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Nematode Genera in Forest Soil Respond Differentially to Elevated CO_2

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Abstract: Previous reports suggest that fungivorous nematodes are the only trophic group in forest soils affected by elevated CO_2. However, there can be ambiguity within trophic groups, and we examined data at a genus level to determine whether the conclusion remains similar. Nematodes were extracted from roots and soil of loblolly pine (*Pinus taeda*) and sweet gum (*Liquidambar styraciflua*) forests fumigated with either ambient air or CO_2-enriched air. Root length and nematode biomass were estimated using video image analysis. Most common genera included *Acrobeloides*, *Aphelenchoides*, *Cephalobus*, *Ditylenchus*, *Ecphyadorphora*, *Filenchus*, *Plectus*, *Prismatolaimus*, and *Tylencholaimus*. Maturity Index values and diversity increased with elevated CO_2 in loblolly pine but decreased with elevated CO_2 in sweet gum forests. Elevated CO_2 treatment affected the occurrence of more nematode genera in sweet gum than loblolly pine forests. Numbers were similar but size of *Xiphinema* decreased in elevated CO_2. Abundance, but not biomass, of *Aphelenchoides* was reduced by elevated CO_2. Treatment effects were apparent at the genus levels that were masked at the trophic level. For example, bacterivores were unaffected by elevated CO_2, but abundance of *Cephalobus* was affected by CO_2 treatment in both forests.

Key words: climate change, ecology, FACE, interaction, nematode biomass, soil function, soil productivity.

Belowground responses to atmospheric CO_2 manifest quickly in the soil food web. For example, microbial biomass increased, albeit no change in its community composition, in soils of *Populus grandidentata* stands within 2 yr of exposure to elevated CO_2 (Zak et al., 1996). Even quicker increases in extracellular enzyme activities of soil microbes have been reported (Finzi et al., 2006). However, few studies have focused on C fluxes in the soil food web beyond microbes, particularly in forests, and even fewer have explored detailed changes at the genus resolution in nematode communities (Nagy et al., 2008). Previous investigations of soil invertebrates in response to elevated CO_2 have focused primarily on grassland soils (Yeates and Orchard, 1993; Yeates et al., 1997, 1999; Blair et al., 2000; Hungate et al., 2000; Niklaus et al., 2003; Sonnemann and Wolters, 2005; Ayres et al., 2008; Nagy et al., 2008; Kardol et al., 2010; Eisenhauer et al., 2012) and/or identified nematodes to trophic group in forest soil (Lussenhop et al., 1998; Romanyà et al., 2000; Neher et al., 2004b). Notable exceptions were the identification of nematodes sometimes to genus in soils beneath trembling aspen (*Populus tremuloides*) in boreal forests (Hoeksema et al., 2000). Besides, trees are more responsive than herbaceous species to elevated CO_2 (Ainsworth and Long, 2005).

Plant-parasitic nematodes can alter grassland C and N dynamics, at least in the short term by influencing root exudation rates (Bardgett et al., 1999; Yeates et al., 1999). Positive associations between soil species richness and C cycling have been observed in 77% to 100% of low-diversity ($\leq$ 10 species) experiments; whereas positive relationships occurred less frequently (35% to 64%) in studies with soil species richness exceeding 10 species (Nielsen et al., 2011). In a 13-yr multifactor experiment in grasslands, nematode taxa richness was least at elevated CO_2 and elevated N (Eisenhauer et al., 2012). In low-N soil with trembling aspen trees (*Populus tremuloides*), twice-ambient CO_2 was associated with increases of the most abundant plant-parasitic taxon (Trichodoridae), lower density of one bacterivore taxon (Rhabditidae), and lower evenness of the community, compared with ambient CO_2 (Hoeksema et al., 2000). In high-N soil, twice-ambient CO_2 was associated with greater abundance of predator/omnivores, smaller diversity values, and larger Maturity Index values, compared with ambient CO_2 (Hoeksema et al., 2000).

Previous reports on these study sites focused on the response of the nematode food web at a community or trophic group level. For example, total respiration of soil nematode communities ranged from 2.9 to 11.2 g C m^{-2} $year^{-1}$ in pine soils and 0.6 to 4.7 g C m^{-2} $year^{-1}$ in sweet gum soils, representing $\leq$ 1% of net primary production in these forests (Neher et al., 2004b). Fungivores were the only trophic group consistent in both forest sites with reduced abundance, biomass, and respiration at elevated CO_2 (Neher et al., 2004b). At the loblolly pine site, CO_2 generally increased the abundance of fungivores and decreased abundance of bacterivores. However, feeding-habit groupings may be ambiguous and/or not mutually exclusive in some cases (Neher et al., 1998; Neher, 2001) and examination at a finer taxonomic resolution can give a different answer (Neher et al. 2004a).

In this article, we examined responses of nematode community free air carbon enrichment (FACE) at the genus resolution for both occurrence and biomass estimations. We predicted different responses among genera within a trophic group to elevated CO_2 and that seasonal fluctuations would be more apparent in

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a deciduous sweet gum plantation than coniferous loblolly pine plantation in response to seasonal fluctuations in primary productivity (Ainsworth and Long, 2005).

