

THESIS

THE EFFECTS OF GRASSHOPPERS ON SOIL ANIMAL COMMUNITIES IN THE
SHORTGRASS STEPPE OF NORTHERN COLORADO

Submitted by

Keith Harrison Post

Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2015

Master's Committee:

Advisor: Diana H. Wall

Alan K. Knapp

Boris C. Kondratieff

Troy W. Ocheltree

Copyright by Keith Harrison Post 2015

All Rights Reserved

ABSTRACT

THE EFFECTS OF GRASSHOPPERS ON SOIL ANIMAL COMMUNITIES IN THE SHORTGRASS STEPPE OF NORTHERN COLORADO

A burgeoning area of research in ecology is on the linkages between aboveground and belowground components of terrestrial systems. Leaf-feeding insects can affect soil communities directly via frass deposition or indirectly through alterations in the quantity or composition of plant roots or the amount of labile carbon exuded belowground. These pathways can affect the three soil energy channels (i.e., root, soil bacterial, and soil fungal) by altering the absolute and/or relative amounts of their source materials and, in turn, impact soil microbial community composition and higher trophic levels, including soil nematodes and microarthropods. This aboveground-belowground interaction is important to fully understanding the functioning of terrestrial ecosystems, especially in the context of global climate change.

This study investigated the effects of short-term grasshopper exclusion in the shortgrass steppe of northern Colorado on plant abundance and temporal changes in trophic groups of soil animals. Above- and belowground plant biomass, soil nematode, and soil microarthropod responses to altered grasshopper abundances were determined using grasshopper exclosures and caged controls from late summer–early fall 2014. Plant community composition during the study was drastically different than long-term data. *Bouteloua gracilis*, a co-dominant grass, was reduced to an average of 5.75% of total aboveground biomass, whereas the typically rare, annual grass *Vulpia octoflora* exploded to

over 93%. Total above- and belowground plant biomass and aboveground biomass from *V. octoflora* and other grasses (mainly *B. gracilis*) were unaffected by grasshopper exclusion. Grasshopper feeding enhanced the ratio of bacterivorous to fungivorous nematodes, which remained similar through time in exclosures. Proportions of bacterial-feeding nematodes increased in caged controls but decreased in exclosures, while there was a trend for the opposite pattern for plant parasitic nematodes. Temporal changes in the densities of soil microarthropods, mites, and mite trophic groups were similar between cage types.

Results indicate that grasshoppers enhanced the relative dominance of the soil bacterial energy channel, likely through greater frass deposition. Apparent exclosure effects on plant parasitic nematodes suggest a possible belowground plant response to altered grasshopper populations, which could have been weak because these effects were specific to the then-rare *B. gracilis*, which was about to enter senescence. Implications of this research in the context of global climate change, particularly droughts in the shortgrass steppe, are discussed.

ACKNOWLEDGMENTS

First and foremost, I thank Dr. Diana Wall for giving me the opportunity to join her laboratory and pursue a project that I had been conceiving for some time. I am also greatly appreciative of Diana's guidance and support throughout my time at Colorado State University, and I enjoyed working with someone so enthusiastic about soil ecology. I thank the remaining members of my advisory committee, Dr. Alan Knapp, Dr. Boris Kondratieff, and Dr. Troy Ocheltree, for providing feedback on proposals and advice for this research.

I am grateful for all the help provided by members of the Wall lab, past and present. I also couldn't have asked to work in a friendlier and more welcoming environment. Amber Cavin helped with field work, soil animal extractions, and plant sample processing. Dr. Matt Knox and Andre Franco assisted me in the field and with statistics. Cecilia Tomasel taught me identification of nematode trophic groups and organized help from undergraduate assistants that contributed to this research: Emily Bernier, Deanna Cox, Brooke Davis, and Diana Granados. I also want to thank Dr. Mark St. John for his help optimizing microarthropod extractions and with all his knowledge of these soil organisms. Finally, a number of people from the lab provided feedback and guidance for this study, both informally and at lab meetings: Ashley Shaw, Dr. Zachary Sylvain, Dr. Pilar Andrés, Marie Dam, Dr. Martijn Vandegehuchte, Dr. Kelly Ramirez, Dr. Pablo García-Palacios, and Dr. Tandra Fraser.

I would also like to thank members of the Knapp lab for help with the plant component of this project. Kevin Wilcox helped me develop sampling strategies for above- and belowground biomass, showed me how to use the elutriator, and provided invaluable

assistance locating data and references regarding precipitation and plant productivity of the shortgrass steppe. Robert Griffin-Nolan gave me advice on sorting aboveground plant biomass, and Melissa Johnston helped identify plant specimens to functional group.

I appreciate the guidance of John Field and my father, Arthur Post, in designing grasshopper exclosures. I thank Greg Selby, who helped immensely with soil microarthropod extractions and identification, and Ram Gurung and Dr. Anne Hess for their assistance with data analysis. I thank Mark Lindquist and Jeff Thomas for their help in setting up the experiment at the Central Plains Experimental Range and Colin Pinney and Dan Reuss, who assisted with burning root samples. I also appreciate help from Allison Burford and Jarvis Choury for facilitating travel related to this project.

I express my sincerest gratitude to my family for their love and support throughout my time in this program. I also want to thank my two closest friends, Trevor Sammis and Brian Hoven, for backing my decision to come to Colorado State and for helping me complete my second master's degree.

Funding for this project was provided by the Department of Biology, the Graduate Degree Program in Ecology, the Colorado State University Graduate School, and Wall Lab Biology Funds.

TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGMENTS iv

1. INTRODUCTION 1

 1.1 SOIL FOOD WEBS AND ENERGY CHANNELS..... 2

 1.2 HOW ABOVEGROUND INSECT HERBIVORES CAN AFFECT SOIL FOOD WEBS..... 3

 1.3 EFFECTS OF ABOVEGROUND INSECT HERBIVORES ON SOIL ANIMALS 5

 1.4 THE SHORTGRASS STEPPE 8

 1.5 THIS STUDY.....12

 1.6 STUDY IMPLICATIONS IN THE CONTEXT OF GLOBAL CLIMATE CHANGE12

REFERENCES.....17

2. SOIL ANIMAL RESPONSES TO GRASSHOPPERS IN THE SHORTGRASS STEPPE OF
NORTHERN COLORADO25

 2.1 INTRODUCTION25

 2.2 METHODS.....29

 2.3 RESULTS36

 2.4 DISCUSSION46

REFERENCES.....53

3. CONCLUSION61

 3.1 SYNTHESIS.....61

 3.2 FURTHER POINTS OF DISCUSSION62

 3.3 FUTURE DIRECTIONS.....64

REFERENCES.....66

1. INTRODUCTION

The past 30 years of research has shown that above- and belowground components of terrestrial environments are more intimately connected than initially thought, encouraging more integrated studies to yield a deeper understanding of ecological interactions and ecosystem function in terrestrial systems (Masters et al. 1993, Wardle 2002, Wardle et al. 2004, Bardgett and Wardle 2010). For example, the properties and interactions of aboveground communities can affect the diversity of belowground organisms and processes (Wardle 2006). Of particular interest are belowground responses to leaf-feeding insects, which have greater global species richness than their mammalian counterparts (Strong et al. 1984). Aboveground insect herbivores can directly and indirectly, through interactions with plants, affect the quantity and quality of inputs to soil food webs and, in turn, soil communities and ecosystem processes (Bardgett et al. 1998, Hunter 2001, Bardgett and Wardle 2003). Despite a growing body of literature on this topic, there is a disproportionate focus on vertebrate herbivores, greenhouse environments, agricultural systems, and responses by individual soil microarthropod species. My study addresses these deficits by manipulating natural grasshopper populations in field plots on the shortgrass steppe of northern Colorado and quantifying responses by communities of soil nematodes and microarthropods. Grasshoppers are significant herbivores in this ecosystem because they can consume more than 20% of aboveground net primary production during outbreaks (Mitchell and Pfadt 1974). Results from this research are important in the context of global climate change, particularly with respect to droughts, which are becoming more frequent in the western United States

(Strzepek et al. 2010) and are related to plants (Evans and Burke 2013) and grasshopper populations (Capinera and Horton 1989).

1.1 SOIL FOOD WEBS AND ENERGY CHANNELS

Soil biodiversity can be so rich, sometimes referred to as the 'poor man's tropical rainforest' (Usher et al. 1979), that ecologists often conceptualize belowground food webs as interactions among organisms with shared feeding habits (Hunt et al. 1987) that compose three energy channels: root, bacterial, and fungal (Moore et al. 1988). Living roots, symbiotic microbes (e.g., mycorrhizal fungi), plant parasitic nematodes, herbivorous insects, and predaceous meso- and macrofauna mainly compose the root energy channel; labile detritus, bacteria, protozoa, bacterivorous nematodes and mites, and their consumers, the bacterial channel; and recalcitrant detritus, saprophytic fungi, fungivorous nematodes and mites, and their predators, the fungal energy channel (Moore et al. 2003). Various ecosystem properties are often associated with the dominant microbial energy channel (i.e., bacterial or fungal). Low carbon: nitrogen ratios, greater nutrient availability to plants, and fast, leaky nutrient cycling frequently characterize bacterial-dominated systems, such as grasslands, whereas fungal-based environments, such as coniferous forests, typically have the opposite properties (Coleman et al. 1983, Moore et al. 2003). Since the bases of the three energy pathways are frequently plant-derived, aboveground factors that affect plant traits and communities, such as herbivory, could have implications for soil food webs (Bardgett and Wardle 2003, Wardle et al. 2004).

1.2 HOW ABOVEGROUND INSECT HERBIVORES CAN AFFECT SOIL FOOD WEBS

Aboveground herbivores, both invertebrate and vertebrate, can affect soil biota in numerous ways. Hunter (2001) and Bardgett and Wardle (2003) lay out the mechanisms that drive these effects. I will expand on the four most common mechanisms for leaf-feeding insects: alterations in the 1) quantity or 2) quality of resources that enter the soil; 3) deposition of waste and other compounds; and 4) shifts in plant community composition. Examples using artificial and vertebrate defoliation are also provided.

Quantitative Changes in Soil Inputs. Insect defoliation may cause plants to exude more organic compounds through their roots, which can be transferred to soil food webs. In general, root exudation primes the soil for rhizospheric microbes, which convert nearby organic matter to inorganic nutrients that are taken up by plants (Dakora and Phillips 2002). The exudates are also assimilated by microbes as biomass (Merckx et al. 1985, Helal and Sauerbeck 1986, Martens 1990). Aboveground herbivory may increase the amount of organic compounds exuded. For example, in short-term greenhouse (Hamilton and Frank 2001) and field (Hamilton et al. 2008) experiments involving carbon-13 tracers, artificial defoliation of *Poa pratensis* plants enhanced levels of microbial biomass through pulses in root exudation.

Leaf herbivory could also affect the quantity of resources that enter the soil via changes in fine root dynamics or shifts in allocation of carbon to shoot and root tissues. Fine plant roots, like leaves, turn over (Gill and Jackson 2000). Thus, root mortality provides detrital substrates for microbial activity (Fekete et al. 2011), suggesting that herbivore-induced changes in fine root turnover rates could have implications for belowground carbon flows. In a 3-yr field experiment, moose (*Alces alces*) and snowshoe

hare (*Lepus americanus*) browsing in an Alaskan taiga forest reduced annual fine root production and longevity (Ruess et al. 1998), providing a possible connection between aboveground herbivory and soil food webs. Defoliation may also alter how plants allocate newly assimilated carbon to their tissues. For example, in a short-term growth chamber experiment using carbon-13 tracers, red oak (*Quercus rubra*) seedlings damaged by fourth-instar white-marked tussock moth larvae (*Orgyia leucostigma*) allocated less carbon to fine roots but more carbon to leaves than undefoliated controls (Frost and Hunter 2008).

Qualitative Changes in Soil Inputs. Aboveground herbivory may yield qualitative differences in the resources deposited by plants into soil environments. Here the focus will be on root tissue because most carbon assimilated by soil biota is derived from plant roots, in contrast to the widespread assumption that it comes from leaf litter (Pollierer et al. 2007). First, defoliation may alter root nutrient levels. For example, roots of manually clipped big bluestem (*Andropogon gerardii*) had higher nitrogen concentrations than unclipped plants (Seastedt et al. 1988). Second, defoliation could induce a systemic chemical defensive response in plants (Karban and Baldwin 1997, Orians et al. 2000), thus potentially enhancing levels of secondary compounds in roots. For instance, black mustard (*Brassica nigra*) plants consumed by *Pieris brassicae* caterpillars had greater levels of indole glucosinolates, a type of secondary chemical, in roots than undefoliated controls (Soler et al. 2007). In contrast, *Mamestra brassicae* caterpillar feeding reduced pyrrolizidine alkaloid concentrations in ragworts (*Senecio jacobaea*) (Hol et al. 2004), suggesting that systemic responses are plant-species dependent.

Frass and Honeydew Deposition. Defoliating insects can have direct effects on soil food webs through waste production. By depositing frass onto soils, herbivorous insects

provide belowground environments with a highly labile form of nutrients (Wardle and Bardgett 2004), which could stimulate soil biotic communities. For instance, red oak saplings exposed to eastern tent caterpillar (*Malacosoma americanum*) frass had greater soil microbial biomass than controls (Frost and Hunter 2004). Many plant-sucking insects excrete carbohydrate-rich honeydew onto leaves, which can also end up in soil (Sinka et al. 2009). In one study, plots of *Populus canescens* saplings and three grass species receiving weekly, simulated honeydew amendments had greater soil microbial biomass than control plots for one of two time periods assessed (Seeger and Filser 2008).

Plant Community Shifts. On longer time scales, insect herbivores can affect soil food webs through plant community changes. Since insect grazing may alter the structure of plant communities (Olf and Ritchie 1998, La Pierre et al. 2014) and since belowground biotic responses to defoliation may depend on plant species identity (Mawdsley and Bardgett 1997, Guitian and Bardgett 2000), long periods of insect defoliation could shape soil communities. A long-term grazing experiment on a floodplain grassland in The Netherlands showed that cattle (*Bos taurus*) and European rabbit (*Oryctolagus cuniculus*) herbivory affected nematode feeding diversity through alterations in plant community composition (Veen et al. 2010).

1.3 EFFECTS OF ABOVEGROUND INSECT HERBIVORES ON SOIL ANIMALS

Nematode Responses. The literature covering the effects of insect defoliation on soil communities is scant, but studies have shown diverse responses by nematodes. For example, high levels of corn (*Zea mays*) plant herbivory by *Romalea guttata* grasshoppers yielded greater abundances of bacterivorous and fungivorous nematodes than low and no herbivory treatments 24 hr after feeding terminated (Fu et al. 2001). Using artificially

established plant communities of 16 forb and grass species, 2 yr of grasshopper (*Chorthippus parallelus*) defoliation had no effect on abundances of bacterivorous, fungivorous, and omni-predaceous nematodes but tended to reduce the number of nematode taxa (either genera or families) (De Deyn et al. 2007). In a field survey, caterpillar-damaged tobacco (*Nicotiana tabacum*) plants had a greater abundance of plant parasitic nematodes in their rhizospheres than undamaged controls, whereas plants with aphid outbreaks (> 10,000 individuals/plant) tended to have fewer plant parasites than non-aphid outbreak plants (< 1,000 individuals/plant; Kaplan et al. 2009). In another study, barley (*Hordeum vulgare* cv. Scarlett) plants were grown with a different nutrient amendment (micronutrients only, micronutrients + N, or micronutrients + NP) and with or without one bird cherry-oat aphid (*Rhopalosiphum padi*) nymph for 16 d to investigate the effects of these two factors on soil nematodes during three time periods (Vestergård et al. 2004). Aphids had an interactive effect with harvest time on fungivorous nematodes, reducing abundance 1 wk prior to spike emergence but stimulating it 1 wk after (in the micronutrient- and micronutrient + N-treated pots only) but had no effect on bacterivorous or plant parasitic nematodes. In another aphid study, short-term feeding (up to 16 d) by *Schizaphis rufula* aphids on potted Marram grass (*Ammophila arenaria*) depressed populations of endoparasitic nematodes, but abundances of the two herbivores were uncorrelated in a field survey (Vandegheuchte et al. 2010).

Microarthropod Responses. The literature on soil microarthropod responses to aboveground insect herbivores is even less developed than that of nematodes. Although studies that quantify the effects of shoot-feeding insects on litter microarthropod communities (e.g., Schowalter and Sabin 1991, Reynolds et al. 2003, Classen et al. 2006,

Classen et al. 2007) and vertebrate herbivores on soil microarthropod communities (e.g., Clapperton et al. 2002, Schon et al. 2008) exist, I have found only two studies on soil microarthropod community responses to aboveground insect herbivores. In one investigation, soil mites and springtails were enumerated from potted *Dactylis glomerata* seedlings exposed to aphids (*Ropalosiphon* sp.), grasshoppers (*Chorthippus* sp.), or no herbivores in mineral or sandy soil for 3 yr (Andrzejewska 1995). Mite densities were similar among treatments in mineral soils (springtail data not reported). In sandy soils, mite and springtail densities in grasshopper-treated pots were greater, fewer, or similar to those in controls, depending on the year. Further, aphid-treated pots yielded greater mite densities in two of three years and fewer springtail densities in one of three years. In a field study that assessed the effects of progressive exclusion of four aboveground herbivores size classes (invertebrate; small, medium, and large vertebrate) on soil springtail and mite richness, community composition, and abundance, no effects of only invertebrate herbivores on soil microarthropods were found, but invertebrate and small vertebrate herbivory combined yielded greater springtail richness than no herbivory (Vandegheuchte et al. 2015). In pairwise pot studies, aphids stimulated abundances of *Folsomia candida* springtails across all depths (Sinka et al. 2007), in the upper soil layer only (Sinka et al. 2009), or in high nutrient conditions only (Haase et al. 2008), whereas grasshoppers reduced springtail numbers at the deepest layer of pots but had no effect across all depths (Sinka et al. 2007).

