



Contents lists available at ScienceDirect

## Soil Biology &amp; Biochemistry

journal homepage: [www.elsevier.com/locate/soilbio](http://www.elsevier.com/locate/soilbio)

## Short Communication

Elevated CO<sub>2</sub> and warming shift the functional composition of soil nematode communities in a semiarid grassland

Kevin E. Mueller<sup>a,\*</sup>, Dana M. Blumenthal<sup>a</sup>, Yolima Carrillo<sup>b</sup>, Simone Cesarz<sup>c,d</sup>,  
Marcel Ciobanu<sup>e</sup>, Jes Hines<sup>c,d</sup>, Susann Pabst<sup>f</sup>, Elise Pendall<sup>b</sup>, Cecilia Milano de Tomasel<sup>g</sup>,  
Diana H. Wall<sup>g</sup>, Nico Eisenhauer<sup>c,d</sup>

<sup>a</sup> Rangeland Resources Research Unit, Agricultural Research Service, Fort Collins, CO, USA

<sup>b</sup> Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia

<sup>c</sup> German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

<sup>d</sup> Institute of Biology, Leipzig University, Leipzig, Germany

<sup>e</sup> Institute of Biological Research, Branch of the National Institute of Research and Development for Biological Sciences, Republicii Str. 48, Cluj-Napoca, Romania

<sup>f</sup> Institute of Ecology, Friedrich Schiller University Jena, Dornburger Str. 159, 07743 Jena, Germany

<sup>g</sup> Department of Biology, Colorado State University, Fort Collins, CO, USA

## ARTICLE INFO

## Article history:

Received 2 May 2016

Received in revised form

7 August 2016

Accepted 8 August 2016

## Keywords:

Climate change

Soil food web

Structure

Colonizer-persister

Trophic groups

Functional guilds

## ABSTRACT

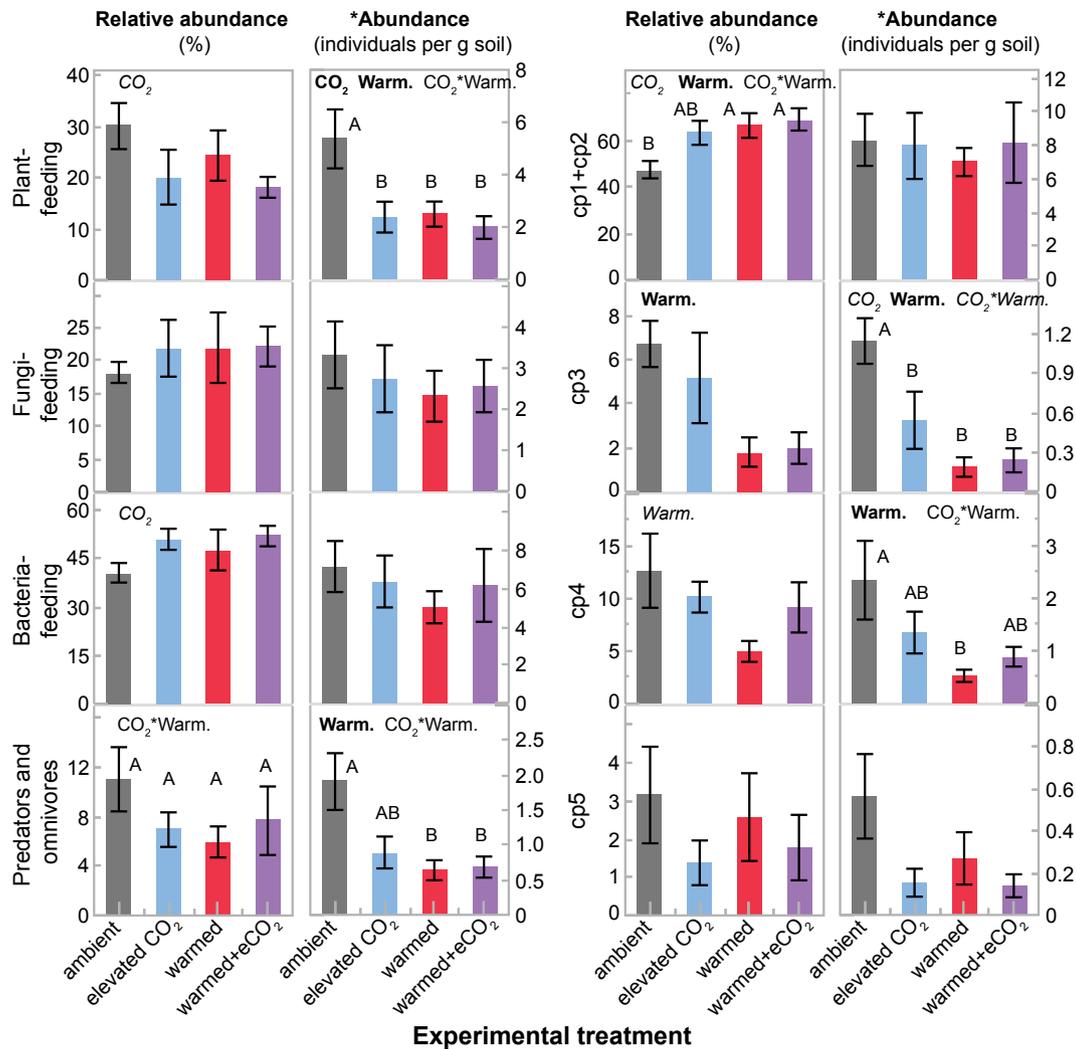
Climate change can alter soil communities and functions, but the consequences are uncertain for most ecosystems. We assessed the impacts of climate change on soil nematodes in a semiarid grassland using a 7-year, factorial manipulation of temperature and [CO<sub>2</sub>]. Elevated CO<sub>2</sub> and warming decreased the abundance of plant-feeding nematodes and nematodes with intermediate to high values on the colonizer-persister scale (cp3-5), including predators and omnivores. Thus, under futuristic climate conditions, nematode communities were even more dominated by *r*-strategists (cp1-2) that feed on bacteria and fungi. These results indicate that climate change could alter soil functioning in semiarid grasslands. For example, the lower abundance of plant-feeding nematodes could facilitate positive effects of elevated CO<sub>2</sub> and warming on plant productivity. The effects of elevated CO<sub>2</sub> and warming on nematode functional composition were typically less than additive, highlighting the need for multi-factor studies.

