DISSERTATION

SOIL NEMATODE COMMUNITY RESPONSE TO CLIMATE CHANGE AND ASSOCIATED ALTERATIONS TO PRECIPITATION AND VEGETATION

Submitted by

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ABSTRACT

SOIL NEMATODE COMMUNITY RESPONSE TO CLIMATE CHANGE AND ASSOCIATED ALTERATIONS TO PRECIPITATION AND VEGETATION

Understanding of the belowground grassland response to climate change is much more limited than aboveground responses. This disparity in knowledge is partially due to the vast diversity in species in belowground ecosystems and the overwhelming task of identifying the roles and processes associated with each. Soil nematodes represent the most abundant soil fauna on earth and are exceptional in that they occupy every trophic level, contain multiple life history strategies, and are relatively easy to extract and identify from soil. Moreover, nematode activity (e.g. feeding) directly regulates the size and function of fungal and bacterial populations thus indirectly impacting the rates of carbon and nitrogen turnover. Determining the abundance of each nematode genera in a soil sample can allow for calculation of ecological indices that can further explore the trophic complexity, energy pathways, and both the sensitivity and resilience of soil nematode communities to stress and disturbance. Therefore studying soil nematode communities provides a means for gaining important insights about poorly understood belowground responses to altered environments.

The aim of this dissertation is to expand our knowledge of soil community dynamics in grasslands in the face of extreme precipitation changes and possible vegetation shifts. In the first chapter of this dissertation, I introduce the importance of grassland ecosystems and the challenges looming from climate change. Next I highlight the two scenarios in which my research is based and give the details on how utilizing nematode data can answer these questions. The second chapter of this dissertation addresses the question: Can nematode trophic analysis reveal associations between vegetation cover types? This study revealed striking differences in the abundances of fungivores and the combined omnivore/predator trophic groups found under the dominant grass compared to both an invasive forb and bare soil cover types.

In the third chapter a focus on the most well-studied nematode trophic group; plant parasitic nematodes (PPN) sought to determine if different feeding strategies lead to distinct responses in precipitation treatments across three grassland sites. This research aimed to understand if host plants will have an increased burden harboring greater PPN populations along with increased water stress. Our results showed that the response of PPN feeding type abundance, functional guild, and herbivory index to precipitation was site dependent, a finding not previously studied.

Building on the findings of Chapter 3, Chapter 4 utilized the entire soil nematode community and calculated indices to see how the different grassland types; arid, semiarid, and mesic would respond to the same precipitation treatments. Specifically, I tested if nematodes would be effective indicators of the soil community to changes in rainfall events. The results of this study showed the importance of genera level resolution and suggests that the sensitivity of these indices allows for ecological interpretation of belowground function and status in a natural setting. A finding that is especially pertinent, as these grasslands will not respond to precipitation alterations similarly and will therefore require unique mitigation strategies.

In summary, with both field and laboratory work my PhD project has: 1) found associations between nematode trophic group abundance and vegetation cover types; 2) revealed the different response of grassland ecto-and endoparasitic nematodes to manipulated rainfall across a precipitation gradient; 3) quantified the herbivory index of a PPN population in response to precipitation treatments across three grassland sites; and 4) demonstrated the sensitivity of nematode ecological indices and found indicator genera in three grassland sites with manipulated precipitation treatments. Together these results bolster our knowledge of how soil nematode communities will respond to climate change and highlight their potential role for monitoring and influencing grassland ecosystem dynamics into the future.

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CHAPTER 1: INTRODUCTION

Grasslands account for a quarter of the terrestrial surface and apart from Antarctica occur on every continent (Wilsey 2018). The ecological importance of grasslands cannot be overstated; providing food and forage for humans and livestock, housing biodiversity, supporting a multitude of ecosystem services (Gibson and Newman 2019), and perhaps most importantly sequestering and storing tremendous amounts of carbon belowground (Conant et al. 2017). However despite the vital role of this ecosystem, grassland area is declining while arable land increases due to the continued conversion to croplands (FAO 2015). Nearly a fifth of global grazing lands have been converted for agriculture (Ramankutty et al. 2008) leading to major losses, roughly 60%, of soil carbon stocks (Guo and Gifford 2002). Additionally, grasslands are threatened by climate change, where models predict large variation in the overall amount and frequency of extreme precipitation events (Melillo et al. 2013). In the Central Plains of the United States, there is likely to be an increase in the frequency of drought and intensity of rainfall events leading to synergistic stresses for plant life. Altered precipitation is not the only climate induced threat, the Front range of Colorado has been the center of many global change studies; experiments focused on temperature (Ray et al. 2008) as well as alterations to N deposition(Baron et al. 2000), and elevated carbon dioxide (Morgan et al. 2007). These combined ecological threats have also led to shifts in plant communities (Seastedt et al. 2008, Beals et al. 2014) with little understanding of the effects of ecosystem functioning that may coincide with the expansion of invasive species and decline of dominant grasses.

The response of grasslands to climate change has been well studied aboveground; namely the response of vegetation to altered precipitation (Fay et al. 2003, Heisler-White et al. 2008, Notaro et al. 2010, Felton et al. 2020), but less focus has been devoted to grasslands soil or their diverse biota. Soils not only sequester carbon but they house a wide range of belowground organisms responsible for a suite of ecological services including disease suppression, decomposition, and nutrient cycling (Wall et al. 2015, Bender et al. 2016, Bach et al. 2020). Few studies have been devoted to testing the effects of altered precipitation on soil fauna and those that have (Landesman et al. 2011, Siebert et al. 2019) do not consider the potential variability of responses across different ecosystems.

Soil nematodes are an ideal animal to focus on as they are the most abundant soil fauna on earth (van den Hoogen et al. 2019, 2020), they are unique to other biota in that they occupy multiple trophic levels, encompass many life history strategies allowing for differential sensitivity to stress and disturbance (Pattison et al. 2005, Ney et al. 2019), and can be relatively easily extracted and identified from the soil. The activity of certain nematode trophic groups, such as fungivores and bacterivores also indirectly impact the rates of carbon turnover in the soil by control of soil microbes. The abundance of each nematode genera in a given community can also be transformed into ecological indexes (Bongers 1990, Ferris et al. 2001), allowing for an assessment of trophic complexity, energy/C pathways, as well as the sensitivity and resilience of soil communities as grasslands adapt to shifts in climate. Soil nematodes are also moisture dependent organisms, and their populations will change directly due to alterations in the soil water content as their movement is dependent on soil water films (Pan et al. 2016, Olatunji et al. 2019).

The aim of my PhD work has been to answer the following questions: 1) Can nematodes effectively be used as indicators of soil communities to altered precipitation across three grasslands placed along an aridity gradient? 2) Will different feeding strategies employed by plant parasitic nematodes uniformly respond to altered precipitation across three unique grasslands? 3)In a single grassland site will the nematode trophic community differ significantly between vegetation cover types indicative of possible climate change scenarios?

Climate change will reshape grassland ecosystems with warming, shifts in vegetation communities and more erratic precipitation, among other factors. Few experiments have adequately focused on the belowground response to such environmental shifts and even fewer have done so in field conditions that can accurately simulate future scenarios. Utilizing nematode functional and trophic groupings can help measure the direct and indirect effects of key climate change impacts allowing for improved understanding of the response of the soil community at large.

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CHAPTER 2: NEMATODE COMMUNITIES ASSOCIATED WITH DIFFERENT VEGETATION COVERS IN A SEMIARID GRASSLAND

2.1 SUMMARY

Nematode-plant interactions and how they respond to global change remains understudied, especially in natural systems. In the western US, range expansion by invasive plant species is an increasing threat in arid and semiarid ecosystems. Transitions toward increasing forbs and bare soil at the expense of native grasses have been documented in the shortgrass prairie of Colorado. A reduction in the dominant grass, *Bouteloua gracilis* or shift toward more invasive species or bare soil could impact belowground processes and therefore grassland stability. We studied the soil nematode community under three different vegetation cover types: *B. gracilis*, *Erodium cicutarium*, and bare soil in the shortgrass prairie. We examined nematode soil food webs under these vegetation covers by analyzing individual nematode trophic group abundances and the fungal-bacteria ratio. While the overall nematode abundance did not differ between types of vegetation cover, fungivores were 5-fold more abundant under grass than under bare soil and 3.4 times greater than under forbs. The combined omnivore/predator abundance trophic group was 3-fold higher, on average, under the native *B. gracilis* than under bare soil, and 3.6 times greater than under forbs. The ratio of fungivores to bacterivores was also found to be higher under *B. gracilis*. These findings suggest that local effects of plant cover types, which may exist in future disturbance scenarios could simplify the nematode community, with implications for decomposition processes and food web stability.

2.2 INTRODUCTION

A crucial consequence of climate change is range expansion by invasive annuals, especially across more arid regions in the western US (Archer et al., 2008; Bradley and Mustard, 2005). While this topic is well studied, few experiments have specifically aimed to show the effects of exotic plants on soil food webs (Belnap and Phillips, 2001; Ehrenfeld et al., 2001). Findings from the shortgrass prairie in the western Great Plains region of North America suggest an alteration of plant communities with transitions toward increasing forbs and bare soil cover at the expense of native grasses (Alward et al., 1999). Different plant types support unique soil organisms and associated food webs (Wardle, 2002), thus shifts in plant community composition associated with invasive species are likely to generate important alterations to soil food webs (Samson et al., 1990). Soil fauna are critical to maintaining the rates and stability of many soil processes (e.g. decomposition, nutrient cycling), and altering the composition and structure of these belowground organisms can have important consequences for ecosystem functioning (Bach et al., 2020; Bardgett and Van Der Putten, 2014; Wall et al., 2015).

Nematodes are the most abundant and functionally diverse soil animals (van den Hoogen et al., 2019) and offer an important link between belowground ecosystem functioning and plant performance aboveground (Wardle et al., 2004). Soil nematodes occupy every trophic level of the soil food web (Bongers and Ferris, 1999), are sensitive to environmental conditions (Neher, 2001), and have a range of life history strategies and life spans, from short-lived (days) to long-lived (months), which has made nematode communities ideal indicators of disturbance (Wilschut and Geisen, 2020; Yeates, 1999). Once extracted from the soil, nematodes can be identified into distinct feeding guilds (Yeates et al., 1993): bacterivores, fungivores, omnivores, predators, and the most studied group, plant parasites (van den Hoogen et al., 2019). Plant parasites are recognized to be particularly important in the context of plant range expansion due to climate warming (Morriën et al., 2012; Viketoft and van der Putten, 2015; Wilschut et al., 2017). Free-living soil nematodes are also extremely valuable to ecosystem function, as they play a vital role in energy transfers and nutrient cycling (Hunt et al., 1987). These nematodes contribute to microbial and faunal turnover, which makes nutrients available to the soil-plant system. Omnivores and predators, in particular, play a critical role in top-down regulation of plant parasites (Bonkowski, 2004; Franco et al., 2019; Thakur and Geisen, 2019). Thus, an analysis of nematode trophic groups and food web structure can provide key insights into the association between vegetation cover, soil biota, and associated processes.

Although *Bouteloua gracilis* dominates the shortgrass steppe (Dufek et al., 2018), variable precipitation characteristic of this region (Milchunas et al., 1994) influences the abundance and distribution of exotic plants and their ability to establish (Milchunas and Lauenroth, 1995). *Erodium cicutarium*, an exotic annual geranium found in disturbed areasclassified as a noxious weed in some states(USDA, NRCS 2010), has been shown to be a strong competitor (Kimball and Schiffman, 2003) that can affect the vegetation structure of arid environments (Klinger and Brooks, 2017), and is a dominant forb in parts of the Colorado High Plains (Concilio et al., 2016). Given the continuing shift in plant communities in the shortgrass steppe in response to climate change, our goal was to understand the belowground implications for nematode trophic groups and associated soil functions. To address this goal, we studied soil nematode communities under different types of vegetation cover in the same semiarid grassland; these included: 1) the dominant grass *B. gracilis,* 2) an invading forb, *E. cicutarium*, and 3) bare soil. We sought to understand the extent to which different types of vegetation cover are associated with different nematode food web structures by examining trophic abundance, fungal-bacterivore ratio, and the overall composition of the nematode community.

2.3 METHODS

<u>*Study site and experimental design.*</u> This research was conducted at the USDA-ARS Central Plains Experimental Range (CPER; lat. 40°40′ N, long. 104°45′ W) in northeastern Colorado. Annual precipitation at the site averages 340 mm, with a markedly wetter period occurring April through August. Mean air temperatures range from 15.6 °C in the summer months to 0.6 °C in winter in this semiarid climate (Del Grosso et al., 2018). Vegetation at the CPER consists mostly of a mixture of warm and cool-season native grasses, native forbs, and a few exotic forbs and grasses that are separated by bare ground patches (Vinton and Burke,

1995). Soils in the region are dominated by fine sandy loams; Aridic Argiustolls (Mollisols) and Ustic Haplargids (Aridisols) formed in alluvium (Morgan et al., 2001).

Soil sampling was conducted in mid-April 2019 in two relatively flat fields, one with the exclusion of cattle for 80 years and the other with moderate grazing for the past 38 years. The two fields were similar in terms of soil texture, pH, and soil organic matter content (Table S1). In each field, vegetation patches were selected with three cover types: 1) the dominant native grass (*B. gracilis*), 2) an exotic forb (*E. cicutarium*), and 3) bare soil. Five patches were selected in each field (grazed and ungrazed) for soil sampling and nematode extraction. Each cover type was at least1 m from the edge of the next cover type, with each patch sampled at roughly 2 m intervals within each field.

Nematode and soil analysis. Nematode samples were taken with a soil corer (2.5 cm diameter) to a depth of 10 cm, as the majority of nematodes are located at this depth (Smolik and Dodd, 1983). Four sub-samples per cover type were collected. The soil corer was cleaned with alcohol wipes between each patch to avoid cross-contamination. Sub-samples collected from each cover type were combined and mixed gently in a plastic bag to form one composite sample per cover type, for a total of 30 samples across the two fields. Soil samples were refrigerated and taken to the lab for nematode extraction the same day. An additional soil sample was taken with a shovel adjacent to each set of cores, also to a depth of 10 cm, for analysis of soil physiochemical properties to explain potential variability observed in nematode communities.

Nematode extraction was performed from 100-g aliquots of the fresh composite sample using Baermann funnels, from which daily samples of 20 ml solution were collected for 72 hours, for a total of 60 ml, and stored at 4°C, according to Hooper (1970). The nematode solution was reduced to 5 ml and poured onto counting dishes for trophic group identification. The total number of nematodes were counted and trophic groups identified based on Yeates et al., (1993) using an inverted microscope (Olympus CKX41, 400X magnification). Nematode abundances were calculated as the number of individuals per kg of dry soil; soil moisture was calculated by drying 50 g of soil from each composite sample at 105°C.

The analysis of key soil chemical and physical parameters was performed using 100 g of soil from the shovel samples previously homogenized, air-dried, and passed through a 2 mm sieve. Soils were extracted with a weak acid (H3A; Haney et al. 2010) analyzed for NH4-N, NO3-N, and PO4-P by flow injection analysis (Lachat QuieChem 8000, Milwaukee, WI, USA) and phosphorus (P), potassium (K), and calcium (Ca) via inductively coupled plasma atomic emission spectroscopy (Thermo Scientific inc., Waltham, MA, USA). Additionally, pH (1:1 v/v) and organic matter via loss-on-ignition method were quantified and soil texture was determined via the hydrometer method (Gee and Bauder, 1979; Ward Laboratories, 2019).

Statistics. Linear mixed model effects with post-hoc Tukey HSD (honestly Significant Difference) test was performed to assess differences between vegetation types for nematode trophic groups and the fungivore to bacterivore ratio (Freckman and Ettema, 1993), with grazing type (i.e., field) considered a random effect (Bates et al., 2015). The data were tested to meet assumptions for homogeneity and normality using visual inspection of predicted plots, Levene's test, QQ plots, Shapiro-Wilk test, and log transformations were applied as needed.

