Invasive plants, herbivores and the underground: Feedbacks with soil biota and their influence on grassland plant communities in the Great Plains

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Dustin F. Haines

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Diane L. Larson and Linda L. Kinkel, co-advisors

April 2013

Acknowledgements

I wish to acknowledge the help given to me by many people during my graduate studies. First and foremost, I thank my two co-advisors, Diane Larson and Linda Kinkel, for their mentoring, sage advice, wise insights, positivity and seemingly endless patience. I am a much better scientist and writer thanks to their tireless efforts. I also thank my committee members Sue Galatowitsch and Nick Jordan, and previous committee member Sarah Hobbie, who were very helpful in developing my research questions.

I also received many hours of assistance with research and writing from a multitude of individuals. Sheri Huerd was particularly helpful in assisting me with field and greenhouse work, and with the T-RLFP component of my research. Jen Larson was instrumental in field, greenhouse and laboratory aspects of my research, and in logistical support. Nick Jordan was also very helpful in my field research, and he provided lots of helpful insights and writing advice during the Larson-Jordan lab meetings. Other members of the lab group provided research and writing advice, including Laura Philips-Mao, Travis Henspeter, and Laura Van Riper. Special thanks go out to Robert Endermuhle, who selflessly provided many hours of volunteer work during my greenhouse and lab research. Field and greenhouse assistance was also given by Katy Adams, Doug Johnston, Bonnie Sample, Peter Ebertowski, David Campbell, Jon Anderson, Sarah Busch, Dominik Halas, and Joey Reid.

A number of individuals were also instrumental in the success of my research projects by providing supplies, lab space and equipment, and training. Carol Windels (University of Minnesota, Crookston) and Jim Kurle (University of Minnesota, Twin Cities) provided all of the plant pathogenic fungal isolates I used – their contributions

helped broaden the scope of my research considerably. Christine Solomon very kindly allowed me to use her lab facilities for liquid-liquid extractions, and Jon Henriks provided training and helped process my samples, for which I am grateful. I thank Monika Chandler and Anthony Cortilet from the Minnesota Department of Agriculture, who helped with flea beetle collection and sorting. Adrian Stec provided use of the large lyophilizer I used for freeze drying my root exudate samples. Bob Sterner was helpful in giving me access to his CHN analyzer, and Sandy Brovold trained me in sample preparation and helped run my samples. Finally, I thank the Bismarck Plant Materials Center (U.S. Department of Agriculture, Natural Resources Conservation Service) for providing free native plant seeds which I used in multiple research projects.

Various individuals provided excellent logistical support over the years. Lisa Wiggins and Sue Wenker in the Ecology, Evolution and Behavior Department were particularly helpful in all manifestations of administrative tasks. Pam Warnke and Roger Meissner run a tight ship at the Plant Growth Facilities, and because of their dedication to the task, I always had greenhouse space when I needed it.

Information on the history and distribution of leafy spurge at Theodore Roosevelt National Park was also graciously provided by Chad Sexton and Karl Brown (National Park Service), Jack Norland (North Dakota State University), Jack Butler (USDA Forest Service) and Raymond Kokaly (U.S. Geological Survey). Internal U.S. Geological Survey reviews of my seed bank manuscript were provided by Deborah Buhl, Margherita Gioria, Amy Symstad and Sue Galatowitsch, which were incredibly helpful.

I also thank the members of the Restoration Rapid Assessment Tool team, especially Ron Hiebert, Diane Larson, and Kathryn Thomas, for their help in my

Research Assistantship during the first two years of my graduate studies. I gained substantial knowledge during the assistantship, and I have the members of the team to thank for that opportunity. I also want to thank Sue Galatowitsch, who provided valuable knowledge about restoration ecology and management practices, and Anthony Starfield, who helped me understand the beauty, usefulness, and pitfalls of modeling.

I was also extremely lucky to be a Teaching Assistant under the fantastic mentorship of Don Alstad, Jeannine Cavender-Bares, David Tilman, Clarence Lehman, and Bruce Fall. My time spent with them during meetings, lectures, coffee hours, labs, and yes, even the many hours of grading exams, were incredibly rewarding and critical to my development as an educator.

Funding for living and for research came from a variety of sources, including the National Park Service, Northern Prairie Wildlife Research Center, Dayton Natural History Funds, the University of Minnesota Thesis Research Grant, the Summer Block Grant through the Department of Ecology, Evolution and Behavior, and Sigma Xi Grants-in-Aid of Research. None of this would have been possible without these sources of support.

Very special thanks go out to Sheri Huerd and Ray, Leo and Rory Hirsch, to Leslie Brandt and Andrew and Thora Barry, and to Kent and Jeannine Cavender-Bares. These selfless families generously opened up their homes to the “weird bearded guy” who slept in their homes, ate their food, and generally got in their way for months on end. Words can’t describe how lucky I am to have such amazing friends.

My sanity would long ago have been forsaken if it wasn’t for the brilliant creativity, comedy, and penchant for the sublime of many talented minds. To the

podcasters and comedians deluxe, including Chris Hardwick, Jonah Ray, Matt Myra, Josh Clark, Charles W. Bryant, Paul F. Tompkins, Scott Aukerman, Doug Benson, John Hodgeman, Aisha Tyler, Ben Acker and Ben Blacker: my many, MANY hours of greenhouse, lab, and statistics work would have been much less amusing without your peerless abilities to entertain. It is also difficult to imagine a life without Joss Whedon, Felicia Day, Amy Poehler, Tina Fey, Mitchell Hurwitz, Dan Harmon, the Sunny Gang (Rob McElhenney, Glenn Howerton, Charlie Day, Kaitlin Olson and Danny DeVito) Gene Rodenberry, Rick Berman, Rod Serling, J. Michael Straczynski, Joel Hodgson (and the whole MST3K crew), Stephen King, C.S. Lewis, and J.K. Rowling – you all delighted me to no end during my graduate school years.

My family has been infinitely supportive through the entire process, and I could not have accomplished my goals without their unending encouragement, love, and emotional and financial support.

Lastly, I wish to thank my wonderful wife and best friend, Jessica Savage. I am so lucky, in so many ways, that I get to spend my life with her. None of this would have been remotely possible without her at my side. Thank you for always being there for me.

Dedication

This dissertation is dedicated to my mother, Fran Mitchell, for her boundless love and support, and to my father, Carl Haines (1939–2010), for always believing in me and encouraging me to follow my own path.

Abstract

Exotic plants have the ability to modify soil seed banks and the soil biotic community in habitats they invade, but little is known about the legacy of invasion once an exotic plant has successfully been controlled. Natural areas previously invaded by leafy spurge in the northern Great Plains typically have one of two fates following control of spurge: a return of native plants, or a secondary invasion of other exotic plants. Furthermore, reduced native plant recruitment has been observed in areas where insect biocontrol has been used for leafy spurge control. It is unknown, however, if the reduced recovery of natives is due to a depauperate native plant seed bank, altered soil biotic communities, or interactions of leafy spurge with biocontrol organisms. To address the seed bank question, I monitored soil seed banks and standing vegetation for two years in mixed-grass prairies that were previously invaded by leafy spurge. I found that native plant seed banks were largely intact in areas previously invaded by leafy spurge, regardless of the current living plant community. I conducted a glasshouse experiment to investigate interactive effects of leafy spurge soil conditioning and flea beetle biocontrol insects on native plants. My results indicate that leafy spurge soil conditioning inhibits native plant germination and growth, and that flea beetles have similar inhibitory effects but only on particular native plant species. Lastly, I investigated the interactive effects of leafy spurge root exudates and fungal pathogens on native plant growth in a glasshouse, and the degree to which fungal pathogen growth varies with leafy spurge and native plant root exudates in petri dishes. I found that leafy spurge root exudates and fungal pathogens, when applied together, tended to enhance native plant germination and growth, and that fungal pathogen growth response to root exudates varied between fungal

genera, and among native species, but that fungal growth did not respond strongly to leafy spurge exudates. Therefore, regardless of the relatively unaltered native plant seed banks, leafy spurge soil occupancy and fungal pathogens may have significant impacts on native plant recovery.

Table of Contents

List of Tables

List of Figures

Chapter 1:

Introduction

Charles Elton, in his classic treatise *The Ecology of Invasions by Animals and Plants*, ominously described species invasions as "ecological explosions" (Elton 1958). This dramatic description underscores the urgency that is often associated with understanding the ecology of species invasions and the impacts they have on invaded communities, and with developing effective strategies for their control. This urgency is not unfounded. Invasive species are responsible for species extinctions, negative impacts to ecosystem services, and an estimated $1.4 trillion per year in economic damages worldwide (Pimentel 2002). Unfortunately, even if an invasive species is successfully controlled, long-term legacy effects may continue to have negative consequences for the formerly invaded community (Corbin and D'Antonio 2004, Elgersma et al. 2011), and much is still unknown about why invasive species are successful in the habitats they occupy and the long-term effects of those invasions.

A considerable body of theory, experimentation, and knowledge has followed the publication of Elton's book. An especially active area of research has focused on evaluating hypotheses posed to explain the success (or lack of success) of invasive organisms into new areas and their persistent dominance in invaded areas following the initial establishment. One of these, the enemy release hypothesis, suggests that invasive species escape their specialist pests and pathogens present in their native range when

invading new areas (Keane and Crawley 2002). Lack of pests and pathogens can reduce resources invested in defense, leaving more energy for growth and competition with native species. Another hypothesis, the accumulation of local pathogens hypothesis, proposes that an exotic plant accumulates generalist pathogens in the invaded range that subsequently inhibit native plants relatively more than the invasive species (Eppinga et al. 2006). These two hypotheses are not mutually exclusive and a native plant could, in theory, escape its native pathogens and accumulate new ones. The success of an invasive species may also result from direct interactions with plant competitors rather than via interactions with pests and pathogens. This is the premise of the novel weapons hypothesis, which posits that invasive species possess biochemicals (e.g., allelochemicals) that inhibit native species (Callaway and Ridenour 2004). Furthermore, the negative impacts of an invasive species on native species may be enhanced when the invasive species is attacked by a pest. For example, herbivores may stimulate an invasive plant to produce secondary chemicals (induced defense) that increase allelopathic effects on nearby plants (Newingham and Callaway 2006), or herbivory of the invasive plant may stimulate compensatory growth of the invasive (Ridenour and Callaway 2003). Root herbivory may also stimulate pathogen accumulation in soils or roots compared to non-grazed plants. Soils adjacent to *Euphorbia* spp. roots that were subject to root-feeding insects had higher populations of fungal plant pathogens (*Fusarium* spp.) than soils of plants without root-feeding insects (Caesar 2003). All four of these hypotheses contribute to our conceptual understanding of species invasions, but the extent to which distinct mechanisms are responsible for invasion success or negative impacts of invaders on

native species remains unknown. This is especially the case for the compensatory growth/induced defense hypotheses, which have been examined predominantly on the knapweeds (*Centaurea* spp.) (Thelen et al. 2005, Newingham and Callaway 2006, Norton et al. 2008), and for the accumulation of local pathogens hypothesis, which has only been tested on marram grass (*Ammophila arenaria*) and Siam weed (*Chromolaena odorata*) (Eppinga et al. 2006, te Beest et al. 2009).

In this research, I investigated several mechanisms by which the invasive plant leafy spurge (*Euphorbia esula*) may both maintain dominance in its invaded range and impact native plant communities after its control. *Euphorbia* has been a highly successful invader of the northern Great Plains since its accidental introduction in the 1800's, and it currently occupies about 1.9 million ha in the US (Duncan et al. 2004). Economic impacts from *Euphorbia* in agricultural and wildland settings approach $120 million annually (Leistritz et al. 2004), and the ecological effects of its invasion and dominance include reduced native plant species diversity (Belcher and Wilson 1989, Butler and Cogan 2004, Cornett et al. 2006). Various management practices have been used in attempts to control *Euphorbia*, including herbicides, biocontrol insects, grazing, and burning (Lym 2005), but its control has been particularly successful following the application of flea beetles (*Aphthona* spp.) (Butler et al. 2006). However, the recovery of native plant communities in areas once dominated by *Euphorbia* has been very slow following control via flea beetles (Lesica and Hanna 2009, Butler and Wacker 2010). To date, the reasons for reduced native plant recovery in these areas have not been

investigated, but the hypotheses outlined here suggest specific lines of research to explain the poor recovery of native species following *Euphorbia* infestation.

The primary objective of my work is to elucidate mechanisms leading to reduced native plant recruitment in Great Plains areas invaded by *Euphorbia*, and to examine why recruitment remains low following *Euphorbia* removal. I examined four specific hypotheses in accomplishing this objective.

In Chapter 2, I tested the hypothesis that *Euphorbia* invasion results in depauperate native plant seed banks. The slow native plant recovery in areas formerly dominated by *Euphorbia* may occur if *Euphorbia* occupation results in fewer native plant seeds entering the soil seed bank, which could reduce opportunities for native plant recruitment. In this scenario, a reduced native seed bank could result from *Euphorbia* stands being dense enough to preclude native plant occurrence, or from reduced native plant seed production via competitive interactions with *Euphorbia*. To evaluate these hypotheses, I examined soil seed banks in areas of varying *Euphorbia* invasion history (never invaded and previously invaded) and standing vegetation (dominated by natives or dominated by exotics other than *Euphorbia*).

In Chapter 3, I evaluated support for the accumulation of local pathogens and induced defense hypotheses by examining how flea beetles modify the effect of *Euphorbia* on native plants. The slow native plant recovery in areas where *Euphorbia* was controlled using flea beetles (Lesica and Hanna 2009, Butler and Wacker 2010) may be related to increased soilborne fungal pathogen populations that can occur in *Euphorbia*-occupied soils when root-attacking herbivores are present (Caesar 2003)

(accumulation of local pathogens). Alternatively, flea beetles may stimulate *Euphorbia* to produce compounds that inhibit native plant germination and growth (induced defense). To address these hypotheses, I quantified the effects of historical and recent *Euphorbia* soil conditioning, and of flea beetles applied to *Euphorbia*, on subsequent native plant germination and growth in a glasshouse.

In Chapter 4, I tested the potential for *Euphorbia* root exudates alone (as a novel weapon) or in combination with pathogens (pathogen accumulation) to reduce native plant recruitment. For example, *Euphorbia* root exudates have compounds that can inhibit the growth of *Arabidopsis thaliana* seedlings (Qin et al. 2006), but whether or not this influences native prairie plants is unknown. In addition, the accumulation of local pathogens hypothesis posits that an invasive plant species will stimulate soil pathogen presence (presumably through root exudation), and that native plants will be negatively impacted by the resulting enriched pathogen populations (Eppinga et al. 2006). There is evidence that fungal pathogens can accumulate in the rhizosphere of *Euphorbia* (Caesar 2003), but the impact of these pathogens on native species has not been tested. To evaluate the potential for novel weapons and pathogen accumulation of *Euphorbia* to impact native plants, I examined native plant germination and growth when exposed to *Euphorbia* root exudates alone or in combination with soilborne fungal plant pathogens.