Though soil animal responses to aboveground herbivores are often attributed to plant physiological responses to defoliation, non-consumptive effects can also play a role. For example, plots of *Populus canescens* saplings and three grass species exposed to

synthetic or natural honeydew had greater abundances of the most dominant collembolan family (Bourletiellidae) than controls, but only in one of two sites and for one of four weeks tested. Numbers of the most abundant springtail species (*Hemisotoma thermophila*) were unaffected by honeydew inputs, regardless of type (Seeger and Filser 2008). Similarly, *F. candida* springtail abundances did not differ between control and synthetic honeydew amended pots (Sinka et al. 2009). Despite documentation of soil nematode and microarthropod responses to aboveground insects via consumptive and non-consumptive means, frequent use of greenhouse environments, agricultural plants, and single species responses compels the need for field research on community responses by both soil animal groups in natural systems such as the shortgrass steppe of northern Colorado.

1.4 THE SHORTGRASS STEPPE

Location, Climate, and Plant Community. This study took place at the Central Plains Experimental Range (CPER) near Nunn, Colorado, in the northern portion of the shortgrass steppe biome. The shortgrass steppe extends from the Front Range of the Rocky Mountains east to the panhandle of Oklahoma and from west Texas north to the Colorado-Wyoming border (Lauenroth and Milchunas 1992). This site has a mean annual precipitation of 321 mm and a mean temperature of 8.6 °C (Lauenroth et al. 2008). The plant community at the CPER is dominated by two shortgrasses: *Bouteloua gracilis* and *Bouteloua dactyloides* (Lauenroth 2008). These two species combined make up 46–87% of basal cover of vegetated sites, but 60–80% of the CPER is bare soil (Coffin et al. 1996). Roughly 78% of all plant species are native, and among these, 70% are forbs and 17%, graminoids (Hazlett 1998).

Grasshoppers. Grasshoppers are an important arthropod group in the CPER because of their abundance and effect on primary production (Crist 2008). Previous studies have collected 25–33 species from this site (Capinera and Sechrist 1982, Przybyszewski and Capinera 1990, Welch et al. 1991). Grasshopper abundances are highly variable both spatially and temporally. During a 6-year experiment (1980–1985), which included fertilization and herbicide treatments in a factorial design, mean grasshopper densities per year ranged from 0.48–4.83 individuals/m², but grasshopper population sizes from individual samples (one sampling event per treatment per pasture) were more variable at 0.14–15.4 grasshoppers/m² (Capinera and Thompson 1987). Grasshopper assemblages at the CPER are dominated by few species. Five species composed greater than 80% of the abundance of the total grasshopper community in 5 of 6 yr of the above study (Capinera and Thompson 1987) and more than 75% in a 1986 study (Przybyszewski and Capinera 1990). Further, from 1933–1986, grasshopper abundances in shortgrass prairies of eastern Colorado were negatively correlated with droughts, indicating the importance of abiotic factors in grasshopper population regulation (Capinera and Horton 1989). During outbreaks, shortgrass steppe grasshoppers can consume at least 20% of aboveground net primary production, illustrating their connection to ecosystem function (Mitchell and Pfadt 1974).

Nematode Responses to Cattle Grazing. Though I have not found a study that examined responses by soil nematode communities to insect herbivory in the shortgrass steppe, field research investigating the effects of cattle grazing on these organisms has yielded informative patterns. In a study that assessed the effects of short- and long-term cattle grazing on nematode communities, grazing regimes had no effect on total

abundances, feeding group abundances, or feeding group proportions of nematodes, except that greater proportions of fungivorous nematodes were found in long-term grazed and short-term ungrazed plots than long-term ungrazed plots (Wall-Freckman and Huang 1998). In an experiment that measured responses of soil nematode communities from 0–60 cm at 10 cm depth increments to heavily cattle grazed pastures, ungrazed pastures, and ungrazed pastures supplemented with nitrogen and water, abundances of predaceous and microbial-feeding nematodes were enhanced by nitrogen and water inputs over the other two treatments at 0–10 cm only. Plant-feeding nematode numbers were greatest in amended areas, intermediate in grazed areas, and smallest in ungrazed areas independent of depth (Smolik and Dodd 1983).

Microarthropod Responses to Cattle Grazing. Like nematodes, there is no published research on the effects of aboveground insects on soil microarthropods in the shortgrass steppe, but responses by this soil animal group to cattle grazing have been quantified in this system. Biweekly sampling of pastures with increasing grazing intensity, from ungrazed through heavily cattle grazed, from May–August 1970 yielded marginally significant differences in total microarthropod biomass among grazing regimes, with ungrazed and lightly grazed pastures having greater biomass than heavily grazed pastures. No effect of cattle grazing on the distribution of biomass among microarthropod groups (springtails, mite suborders, and other microarthropods) was found (Crossley et al. 1975). In a similar study, which assessed responses by both trophic groups and multiple taxonomic levels of microarthropods to light and heavy cattle grazing, trophic group biomass did not differ between grazing regimes, and grazing intensity affected only three taxonomic groups: larger populations of Linotetranidae (Prostigmata) and Pseudococcidae

(Hemiptera) and smaller populations of Bdelliade (Prostigmata) were found in heavily grazed pastures (Leetham and Milchunas 1985). The authors also found that both root biomass and herbivorous microarthropods were concentrated near the surface and that the vertical zone with high mean and low variance of soil water coincided with a peak of total microarthropod biomass at 25–40 cm.

Plant Physiological Responses to Defoliation. Though the effects of insect defoliation on soil nematode and microarthropod communities in the shortgrass steppe have yet to be explicitly tested, physiological responses by *B. gracilis* to clipping and grasshoppers suggest that soil animal communities may be affected by aboveground insects. As mentioned above (Section 1.2), herbivory frequently stimulates root exudation of labile carbon by host-plants. Using hydroponically grown *B. gracilis*, *Melanoplus sanguinipes* grasshopper feeding over 18 d generated significant differences in pH between control and defoliated plants, which the authors attributed to greater root exudation by damaged plants (Dyer and Bokhari 1976). Further, artificial defoliation of hydroponically grown *B. gracilis* seedlings allocated 53% of new production to leaf blades (vs. 33% in controls) and only 18% to new roots (vs. 29% in controls) after 10 d, illustrating a compensatory growth response to defoliation (Detling et al. 1979). However, a study using *B. gracilis* potted in a soil-based medium exhibited opposing exudation and biomass allocation responses to clipping (Augustine et al. 2011). Regardless of the direction, herbivore-induced changes in the amount of inputs to soil environments could affect microbial communities and higher trophic levels, including soil animals.

1.5 THIS STUDY

My study builds on the above ideas regarding soil food webs, herbivore-driven linkages between above- and belowground components of terrestrial systems, and foundational work in the shortgrass steppe of northern Colorado to quantify the effects of natural grasshopper populations on soil nematode and microarthropod communities in this ecosystem. I seek to understand these interactions through a short-term field experiment with grasshopper exclosures and by measuring temporal changes in the abundance and structure of each animal community. Thus, this research addresses the following questions:

- 1) Can exclusion of grasshoppers on small plots (50 × 100 cm) in the shortgrass steppe yield differences in aboveground plant biomass?
- 2) Do soil nematodes, microarthropods, and their feeding groups respond to the presence/exclusion of grasshoppers?
- 3) Can root biomass explain, at least in part, soil animal responses to grasshoppers?

1.6 STUDY IMPLICATIONS IN THE CONTEXT OF GLOBAL CLIMATE CHANGE

This study is better understood in the context of global climate change, particularly the importance of precipitation to the central grassland region of the United States. The central grassland region includes the shortgrass steppe along with the northern and southern mixed grass prairies and the tallgrass prairie (Singh et al. 1983). This region is characterized by a west-east precipitation gradient, which is strongly and positively related to aboveground net primary productivity (Sala et al. 1988). At the biome scale, long-term data from sites throughout the shortgrass steppe showed that frequency distributions of

annual aboveground productivity were shifted towards smaller, middle, and larger values in dry, average, and wet years, respectively (USDA 1967). At the site level, specifically the CPER, 52 yr of observational data indicated a positive relationship between precipitation and annual aboveground primary production (Lauenroth and Sala 1992). Investigations of belowground plant responses to altered precipitation at the CPER have yielded similar patterns to those aboveground. Plots supplemented with water (15–50% greater than controls) from late May through August in 2011 and 2012 had, on average, 102% greater belowground net primary productivity than control plots (Wilcox et al. 2015). In a study in which plots were subjected to either 25, 50, or 100% of ambient rainfall for 11 yr then allowed to recover for 2 yr, belowground productivity in 25% plots was 16% lower than in controls, and both drought treatments had significantly less total root biomass than controls in both recovery years (Evans and Burke 2013).

Droughts highlight the effect of global climate change on the northern shortgrass steppe. Droughts have been common in the western United States over the last 1200 yr (Cook et al. 2004) and are projected to be more frequent and severe in Colorado (Strzepek et al. 2010). In fact, the CPER experienced its most extreme drought over the last 50 yr in 2012 (USDA-ARS 2015).

Alterations in precipitation patterns, including droughts, can impact soil communities indirectly through their effect on plants. As mentioned above, belowground productivity in the shortgrass steppe is inversely related to precipitation (Evans and Burke 2013, Wilcox et al. 2015), so a climate with greater drought frequency would reduce root biomass amounts and depress the absolute size of all three soil energy channels through less living and detrital root biomass (Moore et al. 1988, 2003). Further, field sampling of

plants over 8 yr, a period that included a severe drought and 5 yr of above-average precipitation, at the CPER yielded a close relationship between plant community composition and the amount and temporal pattern of rainfall (Hyder et al. 1975). In a manipulative experiment, 4 yr of water addition at the CPER led to drastic changes in aboveground biomass of plant functional groups such as a 2.5-fold increase in C4 grass biomass, which included *B. gracilis*, and a large reduction in succulent biomass (Lauenroth et al. 1978). Though no studies have explicitly assessed the effects of plant species identity on soil communities at the CPER, this is a widespread phenomenon: bacteria and herbaceous plant species in France (Loranger-Merciris et al. 2006), mites and agroforestry tree species in Nigeria (Badejo and Tian 1999), nematodes and herbaceous plant species in The Netherlands (De Deyn et al. 2004), and arbuscular mycorrhizal fungi and tallgrass prairie species in eastern Kansas, United States (Eom et al. 2000). Thus, plant community shifts due to climatic changes could generate responses by soil organisms.

Soil communities in the northern shortgrass steppe may also respond to precipitation-driven changes in grasshopper communities or their interactions with plants. As mentioned in Section 1.4, long-term data (54 yr) from shortgrass prairies of eastern Colorado showed that statewide grasshopper infestations were negatively correlated with a drought index (combination of heat and low precipitation) measured from June–August each year (Capinera and Horton 1989). Thus, greater drought frequency would reduce grasshopper populations and, in turn, decrease the amount of defoliation and frass that reached the soil. Grasshopper herbivory can stimulate root exudation (Dyer and Bokhari 1976), a labile carbon source (de Graaff et al. 2010), and reduce allocation of new photosynthate to belowground biomass (Detling et al. 1979) in the abundant grass *B.*

gracilis; thus, *less* grasshopper grazing would depress the soil bacterial energy channel but promote the root-based energy channel, respectively. Moreover, in an ecosystem like the shortgrass steppe, which is fertile with high herbivory (Lauenroth and Milchunas 1992), frass is a labile resource (Wardle et al. 2004). Hence, less fecal deposition by a smaller grasshopper community would further reduce the relative dominance of the soil bacterial energy pathway. However, *B. gracilis*, which is drought-resistant, enters dormancy during prolonged dry periods (Riegel 1941). So soil communities may have little to no response to grasshopper feeding if a preferred host-plant by many grasshopper species (Pfadt 1994) is physiologically inactive.

Future precipitation patterns may affect soil food webs directly, without or with minimal mediation by plants or grasshoppers. In an 11 yr field study at the CPER, microbial biomass carbon was similar among precipitation levels (25, 50, and 100% ambient precipitation), but compositional differences in final bacterial communities at the class, order, family, genus, and species levels among water treatments were found (Evans et al. 2014). Additionally, in a 3-yr study at the northern shortgrass steppe in which soil moisture was manipulated through supplemental irrigation and use of different slope positions, total nematode abundances and abundances of all nematode trophic groups were inversely related to precipitation-based variables, whereas proportions of nematode feeding groups and generalist and predatory mite abundances were unrelated to precipitation (Sylvain et al. 2014).

Though this study focuses on the importance of biotic factors, namely plants and grasshoppers, to soil animal communities, it is vital to understand how these interactions are set against a changing climate. Alterations in precipitation patterns, particularly more

frequent and severe droughts, in the shortgrass steppe could have direct and/or indirect effects on soils. Thus, the results of this research could help elucidate how belowground communities may respond to grasshopper herbivory under future climates and inform ensuing studies that explicitly test these abiotic and biotic factors simultaneously.

REFERENCES

- Andrzejewska, L. 1995. Influence of herbivore plant feeding on development of soil mesofauna. *Acta Zoologica Fennica* **196**:132-135.
- Augustine, D. J., F. A. Dijkstra, E. W. Hamilton, III, and J. A. Morgan. 2011. Rhizosphere interactions, carbon allocation, and nitrogen acquisition of two perennial North American grasses in response to defoliation and elevated atmospheric CO₂. *Oecologia* **165**:755-770.
- Badejo, M. A. and G. Tian. 1999. Abundance of soil mites under four agroforestry tree species with contrasting litter quality. *Biology and Fertility of Soils* **30**:107-112.
- Bardgett, R. D. and D. A. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* **84**:2258-2268.
- Bardgett, R. D. and D. A. Wardle. 2010. Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford University Press, New York, New York, USA.
- Bardgett, R. D., D. A. Wardle, and G. W. Yeates. 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. *Soil Biology and Biochemistry* **30**:1867-1878.
- Capinera, J. L. and D. R. Horton. 1989. Geographic variation in effects of weather on grasshopper infestation. *Environmental Entomology* **18**:8-14.
- Capinera, J. L. and T. S. Sechrist. 1982. Grasshopper (Acrididae)-host plant associations: response of grasshopper populations to cattle grazing intensity. *Canadian Entomologist* **114**:1055-1062.
- Capinera, J. L. and D. C. Thompson. 1987. Dynamics and structure of grasshopper assemblages in shortgrass prairie. *Canadian Entomologist* **119**:567-575.
- Clapperton, M. J., D. A. Kanashiro, and V. M. Behan-Pelletier. 2002. Changes in abundance and diversity of microarthropods associated with fescue prairie grazing regimes. *Pedobiologia* **46**:496-511.
- Classen, A. T., J. DeMarco, S. C. Hart, T. G. Whitham, N. S. Cobb, and G. W. Koch. 2006. Impacts of herbivorous insects on decomposer communities during the early stages of primary succession in a semi-arid woodland. *Soil Biology and Biochemistry* **38**:972-982.

- Classen, A. T., S. T. Overby, S. C. Hart, G. W. Koch, and T. G. Whitham. 2007. Season mediates herbivore effects on litter and soil microbial abundance and activity in a semi-arid woodland. *Plant and Soil* **295**:217-227.
- Coffin, D. P., W. K. Lauenroth, and I. C. Burke. 1996. Recovery of vegetation in a semiarid grassland 53 years after disturbance. *Ecological Applications* **6**:538-555.
- Coleman, D. C., C. P. P. Reid, and C. V. Cole. 1983. Biological strategies of nutrient cycling in soil systems. *Advances in Ecological Research* **13**:1-55.
- Cook, E. R., C. A. Woodhouse, C. M. Eakin, D. M. Meko, and D. W. Stahle. 2004. Long-term aridity changes in the western United States. *Science* **306**:1015-1018.
- Crist, T. O. 2008. Insect populations, community interactions, and ecosystem processes in the shortgrass steppe. Pages 215-247 in W. K. Lauenroth and I. C. Burke, editors. *Ecology of the shortgrass steppe: a long-term perspective*. Oxford University Press, New York, New York, USA.
- Crossley, D. A., Jr., C. W. Proctor, Jr., and C. Gist. 1975. Summer biomass of soil microarthropods of the Pawnee National Grassland, Colorado. *American Midland Naturalist* **93**:491-495.
- Dakora, F. D. and D. A. Phillips. 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil* **245**:35-47.
- De Deyn, G. B., C. E. Raaijmakers, J. van Ruijven, F. Berendse, and W. H. van der Putten. 2004. Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. *Oikos* **106**:576-586.
- De Deyn, G. B., J. van Ruijven, C. E. Raaijmakers, P. C. de Ruiter, and W. H. van der Putten. 2007. Above- and belowground insect herbivores differentially affect soil nematode communities in species-rich plant communities. *Oikos* **116**:923-930.
- de Graaff, M.-A., A. T. Classen, H. F. Castro, and C. W. Schadt. 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. *New Phytologist* **188**:1055-1064.
- Detling, J. K., M. I. Dyer, and D. T. Winn. 1979. Net photosynthesis, root respiration, and regrowth of *Bouteloua gracilis* following simulated grazing. *Oecologia* **41**:127-134.
- Dyer, M. I. and U. G. Bokhari. 1976. Plant-animal interactions: studies of the effects of grasshopper grazing on blue grama grass. *Ecology* **57**:762-772.
- Eom, A.-H., D. C. Hartnett, and G. W. T. Wilson. 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia* **122**:435-444.

