Soil Biology & Biochemistry 108 (2017) 78–83

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Nematode exclusion and recovery in experimental soil microcosms

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ARTICLE INFO

Article history:
Received 2 December 2016
Accepted 1 February 2017
Available online 6 February 2017

Keywords:
Nematode removal
Soil defaunation

ABSTRACT

Experimental manipulations of soil fauna are a powerful tool for assessing causal relationships between belowground biodiversity and key ecosystem properties. However, preparing soil microcosm treatments without soil fauna for ecological experiments can be problematic. Methods to exclude nematodes, a ubiquitous and functionally important component of terrestrial ecosystems, have been developed for a few specific ecosystems, some of them involving the application of nematicides that may have interactive effects throughout the soil food web. Our goal was to develop a method to remove nematodes from soils of three Long Term Ecological Research (LTER) grassland sites, ranging from desert to moist, without use of chemicals and with moderate disturbance. Moreover, we aimed at testing whether the nematode removal would remain effective up to several weeks later. The following treatments were applied to ~3-kg soil microcosms in the laboratory: (1) a 72 h heating (65 °C) - freezing (−20 °C) - heating (65 °C) cycle using soil maintained at its original water content, and pre-wetting soil 24 h before heating (65 °C) for either (2A) 48 h or (2B) 24 h. We measured treatment effects on total abundance and trophic structure of the nematode community. To investigate whether nematodes would recolonize eight weeks after treatments, we conducted a greenhouse experiment where individual seedlings of the dominant grass species for each ecosystem were transplanted to treated and non-treated (control) soils. A heat-freeze-heat cycle of 72 h using soil in its original field water content killed 60, 95, and 99% of the nematodes for the desert, semi-arid, and moist tallgrass prairie soils, respectively. Pre-wetting soil before heating increased mortality to 99% for all ecosystems after only 24 h at 65 °C. Root-feeders were the most resistant nematode trophic group. Eight weeks after treatments, there was no significant nematode recolonization for the pre-wetted 48 h heated soils from the three sites, while for the semi-arid and moist sites there was a slight recovery in abundance in soil from the 24 h heating treatment. Therefore, a treatment at 65 °C for 48 h using pre-wetted soil is recommended for eight-week long manipulative experiments in order to assure the effectiveness of the nematode removal throughout the experiment.

1. Introduction

Nematodes are among the most abundant and diverse multicellular organisms inhabiting soils (Bongers and Ferris, 1999) and represent key connections across soil food webs through their remarkable range of feeding strategies as herbivores, bacterivores, and fungivores, to omnivores and predators (Yeates et al., 1993). Although the understanding of how nematodes interact within extremely complex soil food webs has rapidly grown (Ferris, 2010; Cesarz et al., 2015), there is an immediate need for further experiments studying their responses to global change, and possible consequences for soil and ecosystem function. The manipulation of nematode functional diversity in soil is key to answering such questions (Xiao et al., 2010; Gebremikael et al., 2015).

Experimental manipulation of soil fauna is a powerful tool for assessing causal relationships between belowground biodiversity and key ecosystem properties (Bardgett and van der Putten, 2014; Wagg et al., 2014). However, one of the biggest challenges lies in establishing treatments without nematodes (Ingham et al., 1985), which are used in laboratory or field microcosm manipulations to contrast responses of an ecosystem process with the native nematode community. Exclusion of nematodes and other soil fauna

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http://dx.doi.org/10.1016/j.soilbio.2017.02.001
0038-0717/

