were significantly different from the other matric potentials tested. Changes in microbial community composition appeared to affect C and N mineralization rates, but dissolved substrate availability could not directly explain differences in C and N mineralization.

Keywords: Nutrient mineralization; Carbon; Nitrogen; Matric potential; Microbivorous grazing; Free-living nematodes

1. Introduction

Degradation of soil organic matter (O.M.) and the mineralization of C and N are mediated primarily by soil microorganisms. Seasonal variations in temperature and moisture affect microbial activity, which in turn influence the time course and magnitude of nutrient mineralization processes. Both C and N mineralization increase with temperature up to about 35°C (Stanford et al., 1973; Edwards, 1975; Addiscott, 1983; Blet-Charaudeau et al., 1990; Grundmann et al., 1995; Sierra, 1997) and decrease with decreasing matric potential (Miller and Johnson, 1964; Wilson and Griffin, 1975; Orchard and Cook, 1983; Sierra, 1997).

Substrate quality also affects C and N mineralization. N mineralization is generally considered to be coupled to C mineralization by the C:N ratios of specific substrate and microbial pools (Smith, 1994; Mary et al., 1996). In other words, microbes mineralize N at a rate proportional to respiration when growing on a substrate with a C:N ratio less than the microbial C:N ratio (Myrold, 1998). This relationship has been reported for steady state conditions in laboratory studies (Gale and Gilmour, 1986, 1988). The microbial C:N ratio depends on community composition as bacteria (C:N ratio of 5:1) require greater amounts of N per unit C biomass than fungi (C:N ratio 10:1).

Food web interactions among microorganisms and microfauna, i.e. nematodes and protozoa, also influence C and N mineralization (Woods et al., 1982; Ingham et al., 1985; Griffiths, 1986; Hunt et al., 1987; Verhoef and Brussaard, 1990), with increases in N mineralization of up to 30% resulting from the activities of soil microfauna (Verhoef and Brussaard, 1990). Microfauna can affect nutrient mineralization directly, by excreting mineral nutrients (Woods et al., 1982; Anderson et al., 1983), and indirectly, by causing shifts in the microbial community structure (Griffiths, 1994b) and growth rates (Bengtsson et al., 1993). Grazing

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by nematodes and protozoa can increase microbial turnover and stimulate compensatory growth of surviving microbial populations by reducing microbial competition and increasing nutrient availability (Anderson et al., 1981). These trophic interactions are influenced by soil physical properties such as soil moisture, temperature, and pore size distribution (Elliott et al., 1980; Kuikman et al., 1989; Hassink et al., 1993; Bouwman and Zwart, 1994; Wright et al., 1995).

The habitable pore space hypothesis states that access of microbivorous nematodes and protozoa to microbial food resources is constrained by the water-filled soil pore size distribution (Elliott et al., 1980; Hassink et al., 1993; Killham et al., 1993). Nematodes in particular cannot enter water-filled pores with openings smaller than their body diameters, generally considered to be greater than 15 μm (Jones et al., 1969). Matric potential determines what size pores remain water-filled (Hillel, 1971), affecting the ability of aquatic biota to access pores. The threshold matric potential is −20 kPa, equivalent to water-filled pore diameters less than 15 μm. A similar effect of matric potential on protozoan habitat can be expected, although with a lower threshold because protozoa can fit into pore openings down to 6 μm (Elliott et al., 1980). Thus, the exclusion hypothesis predicts that microfaunal access to their microbial food resources decreases as matric potential decreases because microfauna need to search for their food in water films (Wallace, 1958; Elliott et al., 1980).

There is an additional hypothesis, called the enclosure hypothesis (Görres et al., 1999), which states that grazing pressure increases as matric potential decreases due to enclosure of microfauna with their microbial food resources in isolated pockets or water-filled pores. Soil pores are not cylindrical, and pores of all sizes may have small or large openings controlling pore drainage. Aggregation may produce regions with immobile water that become isolated under dry conditions (Addiscott, 1977). Consequently, nematodes and protozoa may become trapped within water-filled pores, as expressed by the micro-habitat concept of Hattori (1994), at matric potentials that are otherwise expected to restrict microbivores access to their forage (Griffiths et al., 1995; Görres et al., 1999).