Multivariate comparisons of nematode trophic groups and soil parameters were conducted in order to better understand differences between vegetation types and associations between soil nematode communities and soil physicochemical properties. Nematode trophic groups were related to a constrained set of soil physicochemical variables using Canonical Correspondence Analysis (CCA) from the vegan package (Oksanen et al., 2019). The constrained parameter set was determined by performing a stepwise selection using the 'ordistep' function in vegan where unhelpful and highly correlated variables were removed from the final model. The statistical significance of this relationship was assessed by a permutation test of both the first constrained ordination axis and the combination of the first and second axes. Data visualization was performed with package ggplot2 (Wickham, 2009). All analyses were conducted using R software, version 3.2.4 (R Development Core Team, 2016).

2.4 RESULTS AND DISCUSSION

Overall nematode abundance did not vary among vegetation types, but several trophic groups were found to be highest under *B. gracilis*. Fungivore abundance was much higher under *B. gracilis* (256 ± 73 kg -1 dry soil) than the other cover types while no difference was observed between forbs (*E. cicutarium;* 76 ± 59 kg -1 dry soil) and bare soil (48 ± 17 kg -1 dry soil; p = 0.002; Fig. 1b). The omnivore/predator group was also more abundant under *B. gracilis* (299 ± 77 kg -1 dry soil) while not differing between forbs (83 ± 30 kg -1 dry soil) or bare soil (102 ± 28 kg -1 dry soil; p = 0.011, Fig. 1d). Neither the total amount of bacterivores, nor plant parasite abundance differed between vegetation covers (Fig. 1). The small difference between r2 marginal (i.e., the proportion of variance explained by the moderator variables) and r2 conditional (that of the whole model, including the random effect) indicates that little of the data variability resided in the random effect, i.e. grazed vs. ungrazed fields.

Fungivore species are known to show preferences for different fungal taxa species (Ruess et al., 2000), suggesting that these nematodes could be affected by the mycorrhizal fungal symbionts, pathogens, and saprotrophs associated with each cover type. Pleosporales and other dark septate fungi (DSF) have been found in great abundance in arid grasslands, are associated with mitigating environmental stress, and are common in the rhizosphere of *B. gracilis* (Bell and Wheeler, 1986; Porras-Alfaro et al., 2011). We suspect that the higher fungivore abundance under *B. gracilis* may be related to the dominant fungal groups associated with this species (Porras-Alfaro et al., 2007). Additionally, *E. cicutarium* has been reported to contain specialized metabolites that inhibit microbes which could explain the lowered fungivore abundances (Fecka et al. 2001, Stojanovic-Radic et al. 2010). Our result aligns with a glasshouse study that showed grasses promoted greater fungivore populations compared to forbs (Viketoft, 2008). In contrast to our results, other studies have shown fungivores to be less responsive than other trophic groups to vegetation types (De Deyn et al., 2004; Porazinska et al., 2003). Additionally, a study from northern Sweden reported fungivore abundance to be enhanced by forb vegetation cover compared to grasses (Viketoft et al., 2005), however, this research considered mainly perennial forbs that likely have more belowground investment in the root systems and associated fungal connections (Gibson, 2009). The inconsistent responses of nematode communities across these studies suggests that some caution is needed in generalizing nematode responses to climate change induced vegetation shifts.

Similar to fungivores, the omnivore/predator group also had considerably higher abundances under *B. gracilis* relative to *E. cicutarium* or bare soil. This result was not entirely unexpected as omnivores are known to feed on fungi so they would likely follow a similar abundance trend as fungivores (Bongers and Bongers, 1998; Yeates et al., 1993). The dominant grass (*B.* gracilis) has a high root to shoot ratio (Coupland, 1979), translocating significant C belowground as biomass and via root exudation, both of which provide more shortterm C availability than forbs (Warembourg et al., 2003). This high belowground C allocation by *B. gracilis* may support greater abundances of microbial consumers compared to the other vegetation covers. Greater abundance of fungivores and bacterivores under grasses would also benefit omnivorous and predatory nematodes by providing additional food and energy resources to these higher trophic levels (Neher et al., 2004). Further, the omnivore/predator results may be explained by the fact that these long-lived nematodes are generally more sensitive than short lived species to disturbance (Ferris et al., 2001; Neher, 2010). Given the shortened life cycle of the annual species, belowground resources supplied by *E. cicutarium* may be more ephemeral in nature, thus contributing to a less stable environment. However, this finding is contrary to many studies that show no significant effects of plant species on omnivore/predators (Balvanera et al., 2006; De Deyn et al., 2004; Viketoft et al., 2009; Wardle et al., 2003).

The ratio of fungivores to bacterivores provides an interpretation of the soil environment status as well as the contribution of each trophic group to the decomposition process, giving important insight about shifts in the decomposition pathway (Ferris et al., 2001; Porazinska et al., 1999). A greater fungal role in decomposition can indicate a slower rate of soil C turnover as opposed to bacterial decomposition channels (Ruess, 2003). Despite bacterivores not varying between vegetation covers, the fungivore-bacterivore ratio was highest under *B. gracilis* ($p < 0.001$, Fig. 2), while no difference was observed between the *E. cicutarium* and bare soil. This result differs from previous studies that have reported fungivore to bacterivore ratios to be higher under forbs than other vegetation cover (Wardle et al. 2003, Viketoft et al. 2008), but as these studies were completed in green and glass-houses respectively a direct comparison to a field study is difficult. The marked impacts of plant species on the fungivore-bacterivore ratio suggest that encroachment of *E. cicutarium* in the shortgrass steppe may have pronounced impacts on nematodes involved in decomposition processes, with implications for long-term soil C dynamics and soil food web structure. Overall, our results indicate that the native *B. gracilis* cover supports more complex food webs with greater top-down regulation and slower nutrient cycling (Williamson et al., 2005), however, temporal factors may be major drivers of this variability through plant and nematode responses to seasonal variation. The higher than usual precipitation experienced by the shortgrass steppe at the time of sampling would have allowed *E. cicutarium* to germinate and establish early as annuals respond to seasonal precipitation much stronger than perennials (Guo et al., 2002).

Multivariate CCA analysis highlights vegetation cover differences between nematode community composition. The first two CCA axes explained 54.4% of the trophic group- soil parameters relations (34.3 and 20.1% for the first and second axes, respectively). Samples taken from under grass were tightly clustered and associated with fungal feeders (Fig. 3). Bare soil communities were somewhat intermediate between grass and forbs, though both groups had more variable community composition. Soil properties did not appear to explain much variability in nematode community composition. We suspect this is related to the fact that areas sampled were selected for homogeneity in land surface and thus little variability was present in the soil physicochemical properties (Table S1).

Invasive plants are known to exert changes to soil biological and chemical properties in the rhizosphere, affecting the microbial community structure (Kourtev et al., 2003), which may indirectly affect free living soil nematodes via alterations in resource quality and quantity (Saj et al., 2009). However, soil-plant feedbacks are not unidirectional; nematode community makeup also drives plant composition as well as plant responses to climate change (Franco et al. 2020). While less studied in natural systems, the impacts of plant parasites on plant performance have been well documented in coastal dune grasslands, where endoparasites, which tend be specialist feeders, can drive natural succession by contributing to reduced fitness of the dominant plant species (Van Der Putten et al., 1993). It should also be noted that alterations to nematode communities can be long-lived, such that the impact of introduced plants and biota on soil communities can persist for multiple decades (Wubs et al., 2019), which further highlights the need to understand nematode communities response to global change and how it will impact plant performance.

Based on our results, increased growth of *E. cicutarium* could be associated with lower abundances of fungivores and omnivores/predators. This would negatively impact the regulatory function of these groups, especially in regard to predator control of plant parasites, a significant soil function (Franco et al., 2019; Yeates et al., 2007) and decomposition and nutrient cycling by fungivores (Bardgett et al., 1998; Ferris et al., 1998). While soil properties appear to be important in previous research from the same site, they did not appear to be a major driving variable in this experiment (Hook et al., 1991). Our results reinforce the variable associations of nematodes with vegetation cover seen in other ecosystems, implying that functional groups important for nutrient cycling are linked to different vegetation cover in semi-arid grasslands. Continued shifts in the vegetation structure of water-limited grasslands like the shortgrass steppe could lead to important changes in soil nematode food web structure and function, likely impacting carbon and nutrient cycling and productivity of a globally important ecosystem.

2.5 CONTRIBUTIONS: We are grateful for the assistance and access to the Central Plains Experimental Range and for the help of the Wall and Fonte labs for soil sampling and processing.

2 FIGURES AND TABLES

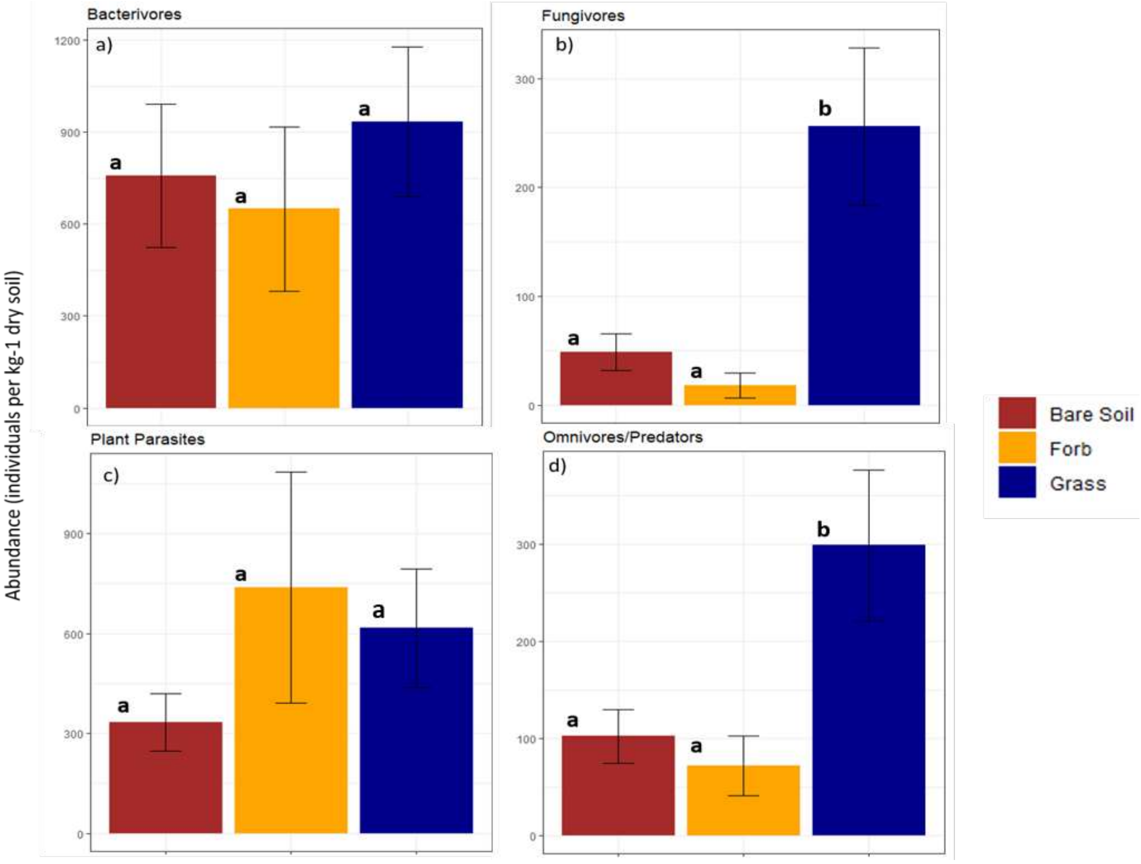

Figure 2-1: Mean abundance of four nematode trophic groups: a) bacterivores, b) fungivores, c) plant parasites and d) omnivores + predators under three vegetation covers (bare soil, *E. cicutarium* (forb), and *Bouteloua gracilis* (grass)) (n=10). Error bars represent standard error. Different lowercase letters mark significant differences ($P < 0.05$) after Tukey tests between plant cover types. Samples were collected in a shortgrass steppe ecosystem from two fields in April 2019 at the USDA-ARS Central Plains Experimental Range, in northeastern Colorado.

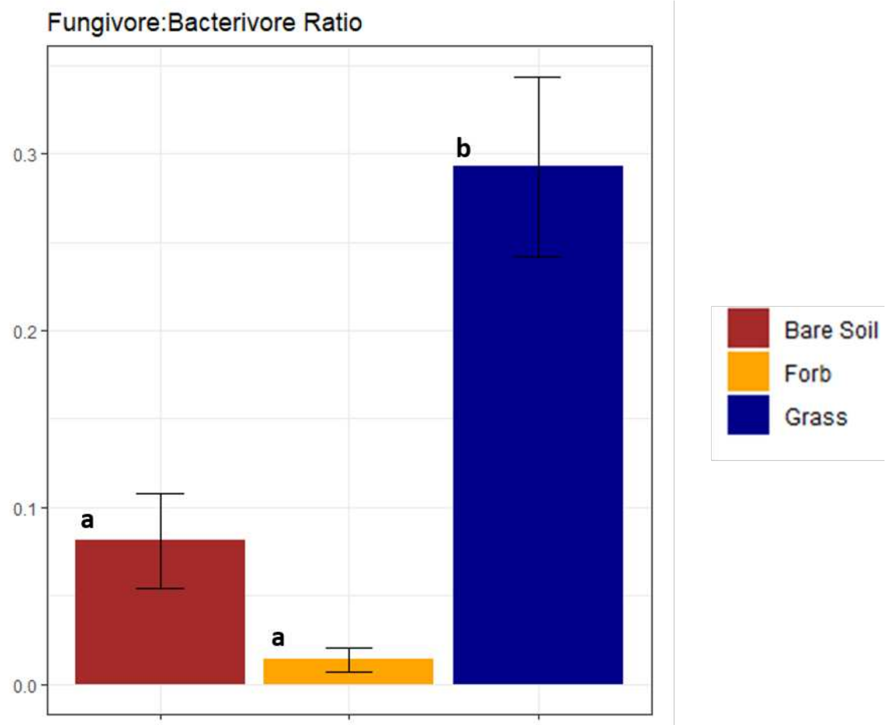

Figure 2-2: Mean fungal-to-bacterivore ratio under three vegetation covers (bare soil, *E. cicutarium* (forb), and *Bouteloua gracilis* (grass)) (n=10). Error bars represent standard error. Different lowercase letters mark significant differences (P < 0.05) after Tukey tests between plant cover types. Samples were collected in a shortgrass steppe ecosystem from two fields in April 2019 at the USDA-ARS Central Plains Experimental Range, in northeastern Colorado.

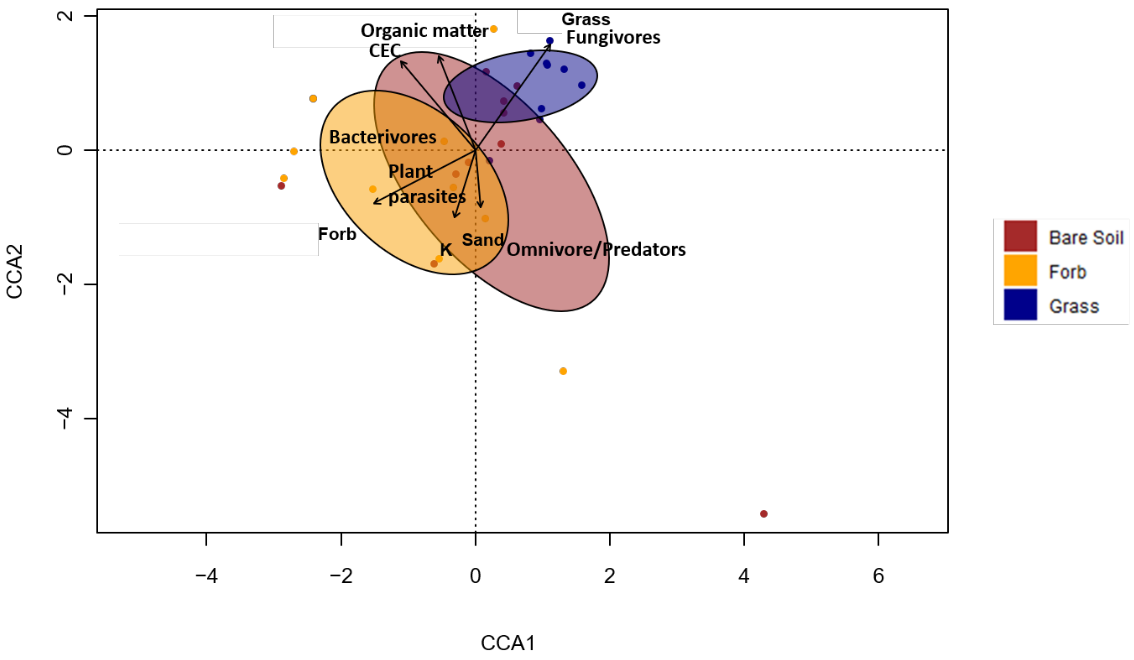

Figure 2-3: Canonical correspondence analysis (CCA) ordination of nematode trophic groups in the shortgrass steppe under three vegetation covers (bare soil, *E. cicutarium* (forb), and *Bouteloua gracilis* (grass)) (n=10). Nematode trophic groups are the response matrix, and all variables displayed on the ordination are included in the model (full model p = 0.044). Cation exchange capacity (CEC), and vegetation cover type were determined to be significant ($p<0.05$). Ellipses represent the standard deviation of the treatment groups. Sample points represent the nematode trophic groups sampled at a single time point and related to corresponding plot soil factor data. The eigenvalues for the first and second axis were 0.143 and 0.045, respectively.