groups has usually been performed by physical or chemical methods, with the latter becoming less frequently used because of well documented, non-target effects of biocides or nematicides on the soil food web (Wall and Reichman, 2000). Physical exclusion methods incorporate variations of timing of temperature extremes to kill nematode communities in soil (e.g. freeze/thaw cycles and heating treatments), and are generally developed for the specific ecosystem to be studied (Bruckner et al., 1995; Bardgett et al., 1998; Jaffee, 2006; Lopez et al., 2009). Examples of distinct exclusion methods tested in a particular ecosystem range from deep-freezing soil using dry ice at −78.5 °C (Bruckner et al., 1995), to heating for 24 h at 80 °C followed by freezing for another 24 h at −80 °C (Bardgett et al., 1998). These methods provide a useful guide for work within a specific ecosystem but are of limited value for experiments across several ecosystem types and at broader geographic scales. Physical exclusion can also occur by autoclaving or use of gamma irradiation (Wall and Reichman, 2000). An issue with autoclaving is that it may produce high nutrient release and loss of soil structure (Trevors, 1996), potentially compromising the representativeness of microcosm experiments to actual field conditions, while gamma irradiation becomes costly for experiments using large amounts of soil.

Here we performed a series of temperature extremes experiments in order to develop a single physical method for soil nematode exclusion that could be applied across ecosystems varying in climate (annual precipitation), soil types, and productivity, thus providing a tool for soil ecologists conducting manipulative experiments. Soils from three different grassland ecosystems, ranging from desert to humid, were used to test for water content influences on nematode exclusion efficiency. We hypothesized that soil nematodes from ecosystems with contrasting water regimes would respond differently to nematode exclusion treatments. Nematodes live in water films around soil particles and thus changes in soil water (chemicals, freezing, desiccation, seasonality) affect survival (Sylvain et al., 2014). At present there is no nematode exclusion technique tested in ecosystems across a spatial gradient of water availability. We also expected that nematode trophic groups would respond dissimilarly to exclusion treatments, with higher trophic levels being most sensitive to treatments as predaceous nematodes have been shown to be more sensitive to disturbance (Bongers, 1999).

Specifically, this study aimed to: (i) test nematode exclusion methods by examining its effect on total nematode abundance and the trophic structure of the nematode community; (ii) determine whether exclusion effectiveness varies among ecosystems across a gradient of water availability; (iii) determine how long a treated soil remains nematode-free by investigating temporal patterns in nematode community recovery; and (iv) investigate the effect of exclusion treatment intensity on nematode community recovery.

2. Materials and methods

2.1. Site description and soil collection

Soil was collected during summer 2015 in three types of US grassland ecosystems: desert grassland, semiarid shortgrass, and mesic tallgrass. The desert grassland site was located in the Jornada Basin Long-Term Ecological Research site (JRN), in New Mexico. This site receives on average, 247 mm of precipitation annually, and vegetation is dominated by *Bouteloua eriopoda*, with the presence of *Prosopis glandulosa* (Havstad and Schlesinger, 2006). The semiarid shortgrass site was located at the Semiarid Grasslands Research Center (SGRC), Colorado, formerly Shortgrass Steppe LTER. Mean annual precipitation is 321 mm, and vegetation is dominated by *Bouteloua gracilis* (Lauenroth and Burke, 2008). The tallgrass prairie site was located in Kansas at the Konza Prairie LTER (KNZ). Average annual precipitation is 835 mm, and vegetation is dominated by *Andropogon gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium* (Knapp, 1998). At each site three soil blocks measuring 20 × 20 cm were taken from the top 20 cm soil from directly beneath the dominant vegetation type. Soil was returned to laboratories at Colorado State University, stored at 4 °C and used within 7 days.