The goals of the present study were to assess the effect of temperature, matric potential, and nematodes on C and N mineralization. Nematodes were chosen to represent the bacterivorous microfauna because they actively migrate through the soil in search of bacterial food (Kuikman et al., 1990; Young et al., 1998). If the exclusion hypothesis dominates trophic interactions in soil, we expected nematode abundance to decrease with decreasing matric potential. If the enclosure hypothesis applies, we did not expect a reduction in nematode abundance. It is generally considered that C and N mineralization are coupled. Therefore, if grazing is of little consequence in C and N mineralization, then the ratio of C-to-N mineralization was expected to be constant regardless of changes in matric potential. However, microbivorous grazing may weaken that coupling, and the level of uncoupling may depend on whether the exclusion or enclosure becomes dominant as matric potential decreases. If exclusion dominated, then we expected higher microbial activity and N mineralization at high matric potentials (in wet soil) with decreased activity measured as C mineralization (per unit microbial biomass) and N mineralization as soil dried, due to decreased grazing pressure. If enclosure dominated, then we expected microbial activity to remain high in dry soil and to be dependent on the density of grazers in dry soil. In wet soil, mineralized N can diffuse away from sites of mineralization and we expected to measure an increase in net N mineralization in the presence of grazers (similar to conditions under exclusion). In dry soil in enclosures, both N mineralization and immobilization will increase with increased grazer density, with a potential net result of no change in mineral N. On the other hand, C mineralization (per unit microbial biomass) will be high because of the increase in microbial activity. Consequently, there should be a greater increase in the ratio of C-to-N mineralization in soil with enclosures.

C and N mineralization were measured during the course of a 28-day incubation. Undisturbed soil cores were obtained from an old field at four different dates during the course of a year. Nematode abundance and trophic group distribution and microbial biomass measurements were used to infer the effects of grazing on C and N mineralization as a function of water-filled pore diameter and sampling date.

2. Materials and methods

2.1. Study area

Intact soil cores were collected from a 20×40 m plot, subdivided into 50 4×4 m plots in an old field at the Peckham Farm Research Area of the University of Rhode Island in Kingston, RI. The soil is a Hinckley sandy loam (sandy-skeletal, mixed, mesic Typic Udorthent) and had not been cultivated for 9 years prior to the start of the study. Dominant vegetation included timothy (*Phleum pratense* L.), brome grass (*Bromus inermis* Leyss.), orchard grass (*Dactylis glomerata* L.), Kentucky blue grass (*Poa pratensis* L.), rose (*Rosa multiflora* Thunb. ex Murr.), cinquefoil (*Potentilla recta* L.), brambles (*Rubus* spp.), and golden rod (*Solidago* spp.).

2.2. Sample collection

Field sampling occurred on 13 May, 12 August, and 11 November, 1997, and 2 March, 1998. Soil temperature to a depth of 5 cm and air temperature were determined in the late morning (between 10:00 and 12:00 EST). Soil temperatures were 14, 22, 6, and 6°C and air temperatures were 18, 29, 7, and 12°C in May, August and November, 1997 and March, 1998, respectively.

Sets of (x, y) coordinates were randomly generated for

40 randomly selected plots. Adjacent 10-cm cores (5-cm dia.) were removed at each point. Because variability of soil properties exhibits spatial dependence (Cambardella et al., 1994; Görres et al., 1998), adjacent cores were used to minimize initial variation among cores. One core was used to establish conditions at the beginning of the incubation and one core was used to establish conditions at the end of the incubation. Aluminum cores were pounded into the soil and dug out by hand. Roots at the bottom of the cores were clipped and core bottoms were covered with a nylon mesh (30 μm mesh size), which was secured with a rubber band. The cores were brought into the laboratory within 2 h of collection for adjustment of matric potential.

2.3. Adjustment of matric potential

A sand and a sand/kaolin table (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) were used to adjust the matric potential of the soil cores. The tables were kept in the dark inside an environmental chamber set to the field soil temperature. Matric potential was adjusted to −3 and −10 kPa using the sand table, and to −20 and −50 kPa using the sand/kaolin table. Pairs of abutting cores were randomly assigned to one of four groups of water potential: −3, −10, −20, or −50 kPa, equivalent to maximum water-filled pore diameters of 100, 30, 15, and 6 μm, respectively. Because of logistical constraints, two groups (−3 and −20 kPa) were placed immediately on sand tables while the other two groups (−10 and −50 kPa) were stored at field moisture and temperature in an environmental chamber in the dark for one week before adjustment of matric potential.

To adjust soil matric potential, the cores were placed on the sand tables, the table saturated with water, and the cores allowed to saturate for two days. Saturation was confirmed gravimetrically when the change in total core mass was less than 1% for two consecutive mass determinations. Following saturation, the prescribed tension was applied using either a water column (sand table) or vacuum pump (sand/kaolin table). The cores were allowed to equilibrate and were removed from the tables when the change in mass was less than 1% for two consecutive mass determinations (about 5 days). Plants growing in the cores were clipped at the soil surface and removed each time the cores were weighed. Cores were kept at the soil temperature at the time of collection during the equilibration and incubation periods.

2.4. Incubation of equilibrated cores

After equilibrating at the desired tension, cores were placed in 900-cm^3 glass Mason jars, sealed with a screw cap fitted with a rubber septum for gas sampling, and incubated in the dark. For the May and August sampling periods, CO_2 evolution was measured every 3 or 4 days to establish the time it took for the effects of disturbance to subside (Blet-Charaudeau et al., 1990) and for the rate of CO_2 evolution to become linear (21 days; data not shown). For the remaining sampling periods, cores were pre-incubated for 21 days, and the jars were periodically flushed with an air stream during this period. Plants growing in the cores during the pre-incubation period were clipped at the soil surface and removed every time the headspace was flushed. All above-ground plant growth ceased by the end of the pre-incubation period in the dark.