- Evans, S. E. and I. C. Burke. 2013. Carbon and nitrogen decoupling under an 11-year drought in the shortgrass steppe. *Ecosystems* **16**:20-33.
- Evans, S. E., M. D. Wallenstein, and I. C. Burke. 2014. Is bacterial moisture niche a good predictor of shifts in community composition under long-term drought? *Ecology* **95**:110-122.
- Fekete, I., C. Varga, Z. Kotroczó, J. A. Tóth, and G. Várbiró. 2011. The relation between various detritus inputs and soil enzyme activities in a Central European deciduous forest. *Geoderma* **167-68**:15-21.
- Frost, C. J. and M. D. Hunter. 2004. Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. *Ecology* **85**:3335-3347.
- Frost, C. J. and M. D. Hunter. 2008. Herbivore-induced shifts in carbon and nitrogen allocation in red oak seedlings. *New Phytologist* **178**:835-845.
- Fu, S., K. W. Kisselle, D. C. Coleman, P. F. Hendrix, and D. A. Crossley, Jr. 2001. Short-term impacts of aboveground herbivory (grasshopper) on the abundance and ¹⁴C activity of soil nematodes in conventional tillage and no-till agroecosystems. *Soil Biology and Biochemistry* **33**:1253-1258.
- Gill, R. A. and R. B. Jackson. 2000. Global patterns of root turnover for terrestrial ecosystems. *New Phytologist* **147**:13-31.
- Guitian, R. and R. D. Bardgett. 2000. Plant and soil microbial responses to defoliation in temperate semi-natural grassland. *Plant and Soil* **220**:271-277.
- Haase, J., R. Brandl, S. Scheu, and M. Schädler. 2008. Above- and belowground interactions are mediated by nutrient availability. *Ecology* **89**:3072-3081.
- Hamilton, E. W., III and D. A. Frank. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* **82**:2397-2402.
- Hamilton, E. W., III, D. A. Frank, P. M. Hinchey, and T. R. Murray. 2008. Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. *Soil Biology and Biochemistry* **40**:2865-2873.
- Hazlett, D. L. 1998. Vascular plant species of the Pawnee National Grassland., U. S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, Colorado, USA.
- Helal, H. M. and D. Sauerbeck. 1986. Effect of plant roots on carbon metabolism of soil microbial biomass. *Zeitschrift für Pflanzenernährung und Bodenkunde* **149**:181-188.

- Hol, W. H. G., M. Macel, J. A. van Veen, and E. van der Meijden. 2004. Root damage and aboveground herbivory change concentration and composition of pyrrolizidine alkaloids of *Senecio jacobaea*. *Basic and Applied Ecology* **5**:253-260.
- Hunt, H. W., D. C. Coleman, E. R. Ingham, R. E. Ingham, E. T. Elliott, J. C. Moore, S. L. Rose, C. P. P. Reid, and C. R. Morley. 1987. The detrital food web in a shortgrass prairie. *Biology and Fertility of Soils* **3**:57-68.
- Hunter, M. D. 2001. Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. *Agricultural and Forest Entomology* **3**:77-84.
- Hyder, D. N., R. E. Bement, E. E. Remmenga, and D. F. Hervey. 1975. Ecological response of native plants and guidelines for management of shortgrass range. Technical Bulletin No. 1503, USDA-ARS, Washington, District of Columbia, USA.
- Kaplan, I., S. Sardanelli, and R. F. Denno. 2009. Field evidence for indirect interactions between foliar-feeding insect and root-feeding nematode communities on *Nicotiana tabacum*. *Ecological Entomology* **34**:262-270.
- Karban, R. and I. T. Baldwin. 1997. Induced responses to herbivory. University of Chicago Press, Chicago, Illinois, USA.
- La Pierre, K. J., A. Joern, and M. D. Smith. 2014 Invertebrate, not small vertebrate, herbivory interacts with nutrient availability to impact tallgrass prairie community composition and forb biomass. *Oikos*:DOI: 10.1111/oik.01869.
- Lauenroth, W. K. 2008. Vegetation of the shortgrass steppe. Pages 70-83 in W. K. Lauenroth and I. C. Burke, editors. *Ecology of the shortgrass steppe: a long-term perspective*. Oxford University Press, New York, New York, USA.
- Lauenroth, W. K., I. C. Burke, and J. A. Morgan. 2008. The shortgrass steppe: the region and research sites. Pages 3-13 in W. K. Lauenroth and I. C. Burke, editors. *Ecology of the shortgrass steppe: a long-term perspective*. Oxford University Press, New York, New York, USA.
- Lauenroth, W. K., J. L. Dodd, and P. L. Sims. 1978. The effects of water- and nitrogen-induced stresses on plant community structure in a semiarid grassland. *Oecologia* **36**:211-222.
- Lauenroth, W. K. and D. G. Milchunas. 1992. The shortgrass steppe. Pages 183-226 in R. T. Coupland, editor. *Natural grasslands: introduction and western hemisphere*. Elsevier Scientific Press, Amsterdam, The Netherlands.
- Lauenroth, W. K. and O. E. Sala. 1992. Long-term forage production of North American shortgrass steppe. *Ecological Applications* **2**:397-403.

- Leetham, J. W. and D. G. Milchunas. 1985. The composition and distribution of soil microarthropods in the shortgrass steppe in relation to soil water, root biomass, and grazing by cattle. *Pedobiologia* **28**:311-325.
- Loranger-Merciris, G., L. Barthes, A. Gastine, and P. Leadley. 2006. Rapid effects of plant species diversity and identity on soil microbial communities in experimental grassland ecosystems. *Soil Biology and Biochemistry* **38**:2336-2343.
- Martens, R. 1990. Contribution of rhizodeposits to the maintenance and growth of soil microbial biomass. *Soil Biology and Biochemistry* **22**:141-147.
- Masters, G. J., V. K. Brown, and A. C. Gange. 1993. Plant mediated interactions between above- and below-ground insect herbivores. *Oikos* **66**:148-151.
- Mawdsley, J. L. and R. D. Bardgett. 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the composition and activity of the microbial population of an upland grassland soil. *Biology and Fertility of Soils* **24**:52-58.
- Merckx, R., A. den Hartog, and J. A. van Veen. 1985. Turnover of root-derived material and related microbial biomass formation in soils of different texture. *Soil Biology and Biochemistry* **17**:565-569.
- Mitchell, M. J. and R. E. Pfadt. 1974. A role of grasshoppers in a shortgrass prairie ecosystem. *Environmental Entomology* **3**:358-360.
- Moore, J. C., K. McCann, H. Setälä, and P. C. de Ruiter. 2003. Top-down is bottom-up: does predation in the rhizosphere regulate aboveground dynamics? *Ecology* **84**:846-857.
- Moore, J. C., D. E. Walter, and H. W. Hunt. 1988. Arthropod regulation of micro- and mesobiota in below-ground detrital food webs. *Annual Review of Entomology* **33**:419-439.
- Olf, H. and M. E. Ritchie. 1998. Effects of herbivores on grassland plant diversity. *Trends in Ecology and Evolution* **13**:261-265.
- Orians, C. M., J. Pomerleau, and R. Ricco. 2000. Vascular architecture generates fine scale variation in systemic induction of proteinase inhibitors in tomato. *Journal of Chemical Ecology* **26**:471-485.
- Pfadt, R. E. 1994. Field guide to common western grasshoppers. University of Wyoming, Laramie, Wyoming, USA.
- Pollierer, M. M., R. Langel, C. Körner, M. Maraun, and S. Scheu. 2007. The underestimated importance of belowground carbon input for forest soil animal food webs. *Ecology Letters* **10**:729-736.

- Przybyszewski, J. and J. L. Capinera. 1990. Spatial and temporal patterns of grasshopper (Orthoptera: Acrididae) phenology and abundance on a shortgrass prairie. *Journal of the Kansas Entomological Society* **63**:405-413.
- Reynolds, B. C., D. A. Crossley, Jr., and M. D. Hunter. 2003. Response of soil invertebrates to forest canopy inputs along a productivity gradient. *Pedobiologia* **47**:127-139.
- Riegel, A. 1941. Life history and habits of blue grama. *Transactions of the Kansas Academy of Science* **44**:76-85.
- Ruess, R. W., R. L. Hendrick, and J. P. Bryant. 1998. Regulation of fine root dynamics by mammalian browsers in early successional Alaskan taiga forests. *Ecology* **79**:2706-2720.
- Sala, O. E., W. J. Parton, L. A. Joyce, and W. K. Lauenroth. 1988. Primary production of the central grassland region of the United States. *Ecology* **69**:40-45.
- Schon, N. L., A. D. Mackay, M. A. Minor, G. W. Yeates, and M. J. Hedley. 2008. Soil fauna in grazed New Zealand hill country pastures at two management intensities. *Applied Soil Ecology* **40**:218-228.
- Schowalter, T. D. and T. E. Sabin. 1991. Litter microarthropod responses to canopy herbivory, season and decomposition in litterbags in a regenerating conifer ecosystem in Western Oregon. *Biology and Fertility of Soils* **11**:93-96.
- Seastedt, T. R., R. A. Ramundo, and D. C. Hayes. 1988. Maximization of densities of soil animals by foliage herbivory: empirical evidence, graphical and conceptual models. *Oikos* **51**:243-248.
- Seeger, J. and J. Filser. 2008. Bottom-up down from the top: honeydew as a carbon source for soil organisms. *European Journal of Soil Biology* **44**:483-490.
- Singh, J. S., W. K. Lauenroth, and D. G. Milchunas. 1983. Geography of grassland ecosystems. *Progress in Physical Geography* **7**:46-80.
- Sinka, M., T. H. Jones, and S. E. Hartley. 2007. The indirect effect of above-ground herbivory on collembola populations is not mediated by changes in soil water content. *Applied Soil Ecology* **36**:92-99.
- Sinka, M., T. H. Jones, and S. E. Hartley. 2009. Collembola respond to aphid herbivory but not to honeydew addition. *Ecological Entomology* **34**:588-594.
- Smolik, J. D. and J. L. Dodd. 1983. Effect of water and nitrogen, and grazing on nematodes in a shortgrass prairie. *Journal of Range Management* **36**:744-748.

- Soler, R., T. M. Bezemer, A. M. Cortesero, W. H. Van der Putten, L. E. M. Vet, and J. A. Harvey. 2007. Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. *Oecologia* **152**:257-264.
- Strong, D. R., J. H. Lawton, and T. R. E. Southwood. 1984. *Insects on plants: community patterns and mechanisms*. Blackwell, Oxford, UK.
- Strzepek, K., G. Yohe, J. Neumann, and B. Boehlert. 2010. Characterizing changes in drought risk for the United States from climate change. *Environmental Research Letters* **5**:044012.
- Sylvain, Z. A., D. H. Wall, K. L. Cherwin, D. P. C. Peters, L. G. Reichmann, and O. E. Sala. 2014. Soil animal responses to moisture availability are largely scale, not ecosystem dependent: insight from a cross-site study. *Global Change Biology* **20**:2631-2643.
- USDA. 1967. *National handbook for range and related grazing lands*. Soil Conservation Service, U.S. Department of Agriculture, Washington, District of Columbia, USA.
- USDA-ARS. 2015. *Rangeland resource research*. USDA-ARS, Cheyenne, Wyoming, USA.
- Usher, M. B., P. Davis, J. Harris, and B. Longstaff. 1979. A profusion of species? Approaches towards understanding the dynamics of the populations of microarthropods in decomposer communities. Pages 359-384 *in* R. M. Anderson, B. D. Turner, and L. R. Taylor, editors. *Population dynamics*. Blackwell Scientific Publications, Oxford, UK.
- Vandegheuchte, M. L., E. de la Peña, and D. Bonte. 2010. Interactions between root and shoot herbivores of *Ammophila arenaria* in the laboratory do not translate into correlated abundances in the field. *Oikos* **119**:1011-1019.
- Vandegheuchte, M. L., U. Raschein, M. Schütz, D. J. Gwiazdowicz, and A. C. Risch. 2015. Indirect short- and long-term effects of aboveground invertebrate and vertebrate herbivores on soil microarthropod communities. *PLoS ONE* **10**:e0118679.
- Veen, G. F., H. Olf, H. Duyts, and W. H. Van der Putten. 2010. Vertebrate herbivores influence soil nematodes by modifying plant communities. *Ecology* **91**:828-835.
- Vestergård, M., L. Bjørnlund, and S. Christensen. 2004. Aphid effects on rhizosphere microorganisms and microfauna depend more on barley growth phase than on soil fertilization. *Oecologia* **141**:84-93.
- Wall-Freckman, D. and S. P. Huang. 1998. Response of the soil nematode community in a shortgrass steppe to long-term and short-term grazing. *Applied Soil Ecology* **9**:39-44.
- Wardle, D. A. 2002. *Communities and ecosystems: linking the aboveground and belowground components*. Princeton University Press, Princeton, New Jersey, USA.

- Wardle, D. A. 2006. The influence of biotic interactions on soil biodiversity. *Ecology Letters* **9**:870-886.
- Wardle, D. A. and R. D. Bardgett. 2004. Human-induced changes in large herbivorous mammal density: the consequences for decomposers. *Frontiers in Ecology and the Environment* **2**:145-153.
- Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. *Science* **304**:1629-1633.
- Welch, J. L., R. Redak, and B. C. Kondratieff. 1991. Effect of cattle grazing on the density and species of grasshoppers (Orthoptera: Acrididae) of the Central Plains Experimental Range, Colorado: a reassessment after two decades. *Journal of the Kansas Entomological Society* **64**:337-343.
- Wilcox, K. R., J. C. Von Fischer, J. M. Muscha, M. K. Petersen, and A. K. Knapp. 2015. Contrasting above- and belowground sensitivity of three Great Plains grasslands to altered rainfall regimes. *Global Change Biology* **21**:335-344.

2. SOIL ANIMAL RESPONSES TO GRASSHOPPERS IN THE SHORTGRASS STEPPE OF NORTHERN COLORADO

2.1 INTRODUCTION

Three decades of research has shown that above- and belowground components of terrestrial ecosystems should not be studied in isolation, but instead require an integrated approach (Masters et al. 1993, van der Putten et al. 2001, Wardle 2002, Bardgett and Wardle 2010). Early work in the late 1980s and early 1990s demonstrated that spatially separated insect herbivores (e.g., a defoliator and a root feeder) can affect each other through physiological changes in shared host-plants (Gange and Brown 1989, Moran and Whitham 1990, Masters and Brown 1992). Later research has investigated modification of aboveground-belowground linkages by global change drivers, such as elevated CO₂ (Jones et al. 1998), nitrogen deposition (Suding et al. 2008), and invasive species (Wardle et al. 2001). Currently, research has shifted towards understanding how soil communities conditioned by abiotic and biotic factors, often aboveground, affect succeeding plant communities (e.g., Rodríguez-Echeverría et al. 2013) and ecosystem functioning (e.g., Göransson et al. 2013, Sonnemann et al. 2013), a concept referred to as ‘carry-over effects’ (Bartelt-Ryser et al. 2005) or ‘legacy effects’ (Kardol et al. 2007).

Soil biodiversity can be so immense (Bardgett and van der Putten 2014) that ecologists frequently simplify complex, belowground food webs into three energy channels—root, soil fungal, and soil bacterial—which are often plant-derived (Moore et al. 1988). These models are developed by grouping species of soil organisms by food source and indicating material flows with arrows, originating from one of three sources: living

root tissue, recalcitrant or resistant detritus (fungi), and labile detritus (bacteria) (Fig. 2.1). Since plants provide these three resources and possess aerial structures, plants can serve as conduits between above- and belowground components of terrestrial ecosystems. Thus, aboveground factors, either abiotic or biotic, could promote or depress one or more pathways (Wardle et al. 2004). Alteration in the energetic structure of soil food webs can have ramifications for ecosystem functioning because bacteria and fungi differ in their nutrient cycling rates (Coleman et al. 1983, Moore et al. 2003).