2.2. Nematode exclusion - experiment 1

Experiment 1 tested the exclusion of nematodes from soils of the three grassland ecosystems with heating/freezing cycles. Samples from each site were homogenized by a coarse-mesh sieving (6.25 mm), and a portion of each sample was placed into aluminum dishes (33 × 23 cm) to a depth of 5 cm. One dish was obtained per soil block, yielding a total of 3 dishes per site. Due to differing soil bulk densities among sites, the total weight of soil in the aluminum dishes used for nematode exclusion differed (e.g. ~4 kg of JRN soil per dish, compared to 2.5 kg of SGRC soil, and 2.2 kg of KNZ soil). The aluminum dishes were then subjected to three days of treatment: 24 h at 65 °C, followed by 24 h at −20 °C, then a further 24 h at 65 °C. Soil in the aluminum dishes was subsampled (100 g for nematode analyses, 50 g for soil moisture) four times from different quadrants of the dish at the following time intervals: control (T = 0 h), after heating (T = 24 h), after heating and freezing (T = 48 h) and after heating, freezing and heating (T = 72 h). Nematodes in soil subsamples were extracted using Baermann funnels (Hooper, 1970), and nematodes removed daily for 3 days, and stored at 4 °C. Nematodes were counted and identified using an inverted microscope (Olympus CKX41, 200X magnification) within 3 days of extraction. The first 150 nematodes per sample were identified to five different trophic groups: bacterial-feeders, fungal-feeders, root-feeders, omnivores, and predators (Yeates et al., 1993), and total numbers per feeding group were extrapolated based on full sample counts. Standardized nematode population abundances were calculated as individuals per kg of soil (corrected to oven dry weight equivalent). Gravimetric soil water content (w/w) and oven dry weight equivalents were determined from mass loss of soils heated to 105 °C for 48 h (Barrett et al., 2004).

2.3. Nematode exclusion - experiment 2

Experiment 2 aimed to reduce any nematode survival rates observed in experiment 1. Sieved soil (6.25 mm) was placed into aluminum dishes as in experiment 1. Soil was then pre-wetted and left at 4 °C for 24 h. To wet the soil, water was gently sprayed over the soil surface until it passed through small holes on the bottom of the dishes and wet an absorbent paper placed beneath the dishes. Then, samples were transferred to an oven at 65 °C for either 24 or 48 h (experiment 1 results showed that the freezing step was redundant). Soil subsamples (100 g for nematode analyses, 50 g for soil moisture and 100 g for experiment 3) were taken from different quadrants of the tray at the following time intervals: control (T = 0 h) and after heating for 24 and 48 h. Nematode and soil moisture samples were processed identically to experiment 1.

2.4. Nematode community recovery – experiment 3

A greenhouse experiment was design to examine the longevity of the exclusion treatments effects observed in experiment 2 and temporal patterns of nematode recovery. The environmental conditions in the greenhouse were 15–21 °C, 30%–50% humidity, and photoperiod of 16 h light / 8 h dark. To replicate a potential

microcosm, the following dominant grass species for each site were grown from seeds in nematode-free vermiculite: black grama (*Bouteloua eriopoda*) for JRN; blue grama (*Bouteloua gracilis*) for SGRC; and big bluestem (*Andropogon gerardii*) for KNZ. Three weeks after germination, seedlings were transplanted to pots containing 100 g soil subsamples from the treatments applied in experiment 2 (0, 24 and 48 h at 65 °C). All pots were watered daily (25 mL water) for the duration of the experiment in order to keep plants alive.

Two approaches were used to investigate community recovery. First, to investigate temporal patterns in nematode recovery a total of nine replicate samples (100 g) from each site were placed into greenhouse pots using the remaining soil from the 48 h heating exclusion treatment of experiment 2 only. Nematodes were extracted for each site at 2, 4 and 8 weeks (three replicates each) after seedling planting. Secondly, to investigate the effect of exclusion treatment intensity on recovery (24 vs 48 h heating), three replicate samples for each site were placed into greenhouse pots using the remaining soil from experiment 2 (0, 24 and 48 h at 65 °C). Eight weeks after planting seedlings, nematodes were extracted. All nematode extractions for experiment 3 followed the same methods described in experiment 1.

2.5. Statistical analysis

For all experiments, comparisons among exclusion and recovery treatments were carried out for total nematode abundance and trophic structure using one-way ANOVA on linear models. The assumptions of homogeneity and normality were validated. When differences were significant ($p < 0.05$), we compared means using Tukey's test ($p < 0.05$). For experiment 2, to visualize major patterns structuring the community, we performed ordination on sites and treatments based on group composition with non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarity matrix of nematode trophic structure data, followed by non-parametric multivariate analysis of variance (npMANOVA) to test the effect of site and treatment. All analyses were conducted using the software R, version 3.2.2 (R Core Team, 2015), and packages vegan (Oksanen et al., 2016) and ggplot2 (Wickham, 2009).