Following the pre-incubation period (21 days after establishment of matric potential), one core from each pair was randomly selected and used to establish initial soil conditions. Cores were sampled destructively. Soil properties measured included pH, bulk density, moisture, soil O.M., total C and N, NO_3^- and NH_4^+, dissolved organic C, dissolved total N, and microbial biomass C and N. All measurements were completed within 3 days of sampling and the remaining soil (120–250 g) was shipped by overnight courier to the University of Toledo for determination of nematode abundance and trophic groups. At the conclusion of the 28-day incubation period (49 days at established matric potential, 56 days from initial saturation), the same measurements were made on the remaining cores.

Soil cores were incubated at 14°C in May, 22°C in August, and 6°C in November and 6°C in March. An environmental chamber malfunctioned for two days during the incubation of cores collected in March. The temperature rose from 6 to 26°C during that period. The cores incubated at −3 and −20 kPa were removed from the chamber and extracted immediately after discovering the malfunction. The cores incubated at −10 and −50 kPa were transferred to another chamber kept at 6°C and incubated for another week prior to extraction to complete the 28-day incubation.

2.5. Carbon mineralization

CO_2 evolution rates were determined by gas chromatography (Görres et al., 1997) over a 28-day incubation period. A gas sample (21 ml) was removed once a week from the headspace of the Mason jars using a gas-tight syringe. Samples were injected at a slightly positive pressure into previously evacuated, 20-ml glass headspace vials that were sealed with a rubber septum and an aluminum crimp collar, and analyzed immediately. After the headspace samples were taken, the Mason jars were uncapped and flushed with an air stream for at least 5 min. After flushing, the jars were sealed and incubation resumed under the conditions described previously.

2.6. Soil physical and chemical properties

Soil water content was determined gravimetrically after oven-drying a known mass of moist soil (10 g) at 105°C overnight. Bulk density was determined from the mass of dry soil in cores of known volume (196 cm^3). Volumetric moisture was calculated as the mass water content multiplied by the bulk density divided by the density of water. Percent water-filled porosities were calculated as volumetric water content at a particular matric potential divided by the

Table 1
Mean values (s.d.) of volumetric moisture (θ_v) and percent water-filled porosity (% WFP) at different matric potentials ($n = 20$) in an old field soil for May, August and November 1997, and March 1998

Matric potential (−kPa)	May		August		November		March	
	θ_v	% WFP	θ_v	% WFP	θ_v	% WFP	θ_v	% WFP
3	0.30 (0.04)	50	0.35 (0.07)	56	0.34 (0.06)	55	0.31 (0.03)	51
10	0.22 (0.02)	37	0.19 (0.02)	31	0.23 (0.04)	37	0.21 (0.03)	34
20	0.19 (0.03)	32	0.17 (0.02)	27	0.21 (0.02)	34	0.22 (0.03)	36
50	0.18 (0.03)	30	0.13 (0.02)	21	0.16 (0.02)	26	0.18 (0.03)	30

volumetric water content at saturation. Volumetric moisture and percent water-filled porosities (% WFP) for each matric potential at each sampling date are presented in Table 1.

Pore sizes were calculated from matric potential using the following generalized form of the capillary rise equation:

$$d = 300/\psi_m \quad (1)$$

where d is pore diameter in μm and ψ_m is tension or matric potential in kPa (Hillel, 1971).

Values obtained at the beginning and end of the 28-day incubation period for bulk density, pH (determined using a 1:10 soil:water (wt:vol) ratio; Hendershot et al., 1993), soil C:N (measured using a Carlo Erba Instruments Nitrogen/Carbon Analyzer, model NA 1500 series 2, Milan, Italy) and soil O.M. (calculated from mass loss-on-ignition at 550°C for 4 h) were averaged across matric potentials for each sampling period (Table 2).

2.7. Nitrogen mineralization/immobilization

A 1:10 soil:extract (wt:wt) ratio was used to extract NH_4^+ and NO_3^- from 1 g soil with a 2 N KCl solution (Keeney and Nelson, 1982). The soil suspension was shaken for 1 h, passed through a Whatman #42 filter, and the filtrate analyzed colorimetrically using an Alpkem Rapid Flow Analyzer (RFA-300, Alpkem Corp., Clackamas, OR).