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CHAPTER 3: ECTO-AND ENDOPARASITIC NEMATODES RESPOND DIFFERENTLY ACROSS SITES TO CHANGES IN PRECIPITATION[1]

3.1 SUMMARY

Plant parasitic nematodes are among the greatest consumers of primary production in terrestrial ecosystems. Their feeding strategies can be divided into endoparasites and ectoparasites that differ substantially, not only in their damage potential to host tissue and primary production, but also in their susceptibility to environmental changes. Climate change is predicted to increase variability of precipitation in many systems, yet the effects on belowground biodiversity and associated impacts on primary productivity remain poorly understood. To examine the impact of altered precipitation on endo- and ectoparasitic soil nematodes, we conducted a 2-year precipitation manipulation study across an arid, a semiarid, and a mesic grassland. Plant parasite feeding type abundance, functional guilds, and herbivory index in response to precipitation were evaluated. Responses of endo- and ectoparasites to increased precipitation varied by grassland type. There was little response of ectoparasites to increased precipitation although their population declined at the mesic site with increased precipitation. The abundance of endoparasites remained unchanged with increasing precipitation at the arid site, increased at the semiarid, and decreased at the mesic site. The herbivory index followed closely the trends seen in the endoparasites response by stagnating at the arid site, increasing

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at the semiarid, and decreasing at the mesic site. Our findings suggest that altered precipitation has differing effects on plant parasite feeding strategies as well as functional guilds. This may have important implications for grassland productivity, as plant parasite pressure may exacerbate the effects of climate change on host plants.

3.2 INTRODCUTION

Like many terrestrial ecosystems, grassland primary production is most limited by water availability (Sala et al. 1988). Climate change studies that assess grassland ecosystem precipitation regimes predict variation in the overall amount and increased frequency of extreme events (Melillo et al. 2014). These precipitation changes can have a major effect on the functioning of grasslands above and belowground (Sala et al. 2012; Wilcox et al. 2017), and may also impact an important driver of plant productivity – their obligate nematode parasites (Todd et al. 1999).

Plant parasitic nematodes (PPN) are affected by soil physical conditions such as soil aggregation and available water films, as well as by biological interactions including microbial pathogens, predators, host plant suitability and sensitivity, and the nutritional quality of roots (Khan 1993; Yeates and Bongers 1999). Through their feeding, PPN can directly cause considerable losses in plant growth and biomass to susceptible hosts (Ingham and Detling 1990; Brinkman et al. 2008; Jones et al. 2013) and in some ecosystems PPN consume more primary production that all other herbivores combined (Lauenroth and Burke 2008). PPN also have indirect ecological effects. For example, Bardgett (2005) suggests nematode herbivory not only affects many ecosystem functions, but also serves as an ecosystem connection by driving plant productivity (De Deyn et al. 2003), altering soil microbial communities (Khan 1993; Grayston et al. 2001), and exerting control on aboveground organisms by influencing plant community diversity (Van der Putten 2003; Wardle et al. 2004).

Feeding strategies of PPN determine their population responses to environmental conditions and their interactions with other organisms. Ectoparasites are more likely to be exposed to environmental fluctuations (e.g. drying), predation and pathogens, as they move freely between plant roots to feed. In contrast, endoparasites are relatively buffered from environmental variation, carrying out most of their life cycle within the root itself - with the exception of their infective, soil dwelling second stage juvenile form - and are more susceptible to plant defenses (Macguidwin and Forge 1991; Holbein et al. 2016). While both ecto- and endoparasites cause localized damage during feeding that can make hosts vulnerable to bacterial and fungal infection (Henderson and Clements 1977; Back et al. 2002), some endoparasitic species are often seen as more harmful to plants because they enter and migrate through host tissue, causing extensive damage as well as limiting the plant host's ability to induce defense mechanisms (Bird and Bird 2001; Jones et al. 2013).

The interplay between changing precipitation and PPN feeding is an important, but poorly understood aspect of grassland responses to climate change. One field study that tested total PPN abundance to changes in precipitation found little measurable effect (Torode et al., 2016), while other studies found PPN populations to be positively affected by greater long-term mean annual precipitation (MAP) and primary productivity allowing for more basal resources in plant roots (Sylvain et al. 2014; Nielsen et al. 2014; Vandegehuchte et al. 2015) as well as being negatively affected by greater top-down control via increased predation (Franco et al. 2019). Potential differences due to the feeding strategies were not assessed in any of these studies. The different life strategies or functional guilds of PPN are also important as this informs the duration and survivability of key nematode groups. Whether a nematode is capable of quickly establishing a base population in a disturbed environment, or is slower-reproducing, longer lived, and more sensitive to environmental fluctuations (i.e. drought), determines the assigned colonizer-persister (cp) value (Bongers 1990; Ferris et al. 2001; Preisser and Strong 2004). Identifying the feeding strategy, cp value and genus offers the potential estimate of the given impact of different PPN groups on plants and overall ecosystem productivity. Therefore, not only genera identification, but feeding and life strategy knowledge, can help to elucidate the response of PPN abundance to changing precipitation regimes.

The aim of this paper was to investigate the effects of incoming precipitation on the abundance of ecto- and endo-parasites across three distinct grasslands that represent differing MAP levels across the Great Plains of North America spanning over 1000 km. We explored the patterns of PPN feeding group abundances across the arid, semiarid, and mesic grasslands, and tested two hypotheses: first, we hypothesized that PPN abundance would respond differently to the precipitation treatments based on feeding strategy. This hypothesis is based on the idea that stronger top-down forces under increasing precipitation would have greater impacts on ectoparasites vs. endoparasites (Eisenback 1993; Bird and Bird 2001). And second, we hypothesized that greater cp value nematodes would increase with increasing precipitation across sites and with water addition treatments. The first hypothesis builds from our previous results suggesting that predation with increases in precipitation over time can suppress PPN populations (Franco et al. 2019). We expected ectoparasites to be more susceptible to increased predation (in wetter treatments) since endoparasites have a more protected, withinroot life cycle and are therefore less vulnerable to predation (Macguidwin and Forge 1991). The second hypothesis followed the logic that greater cp nematodes require more suitable microhabitats for reproduction, and that the plant damage potential expressed as an herbivory index (HI) would also increase, as there would be a greater population of the more damaging endoparasites with higher available soil water.

3.3 METHODS

Sites description and experimental design

This research was conducted across three distinct grassland ecosystems in North America: a desert grassland, a semiarid shortgrass steppe, and a mesic tallgrass prairie. These sites comprise an annual precipitation gradient, as well as variation in other climatic characteristics, soil types, and vegetation composition (Table 1). The desert grassland – located in the Jornada (JRN) Basin Long-term Ecological Research (LTER) in Southern New Mexico – has a long-term MAP of 245 mm, with vegetation dominated by the perennial grass *Bouteloua eriopoda* (Havstad and Schlesinger 2006). The Semiarid Grassland Research Center (SGRC) formally known as the shortgrass steppe LTER, located in northern Colorado, has a MAP of 321 mm, and is dominated by the warm season perennial grass, *Bouteloua gracilis* (Lauenroth and Burke 2008). The tallgrass prairie mesic site – located in Eastern Kansas at the Konza Prairie LTER (KNZ) – averages 835 mm of precipitation annually, with *Andropogon gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium* as the dominant vegetation species (Knapp 1998).

Experiments at each site were established on a relatively flat area, with vegetation representative of the larger ecosystem and with the exclusion of livestock grazing. Rainout shelters for the experiment were set-up at each site in 2016 to manipulate rainfall inputs during the growing season (Gherardi and Sala 2013); see Franco et al. (2019) for additional details. Shelters intercepted incoming precipitation, temporarily storing the water in an accompanying tank, and distributing the water through solar-powered irrigation systems (Gherardi and Sala 2013). Five levels of precipitation manipulations were determined based on long-term, sitebased historical precipitation extremes. Extreme and moderate water reduction, extreme and moderate water addition, and an ambient control were randomly assigned to plots (5 x 2.5 m) based on proportional changes in precipitation specific to the site considered. We applied a drought equivalent to a 100-year drought and an irrigation equivalent to 1 in 100-year deluge at each site. For the arid, semiarid, and mesic sites, respectively, this resulted in 80%, 70%, and 60% of incoming precipitation being diverted from the extreme drought to the extreme water addition treatments, while moderate water reduction treatments diverted 50%, 40%, and 30% of incoming precipitation to the moderate water addition treatments. Eight replicates of all treatments were carried out at each site, for a total of forty plots per site. Treatments were interspersed and spaced at least 5 m apart. Rainfall manipulations were maintained for both the 2016 and 2017 growing seasons.

Soil and nematode collection

Soil samples were collected from each site in September, except for the semiarid site in 2016 that was not collected until October. Sampling was conducted using a soil corer (2.5 cm dia.) to collect four sub-samples to a depth of 10 cm directly beneath the plants of the dominant vegetation type within each experimental plot. The four sub-samples collected from each plot were combined and gently mixed in a plastic bag to form one composite sample per treatment. The soil corer was cleaned with alcohol wipes between each plot to avoid cross-contamination. Soil samples were placed in a cooler with ice packs to prevent overheating during transportation to the lab at Colorado State University for nematode extraction. Upon arrival at the lab, samples were stored at 4°C and extracted within 5 days.

Nematode extraction, counting, and identification

Nematode extraction was performed with 100 g aliquots of the composite sample using Baermann funnels, from which daily samples of 20 ml solution were collected for 3 days, for a total of 60 ml, and stored at 4°C (Hooper 1970). The nematode solution was reduced to 5 ml and poured onto counting dishes for identification of PPN at the genus level. The total number of nematodes were counted, trophic groups were identified, and PPN feeding strategies (ecto- and endoparasites) were determined based on Yeates et al. (1993) using an inverted microscope (Olympus CKX41, 200X magnification). Nematodes in the genus *Tylenchus* and *Ditylenchus*, known to be fungivores, plant parasites, or facultative plant parasites were considered to be fungivores and plant parasites, respectively (Yeates et al. 1993). Nematodes were preserved with 5% formalin (Southey 1986) and at least 100 PPN were identified to the genera level. Nematode abundances were calculated as the number of individuals per kg of dry soil (soil moisture was calculated by drying 50 g of soil for each sample at 105°C for 72 hours).

All identified PPN were classified into one of the five groups along the cp scale (Bongers 1990). The cp scale considers a range from 1 (extreme r strategists) to 5 (extreme k strategist) where nematodes assigned to group 1 are enrichment opportunists that increase their

population quickly after soil disturbance and enrichment processes, nematodes belonging to cp 2 and cp 3 groups have progressively longer life cycles and are more sensitive to soil disturbances, while nematodes in groups 4 and 5 are mostly composed of k-strategists that are very sensitive and slow to recover following disturbance (Bongers and Bongers 1998). Potential nematode impacts on grass productivity were estimated via indirect means using an herbivore impact (HI) factor assigned to each genus based on evidence of known relationships of host plant damage severity by different genera (Ferris 1980; Freckman and Virginia 1989); on a scale of 1-0, where 1 is assigned to the most damaging PPN genus *Meloidogyne,* which was not found in our samples (Table 1). The herbivore impact factor was multiplied by the density of each genus and impact factors for all genera were summed for determination of the HI, interpreted as the potential feeding damage by the PPN taxa found at the three grassland sites.

Statistical analysis

Both years were combined in our analysis which allowed ten levels of received precipitation to be used as a continuous explanatory variable. Linear mixed-effect models (LME) for PPN feeding group abundances were generated using received growing-season precipitation, site, and their interaction, with plot included as a random effect to account for any interdependency of repeated measurements. The assumptions for homogeneity of variance and normality of residuals were met and no transformations to the data were necessary. The conditional r^2 was achieved by the methods of Nakagawa and Schielzeth (2013). These same LME models were applied to the cp grouping of the PPN genera and to the HI of each sample collected. Due to low abundance of cp 4 and 5 groupings these nematodes were combined in the analysis. One sample collected resulted in an exceptionally high number of endoparasites and was removed in all analyses to avoid any influence from outlier data.

To visualize if the individual site and precipitation treatments influenced the PPN community composition, we used non-metric multidimensional scaling (NMDS). As the data contained a high number of absences within genera – which is common for nematode data – Bray-Curtis was chosen as the dissimilarity metric, as it does not equate absences. Relationships of the sites and precipitation levels to the nematode genera abundances were revealed by superimposing data for one genus at a time on the NMDS plot (Ida and Kaneda 2015). The dissimilarity measure and projected distance between genera was calculated. A non-parametric multivariate statistical test of variances (npMANOVA) was used to test the effect of site and seasonal precipitation on community composition. All analyses were conducted using R software, version 3.2.2 (R Core 2014), with the following packages; vegan (Oksanen et al. 2010), nlme (Bates et al. 2015), piecewiseSEM (Bartoń 2013), and package ggplot2 was used for data visualization (Wickham 2009).

3.4 RESULTS

Precipitation variation and nematode response

Plant parasitic nematodes were detected in all samples and PPN populations generally were lowest in the arid site and greater in the semiarid site, with the greatest populations in the mesic site. Feeding groups responded uniquely by site to precipitation treatments (Ectoparasites: $P_{\text{Interaction}} < 0.001$, $R^2 = 0.79$, Fig. 1a; Endoparasites: $P_{\text{Interaction}} < 0.001$, $R^2 = 0.39$, Fig. 1b). Both ecto- and endoparasites decreased in response to increasing precipitation in the mesic grassland (Fig. 1). However, the groups differed in their response to altered precipitation in the semi-arid site, such that endoparasites increased with precipitation, while ectoparasites displayed no trend with precipitation. Meanwhile, neither group responded to increasing precipitation at the arid site.

Nematode life history strategies

The PPN functional data (Table 2) suggested cp groups abundances had variable responses to the different precipitation regimes from each site. Abundance of genera representing cp2 life strategies decreased with increasing seasonal precipitation at the mesic site and increased in abundance at the other two sites ($P_{\text{Received precip.}} < 0.001$, $P_{\text{Site}} < 0.001$, $P_{\text{Interaction}} = 0.491$, $R^2 = 0.24$, Fig. 2a). The cp3 nematodes responded to increasing seasonal precipitation differently at each site ($P_{\text{Interaction}} < 0.001$, $R^2 = 0.22$); with abundance responses changing from positive to negative from the semiarid to mesic sites, and a very slight increase in cp3 nematodes with increased received precipitation at the arid site (Fig. 2b). The nematodes having longer life cycles and belonging to the grouped cp4 - cp5 classifications decreased in abundance with increasing seasonal precipitation at the mesic and semiarid sites but increased in abundance at the arid site ($P_{\text{Interaction}} = 0.003$, $R^2 = 0.12$, Fig. 2c). The HI followed closely the trends seen in the endoparasitic abundance response (Fig. 1b) by decreasing at the mesic site, increasing at the semiarid site, and stagnating at the arid site ($P_{\text{Received precip.}} < 0.001$, $P_{\text{Site}} < 0.001$, $P_{\text{Interaction}} = 0.67$, $R^2 = 0.21$, Fig. 3).

