

Molecular approach for assessing responses of microbial-feeding nematodes to burning and chronic nitrogen enrichment in a native grassland

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Abstract

A substantial proportion of the primary productivity in grassland ecosystems is allocated belowground, sustaining an abundant and diverse community of microbes and soil invertebrates. These belowground communities drive many important ecosystem functions and are responsive to a variety of environmental changes. Nematodes, an abundant and diverse component of grassland soil communities, are particularly responsive to altered environmental conditions, such as those associated with reduced fire frequency and nitrogen enrichment, with the most consistent responses displayed by microbial-feeding nematodes. However, much of the available research characterizing nematode responses to environmental change has been carried out at the taxonomic level of family or by broad trophic categories (e.g. fungivores, bacterivores). The extent to which differential responses to environmental change occurs at the genus level or below is unclear. Therefore, the objective of this study was to use molecular methods to quantify the response of microbial-feeding nematodes, at the lowest levels of taxonomic resolution, to nitrogen enrichment and changes in fire frequency. Using sequencing and quantitative polymerase chain reaction (PCR) probes for the 18S ribosomal RNA gene and the ITS1 region, we identified 19 microbial-feeding nematode taxa across four families. When nematodes were sampled across treatments, we found that some nematode taxa within a family responded similarly to nitrogen and burning treatments, while other taxa within the same family respond quite differently. Additionally, although nematodes from different families on average responded differently to nitrogen enrichment and burning, similar responses were seen in nematode taxa that span three taxonomic families. Thus, if nematodes are to be used as indicators of environmental change, care should be taken to assess the response at the lowest taxonomic level possible.

Keywords: 18S rRNA, community response, ecological genomics, nematoda, quantitative PCR

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Introduction

Belowground communities and the ecological processes they mediate are increasingly recognized as critical, but poorly understood, components of terrestrial ecosystems (Mosier 1998; Wall 1999; Lavelle 2000). This is especially relevant for grassland ecosystems, where a substantial proportion of primary productivity is allocated belowground

in the form of rhizomes, roots, and root exudates (Jackson *et al.* 1996, 1997; Rice *et al.* 1998), leading to large accumulations of soil organic matter and nutrients (Seastedt 1995). Due to the wealth of belowground resources, grasslands contain an abundant and diverse community of soil invertebrates (Hunt *et al.* 1987; Ransom *et al.* 1998). These invertebrate communities play important roles in many key ecological processes (Coleman *et al.* 2004), and are known to be responsive to a variety of environmental factors. For example, research in tallgrass prairie has revealed changes in the abundance and

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composition of the soil invertebrate community in response to changes in natural disturbance regimes, climatic variability, and nutrient enrichment (Todd 1996; Todd *et al.* 1999; Blair *et al.* 2000; Callaham *et al.* 2003), suggesting that soil invertebrates can serve as sensitive indicators of environmental change.

Nematodes are the most abundant of the soil Metazoa, and an important component of the microfauna in grasslands (Curry 1994). Nematode species occurring in soils encompass a wide variety of feeding strategies (Yeates *et al.* 1993), including many free-living species that feed on soil microbes (bacteria or fungi). Microbial-feeding nematodes are among the most important consumers of bacteria and fungi in many systems, including grasslands (Elliot *et al.* 1988), and their interactions with microbial decomposers affect ecosystem processes such as decomposition and nutrient cycling (Freckman 1988; Coleman *et al.* 1991). Nematodes also are known to be responsive to changing environmental conditions (Freckman & Ettema 1993; Todd 1996; Todd *et al.* 1999), and their potential as indicators of soil disturbance is increasingly being recognized (Blair *et al.* 1996; Neher 2001; Fiscus & Neher 2002; Yeates 2003). Changes in natural disturbance regimes, such as reduced fire frequency and chronic nitrogen enrichment, have significant impacts on soil-dwelling nematodes (Todd 1996). Of the trophic groups assayed, Todd (1996) showed that the most consistent responses were displayed by microbial-feeding nematodes, which were strongly favoured by frequent burning and nitrogen addition.

Numerous characteristics favour nematodes as indicators of ecological condition (Freckman 1988; Ferris *et al.* 2001; Neher 2001). However, the adoption of nematodes as bioindicators is currently limited by, among other factors, the difficulty of obtaining fine-scale taxonomic resolution (Neher 2001). Recent evidence suggests that adequate assessment of nematode community changes in response to disturbance requires assessment at the level of genus, and perhaps, species (Fiscus & Neher 2002; Yeates 2003). Because morphological identification to the level of genus or below is often too difficult or limited by systematic expertise (Yeates 2003), molecular assays utilizing the conserved 18S rRNA gene to determine the phylogenetic/taxonomic similarity across taxa have been developed as viable alternative to morphometric assay (Blaxter *et al.* 1998). Recent advancements in DNA technologies have facilitated the use of this method on larger scales. Of particular interest is the use of high-throughput quantitative PCR (qPCR) methods (e.g. TaqMan assay, Heid *et al.* 1996) to diagnose the presence or absence of taxonomically informative sequences. Utilizing the information gained from conserved 18S rRNA sequence, qPCR with species-specific primers and probes provides the ability to assess nematode taxonomy at the most basal level across an entire community.