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, NC. Trees were planted in 1983 as 3-yr-old seedlings at a spacing of 2.4 × 2.4 m. Gassing began on 27 August 1996, when trees were approximately 14-m tall with closed canopy; pine accounted for 98% of the basal area. CO_2 enrichment was continuous (24 hr per day, every day of the year) except in extreme weather. The soil was an unfertilized Ultic Alfisol of the Enon Series, pH 5.75 (Schlesinger and Lichter, 2001). The experimental design consisted of three rings with an approximate interior CO_2 concentration of 180 ppm above ambient (target concentration is 550 ppm) and three rings as controls.

Oak Ridge field site: The second site was part of the FACE experiment in a 10-yr-old sweet gum (*Liquidambar styraciflua*) plantation near Oak Ridge, TN. At this site, two rings received elevated CO_2 concentrations (target concentration was 565 ppm CO_2) and three rings remained at ambient conditions. The plantation was established in autumn 1988 with 1-yr-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 per ha with an average tree height of 12.4 m and stem diameter of 13 cm. Gassing continued annually during the growing season (24 hr per day, every day between April and November). The soil was an unfertilized Aquic Hapludult with a silty clay loam texture, pH 5.5 to 6.0, and bulk density 1.5 g cm^{-3}.

Extraction and enumeration: Six soil cores (2-cm diam, 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 (see Hendrey and Kimball, 1994; Lewin et al., 1994; Hendrey et al., 1999). Sampling dates were May, June, and September 1999, and May, July, and October 2000. On each occasion, samples were pooled and one composite soil sample per ring was analyzed for nematodes. A separate composite litter sample per ring was processed for loblolly pine soils. For each of six sampling dates, a total of 12 composite samples (2 forests × 2 treatments × 3 replicate rings) of soil were collected from each site and six composite samples of litter from loblolly pine soils.

Each composite soil sample (300 to 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 root and soil organic fraction or litter in a mist chamber for 4 d. Nematodes from mineral soil were extracted by a cotton-wool filter method followed by sucrose centrifugal-flotation (Oostenbrink, 1960; Townshend, 1963). Twenty percent 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), and Hunt (1993). Taxonomic families were assigned a trophic grouping based on Yeates et al. (1993). Abundance was standardized to gravimetric soil moisture and root length. An estimate of total abundance of nematodes in the combined organic and soil fractions of each subsample was calculated proportional to the mass of each soil fraction.

Numbers of plant-parasitic nematodes associated with roots and soil were expressed per cm of root length, a measure of their habitat or food base. All roots per sample were spread uniformly in 15-cm-diam. 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 and 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).

Community structure: Indices were estimated of diversity, richness, evenness, similarity, and successional maturity for nematode communities. Community diversity was computed at three levels of resolution: (i) diversity based on abundance of individuals within each genus (Hills N1 and N2 diversity), (ii) trophic diversity based on abundance of individuals within each trophic group (trophic diversity; Hills N1), and (iii) diversity of genera within each trophic group (trophic richness; Hills N1). Hills N1 index is computed as $\exp[-\Sigma P_i(\ln P_i)]$, where P_i is the proportion of group (genus or trophic level) i in the total nematode community and reflects the number of abundant species (N1 is eH', where e is the natural log and H' is Shannon index (Neher and Darby, 2006)). Hills N2 is computed as $1/\lambda = 1/[\Sigma(n_i/N)^2]$, where n_i/N is the proportion of genus i in the total nematode community and reflects the number of very abundant species; and N2 is essentially the reciprocal of the Simpson index. Hills indices are simpler to interpret ecologically than commonly used Simpson or Shannon forms (Peet, 1974). Richness was computed as the number of genera present per sample. Evenness (E5, modified Hill's ratio) was computed as (N2-1)/(N1-1), chosen for its reputation as one of the least ambiguous and most interpretable indexes of evenness (Ludwig and Reynolds, 1988).

Families of nematodes were assigned CP values (Bongers, 1990; Bongers et al., 1991, 1995), based on life history characteristics on a scale ranging from 1 to

TABLE 1. Classification of nematode genera by trophic group, CP value, percentage of samples with nonzero abundance of each ambient and elevated CO_2 treatments in pine and sweet gum forest soil ($n = 61$). Sample size estimates represent numbers of lab replicates × field treatment replicates × time periods sampled.