Published by Elsevier Ltd.

Nematodes are a morphologically and functionally diverse group of soil organisms (Bongers and Bongers, 1998; Ferris, 2010; Ferris et al., 2001; Neher, 2010; Yeates et al., 1993), so shifts in nematode community composition can provide clues about the sensitivity of soil food webs and soil functions to climate change. However, the impacts of climate change on nematode abundance have been idiosyncratic across studies, ranging from positive to negative for unknown reasons. Even when focusing on specific nematode functional groups, such as plant-feeders, the impacts of climate change on nematode abundance vary considerably across studies (A'Bear et al., 2014; Ayres et al., 2008; Blankinship et al., 2011; Cesarz et al., 2015; Ferris et al., 2012). The lack of predictability of nematode responses to climate change likely reflects

three sources of variation among studies: i) biogeographic variation in how climate change influences soil abiotic conditions (e.g., moisture) and the basal resources of soil food webs, including roots, bacteria, and fungi (García-Palacios et al., 2015), ii) variation in the extent to which various factors regulate soil food web structure (e.g., abiotic vs. biotic factors, top-down vs. bottom-up factors, indirect vs. direct effects of climate change) (Kardol et al., 2010; Shao et al., 2015), and iii) variation in the taxonomic and functional resolution of nematode identification (Cesarz et al., 2015; Neher and Weicht, 2013). Thus, additional studies are required to generate hypotheses and better understand the impacts of climate change on soil nematodes. Multi-factor studies are especially needed because of their rarity and potential for interactions among multiple aspects of climate change (e.g., warming and elevated CO<sub>2</sub>; Eisenhauer et al., 2012). There are only two reports of the combined, *in situ* effects of elevated CO<sub>2</sub> (eCO<sub>2</sub>) and warming on nematode communities, both for rice paddies in Asia (Chen et al.,

\* Corresponding author. 1701 Center Avenue, Fort Collins, CO 80526, USA.  
E-mail address: [kevin.e.mueller@gmail.com](mailto:kevin.e.mueller@gmail.com) (K.E. Mueller).