3. Results

3.1. Experiment 1

After 72 h of heat and freeze cycles, mortality of nematodes increased across ecosystems following the gradient of less to more water availability from desert to humid grassland (Fig. 1a). This pattern of response to treatments occurred in all trophic groups, except for predators, which were highly sensitive to treatments regardless of the ecosystem type (Table 1). Total abundance of nematodes was reduced by 60% in JRN soil (from 3348 ± 159 to 728 ± 108 nematodes kg^{-1} dry soil), where soil moisture content was as low as 1.1%. At KNZ, however, where soil moisture was 34.1%, there was a 99% reduction in nematode abundance (reduction from 5868 ± 1434 in control to 3 ± 3 nematodes kg^{-1} dry soil in the 72 h treatment). Intermediate soil moisture content (9.3%) and mortality rate (95%) occurred for the semi-arid grassland (SGRC) (reduction from 5074 ± 1067 in control to 104 ± 44 nematodes kg^{-1} dry soil in the 72 h treatment) (Fig. 1a). Within each site there were no significant differences between nematode exclusion treatments (Fig. 1), suggesting that the freezing step was redundant.

3.2. Experiment 2

In experiment 2, nematodes were reduced nearly 100% at all ecosystems after pre-wetting soil and heating for 24 h at 65 °C (Fig. 1b). Thus, the 48 h heating treatment did not produce any additional effect on nematode mortality. Trophic group structure of the control treatment did not differ among sites (Table 1 and Fig. 2). After treatments, however, trophic structure significantly changed as tested trough npMANOVA ($F = 7.21$, $p < 0.001$, $R^2 = 0.47$) (Fig. 2), with root-feeders being the most resistant group to treatments. Resistance here is defined as the population that has the largest number of individuals surviving the exclusion treatments.

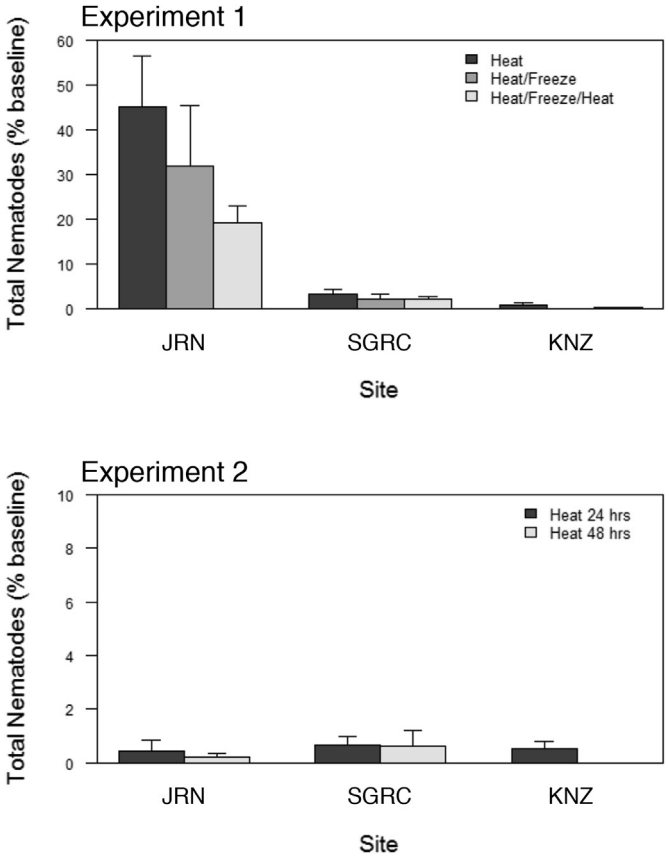

Fig. 1. Nematode percent abundance based on total abundances of control soil in experiments 1 and 2 for soil from Jornada LTER (JRN), Semiarid Grassland Research Center (SGRC), and Konza LTER (KNZ). Error bars represent standard errors. Tukey test did not show significance differences ($p < 0.05$) between treatments.