Net N mineralization (N_{min}) was assumed to be linear (Addiscott, 1983) and was calculated using the equation:

$$N_{min} = \frac{1}{n}\sum_{i=1}^{n}\frac{(NO_{3_f} - NO_{3_i})}{t} + \frac{1}{n}\sum_{i=1}^{n}\frac{(NH_{4_f} - NH_{4_i})}{t} \quad (2)$$

where n is the sample size, NO_{3_f} and NH_{4_f} are the NO_3^- and NH_4^+ concentrations at the end of the 28-day incubation, NO_{3_i} and NH_{4_i} are the initial NO_3^- and NH_4^+ concentrations following the pre-incubation period, and t is the incubation time (28 days).

2.8. Dissolved organic carbon and total dissolved nitrogen

For the determination of dissolved organic carbon (DOC), 10 g wet soil was weighed into a plastic centrifuge tube, 20 ml distilled water added, and allowed to equilibrate without shaking at room temperature overnight. The suspension was then centrifuged for 5 min at 190 *g* and the supernatant solution filtered through a 0.2-μm-pore-size glass fiber filter. The filtrate was analyzed using a Total Organic Carbon Analyzer (Model TOC-5000A, Shimadzu Scientific Instruments, Inc., Columbia, MD). For dissolved total nitrogen (DTN), a portion of the DOC filtrate was oxidized to NO_3^- (Cabrera and Beare, 1993) and analyzed colorimetrically on an automated nutrient analyzer (RFA-300, Alpkem, Clackamas, OR).

2.9. Microbial biomass

The fumigation-extraction method (Vance et al., 1987) was used to measure the microbial biomass C content of the soil. Unfumigated soil (20 g moist) was extracted with a 0.5 M K_2SO_4 solution using a 1:2 soil:extract (wt:vol) ratio, shaken for 30 min, and passed through a Whatman #42 filter. At the same time, 20 g soil was fumigated with ethanol-free chloroform for 24 h and extracted in the same manner as unfumigated soil. Dissolved organic carbon in the filtrate was analyzed using a Total Organic Carbon Analyzer TOC-5000A (low sensitivity platinum catalyst; Shimadzu Scientific Instruments, Inc., Columbia, MD). Microbial biomass C was calculated from the difference

Table 2
Mean values (s.d.) of bulk density ρ, pH, soil C:N and % O.M. for May, August and November 1997, and March 1998

Soil property	Mean (s.d.)			
	May	August	November	March
ρ_B (g cm^{-3}) $n = 80$	1.05 (0.09)	1.02 (0.09)	1.00 (0.09)	1.02 (0.09)
pH $n = 23$–24	5.3 (0.5)	5.6 (0.3)	4.9 (0.2)	4.8 (0.2)
Soil C:N $n = 79$–80	17.1 (1.9)	18.0 (3.8)	ND[a]	ND
%O.M. $n = 80$	5.4 (1.2)	5.7 (1.2)	5.5 (1.2)	5.2 (1.2)

[a] Not determined.

between C in fumigated and unfumigated soil extracts, expressed per kg dry soil and multiplied by a correction factor of 2.64 (Vance et al., 1987). To determine microbial biomass N, an aliquot of the filtrate was oxidized to NO_3^- (Cabrera and Beare, 1993) and analyzed colorimetrically using an automated nutrient analyzer (RFA-300, Alpkem, Clackamas, OR).

The amount of C respired per unit microbial biomass C is known as the metabolic quotient (qCO_2). The value of qCO_2 increases under conditions which stress the microorganisms because relatively more C is allocated to energy and maintenance requirements, instead of growth, and is released as CO_2 (Killham, 1985; Anderson and Domsch, 1993). The C mineralization rate (C_{min}) was divided by the microbial biomass C (C_{mic}) both at the beginning (C_{mic} at $t = 0$ days) and at the end (C_{mic} at $t = 28$ days) of the incubation to determine initial and final qCO_2 as shown in the equation below:

$$qCO_2 = \frac{C_{min}}{C_{mic}} \qquad \text{for all } C_{mic} > 0 \tag{3}$$

2.10. Nematode abundance and classification

Prior to extraction at the University of Toledo, soil samples were stored at particular incubation tensions and 15°C. Nematodes were extracted from soil (140–200 g^{-1} moist soil) using Cobb's sifting and gravity method (Thorne, 1961; Ayoub, 1980) modified by triplicate passes through 710, 250, 150, 75, and 45-μm-mesh sieves. The final pass through the sieves was followed by centrifugal flotation (Caveness and Jensen, 1955) modified by using a 1:1 (vol:vol) aqueous sugar solution and centrifuging for 1 min (Neher and Campbell, 1994). Taxonomic families were assigned to a trophic group (plant parasites, bacterivores, fungivores, omnivores, and predators) according to Yeates et al. (1993). Nematodes classified as omnivore/predators were assigned to the predator group. There were no other omnivores identified in these samples and plant parasitic nematode data are reported elsewhere (Neher et al., 1999). Voucher specimens were preserved in 10% formalin and 1% glycerin, and sealed with Parafilm®.