Finally, in Chapter 5, I compared the abilities of *Euphorbia* and native plant root exudates to stimulate fungal plant pathogen growth. An implicit assumption of the accumulation of local pathogens hypothesis is that an invasive plant species will stimulate soilborne pathogens more than native plant species, but this assumption has not

been experimentally evaluated. If *Euphorbia* negatively impacts native plants via pathogen accumulation, then it would need to have a stronger stimulatory effect on these pathogens than native plant species, and the native species would need to have a greater negative response to pathogens than the invasive species. To test this assumption, I measured the growth of several common soilborne fungal plant pathogens when subjected to root exudates of *Euphorbia* and eight native plant species.

This work used the South Unit of Theodore Roosevelt National Park (TRNP), North Dakota as a setting for field research, and as a basis for selection of the native plant species used in glasshouse and lab research. TRNP is the site of recent and extensive *Euphorbia* infestations. *Euphorbia* was first observed in TRNP in the late 1960s (Anderson et al. 1996), and had come to occupy approximately 10% of the South Unit by 1999 (O'Neill et al. 2000). In response to the infestation, the National Park Service established comprehensive *Euphorbia* control and monitoring programs, which have successfully reduced its prevalence. TRNP provides ready availability of wildlands that are undisturbed by agricultural practices, and there is a long history of vegetation studies conducted in the Park and surrounding grasslands which provide useful context for this research.

My research addresses several important ecological questions and hypotheses regarding exotic plant invasions, and also provides important insight into potential land management strategies for areas currently or recently occupied by *Euphorbia*. In addition to stimulating further research into these topics, these data will contribute to the

development of more effective control of *Euphorbia* and other invasive plants in diverse natural and disturbed habitats.

Chapter 2:

Leafy spurge (*Euphorbia esula*) affects vegetation more than seed banks in mixed-grass prairies of the northern Great Plains, USA

Exotic plants have the ability to modify soil seed banks in habitats they invade, but little is known about the legacy of invasion on seed banks once an exotic plant has successfully been controlled. Natural areas previously invaded by leafy spurge in the northern Great Plains typically have one of two fates following its removal: a return of native plants, or a secondary invasion of other exotic plants. It is unknown, however, if this difference in plant communities following leafy spurge control is due to seed bank differences. To answer this question, I monitored seed banks and standing vegetation for two years in mixed-grass prairies that were previously invaded by leafy spurge but controlled within five years of my study. I found that native plant seed banks were largely intact in areas previously invaded by leafy spurge, regardless of the current living plant community, and leafy spurge invasion history had a larger impact on cover and diversity of the vegetation than on the seed banks. Differences in plant communities following leafy spurge control do not appear to be related to the seed banks, and soil conditions may be more important in determining trajectories of these post-invasion communities.

Introduction

Soil seed banks contribute to vegetation dynamics in plant communities, and they can help maintain species diversity and genetic variability (Fenner 1985) following disturbance and subsequent habitat restoration (Grime 1981, van der Valk and Pederson 1989). Intact, diverse native plant seed banks may persist through habitat change and disturbance, and can play an important role in plant community restoration (Wetzel et al. 2001, Richter and Stromberg 2005, Plassmann et al. 2009, Wang et al. 2010, Kalamees et al. 2012). However, if floristic homogenization occurs through invasive plants replacing diverse native plant communities (McKinney 2004), seed banks may also be homogenized, reducing the habitat restoration potential. Indeed, the general pattern following invasion includes an increased presence of exotic seeds of a few species (Henderson and Naeth 2005, Giantomasi et al. 2008, Fisher et al. 2009) and a reduced presence of native seeds (Henderson and Naeth 2005, Cox and Allen 2008, Fisher et al. 2009) when compared to uninvaded habitats (but see Mason et al. (2007) and Robertson and Hickman (2012)). The restoration potential of communities dominated by invasive plants depends, in part, on the seed bank (Bakker and Berendse 1999), and if the presence of native species in the seed bank is impoverished, restoration can be slow (Dutoit and Alard 1995, Hutchings and Booth 1996, Holl et al. 2000, Bai et al. 2010) or unachievable without the addition of propagules (Aerts et al. 1995, Bekker et al. 1997, Bossuyt and Hermy 2003), especially when invasive plants cause the impoverishment (Cione et al. 2002, Ogden and Rejmánek 2005, Loh and Daehler 2008, Fisher et al. 2009).

In the northern Great Plains, the invasive exotic plant leafy spurge (*Euphorbia esula* L.) has negative economic and ecological effects, and often presents a challenge to restoration. However, it is unclear what role the seed bank plays in the return of native vegetation following its invasion. Introduced accidentally to North America in the 1800's (Dunn 1985), leafy spurge has come to occupy nearly 1.9 million ha in the US (Duncan et al. 2004) and the estimated economic impacts are around $120 million annually, making it one of the worst weeds in the US (Leistritz et al. 2004). Ecologically, leafy spurge can reduce native plant species richness (Belcher and Wilson 1989, Butler and Cogan 2004, Cornett et al. 2006), conspecific pollen presence on native plants (Larson et al. 2006), and presence of native ungulates (Trammell and Butler 1995). It can also produce large, persistent seed banks (Laufmann 2006, Cline et al. 2008, Setter and Lym 2013), with its seeds remaining viable in soils up to eight years (Bowes and Thomas 1978). Biocontrol agents, such as flea beetles (*Aphthona* spp.), and various combinations of biocontrol, herbicides, grazing, and interseeding of native grasses have recently been successful in controlling leafy spurge (Lym 2005, Cornett et al. 2006, Joshi 2008, Richardson et al. 2008, Progar et al. 2010). Following control, some previously invaded sites have returned to their native plant assemblages naturally (without sowing native seeds) (Lesica and Hanna 2009). However, other sites have experienced long-term reductions in native forbs (Rinella et al. 2009) and/or secondary invasions of other invasive plants, especially exotic grasses (Lesica and Hanna 2009, Larson and Larson 2010). Leafy spurge control has also not been successful in all habitats. Riparian zones, for example, are highly susceptible to leafy spurge invasion (Anderson et al. 1996) but have restricted herbicide use due to

potential negative impacts on aquatic organisms (Folmar et al. 1979), limiting the control options available to managers (Progar et al. 2010).

There are conflicting reports of leafy spurge impacts on seed banks in the northern Great Plains. Leafy spurge-invaded areas in the Little Missouri National Grasslands in southwestern North Dakota, USA had higher percentages of native plant seeds compared to leafy spurge seed in the seed bank five (Cline et al. 2008) and 10 years (Setter and Lym 2013) following flea beetle release. Conversely, the density of the leafy spurge seed bank was not different 4–7 years following flea beetle treatment compared to untreated infested areas in Theodore Roosevelt National Park (Laufmann 2006), which is surrounded by the Little Missouri National Grasslands. One commonality among these studies is that the presence of native species in seed banks was improved by flea beetle treatments, and that this will improve native plant recovery potential. However, the relationship between aboveground vegetation and seed banks was not analyzed in the National Grasslands.

I investigated the impact of leafy spurge invasion and subsequent control on the seed bank and compared the seed bank with standing vegetation of mixed grass prairies at Theodore Roosevelt National Park, North Dakota, USA, an area that has been subjected to intense leafy spurge invasions and an active invasive species management program (National Park Service 2005). I identified three categories of leafy spurge invasion history: (1) never invaded, (2) previously invaded but now dominated by native species, and (3) previously invaded but now dominated by exotic species other than leafy spurge. I addressed two primary questions: (1) do sites with different leafy spurge invasion

histories differ in seed bank composition; and (2) are species composition and diversity similar within and between seed banks and vegetation, and is this influenced by leafy spurge invasion history? The presence of germinable native plant seed banks that have persisted with minimal differences among invasion histories would suggest a different restoration strategy than would a depleted seed bank.

Materials and Methods

Study Sites

This research was conducted from 2006 to 2008 in the South Unit of Theodore Roosevelt National Park (TRNP), an 18,600-ha reserve adjacent to Medora, North Dakota, USA (47°N, 104°W). The climate is characterized by long, cold winters and short, hot summers; the long-term mean annual precipitation (1948 – 2010) is 379 mm, a majority of which occurs between April and September (NCDC 2012). Precipitation was 140 mm below the long-term mean in 2006 (239 mm) but it was close to normal in 2007 (369 mm) and 2008 (360 mm) (NCDC 2012).

Three study sites (Bad Buffalo, Petrified Forest, and Knutson Creek) were established in the western wheatgrass-green needlegrass vegetation type (Von Loh et al. 2000), dominated by grasses (primarily *Pascopyrum smithii* (Rydb.) Á. Löve, *Nassella viridula* (Trin.) Barkworth, and *Bouteloua gracilis* (H.B.K.) Lag. ex Griffiths) and forbs (such as *Artemisia frigida* Willd., *Achillea millefolium* L., and *Sphaeralcea coccinea* (Nutt.) Rydb.) (Table 2-1 and Figure 2-1). Separate areas nested within each site were

classified according to the leafy spurge invasion history, determined by National Park Service personnel (Paula Anderson and Laurie Richardson, TRNP, personal communication), and current vegetation: never invaded and dominated by natives (hereafter, uninvaded), previously invaded but now dominated by natives (hereafter, invaded-native), and previously invaded but now dominated by invasives other than leafy spurge (hereafter, invaded-exotic). Current vegetation (native-dominated vs. exotic-dominated) was determined via visual assessment in 2006. Exotic species were present in the vegetation of all native-dominated areas, but invaded-exotic areas were dominated by smooth brome (*Bromus inermis* Leyss.) and Kentucky bluegrass (*Poa pratensis* L.).

Two additional sites (Peaceful Valley and Mike Auney) in the silver sagebrush / western wheatgrass shrublands vegetation type (Von Loh et al. 2000), characterized by a matrix of shrubs (primarily *Artemisia cana* Pursh), grasses (primarily *P. smithii* and *N. viridula*) and forbs (including *A. frigida* and *A. millefolium*), were included in the 2007 and 2008 sampling (Table 2-1 and Figure 2-1). Data collected at these two sites were only used for the descriptive canonical discriminant analyses, as I could only find invaded-exotic areas within this habitat type (they were not used in the density, diversity, or similarity analyses). I chose to include these sites in the study because silver sagebrush / western wheatgrass shrubland vegetation had been heavily invaded by leafy spurge and is therefore of great interest to resource managers.

Nine to 15 plots were established, as space permitted, within each invasion history at each site (Table 2-1). The plots were 2 × 6 m and distributed 10 m apart along several transects 15 m apart. The starting location for plot placement on each transect was

randomly assigned. All plots within each nested invasion history at each site were contained within $\leq$ 50 × 50 m area (0.25 ha), and all plots at each site containing multiple invasion histories were within 400 m of each other. The seed bank was sampled in 2006 and 2007, and the standing vegetation in 2007 and 2008. I was limited to an unbalanced incomplete block design, as there was only one site that contained all three leafy spurge invasion histories, and two sites had two invasion histories (Table 2-1).

Site Histories

Leafy spurge was first noted in the South Unit in the late 1960's (Anderson 1995), and the area infested since then rose steadily from 13 ha in 1970 (Anderson 1995) to 1700 ha (~10% of the area of the South Unit) in 1999 (O'Neill et al. 2000). The duration and intensity of infestation is not precisely known for my study sites, but their relative historic infestation levels can be estimated. Leafy spurge was first observed in the Knutson Creek area in the late 1960's (Andrascik 1994), but there are no records of when the other study sites were first infested. All of the previously infested plots were occupied by leafy spurge, and subjected to biocontrol insects and/or herbicide, at least several years in the 1990's and/or early 2000's (Paula Anderson and Laurie Richardson, TRNP, personal communication). Areas close to creeks or floodplains (such as Knutson Creek, Peaceful Valley, and Mike Auney) typically had denser leafy spurge cover than more upland sites (such as Bad Buffalo and Petrified Forest) (Anderson et al. 1996), but the severity of infestation (% cover or density) was not quantified at my sites. Leafy spurge coverage at TRNP was also estimated using remote sensing data (aerial and satellite imagery) on several occasions in the 1990's and 2000's (Anderson et al. 1996, Mladinich

et al. 2006, Stitt et al. 2006). My plot locations were compared with leafy spurge remote sensing coverage data from 1993 and 2002 to determine if study plots were occupied during these years. According to aerial photography from 1993 (Anderson 1998), the only sites that had plots within leafy spurge stands were Knutson Creek and Mike Auney, while plots at the other sites were adjacent to leafy spurge stands (Table 2-1). Low-density infestations had a lower probability of detection (Anderson et al. 1996), therefore the other previously infested sites may have been occupied at densities below the detection threshold. Aerial imagery from 2002 using the Compact Airborne Spectrographic Imager (CASI) indicate that invaded-exotic plots had the highest levels of leafy spurge occupation, but a few uninvaded plots also showed signs of leafy spurge cover (Table 2-1) (Kokaly et al. 2004). However, TRNP personnel indicated that areas in which I placed uninvaded plots had never been occupied by leafy spurge, and the accuracy of the remote sensing data in properly representing leafy spurge cover was 74% (Kokaly et al. 2004). Since these specific uninvaded plots were either inaccurately classified as having leafy spurge cover, or the leafy spurge plants were only briefly present, I retained their uninvaded designation. Based on the above cover estimates, the sites in my study have been in recovery 9–15 years (duration after leafy spurge control).

Herbicides have been used for leafy spurge control in TRNP since 1960, but use escalated in the mid-1990s, with up to 210 ha per year being treated with over a dozen formulations (Larson et al. 2007). Multiple herbicide treatments were used in the Knutson Creek drainage from 1985–1998 (Samuel and Lym 2008), but the specific locations of these treatments are unavailable. Detailed records of herbicide application are only

available for the 2000's at Knutson Creek, Peaceful Valley, and Mike Auney (Table 2-1) (Chad Sexton, TRNP GIS Analyst, personal communication).

Biocontrol organisms were first released at TRNP in 1987, but large releases started in 1992 (Andrascik 1994), with more than 18 million biocontrol flea beetles released between 1989–2004 (Prosser 2004). These control efforts eventually reduced cover of leafy spurge in the park (Larson and Grace 2004, Larson et al. 2007) to the point that only a few scattered patches were identifiable via hyperspectral remote sensing in 2002 (Anderson et al. 2004). I identified all biocontrol release locations (Hager 1998) within 200 m of my plots, a distance that flea beetles can travel in two weeks (Jonsen et al. 2001), using GIS software (Quantum GIS v. 1.8.0) (Table 2-1).

