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Belowground nematode herbivores are resistant to elevated atmospheric CO$_2$ concentrations in grassland ecosystems

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Abstract

Grasslands are considered to be one of the most sensitive ecosystems to rising atmospheric CO$_2$ concentrations, since, in addition to direct effects of elevated CO$_2$ on plant growth, indirect increases in water availability as an effect of elevated CO$_2$ may enhance primary production and alter plant community composition in these typically dry ecosystems. Moreover, grasslands support large populations of belowground herbivores that consume a major portion of plant biomass. The direct trophic link between herbivores and plants suggests that primary consumers may be particularly sensitive to rising CO$_2$ concentrations; however, the responses of belowground grassland herbivores have rarely been investigated. Here, we report the response of a range of herbivorous nematode populations to elevated CO$_2$ concentrations from three distinct grassland experiments. The three studies each involved 5–6 years of CO$_2$ fumigation, utilized natural or representative plant and soil communities, and were sampled at the end of the growing season. In the vast majority of cases, elevated CO$_2$ did not affect the abundance of nematode families; only two nematode families were significantly influenced by CO$_2$ enrichment (Anguinidae increased in one case and Hoplolaimidae decreased in another). Similarly, elevated CO$_2$ did not influence the total abundance, family richness, diversity or plant parasitic index of the herbivorous nematode community. These neutral responses to CO$_2$ enrichment occurred despite increased root production in all three experiments, suggesting a simultaneous antagonistic mechanism may have operated, possibly decreased root quality and/or changes in the soil environment. Whatever the mechanism, our findings suggest that herbivorous nematodes in grassland ecosystems are resistant to rising atmospheric CO$_2$ concentrations.
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1. Introduction

Grasslands typically occur in relatively arid regions and occupy approximately 40% of Earth's terrestrial landmass (IPCC, 2000). Grasslands, as well as other arid and semi-arid ecosystems, might exhibit particularly large responses in terms of primary production, plant community composition, and tissue quality to rising atmospheric CO$_2$ concentrations because, besides direct CO$_2$ fertilization, CO$_2$ enrichment decreases stomatal conductance, resulting in reduced transpirational water loss and increased soil water content and plant water use efficiency (Volk et al., 2000; Morgan et al., 2004b). Elevated CO$_2$ often increases root production in a range of plant types (Rogers et al., 1994; Lou et al., 2006), and grassland communities as a whole (Dhillion et al., 1996; Hungate et al., 1997; Pendall et al., 2004; but see Shaw et al., 2002). Indeed, root production may increase proportionally more than above-ground production, as plants become increasingly nutrient

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limited in response to CO_2 enrichment (Rogers et al., 1994; Lou et al., 2006); however, this does not always occur (Leadley et al., 1999).

Grasslands also support large, herbivore-dominated, nematode populations in the soil (Peterson, 1982; Porazinska et al., 2003) and herbivorous nematodes are major controllers of plant production in these ecosystems, where they are believed to consume more vegetation than cattle (Smolik, 1974; Lauenroth and Milchunas, 1991). Moreover, plant-feeding nematodes can alter grassland C and N dynamics, at least in the short term, by influencing root exudation rates (Yeates et al., 1998, 1999a; Bardgett et al., 1999; Tu et al., 2003). Most soil organisms are unlikely to be directly affected by rising atmospheric CO_2 concentrations, however, plants, which take up CO_2 from the atmosphere, are an exception. Due to the direct trophic link between herbivores and plants and the widely reported elevated CO_2-induced increases in root production (e.g., Rogers et al., 1994; Lou et al., 2006), there are strong theoretical reasons to expect that herbivorous nematodes communities will be indirectly affected by ongoing increases in atmospheric CO_2 concentration, with potential consequences for ecosystem properties and processes. Indeed, positive effects of CO_2 enrichment on the abundance of herbivorous nematodes have been reported (e.g., Yeates et al., 2003), but most studies report neutral effects, often despite increases in root biomass or turnover (e.g., Hungate et al., 2000; Niklaus et al., 2003; Sonnemann and Wolters, 2005). The herbivorous nematode community comprises taxa representing a range of natural histories, e.g., in relation to feeding strategy (Yeates et al., 1993); nematodes can be obligate parasites of a particular plant species (e.g., *Anguina* spp.) or more facultative herbivores that also feed on fungi (e.g., *Tylenchus* spp.). This has raised the suggestion that responses to elevated CO_2 might be taxa-specific and that changes in community composition may be more common than changes in the total abundance of herbivorous nematodes. However, to date only two experimental studies have investigated the effects of CO_2 enrichment in grasslands on herbivorous nematodes beyond the trophic group level, both in New Zealand (Yeates et al., 1997, 2003).

