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Elevated CO_2 alters functional attributes of nematode communities in forest soils

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Summary

1. We tested the effects of elevated concentrations of atmospheric CO_2 on herbivorous nematodes in soil supporting plantations of Loblolly Pine (*Pinus taeda*) or Sweet Gum (*Liquidambar styraciflua*) trees in FACE experiments in the Eastern USA. We expected any net increase in carbon allocation to the rhizosphere to increase the abundance, biomass or respiration of the nematode community.

2. Data were analysed with effect of CO_2 concentration nested within month and year to isolate the maximum potential effect of CO_2 treatment on soil nematode communities.

3. Elevated CO_2 decreased total abundance of nematodes in both forests, but impacts were greater in Sweet Gum than Loblolly Pine forests. Soil nematode community respiration and biomass increased with elevated CO_2 in Loblolly Pine, but decreased with fumigation in Sweet Gum forests.

4. Fungivores were the only trophic group showing a consistent response at both sites, with reduced abundance, biomass and respiration at elevated CO_2.

5. Estimated total respiration of soil nematode communities ranged from 2·9–11·2 g C m^{-2} $year^{-1}$ in Pine soils and 0·6–4·7 g C m^{-2} $year^{-1}$ in Sweet Gum soils, representing ≤1% of net primary production in these forests.

6. Our results indicate that effects of elevated CO_2 on soil nematode communities will not necessarily have a simple functional relationship with rhizosphere carbon allocation.

Key-words: FACE, nematode biomass, soil function, soil productivity, soil respiration

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Introduction

Net ecosystem responses to elevated CO_2 cannot be predicted solely from the physiological responses of plants because carbon inputs have ecological consequences that ramify throughout the ecosystem. For example, increased carbon fixation can increase root production, exudation, root-to-shoot ratios or fine root turnover (O'Neill, Luxmoore & Norby 1987; Conroy, Milham & Barlow 1992; Comins & McMurtrie 1993; Shepherd & Davies 1993; Zak *et al*. 1993; Curtis *et al*. 1996; Allen *et al*. 2000). In turn, these carbon subsidies can stimulate microbial activities in the rhizosphere that increase short-term mineral nutrient availability. However, labile reservoirs of nitrogen and phosphorus will be depleted quickly unless decomposition of dead organic matter also increases (Curtis *et al*. 1996; Reynolds *et al*. 1996). Thus soil processes may ultimately limit the responses of ecosystems to CO_2 enrichment (Leadley & Reynolds 1992; Jones *et al*. 1998).

Relatively few studies of carbon flow in soils have examined the impacts of elevated CO_2 on invertebrate communities, with the exception of a few grassland and agricultural ecosystems (Yeates & Orchard 1993; Klironomos *et al*. 1996, 1997; Yeates *et al*. 1997, 1999, 2003; Blair, Todd & Callaham 2000; Hungate *et al*. 2000). However, the ecological roles of soil invertebrates include plant herbivory and the mineralization of nutrients in the detrital food web, both of which may be affected by change in carbon-flow patterns and, in turn, have important impacts on ecosystem behaviour. Of the major groups of soil invertebrates, nematodes are particularly convenient for investigating impacts of CO_2 on soil food webs (Bongers & Ferris 1999). They are abundant, typically ranging from 10^5–10^7 m^{-2} in the upper 15 cm of forest soils (Sohlenius 1980), and play at least five trophic roles (Yeates *et al*. 1993). As much as 37–59% of the total soil nematode population in forest soils is herbivorous (Ausmus *et al*. 1978; Sohlenius 1980; Popovici 1984; Buttner 1989), with most of the rest consuming microflora. Hence nematodes may be expected to respond both directly to changes in plant carbon allocation to the below-ground environment, and indirectly

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to changes in microbial communities that respond directly to plant carbon inputs.

