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Response of nematode food webs to human induced disturbances

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To the Graduate Council:

I am submitting herewith a dissertation written by Satyendra Kumar Pothula entitled "Response of nematode food webs to human induced disturbances." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Entomology, Plant Pathology and Nematology.

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Response of nematode food webs to human induced disturbances

**A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville**

**Satyendra Kumar Pothula
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Dedication

This dissertation is dedicated to all that have supported me:

My Wife and Daughter: Ratnasri and Riya Pothula

My Mom and Dad: Sesharatnam and Satyanarayana Murthy Pothula

My Brother, his wife and their Son: Subhash, Viharika and Yuvan Pothula

My Brothers-in-law: Hareen, Hemanth Mallipeddi and and their wives

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Acknowledgements

Abstract

Modern human civilization occurs at the expense of biodiversity. Human activity has extensively transformed the land surface by agricultural intensification and urbanization. Notably, agricultural practices mainly tillage have diverse impacts on plants, soils and soil organisms. Tillage changes soil properties and affects organisms that are living in the soil. In addition, human activities such as burning of fossil fuels, urbanization, agriculture, deforestation and desertification are rapidly changing the world's climate through the emission of greenhouse gases. Increase in the emission of greenhouse gases leads to global warming. Increase in air temperature congruently increases soil temperature, which could affect biodiversity in the soil. Nematodes are the most abundant multicellular soil organisms and are morphologically and functionally diverse. The objectives of this study were: 1) to assess the influence of agricultural intensification and urbanization on nematode communities by comparing different ecosystems through meta-analysis of published literature on a global scale, 2) to evaluate the effect of tillage on nematode communities in terms of increasing level of physical disturbance in an undisturbed forest ecosystem and 3) to investigate the response of nematodes to a 5 ^{o}C rise in soil temperature by simulating future global warming using heating cables in forest and agricultural ecosystems. Results from the meta-analyses indicated that overall richness was higher in forest than in natural grassland, disturbed grassland, urban, and agriculture ecosystems. In contrast, overall abundance was highest in disturbed grassland, agriculture and forest ecosystems. Effects of tillage on nematode communities suggested that it

significantly reduced nematode richness but not abundance. Soil warming in agricultural site did not affect nematode abundance, whereas nematode richness was significantly decreased in the warming treatment. On the other hand, nematode abundance and richness were not affected by soil warming in the forest ecosystem. Results from the warming experiment support the idea that nematode communities in the forest ecosystem may be more resilient to environmental fluctuations than to communitites in agricultural ecosystems. Overall, this research strengthens the concept that human interventions adversely impact nematode richness, which is crucial for the maintenance of the full suite of ecosystem services provided by soil food webs.

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Chapter 1

General introduction

Introduction

Biodiversity plays pivotal roles in ecosystem functioning and provision of ecosystem services that are crucial to human well-being. These services include providing food and water, controlling floods, pests, and diseases, and supporting photosynthesis, nutrient cycles, soil formation, and crop pollination that sustain all other services (MEA, 2003). Modern human civilization occurs at the expense of biodiversity. Land transformation is the principal driving force for biodiversity loss. Human activity has extensively transformed the land surface by agricultural intensification and urbanization (Vitousek et al., 1997). Agricultural intensification affects soil structure, biological activity and processes such as decomposition, mineralization and nutrient cycling by altering the physicochemical properties of soil (Stinner et al., 1984; Dick et al., 1988; Fraser et al., 1994). Notably, agricultural practices such as cultivation, crop rotation, tillage and pesticide application have diverse impacts on plants, soils and soil organisms (Elliott and Cole, 1989). Tillage changes soil properties such as moisture, temperature, aeration and organic matter content and affects organisms that are living in the soil (Kladivko, 2001; Golabi et al., 2014; Holland, 2004). Furthermore, tillage disrupts the relationship between soil organisms by either killing or injuring or exposing them to predators (Altieri, 1999 and Roger-Estrade et al., 2010).

In addition, human activities are rapidly changing the world's climate. Accelerated global climate change, primarily warming is an undeniable fact. Global warming is the increase in average global temperature of the atmosphere and the Earth's surface. Global

warming is caused by an increase in the emission of greenhouse gases such as carbon

dioxide, methane and nitrous oxide (Githeko et al., 2000). Emission of greenhouse gases

has been increasing since the beginning of the Industrial Revolution (IPCC, 2013). Increase

in greenhouse gas emissions is mainly due to human activities such as burning of fossil

fuels, urbanization, agriculture, deforestation and desertification (IPCC, 1997). Over the last

century, mean global temperature has increased by 0.74 ^{o}C and it has been predicted that

the temperature will further increase by 1.8-4.0 ^{o}C in the next 50–100 years (IPCC, 2007;

Houghton et al., 2001). This increase is mainly due to a rise in daily minimum temperatures

twice as much as the increase in daily maximum temperatures (Easterling et al. 1997; IPCC,

2001; Lobell et al. 2011). Soil temperature increases congruently with increases in air

temperature (Jacobs et al. 2011). Temperature and moisture in the soil are the main abiotic

factors that regulate many biological processes. Therefore, change in soil temperature

could affect biodiversity in the soil (Farnsworth et al., 1996; Chapin et al., 1996).

Soil is the habitat for most terrestrial organisms (Young and Crawford, 2004). Soil

supports diverse groups ranging from microscopic organisms such as bacteria, fungi and

archaea to complex organisms such as nematodes, mites and earthworms (Brussaard,

1997). Nematodes are at the center of the soil food web by interacting with several other

soil trophic groups in the lower hierarchy of the soil food web. Plants, bacteria and fungi

serve as food for nematodes; in turn, trophic groups in the higher hierarchy of the soil food

web, such as predatory mites, eat nematodes (Moore, 1994 and Roger-Estrade et al., 2010).

Additionally, nematodes are ubiquitous, functionally diverse and abundant. Therefore,

nematodes can be used to gauge the condition of structure and function of soil food webs and ecosystem conditions (Bongers, 1990; Ferris et al., 2001; Neher, 2001; Bongers and Bongers, 1998). Nematodes have been categorized into different trophic groups such as bacterivores, fungivores, herbivores, predators and omnivores based on their feeding habits (Yeates et al., 1993). Trophic groups in the lower hierarchy of the soil food web include bacterivores, fungivores, and plant feeders, while trophic groups in the higher hierarchy of the soil food web include predators and omnivores (Yodzis, 2001). In addition, a colonizer-persister (c-p) scale with one to five classes has been developed for nematodes ranging from colonizers with a c-p value of 1 to persisters with a c-p value of 5 based on life history characteristics. The c-p scale reflects the continuum of r and K-strategists. Nematodes with high fecundity rate, short generation time and toleration of disturbances are assigned to colonizers and nematodes with low fecundity rate, long generation time and sensitivity to disturbances are assigned to persisters (Bongers, 1990). Nematode community indices have been used to monitor ecological conditions of soil and the influence of human-induced disturbances on nematodes (Sohlenius et al., 1987; Bongers, 1990; Freckman and Ettema 1993; Neher et al., 1995; Wardle et al., 1995). Therefore, we tested the following objectives to assess the influence of human-induced disturbances on nematode communities:

1. To assess the influence of agricultural intensification and urbanization on nematode richness and abundance compared to forest and grassland ecosystems through meta-analysis of published literature on a global scale.

2. To evaluate the effect of tillage on nematode communities in terms of increasing level of physical disturbance in an undisturbed forest ecosystem.

3. To investigate the response of nematodes to a 5 $^\circ$C rise in soil temperature by simulating future global warming using heating cables in forest and agricultural ecosystems.

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Chapter 2

Agricultural intensification and urbanization negatively impact soil nematode richness and abundance: a meta-analysis

A version of this chapter was originally accepted by the Journal of Nematology and is titled as follows:

Pothula, S. K., Grewal, P. S., Auge, R. M., Saxton, A. M., and Bernard, E. C. Agricultural intensification and urbanization negatively impact soil nematode richness and abundance: a meta-analysis

This chapter is being added to my dissertation as the original article that was submitted to, and accepted by, the Journal of Nematology. I have received written authorization from Dr. David Shapiro-llan, the editor in chief of the Journal of Nematology, that there are no objections in using the accepted paper as part of my dissertation, and copyright issues are not being violated. I received written authorization from Dr. David Shapiro-llan r on August 2, 2018, stating, "I have consulted with the publisher. You are free to include the article in your dissertation, even as an exact copy. However, you must be sure the JON is properly cited."

The co-authors and myself conducted meta-analyses to generate quantitative summaries from 111 published articles. I was responsible for the data collection, conducting meta-analyses, heterogeneity, sensitivity analysis, publication bias and wrote the chapter. After the chapter/article was written, it was submitted to my committee members and my co-authors before submitting the article to the Journal of Nematology.

Dr. Ernest Bernard is my major professor and was responsible for overseeing this research. He edited the final version, helped with analyses. Dr. Parwinder Grewal is my professor and committee member that designed the objective. Dr. Robert Auge and Dr.

Arnold Saxton are my professors assisted with meta-analyses. Carrie Lykins assisted in

data extraction and Heather D. Toler assisted in development of forest plots.

Abstract

Human activity has extensively transformed the land surface by agricultural intensification and urbanization. In soil, nematodes are the most abundant invertebrates. The effect of human interventions was assessed on overall richness, overall abundance, richness and abundance of nematodes of each trophic group and colonizer-persister (c-p) class by comparing urban, agriculture and disturbed grassland (DGL) with natural grassland (NGL) and forest ecosystems. Meta-analyses were conducted to generate quantitative summaries from 111 published articles that met the inclusion criteria, 91 expressed data in grams and 20 expressed data in cm3. Results from data expressed per 100 g of soil indicated that overall richness was higher in forest than in NGL, DGL, urban, and agriculture ecosystems. The richness of all c-p classes and of all trophic groups except herbivores was highest in forest ecosystems. In contrast, overall abundance was highest in DGL, agriculture and forest ecosystems. The abundance of c-p 1, c-p2 and c-p 3 classes and bacterivores, fungivores and herbivores was highest in disturbed ecosystems, while the abundance of c-p 4 and c-p 5 classes and predators and omnivores was highest in relatively undisturbed ecosystems. Results from data expressed as nematodes per 100 cm3 of soil indicated that abundance followed a similar pattern, but richness often differed between the two methodologies. These meta-analyses strengthen the concept that human interventions adversely impact both richness and abundance, which is crucial for the maintenance of the full suite of ecosystem services provided by soil food webs.

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Introduction

Biodiversity plays pivotal roles in ecosystem functioning and provision of ecosystem services that are crucial to human well-being. These services include providing food and water; controlling floods, pests, and diseases; and supporting photosynthesis, nutrient cycles, soil formation, and crop pollination that sustain all other services (MEA, 2003). Modern human civilization occurs at the expense of biodiversity. Land transformation is the principal driving force for biodiversity loss. Human activity has extensively transformed the land surface by agricultural intensification and urbanization (Vitousek et al., 1997). Urbanization and agricultural practices such as burning, tillage, fertilizer applications, and mono-cultural cropping practices affect below-ground biodiversity and its functions including decomposition, nutrient cycling, degradation of toxicants, and pest and disease regulation (Giller et al., 1997). Despite its diverse benefits, biodiversity in soils is understudied compared to above-ground biodiversity.

Soil is a dynamic system in which organisms interact with each other and form complex food webs (Hunt and Wall, 2002). Nematodes are at the central place in the soil food web because they represent multiple trophic levels including primary, secondary and tertiary consumer levels (Yeates et al., 1993). The structure of a nematode community provides good information on the condition of the soil food web since nematodes are specific in their food sources and are most abundant in all habitats where decomposition occurs (Bongers and Bongers, 1998). Yeates et al. (1993) assigned nematodes to different trophic groups such as bacterivores, fungivores, herbivores, predators and omnivores based on their feeding habits. Bacterivores, fungivores, and herbivores are considered as

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nematode trophic groups in the lower hierarchy of the soil food web and predators and omnivores are considered as nematode trophic groups in the higher hierarchy of the soil food web (Yodzis, 2001). Nematode trophic interactions contribute to regulating nutrient dynamics in soil. Bacterivores and fungivores promote N and C mineralization by feeding on decomposing bacterial and fungal biomass. Nematode trophic groups in the higher hierarchy of the soil food web maintain ecological balance between decomposition and mineralization by regulating bacterivores and fungivores (Ingham et al., 1985). In addition, predators act as biocontrol agents by feeding on plant feeding nematodes (Bilgrami and Brey, 2005). Bongers (1990) developed a colonizer-persister (c-p) scale for nematodes by allocating the nematode taxa to one of five c-p groups ranging from colonizers (c) with a c-p value 1 to persisters (p) with a c-p value 5 through intermediate values based on their life history characteristics and survival strategies. Nematodes with small size, short life span and high fecundity are assigned to c-p 1 and those with large size, longer life span and low fecundity are assigned to higher c-p values, with the longest-lived nematodes with low fecundity and long development times placed in c-p 5. Many useful indices for nematode faunal analysis have been developed based on trophic groups and c-p scale. Consequently, nematodes can be used as indicators of structure and function of soil food webs and overall ecosystem conditions (Ferris et al., 2001).

A plethora of published literature exists on how different ecosystems affect the abundance (number of nematodes) and richness (number of taxa) of nematodes. However, there is no single consensus about the pattern of nematode abundance and richness in different ecosystems across the published literature. Some authors have reported that

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richness is high in forest ecosystems and abundance is high in agricultural ecosystems (Yeates and Bongers, 1999; Ferris et al., 2001; Yeates, 2007; Cardoso et al., 2015) but others have stated the converse (Neher et al., 2005; Briar et al., 2007; Darby et al., 2007; Kimenju et al., 2009). The existence of a large body of literature with diverse results creates the need to synthesize quantitative summaries in order to draw general conclusions across studies and test key hypotheses regarding patterns and processes governing soil biodiversity. Meta-analysis is a tractable and powerful statistical tool developed to generate a quantitative summary of all the published literature and draw conclusions across multiple studies (Arnqvist and Wooster, 1995). Therefore, meta-analysis was chosen to address this issue.

The specific objective of this study was to assess the influence of agricultural intensification and urbanization on nematode richness and abundance compared to forest and grassland ecosystems through meta-analysis of published literature on a global scale. The richness and abundance of nematodes were compared using different moderator levels or explanatory variables. We hypothesized that overall richness, overall abundance (nematodes of either all c-p classes or all trophic groups), and richness and abundance of nematodes of each trophic group and c-p class are greater in forest and natural grassland (NGL) ecosystems (both relatively undisturbed) compared to urban, agriculture and disturbed grassland (DGL) ecosystems (relatively disturbed with human interventions).

Materials and methods

Data collection: The Web of Science core database was systematically searched for relevant publications on October 7, 2016, with the following combination of search terms:

["nematode communities" or "soil nematodes" or "nematode diversity" or "nematode abundance" or "nematode biodiversity"] and ["grassland" or "forest" or "agriculture" or "prairie" or "urban"], which resulted in 1613 articles. Criteria for including an article in the analysis were: studies were conducted in forest, grassland, urban, or agriculture ecosystems; studies identified nematodes to family or genus level; studies reported mean abundance or richness expressed per grams or cm^3 of soil; soil samples were collected from natural conditions; and studies reported sample size. Criteria for excluding an article were: studies conducted in controlled conditions like microcosms, mesocosms, pots or greenhouses; studies expressing abundance of nematodes as relative abundance instead of absolute abundance; studies reporting data for total free-living nematodes instead of each trophic group. Among the 1613 articles, 598 relevant articles that contained data on richness and abundance of nematodes in different ecosystems were selected by examining titles and abstracts. Among the 598 articles, 111 articles (Supplementary Data Sources) met the inclusion criteria and were selected for data extraction. Among the 111 articles, 91 expressed data in grams and 20 expressed data in cm^3. The first 200 articles from a Google Scholar search was examined using the above search terms, which did not produce additional articles. A spreadsheet was constructed by extracting data from each article on authors, title, year of publication, unit of soil, richness and abundance of nematodes of each trophic group and each c-p class, overall richness and overall abundance of nematodes, treatment, sample size, and type of ecosystem. Overall richness and overall abundance of nematodes were calculated by adding the number of genera/families and abundance of nematodes of either all trophic groups or all c-p classes, respectively. Richness and

abundance of nematodes under each trophic group and each c-p class were calculated by adding the number of genera/families and abundance of nematodes corresponding to each class and each trophic group respectively. If there was more than one treatment in an article, they were considered as distinct studies in the meta-analysis. For example, there were two treatments, conventional-conservation tillage and organic-conservation tillage in Sánchez-Moreno et al. (2009), these two treatments were considered as two distinct studies. Based on these criteria, a total of 667 studies were subjected for meta-analysis of which 449 studies conducted in agriculture, 28 conducted in DGL, 74 conducted in forest, 36 conducted in NGL, and 80 conducted in urban ecosystems. Soil units in nematode studies are typically expressed as grams (Briar et al., 2007) or in cm^3 (Wang et al., 2006). Therefore, the richness and abundance of nematodes expressed per 100 g of soil and 100 cm^3 of soil were analyzed separately. Richness and abundance of nematodes per 100 g of soil were compared across all five ecosystems. However, the data expressed per 100 cm^3 of soil was compared across only four ecosystems as no urban ecosystem studies using 100 cm^3 were available. Abundance of nematodes that was not expressed per 100 g or cm^3 of soil converted to 100 g or cm^3 of soil. However, richness of nematodes was not converted because increase in richness cannot be assessed with increase in the quantity of soil.

Effect size: Effect size typically represents the strength of the relationship between two variables or two groups (treatment and control) but can also refer to the estimate of a single group or value such as richness or abundance of each study (Borenstein et al., 2009). Summary effect size is defined as weighted mean of richness or abundance of all studies in each ecosystem. Meta-analyses were conducted to compare the summary effect sizes of

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overall richness and overall abundance of nematodes and nematodes of each trophic group and each c-p class per weight and volume basis among different ecosystems such as forest, NGL, DGL, agriculture, and urban ecosystems. Overall richness and overall abundance of nematodes per weight (grams) and per volume (cm^3) were considered as four main effect sizes; richness and abundance of nematodes per weight and volume in each trophic group and each c-p class were considered as subgroup effect sizes.

Moderator variable: The types of ecosystems, forest, NGL, DGL, agriculture, and urban, were considered as moderator levels. These five ecosystems were assumed to have different regimes of disturbance where forest and NGL are considered less disturbed, whereas agriculture and urban ecosystems are considered highly disturbed from continuous human intervention. The moderator was chosen to determine the influence of disturbance on soil health.

Meta-analysis: The procedures and terminology of Borenstein et al. (2009) were followed in this analysis. Comprehensive Meta-Analysis (CMA) software was used to estimate effects of different levels of moderator on nematodes based on their confidence intervals, P_{hetero} -values, Q statistics, and I^2 values where Q is heterogeneity, and I^2 is a measure of inconsistency across the studies (Version3, Biostat, Englewood, NJ, USA; 2014). Random effects model was used rather than fixed effects model for meta-analyses as it considers within-study variance along with between-studies variance. Each study was weighted by the inverse of non-parametric variance. Non-parametric variance was calculated using the formula 1/n, where 'n' is the sample size adjusted by using the following formula:

$$V = (1/n*(1+(t-1)*0.5))*(m/t)^{0.5}$$

Where m is the number of studies in a paper, and t is number of time-points within a year (Borenstein et al., 2009, equation 24.6). Studies within a paper are generally considered as not independent (Mengersen et al., 2013), therefore, studies were down-weighted by a factor of $m^{0.5}$, (assuming 0.1 correlation among studies). After estimating different summary effects using CMA, the results were plotted in forest plots using SigmaPlot version 13.0 (Systat Software, San Jose, California). The summary effects along with their confidence intervals (CIs) from the meta-analyses were graphically depicted in forest plots.

Heterogeneity: Q is a weighted squared deviation used to evaluate heterogeneity, defined here as real differences among summary effect sizes. It separates observed variation from true variation. Total variation (Qt) consists of Qw (expected variation, within-study variation, or sampling error) and Qm (excess variation, between-study variation) (Borenstein et al., 2009). I^2 is an estimate of the ratio of heterogeneity to total variation across the observed effect sizes (Higgins and Thompson, 2002; Huedo-Medina et al., 2006). It is the proportion of total variation due to heterogeneity in true effect size. I^2 is computed as $100*(Qt-df)/Qt$ %, where degrees of freedom (df) measures within-study variation and $Qt-df$ is true heterogeneity or between-study variation. I^2 reflects the percentage of variation due to real differences in outcomes among studies (Borenstein et al., 2009). I^2 values of 25%, 50%, and 75% may be considered as low, moderate, and high respectively (Higgins et al., 2003). In meta-analysis, a significant heterogeneity P value (P_{hetero} value<0.05) or positive I^2 indicates that there were real differences among studies,

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however, the converse is not true. A non-significant *P* value (P_{hetero} value>0.05) does not indicate that there were no real differences among studies because the non-significance could be due to low statistical power and/or large real dispersion of effect sizes and/or large within-study variance (Borenstein et al., 2009).

Sensitivity analysis and publication bias: A sensitivity analysis was conducted to assess the stability and consistency of the summary effects. The summary effect was recalculated by removing one study at a time. This measures how sensitive the results are to any one study. The potential presence of publication bias was tested using the Begg and Mazumdar rank (Kendall) correlation test and graphically by examining summary effect sizes vs. their standard errors in funnel plots (Begg and Mazumdar, 1994; Borenstein et al., 2009).

Results

Heterogeneity test: A total of 44 summary effect sizes were tested in the meta-analysis performed, of which 40 summary effect sizes were significantly heterogeneous (P_{hetero} < 0.05) and all summary effects had positive I^2 values (Table 1). The four summary effect sizes that were not significantly heterogenous included overall richness, c-p 4 richness, predator richness and omnivore richness from 100 cm^3 soil samples (P_{hetero} > 0.05) (Table 1).

Sensitivity analysis and publication bias: Sensitivity analysis indicates the contribution of each study to the summary effect, which is measured by the change in the summary effect in its absence. The summary effect size of overall abundance per 100 g of

soil was most affected by the removal of treatment B4 at Bohemia in the study conducted

by Cermak et al (2011). This study reduced the summary effect size from 1208.00 to

1186.23 (Supplementary Table 1). Similarly, the summary effect size of overall richness per

100 g of soil was most influenced by the removal of Renčo and Baležentiené (2015),

grassland (control) treatment, reducing the summary effect size from 27.35 to 27.21

(Supplementary Table 2). The summary effect size of overall abundance per 100 cm^3 was

most affected by the removal of the Bulluck et al. (2002), cotton-gin trash (harvest)

treatment. This study reduced the summary effect size from 649.22 to 634.56

(Supplementary Table 3). The summary effect size of overall richness per 100 cm^3 soil was

most influenced by the removal of the control treatment from Kapagianni et al. (2010) from

28.97 to 28.70 (Supplementary Table 1). These results indicated that no single study

changed any of the summary effect sizes to any important degree. Funnel plots did not

show any observable patterns between standard errors and point estimate values,

indicating no publication bias in this meta-analysis. In addition, the Begg and Mazumdar

rank correlation test gave absolute Kendall tau values for all four summary effect sizes of

less than 0.22, suggesting no publication bias.