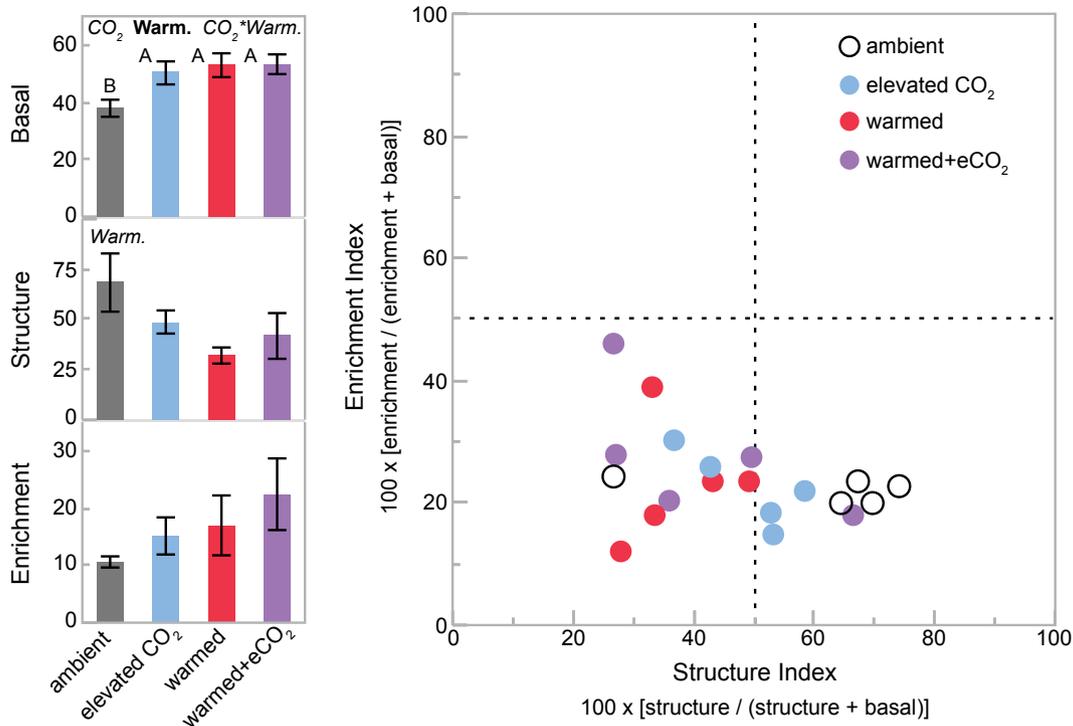
**Fig. 1.** Abundance of nematode functional groups characterized by trophic position (left) and life-history traits (the colonizer-persister scale; right). Bars show treatment means ( $\pm$ SE,  $n = 5$ ). The cp scale ranks nematodes along a spectrum from those with *r*-selected traits (cp1), e.g., short generation times and high fecundity, to those with *K*-selected traits (cp5). Plant-feeding nematodes are not included in the results shown for cp groups because their life-history strategies might not be comparable to other trophic groups (Bongers, 1990). The relative abundance of cp1 nematodes was very low (<2% in all but one plot), so the sum of cp1 and cp2 nematodes are shown. Similarly, the relative abundance of predators was very low (<2% in all but one plot), so the sum of predators and omnivores are shown. **Bold print** indicates model terms with  $P < 0.05$  and *italic print* indicates model terms with  $0.05 < P < 0.1$ . The treatment interaction term is also shown in normal print if  $0.1 < P < 0.2$  because in factorial designs with low statistical power, risk of Type II errors is likely much greater than that of Type I errors (Smith et al., 2002) and the interpretation of main effects is conditional upon the interaction (Stehman and Meredith, 1995). When the interaction term had  $P < 0.2$ , treatment means labelled with different letters were marginally significantly different according to post-hoc Tukey tests ( $\alpha = 0.1$ ). \*Abundance was estimated by multiplying the total number of nematode individuals in each plot by the relative abundance of each functional group (derived from identification of ~100 nematodes per plot).

2014; Okada et al., 2014).

Here, using a factorial field experiment, we describe the *in situ* impacts of eCO<sub>2</sub> and warming on the nematode community in a semi-arid, mixed-grass prairie in Wyoming, U.S.A. Mixed-grass prairies are the most widespread grassland type in North America (Samson et al., 2004). Nematodes could play an important role in shaping the functions of mixed-grass prairies because grasslands typically have high nematode densities and, for example, plant productivity is sensitive to shifts in nematode density (Bardgett et al., 1999; Hunt et al., 1987; Hunt and Wall, 2002; Ingham and Detling, 1990; Stanton, 1988). Reports from this experiment in Wyoming show that warming and eCO<sub>2</sub> have influenced abiotic soil conditions, including temperature, moisture, and nutrient pools, as well as the basal parts of soil food webs, including roots and microbes (Carrillo et al., 2014, 2012; Dijkstra et al., 2012, 2010; Mueller et al., 2016). Given these shifts in resource availability and

microclimate due to eCO<sub>2</sub> and warming, we expected associated shifts in nematode abundance and community composition, i.e., we tested the null hypothesis that eCO<sub>2</sub> and warming do not impact nematodes. The eCO<sub>2</sub> treatment increased [CO<sub>2</sub>] to ~600 ppm using Free-Air CO<sub>2</sub> Enrichment (FACE) technology and was implemented during sunlit hours of the plant growing-season. The warming treatment, implemented using infrared lamps, increased canopy temperatures by ~1.5 °C during the day and 3 °C during the night, through all seasons. The warming and eCO<sub>2</sub> treatments were maintained for 7 and 8 years, respectively, with 5 replicates per treatment (2006–2013) (LeCain et al., 2015; Morgan et al., 2011).