			Pine		Sweet gum	
Genus	Trophic group[a]	CP value[b]	Ambient	Elevated	Ambient	Elevated
Achromadora	8	3	0.0	3.1	5.6	0.0
Acrobeles	3	2	27.6	25.0	0.0[c]	8.0
Acrobeloides	3	2	100.0	100.0	100.0	100.0
Aetholaimus	5	5	3.5	0.0	0.0	0.0
Alaimus	3	4	3.5[c]	31.3	36.1	36.0
Anaplectus	3	2	3.5	6.3	2.8	8.0
Anatonchus	5	4	0.0	0.0	2.8	0.0
Aphanolaimus	3	3	3.5	3.1	0.0	0.0
Aphelenchoides	2	2	100.0	100.0	100.0	100.0
Aphelenchus	2	2	17.2	18.8	72.2	84.0
Aporcelaimellus	5	5	75.9	78.1	61.1[c]	28.0
Aporcelaimus	5	5	10.3	3.1	0.0	8.0
Axonchium	1	5	13.8	21.9	8.3	4.0
Basiria	1	2	6.9	9.4	19.4	28.0
Bastiania	3	3	3.5	3.1	2.8	4.0
Belondira	8	5	41.4[c]	53.1	58.3	52.0
Boleodorus	1	2	31.0	21.9	16.7	32.0
Bunonema	3	1	0.0	0.0	2.8	0.0
Cephalobus	3	2	86.2	78.1	83.3	96.0
Cervidellus	3	2	0.0	15.6	27.8	20.0
Chronogaster	3	2	6.9	15.6	11.1	8.0
Chrysomena	?[d]	5	3.5	0.0	0.0	0.0
Clarkus	5	4	37.9	46.9	25.0	12.0
Coslenchus	1	2	3.5	21.9	22.2	4.0
Criconema	1	3	10.3	21.9	5.6[c]	24.0
Criconemella	1	3	20.7	3.1	5.6	8.0
Cruzenema	3	1	0.0	6.3	13.9	24.0
Dauer larvae	0	0	86.2	87.5	94.4	88.0
Diphtherophora	2	3	44.8	53.1	80.6	72.0
Diploscapter	3	1	0.0	3.1	0.0	0.0
Discolaimus	5	5	0.0	6.3	0.0	0.0
Ditylenchus	2	2	89.7	87.5	94.4	96.0
Ecphyadophora	1	2	72.4	65.6	75.0[c]	52.0
Eudorylaimus	5	4	65.5[c]	68.8	44.4	40.0
Eumonhystera	3	2	3.5	6.3	16.7	4.0
Filenchus	2	2	100.0	100.0	100.0	100.0
Helicotylenchus	1	3	86.2	68.8	41.7	24.0
Hemicycliophora	1	3	100.0	93.8	5.6	4.0
Hexatylus	2	2	48.3	28.1	91.7	84.0
Ironus	5	4	3.5	6.3	0.0	0.0
Lelenchus	1	2	20.7	21.9	16.7	24.0
Macroposthonia	1	3	3.45	12.5	30.6	16.0
Malenchus	1	2	48.3	50.0	11.1	4.0
Meloidogyne juveniles	1	3	6.9	18.8	19.4	16.0
Mesodorylaimus	8	4	31.0	21.9	47.2	56.0
Mesorhabditus	3	1	48.3	28.1	11.1	4.0
Metadiplogaster	3	1	0.0	3.1	0.0	0.0
Miconchus	5	4	0.0	6.3	11.1	0.0
Miculenchus	1	2	3.5	0.0	0.0	0.0
Monhysterella	3	2	10.3	0.0	0.0	0.0
Mylonchulus	5	4	48.3[c]	21.9	47.2[c]	24.0
Nygellus	5	5	10.3	15.6	0.0	0.0
Odontolaimus	3	3	0.0	3.1	0.0	0.0
Oxydirus	1	5	3.5	9.4	83.3	60.0
Panagrolaimus	3	1	44.8	31.3	75.0	76.0
Paramphidelus	3	4	3.5	0.0	2.8	0.0
Paratylenchus	1	2	51.7	62.5	2.8	8.0
Plectus	3	2	96.6	96.9	100.0	100.0
Prismatolaimus	3	3	69.0	84.4	66.7	56.0
Prodesmodora	3	3	13.8	21.9	5.6	8.0
Psilenchus	1	2	6.9	9.4	16.7	16.0
Pungentus	1	4	27.6	25.0	2.8	0.0

(*Continued*)

TABLE 1. Continued.

Genus	Trophic group[a]	CP value[b]	Pine		Sweet gum	
			Ambient	Elevated	Ambient	Elevated
Rhabditella	3	1	0.0	0.0	30.6	28.0
Rotylenchus	1	3	10.3	21.9	0.0	0.0
Sectonema	5	5	0.0	0.0	25.0	12.0
Seinura	5	2	0.0	0.0	2.8	8.0
Teratocephalus	3	3	44.8	50.0	41.7	12.0
Tripyla	5	3	37.9	31.3	38.9	40.0
Trophurus	1	2	0.0	0.0	13.9	20.0
Tylencholaimus	2	4	100.0	93.8	80.6	88.0
Tylenchorhynchus	1	2	10.3	6.3	30.6	8.0
Wilsonema	3	2	55.2[c]	21.9	5.6	0.0
Xiphinema	1	5	31.0	31.3	86.1[c]	56.0

[a] Trophic groups are abbreviated as 1, herbivore; 3, bacterivore; 2, fungivore; 8, omnivore; 5, predator and grouped according to Yeates et al. (1993) except *Filenchus* as fungivore, and *Ecphyadorphora*, *Basiria*, *Boleodorus*, *Lelenchus*, and *Psilenchus* as plant-parasites.
[b] Weighted according to Bongers (1990), Bongers et al. (1991, 1995).
[c] Significant elevated CO_2 treatment on frequency of occurrence in soil or roots ($p \leq 0.05$) using PROC CATMOD.
[d] Uncertain trophic group designation.