In this study we compared soil nematode communities at two Free Air Carbon Enrichment (FACE) experiments: a Loblolly Pine plantation and a Sweet Gum plantation. We expected abundance, biomass and respiration of herbivorous nematode populations to increase with elevated carbon fixation, due to an increase in carbon stocks in soil (e.g. fine roots) (Hoeksema, Lussenhop & Teeri 2000; Yeates *et al*. 2003). Alternatively, bacterivores and fungivores could increase in abundance, biomass and respiration if plants respond to CO_2 fumigation by increasing exudation of labile substrates, fine-root turnover or mycorrhizal colonization. In any case we expected a net increase in carbon allocation to the rhizosphere to result in a net increase in abundance, biomass and/or respiration of the soil nematode community.

Materials and methods

DUKE FIELD SITE

This study was part of the FACE experiment in a Loblolly Pine (*Pinus taeda*) plantation at Duke Forest, Durham, North Carolina, USA. Trees were planted in 1983 as 3-year-old seedlings at a spacing of 2·4 × 2·4 m. Gassing began on 27 August 1996, when trees were ≈14 m tall with closed canopy, with Pine accounting for 98% of the basal area. CO_2 enrichment is continuous (24 h day^{-1} every day of the year) except in extreme weather. The soil is an unfertilized Ultic Alfisol of the Enon Series, pH 5·75 (Schlesinger & Lichter 2001). The experimental design consists of three rings with an interior CO_2 concentration ≈180 p.p.m. above ambient (target concentration is 550 p.p.m.), and three rings as controls.

OAK RIDGE FIELD SITE

The second site was part of the FACE experiment in a 10-year-old Sweet Gum (*Liquidambar styraciflua*) plantation near Oak Ridge, Tennessee, USA. At this site two rings receive elevated CO_2 concentrations (target concentration 565 p.p.m. CO_2) and three rings remain under ambient conditions. The plantation was established in autumn 1988 with 1-year-old, bare-rooted seedlings planted at a spacing of 2·3 × 1·2 m. Gassing began in April 1998 when stand basal area was 29 m^2 ha^{-1} with an average tree height of 12·4 m and stem diameter of 13 cm. Gassing continues annually during the growing season (24 h day^{-1} every year, between April and November). The soil is an unfertilized Aquic Hapludult with a silty clay loam texture, pH 5·5–6·0, and bulk density is 1·5 g cm^{-3} (Van Miegroet *et al*. 1994).

EXTRACTION AND ENUMERATION

Six soil cores (2 cm diameter, 10 cm depth) were collected at each sampling date from random locations within the four zones designated for soil sampling in each treatment ring (ambient or elevated CO_2) at each forest site (Hendrey & Kimball 1994; Lewin *et al*. 1994; Hendrey *et al*. 1999). Sampling dates were May, July and September 1999 and 2000. On each occasion, samples were pooled and one composite soil sample per ring was analysed for nematodes. For each of six sampling dates, a total of 12 composite samples (two forests × two treatments × three replicate rings) of soil were collected from each site.

Each composite soil sample (300–450 g) was subsequently separated into two equal subsamples (laboratory duplicates). From each subsample, roots and organic matter were separated from mineral soil with an 810 μm mesh sieve. Nematodes were extracted from the soil organic fraction in a mist chamber for 4 days. Nematodes from mineral soil were extracted by a cotton-wool filter method followed by sucrose centrifugal flotation (Oostenbrink 1960; Townshend 1963). Twenty per cent of nematodes in soil and organic fractions (including roots) were enumerated and at least 150 individuals per subsample were identified to taxonomic family and genus (Table 1) according to Goodey (1963); Andrássy (1968, 1979, 1980, 1984); Maggenti (1983, 1991); Bongers (1987); Maggenti *et al*. (1987); Nickle (1991); Hunt (1993). Taxonomic families were assigned a trophic grouping based on Yeates *et al*. (1993). Abundance of non-herbivorous and herbivorous nematodes was standardized to g dry soil and cm root length, respectively. An estimate of total abundance of nematodes in the combined organic and soil fractions of each subsample was calculated proportional to the mass of both soil fractions.

To determine total length, all roots per sample were spread uniformly in 15 cm diameter glass Petri dishes on a light table, and digital images were obtained with a Sony CCD video camera equipped with a macro lens. Root length was quantified by the line-intercept method (Newman 1966; Harris & Campbell 1989), calculated from the number of root intercepts along parallel scan lines using KS-300 video imaging software (AXIOVISION 2·0, Carl Zeiss Vision GmbH, Hallbergmoos, Germany).