We sampled nematodes during the experiment's final year. In late July, which is typically within a few weeks of peak plant production aboveground, three soil cores (3 cm diameter; 15 cm deep) were collected for nematode extraction from each experimental plot. Soils were composited at the plot level ( $n = 20$ ) and



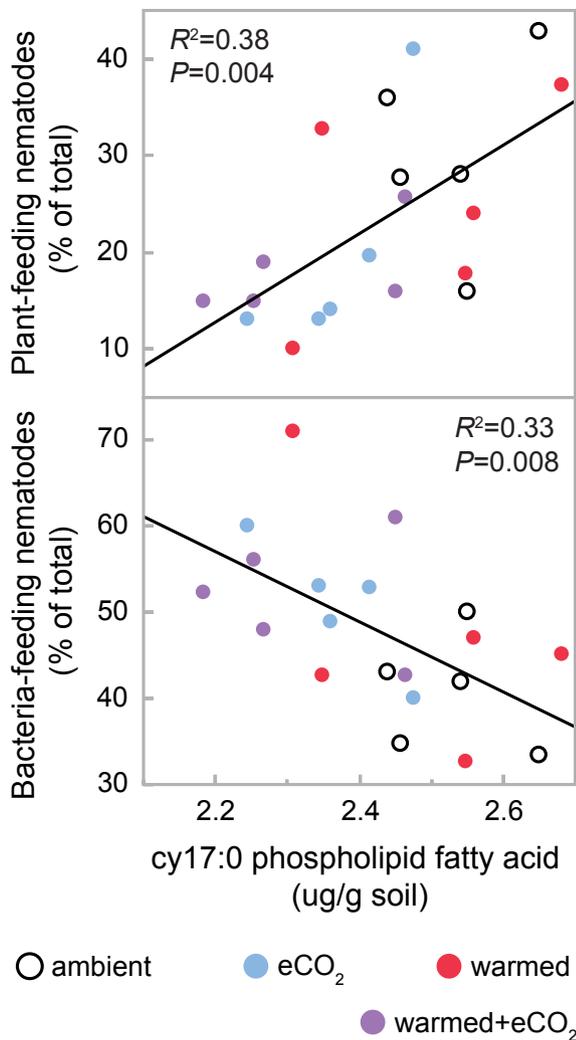
**Fig. 2.** Treatment effects on indices of the functional composition of nematode communities. For the left panel, treatment means are shown with standard error indicated by the error bars ( $n = 5$  for each treatment). For the right panel, the indices for each plot are shown. The basal food web component shows a nearly identical pattern as that for cp1+cp2 nematodes (Fig. 1) because the basal food web component is a weighted function of cp2 nematodes that feed on bacteria or fungi (Ferris et al., 2001) and because cp1 nematodes were uncommon in our samples (Supplemental Table 1). Similarly, the structure food web component and Structure Index show a very similar pattern to that of the cp3 and cp4 nematodes (Fig. 1) because the structure component is a weighted function of the relative abundance of cp3–5 nematodes (Ferris et al., 2001). The Maturity Index (Bongers, 1990; Bongers and Bongers, 1998) was tightly correlated with both structure and the Structure Index ( $R^2 = 0.91$  and  $0.97$ , respectively). **Bold print** indicates model terms with  $P < 0.05$  and *italic print* indicates model terms with  $0.05 < P < 0.1$ . When the interaction term had  $P < 0.2$ , treatment means labelled with different letters were marginally significantly different according to post-hoc Tukey tests ( $\alpha = 0.1$ ).

refrigerated (<48 h) prior to extraction. Nematodes were extracted over 72 h using Baermann funnels (Baermann, 1917) and preserved in a 5% formalin solution. For each sample, nematodes were counted using an inverse Leica DMI 4000B light microscope at 50 $\times$  magnification and at least 100 individuals were randomly identified at 400 $\times$  magnification following Bongers (1988); adults were identified to genus-level and most juveniles were identified to family-level. We identified nematode adults from 44 genera and juveniles from six families. Nematode taxa were then arranged into trophic groups (Bongers and Bongers, 1998; Okada et al., 2005; Yeates et al., 1993), ordered according to the colonization–persistence gradient (cp values) (Bongers, 1990; Bongers and Bongers, 1998), and assigned to functional guilds by combining trophic groups with cp values (Bongers and Bongers, 1998; Ferris et al., 2001) (Supplemental Table 1). We evaluated the role of cp values separately for plant-feeding nematodes (parasitic) and other nematodes (non-parasitic) because the life-history strategies of plant-feeding nematodes might not be comparable to other trophic groups (Bongers, 1990). We used mixed-models to evaluate the significance of treatment effects and interactions (fixed effects), while accounting for the distribution of plots across two blocks (random effect) with slightly different soil types. When treatment interaction terms had  $P < 0.2$  (sensu Stehman and Meredith, 1995), we used post-hoc Tukey tests to compare treatment means using  $\alpha = 0.1$ .