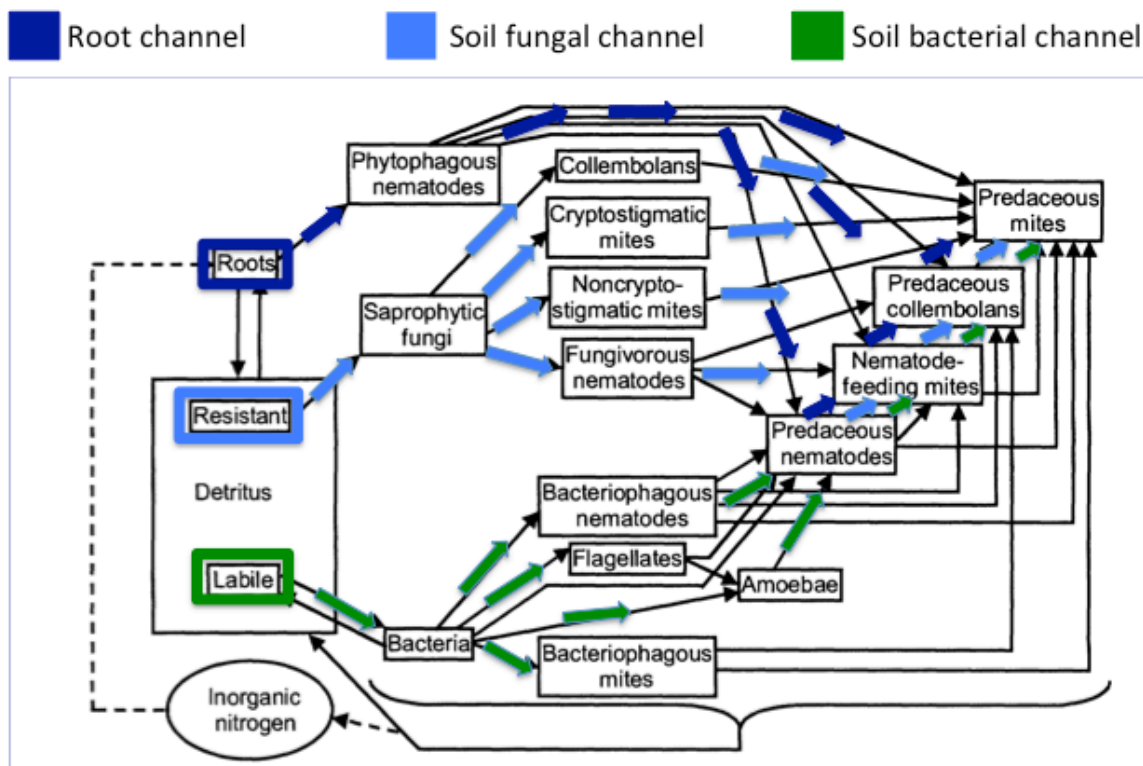


Figure 2.1: This is a conceptual model of a soil food web (adapted from Moore et al. 2003) with the three energy channels (root, soil fungal, and soil bacterial; Moore et al. 1988) illustrated by the author. Species are grouped into functional or trophic groups. Solid, black arrows indicate flows of carbon and nitrogen, and dashed, black arrows represent net nitrogen flows. The bases of the three energy channels are marked with colored boxes, and the general movement of carbon and nitrogen from the bases through the soil organism groups of each energy channel are indicated with colored arrows. Arrow lengths are varied for ease of viewing.

Leaf-feeding insects frequently link above- and belowground components of terrestrial ecosystems (Holland 1995, Fu et al. 2001). For example, grazing insects affect soil food webs through direct means via frass and indirect means via host-plant responses to herbivory (Bardgett and Wardle 2003). Waste deposition can provide soils with a pulse of nutrients that stimulate microbial communities (Frost and Hunter 2004). Defoliation can induce belowground changes in plants, for example, by altering root exudation patterns (Hamilton and Frank 2001, Hamilton et al. 2008), fine root dynamics (Ruess et al. 1998), or the chemical composition of living or dead root tissue (Seastedt et al. 1988, Soler et al. 2007). These changes can affect soil biota through alterations in the quantity or quality of resources that enter the rhizosphere (Bardgett and Wardle 2003, García-Palacios et al. 2013). Leaf herbivory can trigger a pulsed response by microbes (Henry et al. 2008) and nematode trophic groups (Fu et al. 2001, Mikola et al. 2005); thus, it is important to determine whether soil community changes are persistent.

Studies investigating the effects of insect herbivores on soil communities are few but those conducted have shown variable responses by soil microbes, nematodes, and microarthropods. The literature on defoliator-driven changes in soil food webs has focused on vertebrate herbivores (e.g., Stanton et al. 1984, Bardgett et al. 1997, Wardle et al. 2001, Clapperton et al. 2002) and clipping (e.g., Mikola et al. 2005, Sørensen et al. 2008). Experiments with insect grazers frequently were in agricultural systems. For example, corn (*Zea mays*) plants in no-tillage fields consumed by a low density of *Romalea guttata* grasshoppers (5 individuals/plant) for 5 d increased soil microbial biomass 12 d later relative to the other densities (0, 10, and 20 individuals/plant) examined (Holland 1995). Using the same plant and grasshopper species in no-tillage fields, 2 hr of high herbivore

density (8 individuals/plant) led to greater abundances of bacterivorous and fungivorous nematodes 24 hr after feeding relative to controls (0 individuals/plant) and the low density treatment (4 individuals/plant), but no differences in abundances of any nematode feeding group were found at any other post-feeding duration (i.e., 2, 48, and 120 hr) (Fu et al. 2001). A greenhouse study using plants from natural ecosystems resulted in a greater proportion of soil bacteria and diversity of plant parasitic nematodes in the presence of aboveground insect herbivores, but sap-sucking aphids, not leaf-chewing insects, were used (Wardle et al. 2005). When soil microarthropods have been tested in relevant studies, single species responses to insect defoliation were often measured (e.g., Sinka et al. 2007, Bradford et al. 2008, Haase et al. 2008, Sinka et al. 2009). My research differs from the above examples by using field plots of native grassland and foliage-feeding insects, and including responses by soil nematode and microarthropod communities, not single species.

The objectives of this study were to: 1) determine whether exclusion of grasshoppers in the shortgrass steppe of northern Colorado affects aboveground plant biomass; 2) quantify responses by soil nematodes and microarthropods to grasshoppers; and 3) if soil biotic responses are found, determine whether these could be explained by differences in belowground biomass. I hypothesize that: H₁) grasshopper feeding would reduce plant root biomass due to greater allocation of photosynthate to aboveground tissues; H₂) reduced root biomass, and thus, less root litter, would depress root and soil fungal energy pathways; and H₃) grasshoppers would stimulate the soil bacterial energy pathway through frass deposition and enhanced root exudation of labile carbon by defoliated plants.

2.2 METHODS

Study Area. An ungrazed cattle pasture (pasture 15 NW) at the Central Plains Experimental Range near Nunn, Colorado, was selected for this research (Fig. 2.2). This 63 ha pasture was grazed by cattle for more than 50 yr before grazing ceased in 2000 (Jamiyansharav et al. 2011). The Central Plains Experimental Range is located within the shortgrass steppe biome, which extends from the Front Range of the Rocky Mountains east to the panhandle of Oklahoma and from west Texas north to the Colorado-Wyoming border (Lauenroth and Milchunas 1992). This site has a mean annual precipitation of 321 mm and a mean temperature of 8.6 °C (Lauenroth et al. 2008), and its plant community is dominated by *Bouteloua gracilis* and *Bouteloua dactyloides* (Lauenroth 2008). Previous studies have collected 25–33 grasshopper species from the Central Plains Experimental Range (Capinera and Sechrist 1982, Przybyszewski and Capinera 1990, Welch et al. 1991).

Plot Establishment. Twelve 50 × 100 cm plots spaced at least 3 m apart were established in early September 2014. Plots contained similar foliar cover (predominantly *B. gracilis* and *Vulpia octoflora*, no cacti, and minimal forbs and shrubs; see Aboveground Plant Biomass in Results) as determined through visual estimation. *Vulpia octoflora* was particularly abundant in 2014 (K. H. Post, personal observation). Plots were randomly but equally distributed between two treatments: caged controls (grasshopper plots; permitted insect herbivore access) and exclosures (severely limited insect herbivore access; see Monitoring below for grasshopper data).

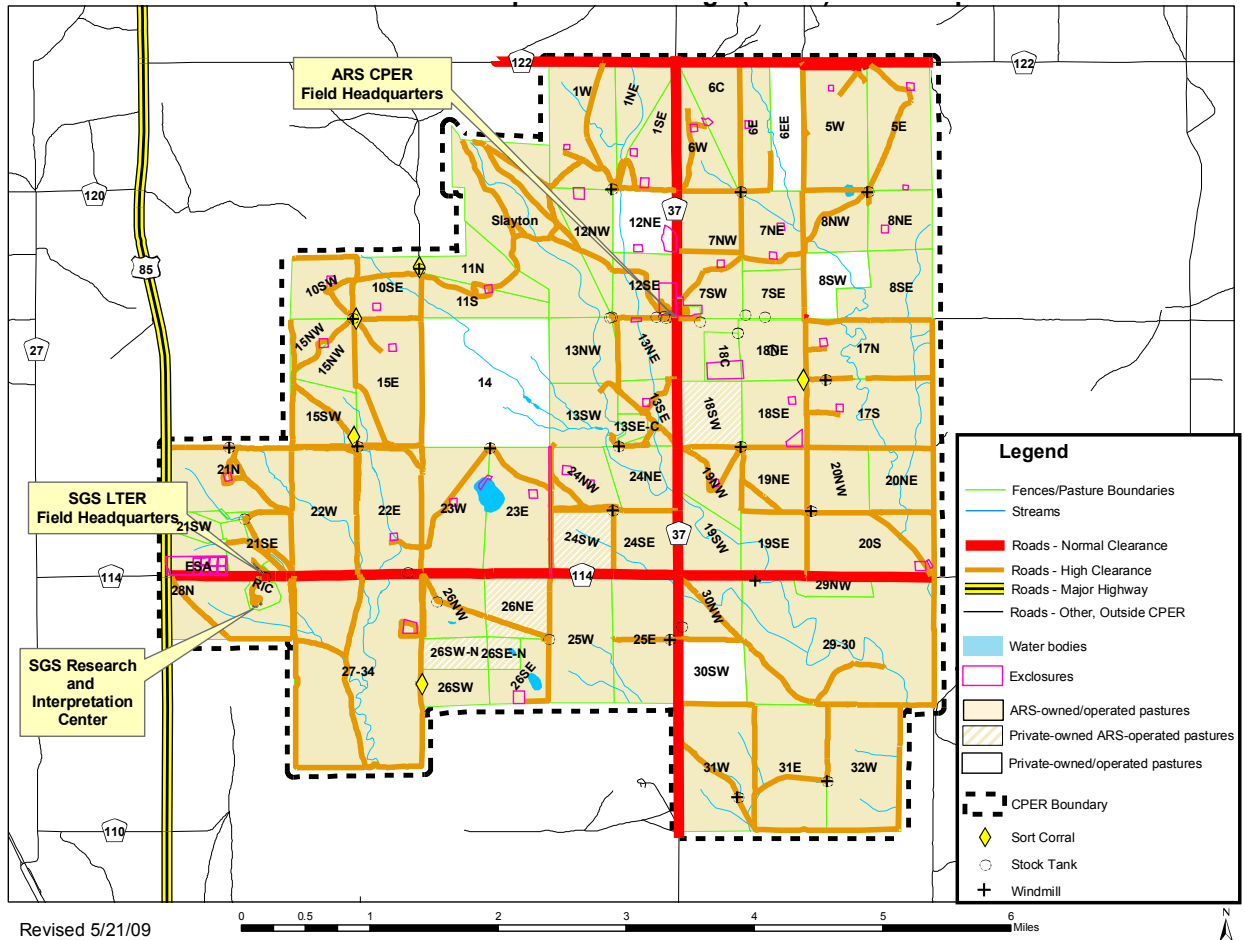


Figure 2.2: This is a map of the pastures at the Central Plains Experimental Range near Nunn, Colorado (from LTER-SGS 2009). Pasture 15NW, which has been ungrazed by cattle since 2000 (Jamiyansharav et al. 2011), was used in this study.

Cage Installation. Three polyvinyl chloride (PVC) pipe support structures consisting of a 50-cm width and two 58-cm vertical pieces attached together with two 90° PVC connectors were installed into each plot prior to cage installation (Fig. 2.3). PVC supports had angled cuts at bottoms and were hammered roughly 7 cm into the ground. Supports were installed widthwise at the 0, 50, and 100 cm points of the longest sides of plots and provided greater stability for the cages to withstand high winds.

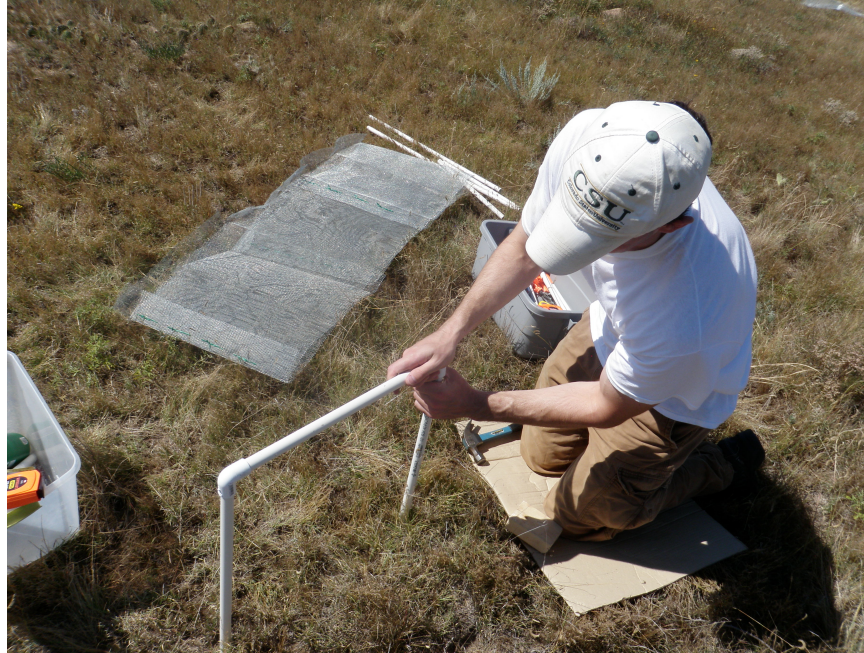


Figure 2.3: Installation of a PVC support (height = 58 cm, width = 50 cm) roughly 7 cm into the ground to increase stability of caged controls and grasshopper exclosures placed in the shortgrass steppe of northern Colorado. Three were installed at equal increments along the long sides of 50 × 100 cm plots. Photo credit: Andre Franco.

Cages (50 × 100 × 55 cm tall) constructed of 8-mesh (0.3175 × 0.3175 cm) galvanized hardware cloth were placed over PVC supports. Cages were attached to the ground by hammering 60 penny (15.24 cm) nails sleeved with 0.635 × 2.54 cm or 0.635 × 3.175 cm fender washers (1 washer per nail) through excess cloth (6 cm) that was folded out from bottoms of side walls of cages (Thompson and Gardner 1996). Cages were joined to PVC supports with plant ties. Any unwanted openings at cage bases were covered with additional hardware cloth and installed using nails and washers. Caged controls were similar to exclosures in all design aspects except that 12-8 × 8 cm holes were cut into sides of hardware cloth to provide grasshoppers and other insect herbivores access to plants (Evans et al. 2012).

Initial Soil Sampling. Four 2.5 cm diameter × 10 cm depth soil samples per plot (two in both bare ground and *B. gracilis*-dominated areas, also referred to as ‘bare’ and ‘plant’, respectively) were collected with a soil probe, bulked, and later allocated for soil moisture determination and nematode analysis. One 6.35 × 10 cm soil core per plot at a *B. gracilis*-dominated area was harvested for microarthropod extraction. Soil samples were transported to Colorado State University in coolers with blue ice then stored at 4°C until processing.

Lid Installation. Enclosures were inspected for insect herbivores, and any insects found within were removed. Vertical slits (3 cm) were cut into all cages at six equidistant points (i.e., four corners and midpoints of 100 cm sides), and hardware cloth was folded out to allow connection to lids. Lids (61 × 106 cm) made of two smaller pieces of hardware cloth attached together with plant ties were connected to cages using standard paper staples and plant ties. Excess cloth was removed. Any large openings were covered with additional hardware cloth and installed using staples, plant ties, and/or metal wire. Closed cage dimensions were 50 × 100 × 52 cm tall (Fig. 2.4).

Monitoring. Plots were monitored weekly for 6.5 wk to (1) count numbers of living grasshoppers in cages for 5 min per cage, (2) assure that cages were upright and stable, and (3) cover any access points to enclosures. Enclosures reduced abundances of live grasshoppers by 143% relative to caged controls over seven, weekly monitoring periods (average total observed grasshoppers during experiment: caged controls = 2.833 individuals/plot and enclosures = 1.167 individuals/plot; one-sided, $t_{10} = -2.936$, $p = 0.007$).

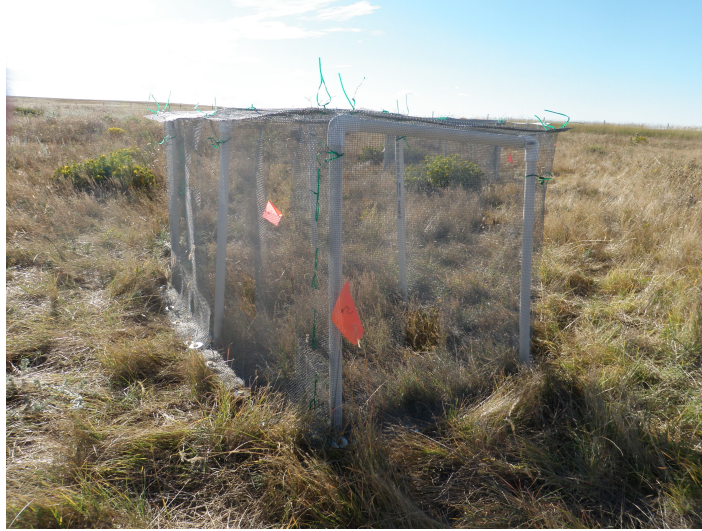


Figure 2.4: This cage (50 × 100 × 52 cm tall) was placed on a 50 × 100 cm plot that provided grasshoppers access to plants in the shortgrass steppe of northern Colorado. Photo credit: Keith Post.