Our evaluation of metabolic characteristics of nematode communities was based on calculated rates of respiration for each genus. Standard calculations for nematode respiration (Andrássy 1956; Klekowski, Wasilewska & Paplinska 1972; Ferris, Lau & Venette 1995) and productivity (Yeates 1979) were based on individual biomass. Fresh weight biomass was calculated according to length and width measurements of representative individual nematodes for all trophic groups, based on digital image analysis. On average, ≈25% of fresh weight is dry weight (Ausmus *et al*. 1978). The numbers of individuals measured per life stage was proportional to age distributions observed in extracted field populations. Total fresh weight was calculated for each genus (Table 1) and a weighted sum computed for the entire community. These analyses were restricted to genera represented in >6% of subsamples.

Table 1. Mean ± SE abundance and biomass of nematode genera in soil pooled across 2 years

Genus	Trophic group*	ng C per worm[c]	Pine		Sweet Gum	
			Ambient	Elevated	Ambient	Elevated
Acrobeloides[b]	3	90 ± 7	2·83 ± 0·43	3·23 ± 0·80	0·67 ± 0·17	0·66 ± 0·22
Alaimus[b]	3	40 ± 10	0·91 ± 0·0	0·03 ± 0·01	0·10 ± 0·03	0·09 ± 0·03
Aphelenchoides[b]	2	50 ± 3	5·69 ± 1·74	2·93 ± 0·60	0·95 ± 0·19	0·93 ± 0·23
Aphelenchus[b]	2	170 ± 30	0·04 ± 0·01	0·09 ± 0·04	0·15 ± 0·04	0·18 ± 0·05
Aporcelaimellus[b]	5	4790 ± 1836	0·27 ± 0·07	0·31 ± 0·13	0·07 ± 0·01	0·04 ± 0·01
Basiria[a]	1	100 ± 16	0·07 ± 0·01	0·03 ± 0·004	0·02 ± 0·01	0·02 ± 0·01
Belondira[b]	8	290 ± 53	0·13 ± 0·03	0·36 ± 0·14	0·21 ± 0·05	0·18 ± 0·05
Boleodorus[a]	1	70 ± 8	0·19 ± 0·11	0·04 ± 0·02	0·16 ± 0·07	0·63 ± 0·29
Cephalobus[b]	3	140 ± 19	0·26 ± 0·06	0·30 ± 0·09	0·38 ± 0·06	0·26 ± 0·06
Clarkus[b]	5	750 ± 175	0·14 ± 0·03	0·13 ± 0·04	0·05 ± 0·01	0·06 ± 0·03
Coslenchus[a]	1	100 ± 14	0·02 ± 0·0	0·06 ± 0·024	0·10 ± 0·04	0·24 ± 0·0
Criconema[a]	1	330 ± 710	0·07 ± 0·03	0·03 ± 0·01	0·01 ± 0·004	0·13 ± 0·05
Criconemella[a]	1	390 ± 106	0·05 ± 0·02	0·02 ± 0·0	0·01 ± 0·001	0·11 ± 0·02
Dauer larvae[b]	0	150 ± 9	0·97 ± 0·27	0·43 ± 0·09	0·88 ± 0·20	0·34 ± 0·09
Diphtherophora[b]	2	210 ± 29	0·14 ± 0·08	0·20 ± 0·07	0·20 ± 0·04	0·19 ± 0·04
Ditylenchus[b]	2	60 ± 3	0·24 ± 0·04	0·20 ± 0·04	0·34 ± 0·07	0·39 ± 0·09
Ecphyadophora[a]	1	30 ± 4	0·06 ± 0·01	0·06 ± 0·01	0·11 ± 0·03	0·26 ± 0·12
Eudorylaimus[b]	5	370 ± 72	0·15 ± 0·03	0·20 ± 0·05	0·11 ± 0·03	0·10 ± 0·04
Filenchus[b]	2	60 ± 3	4·12 ± 1·07	4·95 ± 1·20	1·19 ± 0·20	0·78 ± 0·18
Helicotylenchus[a]	1	200 ± 21	0·27 ± 0·07	0·22 ± 0·07	0·12 ± 0·05	0·11 ± 0·04
Hemicycliophora[a]	1	480 ± 45	0·36 ± 0·06	0·20 ± 0·03	0·05 ± 0·02	0·05 ± 0·0
Hexatylus[b]	2	80 ± 22	0·09 ± 0·03	0·09 ± 0·04	0·27 ± 0·05	0·55 ± 0·14