Seed Bank

I sampled the soil seed bank in the spring of 2006 and 2007 using the seedling emergence method (Roberts 1981). Since I was interested in legacies of leafy spurge infestation, I evaluated the persistent seed bank (seeds that are viable beyond the next germination opportunity). Samples were collected prior to the spring seed rain, which reduced representation of seeds from the transient seed bank (viable only until the next germination opportunity) (Thompson and Grime 1979, Baskin and Baskin 1998) and minimized the impact that research-related disturbance had on the seed banks, as the persistent seed bank is typically deeper in the soil (Thompson and Grime 1979). I collected 10 soil samples every ~ 1.5 meters around the perimeter of each plot, at a distance of 50 cm outside the plot edge. Samples were collected using a 5 cm diameter soil auger to a depth of 10 cm (~190 cm^3 per core), combined to create one composite

sample per plot, placed in plastic bags and kept cool until processed. This method resulted in an area of ~196 cm^2 sampled per plot. I systematically offset the 2007 coring locations ~75 cm from the previous year to avoid resampling the same holes.

Each sample was sieved to remove large roots, litter, and bulbs, and 900 cm^3 of soil was spread 1 cm deep on top of 2700 cm^3 of sterilized sand in nursery trays (25 × 52 cm). Trays were placed in a greenhouse in St. Paul, Minnesota with ambient light April–August (~12–15 hours per day), and 12 hours per day supplemental light September–March. Four separate germination rounds were conducted in the following sequence for all trays: two rounds of water only, one with potassium nitrate solution (KNO_3), and one with gibberellic acid solution (GA) (Roberts 1981). For the KNO_3 and GA treatments, 0.5 L of 10 mmol/L (~1000 ppm) of the former (Young and Young 1986, Baskin and Baskin 1998), and 500 mL of 0.14 mmol/L (50 ppm) GA of the latter (Evans and Young 1975), were applied individually to each tray weekly for four consecutive weeks. The samples were given eight weeks to germinate during each round, and all seedlings were counted every other day. Between rounds the samples were allowed to dry out for at least four weeks to help break dormancy (Roberts 1981), and soil samples were sieved between the second water and the KNO_3 treatments with a 6.35 mm (0.25 inch) mesh to expose light-sensitive seeds that were previously buried. Soils were kept moist through watering daily to every few days, as needed, to stimulate germination and to keep seedlings from drying out. Seedlings that were not immediately identifiable were transplanted into pots for maturation and identification after two weeks. Control trays (with only sterile sand) were not used, as the greenhouse weeds found in the St. Paul facility are not found at TRNP,

and evaporative cooling pads were effective at limiting the ingress of local seeds when windows were open for cooling. I used various sources (Kummer 1951, Chancellor 1966, Stucky et al. 1981, Vaillancourt 1996, Royer and Dickinson 1999, Whitson 2002, Manitoba Agriculture Food and Rural Initiatives 2004, DiTomaso and Healy 2007) to identify seedlings that failed to grow to maturity (~20%).

Vegetation Cover

Percent cover of each species was visually estimated on all field plots of both vegetation types in the spring (May 2007) and the summer (August 2008). Different seasons were sampled to capture the variability in vegetation cover due to differential abundance of annuals (spring and summer) and grass species (cool and warm season) between the seasons. Each plot was divided into six 2 × 1 m subplots to improve accuracy, and cover was averaged among subplots per plot. Cover for each species was compared with cards representing 1%, 5%, and 10% of the area of a subplot (Symstad et al. 2008), and field personnel were calibrated with each other to ensure consistent cover estimates. Plant species nomenclature follows USDA, NRCS (2012).

Seed Density and Vegetation Cover Analyses

Seed densities and vegetation cover were summarized by plot and analyzed for differences among invasion histories separated by year, and separated by origin (native vs. exotic species) and year. I used zero-inflated Poisson nonlinear mixed models (Lambert 1992, Zuur et al. 2009) to analyze seed bank densities (by year and for exotic species) because they had more zeroes than expected under discrete probability

distributions. These were implemented using the NLMIXED procedure in SAS/STAT v. 9.3 (SAS Institute Inc., Cary, NC, 1989-2011), and code was modified from Littell et al. (2006) and Pahel et al. (2011). Native species seed densities for both years were analyzed using a generalized linear mixed model and vegetation cover was analyzed using a linear mixed model (implemented using the GLIMMIX and MIXED procedures in SAS/STAT, respectively) and code from Littell et al. (2006) and SAS Institute, Inc. (2011). Statistical models and further details for these analyses are in Appendix 1.

Species Diversity and Similarity Analyses

To determine if species diversity varied among invasion histories, I performed three separate analyses for each year of seed bank and vegetation data. I examined native, exotic and total species richness among invasion histories to determine if the number of species within histories was dependent upon species nativity. These analyses were conducted via generalized linear mixed models, as described above.

I used Sørensen's similarity index to compare the similarity of species composition within invasion histories for seed banks and vegetation. Similarities within invasion histories (e.g., similarities among uninvaded plots, among invaded-native plots, and among invaded-exotic plots) were examined to determine if plots previously invaded by leafy spurge exhibited floristic homogenization when compared to uninvaded plots, indicated by a higher similarity among previously invaded plots than among uninvaded plots. I also determined if seed bank/vegetation similarities were affected by invasion history. The seed bank and seed bank–vegetation Sørensen data were zero-inflated, with values greater than zero following a continuous gamma distribution. I used a zero-

inflated gamma non-linear mixed model, which was implemented using the NLMIXED procedure in SAS/STAT and code modified from McLerran (2008) and Littell et al. (2006). The vegetation Sørensen values fit a continuous normal distribution, and I used a mixed linear model similar to the vegetation cover model described above for analyzing differences among invasion histories. To compare similarity of seed banks to the vegetation, regardless of invasion history, Sørensen values were pooled across invasion histories. These data did not fit any known distribution, therefore a Wilcoxon nonparametric test was performed using JMP v. 9 (SAS Institute Inc., Cary, NC, 1989-2010). Additional details, including Sørensen's similarity index calculations and statistical models, are in Appendix 1.

Community Composition Analyses

Canonical discriminant analyses (CDAs) were used to describe variation in plant community composition among leafy spurge invasion histories for the seed bank and the vegetation. As noted above, I included the two sites within silver sagebrush / western wheatgrass shrublands (Peaceful Valley and Mike Auney) in the multivariate analyses because of their importance to land managers. The seed bank and vegetation data were analyzed as four separate sets: one for each seed bank collection year (2006 and 2007) and one for each vegetation data collection year (2007 and 2008). I followed the procedures for transforming and adjusting data recommended by McCune and Grace (2002), but I did not relativize the data, as seed and cover abundance were important components with respect to my questions. Canonical correlations for each function (axis) were examined using likelihood ratio tests to determine which axes were necessary to

describe the differences among invasion histories and current vegetation features. Wilks' lambda (λ) and Mahalanobis distances were examined using likelihood ratio tests to identify significant plot and group separations, respectively. The structure coefficients of influential species (those with standardized canonical coefficients greater than one standard deviation from the mean) yielded species correlations with invasion history/vegetation features along a given axis (Can1 & Can2). All CDAs were performed using the CANDISC procedure in SAS/STAT, with code based on SAS Institute, Inc. (2011).

Results and Discussion

Abundance & Diversity

Three lines of evidence show that seed banks are very similar among leafy spurge invasion histories at my study sites. First, seed densities did not vary among invasion histories. This was true whether native and exotic species were analyzed together or separately (Figure 2-2 and Table 2-2). Total seed density ranged from a minimum of 421 ± 69 seeds m^{-2} (uninvaded sites in 2006) to a maximum of 1197 ± 261 seeds m^{-2} (invaded-exotic sites in 2007). These seed bank similarities occurred despite visibly different vegetation cover (native-dominated vs. exotic-dominated; Figure 2-2). Second, native, exotic, and total seed bank species richness did not vary among invasion histories (Figure 2-3 and Table 2-2), and there were no nativity by invasion history interactions in 2006 ($F_{2,1} = 1.02$, $P = 0.574$) or 2007 ($F_{2,1} = 0.27$, $P = 0.805$). The fact that native seed

bank richness was not influenced by invasion history is indicative either of a stable native plant seed bank, or that native seed banks have recovered after leafy spurge control, despite the secondary invasion of exotics. In contrast, native and total vegetation species richness varied in the spring, with richness higher in uninvaded than invaded-exotic plots, and exotic richness varied in the summer, which was highest in invaded-exotic and lowest in uninvaded plots. The former differences were primarily due to a high abundance of native forbs in uninvaded plots in the spring, and the latter difference was caused mostly by the widespread occurrence of the exotic grasses *B. inermis* and *P. pratensis* in invaded-exotic plots compared to uninvaded plots (data not shown). Third, seed bank species similarities did not vary between invasion histories (Figure 2-4 and Table 2-2). While the overall similarity in species composition among plots is lower for seed banks than for vegetation, the fact that seed bank species similarities were not different among histories indicates that seed bank homogenization via exotic plant occupation has not occurred. Meanwhile, differentiation (reduction in species similarity) may have occurred in the vegetation, as invaded-exotic plots had lower species similarities than uninvaded and invaded-native plots. Seed bank-vegetation plot comparisons did not have a consistent pattern with respect to current vegetation or invasion history, as there was a higher similarity in invaded-native plots than in uninvaded and invaded-exotic plots.

Seed banks in my study not only show a high degree of consistency across invasion histories, but are also dominated by native species in both density and species richness in all invasion histories, which was contrary to my expectations and most

published literature. Native was higher than exotic seed richness and density in both years. Most studies show reduced native plant seed densities or richness in invaded compared to uninvaded areas (Drake 1998, Holmes 2002, McLaughlin and Bowers 2007, Giantomasi et al. 2008, Gioria and Osborne 2010, Marchante et al. 2011), and examples of invasive plants having little or no effect on native seed presence are uncommon (but see Mason et al. (2007), Vilà and Gimeno (2009), and Abella et al. (2012)). Only the study by McLaughlin and Bowers (2007) took place in a prairie, but they found an invasive effect on the seed bank. My results were also surprising because seeds of the two most common invasive plants in my study, *B. inermis* and *P. pratensis*, are known to accumulate in the seed bank (Tracy and Sanderson 2000, Otfinowski et al. 2008).

Two scenarios have been suggested to explain the lack of seed bank change via invasion. Invasive species may facilitate native seedling emergence and recruitment via the nurse plant effect, as observed in old fields (Vilà and Gimeno 2009) and in badlands formations in the Great Plains (Van Riper and Larson 2009), or via long-lived native seed banks that persist throughout the invasion and subsequent management (Mason et al. 2007). The former scenario is unlikely, as leafy spurge has been shown to suppress native forbs (Jordan et al. 2008). There is a high diversity and density of native plant seeds in the persistent seed bank at TRNP (Laufmann 2006), so the latter scenario is likely. Seed dispersal probably contributed native seeds to invaded seed banks as well. In a review of seed banks and seed dispersal, Bakker et al. (1996) identified wind, birds and large mammals as capable of long-distance dispersal, while small mammals and insects disperse over shorter distances (several meters). Contiguous monocultures of leafy spurge

were infrequently greater than a hectare and distances to native seed sources were small (personal observation) – therefore, short-distance dispersal would be adequate for many native seeds to infiltrate leafy spurge stands. The existence of multiple granivorous rodent (Reid 2006) and ant species (Wheeler and Wheeler 1944) in the region is suggestive of frequent seed dispersal, and all of my plots were surrounded by similar vegetation as found within the plots, which would make this seed readily available to these granivores.

My study supports previous research that found desirable native plant seed banks in areas where leafy spurge was successfully controlled by flea beetles (Laufmann 2006, Cline et al. 2008, Setter and Lym 2013). Prior to biocontrol, seed banks in the Little Missouri National Grasslands were dominated by leafy spurge seed (between 49–72% of the seed bank) (Cline et al. 2008), but five and 10 years after flea beetle application this was significantly reduced (between 2.3–25% and 2–15% of the seed bank, respectively) (Setter and Lym 2013). I found between 1.6–1.7% (invaded-native plots in 2006 and 2007, respectively) of the seed bank was leafy spurge seeds, which corresponds well with the lower values above. Laufmann (2006) found no significant reduction in leafy spurge seeds 4-7 years following flea beetle application and subsequent leafy spurge control at TRNP, but there was a trend of decreasing density for all habitats sampled. The significant reductions found by Setter and Lym (2013) compared to Laufmann (2006) may be related to the intensity of flea beetle treatments. Flea beetle releases were as few as 1,000 per site in the latter study, while 6,000 per site were released in the former study, and higher flea beetle densities are significantly better at controlling leafy spurge than

low densities (Progar et al. 2010). My study, meanwhile, took place 9–15 years following biocontrol treatments, providing an additional five + years of control compared to Laufmann (2006). Leafy spurge seed can also remain viable up to eight years in the seed bank (Bowes and Thomas 1978), so some seeds produced prior to biocontrol release were likely still viable in the soil when Laufmann (2006) collected seed bank samples. However, fewer pre-control seeds would have been viable upon my collection.

Community Composition

The only analyses that indicated differences in seed banks among invasion histories were the first axes of the CDAs in both years, but differences among pre-determined groups are maximized by a CDA, and there was a clearer separation among histories for vegetation than seed banks (Table 2-3 and Figure 2-5). There was significant separation of plots with respect to invasion history for the seed bank CDAs in 2006 (Wilks' $\lambda = 0.417$, $F_{34,126} = 2.04$, $P = 0.0025$) and 2007 (Wilks' $\lambda = 0.371$, $F_{44,186} = 2.71$, $P < 0.0001$), with invaded-exotic plots being significantly different from uninvaded and invaded-native plots (Mahalanobis distances, Table 2-3). As expected, there was significant separation of vegetation plots for 2007 (Wilks' $\lambda = 0.0098$, $F_{124,120} = 8.78$, $P < 0.0001$) and 2008 (Wilks' $\lambda = 0.0777$, $F_{84,160} = 4.93$, $P < 0.0001$), with significant differences between all invasion history pairs for both years (Mahalanobis distances, Table 2-3). Plots within silver sagebrush / western wheatgrass shrublands exhibited minor grouping in the CDAs, but these groupings are not distinct from the invaded-exotic plots in western wheatgrass / green needlegrass vegetation community (Figure 2-5). The seed banks and vegetation were also consistent in how the CDA plots separated with respect to

invasion histories along the first canonical axis: seed banks and vegetation significantly separated plots according to native-dominated plots (uninvaded and invaded-native) vs. exotic-dominated plots (invaded-exotic; Table 2-3 and Figure 2-5). The second canonical axis for vegetation separated native-dominated plots with different leafy spurge histories (uninvaded vs. invaded-native; Table 2-3 and Figure 2-5). The second axis for seed banks did not significantly separate groups.

The seed bank groupings were driven primarily by differential presence of a few species in native-dominated vs. exotic-dominated plots, which appear to largely reflect differences in the vegetation. For example, six of the 11 species that were influential in the seed bank CDAs were also influential in the vegetation CDAs (Appendix 2) and shared similar correlations: three exotic species (*E. esula*, *Bromus inermis*, and *Poa pratensis*) and one native genus (*Carex* sp.) were mostly correlated with exotic-dominated sites, while two natives (*N. viridula* and *Linum lewisii*) were correlated with native-dominated sites (Appendix 3). This indicates that plots with abundant vegetation of a given species also had abundant seeds from that species. Two of the five species that were only influential in the seed bank CDAs were native, and have vegetation habitat affinities that match the plots with which their seeds were associated. *Draba reptans* is found in sandy soils (McGregor and Barkley 1986), which was a characteristic of invaded-exotic plots, and *Verbena bracteata* is often considered weedy or invasive (Whitson 2002), a characteristic of multiple species that were common in invaded-exotic plots. Beyond *V. bracteata*, none of the native species I found in the seed banks are normally considered weedy (Whitson 2002). Of the 33 species that were only influential

in vegetation CDAs, native species tended to be correlated with uninvaded plots, as these plots were selected based on the presence of native species, but there were no other discernible trends (Appendix 2 and 3).