Overall nematode richness expressed per 100 g soil was highest in forest compared

to NGL, DGL, urban, and agriculture ($P_{hetero} < 0.05$) (Fig. 1). The overall richness expressed

per 100 cm^3 was not significantly heterogenous among ecosystems ($P_{hetero} > 0.05$) (Fig. 2).

The nematode richness of all c-p classes per 100 g of soil was higher in forest

ecosystems than in other ecosystems but richness of c-p 1 nematodes was highest in

agricultural ecosystems along with forest and NGL ecosystems ($P_{hetero} < 0.05$) (Fig. 3). On

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the other hand, the richness of c-p 1 (P_{hetero} < 0.05) and c-p 2 (P_{hetero} < 0.05) nematodes per 100 cm^3 of soil was highest in DGL ecosystems, whereas the richness of c-p 3 (P_{hetero} < 0.05) was highest in DGL and forest ecosystems. However, richness of c-p 4 (P_{hetero} > 0.05) nematodes was not significantly heterogenous among ecosystems and richness of c-p 5 (P_{hetero} < 0.05) class nematodes did not follow any pattern (Fig. 4).

The richness of bacterivores, fungivores and predators per 100 g of soil was higher in forest ecosystems than in the other ecosystems and the richness of omnivores was higher in forest ecosystems than in disturbed ecosystems. However, the richness of herbivores did not follow any pattern (P_{hetero} < 0.05) (Fig. 5). The richness of bacterivores (P_{hetero} < 0.05) and fungivores (P_{hetero} < 0.05) per 100 cm^3 soil was higher in DGL ecosystems, while richness of herbivores was highest in all ecosystems except agriculture (P_{hetero} < 0.05). Richness of predators and omnivores was not significantly heterogenous among ecosystems (P_{hetero} > 0.05) (Fig. 6).

The overall abundance per 100 g of soil was highest in DGL and agriculture ecosystems along with forest ecosystems (P_{hetero} < 0.05) (Fig. 7). Similarly, the overall abundance per 100 cm^3 soil was highest in DGL ecosystems compared to other ecosystems NGL and forest (P_{hetero} < 0.05) (Fig. 8).

The abundance of c-p 1 and c-p 2 classes per 100 g of soil was highest in DGL and c-p 3 was highest in agriculture ecosystems; whereas, the abundance of c-p 4 and c-p 5 classes was highest in undisturbed ecosystems (P_{hetero} < 0.05) (Fig. 9). Likewise, the abundance of c-p 1, c-p 2, and c-p 3 classes per 100 cm^3 soil was higher in disturbed ecosystems while the abundance of c-p 5 class was higher in forest ecosystems, which are

relatively undisturbed (P_{hetero} < 0.05). Abundance of c-p 4 nematodes was not significantly different among ecosystems (P_{hetero} > 0.05) (Fig. 10).

The abundance of bacterivores and fungivores per 100 g of soil was highest in agriculture and abundance of herbivores was highest in DGL ecosystems, whereas the abundance of predators and omnivores was highest in undisturbed ecosystems (P_{hetero} < 0.05) (Fig. 11). The abundance of bacterivores per 100 cm^3 of soil was highest in agriculture and DGL ecosystems and abundance of fungivores and herbivores was highest in DGL ecosystems, whereas the abundance of predators and omnivores was highest in forest ecosystems (P_{hetero} < 0.05) (Fig. 12).

Discussion

Soil nematode assemblages can serve as ecological indicators since different nematode taxa vary in their sensitivity to disturbances in a terrestrial ecosystem (Bongers, 1990; Neher et al., 2005). Extensive research has been conducted on abundance and richness of nematode assemblages in different ecosystems but very few studies have been conducted to compare the impact of disturbances on nematode abundance and richness among two or more ecosystems (Neher et al., 2005; Briar et al., 2007; McSorley and Wang, 2009; Cardoso et al., 2015). Recently, meta-analysis was conducted using the literature published on soil nematodes to analyze soil energy pathways in different ecosystems (Zhao and Neher, 2014) and the effect of organic and inorganic fertilizers on soil nematodes in croplands (Liu et al., 2016). Meta-analysis was conducted to study the collective impact of anthropogenic disturbances on nematode assemblages by comparing five ecosystems with a gradient of human disturbance. Disturbances that are considered anthropogenic include

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physical disturbances such as burning, tillage, soil solarization, and harvesting; chemical

disturbances such as addition of organic amendments and inorganic fertilizers in

agriculture ecosystems; heavy metal pollution; building and road construction in urban

settings; seeding, tillage, harvesting, fertilizer application and grazing rate in DGL were

considered as anthropogenic disturbances. Forests and NGL with little to no direct human

intervention were considered as undisturbed ecosystems.

The results from data expressed per 100 g of soil show that the overall richness of

nematodes was highest in forest ecosystems compared to NGL, DGL, agriculture, and urban

ecosystems. These results supported the hypothesis that the richness of nematodes is

higher in undisturbed ecosystems than in human-disturbed ecosystems (Wasilewska,

1979; Bongers and Bongers, 1998; Briar et al., 2007; Darby et al., 2007). These results were

congruent with the general statement that ecosystems with less or no disturbance support

greater richness of soil biota (Hooper et al., 2005) consistent with the results of Hanel

(1993); Ivezic et al. (2000); Neher et al. (2005); Yeates (2007); Brmez et al. (2007); Jiao et

al. (2008); Cardoso et al. (2012); Cardoso et al. (2015). High richness in forest and NGL

points to the stability of these two ecosystems.

The richness of nematodes of all c-p classes was higher in forest ecosystems due to

little or no disturbance but the richness of c-p 1 was higher in agricultural ecosystems

along with forest and NGL ecosystems. Nematodes in the c-p 1 class are considered

enrichment opportunists as most are bacterial feeders, which are most active in the

presence of abundant resources (De Goede et al., 1993). The high richness of c-p 1 taxa in

agricultural ecosystems may be due to continuous addition and incorporation of fertilizers

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and organic matter. After addition of nutrients or organic matter incorporation into the soil, c-p 1 class nematodes respond immediately and flourish in number due to increased microbial activity, resulting from the newly available nutrients (Ettema and Bongers, 1993). Richness of nematodes in c-p 3, c-p 4 and c-p 5 classes, which are sensitive to disturbance, was higher in forest ecosystem due to little or no disturbance. Nematodes of higher c-p classes were found to be sensitive to disturbances (Park et al., 2010; Cardoso et al., 2015). High richness of higher c-p classes indicates a mature and stable ecosystem (Bongers, 1990; Bongers, 1999).

The richness of nematodes of all trophic groups except herbivores was highest in forest ecosystems. This result is consistent with the reports of Briar et al. (2007), Jiao et al. (2008), and Kimenju et al. (2009). Forests typically support a greater richness of organisms including nematodes due to the absence of human intervention such as tillage, monocultures, cultivated lawns, and application of fertilizers and amendments. Nematode trophic groups in the higher hierarchy of the soil food web such as omnivores and predators are particularly sensitive to disturbances (Korthals et al., 1996) and therefore are rich in undisturbed forest ecosystems. The presence of these nematodes maintains ecological balance by regulating nematode trophic groups in the lower hierarchy of the soil food web including plant feeding nematodes (Bilgrami and Brey, 2005).

Overall nematode abundance was higher in DGL and agriculture ecosystems along with forest ecosystems. Although high nematode abundance in an ecosystem represents high productivity of the ecosystem (Ritz and Trudgill, 1999), the high abundance in DGL and agriculture ecosystems was mostly attributed to high abundance of c-p 2, an indication

of more stressful soil food web populated by recalcitrant bacterivores (Ferris et al., 2001). The higher abundance in forest ecosystems could be contributed by the higher abundance of predators and omnivores, most of which belong to c-p 4 and c-p 5 classes.

The nematodes of c-p 1 and c-p 2 classes were most abundant in DGL and those in the c-p 3 were more abundant in agricultural ecosystems, whereas the abundance of nematodes of c-p 4 and c-p 5 classes was highest in forest and NGL ecosystems. The high abundance of lower c-p classes in disturbed ecosystems may be attributed to the incorporation of plant material and fertilizers, which favor microbial activity; thus, microbivorous colonizers with a high reproduction rate dominate these disturbed ecosystems (Bongers, 1990; Freckman and Ettema, 1993; Brmež et al., 2006; Brmež et al., 2007). Moreover, nematodes of lower c-p classes are tolerant to disturbance (Bongers, 1990). On the other hand, the abundance of nematodes of higher c-p classes, which are sensitive to disturbances, was highest in undisturbed ecosystems, which might be due to the absence of anthropogenic intervention such as tillage and fertilizer applications (Wasilewska, 1995; Grewal et al., 2011). High abundance of higher c-p classes indicates mature soil food webs in an ecosystem (Neher, 1999; Yeates and Bongers, 1999).

The abundance of bacterivores, fungivores and herbivores was highest in DGL and agriculture ecosystems, whereas the abundance of predators and omnivores was highest in forest and NGL ecosystems. These results are consistent with the findings of Ivezic et al. (2000), Hanel, (1993) and Hanel, (2010). The abundance of nematode trophic groups in the lower hierarchy of soil food web is highest in disturbed ecosystems because bacterivores and fungivores with c-p 2 are tolerant and responding to more stressful soil environment

(Bongers, 1990). High abundance of herbivores in disturbed ecosystems may be due to lack of omnivores and predators that potentially feed on herbivores. On the other hand, the high abundance of predators and omnivores in forest and NGL ecosystems may be due to lack of human intervention (Ferris and Ferris, 1974; Wasilewska, 1979; Hanel, 1993; Wasilewska, 1995; Cardoso et al., 2012). Perturbations in an ecosystem may increase the abundance of trophic groups in the lower hierarchy of soil food web (bacterivores, fungivores, and herbivores) but decrease the abundance of nematode trophic groups in the higher hierarchy of the soil food web (predators and omnivores), which play a crucial role in regulating the lower groups including herbivores. Therefore, losing these regulators may be detrimental to nutrient cycling dynamics and agricultural management.

Overall richness, overall abundance, and richness and abundance of each c-p class and each trophic group per 100 cm^3 of soil in all four ecosystems were analyzed as no urban ecosystem studies using 100 cm^3 were available. Summary effect sizes of overall richness, c-p 4 richness, predator and omnivore richness were not significantly different (Table 1). The overall abundance, abundance of nematodes of all c-p classes, and abundance of nematodes of all trophic groups expressed per 100 cm^3 of soil followed a somewhat similar pattern as that of 100 g of soil. However, overall richness, richness of all c-p classes, and richness of all trophic groups expressed per 100 cm^3 differed from those for 100 g of soil. This ambiguity may be due to the fewer number of studies, low statistical power, or the variation in the quantity of soil depending on its compactness, bulk density and soil moisture.

Conclusion

Comprehensive meta-analyses of distinct ecosystems with different schemes of human intervention from 111 publications, using random-effects model and non-parametric variance, confirmed that nematode richness was higher in less disturbed ecosystems (forest and NGL) compared to more disturbed ecosystems (agriculture, DGL, and urban ecosystems), nematode abundance of trophic groups in the lower hierarchy of the soil food web was higher in more disturbed ecosystems and nematode abundance of trophic groups in the higher hierarchy of the soil food web was higher in less disturbed ecosystems, consistent with general findings from previous works in the field of nematode ecology.

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Appendix 2

Table 2.1. Heterogeneity statistics for the summary effect sizes per 100 g and per 100 cm³ of soil.

Summary effect	100 g			100 cm³		
	Q_t [a]	P_{hetero} [b]	I^2 [c]	Q_t [a]	P_{hetero} [b]	I^2 [c]
Overall richness	740.37	0.000	23.37	49.75	0.103	12.42
Overall abundance	525.42	0.007	2.67	320.94	0.000	10.28
Richness of c-p 1	347.88	0.000	8.50	129.66	0.000	66.24
Richness of c-p 2	486.97	0.000	15.43	79.06	0.000	34.16
Richness of c-p 3	453.61	0.000	32.25	147.73	0.000	73.73
Richness of c-p 4	520.05	0.000	29.18	42.39	0.357	7.62
Richness of c-p 5	390.74	0.001	4.56	54.84	0.025	17.00
Abundance of c-p 1	553.15	0.009	2.43	330.58	0.000	6.07
Abundance of c-p 2	422.77	0.028	2.57	186.77	0.000	27.30
Abundance of c-p 3	1299.77	0.000	2.07	224.18	0.000	43.86
Abundance of c-p 4	609.75	0.000	13.95	70.48	0.088	9.27
Abundance of c-p 5	730.70	0.000	9.33	159.32	0.000	14.05

Table 2.1. Continued.

Summary effect	100 g			100 cm^3		
	Q_t [a]	P_{hetero} [b]	I^2 [c]	Q_t [a]	P_{hetero} [b]	I^2 [c]
Richness of bacterivores	584.92	0.000	17.00	76.29	0.000	29.57
Richness of fungivores	392.01	0.000	18.47	105.16	0.000	57.06
Richness of herbivores	358.48	0.000	15.42	69.50	0.000	37.84
Richness of predators	267.55	0.000	18.34	50.88	0.061	14.51
Richness of omnivores	446.01	0.000	18.48	48.12	0.135	11.56
Abundance of bacterivores	519.91	0.001	3.80	396.91	0.000	9.81
Abundance of fungivores	645.08	0.034	1.61	357.16	0.000	17.18
Abundance of herbivores	762.77	0.015	1.62	430.30	0.001	3.92
Abundance of predators	768.10	0.000	6.72	144.93	0.000	18.25
Abundance of omnivores	747.91	0.000	11.09	344.69	0.000	12.77

[a] Q_t, total observed variation among studies

[b] P_{hetero}, probability of true variation among studies

[c] I^2, the proportion of true observed variation.

Figure 2.1. Effect of ecosystem on genus-level nematode richness. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing overall richness of nematodes per 100 g of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. P_{hetero} <0.05 is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels.
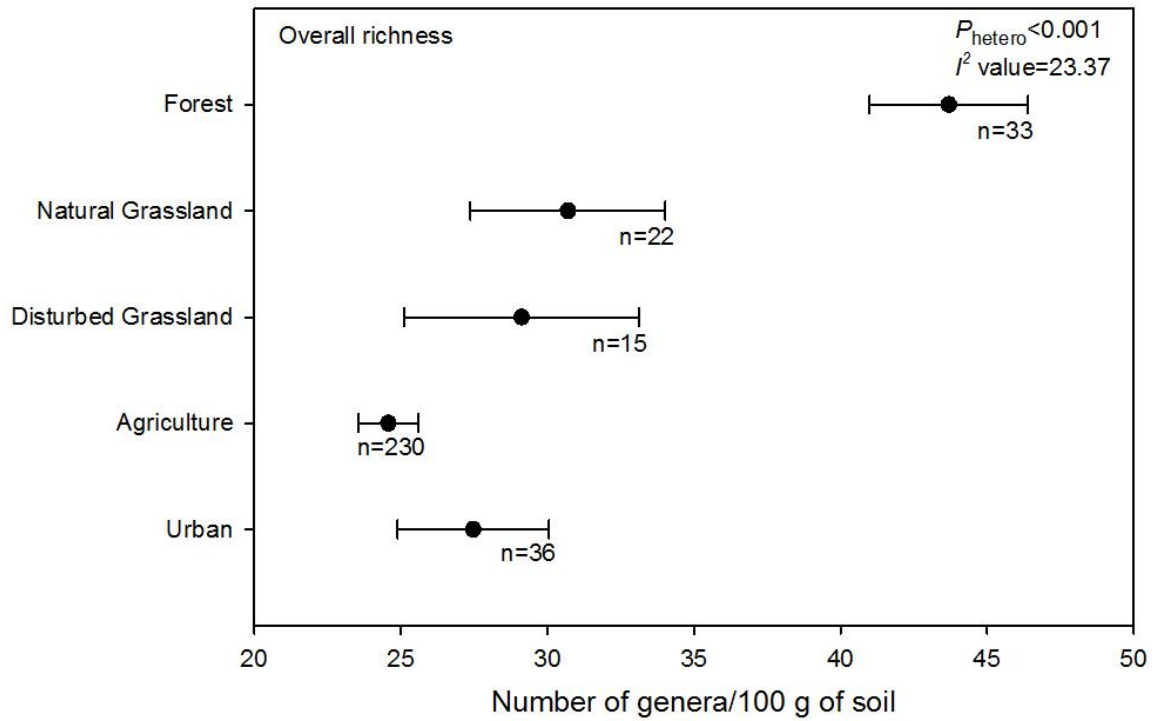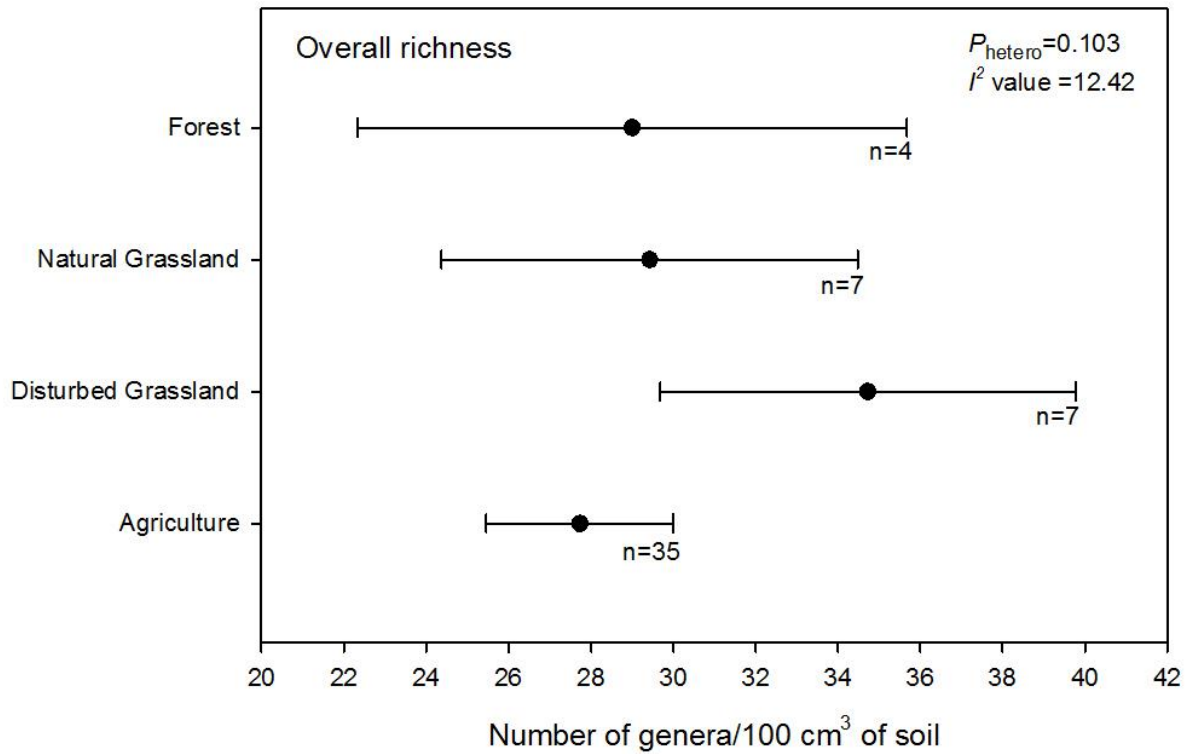
Figure 2.2. Effect of ecosystem on genus-level nematode richness. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing overall richness of nematodes per 100 cm³ of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. P_{hetero} <0.05 is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels.