2.11. Statistical analysis

Confidence intervals (99%) were determined for initial and final values of each measurement for each set of 10 cores and for the rates of change of specific parameters during incubation for every set of 10 pairs of cores equilibrated at a certain tension. Values lying outside the 99% confidence intervals were removed from the data sets. Thus only data within the 99% confidence intervals were included in the calculation of median, mean and standard deviation and in statistical comparisons.

Analysis of variance was used to determine if there were statistically significant differences among means at different tensions for normally distributed data. For non-normally distributed data, the ANOVA on ranks was used to compare median values. Multiple comparison testing, either the Bonferroni *t*-test (for normally distributed data) or Dunn's test (for data non-normally distributed), was used to assess significant differences between groups at the 95% confidence levels.

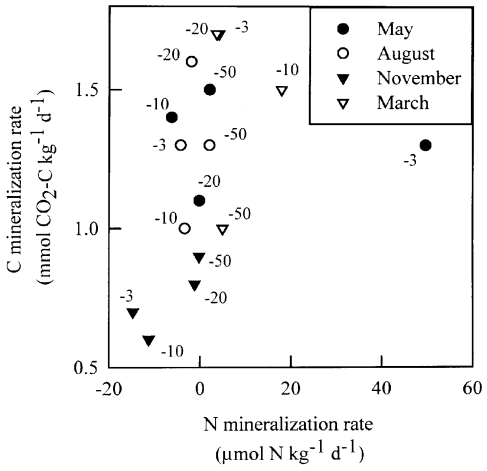

Fig. 1. C and N mineralization rates in an old field for all matric potentials and sampling dates. The numbers next to the symbols indicate matric potential in kPa.

3. Results

Data were pooled for all sampling dates and matric potentials, and C mineralization was plotted against N mineralization (Fig. 1). If the high N mineralization rate at −3 kPa in May was not considered, C and N mineralization were significantly and positively related to one another (Fig. 1, Table 3). However, the relationship between C and N mineralization was not consistent among sampling periods or matric potentials (Fig. 1). There was a significant, positive linear relationship between C and N mineralization only in November (Table 3).

Microbivorous nematodes were present at all four matric potentials in all sampling periods (Table 4). There was no

Table 3
Linear regression analysis of C mineralization versus N mineralization for four different sampling periods in old field soil incubated at four different matric potentials

Sampling month	Slope	r^2	P^a
May	− 0.0003	0.002	0.92
August	0.019	0.13	0.38
November	0.013	0.71	0.009*
March	− 0.003	0.006	0.86
All sampling dates	0.008	0.119	0.19
All dates (without −3 kPa in May)	0.03	0.33	0.03*

[a] *P* values less than 0.05 are statistically significant and indicated with an asterisk (*).

Table 4
Initial abundance, mean number of individuals per kg dry soil, from replicate cores (±standard deviation), of nematode trophic groups at four matric potentials in an old field in May, August and November 1997, and March 1998

		−3 kPa	−10 kPa	−20 kPa	−50 kPa
May	Bacterivores	5900 (3800)	19,000 (4900)	6000 (7700)	7900 (3600)
	Fungivores	390 (310)	270 (480)	350 (470)	170 (150)
	Predators	1800 (500)	490 (660)	2700 (5700)	1800 (2400)
	Total	15,000 (8900)	34,000 (8000)	19,000 (17,000)	18,000 (6300)
August	Bacterivores	9100 (1600)	3220 (990)	4900 (1100)	8100 (1400)
	Fungivores	1400 (1100)	88 (130)	440 (520)	6400 (2100)
	Predators	6500 (3200)	370 (132)	100 (130)	4600 (5300)
	Total	22,000 (3000)	5800 (1200)	6900 (890)	26,000 (4500)
November	Bacterivores	ND[a]	15,000 (3800)	ND	17,000 (6200)
	Fungivores	ND	1200 (480)	ND	3600 (680)
	Predators	ND	3900 (500)	ND	3400 (1200)
	Total	ND	36,000 (5100)	ND	31,000 (5700)
March	Bacterivores	4300 (1200)	16,000 (2700)	7100 (940)	9400 (4000)
	Fungivores	880 (370)	1400 (440)	1000 (500)	2600 (750)
	Predators	160 (72)	3300 (1600)	2000 (610)	1100 (420)
	Total	6800 (2900)	33,000 (4600)	17,000 (3000)	17,000 (6200)

[a] Not determined.

consistent trend in nematode abundance with matric potential, but bacterial-feeding nematodes were always more abundant than fungal-feeding or omnivorous/predaceous nematodes. With two exceptions, average values of qCO_2 calculated for all sampling dates and matric potentials ranged from 0.08 to 0.29 mmol CO_2-C mmol C^{-1} day^{-1}. In August and November, the values of qCO_2 at −50 kPa were approximately three and four times greater than the next highest value of qCO_2. When microbivorous nematode abundances from the beginning and end of the incubation period were averaged and plotted against average qCO_2, there was a significant and positive linear relationship at −50 kPa (Fig. 2, Table 5). Values of qCO_2 were not significantly related to microbivorous nematode abundance at any other matric potential (Table 5).