Assembly rules, which identify the species subset of a given region that can tolerate the environmental conditions of that region (Keddy 1992), may help explain the greater differences in vegetation community composition among invasion histories compared to seed banks. For example, grassland species composition can vary greatly depending on soil conditions (Baer et al. 2005), and soil texture can influence the germination of seeds in the seed bank (Benvenuti 2003). In my study, the soil texture and pH were similar among native-dominated plots, but different between native- and exotic-dominated plots (unpublished data). These different soil conditions may lead to varying germination and seedling establishment rates among species, and therefore might have contributed to the greater similarity in vegetation species composition among native-dominated plots than between native- and exotic-dominated plots (Table 2-3, Figure 2-4 and Figure 2-5).

Site History and Seed Banks

The sites in my study have been in recovery from leafy spurge occupation for 9–15 years, therefore short-term impacts of leafy spurge on seed banks are not represented here. Rigorously testing the effects of leafy spurge management on seed banks was also beyond the scope of my study, but I found no differences among my sites that were clearly attributable to these impacts. This is best illustrated by the lack of separation of the Bad Buffalo and Knutson Creek sites via CDAs for both years, despite differences in

herbicide and biocontrol management (Table 2-1). Furthermore, some differences found among my sites run contrary to expectation based on previous research. For example, Kirby et al. (2003) found that imazapic reduced native forb seed density, and imazapic and 2,4-D reduced exotic forb density in leafy spurge infestations. In my study, native and exotic forb seed densities in 2007 were *greater* in two sites treated with these herbicides (Peaceful Valley and Mike Auney) compared to sites with no herbicide application (Bad Buffalo and Petrified Forest; data not shown). I could not properly examine flea beetle impacts on seed banks as infested areas without flea beetle application were not verifiably present, but previous studies found either no influence on native seed banks (Laufmann 2006), or a positive influence on native species richness (Setter and Lym 2013).

It is also possible that ungulates may impact seed banks at TRNP. For example, elk (*Cervus elaphus*) and bison (*Bos bison*), which are both present in the South Unit, disperse seed via dung and adhesion to fur (Bartuszevige and Endress 2008, Rosas et al. 2008), and they can enhance seedling establishment via creation of germination microsites (Martin and Wilsey 2006). However, there was not a difference in seed banks between bison treatments (grazing vs. exclusion) at Badlands National Park, South Dakota (Fahnestock et al. 2003), and the effect of elk on grassland seed banks has not been studied. Evaluating these effects at TRNP would require additional research

Management Implications

There are only a few published accounts in which native seed banks were used to successfully restore plant communities in grasslands (Dremann and Shaw 2002,

Crimmins and McPherson 2008, Cuevas and Zalba 2010). Most studies indicate the opposite: existing seed banks alone are not adequate for grassland restoration (Rosburg et al. 1994, Seabloom et al. 2003, Vécrin et al. 2007, Wellstein et al. 2007, Zhan et al. 2007, Rosef 2008, Fagan et al. 2010). This is supported by a review of 102 seed bank studies in European plant communities, which revealed that restoration from seed banks alone is not feasible in most cases (Bossuyt and Honnay 2008). However, my study and others in the northern Great Plains (Laufmann 2006, Cline et al. 2008, Setter and Lym 2013) indicate that native plant seed banks are largely unaffected by leafy spurge invasion. This suggests that seed banks of mixed-grass prairies are more resilient to exotic invasion than many other areas. The presence of a relatively unmodified native seed bank may reduce the need for extensive reseeding, but it is unclear how influential the species diversity or seed densities that I observed would be on native plant recruitment or community resistance to invasion following leafy spurge control. As an extreme example, if none of the standing vegetation survived leafy spurge invasion, while 100% of the species that I found in the seed bank successfully established and became part of the vegetation following the control of leafy spurge, 13 new native species would be added to the vegetation, but 55 native species that were only in the vegetation would be lost. At the other end of the scale, losing even a few of the 48 seed bank species would likely have a measurable impact on both species diversity, especially for those species that were not documented in the vegetation, and genetic diversity, as seed banks act as a repository of genetic diversity (Fenner 1995).

More concerning is the lack of native plant recruitment and the abundance of invasive grasses in some previously infested sites. In related research, *P. pratensis* rapidly invaded areas previously infested with leafy spurge, and the authors suggested that this may have been avoided by reseeding selected areas with native species as soon as leafy spurge started to decline via *Aphthona* biocontrol (five years after their release) but before *P. pratensis* invaded (Setter and Lym 2013). Native plant seed banks were recovering at the five year mark in the previous study, especially forbs, but the density and diversity of desirable grass species (including *B. gracilis* and *N. viridula*) had declined (Cline et al. 2008). Seed addition can help overcome seed limitation (Turnbull et al. 2000), and it has been successful in reduction of exotic plant abundance in grasslands (Blumenthal et al. 2003, Wilson and Partel 2003), especially when native species can be established before exotics (Martin and Wilsey 2012). In my study, I did not find strong evidence of native seed limitation, but I do not have seed bank information during the initial years of leafy spurge decline, and it is unknown if seed addition would have prevented the invasion of *B. inermis* and *P. pratensis*. Seed addition experiments may help elucidate the effectiveness of this method in preventing these secondary invasions, or in reducing the presence of established exotic grasses. Unfortunately, suppression of these grasses in the northern Great Plains has been poor, especially when management has been passive (Murphy and Grant 2005). Future research should focus not only on effective ways to encourage native plant establishment while preventing secondary invasion, but also on mechanisms whereby soil characteristics (both biotic (Jordan et al.

2008, Jordan et al. 2011) and abiotic (Bakker and Berendse 1999)) and seed banks interact to influence post-invasion species composition.

Table 2-1. Site characteristics and history. The numbers of plots within specific leafy spurge invasion histories are shown in parentheses, in order of the invasion histories listed. Cover for 1993 and 2002 indicates the number of plots occupied by leafy spurge.

	Sites				
Characteristic	Bad Buffalo	Petrified Forest	Knutson Creek	Peaceful Valley	Mike Auney
Vegetation type	Western wheatgrass/green needlegrass			Silver sagebrush/western wheatgrass shrublands	
Invasion histories	uninvaded, invaded-native, invaded-exotic	uninvaded, invaded-native	invaded-native, invaded-exotic	invaded-exotic	invaded-exotic
Number of plots	15, 15, 15 (45)	9, 15 (24)	15, 10 (25)	15	15
1993 cover[1]	0, 0, 0	0, 0	7, 10	0	2
2002 cover[2]	4, 4, 7	1, 1	0, 1	11	NA
Herbicide application[3]	None recorded	None recorded	Aminopyralid via helicopter (2007, for Canada thistle control)	2,4-D amine / imazapic / picloram mix via backpack sprayer (2003) imazapic via backpack sprayer (2005 & 2007)	Imazapic via helicopter (2007)
Biocontrol application[4]	*A. nigriscutis* (1994–1997)	*A. lacertosa* (1995 & 1997) *A. cyparissiae* & *Spurgia esulae* (1995)	*A. flava* (1992) *A. lacertosa* (1993 & 1995) *A. nigriscutis* (1992, 1994–1996) *Chamaesphecia hungarica* (1994)	*A. lacertosa* (1994–1995) *A. nigriscutis* (1995–1996)	*A. nigriscutis* (1995) *A. lacertosa* (1996)
Analysis notes	Data used for seed density, vegetation cover, species diversity, species similarity, soil characteristics, and discriminant analyses			Data only used for discriminant analyses	

[1](Anderson 1998); [2](Kokaly et al. 2004); [3](Chad Sexton, TRNP, personal communication); [4](Hager 1998)

Table 2-2. Statistical output for mixed models. Degrees of freedom (DF) are displayed as numerator, denominator. P-values less than 0.05 are in bold. Similarity analyses included data from all years.

Variable	Characteristic	Year	Species	DF	*F*	*P*
Seed bank	Density	2006	Native	2,69	1.70	0.191
			Exotic	2,1	8.08	0.232
			All	2,1	1.07	0.564
		2007	Native	2,1	3.53	0.352
			Exotic	2,1	1.64	0.483
			All	2,1	2.09	0.439
	Richness	2006	Native	2,1	0.55	0.690
			Exotic	2,91	2.55	0.084
			All	2,91	0.07	0.936
		2007	Native	2,1	0.15	0.878
			Exotic	2,1	0.49	0.711
			All	2,91	0.87	0.421
	Similarity		All	2,4	3.53	0.131
Vegetation	Cover	2007	Native	2,90.4	14.1	**<0.001**
			Exotic	2,90.8	25.6	**<0.001**
			All	2,91	16.6	**<0.001**
		2008	Native	2,90.7	3.49	**0.035**
			Exotic	2,55	19.9	**<0.001**
			All	2,89	5.28	**0.007**
	Richness	2007	Native	2,91	3.23	**0.044**
			Exotic	2,91	0.37	0.693
			All	2,91	4.26	**0.017**
		2008	Native	2,91	0.60	0.551
			Exotic	2,91	4.38	**0.015**
			All	2,91	0.06	0.940
	Similarity		All	2,1562	78.0	**<0.001**
Seed - Veg.	Similarity		All	2,7	51.3	**<0.001**

Table 2-3. Canonical correlations, Mahalanobis distances, and their multivariate F tests for the seed bank and vegetation. Degrees of freedom (DF) displayed as numerator/denominator. Group pairs designate invasion histories that are being compared for Mahalanobis distances (DM); abbreviations are as follows: U = uninvaded, IN = invaded-native, IE = invaded-exotic. Significant P-values (<0.05) are in bold.

		Canonical correlations						Mahalanobis distances				
Data	Year	Canonical axis	Percent variance	Canonical correlation	*F*	DF	*P*	Group pair	D_M	*F*	DF	*P*
Seed bank	2006	1	64.0	0.645	2.04	34/126	**0.002**	U/IN	2.33	1.51	17/63	0.121
		2	36.0	0.535	1.61	16/64	0.093	U/IE	4.02	2.12		**0.016**
								IN/IE	3.86	2.57		**0.004**
	2007	1	75.8	0.712	2.71	44/186	**<.001**	U/IN	2.64	1.40	22/93	0.133
		2	24.2	0.498	1.47	21/94	0.106	U/IE	5.47	3.17		**<.001**
								IN/IE	4.16	3.60		**<.001**
Vegetation	2007 (spring)	1	71.2	0.966	8.78	124/120	**<.001**	U/IN	49.2	6.16	62/60	**<.001**
		2	28.8	0.923	5.71	61/61	**<.001**	U/IE	90.4	12.1		**<.001**
								IN/IE	50.6	10.0		**<.001**
	2008 (summer)	1	79.9	0.910	4.93	84/160	**<.001**	U/IN	10.0	2.47	42/80	**<.001**
		2	20.1	0.740	2.39	41/81	**<.001**	U/IE	26.9	7.08		**<.001**
								IN/IE	17.8	6.94		**<.001**

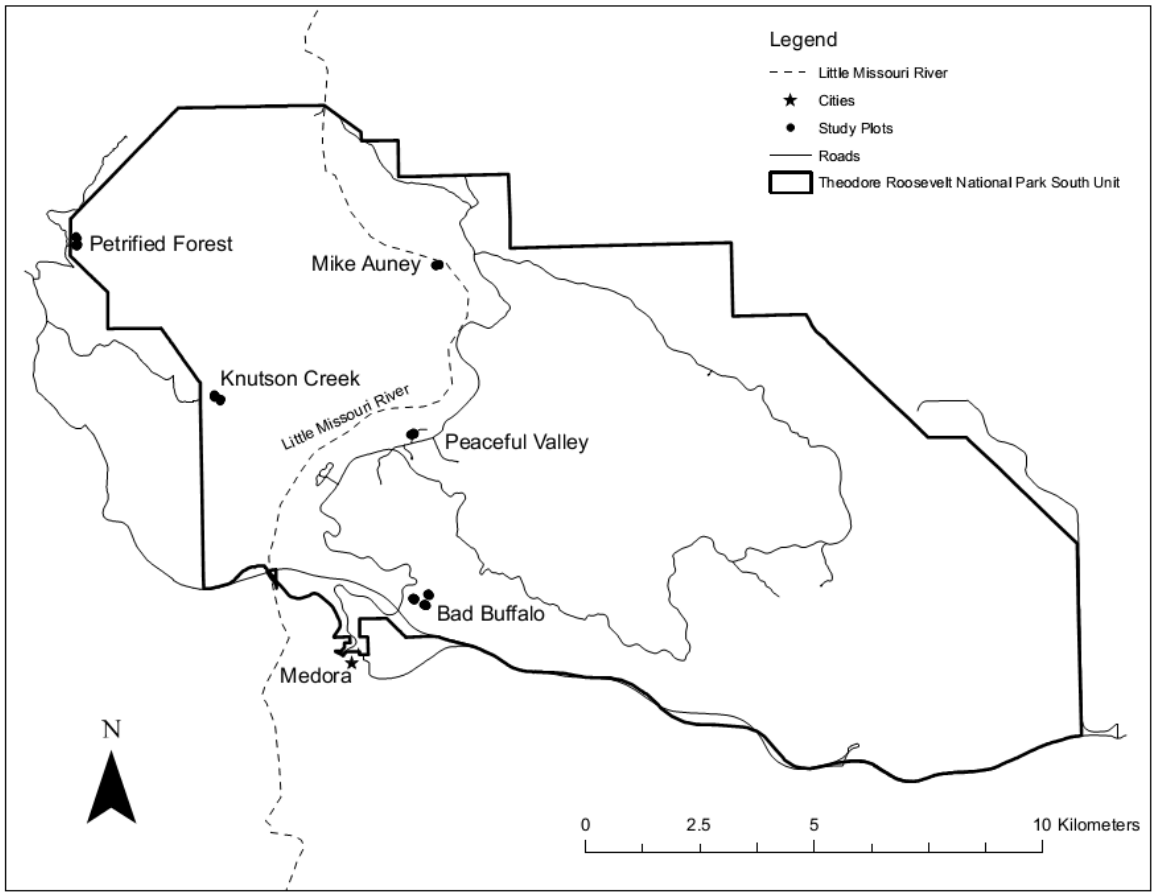


Figure 2-1. Site and study plot locations in the South Unit of Theodore Roosevelt national Park. Groups of study plots are labeled with the site name.