Nematode genera at each site

The composition of the PPN community was affected by site (P =0.001, R2 =0.22, Fig. 4). Twenty-two PPN genera were found across sites. Thirteen genera (Criconema, Ditylenchus, Filenchus, Gracilacus, Helicotylenchus, Hoplolaimus, Merlinius, Paratylenchus, Pratylenchus, Rotylenchus, Subanguina, Tylenchorhynchus, and Xiphinema) were present in all sites sampled. In contrast, one genus was endemic to the semiarid site (Longidorus), while eight genera occurred only in the mesic site (Basiria, Coslenchus, Hemicycliophora, Mesocriconema, Pararotylenchus, Paratrophorus, Psilenchus, and Trichodorus). The arid site was associated with the greatest abundance of the genera Merlinius, whose species feed on many host plants globally, including those found in each grassland site (Navas and Talavera 2002). Basiria, Trichodorus, Mesocriconema, and Hemicycliophora are endemic genera found in the mesic site, and clustered outside the polygon denoting the mesic site. Helicotylenchus and Paratylenchus were found at each grassland, but had greater abundances in our semiarid and mesic sites, respectively.

3.5 DISCUSSION

Plant parasitic nematode differences across sites

Our findings suggest that PPN populations were lowest in the arid site and greatest in the mesic site, however, this trend was not equally observed in ecto- vs. endoparasitic nematodes. Although the mesic site did have the greatest abundances of PPN for both groups, we observed a greater abundance of ectoparasites at the arid site than the semiarid site (Fig. 1). This increase in arid ectoparasitic nematodes follows what was found in our previous study in the same site that showed an increased in community weighted mean of plant parasites body size when exposed to increasing precipitation (Andriuzzi et al. 2020). The finding of increased precipitation supporting more consumers coincides with previous studies suggesting that increased precipitation has a strong, positive effect on PPN abundances within single sites (Jordaan et al. 1989; Bardgett et al. 1999; Todd et al. 1999; A'Bear et al. 2014; Sylvain et al. 2014). There is also evidence to support that at large spatial scales across landscapes and regions, climatic characteristics have greater influences on the soil biota composition (and the local nematode community structure) than inherent soil characteristics (Ettema and Wardle 2002; Blankinship et al. 2011; Nielsen et al. 2014). This may in part explain the greater PPN populations observed at more mesic grasslands as this site had the highest MAP.

Plant parasitic nematode response to precipitation treatments

In agreement with our first hypothesis, the two feeding strategies of plant parasitic nematodes, ecto- and endoparasites, responded differently to precipitation manipulations. This was especially evident at the semi-arid site, where endo-parasites responded positively to increased rainfall, while ectoparasites remained largely flat across the manipulated gradient in precipitation. Contrary to our prediction, ectoparasitic abundance did not significantly change with received precipitation at the drier sites (arid and semiarid) but decreased greatly at the mesic site (Fig. 1a). We suspected that for the more xeric sites (arid and semiarid) that increased soil moisture would allow ectoparasitic nematodes to actively move between root feeding sites, allowing for greater reproduction, and completion of the life cycle. This idea was supported by previous work reporting PPN to increase following one year of irrigation

(Freckman et al. 1987), likely due to increased plant growth and root biomass, but little change in ectoparasitic populations were seen in the drier sites. Aligning with our hypothesis, ectoparasitic abundance was negatively affected by increased water availability in the mesic grassland. This response at the mesic site could be caused by increased top-down control by predator nematodes on ectoparasites since the abundance of predaceous nematodes increased with increasing precipitation in this same site (Franco et al. 2019). Moreover, Franco et al (2019) found the abundance of PPN increased in drought conditions where predators nematodes populations declined; showing a dismantling of the predator-prey balance in which PPN and lower trophic group nematodes are released by the decrease in predator populations in the mesic site.

The endoparasite response to increasing precipitation also differed from our hypothesis in that each site showed a unique outcome to the precipitation treatments rather than remaining unchanged. Endoparasitic nematodes were expected to exhibit a reduced response to environmental changes, as most of their life cycle occurs within roots – providing these nematodes with shelter from environmental fluctuations and predation. The negative response of endoparasitic populations to increased seasonal precipitation seen at the mesic site could be due to increased plant chemical defenses against initial invasion and establishment of endoparasites, since increased soil water could increase plant growth and ability to invest in defensive compounds (Hakes and Cronin 2011). The findings at the semiarid site support previous work reporting a positive response of endoparasitic abundance to increased precipitation in dryland agricultural systems of South Africa (Kandel et al. 2013) as Pratylenchus is known to have inhibited growth in either too little or high soil moisture (Kable and Mai 1986).

Functional diversity of plant parasitic nematodes

It is important to know which genera are present and how they are affected by future precipitation patterns, as the life strategies of different PPN genera vary greatly. Some PPN complete several generations in a year (e.g. Pratylenchus, Paratylenchus, Helicotylenchus), others may only complete one, while still others may have generation times that exceed 12 months (e.g. Xiphinema, Longidorus). This could lead to differences between a thousand, a hundred, or a ten-fold population increase, respectively, within a growing season (Jones and Northcote 1972). Precipitation influenced the structure of the PPN communities in the grasslands studied, as the precipitation treatments significantly impacted abundance and distribution of the functional guilds of the PPN communities identified. Colonizer (r strategist) nematodes (cp2) as well as cp3 nematodes responded negatively to increased precipitation at the mesic site, but increased in the semiarid site, while more persistent cp4-5 nematodes were enhanced by increasing precipitation only in the arid site and decreased in the wetter grasslands. The overall decrease of the longer-lived, slower generating ectoparasitic nematodes at the higher wetter sites could be explained by increased predation pressure on these PPN genera that does not occur at our drier sites. Should the mesic grasslands become wetter in the future these persistent nematodes may become less numerous in abundance and lead to greater increases in colonizer nematodes shifting from more stable trophic structures to less structured ones.

Across our three sites, HI was affected by the precipitation treatments in a different way. While increasing precipitation did not affect HI at the arid site, opposing responses were found for the mesic and semiarid sites showing a decrease and increase, respectively. This finding suggests that in the mesic site more frequent droughts could intensify the overall severity of root herbivory, while the same is true for increasing rainfall at the semi-arid site. Previous field work on cereal cropping systems found the effects of diseases caused by nematodes were more evident under drought conditions that resulted in damaged root systems not efficiently taking in soil moisture and nutrients (Kandel et al. 2013). The increase in available soil water could potentially increase both predation and plant defenses which may offset damage caused by PPN and therefore reduce the impact of infestation. The negative impact of received precipitation on endoparasitic nematodes may also help explain the observed decrease in HI as in general they cause more potential damage (Jones et al. 2013).

Community structure of plant parasitic nematodes

Plant parasitic nematode community structure was affected by site as visualized by the NMDS plot (Fig. 4). Paratylenchus has a drought resistant growth stage, which explains the placement closer to the arid site compared to Helicotylenchus which lacks such resistance and was rarely found in the arid site (Yeates and Lee 2004). The NMDS plot suggests that site can be a predictive factor in determining where some specific genera may be found. Ditylenchus was the most abundant endoparasite at all three sites, but had the greatest quantity in the semiarid site, which is evident from the NMDS results placing Ditylenchus in the polygon signifying the semiarid site. The most abundant ectoparasite genera differed between the three sites (Table 1). Overall, there were more endoparasites present in the arid and semiarid sites and more ectoparasites at the mesic site. This confirms that ectoparasites respond positively to increased precipitation, which aligns with our initial hypothesis.

Implications for grassland ecosystems under climate change

Altered precipitation patterns as a result of climate change pose a challenge to grassland plant species diversity and productivity. If mesic grasslands do get drier as is predicted, these grasses may support a greater population of ectoparasitic nematodes. Given that our results suggest that endoparasites also increase with drought, we suspect that an overall increase in both feeding strategies could further intensify PPN damage within potentially water-stressed mesic grasslands.

Previous work suggests that, in general, endoparasites are superior competitors to ectoparasites (Eisenback 1993; Jones et al. 2013), and while PPN herbivory and plant disease may not always kill their host, they do reduce the plant’s productivity compared to healthier uninfected plants (Grime 1998; De Deyn et al. 2003; Neher 2010). Plant hosts from natural ecosystems have coevolved with PPN much longer than crop-nematode systems, but under climate change endoparasites may have an advantage that could ultimately lead to changes in plant diversity and composition via selection pressure and PPN driven succession in mesic grasslands (De Deyn et al. 2003; Brinkman et al. 2008).

3.6 CONCLUSION

Our results show that plant parasitic nematode abundance, functional guilds, and herbivory index are influenced by seasonal precipitation differently across a regional climatic gradient in grasslands. The varying effects of seasonal precipitation on the structure of plant parasitic nematode communities highlight the vulnerability of mesic and semiarid grasslands to predicted effects of climate change. Mesic grasslands are projected to experience longer durations of drought which will increase both the abundance of the generally more detrimental endoparasitic plant parasite and, consequently, the herbivory impact on plants. Semiarid grasslands may see an increase in wet precipitation events that would lead to increased endoparasite abundance, also resulting in greater herbivory impact. Finally, our results indicate that PPN populations in arid grasslands will remain relatively unchanged despite increases in droughts or deluges. The interaction seen among precipitation treatments and site affecting plant parasitic nematodes communities by specific feeding strategy, functional guild, and herbivory index has not previously been reported. More studies focused on nematode-driven soil processes along climatic gradients with conjunction of plant data (i.e. biomass) will advance our understanding of the ecosystem-wide repercussions of altered precipitation from climate change. Plant parasitic nematode feeding is a dominant control in overall grassland biomass production (Ingham and Detling 1990; Neher 2010) and will increase with drought, thus potentially exacerbating climate change impacts on grassland productivity, more severely in mesic than arid sites.

3.7 CONTRIBUTIONS

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3 FIGURES AND TABLES

Table 3-1. Site characteristics for the Jornada Basin LTER, NM (Arid), Semiarid Grasslands Research Center, CO (Semiarid), and Konza Prairie LTER, KS (Mesic).

	Arid	**Semiarid**	**Mesic**
Geographic location			
Latitude	32°33'N	40°50'N	39°4'N
Longitude	106°49'W	104°45'W	96°34'W
Ecosystem type	Chihuahuan Desert	Shortgrass Steppe	Tallgrass Prairie
Climate			
MAP (mm)[a]	245	321	835
MGSP (mm)[b]	105	204	428
MAT (°C)[c]	14.7	8.4	12.5
Soil Type	Aridisols	Aridisols/Mollisols	Mollisols
Texture[*]	Fine sandy loam	Fine sandy loam	Silty clay loam
Rainfall treatments (relative to ambient)			
Large reduction	-80%	-70%	-60%
Moderate reduction	-50%	-40%	-30%
Moderate addition	+50%	+40%	+30%
Large addition	+80%	+70%	+60%
Most Abundant PPN[**]			

Ectoparasite	*Merlinius*	*Helicotylenchus*	*Helicotylenchus*
Endoparasite	*Ditylenchus*	*Ditylenchus*	*Ditylenchus*

[a]Mean Annual Precipitation [b]Mean Growing Season Precipitation [c]Mean Annual Temperature [a-c] Obtained from NOAA climate data from Las Cruces, NM, Nunn, CO, and Manhattan, KS.

[*] Obtained from Soil Survey USDA

[**]Plant parasitic nematode

(https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx).

Table 3-2. Plant-parasitic nematode taxa detected in each MAP level and their potential impact on plant growth and performance based on literature values. Endoparasites are bolded.

Genera (cp value)	Impact factor	Regional gradient		
		Arid	Semiarid	Mesic
Basiria (2)	0.05			x
Coslenchus (2)	0.05			x
Criconema (3)	0.05	x	x	x
Ditylenchus (2)	0.4	x	x	x
Filenchus (2)	0.05	x	x	x
Gracilacus (2)	0.05	x	x	x
Helicotylenchus (3)	0.4	x	x	x
Hemicycliophora (3)	0.3			x
Hoplolaimus (3)	0.4	x	x	x
Longidorus (5)	0.3		x	
Merlinius (3)	0.07	x	x	x
Mesocriconema (3)	0.4			x
Pararotylenchus (3)	0.07			x
Paratrophorus (3)	0.07			x
Paratylenchus (2)	0.05	x	x	x
Pratylenchus (3)	0.5	x	x	x
Psilenchus (2)	0.05			x
Rotylenchus (3)	0.4	x	x	x
Subanguina (2)	0.5	x	x	x
Trichodorus (4)	0.5			x
Tylenchorhynchus (3)	0.05	x	x	x

Xiphinema (5)	0.07	x	x	x

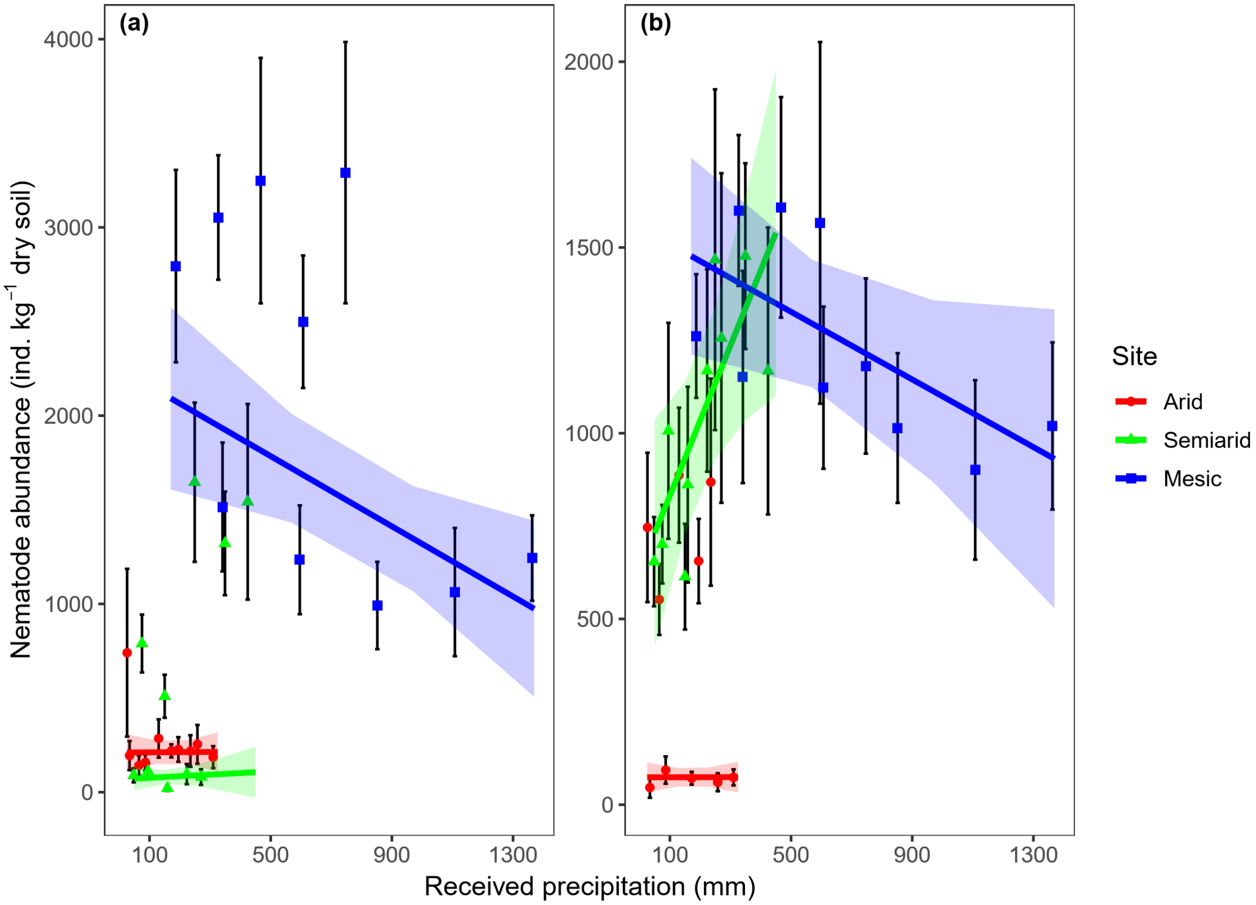

Figure 3-1: Response of plant parasitic nematodes to manipulated precipitation across three ecosystem types. Color-coded trend lines represent effects of manipulated precipitation (*n* = 80) predicted by mixed effects models. Shaded regions indicate 95% confidence limits. Points represent mean abundance of nematodes per precipitation level (n = 16), and error bars represent standard error of the mean. (*a*) Ectoparasitic abundance of nematodes ($P_{\text{Received precip.}} < 0.001$, $P_{\text{Site}} < 0.001$, $P_{\text{Interaction}}$ = < 0.001, $R^2 = 0.793$); (*b*) Endoparasitic abundance of nematodes (P_{Received} precip. < 0.001, $P_{\text{Site}} < 0.001$, $P_{\text{Interaction}} = 0.0154$, $R^2 = 0.387$). For all tests *n* = 238.