Lelenchus[a]	1	40 ± 5	0·15 ± 0·04	0·03 ± 0·01	0·04 ± 0·01	0·24 ± 0·10
Macroposthonia[a]	1	240 ± 46	0·06 ± 0·0	0·07 ± 0·05	0·05 ± 0·02	0·21 ± 0·08
Malenchus[a]	1	60 ± 4	0·07 ± 0·02	0·10 ± 0·03	0·03 ± 0·02	0·01 ± 0·0
Meloidogyne juveniles[a]	1	70 ± 11	0·03 ± 0·002	0·07 ± 0·02	0·04 ± 0·02	0·17 ± 0·12
Mesodorylaimus[b]	8	610 ± 94	0·15 ± 0·05	0·16 ± 0·08	0·12 ± 0·03	0·13 ± 0·04
Mylonchulus[b]	5	900 ± 450	0·11 ± 0·03	0·11 ± 0·03	0·08 ± 0·01	0·05 ± 0·01
Oxydirus[a]	1	730 ± 96	0·02 ± 0·0	0·02 ± 0·01	0·37 ± 0·10	0·29 ± 0·05
Panagrolaimus[b]	3	270 ± 119	0·11 ± 0·03	0·08 ± 0·06	0·12 ± 0·03	0·06 ± 0·01
Paratylenchus[a]	1	60 ± 26	0·23 ± 0·10	0·09 ± 0·02	0·04 ± 0·0	0·04 ± 0·03
Plectus[b]	3	290 ± 51	0·65 ± 0·13	0·74 ± 0·17	0·37 ± 0·08	0·31 ± 0·08
Prismatolaimus[b]	3	60 ± 10	0·24 ± 0·07	0·22 ± 0·07	0·15 ± 0·03	0·14 ± 0·05
Psilenchus[a]	1	170 ± 29	0·19 ± 0·17	0·003 ± 0·0003	0·08 ± 0·04	0·02 ± 0·02
Pungentus[a]	1	970 ± 170	0·07 ± 0·03	0·04 ± 0·01	0·09 ± 0·0	0
Rotylenchus[a]	1	490 ± 126	0·08 ± 0·03	0·12 ± 0·04	0	0
Teratocephalus[b]	3	30 ± 3	0·06 ± 0·01	0·14 ± 0·05	0·08 ± 0·02	0·18 ± 0·07
Trophurus[a]	1	140 ± 28	0	0	0·05 ± 0·02	0·28 ± 0·07
Tylencholaimus[b]	2	130 ± 8	0·50 ± 0·13	0·49 ± 0·16	0·27 ± 0·06	0·09 ± 0·02
Tylenchorhynchus[a]	1	170 ± 48	0·04 ± 0·01	0·02 ± 0·02	0·47 ± 0·19	0·04 ± 0·03
Xiphinema[a]	1	870 ± 66	0·04 ± 0·01	0·05 ± 0·02	0·12 ± 0·02	0·06 ± 0·02

*Trophic group defined according to Yeates *et al*. 1993.
[a]Herbivorous taxa expressed as numbers or ng FW cm^{-1} root length.
[b]Non-herbivorous taxa expressed as numbers or ng FW g^{-1} dry soil.
[c]Mean ± SE (Andrássy 1956). Each value represents ≈10–720 worms depending on relative occurrence.
Restricted to genera represented in >6% of subsamples to optimize precision by avoiding uncommon and highly variable taxa.

Estimates of individual respiration were based on average biomass of an individual of each genus (Klekowski *et al*. 1972). The total respiration of the genus was the product of individual rate and total number of individuals. Total community respiration was calculated as the sum of respiration rates of all genera. Our estimates were converted to maximum potential daily values, expressed as mg C m^{-2} day^{-1} assuming ASTM conditions, adjusted to 20 °C, given a soil bulk density of 1·5 g cm^{-3}, 10 cm depth, respiratory quotient (RQ) 0·95, and 24 h day^{-1}. We assumed optimum environmental conditions supporting maximum rates of respiration, because these calculations were performed with the sole intent of evaluating potential changes in energy flow through the soil nematode community, rather than predicting actual values of soil respiration.