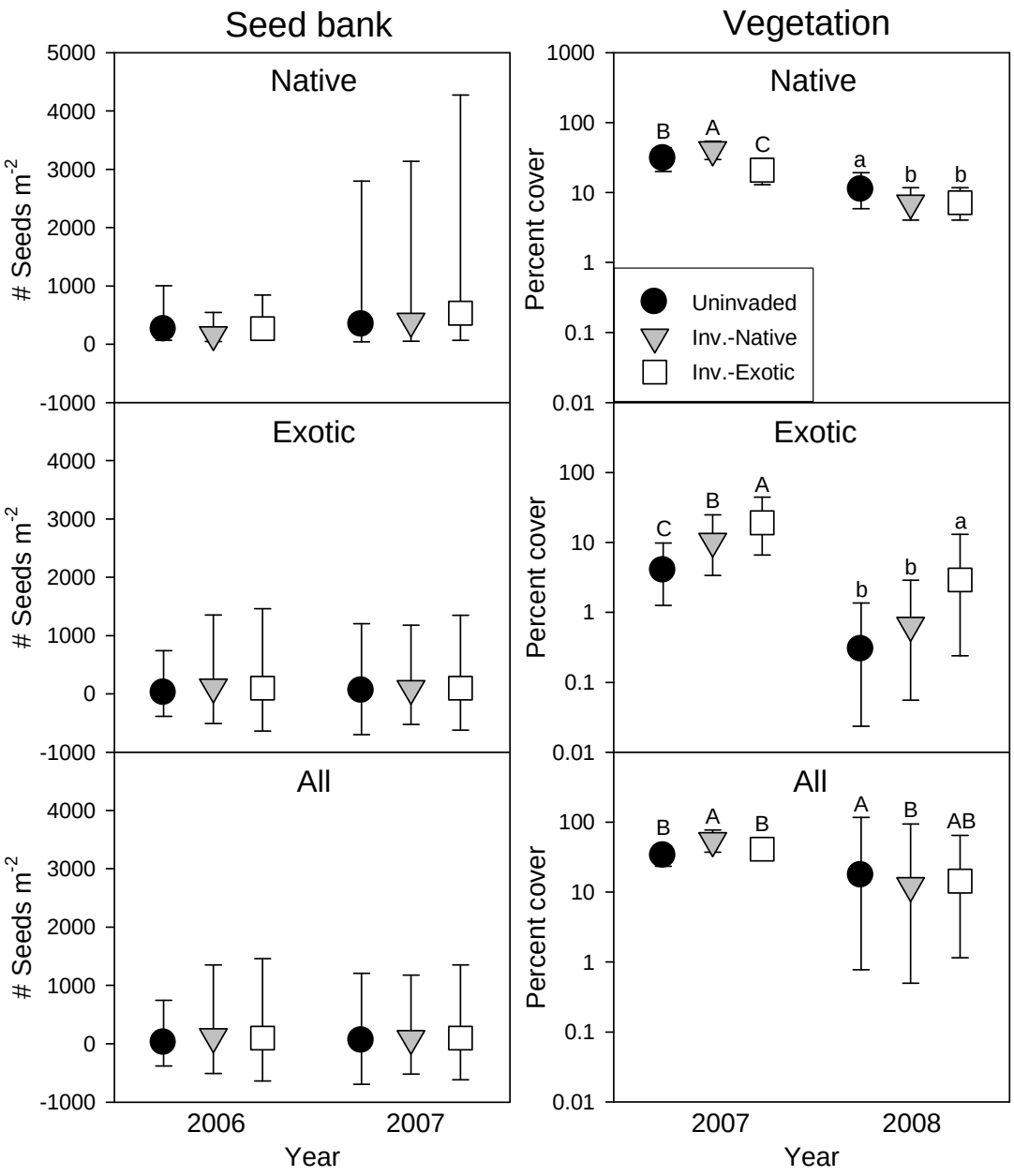


Figure 2-2. Native, exotic, and total seed bank density and vegetation cover across leafy spurge invasion histories. Symbols and error bars represent estimates and 95% confidence intervals, respectively, as determined by generalized linear mixed models and nonlinear mixed models. Analyses were separated by year and nativity for both seed bank and vegetation, and different letters indicate significantly different estimates

via Tukey HSD or Bonferroni ($\alpha = 0.05$) within these groups; lack of letters indicates no significant difference within the grouping. Note: seed density is on a linear scale, vegetation cover is on a logarithmic scale. The number of plots sampled was the same for all years, and for both seed bank and vegetation sampling, in uninvaded (N = 24), invaded-native (N = 45), and invaded-exotic (N = 25) areas.

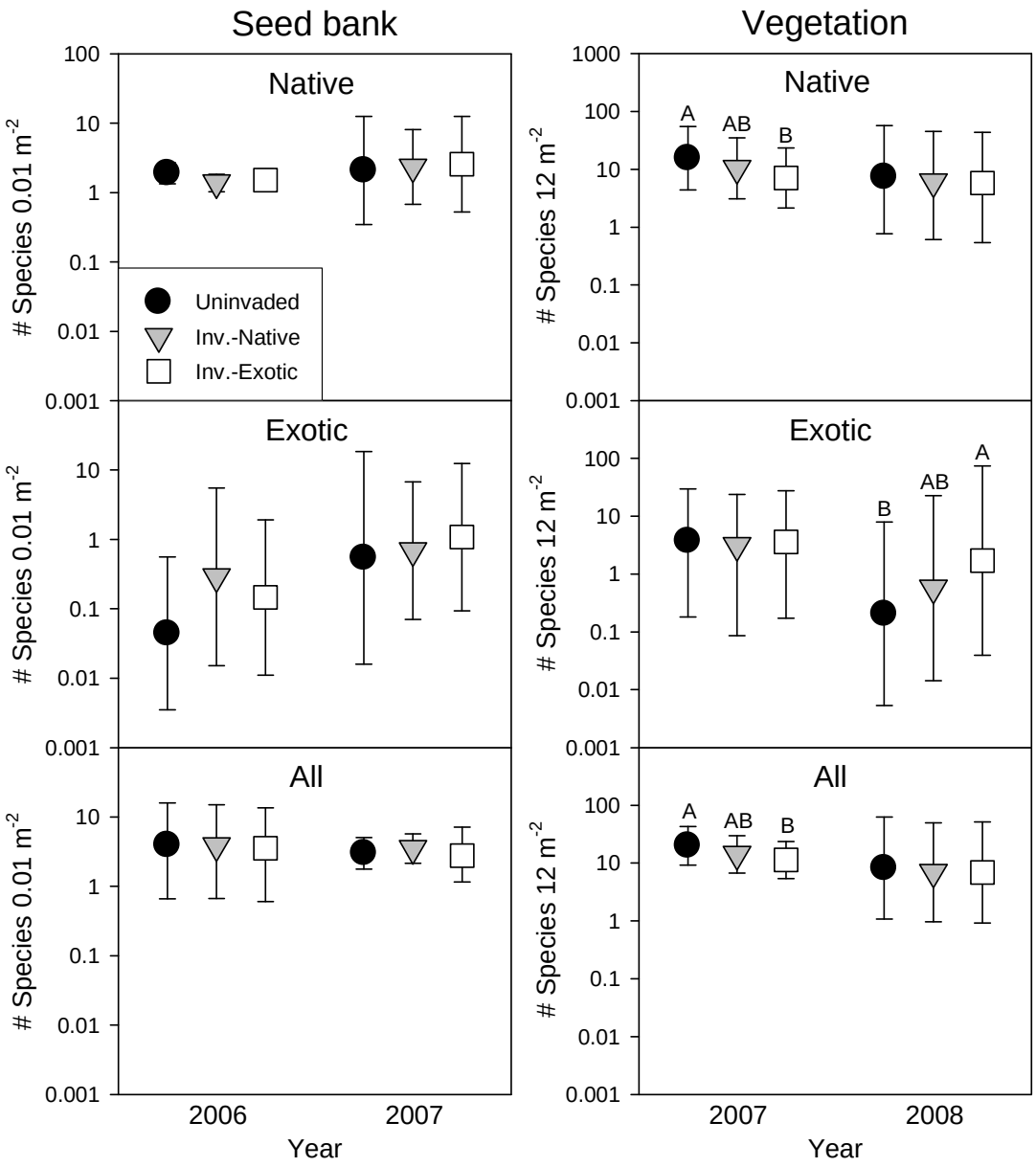


Figure 2-3. Native, exotic, and total species richness across leafy spurge invasion histories. Symbols and error bars represent estimates and 95% confidence intervals, respectively, as determined by generalized linear mixed models. Analyses were separated by year and nativity for both seed bank and vegetation, and different letters indicate significantly different estimates via Tukey HSD or Bonferroni ($\alpha = 0.05$) within these groups; lack of letters indicates no significant difference within the grouping.

Seed bank and vegetation data are reported on the scale in which they were originally recorded; rescaling was not done due to the area/species diversity relationship not being known for the study system. Note: species richness is on a logarithmic scale. The number of plots sampled was the same for all years, and for both seed bank and vegetation sampling, in uninvaded (N = 24), invaded-native (N = 45), and invaded-exotic (N = 25) areas.

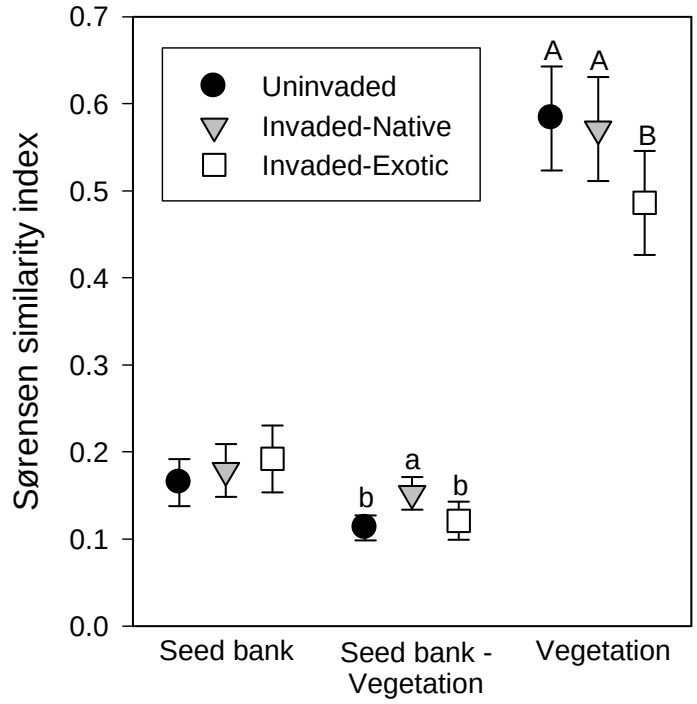


Figure 2-4. Species composition similarity within invasion histories for seed banks and vegetation. Sørensen similarity index values represent the proportion similarity in species within or between invasion histories. Estimates and confidence intervals were determined via a nonlinear mixed model (for the seed bank and seed bank – vegetation) and generalized linear mixed models (for vegetation). Comparisons between invasion histories include the following: uninvaded/invaded-native (U / IN), invaded-native/invaded-exotic (IN / IE), and uninvaded/invaded-native (U / IN). Different letters represent significantly different estimates within a given group of three symbols, determined via Tukey HSD ($\alpha = 0.05$); there were no significant differences in Sørensen values for analyses within or between invasion histories in the seed bank.

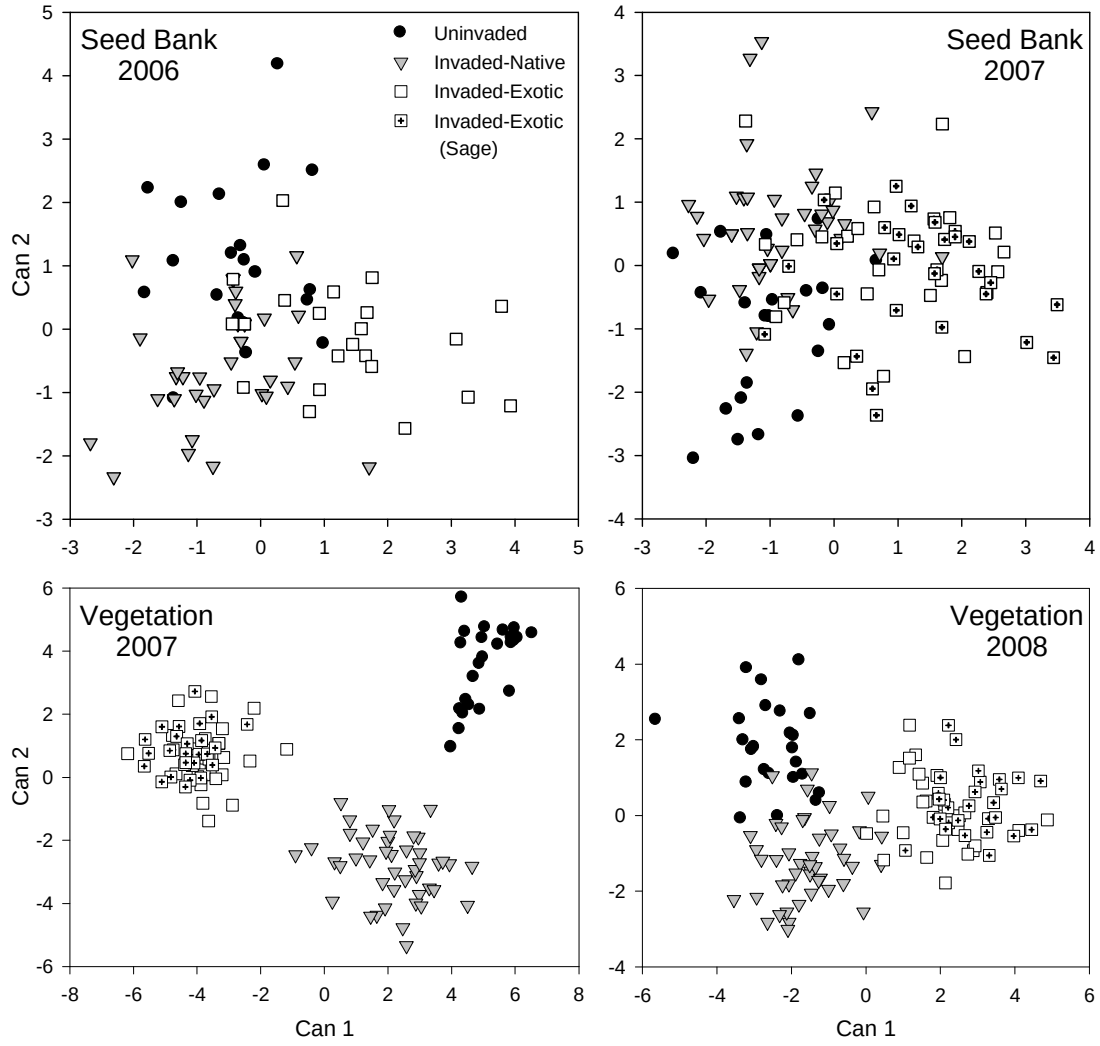


Figure 2-5. Canonical discriminant analysis plots for seed bank and vegetation. Black circles indicate uninvaded plots, gray triangles indicate invaded-native plots, and white squares indicate invaded-exotic plots. Squares with plus signs indicate plots in silver sagebrush / western wheatgrass shrublands.