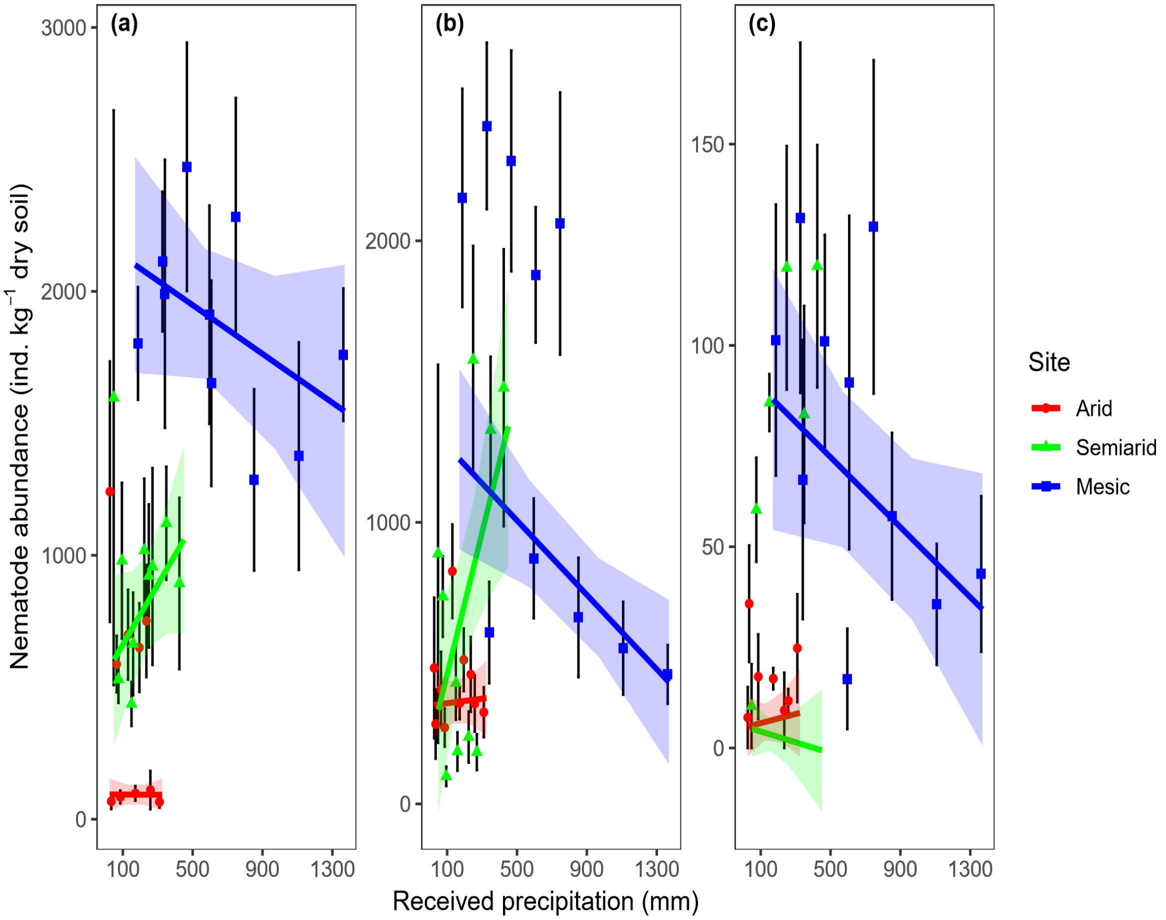

Figure 3-2: Response of plant parasitic nematode life strategies as grouped by colonizer persister scales (cp) to manipulated precipitation across three ecosystem types. Color coded trend lines represent effects of manipulated precipitation (*n* = 80) predicted by mixed effects models. Shaded regions indicate 95% confidence limits. Points represent mean abundance of nematodes per precipitation level (n = 16), and error bars represent standard error of the mean. (*a*) Abundance of cp2 nematodes (*P*Received precip. < 0.0001, *P*Site < 0.001, *P*Interaction = 0.491, *R*2 = 0.242); (*b*) Abundance of cp3 nematodes ($P_{\text{Received precip.}}$ < 0.001, P_{Site} < 0.001, $P_{\text{Interaction}}$ < 0.001, R^2 = 0.219); (c) Abundance of cp4 and 5 nematodes ($P_{\text{Received precip.}}$ < 0.001, P_{Site} < 0.001, $P_{\text{Interaction}}$ = 0.003, R^2 = 0.121. For all tests *n* = 239.

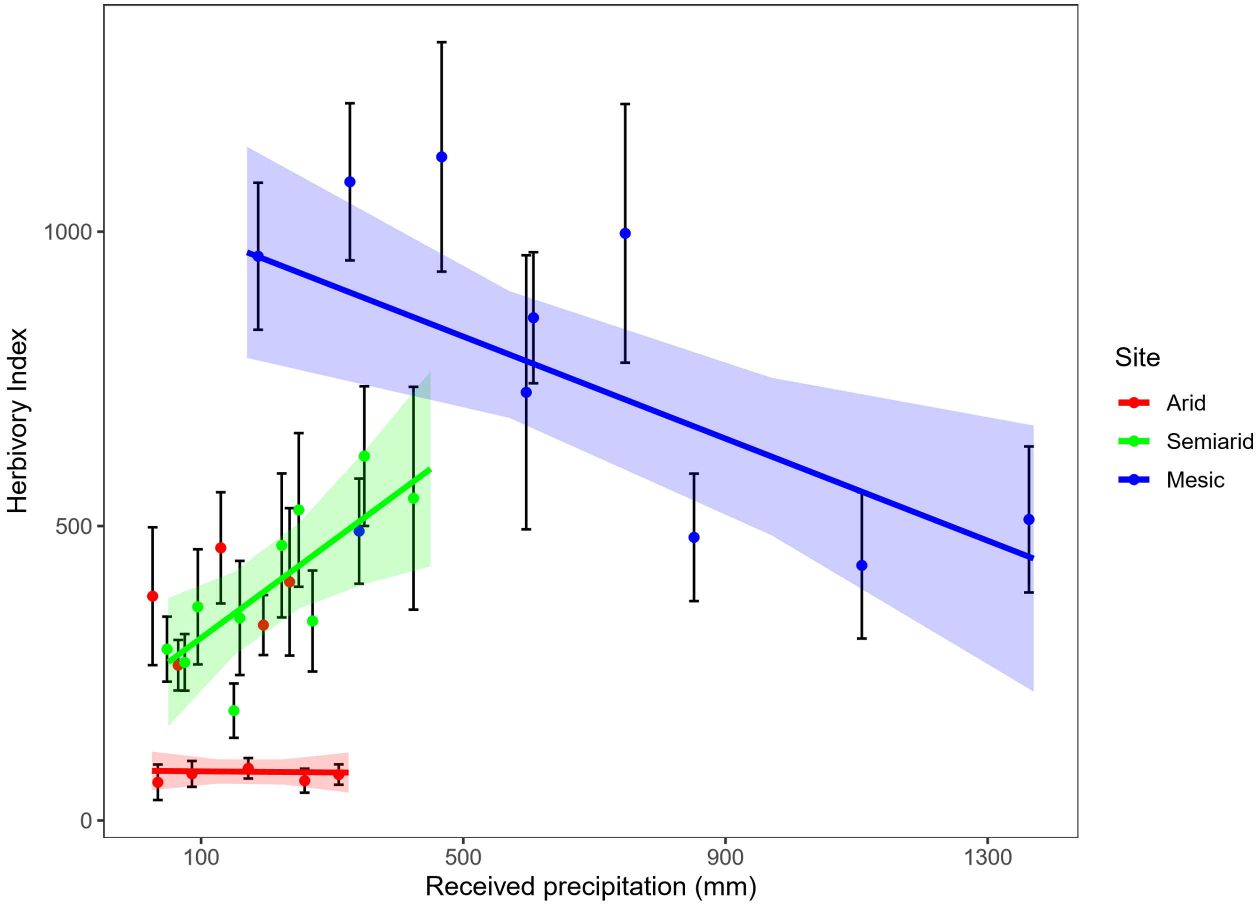

Figure 3-3: Herbivory Index (an indicator of potential severity of herbivorous nematodes on plant growth) response to manipulated precipitation across three ecosystem types. Color-coded trend lines represent local effects of manipulated precipitation (*n* = 80) predicted by mixed effects models. Shaded regions indicate 95% confidence limits. Points represent mean abundance of nematodes per precipitation level (n = 16), and error bars represent standard error of the mean. ($P_{\text{Received precip.}} < 0.0001$, $P_{\text{Site}} < 0.0001$, $P_{\text{Interaction}} = 0.6645$, $R^2 = 0.2129$). *n* = 238. The herbivory impact index was calculated as [(∑(number of nematodes/genus x impact factor)]. Impact factors for each genus are listed in Table 2. (Modified from Freckman and Virginia, 1989).

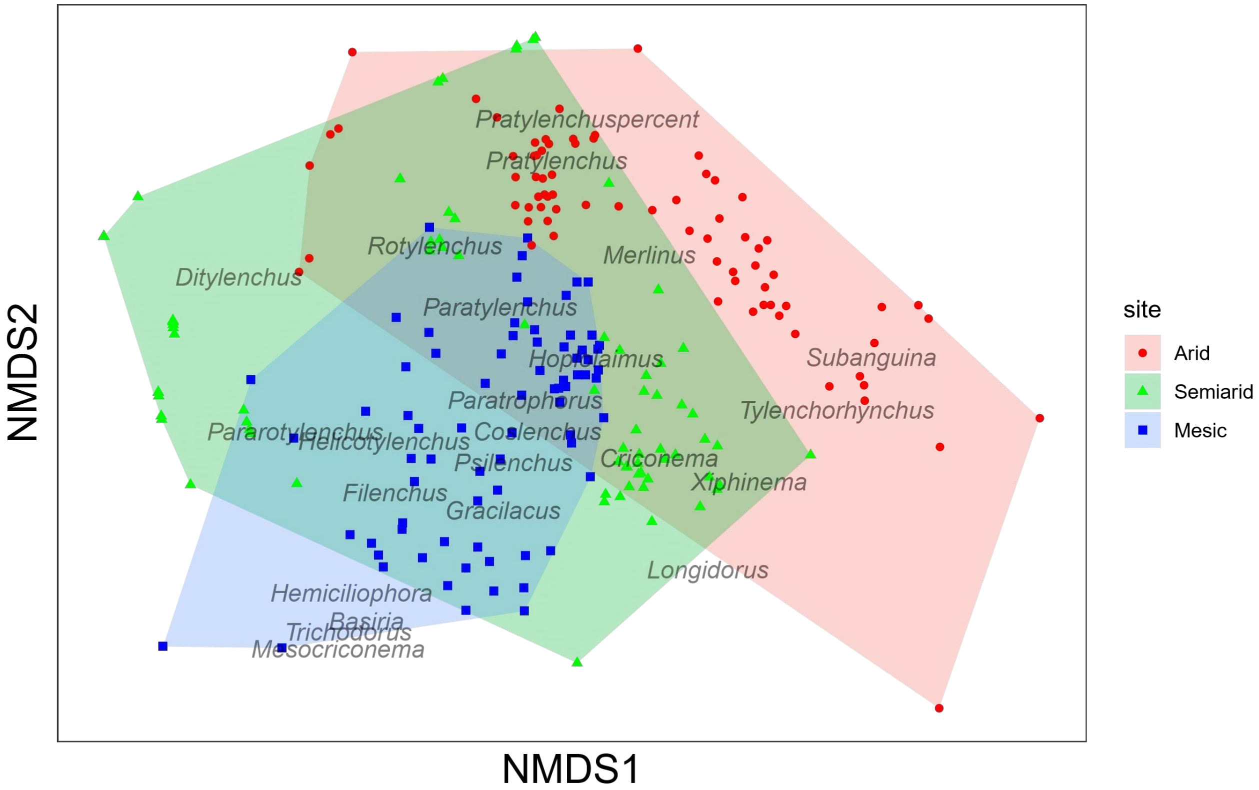

Figure 3-4: Plant parasitic nematode (PPN) composition across the three grassland sites. Nonmetric multidimensional scaling plot of PPN genera as a function of received growing season precipitation (*n* = 239, Bray– Curtis). The genera are grouped by site (shape and color) (npMANOVA: F= 38.86, R^2 = 0.221, *P* = 0.001)

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CHAPTER 4: ECOLOGICAL MATURITY AND STABILITY OF NEMATODE COMMUNITIES IN RESPONSE TO PRECIPITATION MANIPULATIONS IN GRASSLANDS.[2]

4.1 SUMMARY

Climate change is predicted to cause alterations in precipitation patterns in grasslands around the globe. The implications of these changes for soil biota and multiple key ecosystem functions that they regulate in grasslands is little known. We used soil nematodes as biological indicators in grassland ecosystems experiencing large shifts in precipitation in an arid, semiarid, and mesic grasslands. By calculating the nematode plant parasite index, maturity index, and its extensions: the enrichment, basal, channel, and structure index, we assessed grassland ecosystem response to five levels of manipulated precipitation over the course of two years. Nematode community structure described by these indices was sensitive enough to reflect community changes from the altered precipitation treatments and responded uniquely at each of the three sites. With increasing precipitation, nematode communities at the arid site became more enriched in species and switched to a more fungal-dominated decomposition pathway. The semiarid site showed a decline in nematode maturity, structure, and fungally dominated decomposition, but greater levels of enrichment, basal resources, and herbivory. In the mesic site increasing precipitation led to a nematode community with greater maturity and structure and shifted toward a fungal decomposition channel that also reflected a lower level of enrichment and plant parasites. We performed an indicator analysis to identify nematode genera representative of each grassland site and precipitation level. Nematode indicator composition was found to be significantly affected by the levels of applied precipitation and across sites,

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highlighting specific genera that may be affected by future precipitation regimes such as Eucephalobus in the arid site or Trichodorus from the mesic site. Nematode community analysis allowed for the detection of strong influences on the soil food web caused from extreme and moderate precipitation manipulations. This finding stresses the need for more genera level studies to be done to reflect specific ecosystem responses to climate change as well as the need for more cross-site studies as site-specific differences must be accounted for ecological interpretations.