Here, we report the response of herbivorous nematode families to CO_2 enrichment in three grassland experiments. Since most previous studies of herbivorous nematode responses to elevated CO_2 reported no change in total abundance, we similarly expected no change in total abundance. However, we hypothesized that nematode community composition would respond to elevated CO_2. Specifically, we predicted that generalist nematode families (e.g., Dolichodoridae, Sphaeronematidae, and Tylenchidae) would be less responsive to CO_2 enrichment than specialists (e.g., Anguinidae and Meloidogynidae), since their broader resource base would buffer them from changes in plant physiology or community composition. The studies were located in Colorado (USA), California (USA), and France, allowing us to investigate both general

and site-specific responses of herbivorous nematodes to CO_2 in grassland ecosystems. Although the experiments differed in design, they had several similarities. For instance, each study had multi-year (5–6 years) ambient and elevated (700–720 ppm) atmospheric CO_2 treatments, used natural or representative grassland communities, and sampled herbivorous nematodes at the end of the growing season.

2. Materials and methods

2.1. Experimental design

The Colorado study was located at the USDA-ARS Central Plains Experimental Range (40°40′N, 104°45′W) on a shortgrass steppe dominated by three grass species (*Bouteloua gracilis*, *Pascopyrum smithii*, and *Stips comata*; Morgan et al., 2001). The site experiences a dry temperate climate, i.e. low rainfall (~320 mm) that falls predominantly between May and July, hot summers, and cold winters. The experimental design has been reported elsewhere (Morgan et al., 2001). Briefly, nine circular field plots (4.5 m diameter), arranged in three replicate blocks, were assigned one of the three treatments (no chamber-ambient CO_2, chamber-ambient CO_2, and chamber-elevated CO_2). Open-top chambers (4.5 m diameter, 3.8 m high) were placed on the chambered plots each growing season (March to October) between 1997 and 2001 and fans were used to force ambient air (supplemented with additional CO_2 in the elevated CO_2 treatment) into the base of the chamber. Ambient CO_2 concentrations were around 360 ppm and elevated concentrations were maintained at approximately 720 ppm. Soil was collected in October of 1999, 2000, and 2001, i.e. after three, four, and five growing seasons of the treatments. The sampling consisted of the removal of two open-bottom steel cylinders (20.3 cm diameter), inserted into the soil prior to the start of the experiment, from each plot at each sampling date. Nematodes were extracted from the top 20 cm and enumerated (see the following text). Mean values of the various nematode community measures were calculated from the two samples from each plot, and these values were used for statistical analysis (see the following text).

The California study was undertaken in an annual grassland on sandstone-derived soils in central coastal California (Jasper Ridge Biological Preserve; 37°24′N, 122°14′W). The site experiences a Mediterranean climate, i.e. warm wet winters and hot dry summers, and is dominated by three grass species (*Avena fatua*, *Bromus rigidus*, and *Bromus mollis*; McNaughton, 1968). Field et al. (1996) describe the design of the experiment in detail. Briefly, 30 circular field plots (0.65 m diameter), arranged in 10 blocks of three plots, were assigned one of the three treatments (no chamber-ambient CO_2, chamber-ambient CO_2, and chamber-elevated CO_2) such that there were 10 replicates of each treatment. Open-top chambers (0.65 m diameter, 1 m high) were placed on the chambered plots and a blower was used to force ambient air

(supplemented with additional CO_2 in the elevated CO_2 treatment) into the base of the chamber. Atmospheric concentrations of CO_2 averaged approximately 370 ppm in the ambient plots and 720 ppm in the elevated CO_2 plots. The treatments began in January 1992 and soil was sampled after 6 years of CO_2 fumigation during May 1997 (the end of the growing season). Sampling consisted of taking one soil core (8 cm diameter, 7 cm deep) from each field plot.