STATISTICAL ANALYSIS

Data from each forest type were analysed separately to determine the effect of CO_2 concentration on function of soil nematode communities. Measures of abundance, biomass and respiration were analysed by ANOVA with effect of CO_2 concentration nested within month and year. We chose a nested design to account for seasonal changes while focusing on effects of CO_2 concentration. Abundance, respiration and biomass of individual trophic groups were transformed as ln(x), and proportions

of individual trophic groups as arcsine of the square root (x) to meet normality assumptions prior to statistical analysis. Ranks were computed for respiration of each trophic group because it could not be transformed to meet assumptions of normality. ANOVA was performed using the GLM procedure in SAS Version 8 (SAS Institute 2000).

Results

COMMUNITY IMPACTS

The total number of nematodes averaged five to 18 worms g^{-1} dry soil, representing 800–3100 mg C as biomass, and respiring an estimated 4·5–16·8 mg C m^{-2} day^{-1} (Table 2). Total numbers of nematodes were greater in Pine soils, but decreased with elevated CO_2 treatment in both forests. Community respiration and biomass increased with elevated CO_2 in Loblolly Pine, but decreased with elevated CO_2 in Sweet Gum forests. In all cases relative changes in nematode communities were greater in soils with Sweet Gum than Loblolly Pine trees.

TROPHIC IMPACTS

The abundance, respiration and biomass of both bacterivores and fungivores were affected significantly by elevated CO_2 at both sites. Bacterivores showed reductions in all three community characteristics at both sites, but the relative changes were usually greater in Sweet Gum than in Pine plantations. The fungivores also showed a reduction in respiration and biomass with CO_2 fumigation at both sites, but abundance of fungivores actually increased with elevated CO_2 in both forests.

The responses of other trophic groups were more variable. Predator abundance, respiration and biomass declined with elevated CO_2 in Sweet Gum soils. In Loblolly Pine soils, biomass was the only attribute of predatory nematodes affected by elevated CO_2; here biomass increased in response to fumigation. Herbivores responded to elevated CO_2 only at the Sweet Gum site where abundance, respiration and biomass declined with fumigation. Omnivore respiration and biomass showed very small, but significant, reductions in response to CO_2 at the Sweet Gum site, and a significant increase in biomass in Loblolly Pine forest soils.

Discussion

Elevated CO_2 clearly had a variety of effects on soil nematode communities. Impacts were also more pervasive and tended to be greater in soils of Sweet Gum than Loblolly Pine forest. Our original hypothesis anticipated a net increase in nematode abundance, biomass and respiration in response to elevated CO_2 as the

Table 2. Effect of elevated CO_2 and season on nematode community indices in forest soils