Figure 2.3. Effect of ecosystem on genus-level nematode richness of each c-p class. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing richness of nematodes at c-p classes 1–5 per 100 g of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. P_{hetero} <0.05 is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels. The inset in c-p 1 and c-p 5 forest plots is the enlarged view of the respective forest plots.
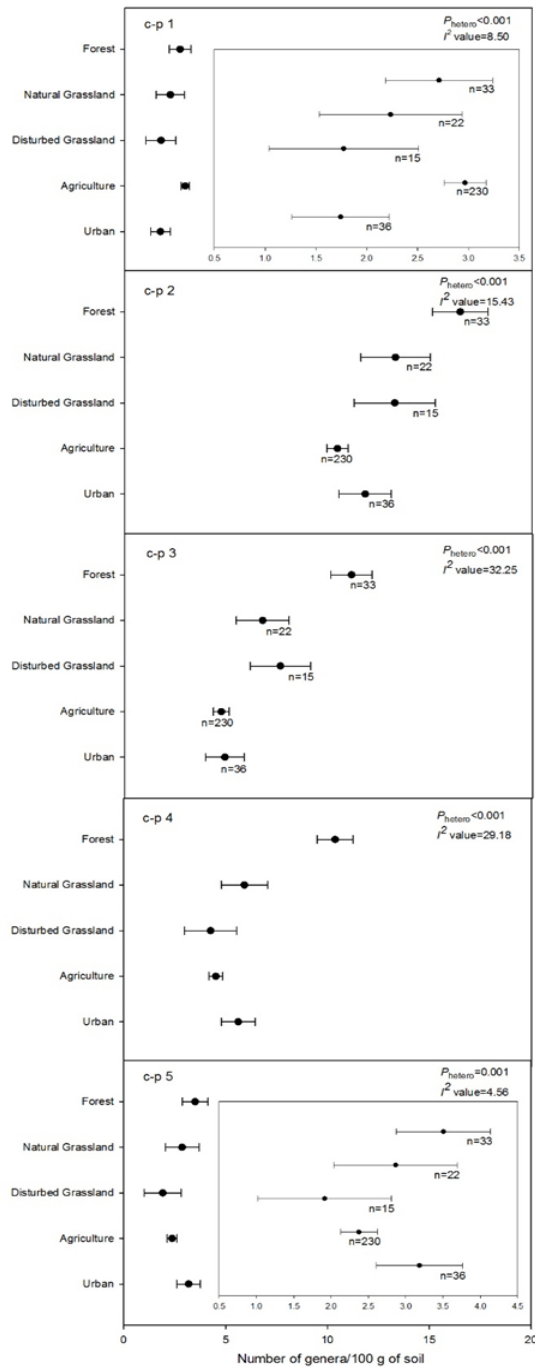
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Figure 2.4. Effect of ecosystem on genus-level nematode richness of each c-p class. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing richness of nematodes at c-p classes 1–5 per 100 cm^3 of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. P_{hetero} <0.05 is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels. The inset in c-p 5 forest plot is the enlarged view of the respective forest plot.
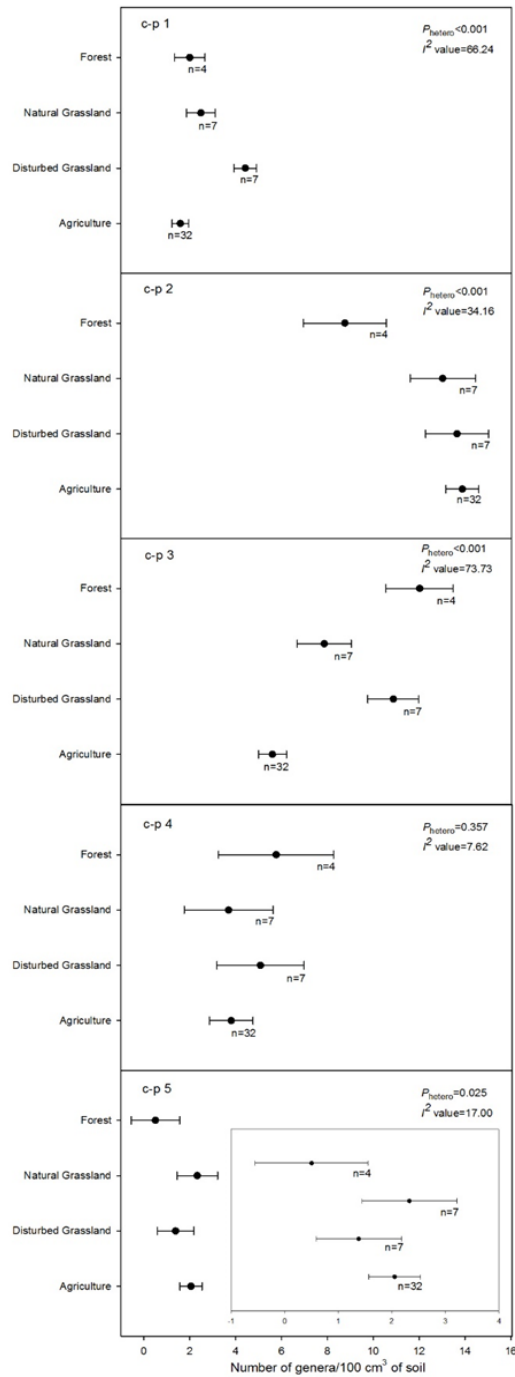
Figure 2.5. Effect of ecosystem on genus-level nematode richness of each trophic group. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing richness of nematodes of each trophic group per 100 g of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. $P_{hetero}<0.05$ is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels. The inset in fungivores and predators forest plots is the enlarged view of the respective forest plots.
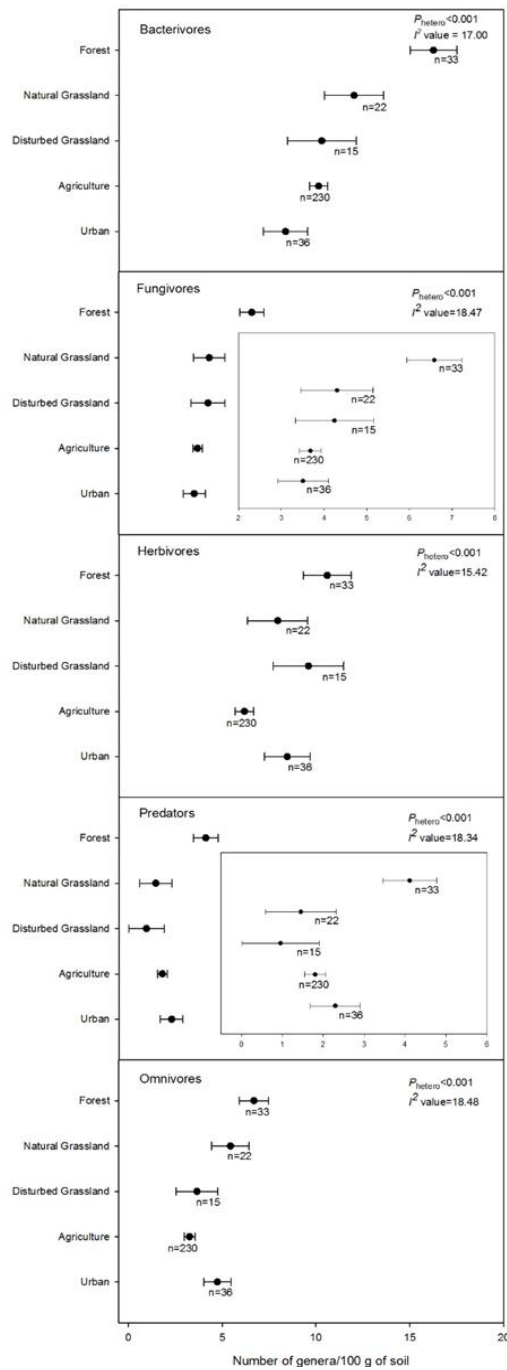
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Figure 2.6. Effect of ecosystem on genus-level nematode richness of each trophic group. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing richness of nematodes of each trophic group per 100 cm^3 of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. $P_{hetero}<0.05$ is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels. The inset in predators and omnivores forest plots is the enlarged view of the respective forest plots

Figure 2.7. Effect of ecosystem on genus-level nematode abundance. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing overall abundance of nematodes per 100 g of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. P_{hetero} <0.05 is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels.
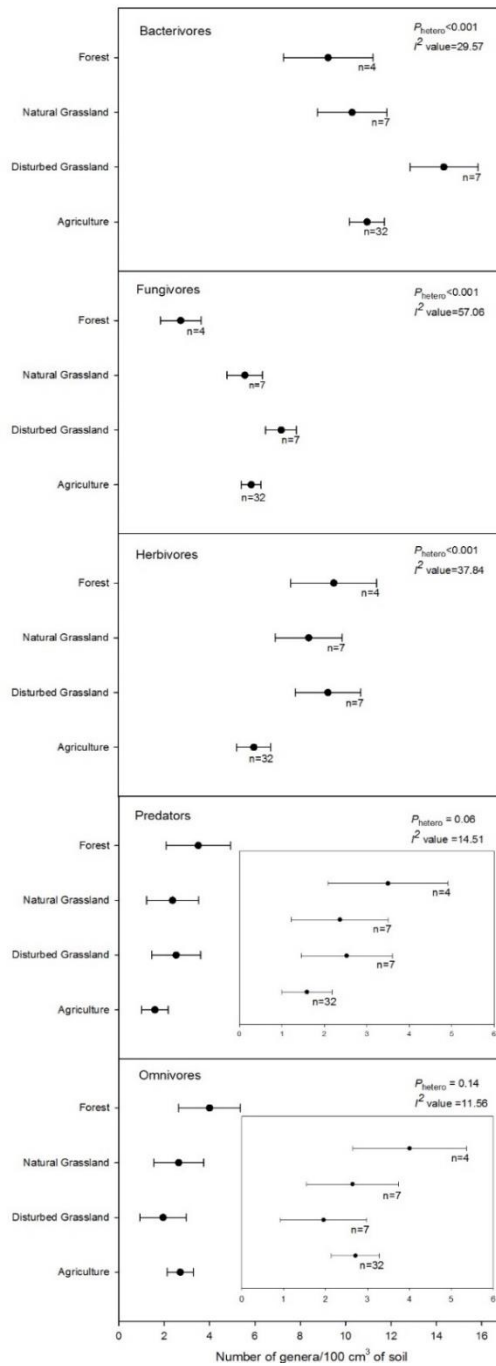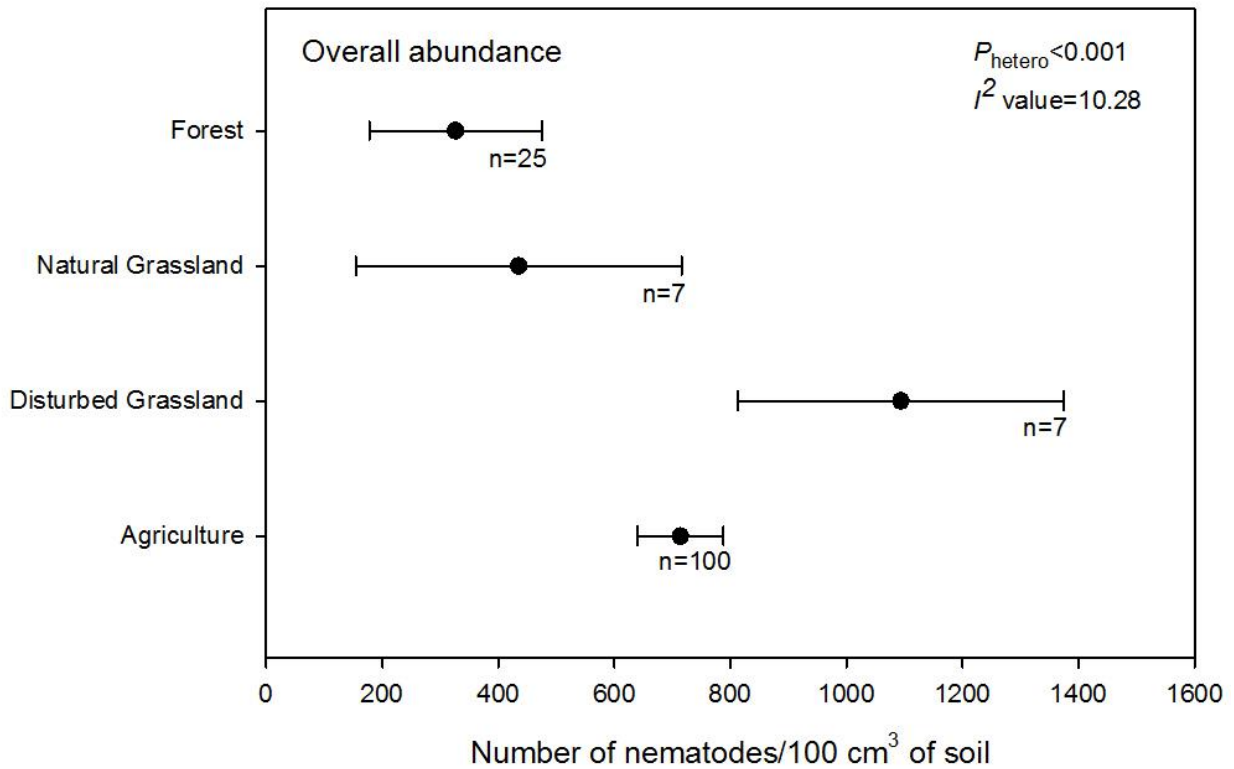
Figure 2.8. Effect of ecosystem on genus-level nematode abundance. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing overall abundance of nematodes per 100 cm³ in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. P_{hetero} <0.05 is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels.

Figure 2.9. Effect of ecosystem on genus-level nematode abundance of each c-p class. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing abundance of nematodes at c-p classes 1–5 per 100 g of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. P_{hetero}<0.05 is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels. The inset in c-p 1, c-p 4, and c-p 5 forest plots is the enlarged view of the respective forest plots.
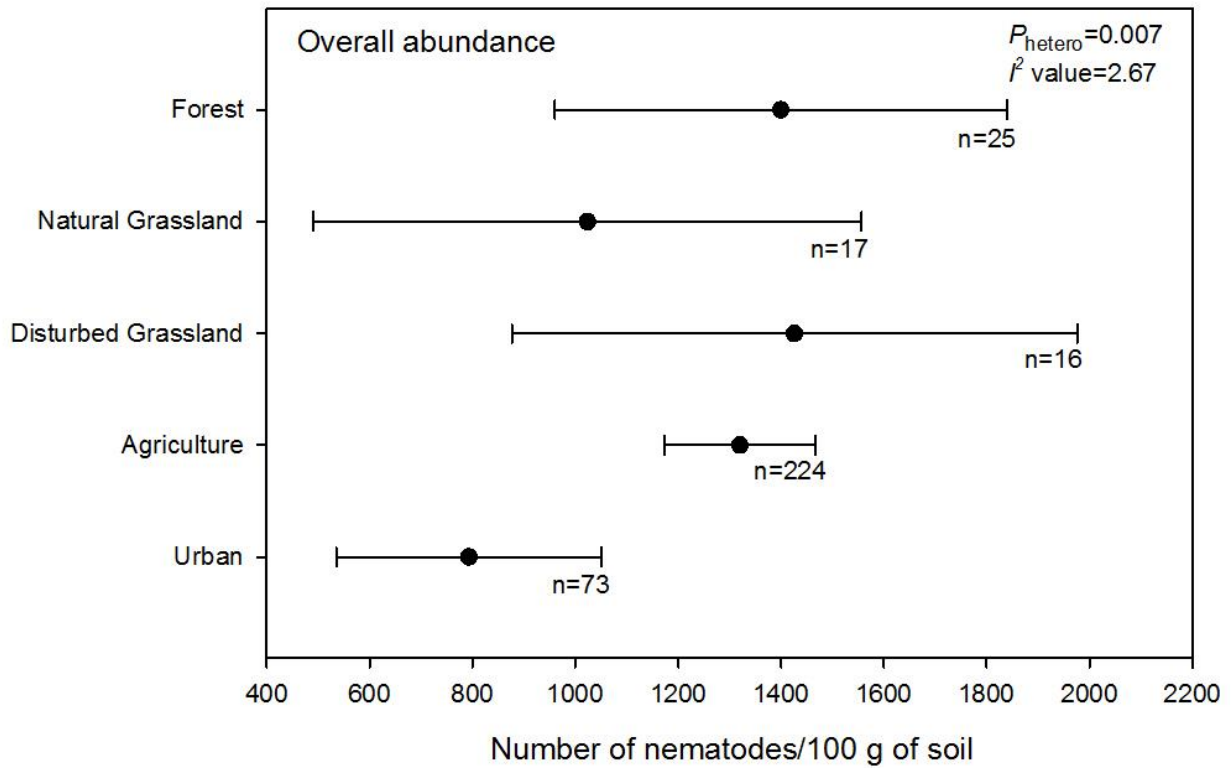
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Figure 2.10. Effect of ecosystem on genus-level nematode abundance of each c-p class. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing abundance of nematodes at c-p classes 1–5 per 100 cm^3 of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. P_{hetero} <0.05 is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels. The inset in c-p 1, c-p 4, and c-p 5 forest plots is the enlarged view of the respective forest plots.

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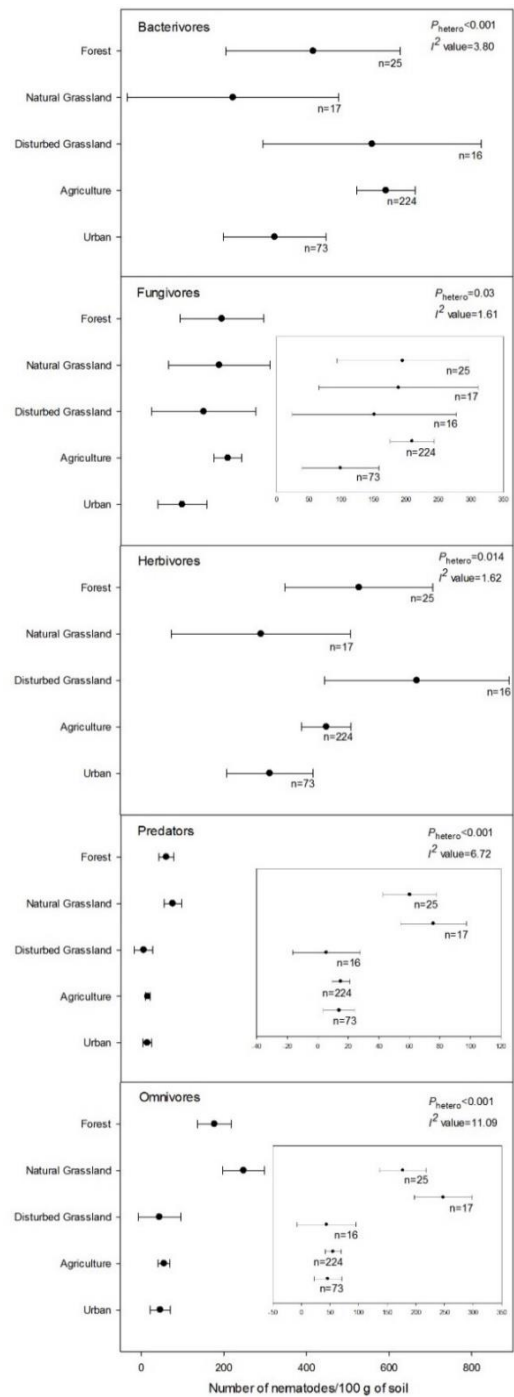
Figure 2.11. Effect of ecosystem on genus-level nematode abundance of each trophic group. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing abundance of nematodes of each trophic group per 100 g of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. P_{hetero} <0.05 is evidence that ecosystem levels differed. I^2 is the 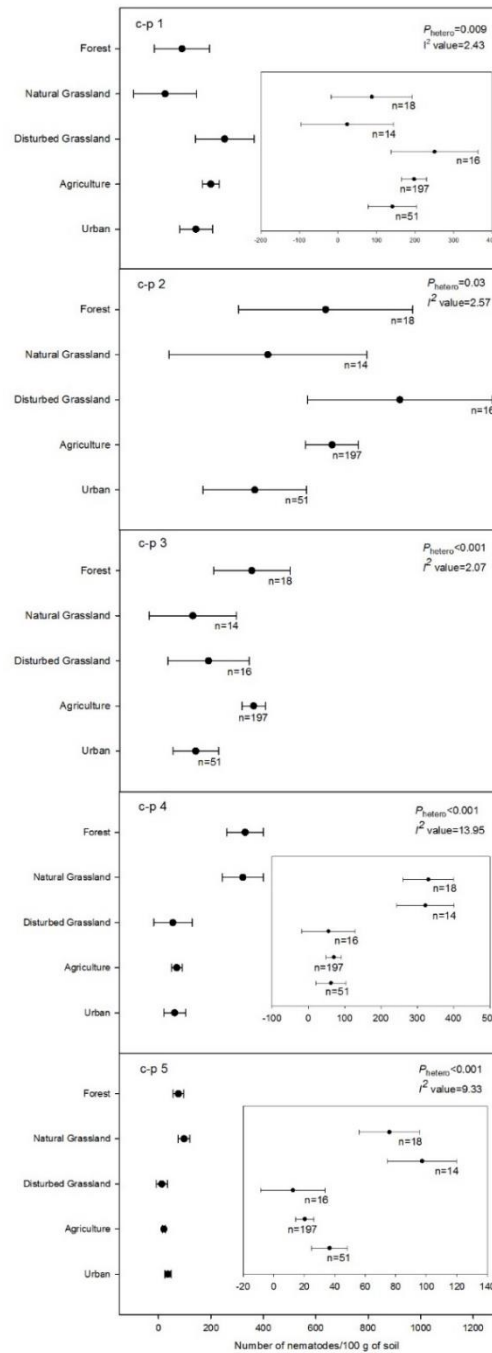percentage of true or real variation among ecosystem levels. The inset in fungivores, predators, and omnivores forest plots is the enlarged view of the respective forest plots.
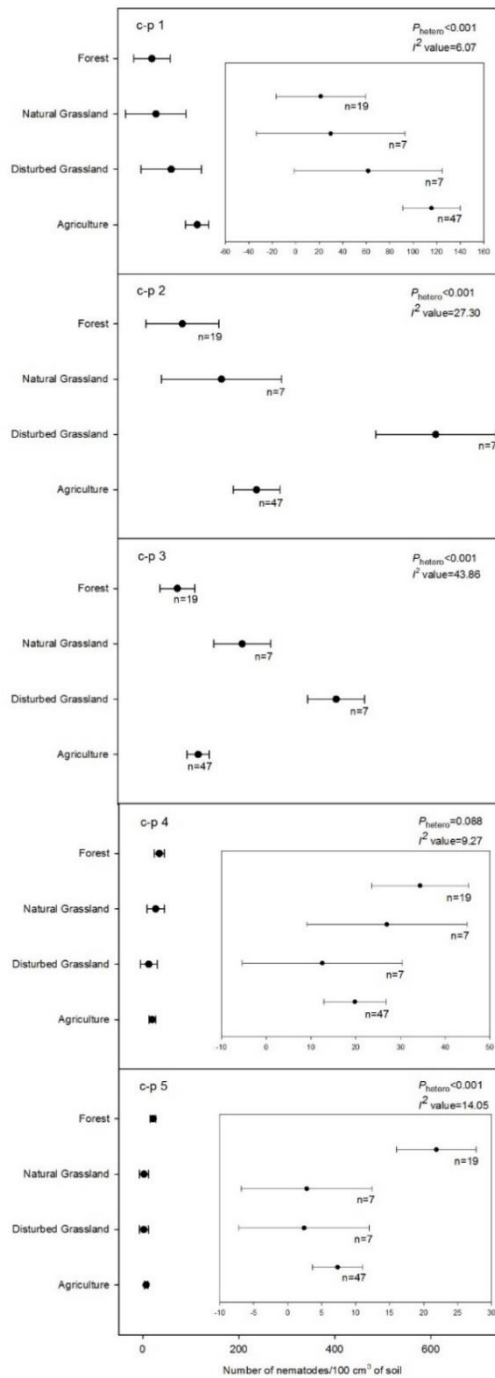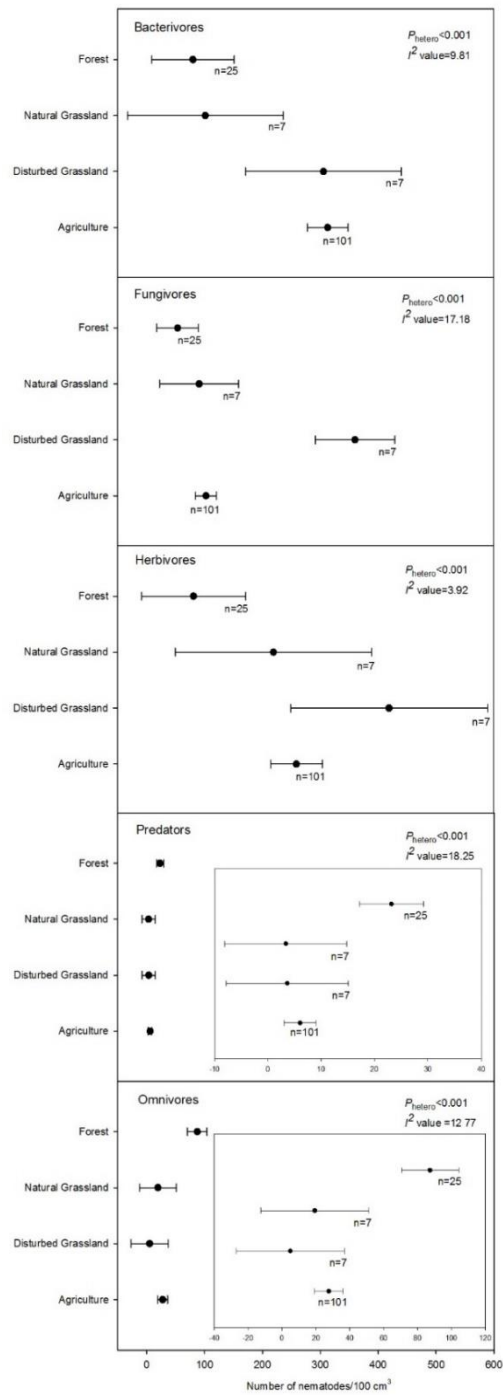
Figure 2.12. Effect of ecosystem on genus-level nematode abundance of each trophic group. Mean values are the weighted summary effect sizes and the bars represent 95% bootstrapped confidence intervals (CIs) for comparing abundance of nematodes of each trophic group per 100 cm^3 of soil in different ecosystems. Letter 'n' is the number of studies reporting data at each ecosystem. P_{hetero} <0.05 is evidence that ecosystem levels differed. I^2 is the percentage of true or real variation among ecosystem levels. The inset in predators, and omnivores forest plots is the enlarged view of the respective forest plots.