The French study involved the extraction of 48 intact soil monoliths (0.71 × 0.71 m, 0.28 m deep) from an experimental field at the Centre National de la Recherche Scientifique, Montpellier, France, in 1993. The site experiences a Mediterranean climate, similar to the California site. The site was tilled and left fallow for 4 years prior to monolith extraction. Plants were removed from the monoliths, but only the top 4 cm of soil was disturbed to minimize changes to soil structure. The monoliths were planted with one of the six different plant communities (eight monoliths for each plant community) composed of grassland species that typically co-dominate Mediterranean old-fields after abandonment and designed to mimic secondary plant succession. The communities varied in richness from 1 to 12 species and also from one to four functional groups (annual grass, annual composite, annual legume, and perennial grass) and were planted at densities of 700 plants m^{-2} (Nijs et al., 2000). Two plant communities consisted of one functional group (annual grasses) with a richness of one or six species; one community consisted of two functional groups (three annual grass species and three annual composite species); two communities consisted of three functional groups (annual grasses, annual composites, and annual legumes) with a total richness of 6 or 12 species (two or four species per functional group, respectively); and one community consisted of four functional groups (annual grasses, annual composites, annual legumes, and perennial grasses) with a total richness of 12 species (four species per functional group). The species within each community were randomly selected from the following species (annual grasses: *Aegilops geniculata*, *Bromus hordeaceus*, *Bromus lanceolatus*, *Bromus madritensis*, *Hordeum murinum*, *Lolium rigidum*; annual composites: *Centaurea solsticialis*, *Galactites tomentosa*, *Tyrimnus leucographus*, *Urospermum picroides*; annual legumes: *Medicago minima*, *Medicago orbicularis*, *Trifolium angustifolium*, *Trifolium scabrum*; and perennial grasses: *Bromus erectus*, *Dactylis glomerata*, *Phleum pratense*) and replicates of each community differed in species composition. The monoliths were placed in glasshouses maintained at ambient or elevated (700 ppm) CO_2 concentrations in a factorial design, resulting in four replicates of each CO_2 × plant community treatment. Glasshouse air temperature and vapor pressure deficit tracked conditions outside (Dhillion et al., 1996; Nijs et al., 2000). Soil cores (1.2 cm diameter, 22 cm deep) were collected in May 1998, towards the end of the growing season, after 5 years of CO_2 enrichment.

2.2. Nematode extraction and community analysis

Nematodes from the California experiment were extracted using the sugar-centrifugation technique (Jenkins, 1964), whereas nematodes were extracted from the Colorado and French experiment using the Baermann funnel technique for 72 h (Freckman and Baldwin, 1990). Both techniques are standard methods for extracting soil nematodes, but they have different efficiencies (Freckman and Virginia, 1993). The Baermann funnel technique extracts live nematodes and may underestimate the abundance of large and/or slow moving nematodes (Barker, 1985). In contrast, the sugar-centrifugation technique extracts both living and dead nematodes from soils. Thus, nematode abundances from the California study are based on live and dead nematodes, whereas abundances from the Colorado and French study are based on live nematodes only. The vast majority of herbivorous nematodes were identified to at least family level. However, 0.1%, 6.0%, and 4.4% of the herbivorous nematodes were not identified to family in the Colorado, California, and French study, respectively. Soil moisture (48 h at 105 °C) was determined, so that nematode abundance could be expressed on a soil dry weight basis. Nematodes were not extracted from plant roots.