3.3. Experiment 3

There was no significant community recovery 2, 4, and 8 weeks in the subsamples from the 48 h heating exclusion treatment of experiment 2 (Table 2). When we kept the full set of treatments from experiment 2 in the greenhouse for eight weeks, results showed different responses for the 24 h and 48 h exclusion treatments: the nematode community in KNZ and SGRC soils heated for only 24 h increased from 18 ± 9 to 1409 ± 1398 and from 22 ± 8 to 774 ± 658 nematodes kg^{-1} dry soil, respectively, producing nematode abundances significantly larger than those for the 48 h treatment in KNZ soil (Fig. 3). In contrast, there was no significant nematode recovery in soils heated for 48 h (Fig. 3). Bacterial-feeding nematodes were almost the only nematode group found in both 24 and 48 h treatments for SGRC, and 48 h for KNZ (Table 2). After 8 weeks in the greenhouse, the number of nematodes in the control treatment decreased for both JRN (from 5645 to 417 nematodes kg^{-1} dry soil) and SGRC soil (from 5581 to 2002 nematodes

Table 1
Nematode trophic group composition (individuals kg^{-1} dry soil) by experiment and site. Standard errors presented, $n = 3$.

Site	Treatment	Bacterial-feeders	Root-feeders	Fungal-feeders	Omnivores	Predators
Exclusion experiment 1						
Jornada	Control	1874.3 ± 185.8	484.3 ± 205.1	602.3 ± 55.4	798.0 ± 102.0	8.3 ± 8.3
	Heat	1014.7 ± 330.3	146.0 ± 96.7	417.0 ± 68.5	102.3 ± 34.7	0.0
	Heat/Freeze	678.3 ± 334.6	256.7 ± 112.9	216.0 ± 77.9	47.0 ± 13.5	0.0
	Heat/Freeze/Heat	380.7 ± 45.9	126.7 ± 60.1	126.7 ± 28.5	46.7 ± 12.0	0.0
Shortgrass	Control	2340.3 ± 689.8	640.3 ± 229.4	1377.3 ± 386.9	606.7 ± 205.4	26.7 ± 13.4
	Heat	20.0 ± 5.8	23.3 ± 18.6	70.0 ± 23.1	30.0 ± 30.0	0.0
	Heat/Freeze	40.3 ± 30.3	13.3 ± 13.3	20.3 ± 15.6	6.7 ± 3.3	0.0
	Heat/Freeze/Heat	46.7 ± 12.0	10.0 ± 5.8	3.3 ± 3.3	6.7 ± 3.3	3.3 ± 3.3
Konza	Control	1500.0 ± 152.5	143.3 ± 77.3	2862.3 ± 920.9	852.0 ± 109.8	279.0 ± 64.0
	Heat	0.0	0.0	3.7 ± 3.7	22.0 ± 22.0	3.7 ± 3.7
	Heat/Freeze	0.0	0.0	0.0	0.0	0.0
	Heat/Freeze/Heat	0.0	0.0	3.3 ± 3.3	0.0	0.0
Exclusion experiment 2						
Jornada	Control	3306.7 ± 576.8	192.7 ± 74.8	1614.7 ± 968.9	339.3 ± 122.9	10.3 ± 10.3
	Heat 24 h	13.3 ± 13.3	0.0	6.7 ± 6.7	0.0	0.0
	Heat 48 h	6.7 ± 6.7	0.0	0.0	6.0 ± 6.0	0.0
Shortgrass	Control	2894.7 ± 1122.0	424.3 ± 222.2	1546.3 ± 812.0	556.7 ± 214.2	3.3 ± 3.3
	Heat 24 h	0.0	10.0 ± 5.8	3.0 ± 3.0	9.0 ± 9.0	0.0
	Heat 48 h	3.0 ± 3.0	6.0 ± 3.0	3.0 ± 3.0	0.0	0.0
Konza	Control	1242.3 ± 171.2	121.3 ± 42.8	1145.0 ± 492.5	603.7 ± 174.1	42.0 ± 22.1
	Heat 24 h	3.7 ± 3.7	14.3 ± 7.2	0.0	0.0	0.0
	Heat 48 h	0.0	0.0	0.0	0.0	0.0