Average values from the beginning and end of the incubation period were calculated for microbial biomass C and C:N ratios and dissolved organic C and C:N ratios. Dissolved C:N ranged from four to 16 and microbial C:N ranged from two to 14 (Table 6). Increases in qCO_2 occurred as microbial biomass C decreased (Fig. 2). This relationship was significant ($P < 0.10$) at −10, −20, and −50 kPa (Table 5). Slope values indicate that the response of qCO_2 to biomass C at −50 kPa was at least an order of magnitude greater than that determined for the other three matric potentials (Table 5). A similar increase in qCO_2 occurred as microbial biomass C:N decreased (Fig. 2). This relationship was significant at −10 and −50 kPa, but the increase in qCO_2 at −50 kPa was an order of magnitude greater than at all other matric potentials, as indicated by slope values (Table 5). In contrast, there were no significant linear relationships between qCO_2 and dissolved organic C or dissolved C:N ratios (Fig. 2, Table 5).

4. Discussion

4.1. Uncoupling of C and N mineralization

Our results show that C and N mineralization in this old field

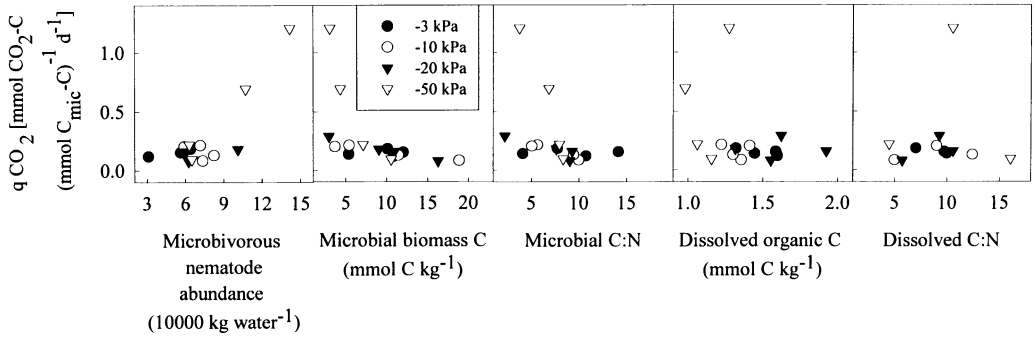

Fig. 2. C mineralization per unit microbial biomass C (qCO_2) at four matric potentials in an old field soil, plotted as a function of: 1. microbivorous nematode abundance (numbers of individuals per kg of water), 2. microbial biomass C (mmol C kg^{-1}), 3. microbial C:N ratios, 4. dissolved organic C (mmol C kg^{-1}), and 5. dissolved C:N ratios. Values given are means of replicate cores from the beginning and end of the incubation.

Table 5
Linear regression analyses of C mineralization per unit microbial biomass C (qCO_2) versus microbivorous nematode abundance, microbial biomass C, microbial C:N, dissolved organic C, and dissolved C:N ratios in old field soil incubated at four matric potentials

Soil property	ψ_m (kPa)	Slope	r^2	P[a]
Microbivorous nematode abundance	−3	0.018a[b]	0.94	0.16
	−10	−0.037a	0.33	0.42
	−20	0.014a	0.38	0.58
	−50	0.133b	0.98	0.009*
Microbial biomass C	−3	0.001a	0.02	0.85
	−10	−0.009a	0.95	0.03*
	−20	−0.016a	0.99	0.003*
	−50	−0.139b	0.84	0.08*
Microbial C:N	−3	−0.000a	0.003	0.94
	−10	−0.024a	0.93	0.04*
	−20	−0.024a	0.82	0.10*
	−50	−0.235b	0.95	0.03*
Dissolved organic C	−3	−0.160a	0.58	0.24
	−10	−0.169a	0.05	0.78
	−20	0.004a	0.00	0.99
	−50	1.73a	0.19	0.57
Dissolved C:N	−3	−0.014a	0.94	0.16
	−10	0.007a	0.17	0.73
	−20	0.026a	0.36	0.59
	−50	−0.008a	0.01	0.95

[a] P values less than 0.10 are statistically significant and indicated with an asterisk (*).
[b] Significant differences among matric potentials for a given soil property are indicated with a different letter.

soil appear to be coupled when data from different sampling dates, obtained at different matric potentials, are analyzed in the aggregate (Fig. 1; Table 3). However, this type of meta-analysis of the data can mask the fact that such coupling does not hold true for all sampling periods (Fig.1; Table 3). Differences in the relationship between C and N mineralization at a given sampling period may be due to differences in microbial community composition, grazing of microbes, substrate quality, or temperature. Although root respiration may also affect measurement of C mineralization, and thus the ratio of C to N mineralization, it probably was not a factor in this experiment because of the long pre-incubation period in the dark (28 days after soil core collection) and the absence of plants from the cores during the incubation period.