4.2 INTRODUCTION

Covering over a third of the Earth's surface, grasslands have great economic and ecological value by supporting multiple ecosystem services such as providing food and forage and supporting biodiversity (Gibson and Newman, 2019; Wilsey, 2018). Grasslands, much like the rest of the planet, will experience changes in temperature and precipitation regimes due to increases in anthropogenic greenhouse gas emissions (Bradford et al., 2020). Climate models predict large shifts in precipitation patterns in the western United States, leading to an increased frequency of drought and intensity of rainfall events to the grasslands of the Great Plains (IPCC, 2013; Paschalis et al., 2020). Alterations in precipitation drive changes in both above and belowground communities. While increased received precipitation have positive effects especially in arid and semiarid environments by stimulating fine root growth (Zhang et al., 2019) or increasing microbial biomass (Zhang et al., 2013). In addition increased precipitation can negatively impact some soil properties by leading to more leaching and erosion (Austin and Vitousek, 1998). Thus for mesic sites an increase in precipitation may be stressful to soil communities, especially higher trophic groups that are sensitive to change (Bongers, 1990; Franco et al., 2017). While the response of vegetation to altered precipitation has been well documented (Fay et al., 2003; Felton et al., 2020; Heisler-White et al., 2008; Jentsch et al., 2011; Notaro et al., 2010), there has been less focus on effects on soil organisms. Soils are an essential component of grasslands and home to a wide range of belowground organisms that regulate multiple ecological functions such as decomposition, nutrient cycling, C sequestration, and disease suppression (Bach et al., 2020; Bender et al., 2016; Wall et al., 2015). When considering global change impacts on soil fauna communities, more emphasis has been on changes in temperature (Thakur et al., 2017) and nutrient gradients (Shaw et al., 2019; Yeates and Newton, 2009) than to alterations in precipitation. While there are some studies that focus on examining the effects of varied precipitation on soil nematodes most have primarily been based on a single field site (Darby et al., 2011; Landesman et al., 2011; Siebert et al., 2019), and thus cannot capture the potential variability of response across different ecosystems and those that do consider multiple sites (Sylvain et al., 2014) lack genera level data.

Soil nematodes, in particular, are known to influence multiple soil functions and have proven extremely useful as indicators of environmental response to stress and disturbance (Neher, 2001). Not only are soil nematodes one of the most diverse and abundant groups of soil organisms (Bardgett et al., 1999; van den Hoogen et al., 2019), they are a critical component of the soil environment, occupying multiple trophic levels and directly affecting primary production, nutrient cycling, decomposition, and influencing the turnover and activity of microbial populations (Cole et al., 2004; Freckman and Baldwin, 1990; Griffiths, 1994). Soil nematodes also span a wide range of life history strategies that allow for unique responses to stress and disturbance (Bongers and Ferris, 1999; Ney et al., 2019; Pattison et al., 2005; Yeates et al., 1993). Based on these characteristics and their vital role in multiple ecosystem processes, nematodes are an ideal model taxon for understanding how changes in precipitation can affect trophic complexity and energy or carbon pathways of soil communities as grasslands under climate change.

Ecological indices based on life history strategy and obligate feeding habits of different nematode genera can provide an assessment of how nematode community structure and function changes with environmental disturbances, and thus can offer valuable insight for understanding grassland ecosystem response to altered precipitation regimes (Bongers 1990; Ferris et al. 2001). The maturity index (MI) has been utilized to show where the free-living nematode community is positioned in ecological succession (Bongers, 1990) while the plant parasite index (PPI) expresses the same ecological interpretation for plant parasites (Bongers and Bongers, 1998). Extensions of the MI; the enrichment (EI), basal (BI), structure (SI), and channel index (CI), indicate the complexity of the nematode soil food web as well as the main decomposition channel (Ferris et al., 2001). The high sensitivity of nematode communities that these indices provide can show a detailed status of the structure and function of soil food webs in response to altered precipitation (Landesman et al., 2011). Employing these indices across distinct grasslands experiencing manipulated droughts and increases in rainfall would provide valuable insight into the status of the soil food web as well as specific details of the nematode community.

With the present work, we tested the sensitivity of nematode ecological communities to climate change in three different grassland ecosystems experiencing five levels of manipulated precipitation. Our first hypothesis was that increased precipitation would result in a more mature and structured community that reflects a less enriched environment as indicated by increases in the MI, SI, and a decrease in PPI, BI, and EI. The rationale for our first hypothesis is driven by previous findings from this same experiment showing an increased number of predator and omnivorous nematode abundances found with increasing precipitation (Franco et al. 2019), which reflects a community composed of higher trophic groups with improved food web linkages, and thus a more resistant nematode community. Second, we hypothesized that greater precipitation would favor a fungal decomposition pathway. This is based on recent research suggesting bacteria abundance and diversity to be less responsive to water additions than fungi (Ochoa-Hueso et al. 2018). Third, we hypothesized that these changes in the nematode community would be more pronounced in the semiarid and mesic sites, compared to the arid site. The third hypothesis follows the findings of previous studies where short-lived changes to water availability in deserts had weak or no effect on nematode abundance and body traits (Andriuzzi et al. 2020; Freckman and Virginia, 1989; Sylvain et al., 2014; Vandegehuchte et al., 2015). Finally, we investigated the potential of individual nematode genera to be used as indicators of changes to precipitation that would complement the community indices mentioned above and provide more specific info about which taxa are driving the nematode community response.

4.3 METHODS

Research sites

This experiment was conducted in three distinct North American grasslands: a desert grassland (Jornada Basin; JRN), a semiarid shortgrass steppe (Semiarid Grasslands Research Center; SGRC), and a mesic tallgrass prairie (Konza Prairie; KNZ). JRN is a long-term Ecological Research site (LTER) located in Southern New Mexico, with vegetation dominated by the perennial grass *Bouteloua eriopoda* (Havstad and Schlesinger, 2006). SGRC is located in northern Colorado, with the warm season perennial grass *Bouteloua gracilis* as the dominant cover (Lauenroth and Burke, 2008). KNZ is an LTER in eastern Kansas, with *Andropogon gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium* as the dominant vegetation species (Knapp, 1998). Each grassland has distinct soil types, mean annual precipitation (MAP) and other climatic characteristics (Table 1).

Experimental design

In order to simulate altered precipitation conditions, we used automated rainfall manipulation systems consisting of rainout shelters paired with irrigation that allowed for reduction and increase of incoming rainfall at each site (Gherardi and Sala 2013). Rainout shelters were constructed at each site in 2016 on relatively flat areas with vegetation representative of the site, and without cattle grazing. These shelters were maintained until 2017, thus allowing for the manipulations to occur for two growing seasons. Each year, the shelters were dismantled at the end of the growing season to avoid deterioration by snow and wind during the winter. The blocked incoming precipitation was stored in an accompanying tank that served as the source for water distribution via a solar-powered irrigation system (Gherardi and Sala, 2013). This design allowed for the implementation of five precipitation levels: extreme and moderate additions, extreme and moderate reductions, and a control (with no modifications to annual rainfall). Based on long-term historical precipitation extremes unique to each site, the first and 10th percentile of long-term precipitation for the rainfall reduction treatments and the 90th and 99th percentile of long-term precipitation for the rainfall addition treatments were distributed following the current manipulative experiment recommendations (Knapp et al., 2017). Thus, the reduction treatments relative to the control resulted in a reduction of 80 and 50% in the arid site, by 70 and 40% in the semiarid site, and 60 and 30% in the mesic site. The addition treatments added increases of 150 and 180%, 140 and 170%, and 130 and 160% in the three sites respectively (Table 1). The five treatments that received growing-season precipitation were randomly assigned to plots (5 x 2.5 m) with eight replications of each treatment resulting in 40 plots in total with the treatments being applied to the same plots during the growing seasons of 2016 and 2017. This research is one part of a larger experiment studying primary productivity, root herbivory, and soil nematode abundance and body size responses to precipitation (Andriuzzi et al., 2020; Ankrom et al., 2020; Franco et al., 2019).

Soil and nematode collection

Using a 2.5 cm diameter soil corer, four sub-samples were taken to a depth of 10 cm directly beneath the dominant vegetation of each plot at the end of the 2016 and 2017 growing seasons starting with the semiarid site, then the mesic, and finally the arid site, to be consistent with the rainfall patterns per site. The sub-samples were combined to form one composite sample for each plot within a site. To avoid cross-contamination, the soil corer was cleaned with 70% ethanol between plots. The soil samples were transported in coolers to Colorado State University for storage at 4°C. Nematodes were extracted from soils within 5 days of arrival.

Nematode abundances were calculated as the number of individuals per kg of dry soil; soil moisture was calculated by drying 50 g of soil for each sample at 105°C for 48 hours.

Nematode extraction and identification

Nematode extraction was conducted with the Baermann method, using approximately 100 g of soil per sample (Hooper, 1970). Solution samples (20 ml) were collected daily for 72 h and stored at 4°C (total of 60 ml nematode solution per plot). Before counting and identification, the nematode solution was allowed to settle for 30 minutes so any nematodes would collect at the bottom, and excess water was vacuumed off until the remaining five ml of solution could be poured into lined counting dishes to be viewed with an inverted microscope (Olympus CKX41) at 200x magnification. The whole dish of nematode taxa was counted and identified to trophic groups (Yeates et al., 1993). For identification to genus, nematode solutions were preserved in 5% formalin (Southey, 1986). Total nematode population density was determined and the first 100 plant parasites and first 150 free living nematodes encountered were identified to genus and then adjusted to the absolute abundance per kg by adding up totals from each nematode genus identified.

The nematodes were classified according to their colonizer-persister (cp) scale to analyze community indices (Bongers, 1990). Life strategies are often categorized by a cp scale that ranges from 1 to 5, where nematodes deemed a '1' represent opportunists that recover quickly when stressed and reproduce rapidly, while those designated as a '5' are, broadly speaking, K-strategists that are intolerant to stress and have longer generation times (Ferris et al., 2001). Nematode genera were assigned to feeding groups based on Yeates et al. (1993).

Nematode genera data was then utilized to calculate the MI (Bongers, 1990), PPI (Bongers et al., 1997) as well as the EI, BI, SI, and CI (Ferris et al., 2001). The value of the MI denotes the disturbance level experienced by the environment; with a low value expressing a highly disturbed system (Bongers, 1990; Neher and Campbell, 1996). Plant parasite index is similar to the MI, but only considers nematodes feeding on higher plant forms. The EI reveals the flow of basal resources (i.e. carbon, nitrogen); wherein a high EI would indicate a large proportion on opportunistic bacterivores and fungivores which respond rapidly to increases in available resources. The BI measures the bacteria- and fungal-feeding nematodes in the cp grouping, thus a high value indicates poor ecosystem health. The SI denotes the food web complexity; where a high SI demonstrates a complex food web with greater representation of long-lived omnivores and predators, reflecting greater stability in the community as well as a more restored system. The CI expresses whether the decomposition pathway is dominated by bacteria (low CI) or fungi (high CI) (Ferris et al., 2001).

Statistical analysis

The effects of altered precipitation on nematode communities were determined by testing received growing-season precipitation, site, and their interaction on nematode ecological indices. The model did not include year as a factor as the variability in precipitation between the two experimental years and five rainfall manipulation treatments were combined to create ten levels of received precipitation to be used as continual explanatory variable without the distinction of years. Data were analyzed using linear mixed-effect models (LME) and plot identity was used as a random effect to account for repeated measurements in each plot, which justifies the lack of independence among years (Bates et al., 2015). All assumptions for homogeneity of variance and normality of residuals were met and visual inspection of predicted plots, Levene's test, QQ plots, Shapiro-Wilk test were done. Methods by Nakagawa and Schielzeth (2013) were used to determine the conditional r2. These analyses were all conducted using R software, version 3.2.2 (R Core, 2014), with the following packages; "vegan"(Oksanen et al., 2019), "piecewiseSEM" (Bartoń 2009), and package "ggplot2" was used for data visualization (Wickham 2016). The effects of altered precipitation on nematode communities were determined by testing received growing-season precipitation, site, and their interaction on nematode ecological indices.

Indicator analysis was done using the “labdsv” package (Roberts 2007) to identify genera that were characteristic of the three sites and the applied precipitation levels (Dufrêne and Legendre 1997). This analysis calculates an indicator value for each genera, where “0” represents no indication and “1” represents the perfect indication of a habitat (site and applied precipitation level). The indicator value is based on the combination of abundance and relative frequency of occurrence of each genera (Dufrêne and Legendre, 1997). Nematode genera that were deemed indicators using this analysis (genera with a significant indicator value ≥ 0.3) were used in the Non-metric multidimensional scaling (NMDS) ordination using the Bray-Curtis distance coefficient and Permutational multivariate analysis of variance (PERMANOVA) analysis conducted in the “vegan” package (Oksanen et al. 2019) to determine dissimilarity among sites, precipitation treatments, and the nematode community. To better visualize the NMDS groupings the applied precipitation levels were grouped into “Reduction”, “Control”, and “Addition” categories.

4.4 RESULTS

Precipitation effects on soil nematode ecological indices

Most nematode ecological metrics were significantly affected by changes in precipitation (Fig. 1 and 2). The MI was significantly influenced by both site and precipitation level as well as the interaction between the two ($P_{\text{Received precip.}} < 0.001$, $P_{Site} < 0.001$, $P_{\text{Interaction}} < 0.001$, $R^2 = 0.448$; Fig. 1A), such that MI increased with precipitation in the mesic site, but was negatively impacted by precipitation in the semiarid site and arid site. The opposite trend was found for the PPI ($P_{\text{Received precip.}} < 0.001$, $P_{Site} < 0.001$, $P_{\text{Interaction}} = 0.027$, $R^2 = 0.56.6$; Fig. 1B), where increased precipitation in the arid site resulted in a decrease in PPI, but increases in the semiarid and mesic sites. The EI varied with both site and precipitation ($P_{\text{Received precip.}} < 0.001$, $P_{Site} < 0.001$, $P_{\text{Interaction}} = 0.096$, $R^2 = 0.765$; Fig 2A), generally decreasing in response to increased precipitation at the arid site and increasing at the semiarid site, with no effect of precipitation at the mesic site. The BI was significantly influenced by the interaction of received precipitation and site ($P_{\text{Received precip.}} < 0.001$, $P_{Site} < 0.001$, $P_{\text{Interaction}} < 0.001$, $R^2 = 0.384$; Fig. 1B), showing higher values with increased precipitation in the two drier sites, but lower values in the mesic site. The CI also responded to site and precipitation and displayed a significant interaction ($P_{\text{Received precip.}} = 0.002$, $P_{Site} < 0.001$, $P_{\text{Interaction}} = 0.001$, $R^2 = 0.687$; Fig. 1D), having values increase in both the arid and mesic sites in response to increasing precipitation, but decreased significantly in the semiarid site. The SI followed a similar trend to the MI, revealing an increase in the index only at the mesic site, but decreased in both the arid and semiarid sites with increasing precipitation ($P_{\text{Received precip.}} < 0.001$, $P_{Site} < 0.0001$, $P_{\text{Interaction}} < 0.001$, $R^2 = 0.310$; Fig. 1C).

The environmental gradient used in this study comprises three grassland types of large area cover (≈ 135 M ha combined) and represents the largest spatial scale in which belowground multitrophic responses to precipitation changes have been experimentally studied. It must be noted therefore that running the experiments over two years to produce a wide response surface (precipitation gradient) that increased statistical power may also have produced possible legacy effects (Fig. S1). Further there were interannual differences in precipitation and nematode responses. For example, in KNZ the second year was much drier than the first, and nematode responses tended to be stronger in that year.

Description of indicator genera results

A total of 72 genera (20 bacterivore, 7 fungivore, 14 omnivore, 23 plant parasitic, and 8 predatory nematodes; Table SI) from 35 families were identified from the three sites. Indicator species analysis identified 39 genera characteristic of the precipitation treatments; 22 genera associated with the arid site, three with the semiarid site, and fourteen with the mesic site. Some endemic genera to the mesic site were found by the analysis to be indicators such as

Trichodorus. Indicator values (IndVal) were computed for each nematode genera and site, and tested by a Monte Carlo test. Table 2 summarizes all the values for the three sites from "IndVal". After 2 years of manipulated precipitation within the three grassland sites, testing based on the Bray-Curtis distance showed indicator nematode communities differed significantly based on site ($P_{Site} = 0.001$, $R^2 = 0.370$; Fig 2). The NMDS also revealed that the applied precipitation treatments significantly affected nematode genera distribution, most easily discerned are the reduction treatments separating nematode genera known to be drought tolerant from the rest of the nematode community ($P_{\text{Recieved precip}} = 0.001$, $R^2 = 0.728$; Fig 2).