Final Harvest. The experiment was terminated in late October 2014. Cage lids were removed, and plots were inspected for living insect herbivores. The same number, size, and location of soil probe samples were collected as for the initial harvest. In addition, two 6.35 × 20 cm soil cores per plot (one in both bare ground and a *B. gracilis*-dominated area) were collected, separated by depth (0–10 and 10–20 cm), and bagged by location and depth (four core samples per plot) for root biomass and microarthropod analyses. Microarthropods were extracted only from 0–10 cm depths from cores taken at *B. gracilis*-dominated areas. All soil samples were transported and stored in the same manner as the initial harvest. All remaining, standing aboveground biomass in each plot was clipped and stored in paper bags at room temperature (~23°C) until processing.

Sample Processing. Bulk soil samples and microarthropod cores were processed within 48 hr of both harvests. Rocks, litter, and living aboveground plant biomass were removed from all bulk samples (initial and final harvests), which were then gently mixed

and separated into 50 g subsamples for analysis of soil moisture and nematodes. Gravimetric soil moisture was measured by quantifying evaporative mass loss after heating soil at 105 °C for 48 hr. Nematodes were extracted at 24 h intervals over 3 d with Baermann funnels (Baermann 1917). Juvenile and adult nematodes were counted and 200 individuals per sample identified to feeding group (bacterial feeders, fungal feeders, plant parasites, predators, and omnivores; Yeates et al. 1993) within 1 wk of extraction. Feeding group densities for each sample were determined by multiplying feeding group proportions of 200-individual subsamples and total abundances, and dividing these estimated abundances by the dry soil mass of each sample using its gravimetric soil moisture content. All nematodes from each sample were preserved in 10% hot formalin solution. Microarthropods were extracted over 6 d using Tullgren funnels (Tullgren 1918; BioQuip, Rancho Dominguez, CA) into 70% ethanol. Soil mites (both juveniles and adults) were separated into two groups: generalist feeders (all juveniles; all adult Astigmata, Endostigmata, and Oribatida; and non-predatory, adult Prostigmata), which mainly feed on detritus and fungi, and predators (predatory, adult Prostigmata and all adult Mesostigmata), which consume nematodes and other microarthropods (Coleman et al. 2004). Remaining soil microarthropods were enumerated and added to mite counts to generate total microarthropod abundances for each sample. All microarthropod counts were divided by the dry mass of soil samples using the gravimetric moisture content of bulk samples to determine densities of microarthropod groups (Cotrufo et al. 2014). Soil microarthropods were preserved in 70% ethanol. All soil animal groups are expressed as individuals/kg dry soil.

Aboveground biomass was dried at 60°C for 48 hr and weighed. *Vulpia octoflora* aboveground biomass was isolated, and the remaining biomass was sorted into functional groups, re-dried, and weighed. Total, *V. octoflora*, and functional group aboveground biomass are expressed in g/m². Aboveground biomass data were corrected for area removed by soil samples collected during the initial harvest.

All aboveground biomass was removed from soil cores used in root analysis before cores were crumbled. Roots were sorted from soil using a hydropneumatic elutriator (Smucker et al. 1982), dried at 60°C for 48 hr, and weighed. Root samples were burned in a muffle furnace at 450 °C for 4 hr to determine ash mass of samples, which was subtracted from ash-inclusive dry mass to determine root biomass per core sample. Root biomass is expressed in g/m². Shoot: root ratios were determined using total aboveground biomass (g/m²) and total belowground biomass (g/m²) at 0–20 cm depth estimated from both cover types (plant and bare).

Statistical Analysis. Observed grasshopper abundances, aboveground plant biomass (total, *V. octoflora*, and other grasses), and shoot: root ratios were analyzed with *t*-tests. Belowground plant biomass was analyzed as a split-split-plot with enclosure as the whole plot factor, cover type as the subplot factor, and depth as the subsubplot factor. The three factors were considered fixed effects in a mixed-effects model. All response data except for root biomass and soil microarthropod densities (see below) met appropriate assumptions (i.e., normality of residuals and homogeneity of variances for *t*-tests and homogeneity of multivariate dispersions (Anderson 2006) for the permutational multivariate analysis of variance (perMANOVA; Anderson 2001) prior to analysis. Root biomass data were square root-transformed to achieve normality and homoscedasticity.

Feeding group distributions of nematode communities between treatments and through time were visualized using non-metric multidimensional scaling (NMDS; Kruskal 1964). Distances between the rank orders of plots were determined using the Bray-Curtis dissimilarity index. A repeated measures perMANOVA was used to ascertain whether enclosures affected the feeding structure of nematode communities. Density and proportion data were assessed with both multivariate methods.

Changes (final – initial) in densities and proportions of soil nematodes were analyzed with sets of *t*-tests. No transformation enabled changes in soil microarthropod densities to achieve normality, so these data were analyzed with Wilcoxon rank-sum tests. Analysis of change data was preferred over using initial and final data in a repeated measures framework because the main effects of time and enclosures were unrelated to the hypotheses tested. One-sided *t*-tests and Wilcoxon rank-sum tests were used when appropriate and are indicated in the text. The proportion of variation in final soil organism data (densities for nematodes and microarthropods and proportions for nematodes) explained by root biomass sampled from the same cover type and depth was determined using Pearson correlations when the assumption of bivariate normality was met. Otherwise, Spearman rank correlations were used. Significance was determined at $\alpha=0.05$ for all analyses, but marginal significance ($0.05 < p < 0.1$) is noted where appropriate. All statistics were computed in R (R Development Core Team 2012).

2.3 RESULTS

Aboveground Plant Biomass. Total aboveground biomass was not significantly different between treatments (one-sided, $t_{10}=-0.699$, $p=0.75$; Fig. 2.5A) despite an average of 8.7% more biomass in grasshopper plots. *Vulpia octoflora* made up most aboveground

biomass in both cage types (average enclosure = 93.47%, average grasshopper plot = 93.94%). Caged controls had 8.45% more *V. octoflora* biomass and 11.82% less biomass from other grasses (predominantly *B. gracilis*), on average, than enclosures, but neither comparison was significant (*V. octoflora*: $t_{10}=-0.735$, $p=0.479$; other grasses: one-sided, $t_{10}=0.544$, $p=0.299$; Fig. 2.5B).

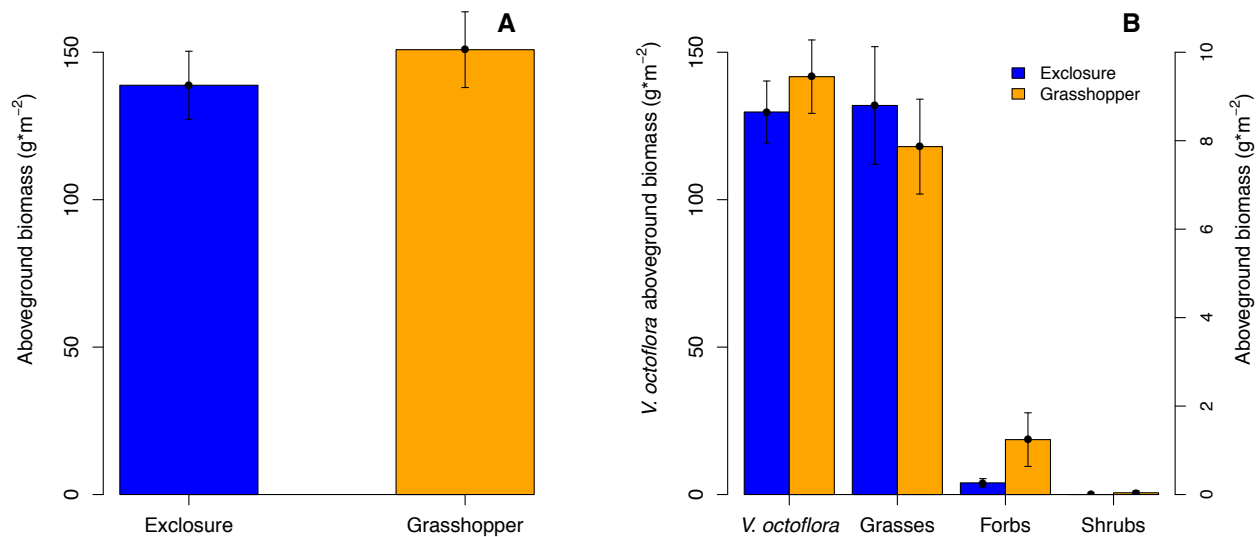


Figure 2.5: Mean final aboveground plant biomass (\pm SE) in 0.5 m² plots in the shortgrass steppe of northern Colorado for (A) all plants and (B) *Vulpia octoflora*, other grasses, forbs, and shrubs. Half of the plots excluded grasshoppers, and half provided grasshoppers access to plants. For (B), note that the left scale applies to *V. octoflora* only. Total aboveground biomass and aboveground biomass of *V. octoflora* and other grasses were not significantly different between cage types ($p>0.05$ in all cases).

Belowground Plant Biomass. Root biomass data from three 10–20 cm soil samples (plant and bare soil cores from plot 1 and plant soil core from plot 2) were not collected due to equipment issues, reducing the analysis to 45 samples. The variance terms of the mixed model, which correspond to the random effects and explain the nested design, were estimated to be 0; thus the model was reduced to a simple 3-way analysis of variance (Table 2.1). The 3- and 2-way interactions were not significant (all $p>0.05$). Of the three

main effects, only depth was significant ($F_{1,37}=56.635$, $p<0.001$), with an average of 176.09% more root biomass found at 0–10 cm than 10–20 cm. No enclosure effect was found ($F_{1,37}=0.133$, $p=0.717$).

Table 2.1: Results from 3-way analysis of variance investigating the effects of grasshopper exclusion, cover type (plant-covered area and bare soil), depth (0–10 and 10–20 cm), and all interactions on square-root transformed root biomass (g/m^2) in the shortgrass steppe of northern Colorado

Factor	<i>df</i>	SS	<i>F</i>	<i>p</i>
Exclosure	1	1.7	0.133	0.717
Cover	1	37.8	3.026	0.090
Depth	1	708.0	56.635	< 0.001
Exclosure × Depth	1	4.8	0.386	0.538
Exclosure × Cover	1	6.2	0.493	0.487
Cover × Depth	1	1.0	0.078	0.781
Exclosure × Cover × Depth	1	4.9	0.391	0.535
Error	37	462.5		

Shoot: Root Ratios. The shoot: root ratios were, on average, 15.78% greater in grasshopper plots, but this difference was not significant ($t_8=-1.468$, $p=0.180$). Data from plots 1 and 2 were excluded from the analysis due to missing samples (see Belowground Plant Biomass above).

Multivariate Analysis of Soil Nematode Communities. NMDS ordination of nematode communities using density data shows considerable overlap between herbivory treatments within each harvest but differentiation across harvests (Fig. 2.6). This is supported by the non-significant exclosure × time interaction (pseudo $F_{1,10}=1.289$, $p=0.294$) and significant time effect (pseudo $F_{1,10}=51.533$, $p=0.001$) in the repeated measures perMANOVA (Table 2.2).

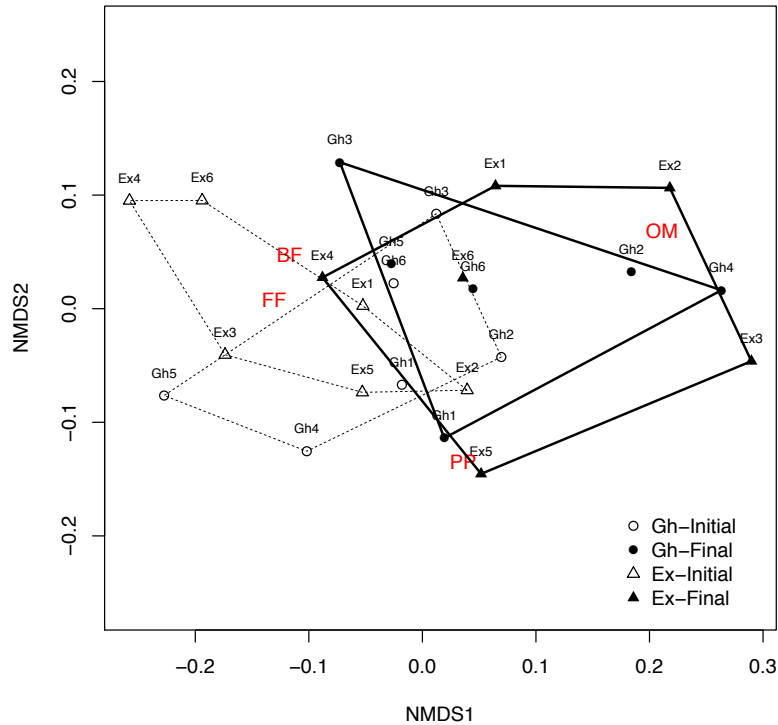


Figure 2.6: Non-metric multidimensional scaling (NMDS; stress value=0.097) ordination plot illustrating feeding group structure of nematode communities in the shortgrass steppe of northern Colorado for both harvests and herbivory treatments using density data. Points that are closer together are more similar in community composition. Points near a feeding group (BF=bacterial feeders, FF=fungal feeders, PP=plant parasites, OM=omnivores) likely contain large numbers of that feeding group. Convex hulls indicate each harvest-herbivory treatment combination.

Table 2.2: Results from repeated measures permutational multivariate analysis of variance (perMANOVA) investigating the effects of grasshopper exclusion, time, and their interaction on densities (number of individuals/dry kg soil) of nematode feeding groups in the shortgrass steppe of northern Colorado

Factor	<i>df</i>	SS	pseudo <i>F</i>	<i>p</i>
Exclosure	1	0.02654	0.772	0.566
Error (1)	10	0.34363		
Time	1	1.27549	51.533	0.001
Exclosure × Time	1	0.03189	1.289	0.294
Error (2)	10	0.24751		

Using proportion data, the NMDS plot shows that nematode communities exhibited separation through time within the enclosure treatment, but Gh5 (final harvest) prevented complete differentiation between herbivory treatments for the final harvest (Fig. 2.7). Data points were more scattered for enclosures than grasshopper plots in the final harvest. Enclosures did not affect proportions of nematode feeding groups (enclosure \times time interaction, pseudo $F_{1,10}=1.989$, $p=0.170$; Table 2.3). However, trophic structure of nematode communities changed through time (pseudo $F_{1,10}=7.066$, $p=0.003$).

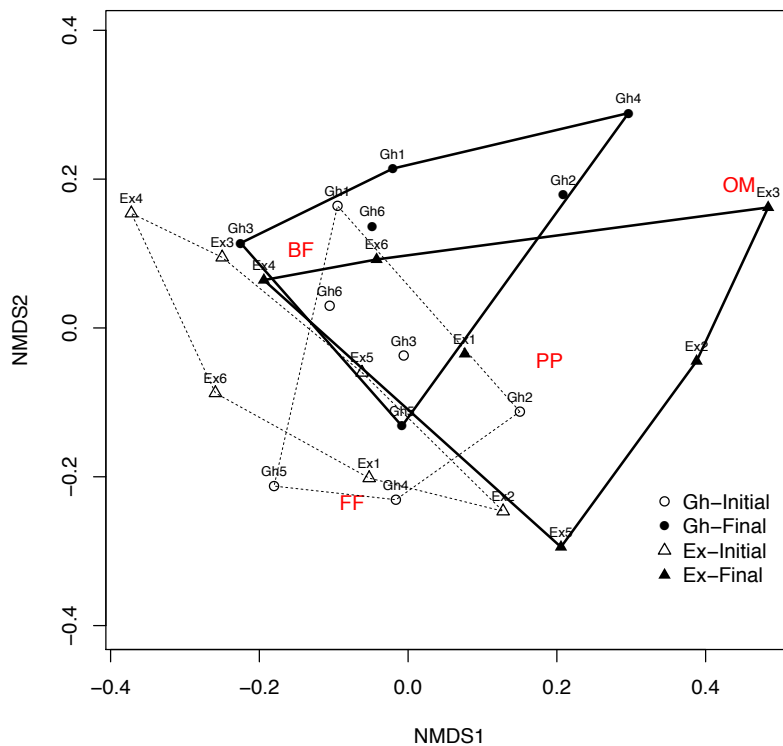


Figure 2.7: Non-metric multidimensional scaling (NMDS; stress value=0.085) ordination plot illustrating feeding group structure of nematode communities in the shortgrass steppe of northern Colorado for both harvests and herbivory treatments using proportion data. Points that are closer together are more similar in community composition. Points near a feeding group (BF=bacterial feeders, FF=fungal feeders, PP=plant parasites, OM=omnivores) likely contain large proportions of that feeding group. Convex hulls indicate each harvest-herbivory treatment combination.

Table 2.3: Results from repeated measures permutational multivariate analysis of variance (perMANOVA) investigating the effects of grasshopper exclusion, time, and their interaction on proportions of nematode feeding groups in the shortgrass steppe of northern Colorado

Factor	<i>df</i>	SS	pseudo <i>F</i>	<i>p</i>
Exclosure	1	0.01048	0.3869	0.733
Error (1)	10	0.27081		
Time	1	0.08201	7.0656	0.003
Exclosure × Time	1	0.02309	1.9894	0.170
Error (2)	10	0.11606		

Univariate Analysis of Soil Nematodes. The total density of nematodes decreased with time in both grasshopper plots (average = -17,476.3 nematodes/kg dry soil) and exclosures (average = -18,528.54 nematodes/kg dry soil), but no treatment effect was found ($t_{10}=-0.213$, $p=0.835$; Fig. 2.8A). Temporal changes in the densities of bacterial feeders, fungal feeders, plant parasites, and omnivores were also similar between cage types (all $p>0.05$; Figs. 2.8B–E). However, the ratio of bacterial feeders to fungal feeders increased significantly over time in grasshopper plots (average = +1.02) relative to exclosures (average = +0.004; one-sided, $t_{10}=-2.092$, $p=0.031$; Fig. 2.8F).