Microbes utilize a portion of the C they ingest for energy, respiring CO_2 and mineralizing excess N. If the C:N ratio of substrate utilized for biomass production is greater than the microbial C:N ratio, then N will be immobilized. In this study, average dissolved C:N ratios, assumed to represent available substrate, were higher in November and March and lower in May than microbial C:N ratios (Table 6). These ratios may help explain the immobilization at high matric potentials and lack of net mineralization at low matric potentials in November (Fig. 1). However, in May, N was only mineralized at −3 kPa, and not at all tensions as expected. Net N mineralization in March may have resulted from a burst of microbial activity due to the temperature increase in the chambers when N immobilization was otherwise likely, based on C:N ratios.

Our study focused on a narrow range of water potentials over which water content should be sufficient for aerobic activity (Table 1). In addition, the effect of trophic interactions among nematodes and microorganisms on C and N mineralization should be most apparent in this range because water-filled pore sizes spanned the range from greater than nematode body diameters (ample habitat available for grazing) to smaller than nematode body diameters (restricted or no habitat available for grazing). The differential effect of microbivorous grazing by microfauna on C and N mineralization may have decoupled C and N mineralization at different sampling dates.

Microbivorous grazing increases nutrient mineralization directly and also indirectly influences it by affecting microbial activity. Indirect effects of microbivorous grazing include compensatory growth of microorganisms (Bengtsson et al., 1993) and changes in microbial community structure (Griffiths, 1994b). The direct contribution of soil fauna to N mineralization can be as high as 30% (Verhoef and Brussaard, 1990; Griffiths, 1994a,b). However, the direct contribution of soil fauna to C mineralization is minimal (Sohlenius, 1980; Hassink et al., 1993). Thus, the observed ratio of C to N mineralization can also depend on the magnitude of microbivorous grazing, which influences the excretion rate of mineral N. The magnitude of grazing is believed to be dependent on the relative density of grazers and their food, which in soil will be controlled by soil moisture and matric potential.

Because soil cores were collected throughout the year, seasonal variation in the relationship between microbivorous

Table 6
C:N ratios for microbial biomass and dissolved substrate of an old field soil incubated at four different matric potentials. Ratios presented are the means of the beginning (time = 21 days from establishment of matric potential) and end (time = 49 days from establishment of matric potential) of the incubation

Sampling date	−3 kPa		−10 kPa		−20 kPa		−50 kPa	
	Microbial C:N	Dissolved C:N	Microbial C:N	Dissolved C:N	Microbial C:N	Dissolved C:N	Microbial C:N	Dissolved C:N
May	7.8	7.0	10.0	5.0	9.0	5.7	8.0	4.4
August	10.8	ND[a]	5.6	ND	7.6	ND	6.8	ND
November	4.1	10.0	5.0	9.0	2.2	9.2	3.7	10.5
March	14.1	9.7	9.4	12.4	9.3	10.6	8.4	16.0

[a] Not determined.

grazing and C and N mineralization with changes in matric potential could be evaluated. However, collection of soil cores at different times of the year led to incubations at different temperatures, and temperature also affects C and N mineralization in several ways. Soil temperature in the field on the date of core collection and the temperature history prior to sampling undoubtedly affected the composition of the community. In fact, nematode and microbial community varied seasonally after preincubation (Neher et al., 1999) and for cores extracted immediately after sampling (Savin et al., 2001). Incubation temperatures affect biochemical reaction rates as evidenced by the increase in C mineralization caused by a perturbation in temperature during one of the incubations. Additionally, different nematode taxa have different temperature optima (Ferris et al., 1995). Therefore, it is important to consider that the relationships developed between C and N mineralization and microbivorous grazing with changes in matric potential depended on the particular incubation temperature and initial field community composition as reported in Savin et al. (2001).

4.2. Evidence for exclusion

The exclusion hypothesis stipulates that nematode habitat comprises water-filled pores with diameters greater than nematode body diameters (Darbyshire, 1976; Elliott et al., 1980). If this mechanism dominated in our soil and trophic interactions were constrained to water-filled pores with openings of appropriate diameter, then nematode numbers were expected to decrease as matric potential decreased. Nematodes have been correlated with water-filled pores in the range of 30–90 μm (Hassink et al., 1993). Bacterial feeding nematodes in this soil have body diameters greater than 9 μm (Görres et al., 1999) and in general nematodes are considered to have body diameters greater than 15 μm (Jones et al., 1969). Matric potentials investigated in this study included −3, −10, −20, and −50 kPa, which correspond to pore openings greater than 100, 30, 15, and 6 μm, respectively. According to the exclusion hypothesis, potential habitat for nematodes, and hence trophic interactions, should only occur at the higher matric potentials (i.e. in water-filled pores with openings >9 μm). However, nematodes were present at all matric potentials in this study (Table 4), indicating the temporal dominance of the enclosure mechanism over the exclusion mechanism.