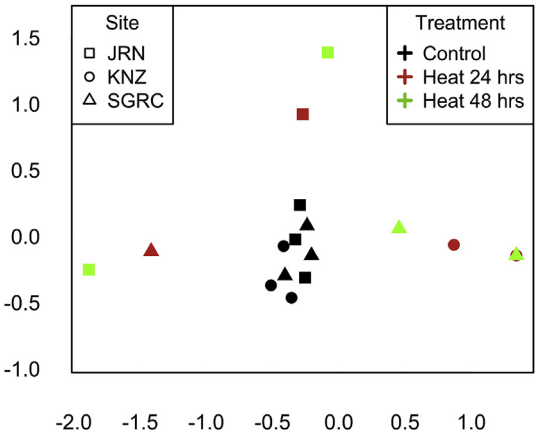

Fig. 2. Non-parametric multidimensional scaling (NMDS) performed for nematode trophic group composition, with observations grouped by site and colored according to treatments in experiment 2. Sites are Jornada LTER (JRN), Semiarid Grassland Research Center (SGRC), and Konza LTER (KNZ). Effect of exclusion treatments on trophic structure among sites is significant (npMANOVA): $F_{2,16} = 7.21$, $p < 0.001$, $R^2 = 0.47$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

kg^{-1} dry soil), but increased threefold in KNZ soil (from 3367 to 12,503 nematodes kg^{-1} dry soil).

4. Discussion

4.1. Reduction in nematode abundance varies among ecosystems

Building on previous studies that established soil fauna exclusion protocols for a single ecosystem (Ingham et al., 1985; Bruckner et al., 1995; Bardgett et al., 1998; Lopez et al., 2009), the present study investigated soil nematode community dynamics following exclusion treatments on soils from three grassland ecosystems with contrasting water regimes. Our results from experiment 1 showed that, as expected, the effectiveness of exclusion treatments varies among ecosystems (Fig. 1a). A 72 h heat-freeze-heat cycle with soils at the original water content (no additions of water) killed only 60 and 95% of nematodes from desert and semi-arid grassland soils, respectively, compared to 99% in humid grassland soils. Knowledge of nematode ecology in desert systems guided the variations in methods used for experiment 2 that resulted in a higher reduction of nematodes from JRN and KNZ soils (Fig. 1b). Evidence shows that nematode communities from desert soils are more resilient to variations in soil moisture, a contrast to the increased population responses that occur in wetter ecosystems (Freckman et al., 1987a, 1987b; Moorhead et al., 1987; Sylvain et al., 2014; Vandegehuchte et al., 2015). Decreases in soil moisture in deserts over short (a few hours) or long periods (days) can induce nematodes to enter a survival, anhydrobiotic, state where they are uncoupled from ecosystem processes until soil moisture becomes favorable for activity again (Freckman et al., 1987a; Moorhead et al., 1987). We reason that a portion of the soil nematode community from the desert site (JRN) was already anhydrobiotic at the sampling time due to dry conditions, which would have allowed them to survive the exclusion treatments in experiment 1, becoming active again when re-hydrated during the extraction procedures. In fact, the increased mortality in experiment 2 (Fig. 1b), where we pre-wetted the soil samples and waited 24 h before applying the treatments, suggests that pre-wetting the drier soils activated anhydrobiotic nematodes and made them more vulnerable to exclusion treatments. The range of survival mechanisms employed by the phylum Nematoda suggests this shift to a more favorable, moist environment in the pre-treatment with water also may have induced reactivation of nematode species using several survival mechanisms and therefore higher mortality occurred during treatment.

4.2. Trophic groups respond dissimilarly to exclusion and recovery treatments

Trophic structure differed significantly among sites after treatments in experiment 2 (Fig. 2), suggesting that trophic groups differed in their responses to exclusion treatments. The higher resistance of root-feeding nematodes to treatments compared to other trophic groups is likely due to survival within protective root fragments (Macguidwin and Forge, 1991; Stevnbak et al., 2012).