Proportions of bacterivorous nematodes, on average, decreased by 19.8% in exclosures but increased by 7.6% in grasshopper plots, indicating a significant exclosure effect (one-sided, $t_{10}=-2.44$, $p=0.017$; Fig. 2.9A). Plant parasites exhibited the opposite pattern, which was marginally significant (one-sided, $t_{10}=1.384$, $p=0.098$; Fig. 2.9C). Their proportions, on average, grew by 28.6% in exclosures but shrunk by 6% in grasshopper plots. The direction of change in proportions was the same between treatments for fungal feeders (decreased) and omnivores (increased), and no exclosure effect was found for either group (both $p>0.05$; Figs. 2.9B and D).

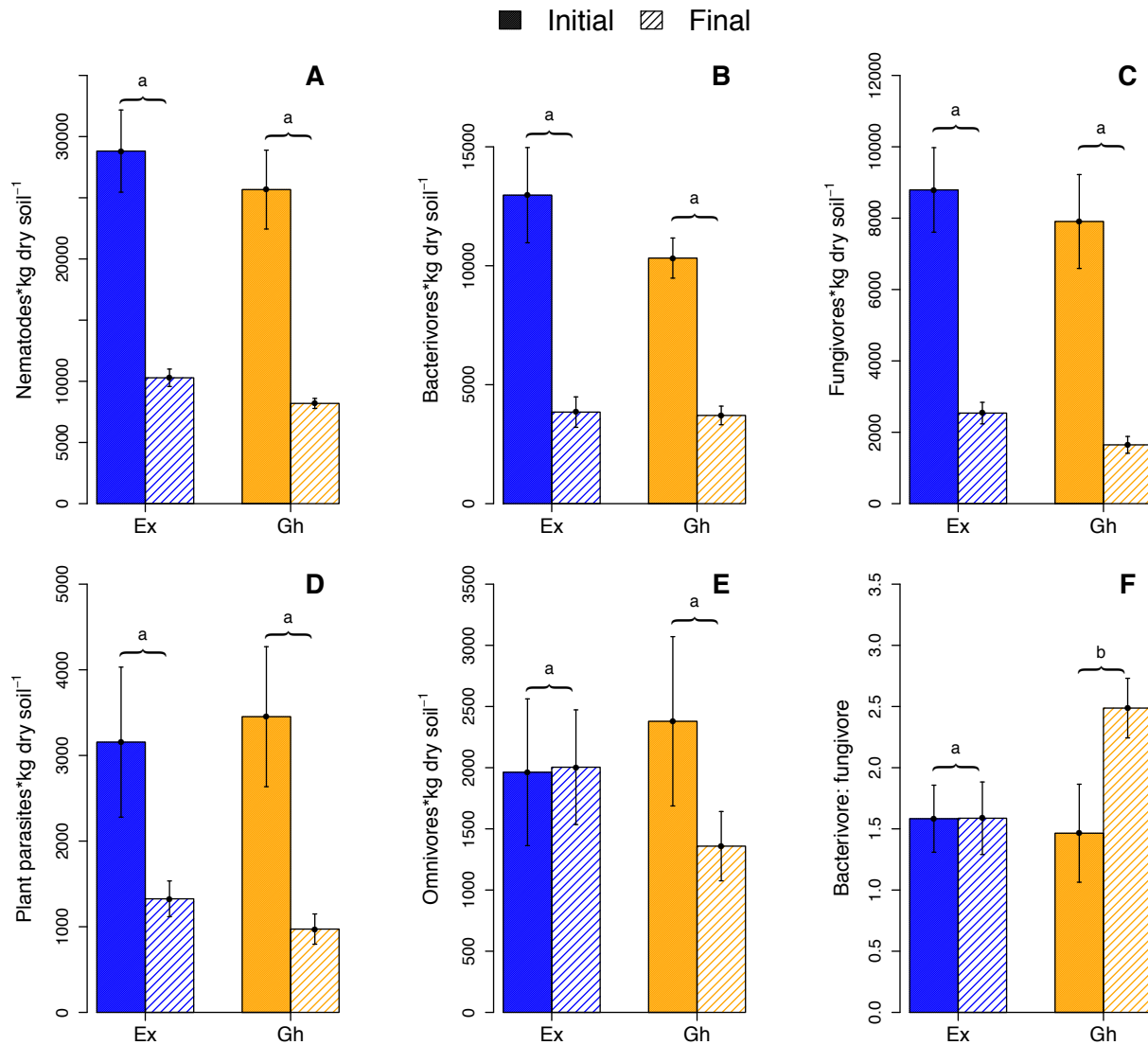


Figure 2.8: Mean (\pm SE) initial and final densities of (A) all, (B) bacterivorous, (C) fungivorous, (D) plant parasitic, and (E) omnivorous nematodes and (F) the ratio of bacterivorous to fungivorous nematodes in plots that excluded (Ex) and permitted (Gh) access to grasshoppers in the shortgrass steppe of northern Colorado. For each feeding group, pairs of bars with the same letters were not significantly different between treatments regarding their temporal change (final – initial) in densities or ratios ($p > 0.05$). Predaceous nematodes were too rare for analysis.

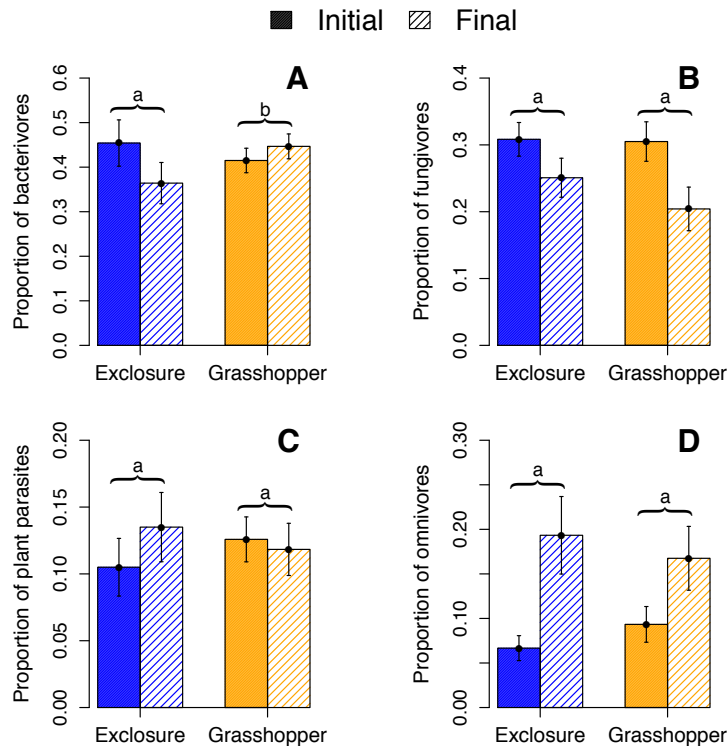


Figure 2.9: Mean (\pm SE) initial and final proportions of (A) bacterivorous, (B) fungivorous, (C) plant parasitic, and (D) omnivorous nematodes in plots that excluded and permitted access to grasshoppers in the shortgrass steppe of northern Colorado. For each feeding group, pairs of bars with the same letters were not significantly different between treatments regarding their temporal change (final – initial) in proportions ($p > 0.05$). Predaceous nematodes were too rare for analysis.

Correlations Between Nematodes and Root Biomass. Root biomass collected at 0–10 cm from both cover types was poorly correlated with final densities of nematode feeding groups using all data and data from grasshopper plots (all $p > 0.1$; Table 2.4). In exclosures only, correlation between root biomass and fungal feeders was positive and marginally significant ($r = 0.746$, $p = 0.088$), whereas root biomass and plant parasites were strongly and negatively correlated ($r = -0.839$, $p = 0.037$). Total densities of nematodes were weakly correlated with root biomass using data from exclosures, grasshopper plots, and both cage types (all $p > 0.1$).

Table 2.4: Pearson correlations (except where indicated) between root biomass (g/m²) at 0–10 cm from both cover types and final densities (number of individuals/kg dry soil) and proportions of nematodes using all data and data from exclosures and grasshopper plots only in the shortgrass steppe of northern Colorado

Densities	All Data	Exclosures Only	Grasshopper Plots Only
Bacterivores	0.259	0.462	0.066
Fungivores	0.308	0.746*	0.228
Plant parasites	-0.415	-0.839**	-0.086 ^a
Omnivores	-0.434	-0.354	-0.579
Total	-0.028	0.230	-0.159
Proportions			
Bacterivores	0.361	0.492	0.219
Fungivores	0.305	0.535	0.248
Plant parasites	-0.362	-0.736*	0.029 ^a
Omnivores	-0.442	-0.384	-0.5

*marginal significance (0.05 < p < 0.1)

**significance (p < 0.05)

^aSpearman rank correlation used

Final proportions of all nematode feeding groups were poorly correlated (p > 0.1) with root biomass using all datasets, except for one relationship (Table 2.4). The correlation between root biomass and the final proportion of plant parasites was negative, which was marginally significant (r = -0.736, p = 0.095).

Soil Microarthropods. Temporal changes in the densities of mite groups (generalists and predators), all mites, and all microarthropods were similar between cage types despite contrasting patterns in three of the four groups (all p > 0.05; Fig. 2.10). All but the predaceous mites increased in densities over time in exclosures (averages: generalists = +124.3%, all mites = +101.37%, all microarthropods = +90.76%), but exhibited small reductions in grasshopper plots (averages: generalists = -4.99%, all mites = -5.41%, all microarthropods = -1.68%). These patterns are likely driven by generalist mites, which composed, on average, 89.17% of all mites and 84.71% of all microarthropods. Non-mite

microarthropods included members of Coleoptera, Collembola, Diplura, Diptera, Hemiptera (Aphididae and Cicadellidae), Hymenoptera (Formicidae), Psocoptera, and Thysanoptera, but no group was common enough for separate analysis.

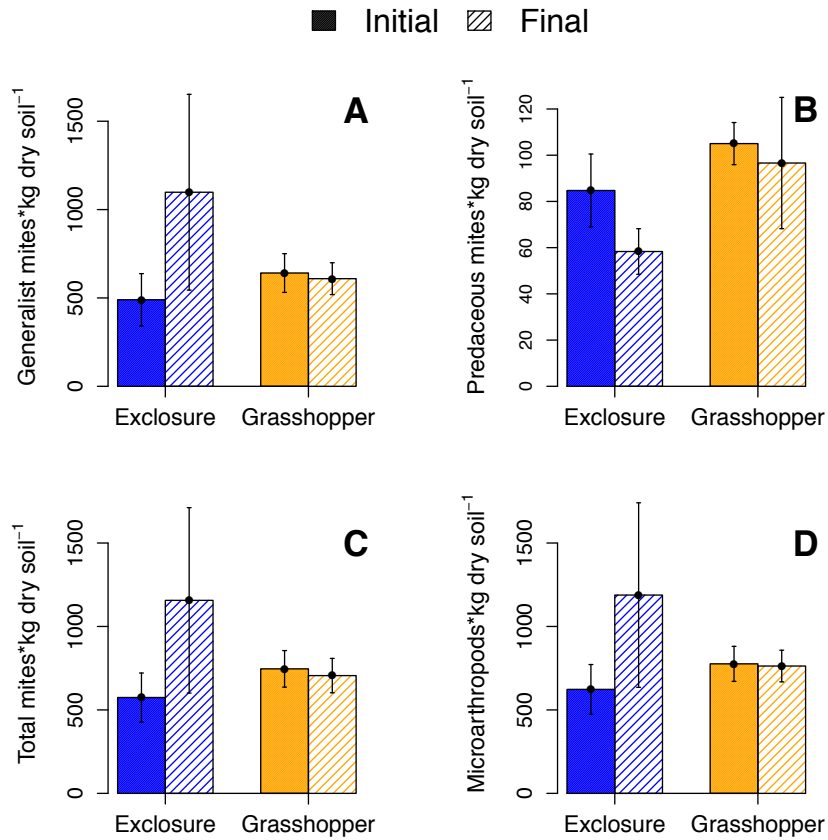


Figure 2.10: Mean (\pm SE) initial and final densities of (A) generalist mites, (B) predaceous mites, (C) all mites, and (D) all microarthropods in plots that excluded and permitted access to grasshoppers in the shortgrass steppe of northern Colorado. Temporal change (final – initial) in microarthropod densities were not significantly different between treatments for any group ($p > 0.05$ in all cases).

Correlations Between Microarthropods and Root Biomass. Root biomass collected from 0–10 cm under plant-covered areas were poorly correlated with final densities of generalist mites, all mites, and all microarthropods in exclosures, grasshopper plots, and all plots (all $p > 0.1$; Table 2.5). Predaceous mites were strongly and positively

correlated with root biomass using all data ($r=0.738$, $p=0.006$). This relationship was still positive but marginally significant in grasshopper plots ($r=0.797$, $p=0.057$) and weakest in exclosures ($r=0.607$, $p=0.202$).

Table 2.5: Pearson correlations (except where indicated) between root biomass (g/m²) at 0–10 cm from plant-covered areas only and final densities (number of individuals/kg dry soil) of microarthropod groups using all data and data from exclosures and grasshopper plots only in the shortgrass steppe of northern Colorado

Microarthropod group	All Data	Exclosures Only	Grasshopper Plots Only
Generalist mites	0.119 ^a	0.143 ^a	0.143 ^a
Predaceous mites	0.738**	0.607	0.797*
All mites	0.14 ^a	0.143 ^a	0.281
All microarthropods	0.168 ^a	0.143 ^a	0.34

*marginal significance ($0.05 < p < 0.1$)

**significance ($p < 0.05$)

^aSpearman rank correlation used

2.4 DISCUSSION

This is the first study to demonstrate that grasshopper populations that graze on plants in a natural ecosystem can affect the feeding structure of soil nematode communities. In contrast, soil microarthropod groups were unresponsive to manipulation of these insect herbivores. The atypical composition of the shortgrass steppe plant community and timing of this study (late summer–early fall) influenced this grasshopper-plant interaction and its effects on soil animals. This research indicates a linkage between grasshoppers and soil nematodes in this ecosystem, which could be modified by a changing climate, particularly one with greater drought frequency.

Though this study was conducted on a site (i.e., an ungrazed pasture at the Central Plains Experimental Range) frequently dominated by *B. gracilis*, 2014 saw an overabundance of the annual grass *V. octoflora*. Using long-term (55 yr) sampling data of

ungrazed areas in the northern shortgrass steppe, *B. gracilis* averaged roughly 15% of total aboveground biomass, and 40% if non-targeted plants (i.e., cacti, shrubs, and subshrubs) are removed (Hart 2001), compared to just 5.75% in this study. By comparison, *V. octoflora* composed, on average, less than 0.05% of both total and targeted aboveground biomass using the same long-term dataset (Hart 2001) but over 93% in this study. Though typically rare, *V. octoflora* achieves high densities in the central Great Plains 1–2 times every decade (Hyder and Bement 1964), which is driven by precipitation during the prior year. Growth, maturation, and seed production of *V. octoflora* in wet springs followed by heavy, early fall rains stimulate germination and large populations the next year (Houston and Hyder 1976), a pattern supported by 2013 precipitation data from the field site (USDA-ARS 2015). This shift in dominant grasses presented an anomalous composition of host-plants for late-season grasshoppers in 2014.

Exclosures did not completely prevent grasshopper access to shortgrass steppe plants, but they did depress their numbers in this study. Grasshopper abundances in exclosures were less than half that of caged controls. However, this is a conservative estimate as grasshopper counts within control plots varied considerably in short time periods (e.g., 1 hr; K. H. Post, personal observation), but weekly observations were made in 5 min throughout the experiment. Therefore, exclosures substantially reduced activity of grasshoppers relative to caged controls.

Despite less activity, grasshoppers had no effect on final aboveground plant biomass from *V. octoflora*, other grasses (mainly *B. gracilis*), or all plants, which could be explained by plant physiology, and plant and grasshopper community composition during the study. *Bouteloua gracilis* employs compensatory growth, as opposed to chemical defense (Mole

and Joern 1993, 1994, Hazlett and Sawyer 1998), in response to defoliation by allocating a greater proportion of new photosynthate to aboveground tissues (Detling et al. 1979, Williamson et al. 1989, but see Augustine et al. 2011). In a greenhouse study, total shoot biomass of *B. gracilis* did not differ between undefoliated controls and grasses subjected to 10% removal of aboveground biomass by *Melanoplus sanguinipes* grasshoppers immediately or 1, 3, 6, or 9 wk post-defoliation (Walmsley et al. 1987). Thus, enhanced regrowth rates may negate the effects of grasshopper herbivory, even in short time periods. Also recall that the study site was dominated by *V. octoflora*, not *B. gracilis*, in 2014. Considering only grasshopper species that were common (0.1–1 individual/10 m²) or abundant (> 1 individual/m²) at the Central Plains Experimental Range in either or both Van Horn et al. (1972) or Welch et al. (1991), only three species consume substantial amounts of *V. octoflora* (*Arphia conspersa*, *Psoloessa delicatula*, and *Xanthippus corallipes*), and they would have been nymphs during the experiment (Pfadt 1994). Further, *V. octoflora* dies by late season, becoming unpalatable to grasshoppers (Pfadt 1994). Therefore, grasshoppers may have fed on *B. gracilis* but imposed little if any damage to *V. octoflora* during this study.