Chapter 3:

Flea beetles (*Aphthona*) alter leafy spurge (*Euphorbia esula*) impacts on native plant germination, establishment, and growth

Proper selection of biocontrol agents for invasive plant management can result in self-sustaining populations that are inexpensive to distribute and that have few negative repercussions. However, even the most careful selection procedure does not eliminate the chance that negative nontarget effects will result from biocontrol use. The non-native invasive plant leafy spurge (*Euphorbia esula*), once occupying over a half million hectares in the northern Great Plains, has been successfully controlled in many areas via integrated pest management, especially via flea beetle (*Aphthona* spp.) biocontrol. Unfortunately, native plants can have a slow, incomplete recovery in areas where leafy spurge has been controlled using flea beetles, which is suggestive of negative nontarget effects from biocontrol application, soil legacy effects from leafy spurge presence, or both. In a factorial glasshouse experiment, I investigated the effects of past and recent leafy spurge soil conditioning and flea beetle application on subsequent native plant germination and growth. I also investigated the effects of these treatments on native plant root infection with arbuscular mycorrhizal fungi using terminal restriction fragment length polymorphism analyses. Recent leafy spurge soil conditioning significantly reduced native plant germination, biomass and height, and

significantly increased root:shoot ratios, when all species were combined. The flea beetle treatment affected individual species, with flea beetle presence significantly reducing the biomass and height, and significantly increasing root:shoot ratios, of some native plants when compared to no beetles. The growth of some native plant species in soils that were previously invaded by leafy spurge and subsequently dominated by non-native grasses was negatively impacted compared to soils dominated by native plants. There were no differences in arbuscular mycorrhizal fungi among the treatments. My results indicate that leafy spurge soil conditioning may reduce native plant establishment. There may also be negative nontarget effects from flea beetle biocontrol of leafy spurge on some native plant species, which supports field observations of slow native plant recruitment in areas treated with flea beetles. Future research should investigate the mechanisms responsible for these effects.

Introduction

The control of invasive plant species, often a primary goal of land managers, can be accompanied by paradox: though control methods are successful, native species often fail to recolonize. Common scenarios following invasive management include reinvasion of the invader or other undesirable species (such as invasive exotics or weedy natives) or recolonization of natives along with undesirables (Reid et al. 2009, Kettenring and Adams 2011). In fact, restoration success has only been moderate following many large-scale invasive plant control programs (Kettenring and Adams

2011). Biological control of invasive plants is actively used in wildlands worldwide (Van Driesche et al. 2010), and many of these biological control programs have been successful at reducing invasive plant presence (Clewley et al. 2012). However, unexpected interactions between biocontrol organisms, their target plants, and soils can result in negative nontarget effects on native plant communities (Louda et al. 2003, Pearson and Callaway 2008). Understanding the mechanisms behind these nontarget effects could greatly improve post-invasion restoration success.

Leafy spurge (*Euphorbia esula* L.; hereafter, *Euphorbia*) is an invasive plant in the northern Great Plains and occupies approximately 1.9 million ha in the US (Duncan et al. 2004) with an estimated $120 million in economic impacts annually (Leistritz et al. 2004). *Euphorbia* can reduce native plant species richness (Belcher and Wilson 1989, Butler and Cogan 2004, Cornett et al. 2006), conspecific pollen presence on native plants (Larson et al. 2006), and presence of native ungulates (Trammell and Butler 1995). *Euphorbia* populations have declined substantially because of flea beetles (*Aphthona* spp.) introduced as biological control from *Euphorbia*'s native range (Kalischuk et al. 2004, Butler et al. 2006, Larson et al. 2008). Along with changes in *Euphorbia* populations, several long-term studies have monitored native vegetation recovery following flea beetle application. In 9- and 14- year studies in Montana, USA, native plant diversity has not fully recovered following *Euphorbia* control by flea beetles (Lesica and Hanna 2009, Butler and Wacker 2010). Native plant biomass and species diversity also did not recover to that of noninvaded sites following *Euphorbia* control by flea beetles in a 10-year experiment in North Dakota, USA (Larson and

Larson 2010). Instead, the vegetation in areas previously dominated by *Euphorbia* had a high proportion of exotic grasses. While I now have a valuable baseline of nonmanaged vegetation changes following *Euphorbia* control in the region, these studies suggest that active post-control management may be required if native plant diversity is to be restored.

Reduced native plant recovery following the biological control of *Euphorbia* is suggestive of potential nontarget effects from flea beetles. Possible mechanisms include enhanced soilborne pathogens or allelochemicals in response to flea beetle-infested *Euphorbia*. Previous work exploring the effects of soilborne plant pathogenic fungi (*Rhizoctonia solani* and *Fusarium oxysporum*) and flea beetles on *Euphorbia* showed that the damage was greatest when flea beetles and fungi were both present, compared to when they were individually present or neither was present (Caesar 2003). The root-attacking larvae of flea beetles are carriers of plant pathogenic fungi, providing a means for inoculating the plants and for dispersing pathogen inoculum. This suggests a mechanism for increasing the infection potential of fungal pathogens in soils where larvae are present, which was demonstrated previously for two *Euphorbia* species in the field (*E. esula* in Europe and USA, and *E. stepposa* in Europe) (Caesar 2003). For generalist pathogens, multiple native species could come under pathogen attack. Furthermore, if the soil carries a high population of persistent pathogens, native plants could continue to be impacted long after *Euphorbia* is controlled.

Alternatively, flea beetle herbivory may enhance the release of compounds from *Euphorbia* roots that inhibit native plant growth. This mechanism has been observed in

Centaurea maculosa, an invasive exotic plant in North America which produces the allelopathic chemical (±)-catechin when attacked by root-boring biological control insects, thereby negatively impacting native plant growth (Thelen et al. 2005). *Euphorbia* has also demonstrated allelopathic effects on other plants (Steenhagen and Zimdahl 1979, Olson and Wallander 2002, Qin et al. 2006). If *Euphorbia* exudes allelopathic chemicals as a response to herbivory, native plants could be negatively impacted following *Euphorbia* biological control, but only if soil microbes do not rapidly break down the allelochemicals first (Kaur et al. 2009).

The arbuscular mycorrhizal fungal (AMF) associations of native plants may also be altered by *Euphorbia* soil conditioning, and indirectly by flea beetle herbivory. In a glasshouse experiment, native plants grown in soils conditioned by three growth periods of *Euphorbia* were hosts to a reduced range of AMF taxa compared to plants grown in native-conditioned soils (Jordan et al. 2012). In the same experiment, the biomass of several native species grown in spurge-conditioned soils was also reduced. While this effect was not directly linked to differences in AMF colonization between conditioning treatments, these findings are consistent with the hypothesis that invasive plants can impact native plant species by changing plant-microbe mutualisms (Inderjit and van der Putten 2010). The invasive plant garlic mustard (*Alliaria petiolata*), for example, releases an allelochemical that inhibits the germination of AMF spores (Cantor et al. 2011), which may be responsible for reduced AMF colonization of sugar maples in areas occupied by garlic mustard (Barto et al. 2011). Additionally, if *Euphorbia* releases compounds that are suppressive to AMF or native plant associations with AMF as a

response to biocontrol herbivory, flea beetles are likely to have indirect, negative effects on native plant communities via associations with AMF.

In a glasshouse experiment, I investigated the effects of *Euphorbia* soil conditioning, flea beetle presence, and presence of native soil microbes on the subsequent growth of plants native to the northern Great Plains. My objectives were to: (1) determine if the conditioning of soils by adult *Euphorbia* plants altered the germination and growth of native plants; (2) evaluate the effects of *Euphorbia* conditioning in the presence of two flea beetle species singly or in combination on subsequent growth of native plants; (3) evaluate the effects of native field soil microbes from varying *Euphorbia* invasion histories (e.g., invaded versus uninvaded) on the germination and growth of native plants; and 4) to examine differences in AMF colonization of native plant roots under the various treatments. If flea beetle presence indirectly enhances soil plant pathogen loads, or stimulates allelochemical exudation, I expected to see negative impacts from flea beetle presence compared to the absence of beetles on native plant germination and growth. Based on previous soil conditioning experiments, I expected to find greater negative impacts on native plants through soil inoculations from sites where *Euphorbia* once dominated, and greater negative impacts from soil from sites dominated by other exotics, compared to soil inoculation from sites dominated by natives.

Methods

Native soil microbiota, *Euphorbia* conditioning, and flea beetle presence were evaluated for their effects on native plant germination, establishment, and growth in a single, factorial experiment.

Experimental soil preparation & inoculation

To test the influence of soil microbial communities resulting from different *Euphorbia* histories on native plants, experimental greenhouse soils were inoculated with soils from field sites of varying *Euphorbia* histories prior to native plant sowing. I collected these field soils from Theodore Roosevelt National Park (TRNP) in June 2008 from sites which had one of three *Euphorbia* histories: never invaded, previously invaded but currently dominated by native plants, and previously invaded but currently dominated by other invasive plants (hereafter, uninvaded, invaded-native, and invaded-exotic, respectively). The invaded sites are in recovery from *Euphorbia* occupation, which were last dominated by *Euphorbia* ~ 10 years prior to this study. Allelopathic compounds from *Euphorbia* are not likely to be present in these soils, as allelochemicals can be broken down quickly by soil microbial action (Kaur et al. 2009). Therefore, the soil inoculation treatments represent the soil microbial component. These soils were collected from within a 300 m × 300 m site (Bad Buffalo) which was used for concurrent research on native vegetation recovery following *Euphorbia* control (see Chapter 2 for site description). I used 2.2 cm diameter soil probes to collect soil to a depth of 10 cm, with 10 cores at 15 sites per invasion history. Soils were air dried for

one week (mean temperature = 27° C), and all soil samples from the same invasion history were combined.

A glasshouse soil mixture composed of 252 liters (l) composted field soil, 126 l peat, 50.4 l river sand, and 25.2 l perlite (Caesar 2003) was thoroughly mixed, sterilized via autoclaving (autoclaved on two consecutive days at 121° C for two hours each), and cooled to room temperature (~24 hours). The soil was divided into four batches, three which were each inoculated with field soil from one *Euphorbia* history (10% of total volume), making the experimental soil history treatments: uninvaded, invaded-native, and invaded-exotic. The remaining batch was a non-inoculated control (hereafter, sterile).

Euphorbia soil conditioning

To test the effect of *Euphorbia* root exudate compounds (including allelochemicals if they are present) and the effect of short-term soil microbial changes from *Euphorbia* on native plants, experimental soils were conditioned by the presence of *Euphorbia* plants prior to native plant sowing. One-year-old *Euphorbia* plants, glasshouse-grown from TRNP seeds in plastic pots with potting soil media, were transplanted into experimental soils in plastic pots (15.25 × 15.25 × 17.75 cm) after thorough root cleaning. Soil was rinsed off the roots in running water, and the roots were soaked in a 10% chloramine T solution for five minutes, which can reduce microbial transfer via plant tissues (Nunez-Palenius et al. 2006). Transplanted *Euphorbia* was grown in the glasshouse for two weeks prior to application of flea beetles. Control pots were not transplanted with *Euphorbia* – they only contained soil.

Flea beetle collection and application

Simultaneous protection of plants and containment of flea beetles was achieved by fitting each pot with an 84 × 30 cm wire tomato cage enclosed by white no-see-um netting (Eastex Products, Inc., Holbrook, MA, USA) with an open top that was clamped shut using a binder clip. No-see-um netting, with 100 holes cm^{-2}, has a mesh size small enough to prevent flea beetles (about 3 mm long) from escaping. All pots, including those without flea beetle or *Euphorbia* treatments, were fitted with cages and netting to control for any effects of netting on the plants.

Flea beetles (*A. lacertosa* and *A. nigriscutis*) were collected from stands of *Euphorbia* along highway roadsides in Hennepin County, Minnesota during peak emergence on 28 June 2008. Beetles were placed in paper cups, kept cool in ice chests for transportation, and sorted to species in the lab. All treatments with flea beetles received 16 beetles/plant, eight each female and male. A similar treatment (15 beetles/plant) was found to demonstrate significant effects on *Euphorbia* in a previous experiment (Caesar 2003).

The two beetle species were applied separately and in combination (as described below). While both species are used extensively as *Euphorbia* biocontrol in the Great Plains, their presence is not equal across the region, and they have varying effects on *Euphorbia* populations (Larson et al. 2008). Flea beetle controls consisted of *Euphorbia* plants without flea beetles.

Experimental design

I used a randomized incomplete block design for the experiment. It is incomplete due to the absence of flea beetles in pots without *Euphorbia* (flea beetles cannot survive in the plant's absence). The four soil treatments, including sterile, uninvaded, invaded-native, and invaded-exotic, each had two *Euphorbia* treatments: present or absent. Each soil treatment with *Euphorbia* had four flea beetle treatments: no beetles, *Aphthona lacertosa* only, *A. nigriscutis* only, and both beetles. This design resulted in twenty different treatment combinations with eight replicate pots in each, for a total of 160 pots in the experiment.

Euphorbia growth and damage assessment

The experiment was conducted in a glasshouse at the University of Minnesota in 2008-2009. The treatments were arranged in a completely randomized design on benches, and, following application of flea beetles, the plants were grown for 3 months under ambient light with temperatures ranging from 15-30° C. Each plant was fertilized with 100 ml full-strength Hoagland's solution (Hoagland and Arnon 1950) every month, and was watered twice weekly. Pots without *Euphorbia* (the *Euphorbia* control) were not fertilized, but were occasionally watered to mimic soil moisture levels found in pots containing plants.

Each plant's condition was monitored one and three months after application of beetles to quantify damage from flea beetles and soilborne pathogens. Plant damage assessment followed the scale used by Caesar (2003), with plant damage ranked from 0-6: 0 = no damage; 1 = wilting; 2 = wilting and chlorosis; 3 = moderate wilting,

chlorosis, and some tissue death; 4 = persistent wilting, chlorosis, and death of one or more stems; 5 = extensive wilting, chlorosis, and tissue death or top dieback with regrowth; and 6 = death of plant (no regrowth). The plants, including aboveground and belowground tissues, were removed from the pots at the end of the experiment and roots were examined for damage from flea beetles or pathogens.

Native plant germination and growth

Following *Euphorbia* harvest, the soil in each pot was thoroughly mixed and allowed to dry completely prior to sowing native seeds. Seeds of eight plant species native to the northern Great Plains were used: *Artemisia frigida* Willd., *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths, *Linum lewisii* Pursh, *Nassella viridula* (Trin.) Barkworth, *Pascopyrum smithii* (Rydb.) Á. Löve, *Ratibida columnifera* (Nutt.) Woot. & Standl., *Symphyotrichum ericoides* (L.) G.L. Nesom, and *Vicia americana* Muhl. ex Willd (nomenclature according to USDA NRCS (2012)). Seeds were acquired from the Bismarck Plant Materials Center (Natural Resources Conservation Service, U.S. Department of Agriculture; Bismarck, ND, USA), Wind River Seed (Manderson, WY, USA), and from Prairie Moon Nursery (Winona, MN, USA).