Table 2
Nematode trophic group composition (individuals kg^{-1} dry soil) by approach and site. Standard errors presented, $n = 3$.

Site	Treatment	Bacterial-feeders	Root-feeders	Fungal-feeders	Omnivores	Predators
Approach 1: temporal patterns in nematode recovery						
Jornada	Control	6.7 ± 6.7	0.0	0.0	6.0 ± 6.0	0.0
	2 weeks	6.7 ± 6.7	6.7 ± 6.7	0.0	0.0	0.0
	4 weeks	474.4 ± 474.7	0.0	0.0	3.3 ± 3.3	0.0
	8 weeks	3.3 ± 3.3	0.0	0.0	3.3 ± 3.3	0.0
Shortgrass	Control	3.0 ± 3.0	6.0 ± 3.0	3.0 ± 3.0	0.0	0.0
	2 weeks	7.0 ± 7.0	6.0 ± 6.0	7.0 ± 7.0	0.0	0.0
	4 weeks	57.0 ± 47.8	6.7 ± 3.3	6.7 ± 6.7	3.3 ± 3.3	0.0
	8 weeks	123.7 ± 50.9	73.0 ± 73.0	3.3 ± 3.3	0.0	0.0
Konza	Control	0.0	0.0	0.0	0.0	0.0
	2 weeks	0.0	0.0	0.0	0.0	0.0
	4 weeks	0.0	0.0	0.0	0.0	0.0
	8 weeks	3.7 ± 3.7	0.0	0.0	0.0	0.0
Approach 2: effects of exclusion treatments on recovery						
Jornada	Control	117.7 ± 63.8	26.0 ± 26.0	176.0 ± 151.2	61.7 ± 56.7	0.0
	Heat 24 h	7.3 ± 7.3	0.0	0.0	3.0 ± 3.0	0.0
	Heat 48 h	3.3 ± 3.3	0.0	0.0	3.3 ± 3.3	0.0
Shortgrass	Control	960.0 ± 291.1	209.7 ± 104.6	437.0 ± 231.4	354.3 ± 89.2	8.3 ± 8.3
	Heat 24 h	688.0 ± 577.0	50.7 ± 50.7	3.7 ± 3.7	18.3 ± 18.3	0.0
	Heat 48 h	123.7 ± 50.9	73.0 ± 73.0	3.3 ± 3.3	0.0	0.0
Konza	Control	10,007.0 ± 823.3	602.0 ± 177.8	776.0 ± 291.8	726.3 ± 178.2	99.0 ± 65.2
	Heat 24 h	972.0 ± 972.0	430.0 ± 430.0	0.0	7.3 ± 7.3	0.0
	Heat 48 h	3.7 ± 3.7	0.0	0.0	0.0	0.0

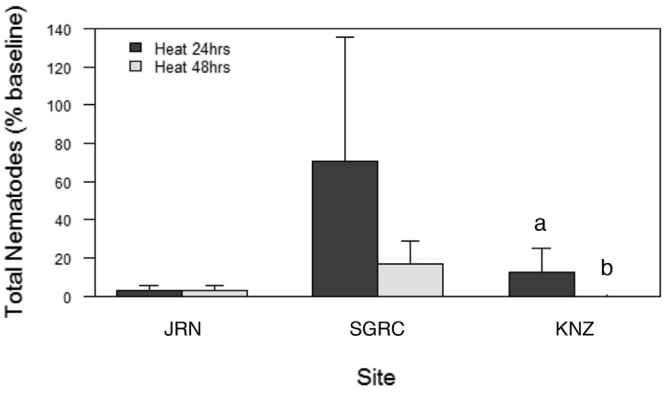

Fig. 3. Percent abundances of nematodes following 8-week recovery experiment, relative to total nematode abundances of controls. Sites are Jornada (JRN), Semiarid Grassland Research Center (SGRC), and Konza (KNZ). Letters are used where statistically significant differences ($p < 0.05$) between treatments occurred according to Tukey test. Error bars represent standard errors.