5, with 1 representing *r*-strategists and 5 representing *K*-strategists (Table 1). Maturity indices were computed two ways, i.e., free-living nematodes with *cp*-1 through *cp*-5 (MI) and plant-parasitic nematodes (PPI). All maturity indices are weighted means computed as Σ [*cp*-value (*i*) * f(*i*)]/[total numbers of nematodes] where (*i*) is the individual taxon, and f(*i*) is the frequency of taxon i in a sample (Bongers, 1990). Three extensions of the maturity index were also computed, i.e., channel index (CI), enrichment index (EI), and structural index (SI) (Ferris et al., 2001).

Statistical analysis: Data from each forest type were analyzed separately to determine the effect of CO_2 concentration on composition and function of nematode communities in soil. Indices of diversity, richness, evenness, similarity, and ecological succession were analyzed by a nested analysis of variance 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. Measures of richness, ecological succession, N2 diversity, and trophic diversity met assumptions of normality or the central limit theorem and were not transformed prior to analysis. Occurrence of nematode genera were analyzed as binomial data defined by assigning a genus present as 1 and absent as 0 and performing a categorical analysis and a chi-squared statistic. Analysis of variance and categorical analysis were performed using GLM and CATMOD procedures, respectively in SAS Version 8 (SAS Institute, 2000).

RESULTS

Root length density: There was greater seasonal variation in root length density in pine than sweet gum soils (Fig. 1). Density of roots in soil tended to be greater earlier than later in the season.

Community impacts: Measures of ecological succession suggested intermediate stages of development, averaging from 2.2 to 3.6 on a scale of 1 (*r*-strategy) to 5 (*K*-strategy). Ecological succession and generic diversity were affected by CO_2 treatment in both forests (Table 2). However, ecological succession (MI) and genus diversity (N1) increased with elevated CO_2 in loblolly pine, but decreased with elevated CO_2 in sweet gum forests. In sweet gum, elevated CO_2 also reduced richness, N2 genus diversity, N1 trophic diversity, and evenness. Values of EI and SI decreased and CI increased with elevated CO_2 in sweet gum soils. There was neither an effect of elevated CO_2 on any of these indices in pine soil nor on richness within trophic groups in either forest type.

Genus composition, abundance, and biomass: A total of 72 genera of nematodes were identified in this study (Table 1). The most common genera were present in at least 50% of samples from both treatments and sites,

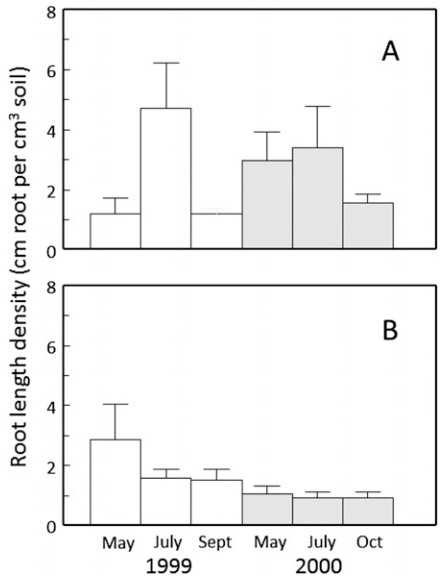

FIG. 1. Mean ± 1 SE root length density (cm root per cm^3 soil) per sampling period in A) pine and B) sweet gum soils. Bars represent the mean of two years (1999 white bars, 2000 gray bars). Root length density was measured by the line-intercept method using video imaging software.

TABLE 2. Effect of elevated CO_2 and season on nematode community indices in forest soils. All mean ± standard error values were computed based on numbers of nematodes per gram of soil for all indices. Sample size (n) estimates represent numbers of lab replicates × field treatment replicates × time periods sampled.

	Pine			Sweet gum		
Index	Ambient (n = 29)	Elevated (n = 32)	% r^2	Ambient (n = 36)	Elevated (n = 25)	% r^2
MI	2.18 ± 0.025[a]	2.21 ± 0.022	22	2.24 ± 0.031[a]	2.21 ± 0.031	34
MI25	2.20 ± 0.027	2.21 ± 0.022	20	2.30 ± 0.030[a]	2.25 ± 0.030	31
PPI	2.77 ± 0.043	2.78 ± 0.039	29	3.60 ± 0.105	3.33 ± 0.181	14
Richness (n = 74)	21.6 ± 0.87	21.7 ± 0.93	29	22.7 ± 0.80[a]	20.2 ± 0.88	49
N1 genus diversity	13.6 ± 1.35[a]	15.3 ± 1.54	33	97.9 ± 60.74[a]	39.6 ± 6.00	37
N2 genus diversity	7.9 ± 0.62	8.5 ± 0.96	12	10.3 ± 0.69[a]	9.6 ± 0.73	41
Evenness (E5)	0.68 ± 0.11	0.65 ± 0.09	22	0.37 ± 0.04[a]	0.34 ± 0.05	73
CI	93.9 ± 1.21	96.3 ± 1.01	20	78.4 ± 3.18[a]	83.4 ± 3.14	58
EI	65.7 ± 2.49[a]	65.8 ± 2.41	41	71.2 ± 2.17[a]	68.8 ± 3.26	49
SI	28.2 ± 7.79	31.0 ± 2.45	20	40.6 ± 2.58[a]	35.2 ± 3.04	38
N1 trophic diversity	2.9 ± 0.10	2.9 ± 0.08	11	3.0 ± 0.08[a]	2.84 ± 0.10	44
Trophic richness	3.96 ± 0.08	4.01 ± 0.09	9	4.06 ± 0.07	3.88 ± 0.11	29