	Pine			Sweet Gum		
Index	Ambient (n = 29)	Elevated (n = 32)	%r^2	Ambient (n = 36)	Elevated (n = 25)	% r^2
Total number	18·3 ± 3·76[a]	16·6 ± 3·40	52	7·3 ± 0·98[a]	5·7 ± 0·96	68
Total respiration[b]	15·89 ± 3·31[a]	16·83 ± 4·33	44	7·46 ± 1·02[a]	4·53 ± 0·62	65
Total biomass[c]	2900 ± 610[a]	3100 ± 800	31	1400 ± 190[a]	800 ± 120	65
Abundance (%)						
Herbivores	11·8 ± 1·3	12·3 ± 1·3	13	14·2 ± 1·7[a]	10·4 ± 1·3	49
Fungivores	50·3 ± 2·5[a]	50·7 ± 2·1	33	40·7 ± 2·1[a]	46·6 ± 3·2	79
Bacterivores	33·4 ± 2·3[a]	32·1 ± 2·1	38	38·4 ± 2·8[a]	36·4 ± 3·7	70
Omnivores	0·8 ± 0·2	0·9 ± 0·2	20	2·3 ± 0·4	2·7 ± 0·4	29
Predators	3·1 ± 0·4	3·1 ± 0·4	14	2·7 ± 0·3[a]	2·2 ± 0·4	49
Respiration[b]						
Herbivores	2·97 ± 0·91	3·17 ± 0·81	23	2·57 ± 0·57[a]	1·00 ± 0·25	64
Fungivores	3·46 ± 0·91[a]	2·95 ± 0·64	41	1·32 ± 0·19[a]	1·25 ± 0·22	64
Bacterivores	3·37 ± 0·47[a]	3·31 ± 0·70	35	2·01 ± 0·28[a]	1·36 ± 0·21	67
Omnivores	0·25 ± 0·08	0·42 ± 0·13	28	0·38 ± 0·09[a]	0·78 ± 0·09	51
Predators	5·92 ± 1·61	6·89 ± 2·90	21	1·38 ± 0·25[a]	0·49 ± 0·13	46
Biomass[c]						
Herbivores	55 ± 17	59 ± 15	23	48 ± 11[a]	19 ± 4	64
Fungivores	63 ± 16[a]	54 ± 12	41	24 ± 4[a]	22 ± 4	64
Bacterivores	62 ± 9[a]	61 ± 13	35	37 ± 5[a]	25 ± 4	67
Omnivores	5 ± 0[a]	8 ± 3	28	7 ± 2[a]	7 ± 2	51
Predators	109 ± 20[a]	126 ± 53	21	26 ± 5[a]	9 ± 3	46

[a]Significant main effect of CO_2 treatment nested within month and year ($P \leq 0·05$).
[b]mg C respired m^{-2} day^{-1} (mean ± 1 SE).
[c]ng C of dominant nematodes (Table 1) g^{-1} dry soil.
All mean ± SE values were computed based on numbers of nematodes g^{-1} soil. Sample size (n) estimates represent numbers of laboratory replicates multiplied by field treatment replicates multiplied by time periods sampled.

direct result of increased allocation of plant carbon to the below-ground environment. Indeed, primary production rates at both the Loblolly and Sweet Gum sites increased by >20% with CO_2 enrichment (DeLucia *et al*. 1999; Norby *et al*. 2002). Early reports noted that root production increased at both sites (Matamala & Schlesinger 2000), but more recent reports indicated that root turnover rates remained unchanged (Matamala *et al*. 2003). Thus we expected greater abundance and biomass for nematode herbivores, bacterivores and fungivores in association with higher plant production and accumulating organic matter.

At the Loblolly Pine site both respiration and biomass of the total nematode community increased, as did the abundance of fungivores and the biomass of both omnivores and predators (Table 2). In contrast, herbivorous nematodes showed no change in abundance, respiration or biomass with CO_2 fumigation. Moreover, CO_2 treatment also reduced the total abundance of nematodes, abundance of bacterivores, respiration of bacterivores and fungivores, and biomass of fungivores and bacterivores. These results suggest that elevated CO_2 influenced soils at the Loblolly site largely though the detrital food web, although most impacts on specific groups emerged as reductions in nematode abundance, biomass and respiration, rather than the increases we expected as a result of greater carbon availability. The greatest change in nematode community at the Loblolly Pine plantation was an increase in predator biomass (Table 2), so that increased predation may have contributed to reduced abundance and biomass of other groups.

In the Sweet Gum forest, responses of soil nematodes to elevated CO_2 were contrary to expectations in almost every way. Total abundance, respiration and biomass of the community declined with treatment (Table 2). Abundance, respiration and biomass declined for herbivores, bacterivores and predators. Only abundance of fungivores increased at elevated CO_2, although respiration and biomass of this group decreased (Table 2). Hence our notion that elevated CO_2 would stimulate soil nematode communities was not supported by experimental results at this site, and suggested reduced carbon flow through both herbivore- and detritivore-based food chains (Table 2).