Total herbivorous nematode abundance, family richness, and Shannon's diversity index were calculated; the unidentified herbivorous nematodes were treated as if they represented a single family and included in these calculations. The plant parasitic nematode index (PPI) was also calculated to determine changes in nematode community structure (Bongers, 1990, 1999). *C–P* values (an index of 'colonizer' versus 'persister' characteristics), which are used to determine the community PPI, were assigned primarily according to Neher et al. (2004). *C–P* values could not be assigned to the unidentified nematodes; therefore, these nematodes were excluded from the PPI calculation. The PPI ranges from one to five. Larger PPI values indicate low levels of disturbance to the community and are associated with dominance by slow reproducing, disturbance-intolerant 'persister' nematodes (analogous to K strategists) (Bongers, 1990, 1999). Conversely, smaller PPI values indicate high levels of disturbance and are associated with rapidly reproducing, disturbance tolerant 'colonizer' nematodes (analogous to r strategists). Although disturbance more commonly refers to physical impacts on soil, e.g., tillage, we feel that elevated CO_2 can also be considered a disturbance, since it can have significant impacts on plant community structure and physiology, with potential consequences for herbivorous nematodes, in particular obligate plant parasites.

2.3. Statistical analysis

The herbivorous nematode community was analyzed using ANOVAs in JMP. Due to the differences in experimental design, each dataset was analyzed separately.

All treatment main effects and interactions were included in the models (i.e. California: CO_2/chamber and block; Colorado: CO_2/chamber, year, and block; France: CO_2, plant community structure, and block). Where significant CO_2/chamber effects were observed in the Colorado or California study, a Tukey's honestly significantly different test was performed to determine whether the effect of the CO_2 treatment was significant. In all cases, significance was assigned at $P < 0.05$. Only the main effect of the CO_2 treatment is presented here, since this was the only treatment that was present in each study; significant interactions with CO_2 were rare, occurring in only two analyses of the Colorado data and one analysis of the French data, thus inclusion of these effects would have had little impact on our conclusions. Data were transformed (log_{n+1}) as necessary to meet the assumption of normality and homogeneity of variance.

3. Results

Plant responses have been reported elsewhere, with all three studies reporting increased root biomass in response to CO_2 enrichment (Dhillion et al., 1996; Hungate et al., 1997; Rillig et al., 1999; Pendall et al., 2004; Milchunas et al., 2005a). Elevated CO_2 increased root biomass by approximately 3–32% in the first 5 years of the Coloradoan

study (Pendall et al., 2004), by 23% after 6 years in the Californian study (Rillig et al., 1999), and by 31% after 6 months in the French study (Dhillion et al., 1996).

At ambient CO_2 concentrations, populations of herbivorous nematodes between the three studies ranged from 5744 (Colorado) to 10,430 kg^{-1} soil (France). The Colorado grassland supported the most diverse nematode community in terms of family richness and Shannon's diversity index, whereas the California grassland was the least diverse (Table 1). The PPI, where greater values indicate increasing dominance by K strategists, was greatest in the French study and lowest in the California study.

A total of 13 nematode families were identified across the three studies (13 in Colorado, 5 in California, and 9 in France), representing a range of natural histories. At ambient CO_2 concentrations, the herbivorous nematode community was dominated (>10% individuals) by Hoplolaimidae, Tylenchidae, Pratylenchidae, Anguinidae, and Paratylenchidae in Colorado; Tylenchidae in California; and Hoplolaimidae, Pratylenchidae, and Dolichodoridae in France. Nematode family occurrence did not differ between soils exposed to ambient or elevated CO_2 concentrations in any experiment, except that Pratylenchidae was only observed in the elevated CO_2 treatments in the California study. Four families were identified across

Table 1
Herbivorous nematode abundance (number kg^{-1} dry soil) and community structure at ambient CO_2 concentrations, % change resulting from CO_2 enrichment, and statistical significance (main effect of CO_2 treatment)