[a] Significant main effect of CO_2 treatment nested within month and year ($p \leq 0.05$).

and consisted of *Acrobeloides*, *Aphelenchoides*, *Cephalobus*, dauer larvae of Rhabditidae, *Ditylenchus*, *Ecphyadophora*, *Filenchus*, *Plectus*, *Prismatolaimus*, and *Tylencholaimus* (Table 1). Abundance of each genus by treatment was presented elsewhere (Neher et al., 2004b). Community composition varied by forest type (Fig. 2). First, plant-parasite *Paratylenchus* was more common in loblolly pine soils and plant-parasites *Oxydirus* and *Xiphinema* were more common in sweet gum soil. Second, bacterivores *Alaimus* and *Cephalobus,* and fungivores *Apehenchus*, *Diphtherophora*, and *Hexatylus* were more abundant in sweet gum than loblolly pine soil. Bacterivores *Plectus* and *Acrobeloides* and fungivore *Filenchus* were more common in pine than sweet gum soil. Third, predator *Eudorylaimus* was more common in loblolly pine soils whereas predator *Sectonema* was more common in sweet gum soils. In addition to forest type, CO_2 treatment altered frequency of occurrence for six genera in sweet gum forests (two increased, four decreased) and five genera in loblolly pine (three increased, two decreased). The effects of CO_2 were more apparent in sweet gum than loblolly pine soils (Fig. 2). Only the loblolly forest had a litter layer, in which the effects of CO_2 treatment showed a consistent, negative impact on all five genera present (Table 3).

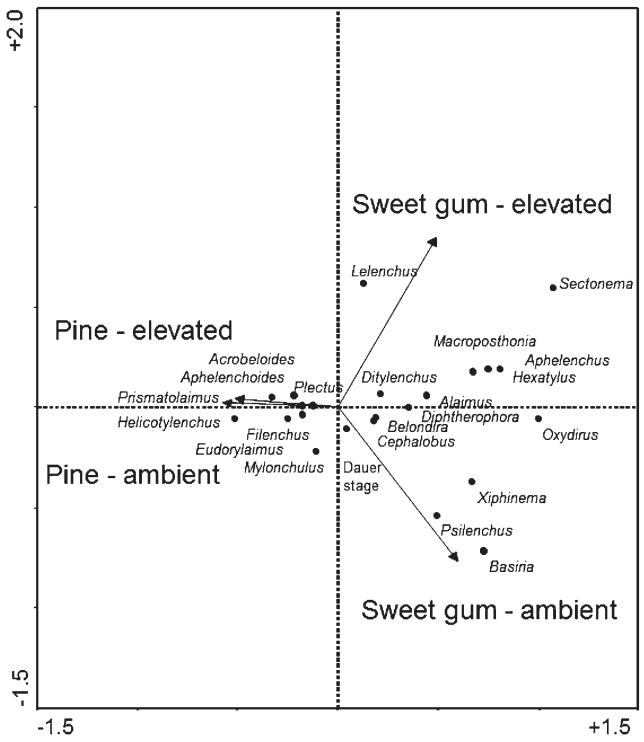

FIG. 2. Canonical correspondence analysis biplot for nematode genera plotted by site and treatment (ambient and elevated CO_2) as vectors. Only genera shown to have a significant main or interaction effect with CO_2 were included (see Table 4). Points represent numbers of nematodes (herbivores expressed per cm root length and non-herbivores as number per gram of dry soil); abundances decrease with increasing distance from each point in a unimodal fashion (ter Braak and Smilauer, 2002). Data represent both sites and all sampling times combined (n = 124). Eigenvalues (lambda) are 0.116 (p = 0.0050), 0.021, 0.005, and 0.091 for the first (horizontal), second (vertical), third, and fourth axes, respectively.

In contrast to a few genera in the loblolly pine soils, there was no indication of major changes in individual size of particular genera that could disproportionately alter the production of the group more or less than expected based on biomass estimates (Table 4). For comparison between forests, *Filenchus* and *Cephalobus* were affected by CO_2 treatment in both forests, but the

TABLE 3. Mean ± 1 standard error of numbers of nematodes per gram of loblolly pine litter (Duke Forest, NC) pooled across two years.

Genus	Ambient	Elevated
Acrobeloides	31.7 ± 14.4[a]	20.0 ± 8.93
Eudorylaimus	3.5 ± 0.40[a]	2.5 ± 0.887
Filenchus	26.7 ± 11.1[a]	15.4 ± 3.97
Teratocephalus	6.6 ± 2.82[a]	2.4 ± 0.73
Tylencholaimus	14.3 ± 5.93[a]	6.5 ± 1.85

[a] Significant main effect of CO_2 treatment nested within month and year ($p \leq 0.05$).