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Chapter 3

Effect of tillage in terms of increasing levels of disturbance on nematode food webs in an undisturbed ecosystem

Abstract

Soil is essential for sustenance of life. Among soil organisms, nematodes are by far the most abundant, ubiquitous and functionally diverse. Tillage affects nematodes directly by altering pore size and disrupting the continuity of water films needed by nematodes and indirectly by affecting the lower trophic groups such as bacteria and fungi. The primary goal of this study was to examine the effect of tillage on nematode communities in terms of increasing level of physical disturbance: control with no disturbance, surface litter removed (SLR) with no litter and no vegetation, soil disturbance with a rototiller every 2 months (R2M), and rototilling every 2 weeks (R2W) in an undisturbed forest ecosystem. Although, the effect of tillage on nematode abundance was not statistically significant, abundance was consistently lowest in R2M and R2W compared to the control and SLR treatment from September 2017 onward. Tillage resulted in significant reduction of nematode richness consistently in the last three samplings. The abundance of bacterial feeders, fungal feeders, plant feeders and predators was not significantly affected by tillage. However, tillage significantly lowered the abundance of omnivores in R2M and R2W compared to control during last sampling. The richness of fungal feeders, plant feeders and predators was not significantly affected by tillage whereas tillage significantly reduced the richness of bacterial feeders and omnivores, especially during the last two samplings. Tillage did not affect the abundance of c-p 1, c-p 2 and c-p 3 class nematodes but significantly affected higher c-p classes. The richness of c-p 1, c-p -3 and c-p 5 class nematodes was not affected by tillage. On the other hand, tillage significantly lowered the

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nematode richness of c-p 2 and c-p 4 class nematodes. Overall, our results indicated that

the rototill significantly reduced the nematode communities in R2M and R2W compared to

control and SLR treatments.

Introduction

Soil is indispensable for sustenance of life. Soil provides essential resources for

human activities such as agriculture, buildings, and industries (Brussaard, 1997). Several

biological processes are continuously active in the soil and play an important role in

replenishment of soil resources and ecosystem maintenance (Young and Crawford, 2004).

Biological processes in the soil are due to the dynamic interactions of diverse assemblages

of living organisms including unicellular bacteria and protozoa to multicellular nematodes,

earthworms and arthropods (Giller et al., 1997). Diverse soil organisms support several

biological processes such as organic matter decomposition, mineralization, nutrient cycling

and controlling pests and diseases (Brussaard, 1997), which directly and indirectly effect

crop growth and quality (Giller et al., 2005; Swift et al., 2004). Among multicellular soil

organisms, nematodes are by far the most abundant. Nematodes are at the center of the soil

food web by interacting with several other soil trophic groups in the lower hierarchy of the

soil food web, Plants, bacteria and fungi serve as food for nematodes; in turn, trophic

groups in the higher hierarchy of the soil food web, such as predatory mites, eat nematodes

(Moore, 1994 and Roger-Estrade et al., 2010).

Nematodes play a pivotal role in organic matter decomposition (Freckman, 1988;

Beare et al., 1992), mineralization (Yeates, 1979; Griffiths, 1989; Neher, 2001), and uptake

of nutrients by plants (Ingham et al.,1985). Nematodes feeding on bacteria and fungi promote mineralization and release nutrients into the soil and thereby regulate decomposition (Ingham et al., 1985). Nematodes are ubiquitous, functionally diverse and abundant. Therefore, nematodes can be used to gauge the condition of structure and function of soil food webs and ecosystem conditions (Bongers, 1990; Ferris et al., 2001; Neher, 2001; Bongers and Bongers, 1998). Nematodes have been categorized into different trophic groups such as bacterivores, fungivores, herbivores, predators and omnivores based on their feeding habits (Yeates et al., 1993). Trophic groups in the lower hierarchy of the soil food web include bacterivores, fungivores, and plant feeders, while trophic groups in the higher hierarchy of the soil food web include predators and omnivores (Yodzis, 2001). In addition, a colonizer-persister (c-p) scale with one to five classes has been developed for nematodes ranging from colonizers with a c-p value of 1 to persisters with a c-p value of 5 based on life history characteristics. The c-p scale reflects the continuum of r and K-strategists. Nematodes with high fecundity rate, short generation time and toleration of disturbances are assigned to colonizers and nematodes with low fecundity rate, long generation time and sensitivity to disturbances are assigned to persisters (Bongers, 1990). Nematode community indices have been used to monitor ecological conditions of soil and the influence of agricultural activities on nematodes (Sohlenius et al., 1987; Bongers, 1990; Freckman and Ettema 1993; Neher et al., 1995; Wardle et al., 1995).

Agricultural activities affect soil structure, biological activity and processes such as decomposition, mineralization and nutrient cycling by altering the physicochemical

properties of soil (Stinner et al., 1984; Dick et al., 1988; Fraser et al., 1994). Notably, agricultural practices such as cultivation, crop rotation, tillage and pesticide application have diverse impacts on plants, soils and soil organisms (Elliott and Cole, 1989). Tillage changes soil properties such as moisture, temperature, aeration and organic matter content and affects organisms that are living in the soil (Kladivko, 2001; Golabi et al., 2014; Holland, 2004). Furthermore, tillage disrupts the relationship between soil organisms by either killing or injuring or exposing them to predators (Altieri, 1999 and Roger-Estrade et al., 2010). Tillage affects nematodes directly by altering pore size and disrupting the continuity of water films needed by nematodes and indirectly by affecting the lower trophic groups such as bacteria and fungi (Wardle, 1995).

The effect of different types of tillage practices on nematode communities has been previously investigated in agricultural ecosystems, that had been previously tilled or disturbed (Zhang et al., 2015; Sánchez-Moreno et al., 2015; Zhong et al., 2017; Okada and Harada, 2007; Lenz and Eisenbeis, 2000; Dong et al., 2013; and Rahman et al., 2007). As an alternative, tillage effect may be better evaluated by conducting an experiment in an undisturbed ecosystem. Therefore, the main objective of this study was to examine the effect of tillage on nematode communities in terms of increasing level of physical disturbance in an undisturbed forest ecosystem. We hypothesized that the increase in level of physical disturbance would negatively affect nematode communities.

Materials and methods

Site description: A field experiment was conducted from April 2017 to May 2018 in a secondary mixed deciduous forest ecosystem in Farragut, TN, USA ($35^0 54'3''$N, $84^0 11'37''$W; 311 m elevation). The experimental site is located in a temperate and seasonal climate with a mean annual temperature of 15.3°C and mean annual precipitation of 1224 mm. The soil at this site is classified as Minvale-Bodine-Fullerton complex (Soil Survey Staff). The experimental site had not been disturbed for at least 50 years before the experiment was laid out. Understory was absent and groundcover was negligible. The site sloped slightly toward the northwest.

Experimental Design**:** The experiment included four treatments with increasing levels of physical disturbance. The first treatment was a control with no disturbance; the second treatment was SLR with no litter and no vegetation; the third treatment was soil disturbance with a rototiller every 2 months (R2M); and the fourth treatment was rototilling every 2 weeks (R2W). Litter and vegetation were cleared every 2 weeks from all the treatments except control. Each treatment was replicated three times. Each plot was 2 m x 2 m plots and were separated by a 2-m distance. The design of the experiment was a completely randomized design with repeated measures. The experiment was started in April 2017.

Soil sampling and nematode analysis: Soil samples were collected from all the plots at zero time before starting the experiment and subsequently samples were collected every two months. At each sampling time, 5 soil cores, each of 2 cm diameter and 20 cm deep

were collected randomly from each plot. Soil samples from each plot were pooled into a plastic bag to prevent drying of soil, transported to the laboratory and stored at $4^{\circ}C$ before nematode extraction. Composite soil samples were thoroughly mixed and $100\ cm^3$ of each soil sample was used for extraction of nematodes by means of a sugar flotation-centrifugation method (Jenkins, 1964). Extracted nematodes from each sample were counted and the first 150 nematodes were identified to genus level using differential interference contrast microscope, proportions of each taxon were extrapolated to the entire sample. The identified nematode genera were assigned to their respective trophic groups: bacterial feeders (BF), fungal feeders (FF), plant feeders (PF), omnivores (OM) and predators (PR), and to a colonizer-persister scale ranging from 1 to 5 (Yeates et al., 1993 and Bongers, 1990)

Statistical analysis: Nematode overall richness and overall abundance were estimated for each sample. In addition, nematode richness and abundance for each trophic group and each c-p class at each time point were estimated. Statistical analyses were performed to compare overall nematode richness and abundance, richness and abundance of each trophic group and each c-p class across different treatments at different time points. Normality of residuals and equal variance were assessed using Shapiro-Wilk statistic and visual observation of histograms. Abundance of omnivores, c-p 1 and c-p 5 class nematodes was ln(x+1)-transformed to normalize data prior to statistical analysis. Analysis of variance with repeated measures was conducted with SAS (Glimmix procedure,

SAS Institute, Cary, NC) and least square means were compared with Tukey's LSD at the 5% significance level.

Results

The effect of increasing levels of physical disturbance (treatment), sampling time and the interaction between treatment and sampling time on nematode abundance was not significant ($P > 0.05$). Although, the effect of tillage on nematode abundance was not statistically significant, nematode abundance was consistently lowest in R2M and R2W compared to the control and SLR treatments from September 2017 onward (Fig. 3.1). In contrast, treatment, sampling time and the interaction between treatment and sampling time significantly affected nematode richness ($P < 0.05$). During the first three samplings, nematode richness did not differ among treatments ($P > 0.05$). However, richness was significantly lower in R2M and R2W than in control during November 2017 ($P < 0.05$). In addition, nematode richness was significantly lower in SLR and R2W than in control during January 2018 ($P < 0.05$). The effect of tillage on nematode richness was more pronounced in the last sampling in May 2018 in which nematode richness was significantly lower in R2M and R2W compared to control and SLR treatments ($P < 0.05$). Tillage resulted in significant reduction of nematode richness consistently in the last three samplings ($P < 0.05$) (Fig. 3.2).

The effect of tillage on nematode abundance and richness of each trophic group was analyzed. The abundance of bacterial feeders, fungal feeders, plant feeders and predators was not significantly affected by tillage ($P > 0.05$). However, tillage significantly lowered

the abundance of omnivores in R2M and R2W compared to control during last sampling in May 2018 ($P < 0.05$) (Fig. 3.3). The richness of fungal feeders, plant feeders and predators was not significantly affected by tillage ($P > 0.05$) whereas tillage significantly reduced the richness of bacterial feeders and omnivores, especially during the last two samplings. The richness of bacterial feeders was lower in R2W than in the control during the last two samplings and ($P < 0.05$). Additionally, the richness of omnivores was lowest in R2M and R2W compared to control and SLR treatments during last sampling ($P < 0.05$) (Fig. 3.4).

The effect of tillage on nematode abundance and richness of each c-p class was also analyzed. Tillage did not affect the abundance of c-p 1, c-p 2 and c-p 3 class nematodes ($P > 0.05$) whereas significantly affected higher c-p classes ($P < 0.05$). The nematode abundance of c-p 4 class was lower in R2W compared to control and SLR treatments and lower in R2M and R2W compared to the SLR treatment in the last sampling ($P < 0.05$). Similarly, the abundance of c-p 5 class nematodes was lower in R2M and R2W than in the control during the last sampling ($P < 0.05$) (Fig. 3.5). The richness of c-p 1, c-p -3 and c-p 5 class nematodes was not affected by tillage ($P > 0.05$). On the other hand, tillage significantly lowered the nematode richness of c-p 2 and c-p 4 class nematodes ($P < 0.05$). The richness of nematodes in c-p 2 class was significantly lower in R2W than in control during January 2018 and significantly lower in R2M and R2W compared to control during last sampling, May 2018 ($P < 0.05$). Moreover, the richness of nematodes in c-p 4 class was significantly lowest in SLR and R2W compared to control in September 2017, January 2018. In the last

sampling, tillage significantly reduced the richness of c-p 4 class nematodes in R2M and R2W compared to control and SLR ($P < 0.05$) (Fig. 3.6).

Discussion

Nematodes play a key role in maintaining and regulating several biological processes, crucial for soil and plant health (Liang et al. 2009; Yeates and Coleman, 1982). Tillage is one of the most intensively used agricultural management strategies. Unfortunately, tillage affects the most important players in soil biological processes such as decomposition, mineralization and nutrient cycling (Stinner et al., 1984; Dick et al., 1988; Fraser et al., 1994). Many studies have been conducted to evaluate the effect of tillage management on nematode communities and other soil organisms in agricultural ecosystems. However, this report is the first on the effect of tillage on nematode populations in a previously undisturbed forest ecosystem.

The results from the analyses indicated that disturbances ranging from a minimal disturbance of removing the litter and vegetation to intensive disturbance by rototilling the soil every two weeks did not result in statistically significant differences on nematode abundance. Nevertheless, a trend of declining nematode abundance was observed consistently in R2M and R2W compared to control and SLR soil treatments in last four samplings (Fig. 3.1). This observed declining trend was not statistically significant due to large standard error, which could be reduced with a higher number of replications or with the prolongation of experiment for longer period. However, tillage significantly lowered nematode richness in both R2M and R2W tillage treatments compared to control and SLR

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treatments (Fig. 3.2). Rototilling directly affects nematode communities by abrasion and indirectly by changing the food supply chain, temperature, moisture and aeration of soil in tillage treatments compared to the control, which was undisturbed (Kladivko, 2001; Golabi et al., 2014; Holland, 2004; Rahman et al., 2007). Our findings are in agreement with the studies conducted by Freckman and Ettema, (1993), Okada and Harada, (2007), Dong et al. (2013), Zhang et al. (2015), Sánchez-Moreno et al. (2015), and Zhong et al. (2017), who reported that tillage reduced the nematode abundance in agricultural ecosystems. Nematode abundance and richness were statistically similar between SLR and control, which indicated that mere removal of litter and vegetation did not seriously affect forest nematode communities.

Among nematode trophic groups, tillage significantly lowered the richness of bacterial feeders. Even though, tillage effect on the abundance of bacterial feeding nematodes was not statistically significant, the abundance was always numerically lowest in R2M and R2W compared to SLR and control treatments (Fig. 3.3). Many studies conducted in agricultural fields have reported that tillage stimulated the bacterial feeding nematodes due to the probable increase in bacterial biomass with the incorporation of organic matter (Andren and Lagerlof, 1983; Parmelee and Alston, 1986; Ettema and Bongers, 1993; Lenz and Eisenbeis, 2000; Liphadzi et al., 2005; Sánchez-Moreno et al., 2006). The decrease in bacterial feeders due to tillage in this case apparently was due to the fact that organic litter was periodically removed from the tillage treatments. On the other hand, the tillage treatments did not have significant effect on the abundance and

richness of fungal feeders. The resistance of fungal feeding nematodes to tillage disturbances may suggest that the experimental site might be dominated by fungi than bacteria. Moreover, there is a discrepancy in the response of fungal feeding nematodes to tillage practices. Some studies reported that tillage increased the fungal feeding nematode communities (Parmelee and Alston, 1986; Liphadzi et al., 2005; Sánchez-Moreno et al., 2006; Dong et al., 2013). However, Okada and Harada, (2007) found that fungal-feeding nematodes increased in a no-till system. This discrepancy may be due to a complex set of factors, including geographic location, type of vegetation, soil type, and ecosystem. Similar to fungal feeders, abundance and richness of plant feeding nematodes did not differ significantly in tillage treatments compared to control. However, both abundance and richness of plant-feeding nematodes were always lower in R2M and R2W compared to control, suggesting a minor effect due to periodic destruction of near-surface feeder roots. This declining trend of plant feeding nematodes was in agreement with Lenz and Eisenbeis, (2000) and Rahman et al. (2007). Among nematodes belonging to the higher hierarchy of soil food web, tillage did not affect predators but significantly reduced the abundance and richness of omnivores, which are sensitive to disturbances (Bongers 1990; Ferris et al., 2001) especially in the last two samplings. Similar results were reported by Dong et al. (2013), Zhang et al. (2015), and Zhang et al. (2017).

The effect of tillage disturbances on nematode communities according to c-p classes were also assessed. The abundance and richness of c-p 1 and c-p 3 class nematodes were not significantly affected by tillage. Nematodes belonging to lower c-p classes are r-

strategists, which are characterized by high fecundity rate, short generation time and tolerance to disturbances (Bongers, 1990; Ferris et al., 2001). Although c-p 2 class nematodes belong to lower c-p classes, the richness of c-p 2 class nematodes was significantly reduced by tillage. The abundance of c-p 2 class nematodes was consistently lower in rototilled treatments than in control though the trend was not statistically significant. The lower c-p 2 class nematodes in tillage treatments could be due to the decrease in bacterial feeding nematodes belonging to c-p 2 class. The abundance and richness of nematodes of higher c-p classes (c-p 4 and c-p 5) were significantly reduced by tillage disturbances as these nematodes are sensitive to disturbances in the soil ecosystem (Bongers, 1990; Lenz and Eisenbeis, 2000; Ferris et al., 2001).

Conclusion

The current study was conducted to evaluate the effect of tillage in terms of increasing levels of physical disturbance on nematode communities in an undisturbed forest ecosystem indicated that tillage reduced the nematode communities, which was consistent with the studies conducted in agricultural ecosystems. However, in this study microbe-feeding nematodes responded differently compared to that of agricultural ecosystem. Tillage reduced the bacterial feeding nematodes and did not affect the fungal feeding nematodes. The effect of increasing levels of disturbance revealed that the rototill significantly reduced the nematode communities compared to control and SLR treatments but the differences between control and SLR on nematode communities were not statistically significant. Similarly, R2M and R2W were not significantly different. Still, there

was a declining trend of nematode communities with increasing levels of physical

disturbance. This trend potentially become statistically significant with the prolongation of

experiment for longer period.

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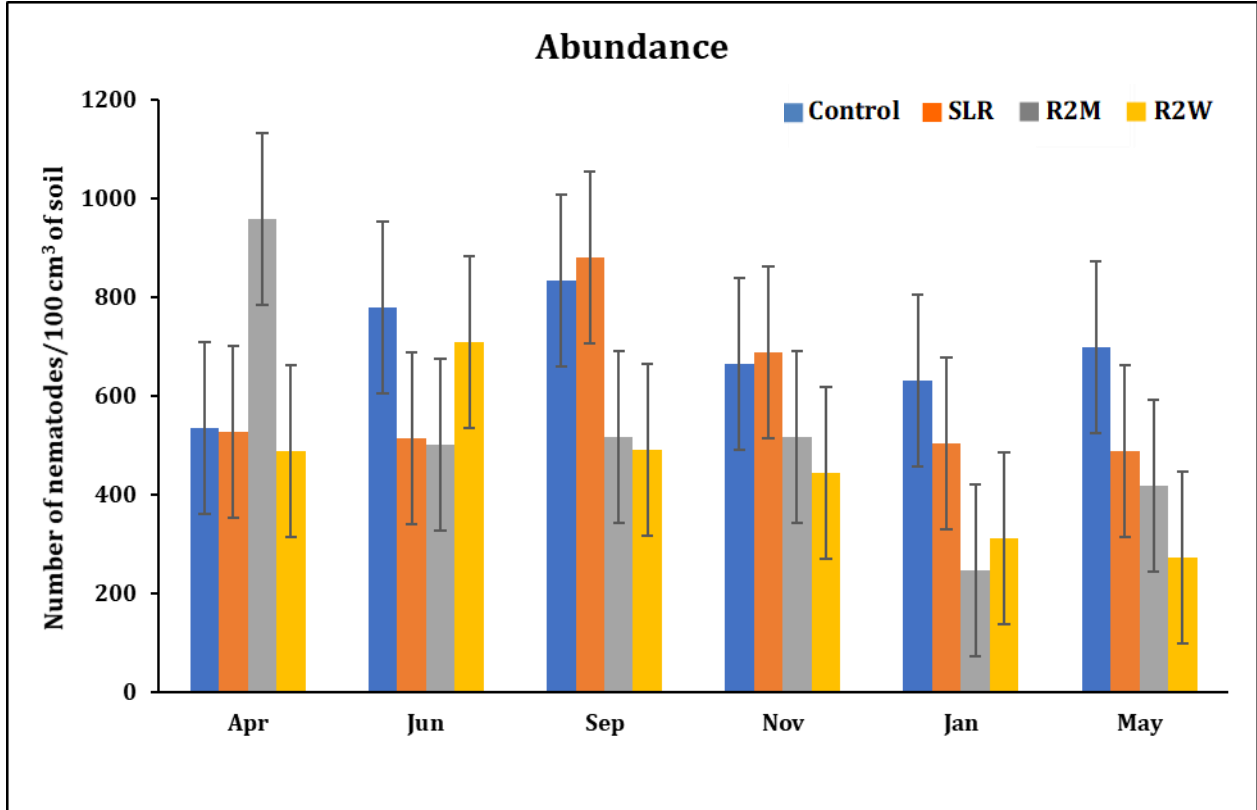
Appendix 3

Fig. 3.1. Effect of tillage on genus-level nematode abundance. Bars indicating number of nematodes (mean±SE) per 100 cm^3 of soil in control, surface litter removed (SLR), rototill for every two months (R2M) and rototill for every two weeks (R2W) at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).