Results from experiment 1 showed that predatory nematodes were more sensitive to treatments than other trophic groups, regardless of ecosystem type (Table 1). These findings are consistent with a previous study reported for the three sites (Sylvain et al., 2014).

Results from our recovery experiments showed that bacterial-feeders are the most resilient nematode trophic group, as their population generally increased better than other groups (Table 2). Resilience here is defined as the capacity of a population to recovery quickly after a disturbance. This suggests that a labile source of soil carbon, such as from decomposing microbes or nematodes following soil treatment, provided a basis for the microbe food source to support the increasing bacterial-feeding nematode population. Bacterial-feeding nematodes are known to have r life history strategies, with short generation times, reproducing faster than other trophic groups after disturbance (Freckman, 1988; Bongers and Ferris, 1999). Nevertheless, it should be stressed that in experiment 2, total numbers in all sites were extremely low compared to control soil (less than 1% of original population).

4.3. Duration of heat treatment affects recolonization of soil by nematodes

Although a number of studies have tested soil defaunation methods for micro and mesofauna manipulations (Ingham et al., 1985; Bruckner et al., 1995; Bardgett et al., 1998; Lopez et al., 2009), the present work is, to our knowledge, the first to follow the progress of nematode community recovery post exclusion treatments. As mentioned, it is well known nematodes have a broad range of survival mechanisms they use when soil conditions are unfavourable for activity, all of which could be reversible when conditions become favourable. Results from our greenhouse experiment suggested that these dormancy mechanisms may have preserved some of the nematodes and their eggs in KNZ and SGRC soils through the 24 h heating treatment of experiment 2, since at 8 weeks in the greenhouse a significant recovery of the nematode community was found in these soils (Fig. 3). The most successful treatment was pre-wetting and heating for 48 h, after which nematodes did not recover at 8 weeks (Fig. 3). Therefore, even though the 24 h heating treatment was as efficient as the 48 h heating to eliminate soil nematodes (Fig. 1b), the latter is recommended for manipulative experiments longer than 8 weeks in order to minimize confounding effects of nematode recovery throughout the experiment.

Results from these three experiments show that responses of soil nematodes to our exclusion treatments were highly variable and ecosystem dependent along a cross-ecosystem moisture gradient when soils were not pre-wetted. Pre-wetting dry soils from arid ecosystems in this study ensured that nematodes were vulnerable to exclusion treatments, resulting in increased mortality. Nematodes at higher trophic levels (K-selected predaceous nematodes) were the most sensitive to exclusion treatments, while root-feeders were the most resistant, and bacterial-feeders the most resilient. Exclusion treatment intensity, or the time interval that soil is exposed to heating, determines the pace of nematode community recovery in treated soil microcosms.

We suggest that the following protocol to exclude nematodes for experimental soil microcosms is feasible for soils from the three ecosystems we examined and prevents recolonization of the

nematode community for at least 8 weeks. This technique will be useful for soil ecologists considering manipulative experiments to study the relationship of ecosystem processes to nematode soil fauna:

Step 1. Collect soil samples from the field (for this experiment we collected soil blocks of 20 × 20 cm, 20 cm depth).

Step 2. Homogenize soil by sieving using a 6.25 mm sieve. Transfer soil samples to aluminum trays (33 × 23 cm) to a depth of 5 cm.

Step 3. Wet soils with a spray bottle using regular tap water. Apply water over the sample surface until it passes through small holes previously opened on the bottom of the trays and until it wets an absorbent paper placed beneath it. Leave soils at 4 °C for 24 h.

Step 4. Transfer trays to an oven at 65 °C for 48 h.

Acknowledgements

We thank Ashley Shaw and William Beaton for their help facilitating laboratory work, as well as Elizabeth Bach for her helpful comments on the manuscript. This work was supported by the National Science Foundation under grant number DEB-1456631 and DEB-1456597 to Diana H. Wall and Osvaldo E. Sala.

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