TABLE 4. Mean ± standard error biomass of nematode genera in soil pooled across two years. Plant-parasitic taxa[a] are expressed as ng fresh weight per cm of root length. Free-living taxa[b] are expressed as ng fresh weight per gram of dry soil. Restricted to genera represented in > 6 % of subsamples to optimize precision by avoiding uncommon and highly variable taxa.

		Pine		Sweet gum	
Genus	Ng C per worm[c]	Ambient	Elevated	Ambient	Elevated
Acrobeloides[b]	90 ± 7	340.8 ± 73.6	302.8 ± 63.1	43.7 ± 15.9	33.3 ± 13.2
Alaimus[b]	40 ± 10	— [d]	0.002 ± 0.001	0.02 ± 0.01	0.01 ± 0.0
Aphelenchoides[b]	50 ± 3	120.6 ± 27.5	95.0 ± 17.7	46.3 ± 13.4[e]	44.5 ± 19.3
Aphelenchus[b]	170 ± 30	4.5 ± 2.8	4.0 ± 1.2	35.5 ± 12.2[e]	39.6 ± 19.2
Aporcelaimellus[b]	4,790 ± 1,836	0.98 ± 0.92	1.0 ± 0.5	0.34 ± 0.22	—
Basiria[a]	100 ± 16	8.5 ± 7.5	2.0 ± 0.0	2.5 ± 0.6[e]	15.0 ± 0.0
Belondira[b]	290 ± 53	27.3 ± 19.6	11.7 ± 2.7	109.2 ± 28.9	62.0 ± 26.1
Cephalobus[b]	140 ± 19	62.8 ± 26.8	19.0 ± 7.8	72.5 ± 9.7	68.5 ± 19.3
Coslenchus[a]	100 ± 14	—	—	0.01 ± 0.005	0.03 ± 0.0
Criconemella[a]	390 ± 106	0.002 ± 0.0	—	—	0.186 ± 0.0
Dauer larvae[b]	150 ± 9	161.8 ± 58.5	54.4 ± 17.7	63.2 ± 10.2	54.0 ± 18.7
Diphtherophora[b]	210 ± 29	7.6 ± 2.6	23.4 ± 6.0	54.6 ± 14.5[e]	47.1 ± 35.6
Ditylenchus[b]	60 ± 3	15.2 ± 2.5	12.0 ± 2.4	15.6 ± 4.1[e]	16.6 ± 3.5
Ecphyadophora[a]	30 ± 4	0.003 ± 0.001	0.004 ± 0.001	0.01 ± 0.001	0.02 ± 0.010
Eudorylaimus[b]	370 ± 72	121.0 ± 0.0[e]	14.0 ± 3.6	64.5 ± 26.5	106.3 ± 80.9
Filenchus[b]	60 ± 3	210.2 ± 60.8[e]	487.8 ± 135.3	62.5 ± 10.7[e]	39.9 ± 9.8
Helicotylenchus[a]	200 ± 21	0.04 ± 0.011	0.05 ± 0.015	0.03 ± 0.011	0.03 ± 0.019
Hexatylus[b]	80 ± 22	2.5 ± 1.5	2.0 ± 0.0	—	—
Lelenchus[a]	40 ± 5	4.6 ± 2.5	1.8 ± 0.4	2.0 ± 0.8	7.4 ± 2.1
Macroposthonia[a]	240 ± 46	0.03 ± 0.0	0.01 ± 0.005	0.02 ± 0.006	0.09 ± 0.026
Mesodorylaimus[b]	610 ± 94	—	—	0.05 ± 0.01	0.04 ± 0.0
Mylonchulus[b]	900 ± 450	426.0 ± 0.0	45.0 ± 0.0	52.3 ± 41.1	18.0 ± 0.0
Oxydirus[a]	730 ± 96	—	—	281.0 ± 75.9[e]	295.5 ± 81.1
Panagrolaimus[b]	270 ± 119	0.01 ± 0.005	—	0.11 ± 0.101	0.01 ± 0.0
Paratylenchus[a]	60 ± 26	8.4 ± 3.5	6.0 ± 2.0	2.0 ± 0.0[e]	1.0 ± 0.0
Plectus[b]	290 ± 51	245.0 ± 79.0	114.7 ± 46.3	82.2 ± 54.1	50.0 ± 19.7
Prismatolaimus[b]	60 ± 10	41.0 ± 0.0	14.8 ± 8.5	28.7 ± 9.1	15.0 ± 11.0
Psilenchus[a]	170 ± 29	54.0 ± 0.0[e]	1.0 ± 0.0	18.2 ± 8.1[e]	3.0 ± 0.0
Teratocephalus[b]	30 ± 3	0.003 ± 0.002	0.004 ± 0.002	0.005 ± 0.001	0.01 ± 0.002
Tripyla[b]	140 ± 28	0.04 ± 0.0	—	0.11 ± 0.0	0.23 ± 0.0
Trophurus[a]	130 ± 8	—	—	0.005 ± 0.001	0.09 ± 0.024
Tylencholaimus[b]	170 ± 48	0.01 ± 0.004	0.02 ± 0.004	0.04 ± 0.015	0.02 ± 0.015
Tylenchorhynchus[a]	870 ± 66	0.001 ± 0.0	—	0.12 ± 0.046[e]	0.003 ± 0.0
Xiphinema[a]	30 ± 3	21.3 ± 8.5	31.2 ± 10.2	123.4 ± 27.6[e]	37.8 ± 11.6