Fig. 3.2. Effect of tillage on genus-level nematode richness. Bars indicating number of genera (mean±SE) per 100 cm^3 of soil in control, surface litter removed (SLR), rototill for every two months (R2M) and rototill for every two weeks (R2W) at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).
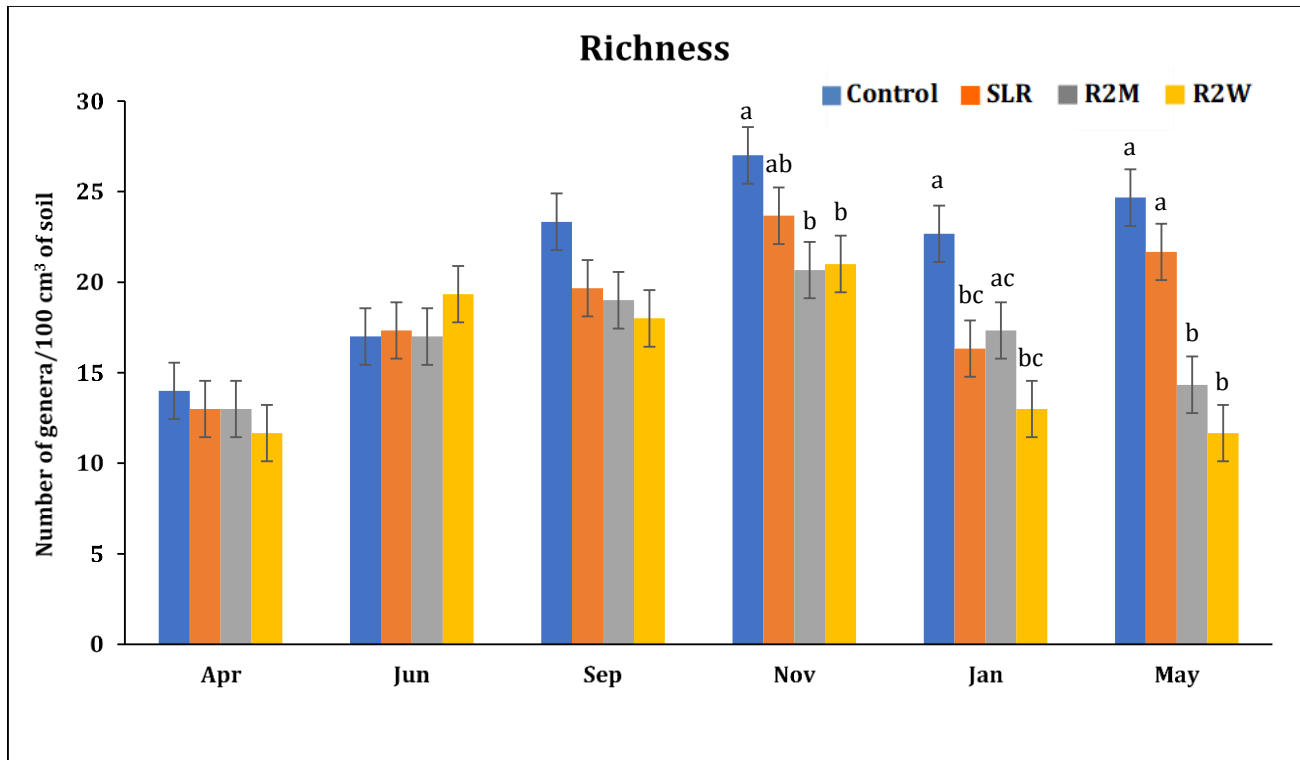
Fig. 3.3. Effect of tillage on genus-level nematode abundance of each trophic group. Bars indicating number of nematodes (mean±SE) per 100 cm^3 of soil in control, surface litter removed (SLR), rototill for every two months (R2M) and rototill for every two weeks (R2W) at each sampling time. Letters indicate significant differences among treatments at each sampling time at P < 0.05 (Tukey-LSD test)
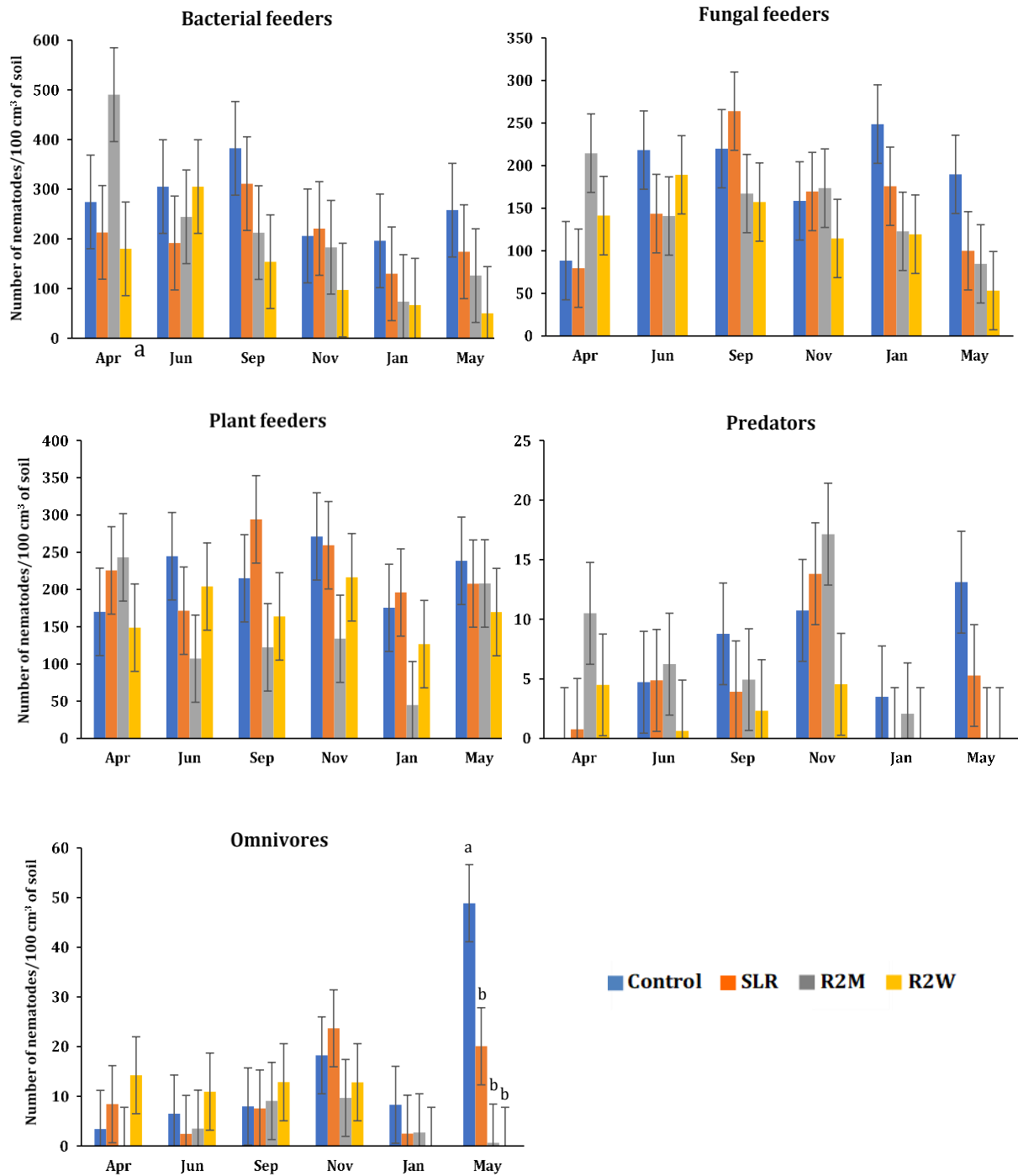
Fig. 3.4. Effect of tillage on genus-level nematode richness of each trophic group. Bars indicating number of genera (mean±SE) per 100 cm^3 of soil in control, surface litter removed (SLR), rototill for every two months (R2M) and rototill for every two weeks (R2W) at each sampling time. Letters indicate significant differences among treatments at each sampling time at P < 0.05 (Tukey-LSD test)

Fig. 3.5. Effect of tillage on genus-level nematode abundance of each c-p class. Bars indicating number of nematodes (mean±SE) per 100 cm³ of soil in control, surface litter removed (SLR), rototill for every two months (R2M) and rototill for every two weeks (R2W) at each sampling time. Letters indicate significant differences among treatments at each sampling time at *P* < 0.05 (Tukey-LSD test)
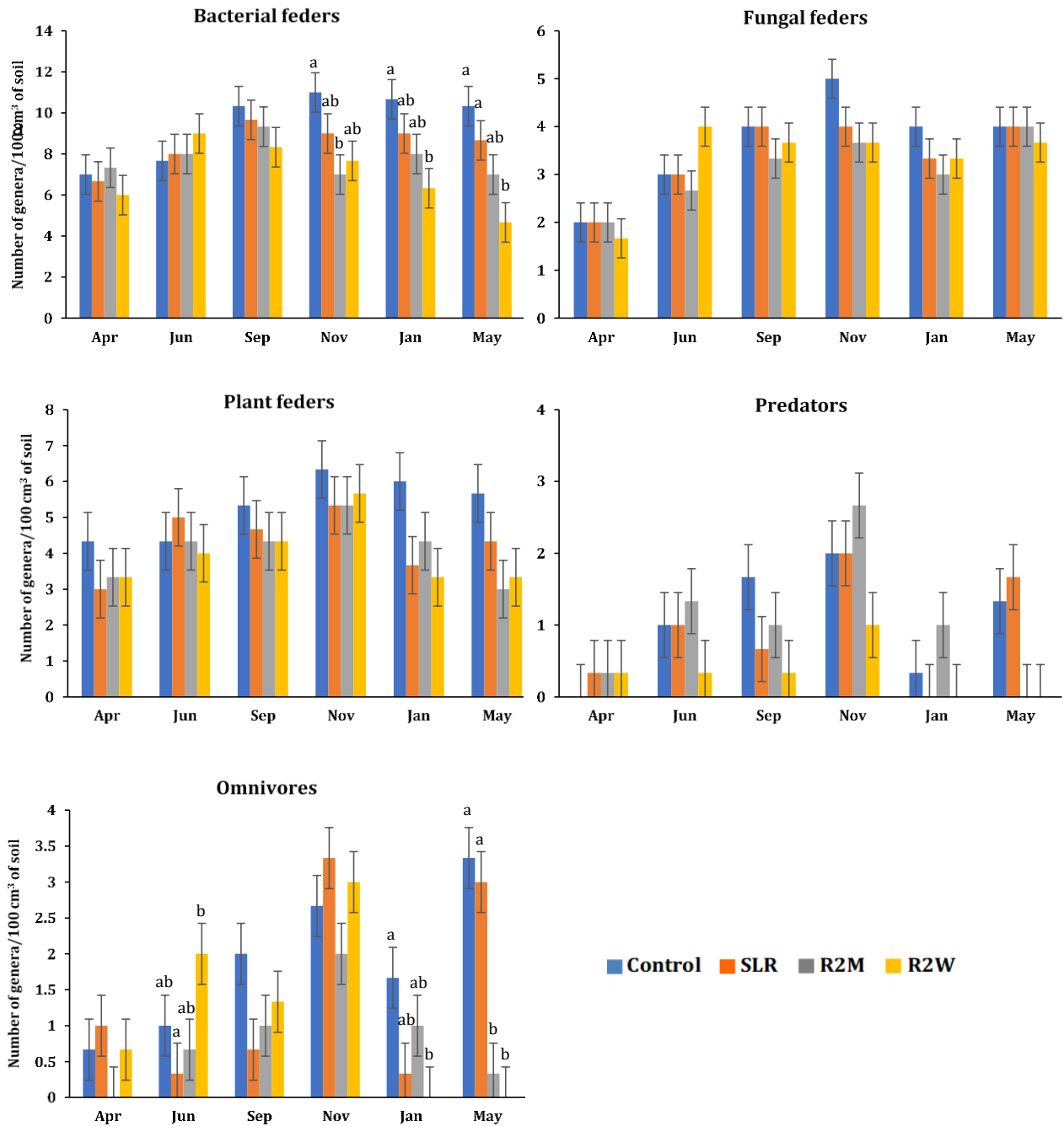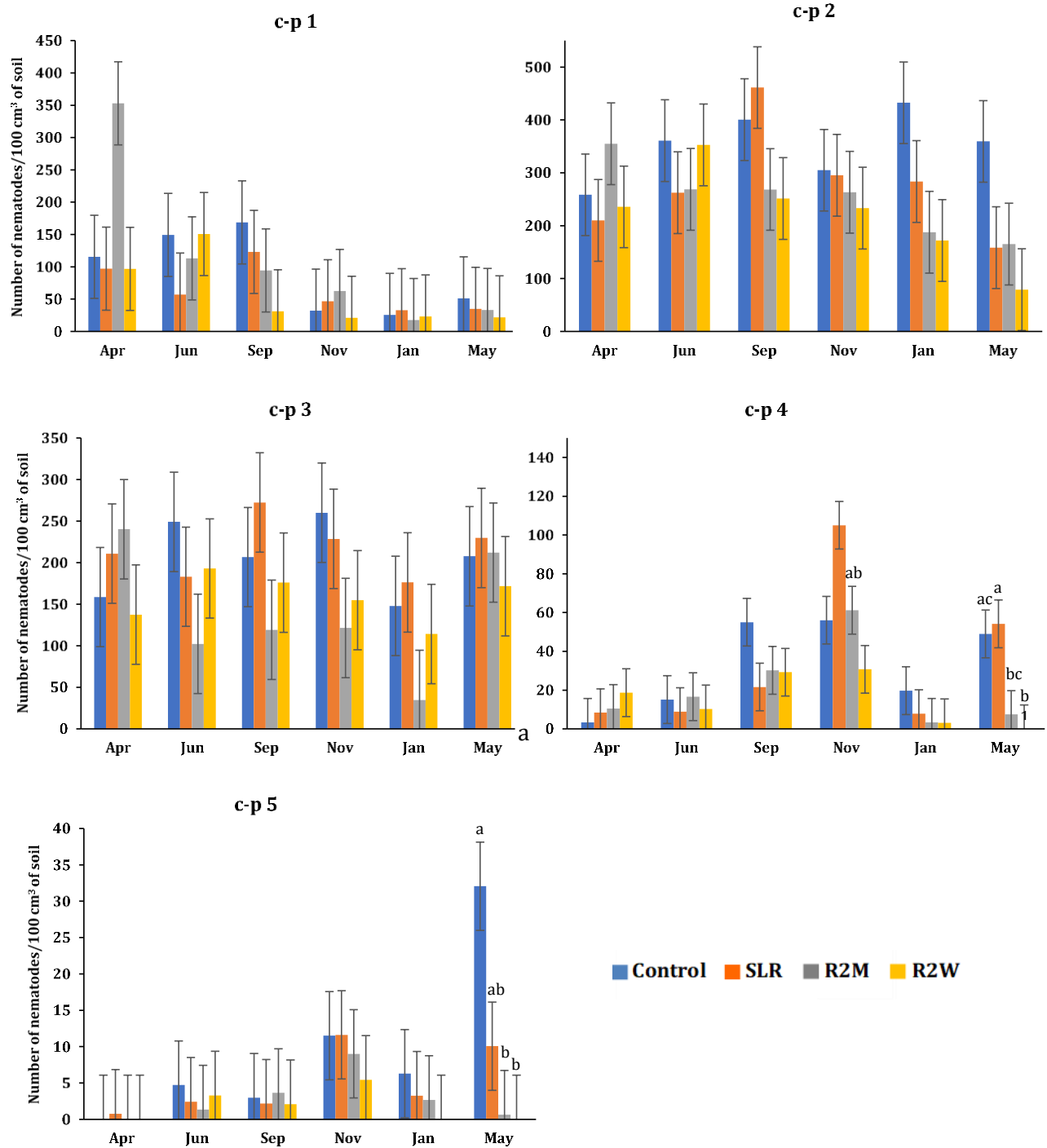
Fig. 3.6. Effect of tillage on genus-level nematode richness of each c-p class. Bars indicating number of genera (mean±SE) per 100 cm³ of soil in control, surface litter removed (SLR), rototill for every two months (R2M) and rototill for every two weeks (R2W) at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test)
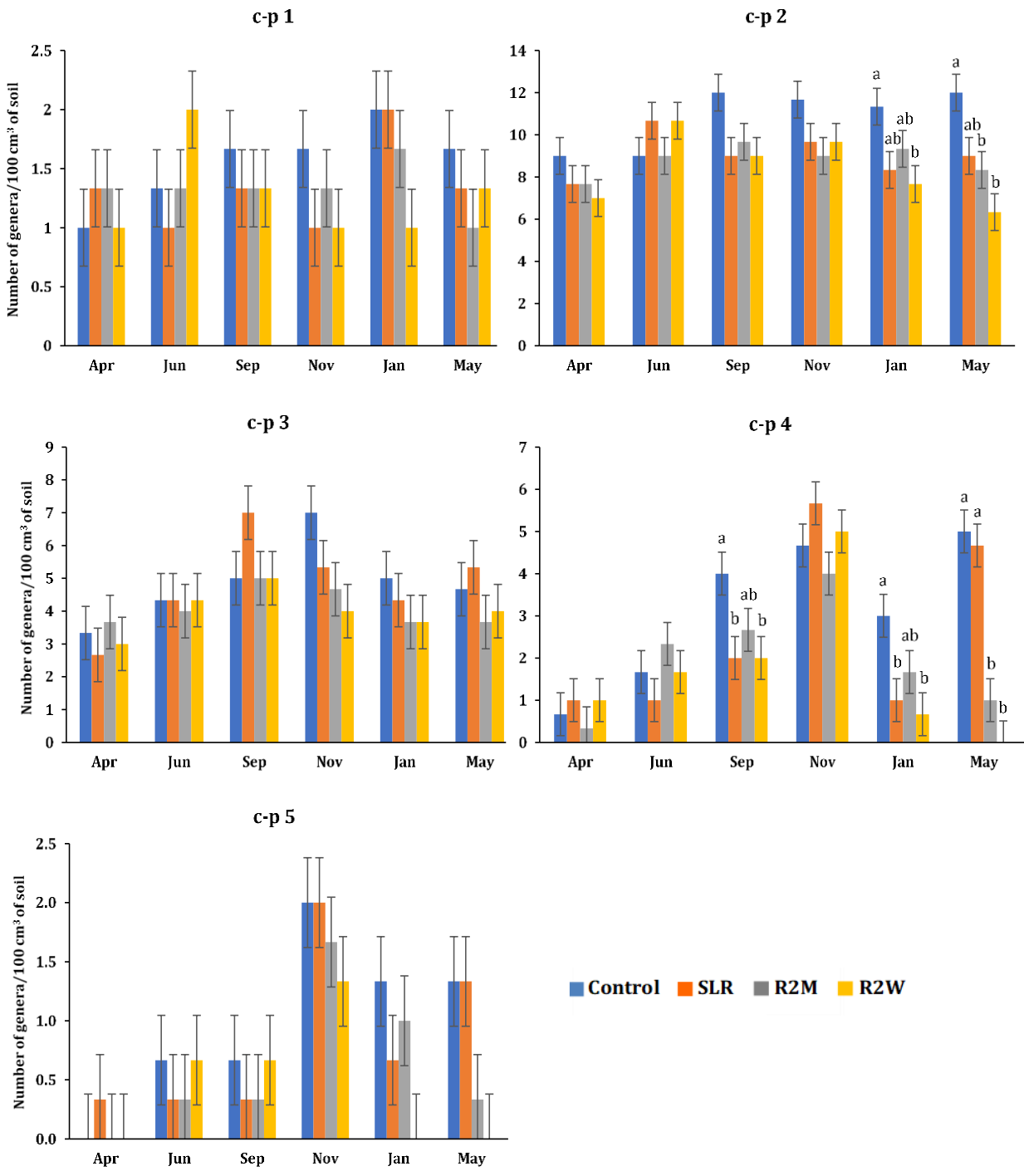
Chapter 4

Response of nematode food webs to temperature stress associated with climage change

Abstract

Accelerated global climate change, primarily warming is an undeniable fact. It is predicted that global temperatures will increase by 1.8-4.0 °C in the next 50–100 years. Soil temperature increases congruently with increases in air temperature. Change in soil temperature affects biodiversity in the soil. Nematodes are the most abundant multicellular soil organisms and are morphologically and functionally diverse. Although nematodes exert a strong influence on soil ecosystem functions, comparatively little is known about the impact of a sustained rise in temperature on nematode communities. Therefore, a one-year soil warming experiment was conducted to investigate the response of nematodes by increasing the average soil temperature by 5 °C in warming plots compared to cabled control (CC) and control using heating cables in forest and agricultural ecosystems. The results from the agriculture site revealed that nematode abundance was not significantly affected by soil warming, whereas richness of nematodes was significantly lowered in the warming treatment. Even though the statistical differences were very few, the abundance and richness of bacterial feeders and the abundance of fungal feeders were always lower in the warming treatment. Soil warming did not have a consistent significant effect on the abundance of plant feeders, predators and omnivores. However, the richness of plant feeders, predators and omnivores was also reduced by soil warming. The abundance of nematodes belonging to all c-p classes and richness of c-p 1 and c-p 2 nematodes were not consistently significantly affected by soil warming. In contrary, higher c-p class (c-p 3, c-p 4 and c-p 5) nematode numbers were lower in the warming treatment than control treatments. Unlike in the agricultural ecosystem, nematode abundance, richness and

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abundance and richness of nematodes of all trophic groups and all c-p classes were not affected by soil warming in the forest ecosystem. Overall, the results from our research indicate that nematode communities in the forest ecosystem may be more resilient to environmental fluctuations compared to that of agricultural ecosystems.

Introduction

Accelerated global climate change, primarily warming is an undeniable fact. Global warming is the increase in average global temperature of the atmosphere and the Earth's surface. Global warming is caused by an increase in the emission of greenhouse gases such as carbon dioxide, methane and nitrous oxide (Githeko et al., 2000). Emission of greenhouse gases has been increasing since the beginning of the Industrial Revolution (IPCC, 2013). Increase in greenhouse gas emissions is mainly due to human activities such as burning of fossil fuels, urbanization, agriculture, deforestation and desertification (IPCC, 1997). Over the last century, mean global temperature has increased by 0.74 ^{o}C and it has been predicted that the temperature will further increase by 1.8-4.0 ^{o}C in the next 50–100 years (IPCC, 2007; Houghton et al., 2001). This increase is mainly due to a rise in daily minimum temperatures twice as much as increase in daily maximum temperatures (Easterling et al. 1997; IPCC, 2001; Lobell et al. 2011). Soil temperature increases congruently with increases in air temperature (Jacobs et al. 2011). Temperature and moisture in the soil are the main abiotic factors that regulate many biological processes. Therefore, change in soil temperature could affect biodiversity in the soil (Farnsworth et al., 1996; Chapin et al., 1996).

Soil is the habitat for most terrestrial organisms (Young and Crawford, 2004). Soil supports diverse groups ranging from microscopic organisms such as bacteria, fungi and archaea to complex organisms such as nematodes, mites and earthworms (Brussaard, 1997). Nematodes are the most abundant multicellular soil animals and are morphologically and functionally diverse (Bongers and Bongers, 1998; Yeates et al., 1993; Ferris et al., 2001). A distinct feature of nematode communities is that they can be categorized into different trophic groups and c-p (colonizer-persister) classes based on their feeding habits and life history characteristics respectively (Bongers, 1990; Yeates et al., 1993). Trophic groups include bacterial feeders, fungal feeders, plant feeders, predators and omnivores (Yeates et al., 1993). The c-p scale ranges from 1 through 5, where c-p 1 comprises of extreme colonizers and c-p 5 consists of long-lived persisters. Nematodes with high colonization ability, short life cycle and tolerantion to disturbances are categorized as colonizers and nematodes with low colonization ability, long life cycle and sensitivity to disturbances are categorized as persisters (Bongers, 1990). By their virtue of diverse feeding habits, nematodes interact with several other soil trophic groups in the lower hierarchy of the soil food web such as bacteria, fungi and plants and trophic groups in the higher hierarchy of the soil food web, such as predatory mites (Moore, 1994 and Roger-Estrade et al., 2010). Such multitrophic interactions contribute to crucial soil processes such as decomposition of soil organic matter, mineralization and nutrient cycling (Bongers and Bongers, 1998; Bongers and Ferris 1999; Liang et al. 2009; Yeates and Coleman, 1982). In addition, nematodes serve as elegant indicators of environmental stress because they are omnipresent, abundant and sensitive to environmental disturbances

(Stone et al., 2016; Bongers and Bongers, 1998; Bongers and Ferris, 1999). Although, nematodes exert a strong influence on soil ecosystem functions, comparatively little is known about the impact of a sustained rise in temperature on nematode communities.