	Colorado			California			France		
	Mean (S.E.)	% change	P	Mean (S.E.)	% change	P	Mean (S.E.)	% change	P
Families									
Anguinidae	817 (273)	79	*	–	–	–	–	–	–
Criconematidae	17 (11)	52	ns	–	–	–	34 (22)	−55	–
Dolichodoridae	465 (129)	−15	ns	230 (126)	261	ns	1766 (280)	54	ns
Heteroderidae	140 (96)	−77	ns	–	–	–	–	–	–
Hoplolaimidae	1349 (235)	−36	ns	104 (47)	138	ns	4946 (703)	−23	*
Longidoridae	14 (9)	343	ns	–	–	–	40 (19)	16	–
Meloidogynidae	133 (53)	−68	ns	42 (42)	363	–	–	–	–
Paratylenchidae	749 (266)	107	ns	–	–	–	156 (107)	30	–
Pratylenchidae	862 (167)	−2	ns	0 (0)	–[a]	–	2942 (381)	−3	ns
Sphaeronematidae	8 (5)	478	–	–	–	–	–	–	–
Trichodoridae	50 (18)	−28	ns	–	–	–	5 (5)	516	–
Tylenchidae	1115 (312)	26	ns	7621 (763)	25	ns	45 (22)	284	–
Tylenchulidae	22 (20)	276	ns	–	–	–	41 (27)	−75	–
Unidentified	3 (3)	−1	–	509 (219)	−17	ns	454 (89)	−25	ns
Community structure									
Total abundance	5744 (766)	19	ns	8507 (907)	32	ns	10430 (988)	−2	ns
Family richness	8.8 (0.4)	−6	ns	2.6 (0.3)	35	ns	4.5 (0.2)	2	ns
Diversity[b]	1.71 (0.04)	−4	ns	0.32 (0.07)	47	ns	1.08 (0.04)	3	ns
PPI[c]	2.55 (0.03)	−6	ns	2.04 (0.02)	3	ns	2.98 (0.02)	4	ns

Where nematode occurrence within a family was low, it was often not possible to test statistically the effect of elevated CO_2.
ns: not significant; "–": absent/not determined.
[a] Cannot be calculated since none were found at ambient CO_2.
[b] Shannon's diversity index.
[c] Plant parasitic nematodes index.
* $P < 0.05$.

all three sites (Dolichodoridae, Hoplolaimidae, Pratylench-idae, and Tylenchidae).

Of the 18 instances where nematodes from a single family, and in two cases other unidentified herbivorous nematodes, were found frequently enough to analyze the effect of CO_2 enrichment, significant effects were observed in only two instances (Table 1). Hoplolaimidae abundance decreased (23%) in response to elevated CO_2 in the French experiment, but their abundance was not affected by CO_2 concentration in the other experiments. The abundance of Anguinidae increased (79%) as a result of CO_2 enrichment in the Colorado study; nematodes from this family were not identified in soil from the other experiments.

Elevated CO_2 also had little effect on the community structure of herbivorous nematodes. The total abundance, number of families, and diversity of herbivorous nema-todes, as well as PPI (which relates to the dominance of r or K strategists within the community), were not affected by CO_2 enrichment in any of the experiments (Table 1).

4. Discussion

We tested the hypothesis that elevated atmospheric CO_2 concentrations alter the community structure, but not total abundance, of herbivorous nematodes in three grassland experiments. Although the experiments were not identical in design, they shared several key characteristics: (1) each study used natural or representative plant and soil communities; (2) atmospheric CO_2 concentrations were increased from ambient to 700–720 ppm; (3) the experi-ments were maintained for either 5 or 6 years and the earliest sampling occurred three growing seasons after the treatments were initiated; and (4) the nematode community was sampled at the end of the growing season and identified to family level.

Elevated atmospheric CO_2 concentrations had very little effect on herbivorous nematodes, with the abundance of only two families significantly influenced by elevated CO_2. Our findings provide little support for the hypothesis that specialist nematode herbivores are more responsive to elevated CO_2 than generalists, since Anguinidae was the only specialist nematode family to exhibit a significant effect; Hoplolaimidae is intermediate in terms of speciali-zation. Only two other experimental studies on grassland communities have reported the response of herbivorous nematodes to elevated CO_2 beyond the trophic group level (Yeates et al., 1997, 2003), both of which were conducted in New Zealand. These studies observed some CO_2-induced changes in herbivorous nematode abundance, but, similar to our findings, neutral effects were the most common response. Yeates et al. (2003) observed increased abun-dances of *Tylenchus* (Tylenchidae) and *Longidorus* (Long-idoridae) in pasture FACE plots after 5 years of CO_2 enrichment, but there was no effect of CO_2 enrichment on the abundance of Hoplolaimidae, *Paratylenchus* (Paraty-lenchidae), and *Trichodorus* (Trichodoridae). Yeates et al. (1997) reported increased *Meloidogyne* (Meloidogynidae)