Previous research on soil nematode communities in native tallgrass prairie ecosystems has demonstrated differential responses of various nematode taxonomic and/or functional groups to long-term treatments, including different fire frequencies and fertilization (Todd 1996). However, the coarse level of resolution (functional group and taxonomic family) employed in this, and other previous studies, precludes the identification of potentially important differential responses among taxa. Therefore, the objective of the present study was to utilize new molecular tools for a more detailed assessment of nematode responses to long-term burning and nitrogen enrichment. Specifically, we focus on microbial-feeding nematodes, a trophic group which was previously shown to exhibit measurable responses to altered fire regimes and chronic nitrogen enrichment. Using molecular methods of taxonomic identification, our aim was to assess the sensitivity of individual nematode taxa to these perturbations, and to determine the extent to which closely related taxa exhibit similarities or differences in their responses to specific treatments.

Methods

Study area

This study was conducted at the Konza Prairie Biological Station, a 3487-ha area of native tallgrass prairie in the Flint Hills of northeastern Kansas, and the primary site for the Konza Prairie Long-Term Ecological Research (LTER) programme. The Konza Prairie LTER programme, established in 1981, is a comprehensive, multidisciplinary programme designed to address long-term research questions relevant to tallgrass prairie ecosystems. In conjunction with the LTER programme, we utilized a long-term experiment, initiated in 1986, designed to assess above- and belowground responses of native tallgrass prairie communities to fire, mowing, and nutrient addition treatments. The experimental design consists of eight large plots (50 × 25 m) subdivided into a total of 64 12.5 × 12.5 m subplots (Fig. 1). Treatments consisted of burning (annually burned vs. unburned); mowing (annually mowed vs. unmowed); ammonium nitrate addition (10 g N/m² annually vs. no addition); and superphosphate addition (1 g P/m² annually vs. no addition). Treatments were arranged in a split-strip plot design with four replications per treatment combination. Burning treatment was assigned at the whole plot level and mowing and fertilization (nitrogen and phosphorus) additions were imposed as strip-plot treatments (Fig. 1). Burning was performed in April of each year and fertilizer treatments were applied in late May to early June. Mowing was conducted in June of each year and was followed by raking to remove above-ground biomass. As previous studies indicated little