Recently, soil nematodes have been gaining importance in predicting future changes in soil ecosystems due to global warming. Changes in nematode communities can provide information about the response of soil food webs and their functions to global warming. Even though, the response of nematode food webs to global warming has been studied considerably, the results have not been consistent across studies. For instance, some studies reported soil warming reduced soil nematode abundance (Simmons et al., 2009; Thakur et al., 2017; Yan et al., 2017) while others reported no effect (Sohlenius and Bostrom, 1999; Dong et al., 2013). Nematode community analysis by trophic group indicated that soil warming resulted a significant increase in bacterial and fungal feeders (Song et al., 2014; Mueller et al., 2016) but Yan et al. (2017) stated the converse and Lee et al. (2013) did not observe any effect of soil warming. In addition, soil warming decreased plant-feeding nematodes (Song et al., 2014; Muller et al., 2016), predators and omnivores (Mueller et al., 2016), but Song et al. (2014) reported that predators and omnivores remain unchanged. Bakonyi et al. (2007) reported that soil warming favored few nematode species. The anomalies in these results may be due to the incorporation of another effect such as plant composition, elevated CO_2, and tillage along with warming; comparing samples from different locations along a temperature gradient; or considering only nighttime warming. Therefore, we conducted an in-situ warming experiment to investigate

the response of nematodes to a 5 ^{o}C rise in soil temperature by simulating future global warming using heating cables in forest and agricultural ecosystems. We hypothesized that 5 ^{o}C rise in soil temperature would reduce nematode abundance and richness of all trophic groups and all c-p classes in agriculture and forest ecosystems.

Materials and methods

Site description: The experiment was carried out at Organic Crops Unit (35^{0}52'23''N, 83^{0}56'10''W; 268.2 m elevation), East Tennessee Research and Education Center, Knoxville, Tennessee, USA. The study was performed from May 2017 to June 2018 in two different ecosystems; one was a disturbed agricultural ecosystem and the other was a relatively less disturbed forest ecosystem. The two sites were approximately 180 m away from each other. The climate at the experimental site is temperate, seasonal with a mean annual temperature of 15.3 ^{o}C and mean annual precipitation of 1224 mm. The soil in the agricultural and forest ecosystems is classified as Decatur silty loam and Dandridge shaly silty clay loam respectively. Before laying out the experiment at the agricultural site, the plots had been cultivated with tomato, cucumber and squash while the forest ecosystem had not been disturbed for about 50 years.

Experimental Design: A one-year soil warming experiment was conducted by increasing the average soil temperature by 5 ^{o}C above ambient soil temperature in warming plots using heating cables. Cables were installed in cabled control (CC) plots but not heated to account for physical disturbances and undisturbed control plots were left in their natural state without any disturbance. A total of nine 2 m x 2 m plots were established

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with a 2-m distance between the plots in each ecosystem. All treatments were replicated three times. The design of the experiment in both ecosystems was a completely randomized design with repeated measures. Heating cables (Greenhouse Megastore, Danville, IL, USA) were installed in February 2017 but soil warming was started in May 2017 to allow nematodes to recover from any potential physical disturbances occurred during the experimental setup.

To install heating cables, eight trenches were made per each plot in warming and CC plots. Trenches were 20 cm deep with a spacing of 20 cm between them. Heating cables were buried at three different depths, 7 cm, 14 cm, and 20 cm with in each trench to uniformly heat the soil. The heating cable in the warming treatments used 8.3 amps at 120 V AC with a power density output of 1250 W m^{-2}. Four thermocouples were installed in warming plots and one thermocouple was installed in CC and control plots at 20 cm deep to constantly monitor and maintain temperature. To monitor moisture content, one watermark was installed in each plot at 20 cm deep. A Campbell CR 1000 datalogger (Campbell Scientific, Logan, UT, USA) was used to monitor 18 thermocouples and 9 watermarks. The data logger maintained the temperature at 5 ^{o}C in the warming plots, by comparing the average temperature of the thermocouples in the warming plot with the average temperature of the thermocouples in the control and CC plots. If the average temperature in a warming plot was < 5 ^{o}C the datalogger turned on a relay that allowed power to heating cables; if it was > 5 ^{o}C then the datalogger turned off the relay. Prior to

sampling the soil temperature was gradually increased by 1 °C each week until it reached 5 °C in the warming plot compared to control plots (Fig. 4.1).

Soil sampling and nematode analysis: Before heating, soil samples were collected from all the plots at zero time and subsequently collected every three months. During every sampling time, 3 soil cores of 2 cm diameter, 20 cm deep were collected randomly from each plot. Soil samples from each plot were packed in a plastic bag to prevent moisture loss and stored at 4 °C to minimize changes in nematode populations prior to examination. Before nematode extraction, composite soil samples were thoroughly mixed and 100 cm^3 of soil sample was used for extraction of nematodes by means of a sugar flotation-centrifugation method (Jenkins, 1964). All nematodes were counted and at least 150 nematodes were identified to genus level using differential interference contrast microscope and extrapolated to the entire sample. After identification, all nematode genera were assigned to a trophic group (plant feederss (PF), fungal feeders (FF), bacterial feeders (BF), omnivores (OM) and predators (PR)) and a colonizer-persister class 1 through 5 (Yeates et al., 1993 and Bongers, 1990)

Statistical analysis: Richness and abundance for overall nematodes and nematodes of each trophic group and each c-p class was calculated at each time point. The significance of effect of treatment on overall nematode richness and abundance, richness and abundance of each trophic group and each c-p class at each time point was analyzed. Data failed to pass Shapiro-Wilk normality test and equal variance were ln(x+1) transformed prior to analysis. ANOVA with repeated measures was used to analyze the nematode

communities using the Glimmix procedure in SAS (Glimmix procedure, SAS Institute, Inc., Cary, NC). Separate analyses were performed for richness and abundance of overall nematodes and nematodes of each trophic group and each c-p class. Least square means were generated using Tukey's LSD option of glimmix procedure. Significant difference was considered at a p value ≤ 0.05.

Results

Agricultural site:

Nematode abundance was not significantly affected by soil warming (treatment) and the interaction between treatment and time of sampling ($p > 0.05$) but time of sampling significantly influenced nematode abundance ($p < 0.05$) (Fig. 4.2). On the other hand, nematode richness was significantly affected by treatment, time of sampling and the interaction between them ($p < 0.05$). Warming significantly reduced nematode richness compared to the control in September 2017, compared to both control and CC in December 2017 and compared to CC in June 2018 ($p < 0.05$). In addition, nematode richness was lower in the warming treatment than in the control in March 2018 ($p = 0.058$) and June 2018 ($p = 0.095$). Nematode richness was significantly lower in control than in CC during the first sampling, May 2017 ($p < 0.05$) (Fig. 4.3).

The soil warming effect on nematode abundance and richness of each trophic group was analyzed at each time point. Soil warming did not have a significant effect on abundance of bacterial feeders and plant feeders ($p > 0.05$). Soil warming significantly

reduced the abundance of fungal feeders, predators and omnivores in the warming treatment compared to the control and abundance of omnivores was lower in CC than in control ($p < 0.05$) in the September 2017 sampling (Fig. 4.4). Similarly, the richness of bacterial feeders, fungal feeders and omnivores was significantly lower in warming treatment than in control during September 2017 ($p < 0.05$). Additionally, soil warming significantly reduced the richness of fungal feeders compared to both controls ($p < 0.05$) during March 2018. The richness of plant feeders was lower in the warming treatment than in CC ($p < 0.05$) during December 2017 and lower than both controls ($p < 0.05$) in June 2018. Additionally, richness of plant feeders significantly differed between control and CC at the initial sampling, May 2017 ($p < 0.05$). The richness of predators was lower in the warming than in CC ($p < 0.05$) during June 2018 (Fig. 4.5).

The effect of soil warming on abundance and richness of each c-p class was also analyzed at each time point. The abundance and richness of c-p 2 and c-p 3; and richness of c-p 5 nematodes were not significantly altered by soil warming ($p > 0.05$). The abundance of nematodes of c-p 1 was lower in warming than in CC ($p < 0.05$) in September 2017 and the richness of c-p 1 nematodes was lower in the control than in the warming treatment ($p < 0.05$) in the first sampling. Soil warming reduced the abundance and richness of c-p 4 and abundance of c-p 5 nematodes compared to the control ($p < 0.05$) during September 2017. Moreover, soil warming resulted in significant decrease of richness of c-p 4 nematodes compared to CC in December 2017 (Fig. 4.6 and 4.7)

Forest site:

Although seasonal fluctuations had significant effects on nematode richness ($p <$ 0.05), soil warming and the interaction between treatment and time did not affect both nematode abundance and richness ($p > 0.05$) (Fig. 4.8 and 4.9).

Unlike in the agricultural site, soil warming in forest site did not affect the abundance of nematodes belonging to lower (bacterial, fungal and plant feeders) and higher (predators and omnivores) hierarchy levels of the soil food web ($p > 0.05$) at any sampling time except for the abundance of fungal feeders. Fungal feeding nematode numbers were lower in the control than in the CC and warming treatments in the last sampling, June 2018 ($p < 0.05$) (Fig. 4.10). Furthermore, the richness of bacterial feeders and omnivores was not significantly affected by soil warming ($p > 0.05$). The richness of fungal feeders was lower in CC than in control and warming only in the last sampling, June 2018 ($p < 0.05$). The richness of plant feeders was lower in CC than in the other treatments during the initial sampling ($p < 0.05$). The richness of predators was significantly lower in the warming treatment than in the control at initial sampling and during December 2017 ($p < 0.05$); these significant differences were not consistent at all sampling periods (Fig. 4.11).

The abundance of nematodes belonging to c-p 1, c-p 2, c-p 3 and c-p 5 and richness of nematodes of c-p 1 and c-p 3 were not significantly affected by soil warming ($p > 0.05$). During the initial sampling, the abundance and richness of c-p 4 nematodes were significantly lower in the warming treatment than in the controls ($p < 0.05$) but these differences were not apparent during subsequent sampling. The richness of c-p 2 nematodes was significantly lower in the warming treatment than in the control during

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September 2017 ($p < 0.05$). The richness of c-p 2 nematodes was lower in control and warming treatments than in CC and, richness of c-p 5 nematodes was lower in CC and warming treatments than in the control during December 2017 (Fig. 4.12 and 4.13).

Discussion

In the present study, we simulated global warming to investigate the response of nematode communities in undisturbed forest and disturbed agriculture ecosystems to future increase in soil temperature by 5 ^{o}C using heating cables. To account for potential physical disturbances occurred during installation of heating cables, a CC treatment was included in this experiment at both the ecosystems. Although significant differences at the initial sampling were observed between control and CC for a very few groups, these differences were not evident in later samplings, which indicate that there was no real effect of physical disturbances on nematode communities.

The results from the experiment conducted in the agriculture site revealed that nematode abundance was not significantly affected by soil warming. However, a declining trend of nematode abundance was observed consistently in warming treatment compared to control and CC treatments in most of the samplings. This observed declining trend was not statistically significant due to large standard error, which could be reduced with a higher number of replications or with the prolongation of the experiment. On the other hand, richness of nematodes was significantly lowered in the warming treatment compared to control in all samplings except in March 2017. Even though the difference was not statistically significant during March 2017, richness of nematodes was lower in the

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warming treatment than control and CC treatments (Fig. 4.3). Results from previous studies also supported that soil warming reduced nematode communitites (Simmons et al., 2009; Thakur et al., 2017; Yan et al., 2017). On the other hand, Sohlenius and Bostrom (1999) reported no effect of warming on nematode communities, which may be due to conducting experiments at different locations along a temperature gradient. Similarly, Dong et al. (2013) did not observe any effect of warming on soil nematode communities, may be due to shorter duration of the experiment and the difference in the soil warming temperature (1.5 ^{o}C) used.

Analyses of the effect of soil warming on nematodes of different trophic groups revealed that soil warming did not have a consistent significant effect on the abundance of nematodes of all trophic groups. However, the abundance of fungal feeders, predators and omnivores was significantly reducing in the warming treatment compared to the control during September 2017 (Fig. 4.4). The richness of nematodes feeding on bacteria and fungi was significantly lower in the warming treatment compared to the control in September 2017 and fungal feeders were also lower in the March 2018 sampling time (Fig. 4.5). Even though, the statistical differences were few, the abundance and richness of bacterial feeders and the abundance of fungal feeders were always lower in the warming treatment compared to control. The lower number of bacterial and fungal feeding nematodes in the warming treatment is indicate that microbial population on which these nematodes feed may not increase at lower warming temperatures. Similarly, Frey et al. (2013) observed higher microbial efficiency in a control compared to a warming treatment at low

temperatures (<10 °C) in a long-term experiment. In contrast, some of the studies have found that soil warming resulted in increased microbial feeding nematodes due to an upsurge in microbial biomass (Song et al., 2014; Muller et al., 2016). The richness of plant feeders and predators was also lowered by soil warming in the warming treatment compared to control, which is consistent with the findings of Song et al. (2014) and Muller et al. (2016). Additionally, the richness of omnivores was always lower in the warming treatment than in control and CC except in the last sampling. The lower richness of predators and omnivores indicates that nematodes that belong to these two groups are sensitive to disturbances (Bongers, 1990 and Ferris et al., 2001). Moreover, the significant reduction of abundance of fungal feeders, predators and omnivores and richness of bacterial feeders, fungal feeders and omnivores especially in September 2017, was due to the highest average temperatures occuring during July and August 2017 (Fig. 4.1) to which the nematodes at this site were never exposed before the experiment.

Analysis of nematode communities based on c-p class categorization indicated that similar to trophic groups, abundance of nematodes belonging to all c-p classes was not consistently affected by soil warming. Likewise, soil warming did not influence the richness of nematodes belonging to c-p 1 and c-p 2 classes. Most of the nematodes in c-p 1 and c-p 2 classes are bacterial and fungal feeders, whose richness was not affected by soil warming. It is well known that c-p 1 and c-p 2 class nematodes are tolerant to environmental disturbances (Bongers, 1990 and Ferris et al., 2001). Higher c-p class (c-p 3, c-p 4 and c-p 5) nematodes, which are sensitive to disturbances, had reduced richness were lower in the

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warming treatment compared to the controls. However, the significant reduction of abundance of c-p 1, c-p 4 and c-p 5 and richness of c-p 4 in September 2017 sampling was due to the highest average temperatures during July and August 2017. Although, the effect of soil warming on richness of nematode communities was not consistently significant at all sampling times, a declining trend was observed, which perhaps would become more consistent become consistent with the prolongation of the experiment.

Unlike in the agricultural ecosystem, nematode communities in the forest ecosystem responded differently to the increase in soil temperature. The nematode abundance and richness were not consistently affected by soil warming. Additionally, the abundance and richness of nematodes of all trophic groups and c-p classes were neither significantly affected by soil warming nor followed any pattern, indicating that nematode communities may be more resilient to temperature changes in the forest ecosystem compared to agricultural ecosystem.

Conclusion

A one-year in-situ soil warming experiment was conducted in a previously disturbed agricultural ecosystem and an undisturbed forest ecosystem to forecast the effect of global warming on nematode communities, which are considered as indicators of environmental disturbances and their consequences on structure and function of soil food webs. Increase in soil temperature reduced nematode richness and abundance in the agricultural ecosystem. On the other hand, nematode abundance and richness were not influenced by soil warming in the forest ecosystem. Warming reduced the richness of all

trophic groups and richness of higher c-p classes in the warming treatment compared to the control in the agricultural ecosystem but did not affect nematodes in the forest ecosystem. In addition, warming during the highest temperature months of the year resulted in significant reduction of all trophic groups except plant feeders especially in the agricultural ecosystem. Although the effects of soil warming on richness of nematode communities was not consistently significant at all sampling times, a declining trend was observed, which perhaps would become consistent with the prolongation of the experiment. Overall, the results from our research indicate that nematode communities in the forest ecosystem may be more resilient to environmental fluctuations than those in agricultural ecosystems.

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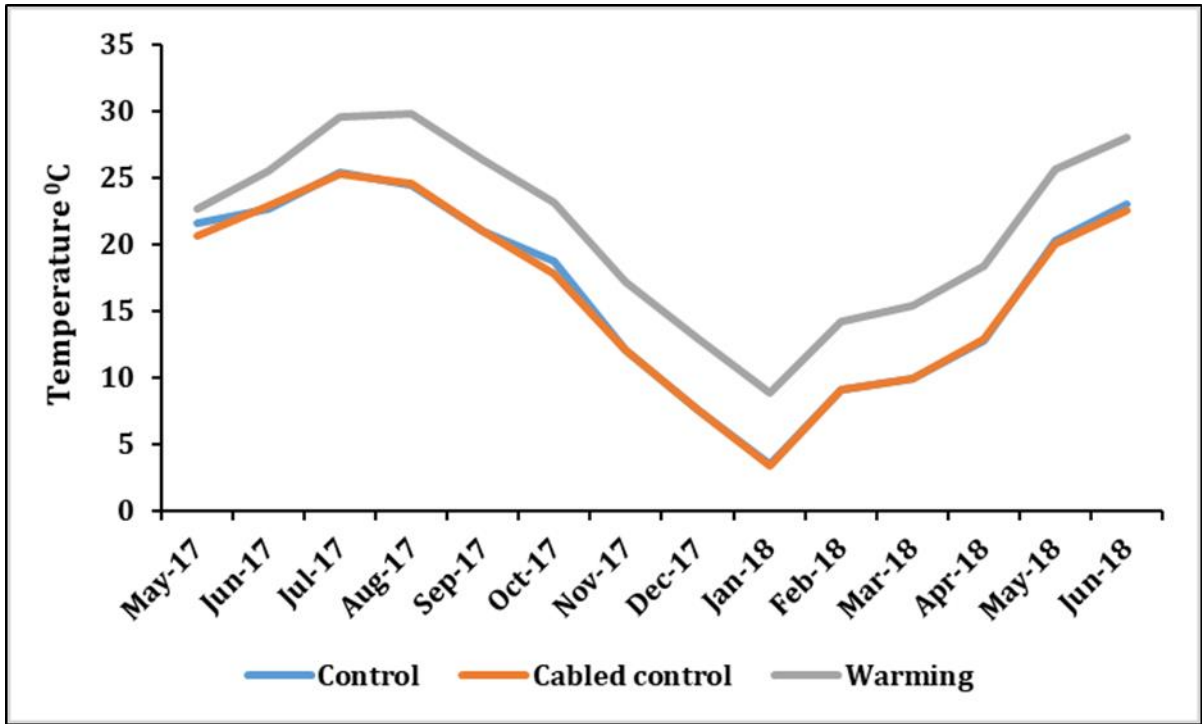
Appendix 4