4.5 DISCUSSION

Ecological indices in the arid site

Our findings did not fully support our expectation that shifts in ecological indices would be absent from the arid site, as desert environments have previously shown little effect on nematode abundance or ecological makeup in response to altered available soil moisture (Freckman et al., 1987; Sylvain et al., 2014; Vandegehuchte et al., 2015). Contrary to our hypotheses, increased precipitation at the arid site resulted in reduced maturity, enrichment, and structure of the nematode communities, as shown by decreases in MI, EI, and SI. This finding indicates a nematode assemblage that is experiencing a disturbed environment and is part of a less complex food web (Ney et al., 2019), a finding bolstered by the increase in BI. In response to greater precipitation the arid site displayed a lower PPI, largely driven by the lower abundances of *Pratylenchus* and *Tylenchorhynchus,* both genera extremely sensitive to moisture (Kandel et al., 2013; Ruan et al., 2012). A lower EI in response to greater precipitation suggests that fewer opportunistic nematodes are present (Ferris et al., 2001); mainly due to the decreases in *Rhabditis* nematodes; a cp-1 bacterivore, (Ferris and Bongers, 2006) deemed an indicator genus in the arid site (Table 2). Despite the decline of enrichment opportunists the BI increased with increasing precipitation, showing a soil food web sensitive to received precipitation (Ferris et al., 2001). This finding contrasts with previous findings from this same site focused on nematode trophic levels (Franco et al., 2019) that showed little change in abundance of nematode trophic groups in response to increased precipitation. The CI did increase slightly with received precipitation, indicating a shift toward the fungal decomposition pathway. This was surprising, given the slight negative response of fungivores to increased precipitation observed at this site (Franco et al., 2019). The lower EI and higher CI indicates that in response to more precipitation more organic matter will be decomposed through the fungal energy channel (Sánchez-Moreno et al., 2010). As no predators were found in the arid site (Franco et al., 2019), the decreases in the MI and SI are driven mostly by the decline of important omnivore genera such as *Eudorylaimus,* which had its greatest abundances in the control and drought treatments (Table SI). The decline in SI (decreased higher trophic nematodes) and slight rise in the CI (decrease in opportunist bacterivores) in response to increased precipitation indicates a food web that is severely disrupted (Yeates and Bongers, 1999). Bacterivores deemed to be enrichment-opportunist are very efficient in nutrient mineralization, especially N (Freckman, 1988), while omnivorous and predatory nematodes are often correlated with concentrations of many other soil nutrients (Ferris and Matute, 2003). Thus, the loss of these groups in the arid grassland due to increased precipitation has consequences for plant health as well as the stability of the ecosystem. Namely, that in the longterm plant parasites without predator pressure could decrease plant health (Franco et al., 2019). Our results in the arid site contrast with previous work where applying water pulses to a desert grassland, resulted in no change in nematode community at the broad trophic-group level, suggesting that such nematodes are resistant and can remain stable despite environmental variability; likely due to survival strategies for dehydration (Freckman 1986). The arid site findings do partially align with results from a climatically similar desert steppe in Inner Mongolia by Zhang et al. (2020), where a switch from the bacterial to fungal decomposition channel occurred when precipitation increased. However; this same study also showed increasing precipitation led to a more connected soil food web with greater food chain lengths

(Zhang et al., 2020), whereas our results show an inverse relationship between SI and precipitation. The changes of the nematode community in response to varied precipitation also differs from previous results from this same arid site that showed no response from nematode trophic groups (Sylvain et al., 2014) and a study that only showed a positive response from plant parasites and omnivores (Vandegehuchte et al., 2015). Nematodes from the arid site may be more tolerant to water stress (Freckman et al., 1987; Vandegehuchte et al., 2015), but with increased precipitation these communities will be less structured in terms of trophic interactions, which may have long-term consequences for nutrient losses in the system as nematode predation releases excess for nutrients, primarily as plant available amino acids, NH_4^+ and PO_4^{-3} (Ingham et al., 1985). The evidence from our arid site, where nematode community composition was modified by a reduction in omnivorous nematodes with increasing precipitation (Franco et al., 2019), suggests that these systems are more susceptible to environmental stress (Bongers and Bongers, 1998). This differential response of ecological indices to trophic groups highlights the value of generic level descriptions for gaining insight to the broader community response to climate change and outlining a more likely scenario for the nematode community that will result from altered precipitation. This insight can lead to more specific management strategies as it could be necessary to manage the cp-1 nematodes in the arid site by applying labile inputs frequently to avoid the shifts in EI, BI, and CI (Ferris and Matute, 2003) that would potentially lower mineralization and lead to lower levels of soil fertility in an already stressed system.

Ecological indices in the semiarid site

In contrast to our first hypothesis, the semiarid site exhibited a nematode community that was less mature, more enriched, less structured, and shifted toward a bacterial decomposition pathway when exposed to increasing precipitation. The rise in PPI in response to greater precipitation aligns with previous studies (Ruan et al., 2012; Zhang et al., 2020) and is reflected in our study by enhanced abundances of *Paratylenchus* and *Helicotylenchus* which represented a large proportion of the total plant parasite population (Ankrom et al., 2020) as well as higher cp plant parasite *Hoplolaimus*. The increase in EI and BI could be associated with the decrease in CI, as both a greater population of cp-1 bacterivore *Acrobeloides* and a lower population of cp-2 fungivore *Aphelenchoides* were found under increased precipitation in the semiarid site. This finding supports previous work from Sohlenius (1985) that found high densities of *Acrobeloides* and a low density of *Aphelenchoides* under wet conditions explaining the different response to be due to a change in the food supply as both genera respond rapidly to increased food availability (Forge et al., 2005; Sohlenius, 1985). Soil bacteria are generally resilient in response to drought and precipitation treatments (Cole et al., 2019; Cruz-Martínez et al., 2009; Zhang et al., 2016), while fungi are relatively sensitive to drought in less arid sites (Ochoa-Hueso et al., 2018), which could also help explain the increase in the aforementioned bacterivores in the wetter treatments. The increase in EI and BI and decrease in CI with increasing precipitation aligns with work in the Inner Mongolian Steppe that reported greater abundances of bacterivores in response to water additions (Ruan et al. 2012). The decrease in SI with increased precipitation reflects a community with fewer omnivores and predators, aligning with findings that showed a lack of response in omnivores and decrease in predators within this same site (Franco et al., 2019). The negative response of the MI and SI to increasing precipitation seems to be driven solely by the abundance of *Aporcelaimellus*, a cp 5 omnivore that declined in abundance under the addition treatments (Table SI). The semiarid site, seeing a reduction in MI and SI with increased precipitation, may actually continue to increase the EI; these higher cp nematodes feed on and regulate the abundance of opportunist nematodes (Ferris, 2010) leading to a positive feedback loop where the decrease in SI increases the EI.

Ecological indices in the mesic site

As predicted, the mesic site became more mature and structured, and shifted toward a fungal decomposition channel when precipitation was increased. Increased precipitation led to a decrease in PPI in the mesic site which opposes a finding in the same site that saw large increases in plant parasites with increased soil moisture (Todd et al., 1999); however, this difference is certainly driven by the lowered abundances *Trichodorus*, *Xiphinema* and,

Longidorus. The weak effect seen in the EI and BI is likely due to the low populations of *Panagrolaimus* and *Panagrellus* as well as the slight alterations in *Rhabditis* populations in response to any precipitation treatments. The mesic site exhibited a greater MI and SI in response to increasing precipitation, indicating an environment that supports enhanced soil food web complexity, and suggests a more stable and resilient soil ecosystem (Bongers, 1990; Neher, 2001). Additionally, increases in abundance of long-lived and higher cp omnivores *Eudorylaimus* and *Paraxonchium*, as well as predatory *Discolaimium* (Table SI) nematodes further point to increased ecosystem stability and resilience. This marked shift in ecological indices at the mesic site is likely due to multiple trophic interactions, associated with the greater abundance of omnivorous and predacious nematodes feeding on lower cp nematodes. Water additions having positive effects on the stability and maturity of the mesic soil food web does align with a study from an agroecosystem with comparable MAP (704.2 mm) in China that showed severe drought to decrease MI and SI (Yan et al., 2018). The decomposition channel switch from bacterial to fungal dominance is also supported by *Aphelenchoides* having a greater abundance in the water addition treatments (Table SI).

Nematode indicators response to precipitation treatments across sites

In this study we found differences in nematode indicator composition between levels of applied precipitation and grassland sites, revealing that specific grasslands will see shifts in certain genera that are characteristic of each site with changing precipitation regimes, which may lead to altered decomposition, nutrient cycling, and even C sequestration. A study focused on the link between nematodes and microorganisms in mediating SOC turnover in soil macroaggregates found that an increase in bacterivores led to greater grazing pressure on microorganisms resulting in microbial-derived SOC turnover (Jiang et al., 2018). This finding of bacterivore driven C dynamics places importance on the indicator results from the arid site; as both *Eucephalobus* and *Acrobeloides* are known to be adapted to dry climates (Griffiths et al. 1995, Bakonyi and Nagy 2000) and *Plectus* has shown variable responses to precipitation, by being enhanced with drought in an agroecosystem (Yan et al. 2018) and by wetter conditions in a citrus field (Porazinska et al. 1999). The indicator bacterivores had their greatest abundance under drought treatments (Table 2; Table SI) and as this arid site is likely to experience more intense rainfall patterns, any decline in important bacterivores would affect C metabolism shifting it toward the fungal channel even more. An indicator omnivore, *Aporcelaimellus*, declined in both drought and addition treatments, a variable response that that aligns with previous work (Porazinska et al., 1998; Yan et al., 2018) and shows its sensitivity to altered precipitation. When *Aporcelaimellus* decreases in abundance the maturity and structure for the semiarid community also decreases; and with its reduction the PPI increases as top down control is lessened (Franco et al., 2019). *Pratylenchus* an indicator genera for the mesic site declined under addition treatments, with this grassland expected to experience greater drought it's likely the rise in PPI associated with greater *Pratylenchus* populations will lower the nematode community stability.

As for site effects on nematode genera there was a clear separation of the mesic site community from the drier grasslands. This finding contrasts with our hypothesis that the arid site would respond uniquely to altered precipitation, and appear to differentiate more from the wetter grasslands. However the arid nematode community and the semiarid nematode community share many more genera as displayed by a large overlapping polygon seen in the NMDS (Fig. 2). There are more unique genera that were found in the mesic site. It is these genera such as *Trichodorus*, a plant parasite known to be sensitive to abiotic factors (Bor and Kuiper 1966) that drive this separation from our other sites displayed in the NMDS.

4.6 CONCLUSION

This experiment as part of a larger collaboration study has now revealed the response of nematode predator-prey interactions (Franco et al., 2019), those nematodes tightly linked to decomposition (Andriuzzi et al., 2020), and herbivory (Ankrom et al., 2020) to climate change across three grasslands. Our current observation shows that altered precipitation affected nematode assemblages as well as nematode indices suggesting key ecosystem functions influenced by these nematodes (e.g., nutrient mineralization, herbivory, C cycling) will also be affected. With more extreme precipitation events likely to occur across these grasslands (Reidmiller et al. 2017), changes in nematode community dynamics and functionality may result in cascading effects for host plants and the soil ecosystem at large. The greater drought periods predicted for the mesic grassland showed an overwhelming effect on the soil nematode community resulting in altered energy channels, simplified food web structures, and a general decrease in maturity. Through nematode community analysis we observed strong influences on the soil food web caused from extreme and moderate precipitation manipulations. This finding features the importance of genera level resolution and suggests that the sensitivity of these indices allows for ecological interpretation of belowground function and status, which is especially pertinent as these grasslands will not respond to precipitation alterations similarly and will therefore require unique mitigation strategies. Including long term observational studies of nematodes at regional grassland sites such as in this study can also be linked to how plant health will respond to nematodes with future climate changes.

4 FIGURES AND TABLES

Table 4-1. Site characteristics for the Jornada Basin LTER, NM, Semiarid Grasslands Research Center, CO, and Konza Prairie LTER, KS. Mean annual precipitation and long-term mean for growing-season precipitation from NOAA climate data of nearby localities (Las Cruces [New Mexico], Nunn [Colorado], and Manhattan [Kansas]).

	Jornada Desert	Shortgrass Steppe	Konza Prairie
Grassland type	Arid	Semiarid	Mesic
Latitude	32°33'N	40°50'N	39°4'N
Longitude	106°49'W	104°45'W	96°34'W
Mean annual precipitation (mm)	245	321	835
Mean growing-season precipitation (mm)	105	204	428
Mean annual temperature (°C) a	14.7	8.4	12.5
Rainfall treatments (relative to ambient) Large reduction Moderate reduction Moderate addition Large addition	-80% -50% +50% +80%	-70% -40% +40% +70%	-60% -30% +30% +60%
Soil texture class	Fine sandy loam	Fine sandy loam	Silty clay loam
USDA soil type	Aridisols	Mollisols	Mollisols

Soil characteristics refer to the top 10 cm and are from the USDA Soil Survey.

Table 4-2. Indicator values for nematode genera for the three grassland sites. The displayed values range from 0 (no indication) to 1 (perfect indicator).

Site	Season Precip	Genera	Indicator Value	P Value	Abbreviation
Mesic	Control	*Achromadora*	0.567	0.005	Ach
Arid	Control	*Acrobeles*	0.338	0.005	Abe
Arid	Reduction	*Acrobeloides*	0.323	0.01	Abo
Arid	Reduction	*Anaplectus*	0.513	0.005	Ana
Arid	Reduction	*Aphelenchoides*	0.305	0.005	Apl
Arid	Reduction	*Aphelenchus*	0.371	0.04	Aph
Semiarid	Addition	*Aporcelaimellus*	0.318	0.04	Apo
Mesic	Addition	*Basiria*	0.440	0.005	Bas
Arid	Addition	*Cephalobus*	0.391	0.005	Cep
Arid	Addition	*Cervidellus*	0.366	0.005	Cer
Arid	Addition	*Chiloplacus*	0.398	0.01	Chi
Mesic	Control	*Coslenchus*	0.520	0.005	Cos
Mesic	Addition	*Criconema*	0.341	0.01	Cyl
Arid	Reduction	*Diphtherophora*	0.457	0.005	Dip
Arid	Reduction	*Dorydorella*	0.366	0.005	Dor
Arid	Reduction	*Ecumenicus*	0.468	0.005	Ecu
Mesic	Addition	*Epidorylaimus*	0.427	0.015	Epi
Arid	Addition	*Eucephalobus*	0.333	0.01	Euc
Arid	Reduction	*Eudorylaimus*	0.420	0.005	Eud
Mesic	Control	*Eumonhystera*	0.419	0.005	Eum
Mesic	Addition	*Gracilacus*	0.332	0.05	Gra
Mesic	Addition	*Helicotylenchus*	0.320	0.01	Hel
Mesic	Addition	*Longidorus*	0.449	0.005	Lon
Mesic	Addition	*Mesocriconema*	0.532	0.005	Mec
Mesic	Control	*Mylonchulus*	0.461	0.005	Myl
Semiarid	Addition	*Panagrolaimus*	0.437	0.005	Pan
Semiarid	Reduction	*Panagrellus*	0.528	0.005	Pag
Arid	Control	*Paraphelenchus*	0.338	0.035	Pap
Semiarid	Reduction	*Paratrophorus*	0.480	0.005	Pat
Mesic	Addition	*Paratylenchus*	0.336	0.03	Par
Arid	Control	*Paraxonchium*	0.492	0.005	Pax
Arid	Addition	*Plectus*	0.450	0.005	Ple
Arid	Addition	*Pratylenchus*	0.302	0.045	Pra
Mesic	Addition	*Prismatolaimus*	0.414	0.005	Pri
Arid	Reduction	*Rhabditis*	0.325	0.03	Rha
Arid	Addition	*Rhabdolaimus*	0.338	0.025	Rhd
Arid	Control	*Subanguina*	0.327	0.005	Sub
Arid	Reduction	*Thonus*	0.314	0.005	Tho
Arid	Addition	*Torumanawa*	0.442	0.005	Tor