Elevated CO<sub>2</sub> and warming altered nematode community composition (Fig. 1). For several nematode functional groups, negative treatment effects were more apparent for absolute abundance than for relative abundance, because total nematode

abundance was lower in all treated plots compared to the controls (11.4 nematodes per g of soil  $\pm 1.2$  SE and 17.8 nematodes per g of soil  $\pm 3.1$  SE, respectively;  $P > 0.1$ ). Both eCO<sub>2</sub> and warming tended to decrease the abundance of three nematode functional groups (Fig. 1), including plant-feeding nematodes, predators plus omnivores, and non-parasitic nematodes with intermediate to high cp values (cp3 to cp5, where high values reflect a K-selected strategy; predators and omnivores were assigned cp values of 4 or 5). Consequently, the relative abundance of other functional groups of nematodes tended to increase in response to eCO<sub>2</sub> and warming, especially bacteria- and fungi-feeding nematodes that are on the ‘colonizer’, r-selected end of the cp scale (cp1 and cp2; Fig. 1). More than 90% of plant-feeding nematodes were characterized as cp2 or cp3 (Supplemental Table 1), and these two groups of plant-feeders had similar responses to the treatments. The relative abundance of some groups of nematodes was more sensitive to eCO<sub>2</sub> (plant-feeding and bacteria-feeding nematodes), while other groups were more sensitive to warming (non-parasitic cp3 and cp4 nematodes; Fig. 1). There was no evidence that the treatments influenced various indices of nematode diversity or relative abundance of the fungal or bacterial decomposition pathways (data not shown).

The treatment effects were less than additive for several groups of nematodes, reflecting potential CO<sub>2</sub>  $\times$  warming interactions (Fig. 1). For plant-feeding nematodes, predators plus omnivores, and non-parasitic nematodes with cp3 or cp4, if the negative effects of eCO<sub>2</sub> and warming alone were additive, the expected mean abundance of these groups in the eCO<sub>2</sub>+warming treatment would be zero; yet, their abundance in the combined treatment was similar to that in the eCO<sub>2</sub> alone and warming alone treatments



**Fig. 3.** Relationships between nematode community composition and the abundance of a phospholipid fatty acid (PLFA) in soil that is commonly attributed to gram negative bacteria (Frostegård et al., 2011). PLFAs were extracted and identified following standard methods (Frostegård et al., 1993) using soil sampled in the same month and year that nematodes were sampled (July 2013).

(Fig. 1). These results suggest the impacts of climate change on soil nematode communities could be difficult to predict from single-factor experiments. In a rice paddy in Japan, Okada et al. (2014) did not observe interactive effects of eCO<sub>2</sub> and warming on the abundance of six nematode genera, but they did not evaluate effects on the relative abundance of those genera or nematode functional groups.

Other results from this experiment provide clues regarding possible causes and consequences of the observed shifts in nematode community composition. In contrast to the nematode results, warming and eCO<sub>2</sub> had opposing effects on soil moisture and nutrient availability (Carrillo et al., 2012; Dijkstra et al., 2012, 2010; Mueller et al., 2016); thus, simple links between shifts in nematode communities, moisture, and nutrients were not apparent. There were, however, some commonalities between the observed impacts of warming and eCO<sub>2</sub> on nematodes, microbes, and plant roots. Similar to the observed effects on ‘basal’ nematodes (i.e., bacteria- and fungi-feeding, sensu Ferris et al., 2001, Figs. 1 and 2), warming and eCO<sub>2</sub> tended to have positive, but sub-additive effects on the number and length of fine roots (Carrillo et al., 2014; Mueller et al., unpublished) and the amount of C and N in microbial biomass

(Carrillo et al., unpublished; Chen et al., 2015; Dijkstra et al., 2010). Also, the relative abundance of bacterial-feeding and plant-feeding nematodes was correlated with the concentration of a single PLFA attributed to gram negative bacteria (Fig. 3), which can be abundant in the rhizosphere (Griffiths et al., 1999). Finally, Suseela et al. (in review) observed positive, and sometimes sub-additive, effects of eCO<sub>2</sub> and warming on the amount of suberin and individual suberin monomers in plant roots. If these plant biomolecules inhibit plant-feeding nematodes (Biederman and Boutton, 2009; Holbein et al., 2016), this result could help explain the negative, sub-additive effects of eCO<sub>2</sub> and warming on the abundance of plant-feeding nematodes (Fig. 1). In turn, the diminished abundance of plant-feeding nematodes could have contributed to observed positive responses of plant productivity, above and below-ground, to both eCO<sub>2</sub> and warming (Carrillo et al., 2014; Mueller et al., 2016). Collectively, these results suggest the impacts of climate change on nematodes might be largely indirect and mediated from the “bottom-up” by plants and microbes.

Additional information was gained by classifying nematodes into functional guilds defined by both trophic position and life history (Fig. 2). Consistent with expectations for natural grasslands (Ferris et al., 2001), the nematode communities in control plots had a relatively high Structure Index, reflecting abundant trophic links and high abundance of predators and omnivores within the nematode community; this structure was diminished by the treatments, particularly by warming alone (Fig. 2). The mean Enrichment Index, reflecting the abundance of opportunistic bacteria- and fungi-feeding nematodes that respond to resource enrichment, was relatively low and similar across control and treatment plots. However, due to a few higher enrichment values in treated plots, the control plots showed less variability in the Enrichment Index (Fig. 2). According to the original interpretation of these functional indices (Ferris et al., 2001), by reducing the number of trophic links within the nematode community and increasing the relative abundance of the basal components of the soil food web (particularly bacteria- and fungi-feeding nematodes), the future climatic conditions implemented in the PHACE experiment have induced a shift toward a soil food web that is “degraded” and “stressed”, and possibly less regulated by top-down effects.