4.3. Evidence for enclosures

The enclosure hypothesis states that microfauna become trapped in isolated water-filled pore pockets when soil dries. Nematode density, measured as nematodes per unit volume of water, increases as soil dries and, as a result, grazing pressure should also increase. Enclosures are akin to the micro-environment of protozoa (Hattori, 1994). We have assumed that changes in matric potential affect protozoan grazing in parallel with bacterivorous nematode grazing because both groups of microfauna are constrained by pore structure. Enclosures may reside in aggregates. The habitable pore space of an aggregate may be limited by an anaerobic center, in particular when the aggregate is large (Revsbeck et al., 1985). However, even when the center of the aggregate is anaerobic, there is enough oxygen diffusing into the aggregate to maintain aerobic activity in considerable aggregate volumes.

Microfauna feed selectively, increase bacterial activity, and are likely to consume actively growing bacteria to maintain their own growth (Griffiths, 1994a). By removing microbial biomass, grazing induces density-dependent growth of microorganisms as long as carbon is not limiting. Therefore, grazing may not result in any changes in the standing crop of the microbial biomass. However, if the enclosure hypothesis is correct, grazing, and consequently microbial activity, would be more intense at low matric potentials because, in enclosures, grazers would be in closer proximity to their forage. Under equilibrium conditions, we then expect more CO_2 to be released per unit microbial biomass C as grazing intensity increases. In other words, qCO_2 would be related to microbivorous nematode density only when both nematodes and microorganisms are trapped within enclosures. We found a strong, positive linear relationship between microbivorous nematodes and qCO_2 at −50 kPa (Fig. 2). In addition, qCO_2 was significantly more sensitive to changes in biomass C at −50 kPa than any other matric potential. The negative relationship between microbial biomass C:N and qCO_2 at −50 kPa was also significantly different from other matric potentials, suggesting a shift towards the predominance of bacteria coinciding with increased microbial activity. Together the relationships of these three variables with qCO_2 support the hypothesis that grazing of microorganisms stimulates bacterial activity at our lowest matric potential.

Alternatively, grazing of fungal hyphae in interaggregate spaces could have decreased fungal biomass and increased microbial respiration per unit biomass preferentially in dry soil. Fungivorous nematodes showed seasonal differences, with abundance at −50 kPa in August and November exceeding abundance in May and March (Table 4). In the absence of grazing, fungal degradation of substrates with high C:N ratios could also lead to a comparatively high qCO_2. Previously dry field conditions during the summer (mean matric potential in the field was approximately −150 kPa in July and −50 kPa in August) could have repressed bacterial activity and selected for the establishment of a predominantly fungal community in August. While bacterial activity becomes limited by decreased substrate diffusion in dry soil, fungi have the ability to search for water and substrates via hyphal extension. However, bacteria located inside water-filled pores in aggregates would not directly benefit from nutrients released in interaggregate spaces while conditions limited substrate diffusion. Consequently, fungal activity does not appear to explain the negative relationship between qCO_2 and biomass C:N at −50 kPa.

Microbial C:N ratios in November were the lowest of all the sampling periods, suggesting bacterial dominance of the microbial biomass. Therefore, the high qCO_2 at −50 kPa in November could not be related to fungal dominance of the microbial biomass. Dissolved organic C was not related to qCO_2 at −50 kPa (Table 5). Decreased diffusion of easily degraded substrates at −50 kPa may have resulted in stressful conditions, increasing qCO_2. However, contradicting this hypothesis, microbial biomass C increased during incubation at −50 kPa in November (data not shown), so that C was apparently available for biomass accretion. Microfaunal grazing of microorganisms could explain the high values of qCO_2 in November. Because microbial C was initially low in November, grazing of microorganisms could increase nutrients and stimulate microbial growth, leading to an overall increase in biomass C during incubation. Microbivorous nematode abundance was quite high in November at −50 kPa compared to other sampling periods, supporting this interpretation.

In conclusion, the relationship between C and N mineralization varied depending on matric potential and sampling date. Decoupling may be enhanced by grazing of microorganisms because of differential effects of grazing on C and N mineralization and effects on microbial community composition. In support of the dominance of the enclosure hypothesis, we found that (1) nematode abundance did not decline with matric potential; and (2) qCO_2 was significantly more sensitive to microbivorous nematode density, microbial biomass C and microbial C:N ratios at −50 kPa than at other matric potentials. Hypotheses involving the quantity and quality of dissolved substrate did not appear to explain qCO_2 at −50 kPa. At some point, as matric potential continues to decrease past −50 kPa, exclusion will most likely become the predominant mechanism. However, at the matric potentials evaluated in this study, grazing appeared to enhance microbial activity per unit microbial biomass more in dry soils than in wet soils, possibly through the formation of enclosures.

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