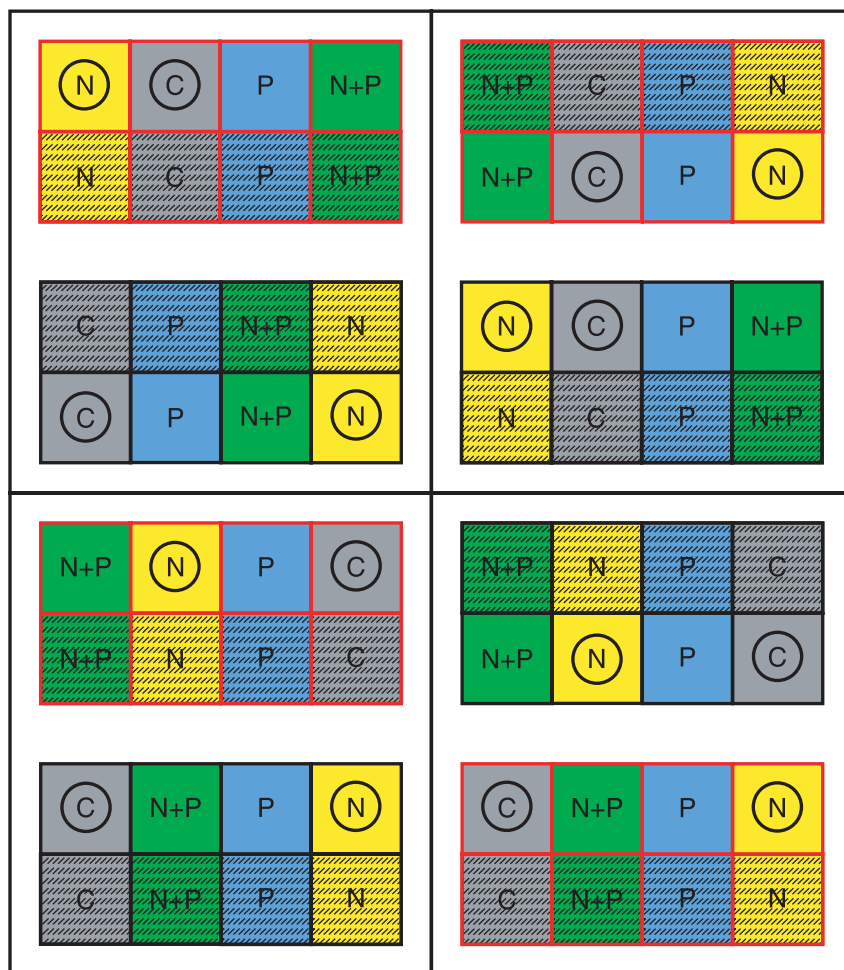


Fig. 1 The field experimental design included eight whole plots grouped into four blocks, with one plot per block that was burned annually (red outline) or left unburned (black outline). A split-strip plot design was obtained by mowing (hatched plots) or not mowing (open plots) one-half of each whole plot (i.e. the whole plots were split by mowing treatment) and using nutrient enrichment [nitrogen (N), phosphorous (P), both (N + P), or neither (C)] as a strip treatment applied perpendicular across the mowing treatments of each block. For this study, we sampled the 16 subplots (circled) that were not mowed, and had either nitrogen enrichment alone or had no nutrient addition. Thus, the four treatment combinations sampled were burned with and without nitrogen addition, and unburned with and without nitrogen addition.

response of microbial-feeding nematodes to mowing and phosphorus additions (Todd 1996), these treatments were not sampled for the present study. Instead, sampling was restricted to the 16 subplots that were either burned or unburned and had either nitrogen or no nitrogen addition.

Soil sampling and nematode extraction

Two 5 cm diameter \times 20 cm deep cores were collected, pooled, and homogenized from each subplot sampled in June and October 2004. For each composite sample, nematodes were extracted from a 100-cm³ subsample using a standard centrifugal-flotation technique (Jenkins 1964). Of the numerous trophic guilds available in the soil (e.g. herbivores, predators, etc.), only microbial-feeding nematodes were censused and collected for further analysis in this study. Microbivores were subsequently identified to morphological genus (using light microscopy and morphological characteristics) and placed singly into 40 μ L worm lysis solution [1 mg/mL proteinase K, 10 mM Tris (pH 8.2), 50 mM KCl, 2.5 mM MgCl₂, 0.45% Tween-20,

0.45% NP-40, 0.05% gelatin]. Nematodes were incubated at 60 °C for 1 h to liberate DNA and denature cellular proteins. A final incubation for 15 min at 95 °C was performed to inactivate the proteinase K. After incubation, the lysate was stored at -80 °C.

Molecular identification of nematode taxa

For all samples, taxonomic identities of individual worms were determined using taxon-specific PCR primers and probes. Quantitative PCRs were run in 20 μ L multiplexed taxa sets using the methods of Jones *et al.* (2006). Where taxonomy was not resolved using existing molecular probes, species determinations were made by sequence analysis of the 18S rRNA gene and its intervening ITS1 region (Jones *et al.* 2006).

Statistical analyses

Nematode abundances were determined by sorting individuals based on 18S rRNA sequence identity and standardizing to 100 g dry soil weight. A student's *t*-test

was used to identify taxa whose abundances were either too rare or too variable to provide accurate inferences on their distribution. Taxa whose mean abundances were not statistically greater than zero on both sample dates were removed from subsequent analyses. Nematode abundances for Spring and Fall sample dates were analysed using the MIXED procedure in SAS (SAS Institute Inc.). For all analyses, a \log_{10} transformation was performed to reduce heterogeneity of variances. In a subsequent analysis, taxon-specific responses were standardized using the following equation: mean adjusted response = (plot abundance – average abundance across plots)/average abundance across plots. As the resulting units of measure are expressed in relation to the proportion of a taxon's own mean, standardizing taxon-specific responses for differences in absolute population densities, allowed for comparisons of taxa whose abundance varied with season, as well as for comparisons across common and rare taxa. Mean adjusted responses for each treatment and season were calculated independently for each taxa and a principal components analysis (PCA) was performed to model the similarities in responses among various taxa.

Results

Molecular identification of nematode taxa

From the 16 subplots sampled, we obtained consistently high numbers of microbial-feeding nematodes on both sampling dates ($n = 547$, and $n = 437$, respectively). For the 984 nematodes sampled across both seasons, qPCR using taxon-specific probes (Jones *et al.* 2006) positively assigned the majority of nematodes to genus/species groups (96% and 83%, respectively). From the remaining samples, sequencing identified three additional taxa: one from the Plectidae family (*Anaplectus* sp.), and two from the Rhabditidae family (*Pellioiditis* sp., and one of unknown genus). Of the 19 microbial-feeding nematode taxa identified (Table 1), the mean abundances of nine taxa were not significantly greater than zero ($P \leq 0.10$). The remaining 10 taxa were selected for further analyses based on their consistent presence in the field plots.