and decreased *Trichodorus* (Trichodoridae) abundance in response to CO_2 enrichment of grassland turfs maintained in controlled environment rooms for two growing seasons, but seven other herbivorous nematode taxa were not affected by elevated CO_2. Similarly, in a spatial study around a natural CO_2 vent in New Zealand, the abundance of *Pratylenchus* (Pratylenchidae) was positively associated with CO_2 concentration in gley, but not organic soil, while none of the other eight herbivorous nematode taxa exhibited a significant relationship to CO_2 concentration (Yeates et al., 1999b). The findings presented here, as well as those reported by Yeates et al. (1997, 1999b, 2003), indicate that while elevated atmospheric CO_2 concentra-tions can significantly affect various herbivorous nematode taxa, the effects are idiosyncratic, presumably depending on site-specific factors. Moreover, in each experiment, neutral effects were the most frequent response to CO_2 enrichment, suggesting that rising atmospheric CO_2 con-centrations may have only modest effects on the commu-nity structure of herbivorous nematodes in grasslands. This is supported by our observation that CO_2 enrichment did not significantly affect the family richness, diversity, or PPI of herbivorous nematodes in the Colorado, California, or French study. Alternatively, teasing out the effects of CO_2 enrichment on the herbivorous nematode community may require species-level resolution, which has not been attempted to date due to the considerable time and expertise this would require. Similarly, since nematodes were not extracted directly from plant roots, changes in the abundance of endoparasitic nematodes may have been masked, although their abundance in the soil should reflect changes in their populations.

We found no evidence that CO_2 enrichment increases the total abundance of herbivorous nematodes in any of the three studies. Six other studies have reported the effect of CO_2 enrichment on the total abundance of herbivorous nematodes (Table 2; the data we present from the California study is derived from the same experiment as the sandstone component of Hungate et al., 2000). These studies report either neutral or positive effects of CO_2 enrichment on herbivorous nematodes; none reported negative effects. Several studies found that the effect of CO_2 enrichment on herbivorous nematodes was dependent on another factor. For instance, positive effects of CO_2 enrichment were found to disappear after more than 1 year of enrichment (Sonnemann and Wolters, 2005) and later in the growing season (Hungate et al., 2000). Surprisingly, the response of herbivorous nematodes to elevated CO_2 does not appear to relate to the response of belowground productivity. For instance, elevated CO_2 increased root biomass in the Colorado, California, and French experi-ment (Dhillion et al., 1996; Hungate et al., 1997; Rillig et al., 1999; Pendall et al., 2004; Milchunas et al., 2005a), but had no effect on the total abundance of herbivorous nematodes. Similarly, root biomass or turnover increased, at least in some cases, in the studies by Yeates et al. (1997; see Newton et al., 1995), Hungate et al. (2000; see Hungate

Table 2
A summary of the effect of CO_2 enrichment on total herbivorous nematodes abundances in grassland studies

Herbivorous[a]	Plant-feeding	Plant-associated	Interacting factor (where significant)	Design[b]	Location	Reference
↑/ns	–	–	Sandstone grassland (early/mid-season)	OTC	California, USA	Hungate et al. (2000)
ns	–	–	Serpentine grassland	OTC	California, USA	Hungate et al. (2000)
–	ns	ns		SACC	Switzerland	Niklaus et al. (2003)
–	ns	↑/ns/ns	Year (1/2/3)	FACE	Germany	Sonnemann and Wolters (2005)
–	ns	↑		CER	New Zealand	Yeates et al. (1997)
–	ns	ns		Vent	New Zealand	Yeates et al. (1999b)
–	↑	↑		FACE	New Zealand	Yeates et al. (2003)

↑: significantly increased; ns: not significant.
[a]Includes both plant-feeding and plant-associated nematodes.
[b]OTC: open-top chamber; SACC: screen-aided CO_2 control; FACE: free air CO_2 enrichment; Vent: natural CO_2 vent; CER: controlled environment room.