TROPHIC RESPONSES

There were few consistencies in the responses of trophic groups either within or between sites. For example, we found that abundance of herbivores declined in Sweet Gum soils at elevated CO_2 but were not influenced in Loblolly Pine soils. In comparison, herbivores increased in a trembling aspen forest (Hoeksema *et al*. 2000), grasslands (Hungate *et al*. 2000) and pasture (Yeates & Orchard 1993; Yeates *et al*. 1997, 2003), but declined in a field of cotton when exposed to elevated CO_2 (Runion *et al*. 1994). Clearly, many herbivorous nematodes are host-specific and sensitive to host phenology. Predicting the responses of herbivorous nematodes to elevated CO_2 may require considerably more understanding of individual plant species responses as well as composite, plant community responses than are generally available.

Much like herbivores, abundance of bacterivores, predators and fungivores also increased or decreased in response to elevated CO_2 in grassland, agricultural and aspen soils (Tate & Newton 1997; Yeates *et al*. 1999, 2003; Hoeksema *et al*. 2000; Hungate *et al*. 2000). However, some further insight into CO_2 effects on bacterivores in our study is suggested by changes in abundance of dauer larvae. Dauer larvae are non-feeding juveniles of Rhabditiae often associated with food scarcity. Jessen *et al*. (2000) reported that emergence from dauer stages can be stimulated by elevated concentrations of CO_2, perhaps signalling greater availability of bacterial prey. Thus lower abundance of dauer larvae on both our sites at elevated CO_2 may indicate more bacteria, or at least more favourable conditions for juvenile development. Moreover, the proportion of dauer larvae in the total Rhabditiae population showed a significant decrease with CO_2 fumigation on the Sweet Gum site.

Predatory nematodes might respond to elevated CO_2 as energy flow either increases or decreases in the food web. In general, we found that both abundance and biomass of predatory nematodes decreased on the Sweet Gum site, and that biomass increased on the Loblolly site with fumigation. These results suggest a possible trophic cascading effect in Loblolly Pine soils, similar to that reported in a fumigated pasture soil in which the largest response in soil nematode communities was an increase in predator abundance (Yeates *et al*. 2003).

ECOSYSTEM RESPONSES

Other ecosystem responses to elevated CO_2 at our study sites suggested that soil communities, including nematodes, should be affected. Andrews & Schlesinger (2001) reported modest increases in soil microbial activity at the Loblolly site, measured as respiration and extracellular enzyme activities, accompanying increased root production, soil organic matter accumulation and soil respiration. These changes suggest a possible positive, bottom-up effect on soil nematodes. However, abundance of other soil invertebrates [including Collembola and mites (Prostigmata, Mesostigmata, and Oribatida)] recently decreased with elevated CO_2 on the Pine site (Hansen *et al*. 2001), which could stimulate nematode communities if these arthropods are predators or competitors of nematodes. Unfortunately too few data exist to define consistent relationships between nematode populations and other reported changes in the below-ground community in the Pine stand.

The Sweet Gum forest has shown less response than the Loblolly forest to increased CO_2. There is no accumulation of litter (Sinsabaugh *et al*. 2003), probably due to rapid turnover (≈1 year; R. J. Norby, personal communication). Carbon allocation to root systems has increased with CO_2 treatment, but there has been

no change in soil microbial activity, measured as extracellular enzyme activity, respiration on BIOLOG plates, or rates of N mineralization (Sinsabaugh *et al*. 2003). Thus it is surprising that the nematode community in Sweet Gum soil was more responsive to CO_2 than that in the Loblolly forest, even if the responses were mostly negative. However, differences between the two sites include the composition of both organic matter and fungi. In contrast to Pine litter, which contains a large fraction of recalcitrant chemical compounds, Sweet Gum litter is very labile. Also, soils in the Loblolly forest contain substantial ectomycorrhizae fungi (mostly Basidiomycota and Ascomycota) whereas Sweet Gum forests have abundant, non-mycorrhizal Oomycota (candidate kingdom Stramenopila). The effects of Oomycota on nematode communities are unknown, but Klironomos, Rillig & Allen (1996) reported a negative correlation between abundance of collembolans and other non-mycorrhizal fungi at elevated CO_2.