Four randomly-selected species were sown in each pot (15.25 × 15.25 × 17.75 cm), with 25 seeds per species per pot. Each species was represented in four pots in each soil treatment. In each pot, the seeds from each species were evenly distributed on the soil surface in one of four quadrants, which were created by two plastic dividers (20 × 2.5 cm) joined at the middle to form an “X” shape and pressed one cm into the soil surface. This allowed seeds from each species to be distinguished during germination

and establishment, which was particularly important for the three grasses, which are difficult to identify at early stages. Seeds were topped with one cm of sterile vermiculite (autoclaved as described previously) to retain soil moisture and stimulate seed germination. Germination and seedling conditions were monitored five times after planting (days 4, 8, 24, 41, and 56), and dead seedlings were removed from pots. Pots were watered daily, or as needed, during this period.

Native plant harvesting and molecular assessment

Native plant survivorship, height, and biomass were determined after 130 days of growth. Because plants were not thinned in the experiment, there were unequal numbers of the eight species at harvest. Roots were thoroughly cleaned with water and separated from the shoots. Roots were partially mixed at the time of harvest, but most remained attached to shoots, and separated roots were usually identifiable via morphological differences, therefore I was confident in assigning roots to the proper species. Both were oven dried at 60° C for one week, and their biomass determined.

I used terminal restriction fragment length polymorphism (T-RFLP) analysis to identify the arbuscular mycorrhizal fungi (AMF) operational taxonomic units (OTUs) and non-AMF OTUs in dried root samples (Jordan et al. 2012). I analyzed 191 root samples, using at least one sample per species per treatment combination where possible. In five instances with *Symphyotrichum* and two instances with *Ratibida*, a lack of available root tissue precluded AMF analyses. Each sample consisted of three to five dried, 1-cm root pieces which were pulverized using steel beads on a plant shaker for 2 min. I used the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), using

manufacturer's instructions, to isolate total cellular DNA from the pulverized root samples. The resulting extracts were stored at -20 °C. I amplified the ITS region of the DNA using nested PCR. The primers SSU-Glom1 and LSU-Glom1 (Renker et al. 2003) were used in the first PCR (PCR1), and fluorescently labeled primers ITS4 and ITS5 were used in the second (PCR2). PCR1 products from each reaction were digested using *Alu*I (New England Biolabs (NEB), Ipswich, MA) to reduce additional amplification of non-AMF DNA (Renker et al. 2003). This digest was used as a template for PCR2. The PCR2 products were separated via electrophoresis on a 0.8% agarose gel, and samples having product sizes consistent with AMF (500-650 bp) (Aldrich-Wolfe 2007) were purified using the UltraClean PCR Clean-Up Kit using manufacturer's instructions (MoBio Laboratories, Carlsbad, CA). These purified products were digested with *Mbo*I and *Hinf*I (NEB), and the PCR2 and digested products for each sample were analyzed by the University of Minnesota BioMedical Genomics Center DNA Sequencing and Analysis Facility to obtain PCR2 product and terminal restriction fragment lengths. Fragment size and strength (strong peaks, as described below) were determined using Peak Scanner Software v1.0 (Applied Biosystems, Carlsbad, CA).

Profiles of PCR2 product length and 5'-*Hinf*I, 3'-*Hinf*I, and 3'-*Mbo*I T-RFLP fragment lengths were constructed to define distinct operational taxonomic units (OTUs). Samples with equivalent product and fragment lengths (± 3 bp) of all four regions were deemed to share an OTU. Strong peaks for all four regions were required for OTU identification, therefore peaks less than 10% of the strongest were discarded (Jordan et al. 2012). My profiles were compared with T-RFLP databases of AMF and

non-AMF root-inhabiting fungi from a broad range of studies (Aldrich-Wolfe 2007) and from plants grown in soils from the same region (Jordan et al. 2012). Further details for this method are outlined in Jordan et al. (2012).

My T-RFLP method yielded identification of OTUs that belong to the phylum *Glomeromycota* (AMF), but the digests I used only reduce the presence of non-AMF DNA in my samples (Renker et al. 2003), including fungi from the phyla *Basidiomycota* and *Ascomycota*, referred to here as non-AMF OTUs. The AMF and non-AMF OTUs combined, however, allowed us to quantify a subset of the fungal diversity inhabiting native plant roots in my experiment.

Analyses

Damage ratings on *Euphorbia* from flea beetle and soil treatments, which did not follow any distinct distribution, were analyzed using Wilcoxon and Steel-Dwass (Steel 1960) nonparametric tests, performed in JMP v. 9 (SAS Institute Inc., Cary, NC, 1989-2010). Seed germination data were analyzed via generalized linear mixed models. Biomass data were analyzed using one of two methods: linear mixed models, or nonlinear zero-inflated mixed models (for species with zero biomass numbers high enough that no transformation could normalize the distribution). Height data were analyzed using linear mixed models. Mixed models were executed in SAS/STAT v. 9.3 (SAS Institute Inc., Cary, NC, 1989-2011) and are described in Appendix 4. The MIXED procedure was used for the linear mixed models, the GLIMMIX procedure for the generalized linear mixed models, and the NLMIXED procedure for the nonlinear zero-inflated mixed models, with code modified from Littell et al. (2006) McLerran

(2008), Pahel et al. (2011) and SAS Institute Inc. (2011). I used Tukey's HSD test to determine differences among treatment levels for all of the mixed models. I only interpreted interaction terms in type III tests of fixed effects that yielded both significant single effects and a significant interaction. *P*-values <0.05 were deemed as significant, and Bonferroni corrections were applied for comparisons among multiple species for a given treatment effect test.

Statistical analyses were not performed on AMF presence data, as the number of AMF found was very small (eight total root samples had AMF). The effects of treatments and plant species identity on number of OTUs was analyzed using Wilcoxon nonparametric tests, because these data could not be transformed to meet any statistical distribution function. Relationships between OTU numbers and log-transformed plant biomass were determined using linear regression analyses, which were conducted in JMP v. 9.

To elucidate broad trends in *Euphorbia* treatment effects on individual species, I graphically summarized positive and negative treatment effects regardless of their statistical significance. Individual *Euphorbia* treatment effects were calculated by subtracting "no *Euphorbia*" from "*Euphorbia*" least square means estimates, which yielded a negative number if the latter mean was smaller, indicating a negative effect. These calculations were done for the "no beetle" treatment (to eliminate potentially confounding effects from beetle presence), and also within each of the soil inoculation treatments (sterile excluded), yielding four separate positive or negative effects. I then calculated the difference in the number of positive and negative effects for a given

treatment by species by response variable combination (hereafter, net effects). Trends in the flea beetle treatment effects were calculated as described for the *Euphorbia* net effects, with the following differences. The "no beetle" estimates were subtracted from estimates of each of the treatments with beetles present, yielding three positive or negative numbers. These calculations were performed for the "*Euphorbia*" treatment, and within each of three soil inoculation treatments (sterile excluded), yielding a total of 12 numbers for the net effects calculation. The net effects for the soil treatments were calculated as previously described, with the following differences. I subtracted the uninvaded treatment least square mean estimate from the invaded-native and invaded-exotic estimates, yielding two positive or negative numbers. This was done across all pots, and also within each *Euphorbia* and beetle treatment level, yielding a total of 14 numbers for the net effects calculation.

To further explore native plant responses to the *Euphorbia*, flea beetle and soil inoculation treatments, I pooled all data within individual pots, making pots the experimental units (hereafter, per-pot analyses). This was done for total germination, biomass and root:shoot ratios, but since I measured the height of each individual plant, I used mean plant heights as the response variable for these analyses.

Larger root:shoot ratios are typically indicative of more resources being spent on root tissue for water and nutrient acquisition (Levang-Brilz and Biondini 2003), and root:shoot ratios are negatively related to shoot biomass and mean annual precipitation (Mokany et al. 2006). Therefore, enhanced root:shoot ratios were interpreted as negative impacts on native plants.

Results

Euphorbia soil conditioning effects on native plants

Native plant germination, biomass, and height were consistently reduced in *Euphorbia* conditioned soils compared to nonconditioned soils (Figure 3-1 and Table 3-1), but *Euphorbia* did not significantly affect any individual species except *Pascopyrum* (Appendix 5). Per-pot native plant biomass, germination and height were significantly reduced in *Euphorbia* conditioned soils compared to nonconditioned soils, but root:shoot ratios were enhanced by *Euphorbia* conditioning (Figure 3-1 and Table 3-1). The effect of *Euphorbia* conditioning on per-pot germination also depended on the soil conditioning treatment (a significant *Euphorbia* × soil interaction; Table 3-1), where germination rates were lower in *Euphorbia*-conditioned soils, but only for sterile (t_{56} = -3.64, P = 0.0129) and uninvaded (t_{56} = -3.89, P = 0.0062) soils. The net effect of *Euphorbia* on native plants was largely negative across most species and most response variables (Figure 3-2). However, five species had positive net effects from *Euphorbia* in one or more response variables, and two species, *Linum* and *Nassella*, only had positive net effects.

Pascopyrum was the only species with negative responses to *Euphorbia* soil conditioning. There was a significant interaction between *Euphorbia* and soil treatments for *Pascopyrum* germination, which was reduced by *Euphorbia* in all soils but invaded-exotic (Figure 3-3 and Appendix 5). *Pascopyrum* root:shoot ratios were also increased by *Euphorbia* regardless of the soil treatments (Figure 3-4 and Appendix 5). After

Bonferroni corrections were applied, however, only root:shoot values were different for *Pascopyrum* (Appendix 5).

Flea beetle application effects on native plants

There were no differences in damage ratings on *Euphorbia* among the flea beetle treatments (χ^2 = 3.382, DF =3, P = 0.336); specifically, there were no differences between no beetle and *A. lacertosa* (Z = -1.11, P = 0.266), *A. nigriscutis* (Z = -1.26, P = 0.205), and both beetle (Z = 0.382, P = 0.703) treatments.

Per-pot native plant germination, height, and biomass were also not significantly impacted by beetle treatments (Figure 3-1 and Table 3-1). However, the two flea beetle species differed in their effects, with *A. nigriscutis* presence more frequently associated with significant negative effects on native plants than *A. lacertosa*. For example, per-pot root:shoot ratios were greater in the *A. nigriscutis* treatment compared to the absence of beetles (t_{108} = 2.74, P = 0.0356), and per-pot plant height and biomass tended to be less in the presence of *A. nigriscutis* than in the presence of *A. lacertosa* (2.48% and 7.47% less for height and biomass, respectively; differences were non-significant) (Figure 3-1).

The net effects of flea beetle treatments among the species indicate that there was a consistent positive association of flea beetle presence with native plant germination, but a consistent negative association of flea beetle presence with native plant height (Figure 3-2). There were no consistencies among the species for flea beetle effects on biomass and root:shoot ratios.

Most of the statistically significant effects of flea beetles on native plant germination and growth were on individual plant species. Four primary patterns

emerged from these analyses by species. First, all of the significant negative effects were due to the presence of flea beetles, and none to the absence of beetles (Figure 3-5, Figure 3-6 and Appendix 5). Second, more negative effects were associated with the presence of both flea beetle species than with an individual beetle species. For example, plant biomass was significantly less when both beetles were present compared to *A. nigriscutis* for *Linum* ($t_{43.8} = 2.89$, $P = 0.0293$) and *Pascopyrum* ($t_{39.5} = 2.88$, $P = 0.0309$), and *Nassella* had less biomass under both beetles than no beetles ($t_{36.4} = 3.58$, $P = 0.0053$). However, *A. lacertosa* did not have any significant negative effects on biomass, and *A. nigriscutis* only affected the biomass of *Nassella*, which was less with *A. nigriscutis* than with no beetles ($t_{35.7} = 4.3$, $P = 0.0007$) (Figure 3-6). Third, more significant effects were attributable to *A. nigriscutis* than to *A. lacertosa*, approximately half of which were positive and half negative towards native plants. Biomass is again a good example of this difference, as can be seen from the previous example, where the biomass under *A. nigriscutis* varied for three plant species, while no species varied due to the *A. lacertosa* treatment (Figure 3-6). Similarly, *Linum* root:shoot ratios were significantly greater with *A. nigriscutis* than with both beetles ($t_{47} = 2.87$, $P = 0.03$), but the only difference in root:shoot ratios attributable to *A. lacertosa* involved an interaction with soil inoculation treatments (Figure 3-6 and Appendix 5). Fourth, the most definitive impacts from flea beetle presence were in plant biomass, as highlighted by the previous two examples. Out of all the species-specific flea beetle treatment effects, only *Nassella* biomass was significantly affected by flea beetles following Bonferroni corrections (Appendix 5). There were also six significant interactions

between flea beetle and soil inoculation treatments, three of which were significant following Bonferroni corrections (Appendix 5), but there were no distinct patterns in the nature of these effects.

Soil history effects on native plants

Pathogen infection signs in *Euphorbia* plants did not vary among soil treatments (χ^2 = 5.99, DF =3, P = 0.112), indicating that if there were pathogens within the inoculated soils, they did not strongly impact *Euphorbia*.

There were few trends in net effects for the soil treatments, but more response variables were negatively impacted than positively impacted when plants were grown in previously-invaded soils compared to uninvaded soils (Figure 3-2).

Despite the lack of differences in pathogen signs among soil inoculation treatments, there were increases in subsequent native plant germination in inoculated compared to sterile soils, but reductions in native plant growth in invaded-exotic compared to invaded-native soils, or in previously-invaded soils compared to uninvaded soils. These patterns indicate that there are negative legacy effects associated with *Euphorbia* soil occupancy in the past and with the recent soil occupancy by other invasive plants. Per-pot root:shoot ratios, for example, were greater in invaded-native soils than in invaded-exotic ($t_{20.3}$ = -5.51, P = 0.0007) or sterile ($t_{16.3}$ = -4.22, P = 0.0178) soils, but only in the presence of both flea beetles. Per-pot total plant biomass was also greater in invaded-native than invaded-exotic soils, but only in pots that had *Euphorbia* plants (t_{107} = -2.87, P = 0.025). *Ratibida* was the species that showed the strongest soil treatment impacts: *Ratibida* biomass (t_{31} = -3.13, P = 0.004) and height

($t_{44.3}$ = -2.8, P = 0.0363) were greater in invaded-native than in invaded-exotic soils (97% and 39% greater for biomass and height, respectively) (Figure 3-4, Figure 3-6 and Appendix 5). Two species were negatively impacted by growing in previously-invaded soils (invaded-native and invaded-exotic) compared to uninvaded soils. Germination rates of *Nassella* (t_{48} = 3.69, P = 0.0413) were lower in invaded-native soils, and the germination of *Vicia* was lower in invaded-exotic soils (t_{48} = 3.02, P = 0.0206), but both results only occurred in the presence of both beetles (Figure 3-5). Soil inoculations did have two positive influences on native plants, however. Native plant per-pot germination was greater in inoculated soils (the uninvaded, invaded-native, and invaded-exotic treatments) compared to sterile soils, but this effect was only in pots where flea beetles were applied to *Euphorbia* plants (a significant flea beetle by soil interaction; Table 3-1). *Bouteloua* germination was also enhanced in uninvaded and invaded-exotic soils compared to sterile soils (Figure 3-3). Among the species-specific tests above, only the soil effects on *Nassella* and *Bouteloua* germination were significant after Bonferroni corrections (Appendix 5).