Effects of burning and nitrogen on absolute abundance of microbial-feeding nematodes

Of the three fixed effects, the burning treatment alone had the least direct effect on nematode abundance (Table 2). Season of sampling and nitrogen addition both directly affected the abundances of multiple taxa. However in contrast to the nitrogen addition treatment, where all significant responses were based on an increase in abundance with nitrogen addition (Fig. 2a), differential changes in abundance were seen in response to season. For

Table 1 Mean abundance (standard error in parentheses) per 100 g soil and Student's *t*-test (H_0 : mean = 0) for microbial-feeding nematode taxa across all plots on two sampling dates

Taxa	Spring 2004		Fall 2004	
	Abundance	t	Abundance	t
All taxa	41.66 (5.70)	7.31***	35.96 (5.83)	6.17***
Cephalobidae	15.59 (2.85)	5.47***	22.61 (4.03)	5.61***
<i>Acrobeles</i> sp.	6.31 (2.00)	3.16***	6.07 (1.76)	3.45***
<i>Acrobeles</i> sp. 1	4.96 (1.90)	2.16**	4.81 (1.58)	3.05***
<i>Acrobeles</i> sp. 2	1.35 (0.51)	2.68**	1.26 (0.47)	2.67**
<i>Acrobeloides</i> sp.	3.11 (0.79)	3.93***	10.37 (2.15)	4.84***
<i>Cephalobidae</i> sp. 1	0.23 (0.17)	1.38 ^{NS}	0.00 (0.00)	0.00 ^{NS}
<i>Cephalobidae</i> sp. 2	0.16 (0.11)	1.46 ^{NS}	0.56 (0.26)	2.14**
<i>Chiloplacus</i> sp.	1.82 (0.71)	2.59**	1.11 (0.54)	2.06*
<i>Eucephalobus</i> sp.	3.95 (0.73)	5.43***	4.49 (0.80)	5.59***
Panagrolaimidae (2 taxa)	0.16 (0.11)	1.46 ^{NS}	0.48 (0.31)	1.52 ^{NS}
Plectidae	17.27 (3.74)	4.61***	4.27 (0.84)	5.11***
<i>Anaplectus</i> sp.	0.69 (0.24)	2.84**	0.43 (0.20)	2.14**
<i>Plectus</i> sp. 1	0.85 (0.24)	3.49**	0.60 (0.25)	2.43**
<i>Plectus</i> sp. 2	13.95 (3.43)	4.06**	2.74 (0.72)	3.82***
<i>Plectus</i> sp. 3	0.45 (0.19)	2.45**	0.24 (0.17)	1.39 ^{NS}
<i>Wilsonema</i> sp.	1.33 (0.39)	3.45**	0.26 (0.14)	1.86*
Rhabditidae	8.64 (3.22)	2.68**	8.61 (2.83)	3.05***
<i>Mesorhabditis</i> sp. 1	1.49 (0.89)	1.67 ^{NS}	0.43 (0.27)	1.60 ^{NS}
<i>Mesorhabditis</i> sp. 2	0.08 (0.08)	1.00 ^{NS}	0.59 (0.30)	1.99*
<i>Oscheius</i> sp.	7.00 (2.78)	2.51**	5.01 (2.00)	2.51**
<i>Pellioiditis</i> sp.	0.08 (0.08)	1.00 ^{NS}	2.32 (1.71)	1.35 ^{NS}
<i>Rhabditidae</i> sp.	0.00 (0.00)	0.00 ^{NS}	0.26 (0.18)	1.43 ^{NS}

* $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; NS $P > 0.10$.

example, *Acrobeloides* sp. (Cephalobidae) had a higher overall abundance overall in the Fall, whereas members of the Plectidae family were more abundant in the Spring (Fig. 2b). In addition to direct effects of season, the interaction of season with burning (Fig. 3a) and nitrogen addition (Fig. 3b) separately, as well as the interaction of season with burning and nitrogen treatments combined (Fig. 3c), all indicated differential responses of various taxa across season. In taxa where the burn–nitrogen interaction was not affected by season (Fig. 3d), most taxa responded favourably to nitrogen addition, and particularly well to the combination of nitrogen addition and burning. *Chiloplacus* sp. was the only taxon that had a significant increase in the unburned plus nitrogen treatment.

Taxon-specific response to burning and nitrogen

To circumvent the confounding effects of different relative abundances among taxa, we calculated taxon-specific responses to the treatments as a function of that taxon's mean (see Methods). A PCA was then performed on mean

Table 2 Analysis of variance of fixed effects for absolute abundances (number per 100 g soil) of microbial-feeding nematode taxa