Final root biomass was also similar between cage types which does not support H₁, but other factors may have been important. A compensatory growth response by *B. gracilis* to grazing reduces allocation of new photosynthate to belowground tissues (Detling et al. 1979); thus, a positive exclosure effect on root biomass was expected. However, *B. gracilis* was approaching or in senescence during the study (Dickinson and Dodd 1976), so its response to defoliation could have been diminished. But the average maximum and minimum air temperatures at the field site during the study period were 6.56% and

27.55% greater (NCDC 2014) than the 30-year averages (NCDC 2010), respectively. A warm late summer and early fall suggests a longer growing season, which could have tempered the effect of senescence on *B. gracilis*. Further, given that more than 93% of aboveground plant biomass was from *V. octoflora*, it is likely that a large proportion of belowground biomass was from this plant. Unfortunately, actual amounts are unavailable because root biomass was not sorted by species. Therefore, any enclosure effect on *B. gracilis* root growth would have been minimal due to reduced physiological activity, and this effect may have been obscured by the abundant *V. octoflora*.

Despite not finding plant biomass responses to altered grasshopper abundances, grasshopper exclusion did affect the root energy channel (Fig. 2.1), partly supporting H₂. There was a trend for the proportion of plant parasitic nematodes to grow in soils of caged controls and decline in enclosures, which was a pattern predicted by H₂. Since these animals are belowground herbivores, enclosures appeared to affect their relative abundances through alterations in plant roots despite similar total root biomass between cage types. Responses by plant parasitic nematodes to grasshopper exclusion could be explained by a lower than average occurrence of *B. gracilis* across all plots and partially senesced root biomass (Dickinson and Dodd 1976). Interestingly, these nematodes were negatively correlated (densities=significant, proportions=trend) with root biomass in enclosures only (Table 2.4). No differences between cage types for fungivorous nematodes and generalist mites were found, which opposes the soil fungal energy channel (Fig. 2.1) component of this hypothesis. The lack of a belowground plant biomass response to grasshopper exclusion, as mentioned earlier, would translate to an even smaller disparity between cage types in root detrital substrates used by soil fungi and consumers of both

resources, including generalist-feeding oribatid and prostigmatid mites (Coleman et al. 2004). Further, such subtle differences in food sources may not affect oribatid mites, which are insensitive to nutrient pulses due to their long generation times and low fecundity (Behan-Pelletier and Newton 1999).

Responses by bacterivorous nematodes to grasshopper exclusion support H₃. The proportion of bacterivorous nematodes and the ratio of bacterivorous to fungivorous nematodes increased through time in grasshopper plots, while a decline and no change, respectively, occurred in exclosures. The growth in the ratio of these two feeding groups in caged controls (Fig. 2.8F) was driven by a larger reduction in the density of fungivorous than bacterivorous nematodes (Figs. 2.8B and 2.8C). These results suggest enhanced dominance of the bacterial energy channel (Fig. 2.1; Moore et al. 1988) either through increased production of root exudates (Hamilton and Frank 2001) or greater deposition of grasshopper frass (Lovett and Ruesink 1995, Fielding et al. 2013), both labile resources. Greater grasshopper activity in caged controls combined with similar above- and belowground plant biomass between cage types suggests a non-consumptive effect of grasshoppers (i.e., frass deposition) on the soil bacterial channel (Fig. 2.1). Thus, the amount of fecal deposition by grasshoppers could alter the energetic structure of the soil food web, particularly the relative dominance of the bacterial versus the fungal energy channel. This is supported by the NMDS ordination plot of proportion data, which shows movement of the grasshopper plot points away from fungivorous nematodes and location of all but one of the final harvest points from this cage type on a plane between bacterivorous and omnivorous nematodes (Fig. 2.7).

Results of this research have implications for the effects of global climate change, specifically droughts, on this ecosystem. Droughts have been common in the western United States since 800 AD (Cook et al. 2004), and climate models project they may increase in frequency (Strzepek et al. 2010). Grasshopper populations in shortgrass prairies of eastern Colorado have been negatively associated with droughts (Capinera and Horton 1989). Thus, more frequent droughts in a changing climate may depress grasshopper abundances, and in turn affect soil food webs. If the findings of this study have predictive power, then smaller grasshopper populations would diminish dominance of the soil bacterial energy pathway (Fig. 2.1) through reduced waste deposition and root exudation. Despite the pattern observed for proportions of plant parasitic nematodes, a smaller root-based energy channel (Fig. 2.1) is expected in a climate with greater drought frequency. Belowground productivity of shortgrass steppe plants is positively related to precipitation (Evans and Burke 2013, Wilcox et al. 2015). Therefore droughts would reduce root biomass available for soil organisms. Further, prolonged dry periods can induce dormancy in *B. gracilis* (Shantz 1923), which could diminish its response to insect defoliation and perhaps alter the quality and/or quantity of substrates available to soil microbes, the food source of many soil nematodes (Yeates et al. 1993) and microarthropods (Schneider et al. 2004). Droughts could also affect soil communities directly (Sylvain et al. 2014), or indirectly through changes in plant community composition (Kardol et al. 2010).

This study showed that exclusion of grasshoppers in the northern shortgrass steppe affected the energetic structure of the soil food web through consumptive means, non-consumptive means, or both. Continued feeding by grasshoppers late into the growing

season favored the dominance of the soil bacterial energy pathway over the fungal pathway and tended to stimulate plant parasitic nematode populations. However, such changes did not affect omnivorous nematodes or soil microarthropods. Though this study suggests the potential effects of global climate change on soil food webs through alterations in grasshopper populations, more research is needed to fully understand this aboveground-belowground linkage under future climates.

REFERENCES

- Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**:32-46.
- Anderson, M. J. 2006. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **62**:245-253.
- Augustine, D. J., F. A. Dijkstra, E. W. Hamilton, III, and J. A. Morgan. 2011. Rhizosphere interactions, carbon allocation, and nitrogen acquisition of two perennial North American grasses in response to defoliation and elevated atmospheric CO₂. *Oecologia* **165**:755-770.
- Baermann, G. 1917. Eine einfache Methode zur Auffindung von Ancylostomum (Nematoden) Larven in Erdproben. *Geneeskundig Tijdschrift voor Nederlandsch-Indië* **57**:131-137.
- Bardgett, R. D., D. K. Leemans, R. Cook, and P. J. Hobbs. 1997. Seasonality of the soil biota of grazed and ungrazed hill grasslands. *Soil Biology and Biochemistry* **29**:1285-1294.
- Bardgett, R. D. and W. H. van der Putten. 2014. Belowground biodiversity and ecosystem functioning. *Nature* **515**:505-511.
- Bardgett, R. D. and D. A. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* **84**:2258-2268.
- Bardgett, R. D. and D. A. Wardle. 2010. Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford University Press, New York, New York, USA.
- Bartelt-Ryser, J., J. Joshi, B. Schmid, H. Brandl, and T. Balsler. 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. *Plant Ecology, Evolution and Systematics* **7**:27-49.
- Behan-Pelletier, V. and G. Newton. 1999. Computers in biology: linking soil biodiversity and ecosystem function--the taxonomic dilemma. *BioScience* **49**:149-153.
- Bradford, M. A., T. Gancos, and C. J. Frost. 2008. Slow-cycle effects of foliar herbivory alter the nitrogen acquisition and population size of Collembola. *Soil Biology and Biochemistry* **40**:1253-1258.
- Capinera, J. L. and D. R. Horton. 1989. Geographic variation in effects of weather on grasshopper infestation. *Environmental Entomology* **18**:8-14.

- Capinera, J. L. and T. S. Sechrist. 1982. Grasshopper (Acrididae)-host plant associations: response of grasshopper populations to cattle grazing intensity. *Canadian Entomologist* **114**:1055-1062.
- Clapperton, M. J., D. A. Kanashiro, and V. M. Behan-Pelletier. 2002. Changes in abundance and diversity of microarthropods associated with fescue prairie grazing regimes. *Pedobiologia* **46**:496-511.
- Coleman, D. C., D. A. Crossley, Jr., and P. F. Hendrix. 2004. *Fundamentals of soil ecology*. 2nd edition. Academic Press, Burlington, Vermont, USA.
- Coleman, D. C., C. P. P. Reid, and C. V. Cole. 1983. Biological strategies of nutrient cycling in soil systems. *Advances in Ecological Research* **13**:1-55.
- Cook, E. R., C. A. Woodhouse, C. M. Eakin, D. M. Meko, and D. W. Stahle. 2004. Long-term aridity changes in the western United States. *Science* **306**:1015-1018.
- Cotrufo, M. F., J. Soong, M. L. Vandegehuchte, T. Nguyen, K. Denef, E. A. Shaw, Z. A. Sylvain, C. M. Tomael, U. N. Nielsen, and D. H. Wall. 2014. Naphthalene addition to soil surfaces: a feasible method to reduce soil micro-arthropods with negligible direct effects on soil C dynamics. *Applied Soil Ecology* **74**:21-29.
- Detling, J. K., M. I. Dyer, and D. T. Winn. 1979. Net photosynthesis, root respiration, and regrowth of *Bouteloua gracilis* following simulated grazing. *Oecologia* **41**:127-134.
- Dickinson, C. E. and J. L. Dodd. 1976. Phenological pattern in the shortgrass prairie. *American Midland Naturalist* **96**:367-378.
- Evans, D. M., N. E. Turley, D. J. Levey, and J. J. Tewksbury. 2012. Habitat patch shape, not corridors, determines herbivory and fruit production of an annual plant. *Ecology* **93**:1016-1025.
- Evans, S. E. and I. C. Burke. 2013. Carbon and nitrogen decoupling under an 11-year drought in the shortgrass steppe. *Ecosystems* **16**:20-33.
- Fielding, D. J., E. Trainor, and M. Zhang. 2013. Diet influences rate of carbon and nitrogen mineralization from decomposing grasshopper frass and cadavers. *Biology and Fertility of Soils* **49**:537-544.
- Frost, C. J. and M. D. Hunter. 2004. Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. *Ecology* **85**:3335-3347.
- Fu, S., K. W. Kisselle, D. C. Coleman, P. F. Hendrix, and D. A. Crossley, Jr. 2001. Short-term impacts of aboveground herbivory (grasshopper) on the abundance and ¹⁴C activity of soil nematodes in conventional tillage and no-till agroecosystems. *Soil Biology and Biochemistry* **33**:1253-1258.

- Gange, A. C. and V. K. Brown. 1989. Effects of root herbivory by an insect on a foliar-feeding species, mediated through changes in the host plant. *Oecologia* **81**:38-42.
- García-Palacios, P., F. T. Maestre, J. Kattge, and D. H. Wall. 2013. Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. *Ecology Letters* **16**:1045-1053.
- Göransson, H., D. L. Godbold, D. L. Jones, and J. Rousk. 2013. Bacterial growth and respiration responses upon rewetting dry forest soils: impact of drought-legacy. *Soil Biology and Biochemistry* **57**:477-486.
- Haase, J., R. Brandl, S. Scheu, and M. Schädler. 2008. Above- and belowground interactions are mediated by nutrient availability. *Ecology* **89**:3072-3081.
- Hamilton, E. W., III and D. A. Frank. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* **82**:2397-2402.
- Hamilton, E. W., III, D. A. Frank, P. M. Hinchey, and T. R. Murray. 2008. Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. *Soil Biology and Biochemistry* **40**:2865-2873.
- Hart, R. H. 2001. Plant biodiversity on shortgrass steppe after 55 years of zero, light, moderate, or heavy cattle grazing. *Plant Ecology* **155**:111-118.
- Hazlett, D. L. and N. W. Sawyer. 1998. Distribution of alkaloid-rich plant species in shortgrass steppe vegetation. *Conservation Biology* **12**:1260-1268.
- Henry, F., M. Vestergård, and S. Christensen. 2008. Evidence for a transient increase of rhizodeposition within one and a half day after a severe defoliation of *Plantago arenaria* grown in soil. *Soil Biology and Biochemistry* **40**:1264-1267.
- Holland, J. N. 1995. Effects of above-ground herbivory on soil microbial biomass in conventional and no-tillage agroecosystems. *Applied Soil Ecology* **2**:275-279.
- Houston, W. R. and D. N. Hyder. 1976. Controlling sixweeks fescue on shortgrass range. *Journal of Range Management* **29**:151-153.
- Hyder, D. N. and R. E. Bement. 1964. Sixweeks fescue as a deterrent to blue grama utilization. *Journal of Range Management* **17**:261-264.
- Jamiyansharav, K., D. Ojima, R. A. Pielke, W. Parton, J. Morgan, A. Beltrán-Przekurat, D. LeCain, and D. Smith. 2011. Seasonal and interannual variability in surface energy partitioning and vegetation cover with grazing at shortgrass steppe. *Journal of Arid Environments* **75**:360-370.

- Jones, T. H., L. J. Thompson, J. H. Lawton, T. M. Bezemer, R. D. Bardgett, T. M. Blackburn, K. D. Bruce, P. F. Cannon, G. S. Hall, S. E. Hartley, G. Howson, C. G. Jones, C. Kampichler, E. Kandeler, and D. A. Ritchie. 1998. Impacts of rising atmospheric carbon dioxide on model terrestrial ecosystems. *Science* **280**:441-443.
- Kardol, P., N. J. Cornips, M. M. L. van Kempen, J. M. T. Bakx-Schotman, and W. H. van der Putten. 2007. Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs* **77**:147-162.
- Kardol, P., M. A. Cregger, C. E. Company, and A. T. Classen. 2010. Soil ecosystem functioning under climate change: plant species and community effects. *Ecology* **91**:767-781.
- Kruskal, J. B. 1964. Nonmetric multidimensional scaling: a numerical method. *Psychometrika* **29**:115-129.
- Lauenroth, W. K. 2008. Vegetation of the shortgrass steppe. Pages 70-83 in W. K. Lauenroth and I. C. Burke, editors. *Ecology of the shortgrass steppe: a long-term perspective*. Oxford University Press, New York, New York, USA.
- Lauenroth, W. K., I. C. Burke, and J. A. Morgan. 2008. The shortgrass steppe: the region and research sites. Pages 3-13 in W. K. Lauenroth and I. C. Burke, editors. *Ecology of the shortgrass steppe: a long-term perspective*. Oxford University Press, New York, New York, USA.
- Lauenroth, W. K. and D. G. Milchunas. 1992. The shortgrass steppe. Pages 183-226 in R. T. Coupland, editor. *Natural grasslands: introduction and western hemisphere*. Elsevier Scientific Press, Amsterdam, The Netherlands.
- Lovett, G. M. and A. E. Ruesink. 1995. Carbon and nitrogen mineralization from decomposing gypsy moth frass. *Oecologia* **104**:133-138.
- LTER-SGS. 2009. Central Plains Experimental Range (CPER) detail map. Nunn, Colorado, USA.
- Masters, G. J. and V. K. Brown. 1992. Plant-mediated interactions between two spatially separated insects. *Functional Ecology* **6**:175-179.
- Masters, G. J., V. K. Brown, and A. C. Gange. 1993. Plant mediated interactions between above- and below-ground insect herbivores. *Oikos* **66**:148-151.
- Mikola, J., M. Nieminen, K. Ilmarinen, and M. Vestberg. 2005. Belowground responses by AM fungi and animal trophic groups to repeated defoliation in an experimental grassland community. *Soil Biology and Biochemistry* **37**:1630-1639.

- Mole, S. and A. Joern. 1993. Foliar phenolics of Nebraska sandhills prairie graminoids: between-years, seasonal, and interspecific variation. *Journal of Chemical Ecology* **19**:1861-1874.
- Mole, S. and A. Joern. 1994. Feeding behavior of graminivorous grasshoppers in response to host-plant extracts, alkaloids, and tannins. *Journal of Chemical Ecology* **20**:3097-3109.
- Moore, J. C., K. McCann, H. Setälä, and P. C. de Ruiter. 2003. Top-down is bottom-up: does predation in the rhizosphere regulate aboveground dynamics? *Ecology* **84**:846-857.
- Moore, J. C., D. E. Walter, and H. W. Hunt. 1988. Arthropod regulation of micro- and mesobiota in below-ground detrital food webs. *Annual Review of Entomology* **33**:419-439.
- Moran, N. A. and T. G. Whitham. 1990. Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. *Ecology* **71**:1050-1058.
- NCDC. 2010. 1981-2010 station normals of temperature, precipitation, and heating and cooling degree days. Asheville, North Carolina, USA.
- NCDC. 2014. Record of climatological observations September-October 2014. Asheville, North Carolina, USA.
- Pfadt, R. E. 1994. Field guide to common western grasshoppers. University of Wyoming, Laramie, Wyoming, USA.
- Przybyszewski, J. and J. L. Capinera. 1990. Spatial and temporal patterns of grasshopper (Orthoptera: Acrididae) phenology and abundance on a shortgrass prairie. *Journal of the Kansas Entomological Society* **63**:405-413.
- R Development Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rodríguez-Echeverría, S., C. Afonso, M. Correia, P. Lorenzo, and S. R. Roiloa. 2013. The effect of soil legacy on competition and invasion by *Acacia dealbata* Link. *Plant Ecology* **214**:1139-1146.
- Ruess, R. W., R. L. Hendrick, and J. P. Bryant. 1998. Regulation of fine root dynamics by mammalian browsers in early successional Alaskan taiga forests. *Ecology* **79**:2706-2720.
- Schneider, K., S. Migge, R. A. Norton, S. Scheu, R. Langel, A. Reineking, and M. Maraun. 2004. Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios ($^{15}\text{N}/^{14}\text{N}$). *Soil Biology and Biochemistry* **36**:1769-1774.