[a] Plant-parasitic taxa.
[b] Free-living taxa.
[c] Mean ± standard error (Andrássy 1956); Each value represents approximately 10 to 720 worms, depending on relative occurrence.
[d] No nematodes measured.
[e] Significant main effect of CO_2 treatment nested within month and year ($p \leq 0.05$).

pattern was opposite between the two forest types (Figs. 3,4). The fungivore *Filenchus* was less numerous in sweet gum than loblolly pine soils and *Cephalobus* were of similar magnitude between forest types. Two common bacterivores, *Cephalobus* and *Plectus*, decreased in sweet gum. Specifically, *Cephalobus* abundance increased 15% with elevated CO_2, biomass decreased by 30% of the control (Fig. 4).

DISCUSSION

As predicted, diversity and ecological succession of soil nematodes decreased in response to elevated CO_2 in sweet gum soils. However, the opposite was observed in loblolly pine soils. This agrees with Hoeksema et al. (2000) who observed MI (but not PPI) values to be 20% greater in elevated than ambient CO_2 in high N soil of boreal forests, but not significant in low-N soil. There are inconsistent reports for grassland soils. For example, MI was the only community index (in comparison with diversity, evenness, richness, N1, N2, NCR) to respond with an increase (2.9 to 3.2) to elevated CO_2 (Yeates et al., 2003). Other grassland studies report insensitivity of nematode community indices to climate change (Ayres et al., 2008; Nagy et al., 2008). Eisenhauer et al. (2012) suggest that elevated CO_2 influences soil biota primarily through increased rhizodeposition and these effects were still pronounced after 13 yr of CO_2 manipulation. Large values of CI, EI, and SI represent a dominance of fungi in the decomposition pathway, reflect the relative abundance and activity of primary detritus consumers, and represent food webs where recovery from stress is occurring, respectively (Ferris et al., 2001).

Plant-parasites: One plant-parasitic genus common in our study, *Xiphinema*, is an unselective parasitic

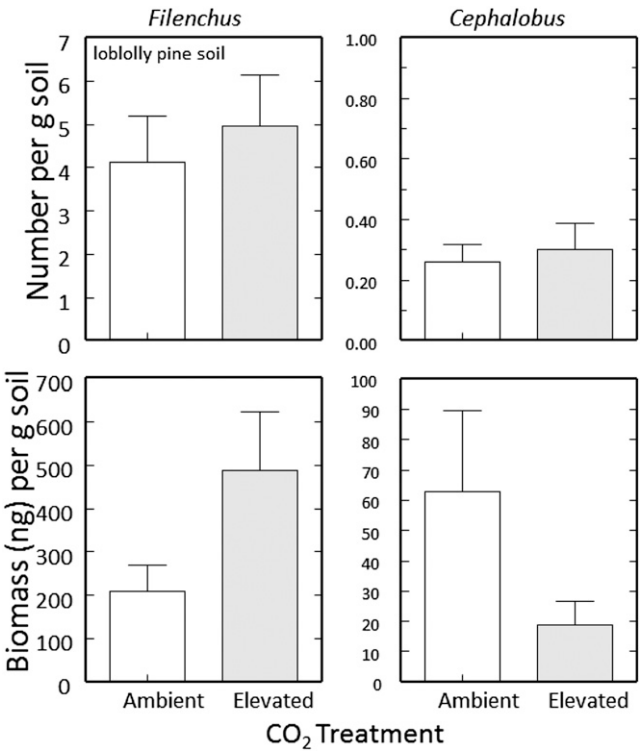

FIG. 3. Number (top row) and biomass (ng) per gram of dry soil (bottom row) for *Filenchus* and *Cephalobus* in loblolly pine soil, Duke Forest, NC. Illustrated are means with 1 SE as the error bar across three seasons in two years (1999, 2000) for ambient (white bars) and elevated (gray bars) CO_2 treatments.