Taxa	F values†						
	S	B	N	S × B	S × N	B × N	S × B × N
All	1.07 ^{NS}	0.01 ^{NS}	18.47 ^{***}	6.23 [*]	0.66 ^{NS}	3.79 [*]	0.93 ^{NS}
Cephalobidae	4.69 ^{NS}	3.93 ^{NS}	4.21 [*]	0.01 ^{NS}	1.36 ^{NS}	2.23 ^{NS}	0.30 ^{NS}
<i>Acrobeles</i> sp.	0.08 ^{NS}	3.27 ^{NS}	0.10 ^{NS}	0.34 ^{NS}	0.06 ^{NS}	0.09 ^{NS}	0.43 ^{NS}
<i>Acrobeles</i> sp. 1	0.19 ^{NS}	3.23 ^{NS}	0.16 ^{NS}	0.35 ^{NS}	0.39 ^{NS}	0.00 ^{NS}	0.01 ^{NS}
<i>Acrobeles</i> sp. 2	0.06 ^{NS}	1.49 ^{NS}	0.02 ^{NS}	0.05 ^{NS}	0.00 ^{NS}	0.00 ^{NS}	0.15 ^{NS}
<i>Acrobeloides</i> sp.	14.25 ^{**}	3.68 ^{NS}	4.96 [*]	0.77 ^{NS}	0.08 ^{NS}	4.82 [*]	0.11 ^{NS}
<i>Chiloplacus</i> sp.	0.90 ^{NS}	9.00 [*]	3.70 [*]	0.03 ^{NS}	1.57 ^{NS}	5.85 [*]	3.20 ^{NS}
<i>Eucephalobus</i> sp.	0.35 ^{NS}	1.06 ^{NS}	5.80 [*]	0.69 ^{NS}	5.72 [*]	3.88 [*]	4.90 [*]
Plectidae	14.13 ^{**}	2.19 ^{NS}	4.12 [*]	1.59 ^{NS}	0.43 ^{NS}	1.51 ^{NS}	1.16 ^{NS}
<i>Anaplectus</i> sp.	1.86 ^{NS}	0.21 ^{NS}	15.81 ^{***}	6.80 [*]	2.30 ^{NS}	1.18 ^{NS}	0.48 ^{NS}
<i>Plectus</i> sp. 1	0.52 ^{NS}	0.11 ^{NS}	0.02 ^{NS}	0.38 ^{NS}	0.18 ^{NS}	0.14 ^{NS}	1.37 ^{NS}
<i>Plectus</i> sp. 2	21.43 [*]	3.01 ^{NS}	1.46 ^{NS}	2.59 ^{NS}	0.67 ^{NS}	0.27 ^{NS}	1.42 ^{NS}
<i>Wilsonema</i> sp.	7.90 [*]	0.30 ^{NS}	1.12 ^{NS}	1.90 ^{NS}	8.54 ^{**}	1.20 ^{NS}	1.05 ^{NS}
Rhabditidae	0.44 ^{NS}	0.86 ^{NS}	6.91 ^{**}	1.71 ^{NS}	3.78 [*]	7.38 ^{**}	6.55 ^{**}
<i>Oscheius</i> sp.	0.01 ^{NS}	0.19 ^{NS}	5.57 [*]	1.31 ^{NS}	1.21 ^{NS}	8.81 ^{**}	2.08 ^{NS}

* $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; NS $P > 0.10$.

†S, season; B, annual burning; N, nitrogen addition.

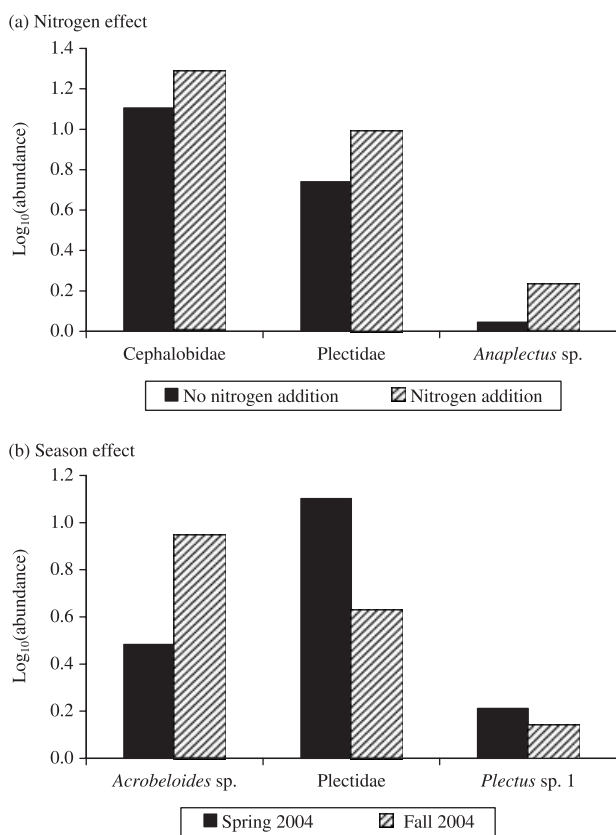


Fig. 2 Log_{10} transformed mean microbial-feeding nematode abundances (number per 100 g soil) for significant (a) nitrogen and (b) season main effects ($P \leq 0.10$).

adjusted taxon-specific responses to assess similarities and/or differences of response across taxa. The eigenvectors of the first three principal components are shown in Table 3. From the PCA, a two-dimensional canonical plot was produced which visualizes only the first and third principal components (Fig. 4). Although the second principal component (PC2) explained 28% of the variance, it was heavily weighted for the unburned plots and was related to a season–burn interaction. While this may be useful in describing the overall variance in the data set, it does not contribute to our goal of grouping taxa based on consistent and predictable responses to the treatments. Thus, PC2 was not utilized in further analyses.