- Seastedt, T. R., R. A. Ramundo, and D. C. Hayes. 1988. Maximization of densities of soil animals by foliage herbivory: empirical evidence, graphical and conceptual models. *Oikos* **51**:243-248.
- Shantz, H. L. 1923. The natural vegetation of the Great Plains region. *Annals of the Association of American Geographers* **13**:81-107.
- Sinka, M., T. H. Jones, and S. E. Hartley. 2007. The indirect effect of above-ground herbivory on collembola populations is not mediated by changes in soil water content. *Applied Soil Ecology* **36**:92-99.
- Sinka, M., T. H. Jones, and S. E. Hartley. 2009. Collembola respond to aphid herbivory but not to honeydew addition. *Ecological Entomology* **34**:588-594.
- Smucker, A. J. M., S. L. McBurney, and A. K. Srivastava. 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. *Agronomy Journal* **74**:500-503.
- Soler, R., T. M. Bezemer, A. M. Cortesero, W. H. Van der Putten, L. E. M. Vet, and J. A. Harvey. 2007. Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. *Oecologia* **152**:257-264.
- Sonnemann, I., S. Hempel, M. Beutel, N. Hanauer, S. Reidinger, and S. Wurst. 2013. The root herbivore history of the soil affects the productivity of a grassland plant community and determines plant response to new root herbivore attack. *PLoS ONE* **8**:e56524.
- Sørensen, L. I., J. Mikola, and M.-M. Kytöviita. 2008. Defoliation effects on plant and soil properties in an experimental low arctic grassland community—the role of plant community structure. *Soil Biology and Biochemistry* **40**:2596-2604.
- Stanton, N. L., D. Morrison, and W. A. Laycock. 1984. The effect of phytophagous nematode grazing on blue grama die-off. *Journal of Range Management* **37**:447-450.
- Strzepek, K., G. Yohe, J. Neumann, and B. Boehlert. 2010. Characterizing changes in drought risk for the United States from climate change. *Environmental Research Letters* **5**:044012.
- Suding, K. N., I. W. Ashton, H. Bechtold, W. D. Bowman, M. L. Mobley, and R. Winkleman. 2008. Plant and microbe contribution to community resilience in a directionally changing environment. *Ecological Monographs* **78**:313-329.
- Sylvain, Z. A., D. H. Wall, K. L. Cherwin, D. P. C. Peters, L. G. Reichmann, and O. E. Sala. 2014. Soil animal responses to moisture availability are largely scale, not ecosystem dependent: insight from a cross-site study. *Global Change Biology* **20**:2631-2643.

- Thompson, D. C. and K. T. Gardner. 1996. Importance of grasshopper defoliation period on southwestern blue grama-dominated rangeland. *Journal of Range Management* **49**:494-498.
- Tullgren, A. 1918. Ein sehr einfacher Ausleseapparat für terricole Tierfaunen. *Zeitschrift für angewandte Entomologie* **4**:149-150.
- USDA-ARS. 2015. Rangeland resource research. USDA-ARS, Cheyenne, Wyoming, USA.
- van der Putten, W. H., L. E. M. Vet, J. A. Harvey, and F. L. Wäckers. 2001. Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology and Evolution* **16**:547-554.
- Van Horn, D. H., R. Mason, P. Grove, D. Maujean, and G. Hemming. 1972. Grasshopper population numbers and biomass dynamics on the Pawnee site. U. S. IBP Grassland Biome Technical Report No. 148. Colorado State University, Fort Collins, Colorado, USA.
- Walmsley, M. R., J. L. Capinera, J. K. Detling, and M. I. Dyer. 1987. Growth of blue grama and western wheatgrass following grasshopper defoliation and mechanical clipping. *Journal of the Kansas Entomological Society* **60**:51-57.
- Wardle, D. A. 2002. *Communities and ecosystems: linking the aboveground and belowground components*. Princeton University Press, Princeton, New Jersey, USA.
- Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. *Science* **304**:1629-1633.
- Wardle, D. A., G. M. Barker, G. W. Yeates, K. I. Bonner, and A. Ghani. 2001. Introduced browsing mammals in New Zealand natural forests: aboveground and belowground consequences. *Ecological Monographs* **71**:587-614.
- Wardle, D. A., W. M. Williamson, G. W. Yeates, and K. I. Bonner. 2005. Trickle-down effects of aboveground trophic cascades on the soil food web. *Oikos* **111**:348-358.
- Welch, J. L., R. Redak, and B. C. Kondratieff. 1991. Effect of cattle grazing on the density and species of grasshoppers (Orthoptera: Acrididae) of the Central Plains Experimental Range, Colorado: a reassessment after two decades. *Journal of the Kansas Entomological Society* **64**:337-343.
- Wilcox, K. R., J. C. Von Fischer, J. M. Muscha, M. K. Petersen, and A. K. Knapp. 2015. Contrasting above- and belowground sensitivity of three Great Plains grasslands to altered rainfall regimes. *Global Change Biology* **21**:335-344.

Williamson, S. C., J. K. Detling, J. L. Dodd, and M. I. Dyer. 1989. Experimental evaluation of the grazing optimization hypothesis. *Journal of Range Management* **42**:149-152.

Yeates, G. W., T. Bongers, G. M. Degoede, D. W. Freckman, and S. S. Georgieva. 1993. Feeding habits in soil nematode families and genera—an outline for soil ecologists. *Journal of Nematology* **25**:315-331.

3. CONCLUSION

3.1 SYNTHESIS

This research can be summarized as follows:

- 1) Anomalous weather patterns in the northern shortgrass steppe in 2013 drastically altered plant community composition during this study the following year (2014) by greatly promoting abundance of the typically rare annual grass *Vulpia octoflora*, while severely depressing abundance of the co-dominant, perennial grass *Bouteloua gracilis*.
- 2) Short-term exclusion of grasshoppers from mixed plant communities in the northern shortgrass steppe had no effect on final belowground biomass of all plants or final aboveground biomass of *V. octoflora*, other grasses (predominantly *B. gracilis*), or all plants.
- 3) Grasshopper feeding of mixed plant communities led to a more bacterially dominant soil energy channel, likely through non-consumptive means (i.e., greater grasshopper frass deposition).
- 4) Grasshopper exclusion tended to enhance proportions of plant parasitic nematodes, while grasshopper grazing tended to reduce their relative abundances. This pattern suggests a decline in root growth by *B. gracilis* in response to grasshopper feeding, which could have been tempered by late-season senescence and obscured by the abundant *V. octoflora*.
- 5) Densities of soil microarthropod groups were unresponsive to grasshopper exclusion during the study period.

- 6) Results from this research can help inform future studies on this aboveground-belowground linkage in the context of global climate change because this ecosystem is sensitive to alterations in precipitation, and climate models project greater frequency and severity of droughts in Colorado.

3.2 FURTHER POINTS OF DISCUSSION

The cage design used in this research was an effective way to manipulate grasshopper abundances and isolate the effects of grazing by these insects compared to other options. Many studies have depressed or excluded insect populations through the use of chemicals (e.g., Brown and Gange 1989, Allan and Crawley 2011, Blue et al. 2011). However, foliar insecticides can have residual, non-target effects on soil organisms, especially microarthropods (Joy and Chakravorty 1991, Endlweber et al. 2006), which precluded their use in this study. Alternatively, researchers frequently employed enclosures (full cages) in field experiments to manipulate herbivory (e.g., Crawley 1990, McShea and Rappole 2000). These cages are used alongside partial or 'half' cages (caged controls) that allow herbivory and control for the confounding effect of shading by full cages (Reader 1992a, b). However, half cages also provide access to rodents and other small mammals (Mills 1983, 1986). Given that this research sought to investigate the effects of grasshoppers on soil food webs, caged controls were constructed by cutting holes into the sides of full cages (Evans et al. 2012) to equalize the shading effect and isolate the impact of grasshoppers on this system. Though enclosures did not completely prevent access to grasshoppers, they severely depressed their populations (Section 2.2 Monitoring).

In addition to cage design, manipulation of entire grasshopper communities as opposed to using single species was an important consideration for this research. Many field studies with grasshoppers use enclosures populated with one species (e.g., Williamson et al. 1989, Thompson et al. 1995a, Thompson et al. 1995b) for three main reasons. First, given that plant species may respond differently to different herbivores (Van Zandt and Agrawal 2004), this approach provides greater control over the insect-plant community interaction under investigation. Second, if grasshoppers are colony derived then their use can compensate for low field populations. And third, enclosures with single grasshopper species allow greater control over the amount of defoliation imposed in a field experiment. In contrast, mixed, natural communities are advantageous because colonies are difficult to start and maintain (K. H. Post, personal observation), and grasshoppers may not transition well from the laboratory to the field (D. H. Branson, personal communication). Moreover, despite the benefit of greater control in using single herbivore species, a large degree of realism is lost by eliminating the entire suite of diet preferences and breadths (Pfadt 1994) of the remaining species in the natural grasshopper community. This can be a major issue if the plant community shifts, as it did in this study, and the experimental grasshopper species specializes on a now rare host-plant.

Finally, identification of soil animals to trophic rather than taxonomic groups was advantageous and crucial to testing the hypotheses of this study. Classification of soil nematodes and microarthropods to feeding groups is much easier and faster than it is to taxonomic categories (K. H. personal observation). Additionally, trophic groups address the focus of this research: understanding how this aboveground-belowground linkage affects soil energy channels. For instance, soil animal community shifts or diversity

changes could occur without alterations to feeding group distributions; thus, no insight into the effects of grasshopper grazing on the relative dominance of soil bacterial versus fungal energy pathways would be obtained if, for example, Simpson's diversity (Simpson 1949) responses of soil nematode communities were found.

3.3 FUTURE DIRECTIONS

A few modifications to this research are recommended if it were repeated in the future. This experiment should be conducted earlier in the growing season to reduce the effect of seasonality on soil nematode densities (Table 2.2, Figs. 2.7A–E) and in a year when *V. octoflora* is rare. Additionally, a reliable, non-destructive aboveground plant sampling method for shortgrass steppe communities is needed to accurately measure the amount of defoliation and regrowth during the experimental period. Belowground net primary productivity should be measured with root ingrowth cores, as opposed to relying only on total root biomass, to better quantify any subtle belowground growth differences between treatments. Finally, amounts of soil bacterial and fungal biomass are needed to fully understand any energetic changes to soil food webs caused by grasshoppers.

Future research should address the findings of my study in the context of global climate change to better understand how grasshopper-plant-soil food web interactions of the northern shortgrass steppe may change under new precipitation regimes. Due to the significant relationships between precipitation and above- (Lauenroth and Sala 1992) and belowground plant productivity (Evans and Burke 2013, Wilcox et al. 2015) in the Central Plains Experimental Range and the projected increase in frequency and severity of droughts in this region (Strzepek et al. 2010), water manipulation, preferably using rainout shelters (Yahdjian and Sala 2002), should become an additional treatment factor. Further,

multiple levels of each treatment (i.e., water and grasshoppers), as opposed to presence-absence of treatments, should be imposed to ascertain response surfaces (e.g., linear, asymptotic, parabolic) of soil biota. These shapes could help predict changes in soil communities and energy channels under multiple precipitation regimes and grasshopper population sizes. Additionally, soil processes, such as root decomposition, should also be measured to understand how the effects of these abiotic and biotic factors on soil food webs carry over to ecosystem function (García-Palacios et al. 2015). Lastly, a future study should cover multiple years, implement repeated sampling, and incorporate at least one recovery phase (i.e., all treatments are removed) to determine whether plant, soil organismal, and ecosystem function responses to water and/or grasshopper manipulations are pulsed (Fu et al. 2001, Henry et al. 2008) or persistent (Mueller et al. 2005) and to ascertain the resiliency (Holling 1973) of the shortgrass steppe ecosystem to these factors.

REFERENCES

- Allan, E. and M. J. Crawley. 2011. Contrasting effects of insect and molluscan herbivores on plant diversity in a long-term field experiment. *Ecology Letters* **14**:1246-1253.
- Blue, J. D., L. Souza, A. T. Classen, J. A. Schweitzer, and N. J. Sanders. 2011. The variable effects of soil nitrogen availability and insect herbivory on aboveground and belowground plant biomass in an old-field ecosystem. *Oecologia* **167**:771-780.
- Brown, V. K. and A. C. Gange. 1989. Differential effects of above- and below-ground insect herbivory during early plant succession. *Oikos* **54**:67-76.
- Crawley, M. J. 1990. Rabbit grazing, plant competition and seedling recruitment in acid grassland. *Journal of Applied Ecology* **27**:803-820.
- Endlweber, K., M. Schädler, and S. Scheu. 2006. Effects of foliar and soil insecticide applications on the collembolan community of an early set-aside arable field. *Applied Soil Ecology* **31**:136-146.
- Evans, D. M., N. E. Turley, D. J. Levey, and J. J. Tewksbury. 2012. Habitat patch shape, not corridors, determines herbivory and fruit production of an annual plant. *Ecology* **93**:1016-1025.
- Evans, S. E. and I. C. Burke. 2013. Carbon and nitrogen decoupling under an 11-year drought in the shortgrass steppe. *Ecosystems* **16**:20-33.
- Fu, S., K. W. Kisselle, D. C. Coleman, P. F. Hendrix, and D. A. Crossley, Jr. 2001. Short-term impacts of aboveground herbivory (grasshopper) on the abundance and ¹⁴C activity of soil nematodes in conventional tillage and no-till agroecosystems. *Soil Biology and Biochemistry* **33**:1253-1258.
- García-Palacios, P., M. L. Vandegehuchte, E. A. Shaw, M. Dam, K. H. Post, K. S. Ramirez, Z. A. Sylvain, C. M. De Tomasel, and D. H. Wall. 2015. Are there links between responses of soil microbes and ecosystem functioning to elevated CO₂, N deposition and warming? A global perspective. *Global Change Biology* **21**:1590-1600.
- Henry, F., M. Vestergård, and S. Christensen. 2008. Evidence for a transient increase of rhizodeposition within one and a half day after a severe defoliation of *Plantago arenaria* grown in soil. *Soil Biology and Biochemistry* **40**:1264-1267.
- Holling, C. S. 1973. Resilience and stability of ecological systems. *Annual Review of Ecology and Systematics* **4**:1-23.

- Joy, V. C. and P. P. Chakravorty. 1991. Impact of insecticides on nontarget microarthropod fauna in agricultural soil. *Ecotoxicology and Environmental Safety* **22**:8-16.
- Lauenroth, W. K. and O. E. Sala. 1992. Long-term forage production of North American shortgrass steppe. *Ecological Applications* **2**:397-403.
- McShea, W. J. and J. H. Rappole. 2000. Managing the abundance and diversity of breeding bird populations through manipulation of deer populations. *Conservation Biology* **14**:1161-1170.
- Mills, J. N. 1983. Herbivory and seedling establishment in post-fire southern California chaparral. *Oecologia* **60**:267-270.
- Mills, J. N. 1986. Herbivores and early postfire succession in southern California chaparral. *Ecology* **67**:1637-1649.
- Mueller, R. C., C. M. Scudder, M. E. Porter, R. T. Trotter III, C. A. Gehring, and T. G. Whitham. 2005. Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. *Journal of Ecology* **93**:1085-1093.
- Pfadt, R. E. 1994. Field guide to common western grasshoppers. University of Wyoming, Laramie, Wyoming, USA.
- Reader, R. J. 1992a. Herbivory as a confounding factor in an experiment measuring competition among plants. *Ecology* **73**:373-376.
- Reader, R. J. 1992b. Herbivory, competition, plant mortality and reproduction on a topographic gradient in an abandoned pasture. *Oikos* **65**:414-418.
- Simpson, E. H. 1949. Measurement of diversity. *Nature* **163**:688.
- Strzepek, K., G. Yohe, J. Neumann, and B. Boehlert. 2010. Characterizing changes in drought risk for the United States from climate change. *Environmental Research Letters* **5**:044012.
- Thompson, D. C., K. C. McDaniel, L. A. Torell, and D. B. Richman. 1995a. Damage potential of *Hesperotettix viridis* (Orthoptera: Acrididae) on a rangeland weed, *Gutierrezia sarothrae*. *Environmental Entomology* **24**:1315-1321.
- Thompson, D. C., L. A. Torell, E. W. Huddleston, and J. H. Davis. 1995b. Forage destruction by *Aulocara ellioti* (Orthoptera: Acrididae) on rangeland in the southwestern United States. *Journal of Economic Entomology* **8**:1455-1460.
- Van Zandt, P. A. and A. A. Agrawal. 2004. Community-wide impacts of herbivore-induced plant responses in milkweed *Asclepias syriaca*. *Ecology* **85**:2616-2629.

Wilcox, K. R., J. C. Von Fischer, J. M. Muscha, M. K. Petersen, and A. K. Knapp. 2015. Contrasting above- and belowground sensitivity of three Great Plains grasslands to altered rainfall regimes. *Global Change Biology* **21**:335-344.

Williamson, S. C., J. K. Detling, J. L. Dodd, and M. I. Dyer. 1989. Experimental evaluation of the grazing optimization hypothesis. *Journal of Range Management* **42**:149-152.

Yahdjian, L. and O. E. Sala. 2002. A rainout shelter design for intercepting different amounts of rainfall. *Oecologia* **133**:95-101.