Mesic Control *Trichodorus* 0.435 0.005 Tri

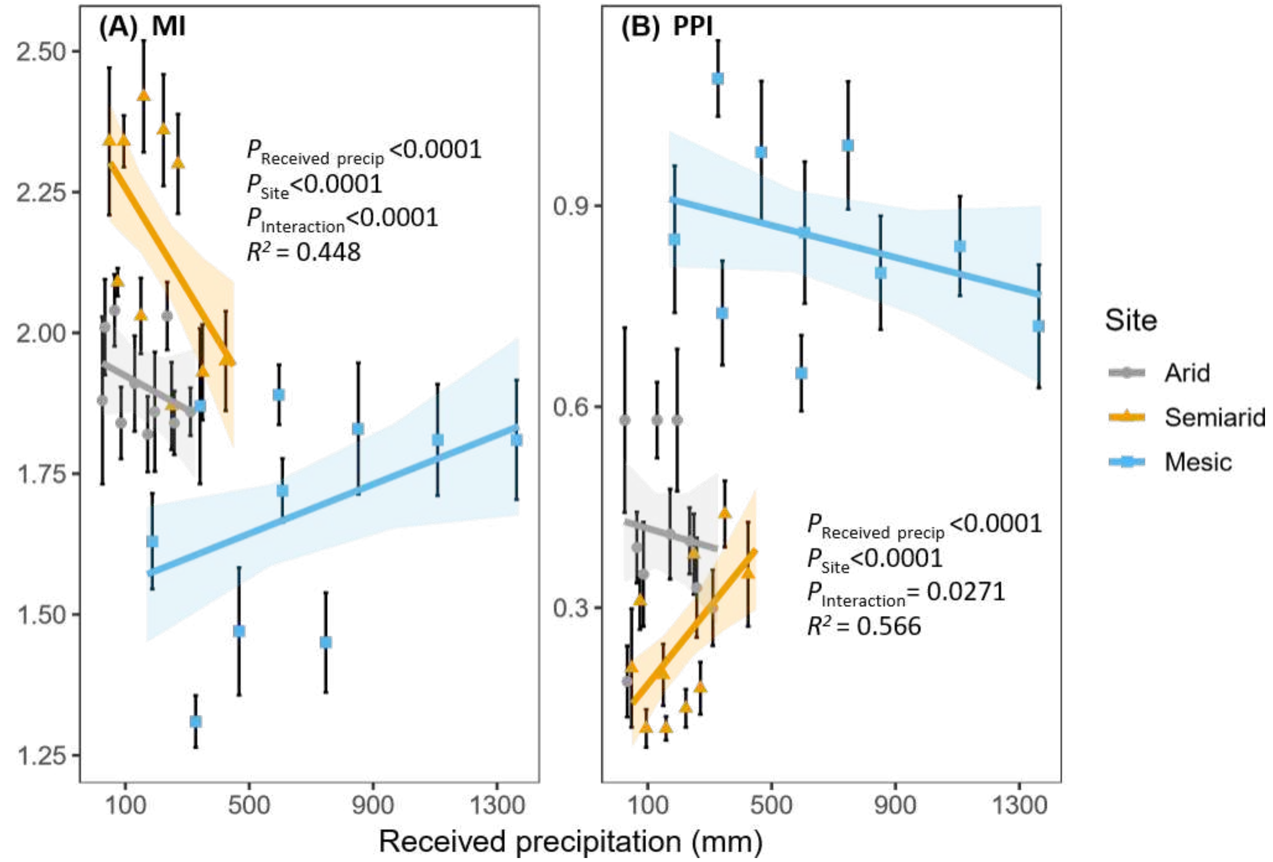

Figure 4-1: Nematode maturity indices in response to increasing precipitation. Functional nematode indices (y axis) in response to precipitation treatments (x axis). Legend represents the grassland sites. Color- and shape-coded trend lines represent effects of manipulated precipitation (n = 8 per treatment in each site) predicted by mixed effects models for the three different sites. Shaded regions indicate 95% confidence limits. Points represent mean abundance of index per precipitation level (n = 16), and error bars represent standard error of the mean. (A) Maturity Index; (B) Plant Parasite Index. For all tests n = 240

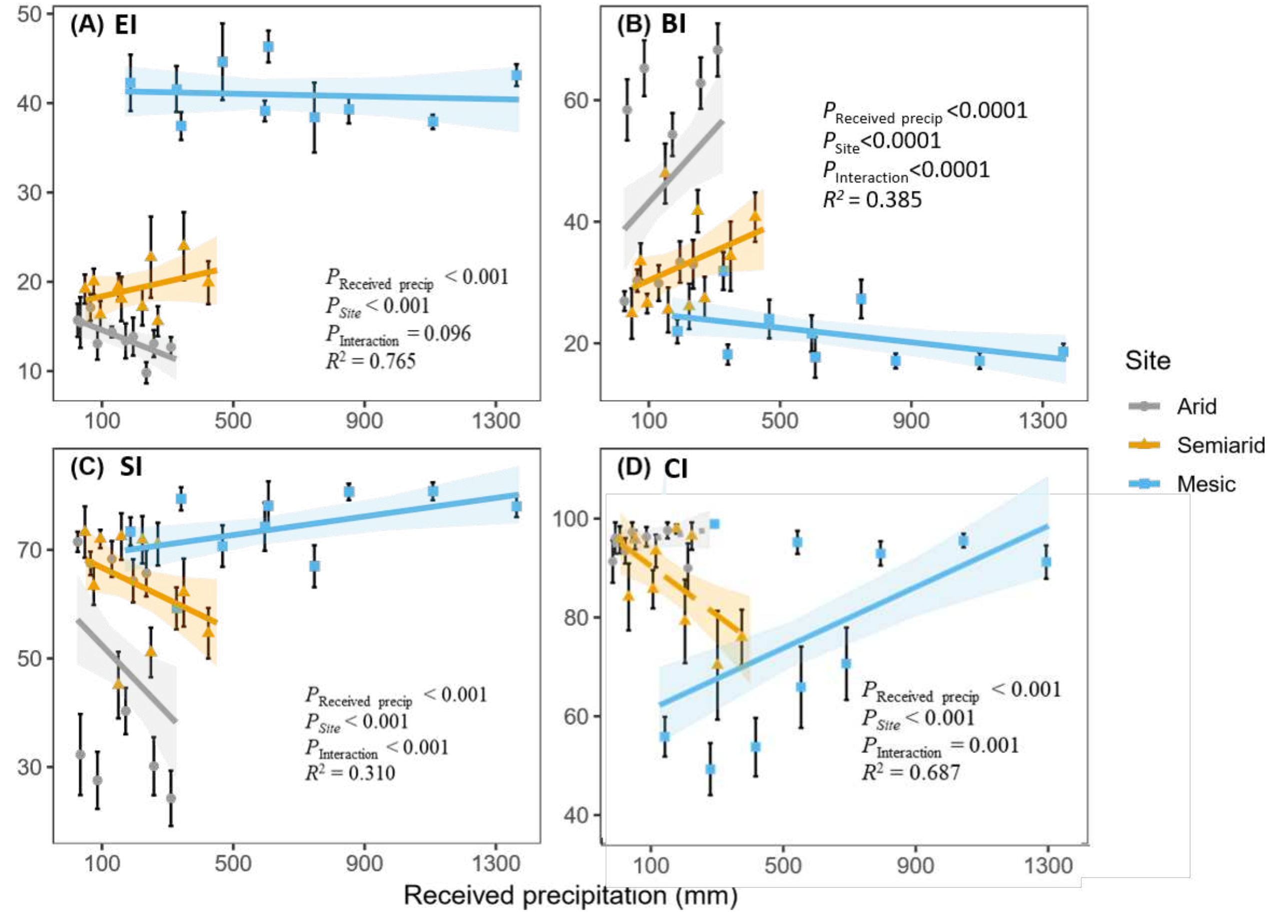

Figure 4-2: Nematode ecological indices in response to increasing precipitation. Functional nematode indices (y axis) in response to precipitation treatments (x axis). Legend represents the grassland sites. Color- and shape-coded trend lines represent effects of manipulated precipitation (n = 8 per treatment in each site) predicted by mixed effects models for the three different sites. Shaded regions indicate 95% confidence limits. Points represent mean abundance of index per precipitation level (n = 16), and error bars represent standard error of the mean. (A) Enrichment Index; (B) Basal Index; (C) Structure Index; (D) Channel Index. For all tests *n* = 240.

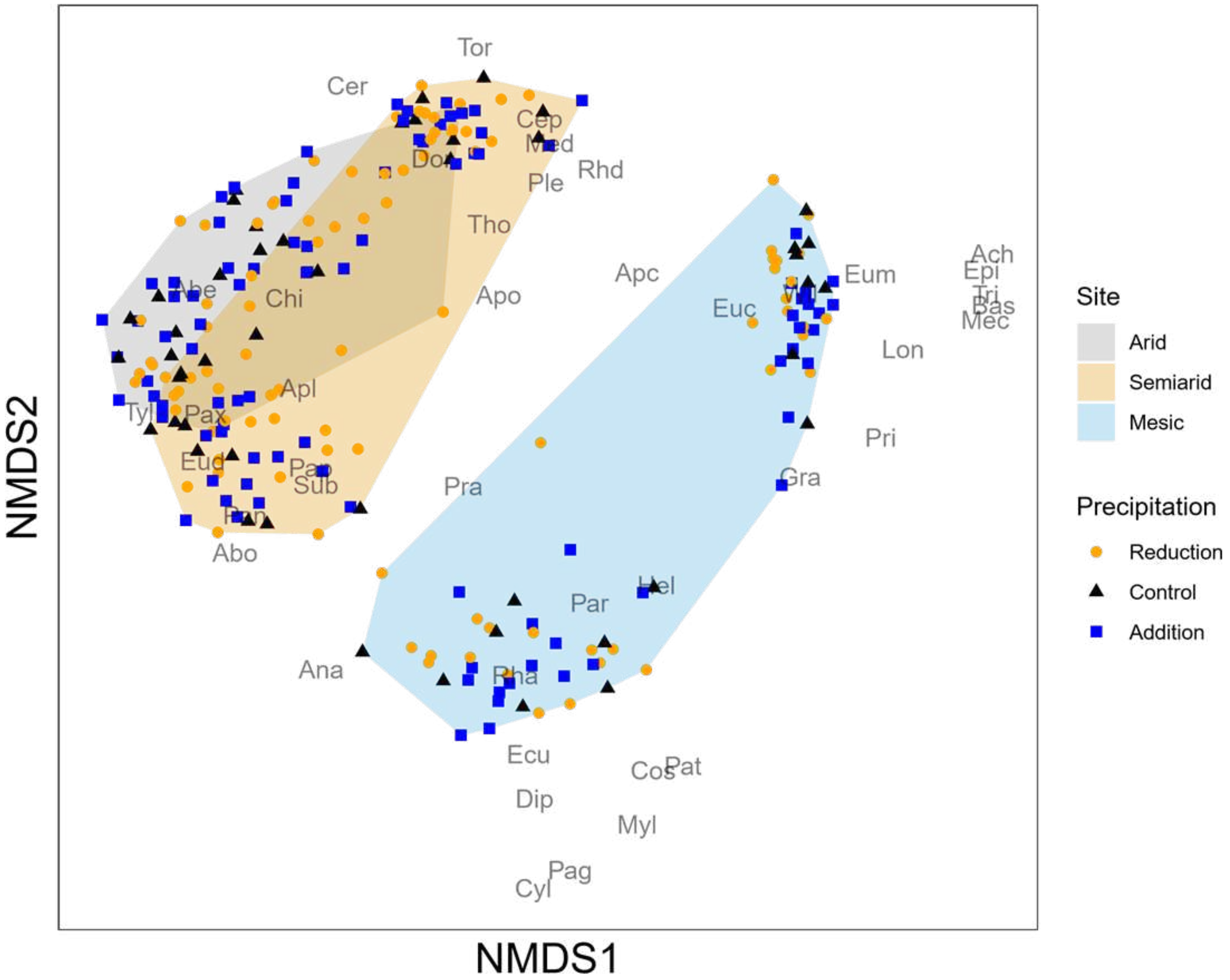

Figure 4-3: Nonmetric multidimensional scaling plot of nematode genera as a function of received growing season across the three sites. Each point reflects the community found in each plot (n = 240) from both years of the study (2016 and 2017). Points that are close together have more similar communities than points that are far apart. Names of nematode genera are overlaid. See Table 2 for abbreviation. Polygons enclose points representing each site (colored). PERMANOVA results ($P_{\text{Recieved precip}}$ = 0.001, F=19.285, r^2 = 0.728, P_{Site} = 0.001, F=141.717, R^2 = 0.370).

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CHAPTER 5 – CONCLUSIONS

This dissertation advances our understanding of the soil nematode community response to climate change in grassland ecosystems. By utilizing trophic and functional groupings of soil nematodes from field experiments across a precipitation gradient, I answered three questions (see Chapter 1) that aimed to reveal the structure of the soil food web and how its status may impact the stability of a vulnerable ecosystem.

Identifying nematodes trophic abundances in the Shortgrass Steppe of Colorado allowed us to understand to what extent differing types of vegetation cover are associated with different nematode food web structures as well as to understand the dominant decomposition pathway (Chapter 2). This study not only showed differences among nematode communities under cover types, but highlighted a potential threat of *E. cicutarium* invasion of the dominant *B. gracilis* is associated with lower abundances of fungivores and omnivore/predators. Lowered abundances of these two trophic groups would of course lead to impacts on the regulation of the soil food web (e.g. decomposition of nutrient cycling), particularly predator control of plant parasites (Yeates et al. 2007, Franco et al. 2019). Should changes to the dominate vegetation makeup occur in water-limited grasslands there will likely be major shifts in nematode community composition affecting not only the soil nematode food web structure and function, but also carbon and nutrient cycling of a globally important ecosystem.

The identification of plant parasite feeding type, functional guild, and calculation of herbivory index (Bongers 1990; Ferris, Bongers, and De Goede 2001; Preisser and Strong 2004; Yeates et al. 1993) presented in Chapter 3 showed varied responses to increased precipitation of endo- and ectoparasites across grasslands, a focus of the nematode community not previously reported. The localized damage caused by PPN feeding increases host vulnerability to bacterial and fungal infection (Henderson and Clements 1977; Bach et al. 2002), with certain endoparasitic species seen as more damaging due to their feeding habit of migrating through host tissue, which further limits the host's ability to induce defense mechanisms (Bird and Bird 2001; Jones et al. 2013). The interplay between changing precipitation and PPN feeding is an important, but poorly understood aspect of grassland responses to climate change. The results of this chapter expands our knowledge of this relationship and outlines important implications for grassland productivity as plant parasitic nematodes contribute greatly to the consumption of primary production in terrestrial systems. This understanding of the status of plant parasite pressure in grasslands is a necessary confirmation to determining the threat of climate change on hosts plants.

By using soil nematodes as biological indicators in arid, semiarid, and mesic grasslands experiencing large shifts in precipitation we confirmed (Chapter 4) the sensitivity of nematode community structure to interpret ecological responses in natural settings. Further exploration of the nematode community analysis revealed that certain genera were indicative of both sites and precipitation treatments; meaning individual genera can reveal strong influences on the soil food web caused from extreme and moderate precipitation manipulations. This experiment as part of a larger collaboration study (Andriuzzi et al. 2020; Ankrom et al. 2020; Franco et al. 2019) shows that altered precipitation affected nematode assemblages as well as nematode indices suggesting key ecosystem functions influenced by these nematodes (e.g., nutrient mineralization, herbivory, C cycling) will also be affected. With more extreme precipitation events likely to occur across these grasslands (Reidmiller et al. 2017), changes in nematode community dynamics and functionality may result in cascading effects for host plants and the soil ecosystem at large. These results along with those found in Chapter 3 provide more evidence that response to climate change in grasslands requires both the distinction of genera level identification and cross-site studies to confidently uncover the ecological status of grassland stability in the face of extreme precipitation alteration.

Overall, the results of this dissertation contribute to the understanding of soil nematode communities in grassland ecosystems and provide substantial data and interpretation to the use of soil nematodes as bioindicators.

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