Using the adjusted response, it was shown that differences in abundance due to seasonality did not alter the responses of individual taxa. For example, in both species that exhibited pronounced seasonality (*Acrobeloides* sp. and *Plectus* sp. 1, Fig. 2b), mean adjusted responses to treatments were similar in both seasons, regardless of whether the populations occurred at high or low abundance. The PCA separated taxa based primarily on differential responses to the interaction of burning and nitrogen. The first principal component (Table 3, PC1), which can be interpreted as an overall contrast of burned vs. unburned plots weighted for nitrogen-enriched conditions (i.e. unburned plus nitrogen vs. burned plus nitrogen), accounted for almost one-half of the total variation (Table 3, Fig. 4). The third principal component (Table 3, PC3), which accounted for one-fourth of the total variation, can be interpreted as an overall contrast of nitrogen enrichment

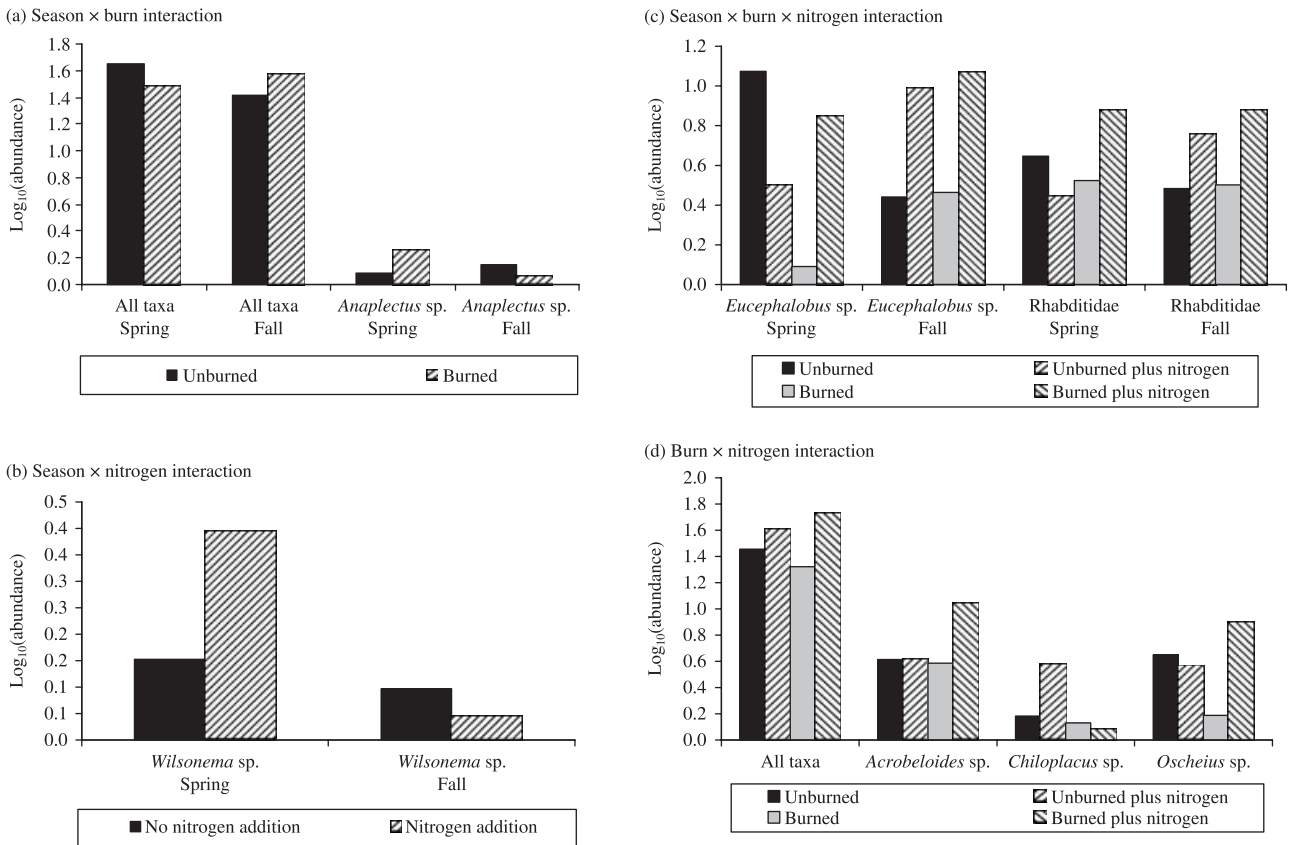


Fig. 3 Log₁₀ transformed mean microbial-feeding nematode abundances (number per 100 g soil) for significant (a) season × burn, (b) season × nitrogen, (c) season × burn × nitrogen, and (d) burn × nitrogen interaction effects ($P \leq 0.10$).