Fig. 4.1. Maintenance of soil temperature. Elevation of average soil temperature to 5 °C in warming (W) plot compared to cabled control (CC) and control (c) plots at 20 cm depth.
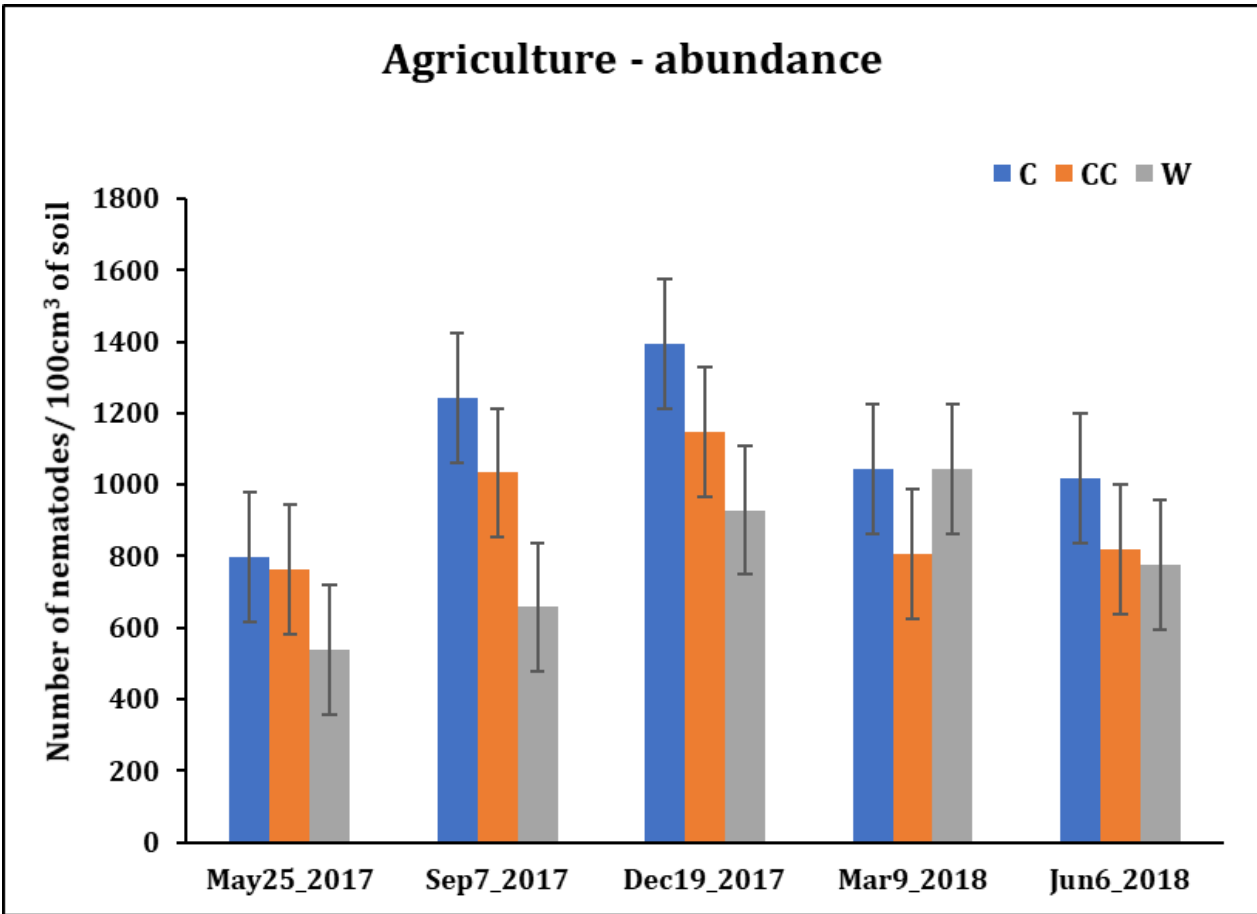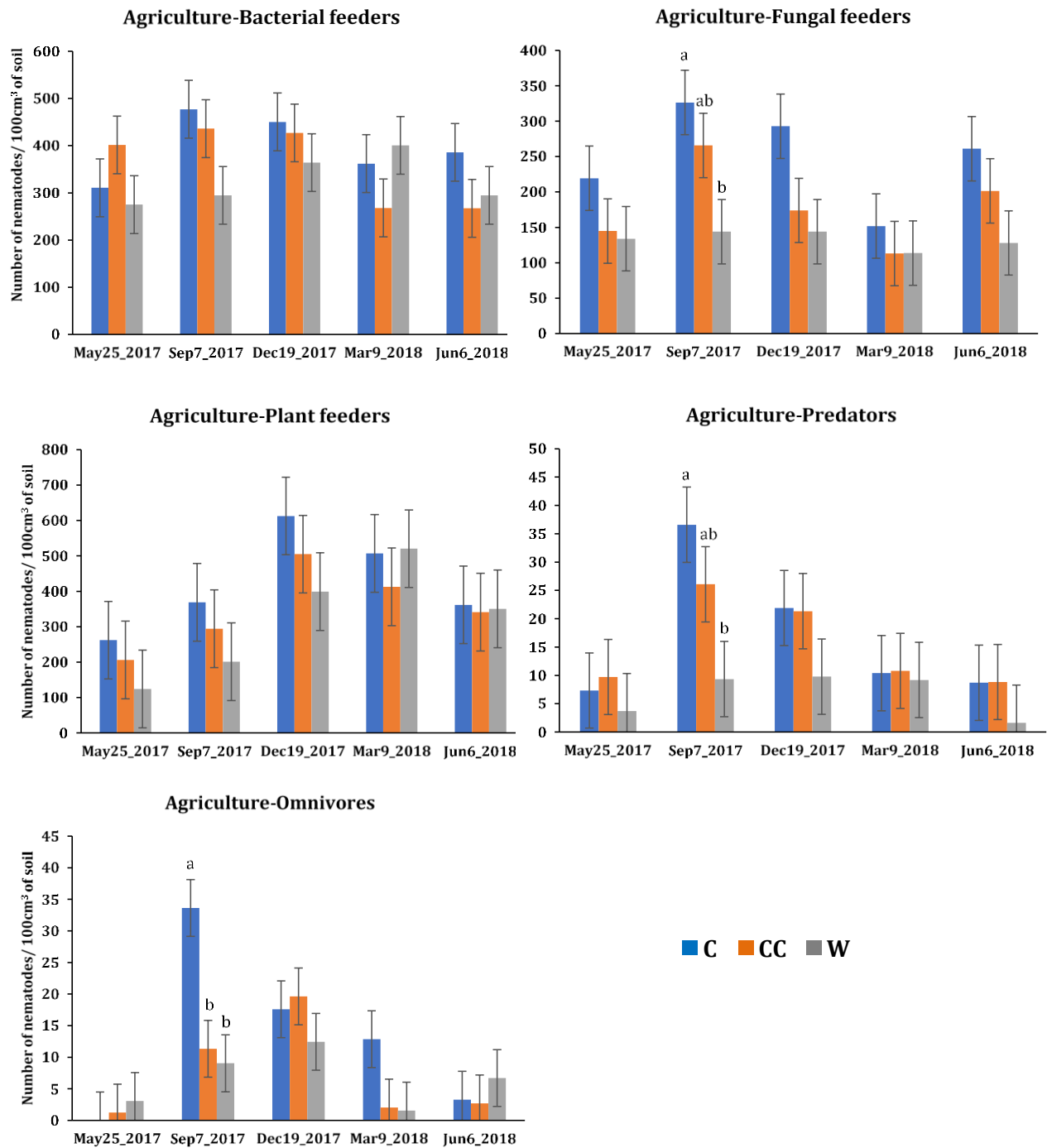
Fig. 4.2. Effect of soil warming on genus-level nematode abundance in agricultural ecosystem. Bars indicating number of nematodes (mean±SE) per 100 cm^3 of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).
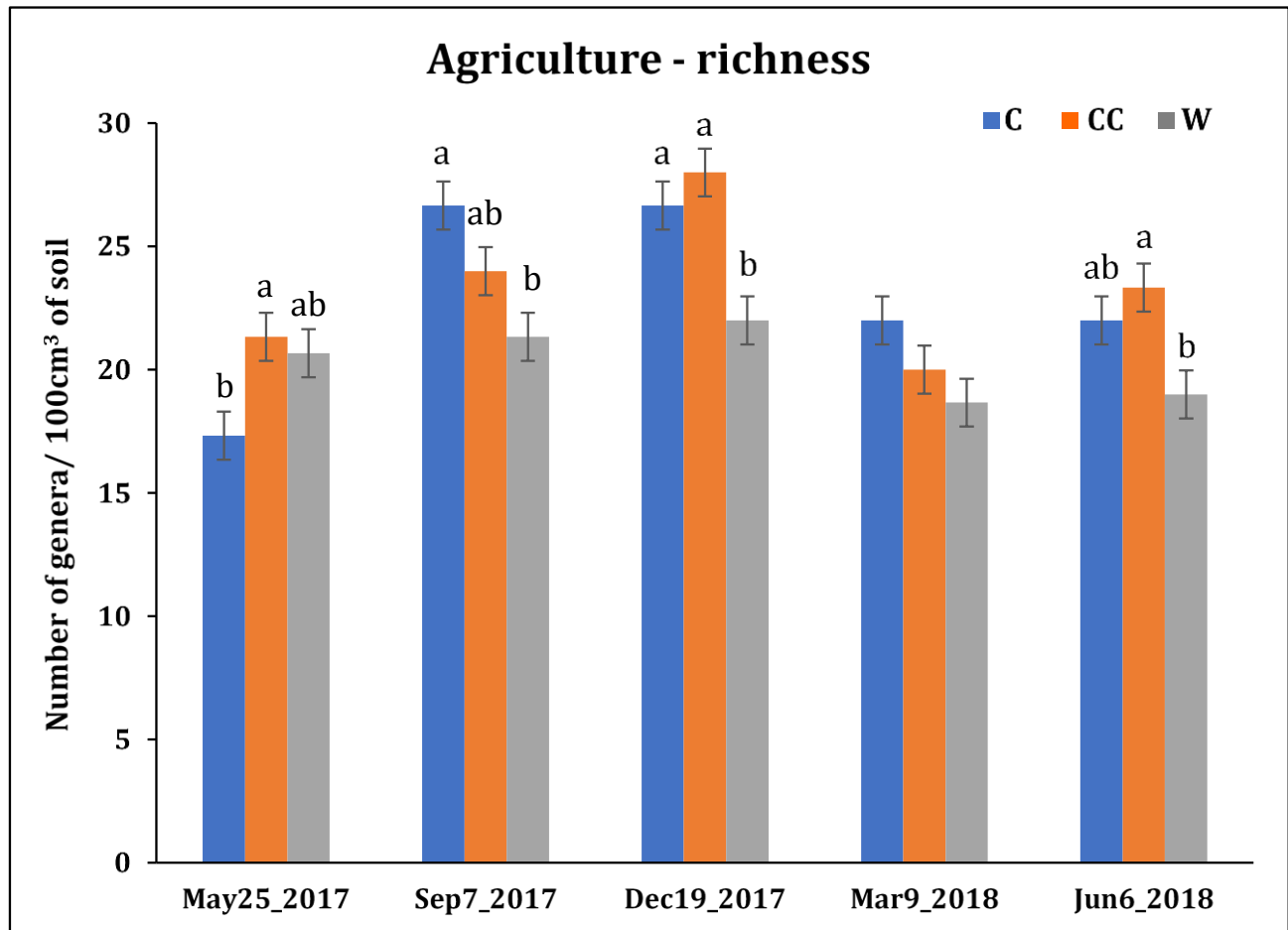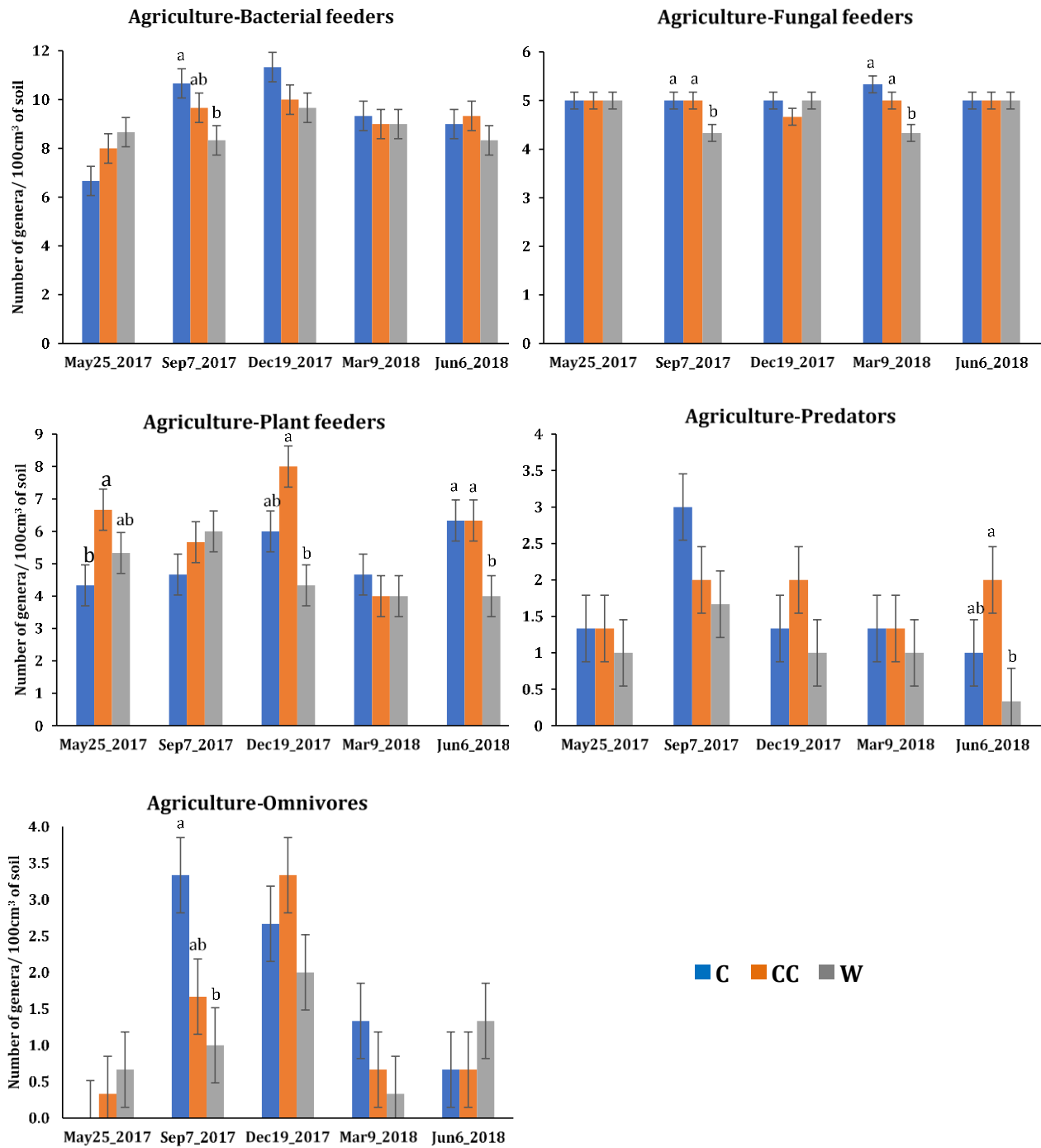
Fig. 4.3. Effect of soil warming on genus-level nematode richness in agricultural ecosystem. Bars indicating number of genera (mean±SE) per 100 cm^3 of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).
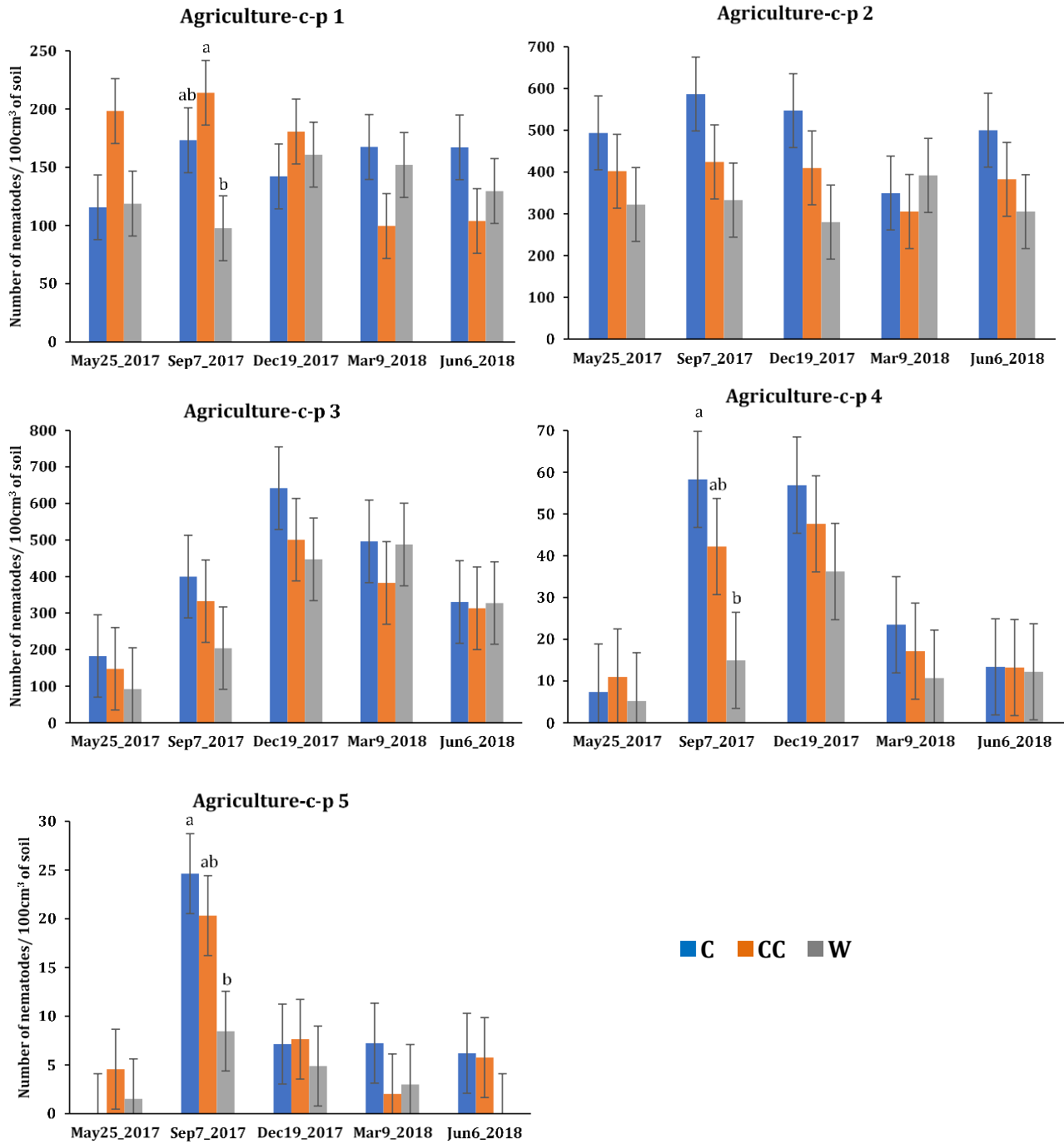
Fig. 4.4. Effect of soil warming on genus-level nematode abundance of each trophic group in agricultural ecosystem. Bars indicating number of nematodes (mean±SE) per 100 cm^3 of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).

Fig. 4.5. Effect of soil warming on genus-level nematode richness of each trophic group in agricultural ecosystem. Bars indicating number of genera (mean±SE) per 100 cm³ of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).
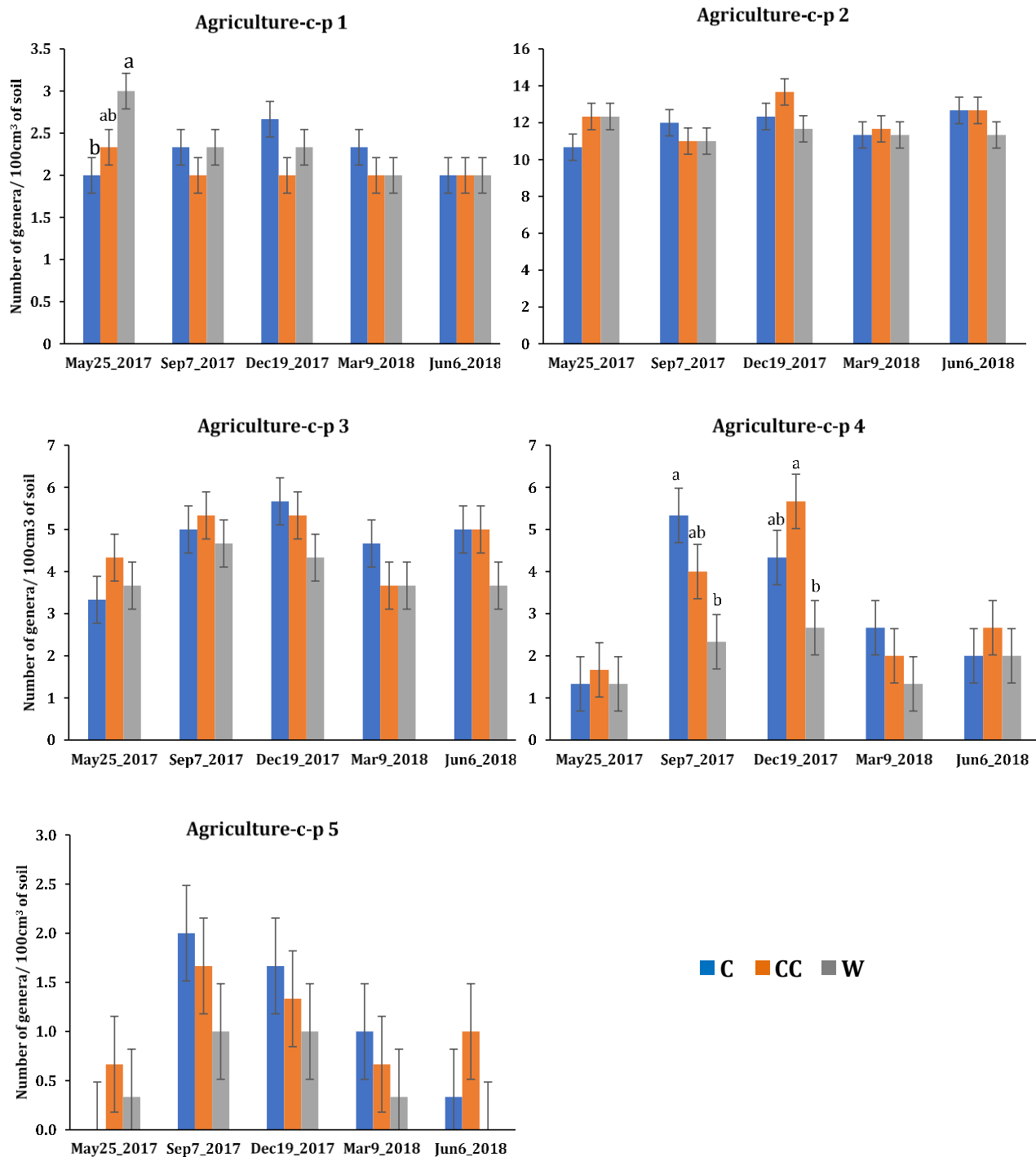
Fig. 4.6. Effect of soil warming on genus-level nematode abundance of each c-p class in agricultural ecosystem. Bars indicating number of nematodes (mean±SE) per 100 cm^3 of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).
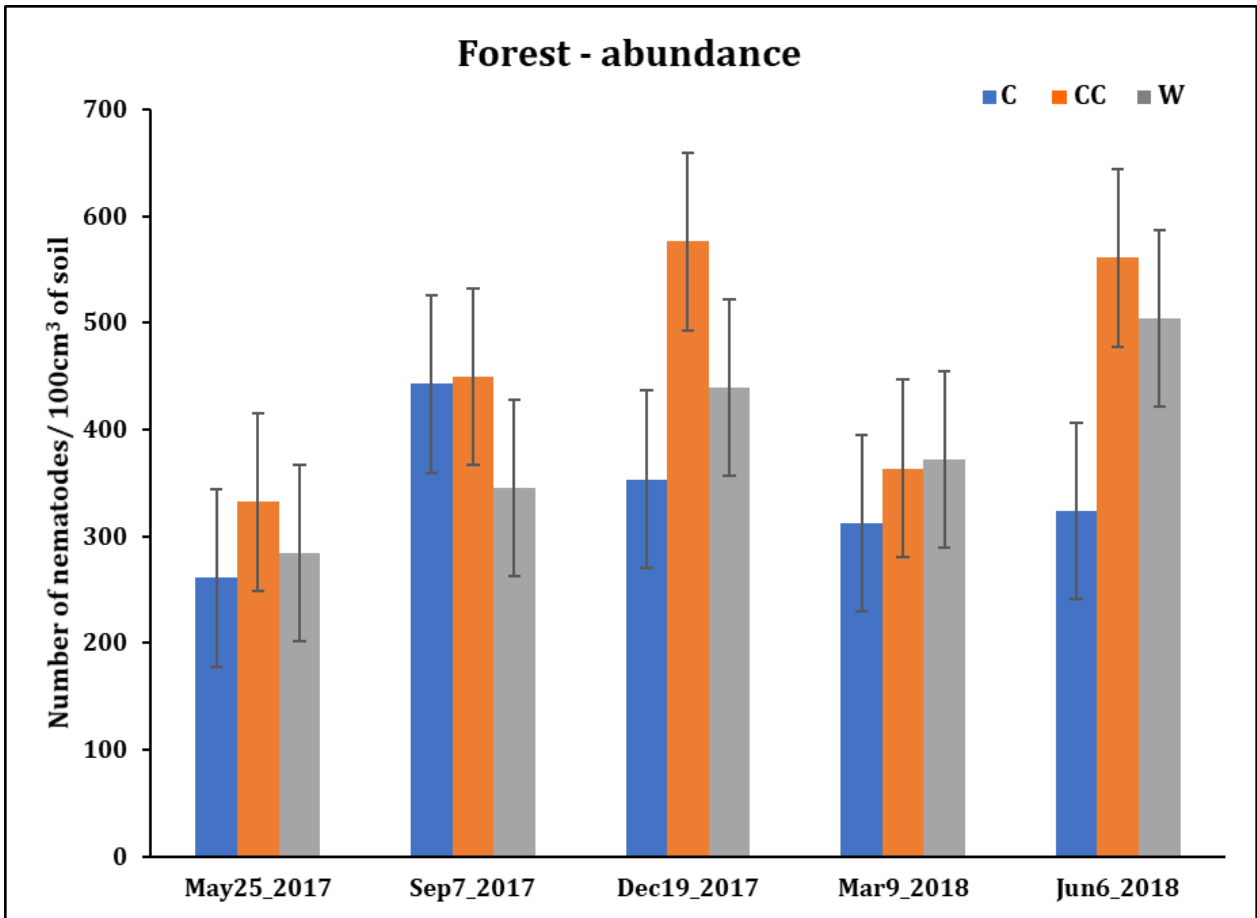
Fig. 4.7. Effect of soil warming on genus-level nematode richness of each c-p class in agricultural ecosystem. Bars indicating number of genera (mean±SE) per 100 cm³ of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).
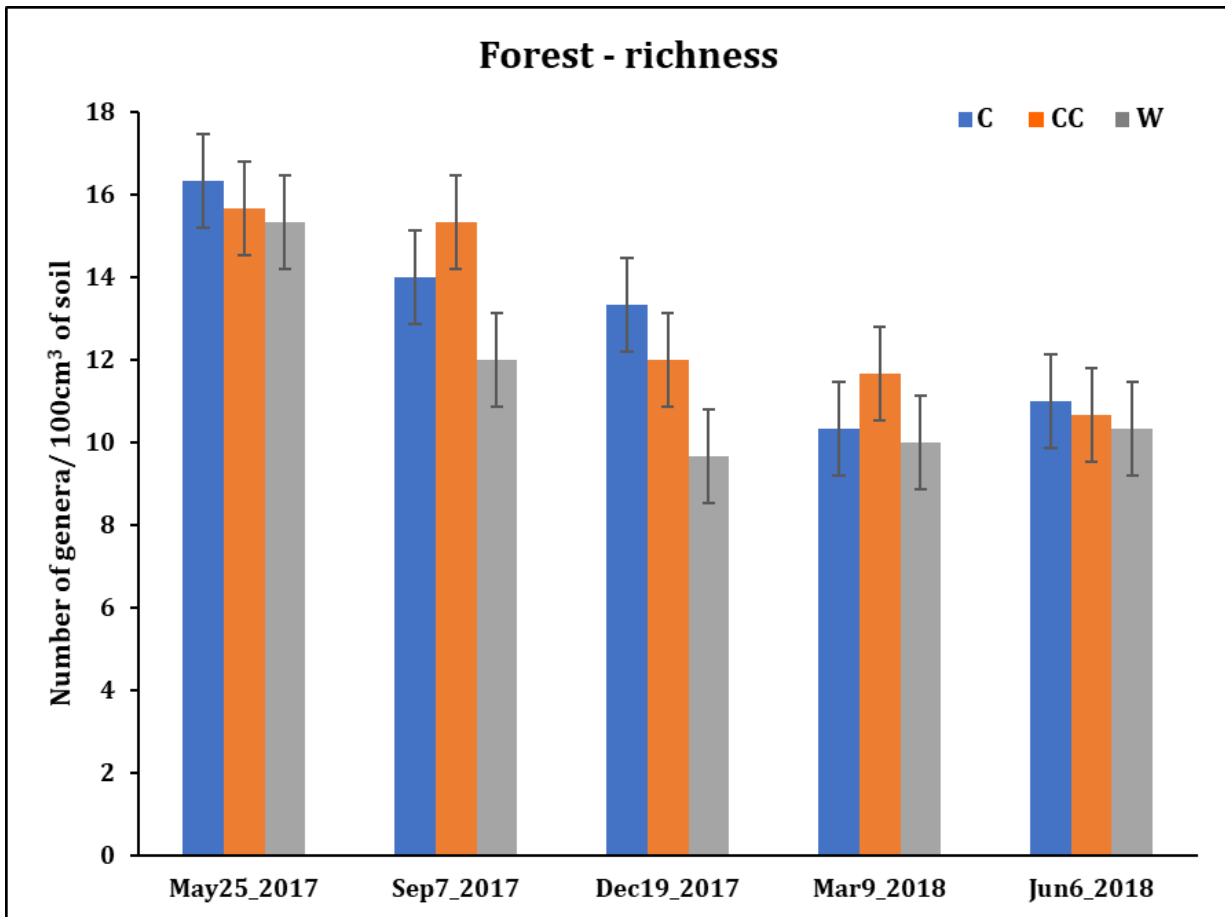
Fig. 4.8. Effect of soil warming on genus-level nematode abundance in forest ecosystem. Bars indicating number of nematodes (mean±SE) per 100 cm³ of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).