NEMATODE ENERGETICS

Despite the equivocal response to CO_2 fumigation that we found in soil nematode communities, our results are among few that have evaluated contributions of soil nematodes to energy flow in forest ecosystems. Sohlenius (1980) provided the first and most comprehensive summary of similar work to date, and reported high variation among studies and ecosystem types. Respiration ranged between 1·4 and 339 kcal m^{-2} $year^{-1}$, with grasslands and pastures having much higher values than forests. For comparison, we calculated total annual respiration by assuming that our daily rates (Table 2) represented average daily rates of community respiration. This yielded total respiration rates of 25–95 kcal m^{-2} $year^{-1}$ (≈2·9–11·2 g C m^{-2} $year^{-1}$) and 5–40 kcal m^{-2} $year^{-1}$ (≈0·6–4·7 g C m^{-2} $year^{-1}$) in soils with Pine and Sweet Gum, respectively. These estimates fall within the range of values reported by Sohlenius (1980), and represent ≤1% of the total annual efflux of carbon from these forest soils (DeLucia *et al*. 1999; Hamilton *et al*. 2002; Norby *et al*. 2002; George *et al*. 2003). Thus our estimates of the relative contribution of soil nematodes to forest soil respiration agree with the conclusion of Sohlenius (1980) that soil nematodes often contribute <1% of total soil respiration. Our maximum estimated daily respiration for the nematode community in the Pine stand was 30 mg C m^{-2} day^{-1}, ≈0·6% of the maximum rate of soil respiration reported by Allen *et al*. (2000): ≈20 g CO_2 m^{-2} day^{-1} (≈5 g C m^{-2} day^{-1}).

Ausmus *et al*. (1978) conducted a detailed analysis of nematode energetics for a 45-year-old hardwood stand dominated by *Liriodendron tulipifera*, located near the Sweet Gum site in our study. The total biomass of the community varied over the year, but averaged 0·38 g DW m^{-2}, which lies within the range of our estimates of 43–351 mg C m^{-2} at the nearby Sweet Gum site. Ausmus *et al*. (1978) found that herbivores represented 19–60% of the total nematode biomass, which is slightly higher than our estimates of 14–43% of the total. They further estimated that herbivores consumed 338 kcal m^{-2} $year^{-1}$ from roots, that assimilation was 100%, and that 85% of the assimilated energy was expended through maintenance respiration (287·3 kcal m^{-2} $year^{-1}$). We estimated that herbivores respired 0·7–16·8 kcal m^{-2} $year^{-1}$, and suggest that a more realistic assessment of the observations of Ausmus *et al*. (1978) would be to consider a 40% respiratory coefficient (cf. Sohlenius 1980), producing 135 kcal m^{-2} $year^{-1}$. In either case, Ausmus *et al*. (1978) suggest a 10-fold greater respiratory output than we calculated, although our estimates fall within the general range of values reported elsewhere (Sohlenius 1980).

It is difficult to estimate production rates of nematodes with much confidence of accuracy, given the range of reported production : assimilation (P : A) relationships. Ferris, Venette & Lau (1997) report values between 0·58 and 0·86 for bacterivores, although Schiemer (1982) notes that values approach zero at low food availabilities. Ausmus *et al*. (1978) estimated that 85% of the carbon consumed by herbivores was respired, suggesting a P : A of about 15%. Sohlenius (1980) reports values ranging between 8 and 23% for a variety of nematode trophic groups. If we assume a P : A value of ≈25% for the nematode communities in our study, then production estimates are ≈6·2–23·9 and 1·2–9·9 kcal m^{-2} $year^{-1}$ in the Pine and Sweet Gum stands, respectively. Sohlenius (1979) reported soil nematode respiration and production rates in a Pine forest of ≈8·1 and 5·1 kcal m^{-2} $year^{-1}$, respectively, slightly lower than our estimates for the Pine stand. However, Sohlenius (1979) also reports a P : A ratio of only 13·7% for his community.

In summary, comparisons demonstrate that our estimates of nematode energetics are within the range reported for forest soil communities found elsewhere, and consistent with larger patterns of ecosystem production. For example, levels of carbon immobilized in or released by nematodes generally appear to be greater in coniferous than in deciduous forests (Petersen & Luxton 1982). However, our study also demonstrates both large variation in soil nematode response to elevated CO_2 and results contrary to predictions that increased carbon flow through the ecosystem would generate an increase in nematode biomass and/or energy flux.

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