The shifts in nematode functional composition we observed suggest that, for the expansive mixed-grass prairie of North America, climate change could have significant impacts on below-ground food webs and functions (e.g., decomposition, respiration, nutrient cycling). To better predict the impacts of climate change on nematode communities and soil functions across ecosystems, our results and those of other recent studies (Cesarz et al., 2015; Kardol et al., 2010; Neher and Weicht, 2013; Thakur et al., 2014) suggest that: *i*) more factorial studies should be conducted (e.g., CO<sub>2</sub> × warming), and *ii*) nematode abundance should be measured for functional groups defined by trophic level and life history.

#### Author contributions

DMB and EP helped design and conduct the experiment; KEM sampled soil nematodes with assistance from DMB, CMT and DW, NE managed the study of soil nematode abundance and composition; MC characterized nematode community composition, SP estimated nematode abundance with assistance from SC and JH, KEM analyzed the data and wrote the first draft of the manuscript, with input and revisions provided by all authors. Except for KEM and NE, authors are listed in alphabetical order.

#### Acknowledgements

This research was supported by the Climate Change, Soils &

Emissions Program of the USDA-ARS, the Soil Processes program of the USDA-CSREES (2008-35107-18655), the Terrestrial Ecosystem Science program of the United States Department of Energy Office of Science (DE-SC0006973), the Western Regional Center of the National Institute for Climatic Change Research, and the National Science Foundation (DEB 1021559). SC, JH and NE acknowledge funding by the German Research Foundation (DFG) in the framework of the Emmy Noether research group (Ei 862/2). Further support came from the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, funded by the German Research Foundation (FZT 118). Dan LeCain and Jack Morgan contributed to experimental design and infrastructure maintenance, with assistance from many others including: B. Kimble, F. Miglietta, D. Smith, and D. Williams. Keith Post assisted with extraction of soil fauna.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2016.08.005>.