Fig. 4.9. Effect of soil warming on genus-level nematode richness in forest ecosystem. Bars indicating number of genera (mean±SE) per 100 cm³ of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).
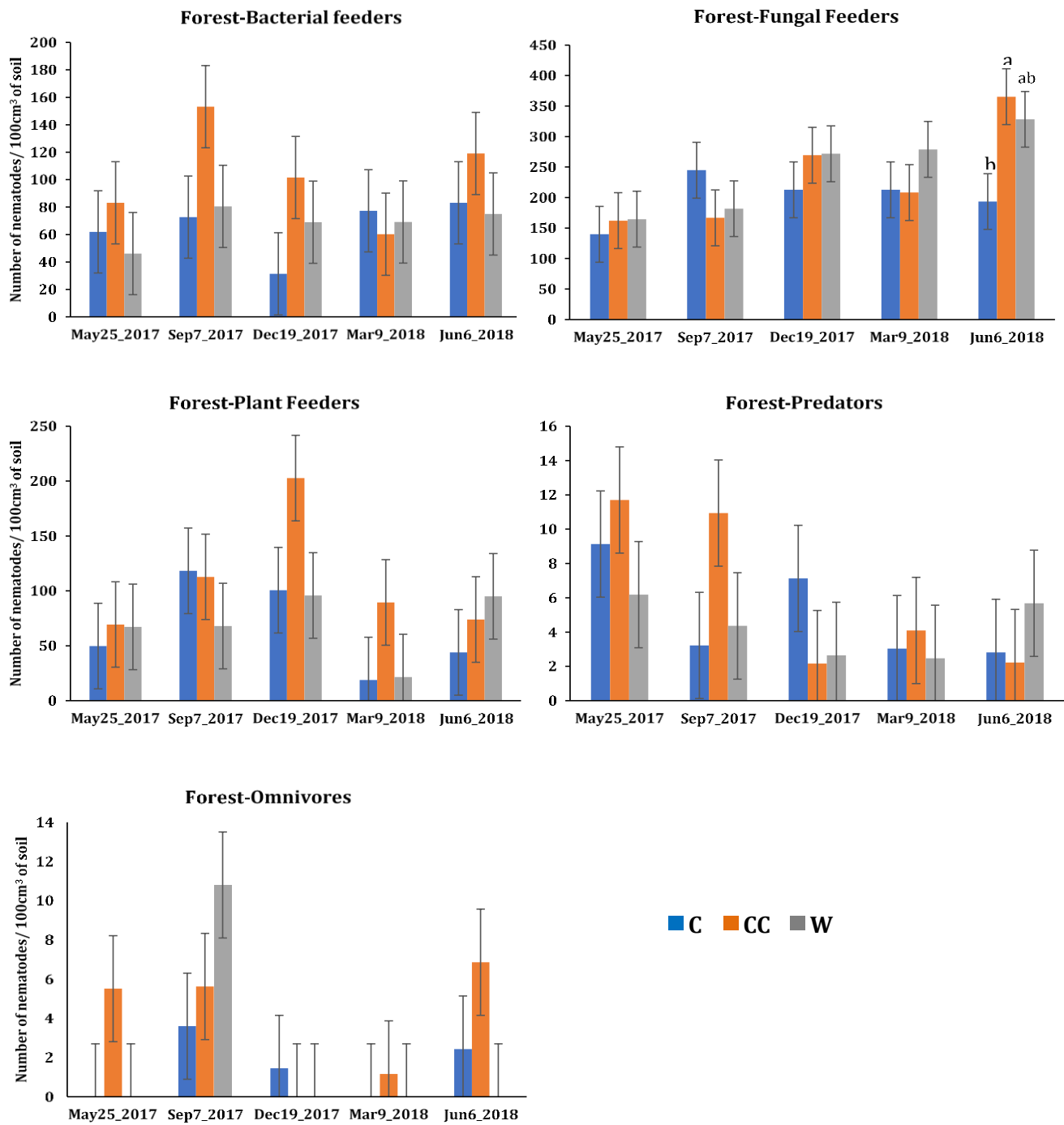
Fig. 4.10. Effect of soil warming on genus-level nematode abundance of each trophic group in forest ecosystem. Bars indicating number of nematodes (mean±SE) per 100 cm^3 of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).
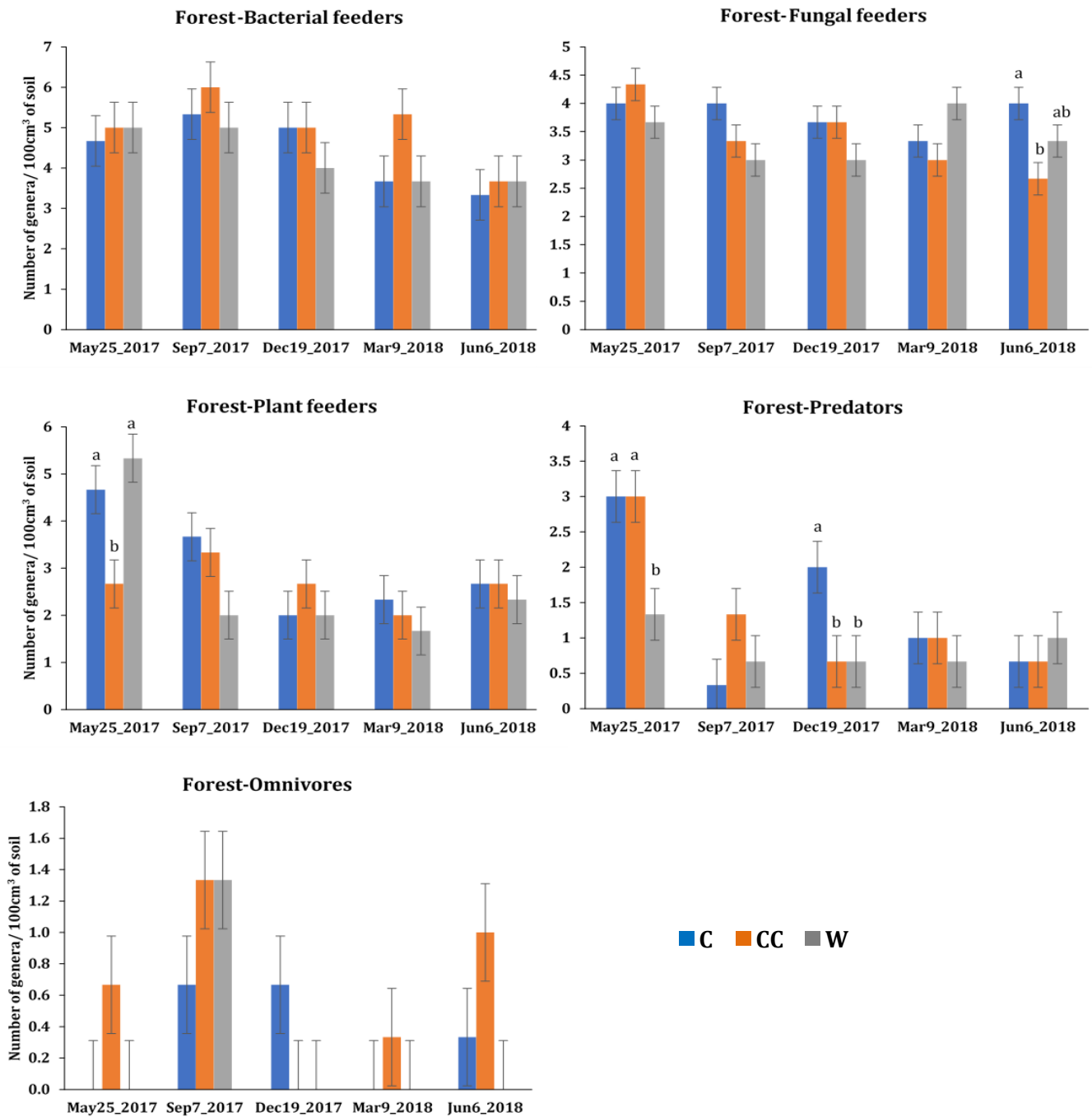
Fig. 4.11. Effect of soil warming on genus-level nematode richness of each trophic group in forest ecosystem. Bars indicating number of genera (mean±SE) per 100 cm³ of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).
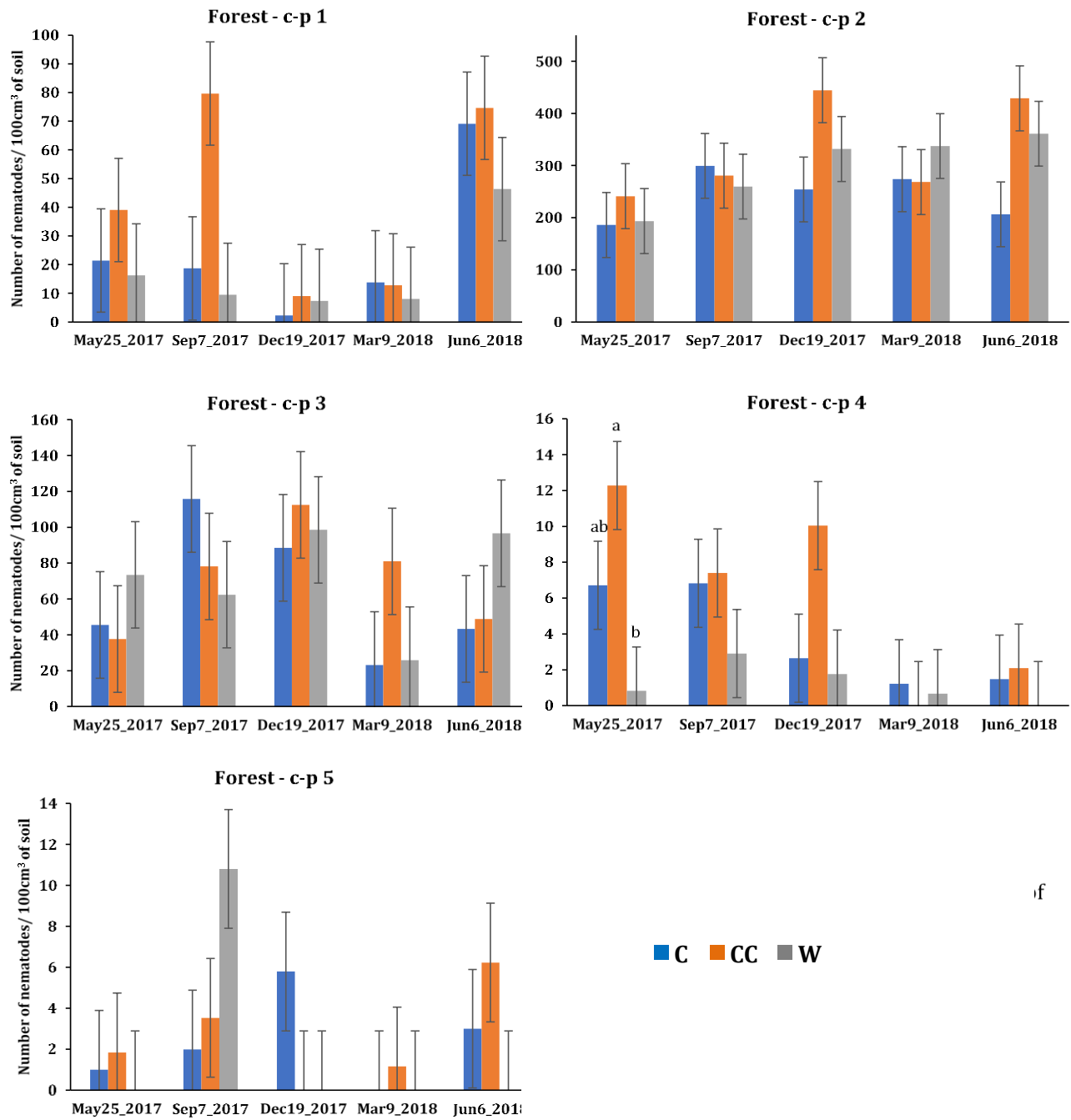
Fig. 4.12. Effect of soil warming on genus-level nematode abundance of each c-p class in forest ecosystem. Bars indicating number of nematodes (mean±SE) per 100 cm^3 of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at $P < 0.05$ (Tukey-LSD test).
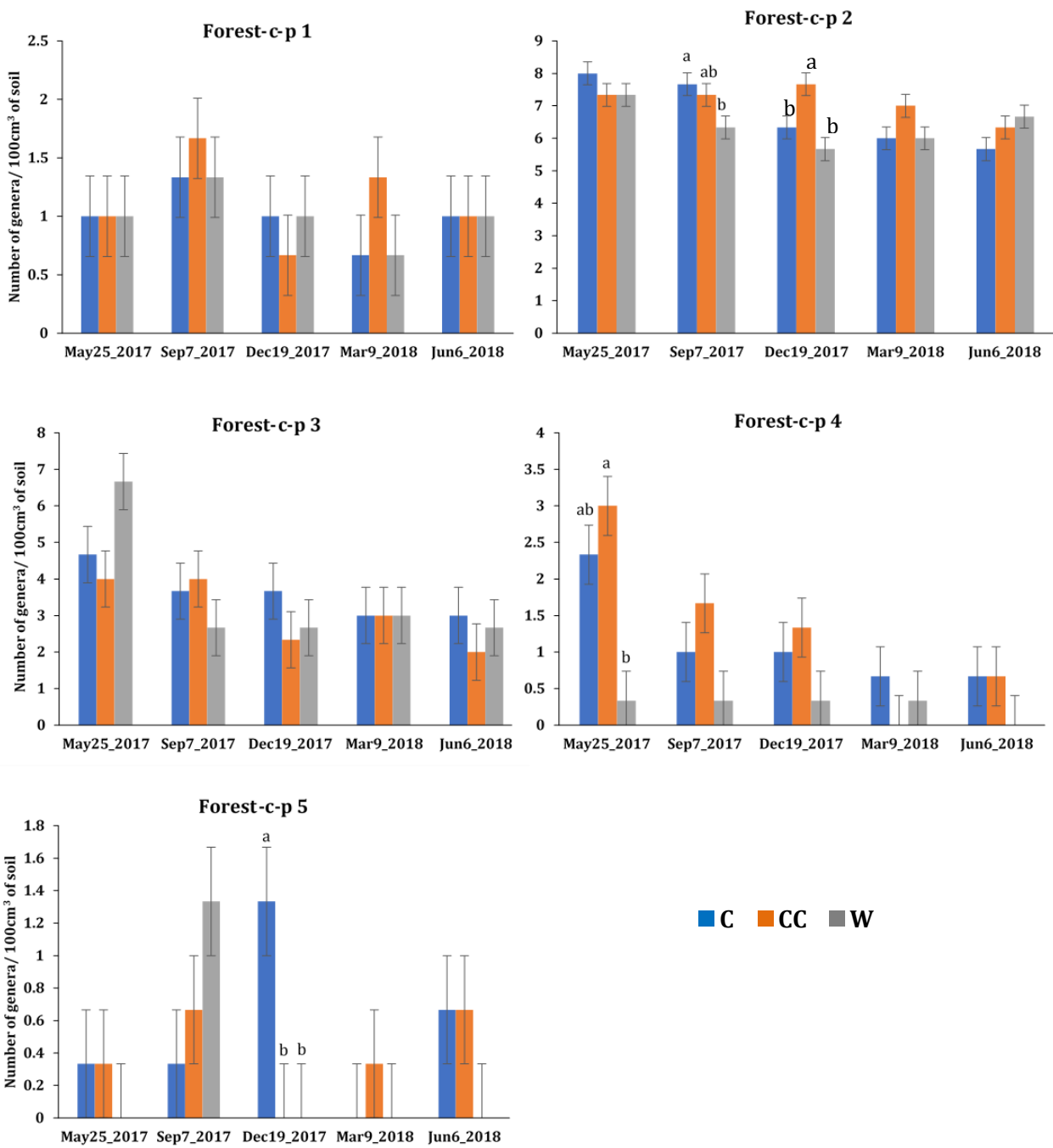
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Fig. 4.13. Effect of soil warming on genus-level nematode richness of each c-p class in forest ecosystem. Bars indicating number of genera (mean±SE) per 100 cm^3 of soil in control (C), cabled control (CC) and warming (W) treatments at each sampling time. Letters indicate significant differences among treatments at each sampling time at P < 0.05 (Tukey-LSD test).

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Chapter 5

General conclusions

Conclusion

The response of nematode food webs to human-induced disturbances were evaluated. In the first objective, comprehensive meta-analyses of distinct ecosystems with different schemes of human intervention from 111 publications, using random-effects model and non-parametric variance, confirmed that nematode richness was higher in least-disturbed ecosystems (forest and Natural grassland) than in more disturbed ecosystems (agriculture, Disturbed grassland, and urban ecosystems). Nematode abundance was not reduced by human interventions, consistent with general findings from previous works in the field of nematode ecology.

In the second objective, the effect of tillage in terms of increasing levels of physical disturbance on nematode communities in an undisturbed forest ecosystem indicated that tillage reduced the nematode communities, which was consistent with the studies conducted in agricultural ecosystems. However, in this study microbe-feeding nematodes responded differently compared to that of agricultural ecosystem. Tillage reduced the bacterial feeding nematodes and did not affect the fungal feeding nematodes. The effect of increasing levels of disturbance revealed that the rototill significantly reduced the nematode communities compared to control and SLR treatments but the differences between control and removal of litter and vegetation on nematode communities were not statistically significant. Similarly, intensity of rototilling (every two months and two weeks) did not significantly different. Still, there was a declining trend of nematode communities

with increasing levels of physical disturbance. This trend could potentially become statistically significant with the prolongation of experiment for longer period.

In the third objective, a one-year in-situ soil warming experiment was conducted in a previously disturbed agricultural ecosystem and an undisturbed forest ecosystem to forecast the effect of global warming on nematode communities, which are considered as indicators of environmental disturbances and their consequences on structure and function of soil food webs. Increase in soil temperature reduced nematode richness and abundance in the agricultural ecosystem. On the other hand, nematode abundance and richness were not influenced by soil warming in the forest ecosystem. Warming reduced the richness of all trophic groups and richness of higher c-p classes in the warming treatment compared to the control in the agricultural ecosystem but did not affect nematodes in the forest ecosystem. In addition, warming during the highest temperature months of the year resulted in significant reduction of all trophic groups except plant feeders especially in the agricultural ecosystem. Although the effect of soil warming on richness of nematode communities was not consistently significant at all sampling times, a declining trend was observed, which perhaps would become consistent with the prolongation of the experiment. Overall, the results from our research indicate that nematode communities in the forest ecosystem may be more resilient to environmental fluctuations than those in agricultural ecosystems. Overall, our research strengthens the concept that human interventions adversely impact nematode richness, which is crucial for the maintenance of the full suite of ecosystem services provided by soil food webs.

Vita

Satyendra Pothula is from India and graduated with a B.Sc. degree in Agricultural sciences and M.Sc. degree in Statistics and Mathematics from the Acharya N. G. Ranga Agricultural University, Hyderabad, India. Upon graduation, he was accepted into the Indian bank as an assistant manager and served 2 years in India. Later, he was accepted into a PhD program at the University of Tennessee at Knoxville under the guidance of Dr. Ernest C. Bernard in Entomology, Plant Pathology and Nematology.