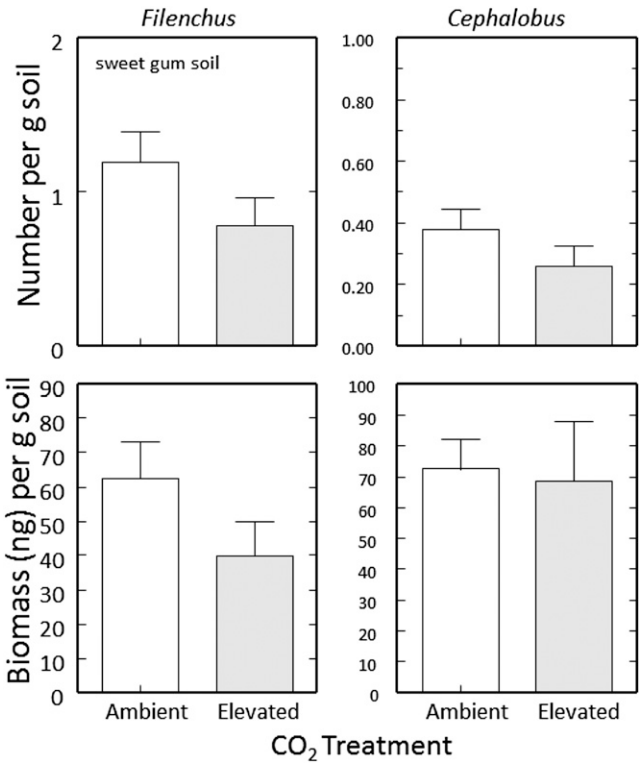

FIG. 4. Number (top row) and biomass (ng) per gram of dry soil (bottom row) for *Filenchus* and *Cephalobus* in sweet gum soil, Oak Ridge National lab, TN. Illustrated are means with 1 SE as the error bar across three seasons in two years (1999, 2000) for ambient (white bars) and elevated (gray bars) CO_2 treatments.

Dorylaimidae (Nicholas, 1975), which may respond more generally to changes in physiology of multiple plant species and, thus, serve as a potential indicator of general plant condition. We found abundance of *Xiphinema* to be unaffected although individual size was smaller at elevated CO_2 in sweet gum soils. Hoeksema et al. (2000) also reported no effect of CO_2 on numbers of *Xiphinema* beneath aspen on N-poor soils, but provided no information on biomass. This response of *Xiphinema* suggests that, although, particular species of plants and their associated nematode parasites may respond variously to CO_2 fumigation, the plant community, as a whole, may show insufficient change to produce a net change in the overall plant-parasitic nematode community. In boreal forest soil, Hoeksema et al. (2000) found an increase in Trichodoridae with *Populus tremuloides*.

Longidorus elongatus increased with elevated CO_2 in soils in pasture for more than 30 yr in New Zealand (Yeates et al., 2003; Yeates and Newton, 2009). At year 4, the growth rate increased but residence time of roots decreased and their turnover increased under elevated CO_2 (Allard et al., 2005). From feeding studies of *Longidorus*, Yeates et al. (2008) concluded that the increased root turnover is probably mostly due to the feeding of *Longidorus* that led to a leakage of photosynthate into the rhizosphere. Specific root length of *Lolium perenne* plants decreased with increased abundance of *Longidorus elongates* (Yeates and Newton, 2009). Metabolism of this nematode and root death increases resources available to the soil microbial biomass. In a temperate grassland study, Anguinidae increased and Hoplolaimidae decreased in response to elevated CO_2 (Ayres et al., 2008).

Root growth: Contrary to our predictions, there was more seasonal fluctuation of root growth in loblolly pine than sweet gum soils. Roots were found deeper in loblolly pine than sweet gum soils, especially in elevated CO_2 treatments (Norby et al., 2004). Our counterintuitive results may be partly because our measures of root length density were limited by the depth of sampling (5 to 10 cm) and would have underestimated fine roots. Furthermore, the ectomycorrhizal sheath may have altered the response of loblolly roots.

Fungivores: Abundance of one of the most common nematodes, *Aphelenchoides*, was reduced nearly 50% by CO_2 fumigation, consistent with trophic level abundance, biomass, and respiration patterns (Neher et al., 2004b). There was a positive correlation between *Aphelenchoides* biomass and quantity of ergosterol, a measure of soil fungal biomass (R. L. Sinsabaugh, unpub. data). Curiously, the abundance of *Filenchus* increased only by 20% with treatment, although the biomass of this genus rose by 232%, becoming the largest biomass contributed by any single genus. These data suggest an interaction of CO_2 fumigation with

life-stage or developmental phenology of *Filenchus*. In contrast, *Aphelenchus* increased in response to elevated CO_2 in low-N but not high-N soil in aspen of northern Michigan (Hoeksema et al., 2000). Similarly, *Aphelenchus* was more prominent in soils with elevated than ambient CO_2 in long-term studies of old-growth pastures (Yeates et al., 2003).

Bacterivores: There was no evidence of a consistent pattern of responses for bacterivorous nematode genera, although we found that the abundance of at least two common bacterivorous nematode genera, *Cephalobus* and *Plectus*, decreased in sweet gum soils at elevated CO_2, similar to the response of *Cephalobus* in an aspen soil with N amendments (Hoeksema et al., 2000). In contrast to Cephalobidae, Rhabditidae are insensitive to elevated CO_2 (Hoeksema et al., 2000; Yeates et al., 2003).

Litter: The standing stock of loblolly litter has increased on CO_2 enrichment plots because rates of production have increased although rates of decomposition have not changed. Sweet gum litter is more labile than recalcitrant loblolly pine resulting in turnover times for litter in loblolly is ~ 3 years and ~1 year in sweet gum. The reduced abundance of all genera could have been a 'dilution' effect, i.e., decreased numbers of nematodes per gram litter because of the increase in total litter.

Conclusions

Opposite of our prediction, elevated CO_2 reduced C flow through both herbivore- and detritivore-based food chains. Variety of effects on structure and function of soil nematode communities at all levels of resolution: Finer resolution of taxonomic identification (i.e., genus) identified responses to elevated CO_2 that were not detectable by community indices of ecological succession, structure, and decomposition channels. Impacts of elevated CO_2 were more pervasive in soil with sweet gum than loblolly pine forests.

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