## References

- A'Bear, A.D., Jones, T.H., Boddy, L., 2014. Potential impacts of climate change on interactions among saprotrophic cord-forming fungal mycelia and grazing soil invertebrates. *Fungal Ecology* 10, 34–43. <http://dx.doi.org/10.1016/j.funeco.2013.01.009>. Fungi in a changing world: The role of fungi in ecosystem response to global change.
- Ayres, E., Wall, D.H., Simmons, B.L., Field, C.B., Milchunas, D.G., Morgan, J.A., Roy, J., 2008. Belowground nematode herbivores are resistant to elevated atmospheric CO<sub>2</sub> concentrations in grassland ecosystems. *Soil Biology and Biochemistry* 40, 978–985. <http://dx.doi.org/10.1016/j.soilbio.2007.11.018>.
- Baermann, G., 1917. Eine einfache Methode zur Auffindung von Ankylostomum-(Nematoden)-Larven in Erdboden. *Meded. Uit Het Geneesk. Lab. Te Weltevreden*, pp. 41–47.
- Bardgett, R.D., Cook, R., Yeates, G.W., Denton, C.S., 1999. The influence of nematodes on below-ground processes in grassland ecosystems. *Plant Soil* 212, 23–33. <http://dx.doi.org/10.1023/A:1004642218792>.
- Biederman, L.A., Boutton, T.W., 2009. Biodiversity and trophic structure of soil nematode communities are altered following woody plant invasion of grassland. *Soil Biology and Biochemistry* 41, 1943–1950. <http://dx.doi.org/10.1016/j.soilbio.2009.06.019>.
- Blankinship, J.C., Niklaus, P.A., Hungate, B.A., 2011. A meta-analysis of responses of soil biota to global change. *Oecologia* 165, 553–565. <http://dx.doi.org/10.1007/s00442-011-1909-0>.
- Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83, 14–19. <http://dx.doi.org/10.1007/BF00324627>.
- Bongers, T., 1988. De Nematoden van Nederland: Een identificatietabel voor de in Nederland aangetroffen zoetwater-en bodembewonende nematoden. Koninklijke Nederlandse Natuurhistorische Vereniging.
- Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. *Appl. Soil Ecol.* 10, 239–251. [http://dx.doi.org/10.1016/S0929-1393\(98\)00123-1](http://dx.doi.org/10.1016/S0929-1393(98)00123-1).
- Carrillo, Y., Dijkstra, F.A., LeCain, D., Morgan, J.A., Blumenthal, D., Waldron, S., Pendall, E., 2014. Disentangling root responses to climate change in a semiarid grassland. *Oecologia* 175, 699–711. <http://dx.doi.org/10.1007/s00442-014-2912-z>.
- Carrillo, Y., Dijkstra, F.A., Pendall, E., Morgan, J.A., Blumenthal, D.M., 2012. Controls over soil nitrogen pools in a semiarid grassland under elevated CO<sub>2</sub> and warming. *Ecosystems* 15, 761–774. <http://dx.doi.org/10.1007/s10021-012-9544-0>.
- Cesarz, S., Reich, P.B., Scheu, S., Ruess, L., Schaefer, M., Eisenhauer, N., 2015. Nematode functional guilds, not trophic groups, reflect shifts in soil food webs and processes in response to interacting global change factors. *Pedobiologia* 58, 23–32. <http://dx.doi.org/10.1016/j.pedobi.2015.01.001>.
- Chen, J., Carrillo, Y., Pendall, E., Dijkstra, F.A., Evans, R.D., Morgan, J.A., Williams, D.G., 2015. Soil microbes compete strongly with plants for soil inorganic and amino acid nitrogen in a semiarid grassland exposed to elevated CO<sub>2</sub> and warming. *Ecosystems* 18, 867–880. <http://dx.doi.org/10.1007/s10021-015-9868-7>.
- Chen, J., Chen, F., Liu, M., Feng, Y., Dand, Z., Li, H., Hu, F., 2014. Short-term effects of CO<sub>2</sub> concentration elevation, warming and transgenic *Bt* rice cropping on soil labile organic carbon and nitrogen, and nematode communities. *Acta Ecol. Sin.* 34 <http://dx.doi.org/10.5846/stxb201210141428>.
- Dijkstra, F.A., Blumenthal, D., Morgan, J.A., Pendall, E., Carrillo, Y., Follett, R.F., 2010. Contrasting effects of elevated CO<sub>2</sub> and warming on nitrogen cycling in a semiarid grassland. *The New Phytologist* 187, 426–437. <http://dx.doi.org/10.1111/j.1469-8137.2010.03293.x>.
- Dijkstra, F.A., Pendall, E., Morgan, J.A., Blumenthal, D.M., Carrillo, Y., LeCain, D.R., Follett, R.F., Williams, D.G., 2012. Climate change alters stoichiometry of phosphorus and nitrogen in a semiarid grassland. *The New Phytologist* 196, 807–815. <http://dx.doi.org/10.1111/j.1469-8137.2012.04349.x>.
- Eisenhauer, N., Cesarz, S., Koller, R., Worm, K., Reich, P.B., 2012. Global change belowground: impacts of elevated CO<sub>2</sub>, nitrogen, and summer drought on soil food webs and biodiversity. *Global Change Biology* 18, 435–447. <http://dx.doi.org/10.1111/j.1365-2486.2011.02555.x>.
- Ferris, H., 2010. Form and function: metabolic footprints of nematodes in the soil food web. *European Journal of Soil Biology* 46, 97–104. <http://dx.doi.org/10.1016/j.ejsobi.2010.01.003>.
- Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Applied Soil Ecology* 18, 13–29. [http://dx.doi.org/10.1016/S0929-1393\(01\)00152-4](http://dx.doi.org/10.1016/S0929-1393(01)00152-4).
- Ferris, H., Griffiths, B.S., Porazinska, D.L., Powers, T.O., Wang, K.-H., Tenuta, M., 2012. Reflections on plant and soil nematode ecology: past, present and future. *Journal of Nematology* 44, 115–126.
- Frostegård, Å., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry* 25, 723–730. [http://dx.doi.org/10.1016/0038-0717\(93\)90113-P](http://dx.doi.org/10.1016/0038-0717(93)90113-P).
- Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. *Soil Biology and Biochemistry* 43, 1621–1625. <http://dx.doi.org/10.1016/j.soilbio.2010.11.021>.