et al., 1997), Yeates et al. (2003; see V. Allard, personal communication therein; Allard et al., 2005), and Niklaus et al. (2003; see Leadley et al. 1999), but the abundance of herbivorous nematodes increased only in some cases (Table 2); the studies by Yeates et al. (1999b) and Sonnemann and Wolters (2005) did not report root responses to CO_2 enrichment. The neutral effects of CO_2 enrichment on herbivorous nematodes observed in many of these studies, despite increased root growth, are surprising and might indicate that simultaneous antagonistic mechanisms were operating. These mechanisms could include reductions in root quality either as a direct result of CO_2 enrichment on plant physiology and/or changes in plant community structure. Indeed, elevated CO_2 increased root C:N, indicative of decreased quality, in the Colorado study (Pendall et al., 2004; Milchunas et al., 2005a) and increased the dominance of the grass *S. comata*, which produces the least digestible aboveground tissue of the three dominant plant species in this ecosystem (Morgan et al., 2004a; Milchunas et al., 2005b). Similarly, elevated CO_2 increased the C:N of root litter from *Lolium multiflorum*, a grass that occurs on serpentine grassland in California (adjacent to the sandstone grassland in our study), but did not influence root litter C:N of three other species (Franck et al., 1997). In general, elevated CO_2 simultaneously increases root production and C:N in a range of plants (Lou et al., 2006), as well as altering grassland plant community composition (Leadley et al., 1999; Zavaleta et al., 2003; Morgan et al., 2004a), which may also influence the root quality. However, it should be noted that changes in nutrient availability (e.g., root quality) may have a greater impact on the biomass of nematode individuals than their abundance (Verschoor, 2002).

Changes in soil moisture in response to CO_2 enrichment might also explain the neutral effect of elevated CO_2 on herbivorous nematodes despite increases in root biomass. Soil moisture increased in response to elevated CO_2 in the California, Colorado, and French study (Hungate et al., 1997; Nijs et al., 2000; Morgan et al.,

2004a), and nematode abundances may be negatively related to soil moisture in grassland soils (e.g., Bakonyi and Nagy, 2000; but see Todd et al., 1999). Alternatively, the abundance of herbivorous nematodes might have been limited by increased predation at elevated CO_2 concentrations. However, since the response of the various predators of nematodes in these ecosystems was not monitored, we cannot determine if this occurred. The factors that limit herbivorous nematodes in the three ecosystems we studied are unknown, however, in other ecosystems, the distribution and abundance of plant-feeding nematodes is controlled by plant community composition and, in some cases, may or may not be modified by environmental conditions (e.g., soil texture and moisture; Freckman and Virginia, 1989; Ettema, 1998; Denton et al., 1999; Todd et al., 1999).

We combined the results from the three elevated CO_2 experiments for this paper, because we felt that the similar findings from each study added weight to our conclusions. As noted in the earlier text, there were differences in experimental design (e.g., natural vs. constructed plant communities), sampling (e.g., soil collection depth), and sample processing (sugar centrifugation vs. Baermann funnel extractions) between the three experiments. Although these differences mean that the absolute values are not directly comparable between the three studies, it is unlikely that they influenced our findings in relation to the effects of elevated CO_2 concentrations on herbivorous nematodes. In addition, presenting the findings of the three studies together, as well as those shown in Table 2, facilitates the identification of general patterns in the response of herbivorous nematodes to elevated CO_2 and allows tentative predictions about their responses to rising atmospheric CO_2 concentrations to be made.

Herbivorous nematodes are major consumers of plant biomass (Smolik, 1974; Lauenroth and Milchunas, 1991) and can influence C and N dynamics (Yeates et al., 1998, 1999a; Bardgett et al., 1999) in grasslands, thus their response to rising atmospheric CO_2 concentrations over

coming decades could have significant implications for grassland processes and community structure. However, our findings show remarkably few changes in the community structure of these nematodes at the family level in three grassland systems exposed to elevated CO_2 concentrations for multiple years. One consequence of increased root production, without changes in belowground herbivore populations, might be greater plant inputs to soil and the detrital food web. This may lead to greater soil organic matter pools in grassland ecosystems, potentially enhancing soil C sequestration, and/or stimulate the biota of the detrital food web (i.e. bacteria, fungi, and microbivores), with consequences for nutrient cycling.

Acknowledgments

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