Table 3 Major principal components for adjusted responses of taxa to burning and nitrogen enrichment

	Principal components		
	PC1	PC2	PC3
Eigenvectors			
Unburned	-0.25	0.79	0.44
Unburned plus nitrogen addition	-0.65	-0.37	-0.22
Burned	0.48	-0.41	0.62
Burned plus nitrogen addition	0.54	0.27	-0.61
Eigenvalues	1.89	1.12	0.98
Proportion of variance explained	0.47	0.28	0.25

vs. nitrogen limitation weighted for the presence of burning (i.e. burned vs. burned plus nitrogen). As both the right half of PC1 and the lower half of PC3 explain an amount of variance due to burning plus nitrogen addition, the taxon continuum from *Acrobeloides* sp. 1 to *Anaplectus* sp. (Fig. 4) represents a gradient of responses to nitrogen in the presence of burning. Conversely, the taxon group from *Plecticus* sp. 1 to *Chiloplacus* sp. responded to effects other than those produced by the burned plus nitrogen treatment, primarily those of the unburned plus nitrogen treatment.

Discussion

Molecular assay of nematode communities

The phylogeny of nematodes is currently in flux (see the Nematode Tree of Life project, <http://nematol.unh.edu/>), due in part to the past use of morphological characters alone to derive nematode taxonomy. The use of the 18S rRNA gene for taxonomic identification in this large and diverse group has provided a less subjective approach to taxonomic identification. However, as DNA sequencing is still quite costly and time consuming when assaying hundreds of individuals, the use of high-throughput methods provide a more efficient alternative for molecular identification of nematode taxa in field experiments. Although molecular probes have previously been used for taxonomic identification of nematodes (Meksem *et al.* 2001a, b; von Samson-Himmestjerna *et al.* 2002; Atkins *et al.* 2003), the large-scale use of this method to assess the diversity of a nematode community in response to environmental perturbations is unique to our study. The high-throughput nature of this method lends itself well to ecological studies at the community level. Using qPCR, we were able to quickly and accurately assess the taxonomic

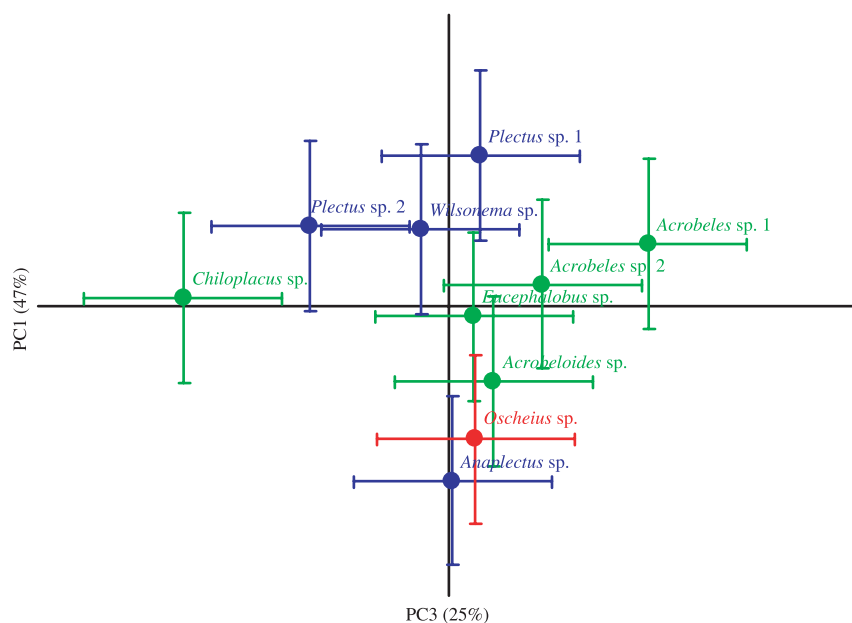


Fig. 4 Canonical plot of the first and third principal components of mean adjusted response. Members within taxonomic families are designated by colour (Cephalobidae, green; Plectidae, blue; Rhabditidae, red). Data are means \pm standard error of the difference.

identity of 16 known taxa across multiple plots and multiple samplings. The only caveat to this approach is that the sequences of all taxa of interest must be known. Within this study, 10% of the 984 samples processed were not identifiable using existing molecular probes. Subsequent sequencing of these individuals provided sequence information on three additional taxa.

Treatment effects and taxon responses

The analyses of treatment effects on nematode communities revealed a pattern of response similar to that reported in previous studies of fire, mowing, and nutrient amendment in tallgrass prairie (Todd 1996). The current data along with those of Todd (1996) suggest that nitrogen addition and burning elicit predictable responses in overall abundance of microbial-feeding nematodes. However, with the more detailed analyses made possible by the application of molecular methods, we show, as have others (Ferris *et al.* 2001; Fiscus & Neher 2002; Yeates 2003; citations within), that trophic-level responses are actually an average of differential responses of individual taxa at the family level and below. Within the nematode families that, in aggregate, compose the microbial-feeding trophic group, we show that individual taxa are differentially affected by season, nitrogen addition, and burning, either in the direction of their response (positive or negative) or in the degree to which they respond.