- García-Palacios, P., Vandegheuchte, M.L., Shaw, E.A., Dam, M., Post, K.H., Ramirez, K.S., Sylvain, Z.A., de Tomasel, C.M., Wall, D.H., 2015. Are there links between responses of soil microbes and ecosystem functioning to elevated CO<sub>2</sub>, N deposition and warming? A global perspective. *Global Change Biology* 21, 1590–1600. <http://dx.doi.org/10.1111/gcb.12788>.
- Griffiths, B.S., Bonkowski, M., Dobson, G., Caul, S., 1999. Changes in soil microbial community structure in the presence of microbial-feeding nematodes and protozoa. *Pedobiologia* 43, 297–304.
- Holbein, J., Grundler, F.M.W., Siddique, S., 2016. Plant basal resistance to nematodes: an update. *Journal of Experimental Botany*. <http://dx.doi.org/10.1093/jxb/erw005>.
- Hunt, H.W., Coleman, D.C., Ingham, E.R., Ingham, R.E., Elliott, E.T., Moore, J.C., Rose, S.L., Reid, C.P.P., Morley, C.R., 1987. The detrital food web in a shortgrass prairie. *Soil Biology and Fertility of Soils* 3, 57–68. <http://dx.doi.org/10.1007/BF00260580>.
- Hunt, H.W., Wall, D.H., 2002. Modelling the effects of loss of soil biodiversity on ecosystem function. *Global Change Biology* 8, 33–50. <http://dx.doi.org/10.1046/j.1365-2486.2002.00425.x>.
- Ingham, R.E., Detling, J.K., 1990. Effects of root-feeding nematodes on aboveground net primary production in a North American grassland. *Plant Soil* 121, 279–281. <http://dx.doi.org/10.1007/BF00012321>.
- Kardol, P., Cregger, M.A., Campy, C.E., Classen, A.T., 2010. Soil ecosystem functioning under climate change: plant species and community effects. *Ecology* 91, 767–781. <http://dx.doi.org/10.1890/09-0135.1>.
- LeCain, D., Smith, D., Morgan, J., Kimball, B.A., Pendall, E., Miglietta, F., 2015. Microclimatic performance of a free-air warming and CO<sub>2</sub> enrichment experiment in windy Wyoming, USA. *Plos One* 10, e0116834. <http://dx.doi.org/10.1371/journal.pone.0116834>.
- Morgan, J.A., LeCain, D.R., Pendall, E., Blumenthal, D.M., Kimball, B.A., Carrillo, Y., Williams, D.G., Heisler-White, J., Dijkstra, F.A., West, M., 2011. C<sub>4</sub> grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature* 476, 202–205. <http://dx.doi.org/10.1038/nature10274>.
- Mueller, K.E., Blumenthal, D.M., Pendall, E., Carrillo, Y., Dijkstra, F.A., Williams, D.G., Follett, R.F., Morgan, J.A., 2016. Impacts of warming and elevated CO<sub>2</sub> on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time. *Ecology Letters* 19, 956–966.
- Neher, D.A., 2010. Ecology of plant and free-living nematodes in natural and agricultural soil. *Annual Review of Phytopathology* 48, 371–394. <http://dx.doi.org/10.1146/annurev-phyto-073009-114439>.
- Neher, D.A., Weicht, T.R., 2013. Nematode genera in forest soil respond differentially to elevated CO<sub>2</sub>. *J. Nematol.* 45, 214–222.
- Okada, H., Harada, H., Kadota, I., 2005. Fungal-feeding habits of six nematode isolates in the genus *Filenchus*. *Soil Biology and Biochemistry* 37, 1113–1120. <http://dx.doi.org/10.1016/j.soilbio.2004.11.010>.
- Okada, H., Sakai, H., Tokida, T., Usui, Y., Nakamura, H., Hasegawa, T., 2014. Elevated temperature has stronger effects on the soil food web of a flooded paddy than does CO<sub>2</sub>. *Soil Biology and Biochemistry* 70, 166–175. <http://dx.doi.org/10.1016/j.soilbio.2013.12.029>.
- Samson, F.B., Knopf, F.L., Ostlie, W.R., 2004. Great Plains ecosystems: past, present, and future. *Wildlife Society Bulletin* 32, 6–15. [http://dx.doi.org/10.2193/0091-7648\(2004\)32\[6:GPEPPA\]2.0.CO;2](http://dx.doi.org/10.2193/0091-7648(2004)32[6:GPEPPA]2.0.CO;2).
- Shao, Y., Bao, W., Chen, D., Eisenhauer, N., Zhang, W., Pang, X., Xu, G., Fu, S., 2015. Using structural equation modeling to test established theory and develop novel hypotheses for the structuring forces in soil food webs. *Pedobiologia* 58, 137–145. <http://dx.doi.org/10.1016/j.pedobi.2015.06.001>.
- Smith, R.A., Levine, T.R., Lachlan, K.A., Feduik, T.A., 2002. The high cost of complexity in experimental design and data analysis: type I and type II error rates in multiway ANOVA. *Human Communication Research* 28, 515–530. <http://dx.doi.org/10.1111/j.1468-2958.2002.tb00821.x>.
- Stanton, N.L., 1988. The underground in grasslands. *Annual Review of Ecology and Systematics* 19, 573–589. <http://dx.doi.org/10.1146/annurev.es.19.110188.003041>.
- Stehman, S.V., Meredith, M.P., 1995. Practical analysis of factorial experiments in

- forestry. *Canadian Journal of Forest Research* 25, 446–461. <http://dx.doi.org/10.1139/x95-050>.
- Suseela, V., Tharayil, T., Pendall, E., 2016. Warming and elevated CO<sub>2</sub> alter the suberin chemistry in roots of photosynthetically divergent grass species. *Annals of Botany* (in review).
- Thakur, M.P., Reich, P.B., Fischelli, N.A., Stefanski, A., Cesarz, S., Dobies, T., Rich, R.L., Hobbie, S.E., Eisenhauer, N., 2014. Nematode community shifts in response to experimental warming and canopy conditions are associated with plant community changes in the temperate-boreal forest ecotone. *Oecologia* 175, 713–723. <http://dx.doi.org/10.1007/s00442-014-2927-5>.
- Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and Genera—An outline for soil ecologists. *Journal of Nematology* 25, 315–331.