Community response to environmental change

The treatment structure used in this study was designed to assess the impacts of changing land use and nitrogen

deposition, and was a surrogate for studying the impacts of important aspects of global change in tallgrass prairie, and other productive grasslands. These treatments had measurable effects on the microbial-feeding nematodes, suggesting that changes in natural disturbance regimes (e.g. frequent fire and grazing) and anthropogenic changes in the environment (e.g. nitrogen enrichment via fertilization) will elicit changes in the soil invertebrate community, and potentially the processes they mediate. The data reported here, along with the results of previous studies, indicate that the dominant groups of microbial-feeding nematodes in tallgrass prairie soils (Cephalobidae and Plectidae), are not adversely affected by the harsher environmental conditions associated with frequent burning (i.e. warmer soil temperatures, drier soil conditions, more extreme temperature and moisture fluctuations). In contrast, population densities of *Oscheius* (Rhabditidae) were significantly reduced in the presence of burning. Removal of burning from the system provides an opportunity for community composition shifts, favouring taxa such as the Rhabditidae that are relatively rare in terms of both abundance and diversity in burned prairie (T. C. Todd, unpublished). Such a shift in the relative abundance of this family is often indicative of resource pulses (Yeates 2003) and may reflect the increase in nitrogen availability and soil moisture associated with fire suppression. Similarly, a positive response to nitrogen enrichment was observed for most microbial-feeding taxa. These responses may be linked to changes in the composition of the microbial biomass, specifically to an increase in the proportion of bacteria relative to fungi, which was documented earlier in this field experiment (Garcia & Rice 1994). As frequent burning decreases both the concentration of soil inorganic

nitrogen and nitrogen mineralization (Blair 1997; Turner *et al.* 1997), nitrogen becomes a more limiting nutrient in frequently burned prairie. Thus, nitrogen supplementation in a nitrogen-limited system should provide greater resources for microbial populations and, subsequently, microbial grazers. However, this hypothesis assumes that all nematode taxa utilize the same food resource. It is likely, that microbial-feeding nematodes exhibit differences in food preference and food utility, as is true in many other broad trophic groups.

Studies are currently underway to assay food preference and subsequently changes in availability of those preferred food resources due to the altered burning and fertilization treatment. It is certainly plausible that changes in the food web may contribute to the observed nematode responses. In contrast, responses of nematodes not correlated to nitrogen addition under annually burned conditions, such as *Chiloplacus* sp., may indicate that their densities are influenced by a different limiting factor (e.g. soil water availability). Similarly, some taxa (*Acrobeles*, *Plectus*) appear to be less responsive to nitrogen enrichment, suggesting that resource availability may not be limiting, or that increases in resource availability are accompanied by unfavourable environmental changes. Additional research is ongoing to determine whether other environmental changes, particularly pH and osmolaric changes, can explain the response of taxa that do not exhibit food related responses. In addition to food and environmental interactions, some of the observed responses may simply be due to inter-specific interactions. Competition and/or mutualistic interactions could account for changes in nematode community composition; however, no significant species–species correlations were observed in this study, either positive or negative. Thus, it seems more likely that some combination of food specificity/availability or environmental sensitivity is responsible for the community change.

Nematodes as bioindicators

Yeates (2003) argued for the necessity of species-level discrimination of nematode taxa in their use as ecological indicators. The data presented here support at least genus-level discrimination and indicate that generalizations based on higher levels of taxonomy are speculative at best. For example, although the responses of microbial-feeding nematodes to nitrogen enrichment generally were similar at the family level, there were individual taxa within families that responded differentially. For each family represented by multiple genera (Cephalobidae and Plectidae), there was a taxon (*Chiloplacus* sp. and *Anaplectus* sp., respectively) that exhibited a significantly different response from the others. Similarly, although most taxa responses tended to group by family, there were three taxa across three families (*Acrobeloides* sp., *Anaplectus* sp., and

Oscheius sp.) that responded similarly to each other, but were not necessarily representative of their respective families. Documentation of differential sensitivity among related nematode taxa to the effects of disturbance has been limited, but our data, together with those of Fiscus & Neher (2002), suggest that generalizations on direction and levels of response to environmental change must be made with caution, and that community-level responses need to be assessed with the maximum precision possible.

Implications for linking ecological responses to their genetic mechanisms

Changes in the natural environment present a multitude of biotic and abiotic challenges to all organisms, requiring both short-term ecological and long-term evolutionary responses. These responses have been the subject of much biological interest, yet their inherent complexity has made genetic and mechanistic dissection empirically difficult. However, recent technical advances in high-throughput sequencing, genotyping, and genome-wide expression profiling, coupled with bioinformatics approaches for handling such data, hold great promise for disarticulating these responses with unprecedented resolution. By combining genomic and ecological approaches, it is now possible to study the genetic mechanisms underlying the responses of organisms to their natural environment (Feder & Mitchell-Olds 2003). While a large database exists concerning the roles genes play in development and physiology in model organisms, relatively little is known about how the environment affects endemic organisms at the level of expression of individual genes (Singh 2003). Organisms have varying genetic capacities to respond to changes in the environment, either as a result of differences in the genes/alleles they possess or in how they are regulated (Mackay 2001). Future studies will attempt to identify specific genes linked to the nematode responses described in this study.

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