AMF and other root-inhabiting fungi

AMF OTUs were found in eight of the 192 root samples analyzed (4.2%) – the remaining 186 samples had no AMF. Within these eight samples I identified six different AMF OTUs, including three *Glomus intraradices* (A, B, and C), two *G. mossae* (A and B) and one *Glomus* sp. These AMF were in root samples of eight different individual plants (five *Linum*, two *Bouteloua*, and one each *Artemisia* and *Pascopyrum*), but there was not equal representation of the different AMF among these

plants. The five *Linum* plants were hosts to the following AMF OTUs: *Linum* #1 = *G. mossae* A, *Linum* #2 = *G. intraradices* A and *Glomus* spp. A, *Linum* #3 = *G. intraradices* A, *Linum* 4 = *G. intraradices* B, and *Linum* #5 = *Glomus* spp. B. The *Bouteloua* plant was host to two AMF (*G. intraradices* C and *G. mossae* B), while the *Pascopyrum* was host to *G. intraradices* A, and *Artemisia* was host to *G. mossae* A. The low frequency of AMF detection prevented statistical analyses from being performed on AMF presence.

However, there were some interesting patterns in the total proportion of pots with plants infected by AMF when compared among treatments. For example, the proportion of pots with AMF was approximately equal between no *Euphorbia* (0.04) and *Euphorbia* (0.0476) treatments, but higher with both flea beetle species (0.182) than no beetles (0.0476), *A. lacertosa* (0.0454) and *A. nigriscutis* (0.05). As expected, pots with sterile soils had no plants infected with AMF, but pots inoculated with uninvaded soil had a lower proportion with AMF (0.037) than pots inoculated with invaded-native (0.115) or invaded-exotic (0.133) soils.

Grasses (*Bouteloua*, *Nassella*, and *Pascopyrum*) had higher OTU diversity (including both AMF and non-AMF) than forbs ($\chi^2 = 5.27$, DF = 1, $P = 0.0217$). However, OUT diversity per pot did not vary with *Euphorbia* ($\chi^2 = 0.087$, DF =1, $P = 0.769$), flea beetle ($\chi^2 = 0.5861$, DF = 3, $P = 0.9$), or soil ($\chi^2 = 5.24$, DF = 3, $P = 0.1552$) treatments, or among species ($\chi^2 = 5.24$, DF = 7, $P = 0.223$). There were also no significant relationships between the total number of OTUs per pot and shoot mass, shoot mass per plant, total mass per plant, and root:shoot ratio (data not shown). There

was, however, a significant biomass - OTU richness relationship for three species: *Bouteloua* (total biomass, root biomass, and root mass per plant were positively correlated with OTU richness), *Pascopyrum* (biomass and root biomass were negatively correlated with OTU richness), and *Artemisia* (biomass was negatively correlated with OTU richness) (Table 3-2).

Discussion

Recent and past *Euphorbia* soil conditioning, and flea beetles applied to *Euphorbia*, can all have detrimental effects on subsequent native plant establishment and growth. These results lend experimental support to field studies that found reduced native plant richness following *Euphorbia* invasion (Belcher and Wilson 1989, Butler and Cogan 2004, Cornett et al. 2006), and reduced native plant recovery after *Euphorbia* control by flea beetles (Lesica and Hanna 2009, Butler and Wacker 2010). However, while *Euphorbia* had broad effects that were not generally species-specific, flea beetles and soil origin generally had species-specific rather than broad impacts on native plant germination and growth.

Euphorbia legacy and flea beetle impacts

Recent *Euphorbia* soil conditioning negatively influenced the germination, biomass, root:shoot ratio and average height of native plant "communities" in my study. This may indicate that there are broadly negative impacts of *Euphorbia* presence on native plant communities, either through modified soil microbial communities or root exudates that inhibit native plant germination and growth. The experimental design did

not allow me to determine if soil microbes or root exudates were responsible for the negative *Euphorbia* conditioning effect. However, *Euphorbia* conditioning suppressed native plant germination in both sterile and uninvaded soils in the per-pot analyses, suggesting that root exudates alone may reduce native plant germination (i.e., an allelopathic effect). Alternatively, the *Euphorbia* conditioning effect may be a side effect of the experimental and statistical designs. If the variability of individual plant species germination and growth was increased by the presence of other species within pots, then this may have resulted in overlapping confidence intervals and non-significant differences among treatments at the species level. As I had no pots that were sown with single plant species, this effect cannot be tested. However, *Pascopyrum* exhibited significant negative responses to *Euphorbia*, and all species did not respond similarly in the non-significant net effects (Figure 3-2). A range of responses to *Euphorbia* presence may therefore be exhibited by native species, with *Pascopyrum* having the strongest decline, others such as *Artemisia* and *Bouteloua* having moderate negative impacts, and others such as *Linum* and *Nassella* not being negatively impacted. There is some evidence for a negative impact of *Euphorbia* on *Pascopyrum* in field settings, as *Pascopyrum* was less frequently associated with *Euphorbia*-infested than with non-infested areas at Theodore Roosevelt National Park, North Dakota (Butler and Cogan 2004).

Jordan et al. (2008) reported that native forbs were suppressed more than native grasses by *Euphorbia*, but I did not find a similar difference among functional groups. There are four methodological differences between the two studies that may account for

this inconsistency. First, the conditioning period in my study was a single period of three months, while previous studies had three conditioning periods of ~two to three months (Jordan et al. 2008, Jordan et al. 2011). In addition, *Euphorbia* plants are perennial with extensive root systems, and flea beetle adults and larvae are each active for months at a time during the growing season (Gassmann et al. 1996), therefore longer conditioning periods for both are recommended for future studies on *Euphorbia* conditioning and flea beetle effects. Second, I used adult *Euphorbia* to condition soils, while *Euphorbia* was grown from seed in the other study. Plant root exudates can vary with plant growth stage, which can, in turn, have varying effects on the rhizosphere microbiota (Nannipieri et al. 2008) – seedling and adult *Euphorbia* may therefore vary in their rhizosphere influence, leading to varying plant responses between studies. Lastly, I used soil mixtures containing 10% field soil, while the other study used 100% field soil. Bartelt-Ryser et al. (2005) found a soil microbial influence on plant growth at 8% soil inoculant by volume, suggesting that native soil addition at low proportions (such as ours) can be effective at inoculating soil with living microbiota. However, soils in the study by Jordan et al. likely had a larger starting population of living organisms which, interacting with the longer time period of conditioning, would have a greater likelihood of influencing native plant germination and growth.

Flea beetle presence in combination with *Euphorbia* had consistent, detrimental effects on subsequent native plant growth in conditioned soils, which may relate to changes in *Euphorbia* root exudation stimulated by flea beetle herbivory. There was ample time in my experiment for adults to mate and lay eggs, for the eggs to incubate

and hatch, and for the larvae to feed on *Euphorbia* roots (Gassmann et al. 1996). While I did not find any larvae inhabiting roots, the larvae are quite small (Fornasari 1993), and difficult to detect. Herbivores can elicit plant root exudation (Holland et al. 1996), which may have effects on the soil microbial community that are detrimental to native plants, or the exudates may be directly harmful to plants. This was implicated in the competitive success of the invasive plant *Centaurea maculosa* against native plants when herbivorized by root-feeding biocontrol insects (Callaway et al. 1999), and one or both of these effects may also be responsible for the reduced native plant growth I observed. In light of my findings, these mechanisms warrant further research.

The two flea beetle species also differed in their effects, with *A. nigriscutis* presence more frequently associated with significant positive or negative effects on native plants than *A. lacertosa*. In field studies, *A. lacertosa* is associated with significant reductions in *Euphorbia* stem counts, while *A. nigriscutis* is not effective at controlling *Euphorbia* (Larson and Grace 2004, Larson et al. 2008), so there are measurable differences in flea beetle effects on *Euphorbia* in the field. While I did not observe differences in *Euphorbia* damage between beetle treatments, the different flea beetle species could elicit different defense responses from *Euphorbia*, which may have led to the varying impacts I observed.

The soil origin effects in my study, while constrained to individual species, are indicative of negative soil biota legacy effects related both to *Euphorbia* presence in the past, and to more recent occupation by other invasive plants. This is different from a previous experiment that investigated the effects of soil origin (field sites that were

invaded by *Euphorbia* or native-occupied) on subsequent plant growth, where soil origin effects were overwhelmed by other treatment effects (e.g., *Euphorbia* and native soil conditioning) (Jordan et al. 2008). Soils conditioned by other invasives like *Bromus inermis*, which was common in invaded-exotic sites in my study, can also be suppressive towards native plants (Jordan et al. 2008, Jordan et al. 2011, Perkins and Nowak 2012). Therefore, the negative effects I observed in invaded-exotic sites compared to invaded-native sites may be a signature of suppression by other invasive plant species through the soil microbial community.

There are two important caveats to consider with my study. First, the scarcity in significant effects may relate to the brief *Euphorbia* and beetle conditioning period employed, as previously mentioned. Second, the negative *Euphorbia* conditioning effects may have been caused by soil nutrient differences between the *Euphorbia* treatments: I fertilized *Euphorbia* pots in an attempt to offset nutrient uptake by *Euphorbia*, but I did not measure soil nutrient levels prior to sowing native plant seeds. If *Euphorbia* consumed more nutrients than I added, this may partially explain the reduced native plant growth in pots with *Euphorbia* conditioning compared to pots without *Euphorbia*, as this would have resulted in fewer nutrients in the former pots than the latter. Future studies should measure and adjust soil nutrient levels between plant conditioning and no plant conditioning treatments, and compare *Euphorbia* conditioning against native plant conditioning, as used in other studies (Jordan et al. 2008, Jordan et al. 2011, Jordan et al. 2012).

AMF colonization and soil microbiota diversity

The colonization rate of AMF on native plant roots in my study was very low (AMF was present on 4.2% of root samples). Other research on AMF using soils from the same region found 28% of roots with AMF (Jordan et al. 2012). The large difference between studies may relate to the proportion of field soils: I used 10%, while Jordan et al. (2012) used 50%, which would have more inoculum potential than in the present study. The lack of AMF from plants growing in sterile, non-inoculated soils was expected, but the proportion of AMF colonization in invaded soils being more than twice that of uninvaded soils was unexpected. *Euphorbia* is a known host of AMF (Harbour 1992, Jordan et al. 2011), but vesicular-arbuscular mycorrhizal inoculum potential did not differ between sites invaded by *Euphorbia* and uninvaded sites in Colorado grasslands (Pritekel et al. 2006). The disproportionate AMF colonization among species was also expected, as AMF in grasslands can display host-specificity (Eom et al. 2000).

The OTU diversity per pot did not significantly vary among any of the treatments, but the significantly higher OTUs among grasses than forbs is not surprising. Soil microbial communities can vary among functional groups, as was demonstrated by higher bacterial diversity in sedge compared to grass and mixed species assemblages in UK grasslands (Johnson et al. 2003). The three grasses in my experiment also had more biomass than most of the forbs, but the high OTU diversity among grasses was not a function of biomass alone, as there was no significant relationship between biomass and number of OTUs per pot in my study (Table 3-2).

The T-RFLP method I used, which was designed to sample AMF species, only captures a fraction of the organisms that can potentially colonize plant roots. Despite this bias, my results can be a starting point for further research, including elucidation of flea beetle and invasion history influences on AMF colonization and soil microbial diversity.

Management implications

The control of *Euphorbia* in the northern Great Plains is a critical objective for land management interests, in which flea beetles have played an important role. However, native plant communities do not always readily return following *Euphorbia* control by flea beetles, as was demonstrated in recent long-term studies (Butler et al. 2006, Lesica and Hanna 2009, Larson and Larson 2010). Based on my research, flea beetles may have nontarget effects on specific plant species, including *Linum*, *Bouteloua*, and *Pascopyrum*, which may be a factor in the lack of native species recovery in field studies. The negative effect of flea beetles on native plants was generally stronger for *A. nigriscutis* than *A. lacertosa*, but the latter is more effective in controlling *Euphorbia* stands in the field (Larson et al. 2008). Populations of *A. nigriscutis* have persisted in the field (Larson et al. 2008), and if the effects I observed in the glasshouse extend to similar effects in the field, this species may be having larger non-target effects on native plant species while, at the same time, not being an effective biocontrol agent. The fact that *A. lacertosa* is more effective at *Euphorbia* control is fortunate, but selective application of one species without the other may be very difficult or impossible on a large scale. Flea beetles are wild-caught from sustaining

populations and redistributed to areas needing treatment, but these two species frequently co-occur and are labor-intensive to separate. If additional research confirms my findings of stronger non-target effects from *A. nigriscutis* than *A. lacertosa*, managers may want to collect flea beetles from areas dominated by *A. lacertosa* to prevent additional negative impacts to native plants.

The *Euphorbia* soil legacy is similarly problematic, but the stronger negative effects associated with sites that have secondary invasions of exotic grasses (invaded-exotic) compared to previously invaded sites where natives recovered (invaded-native) reinforces the fact that *Euphorbia* impacts do not stop once the invasive species is removed. Exotic grasses were also found to have stronger negative effects on native plant richness than *Euphorbia* in a North Dakota field study (Larson and Larson 2010), and other studies have demonstrated similar negative effects from these grasses on native prairies (Grilz and Romo 1995, Murphy and Grant 2005, Vinton and Goergen 2006). Future *Euphorbia* management will therefore face challenges on at least two fronts: removing *Euphorbia* without negative side effects (e.g., from nontarget biocontrol effects), and encouraging subsequent recovery of plants that do not have stronger negative impacts on native plant community than the original invader.

Table 3-1. Per-pot type III tests of fixed effects for germination, biomass, root:shoot, and height. Degrees of freedom (DF) are listed as numerator,denominator. P-values < 0.05 are shown in bold.

		Germination			Biomass			Root:shoot ratio			Height		
Pots	Treatment	DF	*F*	*P*	DF	*F*	*P*	DF	*F*	*P*	DF	*F*	*P*
No beetle	*Euphorbia*	1,56	19	**<.001**	1,50.4	5.53	**0.023**	1,53.3	11.54	**0.001**	1,45.2	4.81	**0.034**
	Soil	3,56	1.6	0.200	3,50.4	1.21	0.316	3,53.3	0.78	0.513	1,45.3	0.81	0.495
	Euphorbia × Soil	3,56	5.24	**0.003**	3,50.4	1.82	0.155	3,53.3	2.52	0.068	3,45.3	0.45	0.718
Euphorbia	Beetle	3,51	2.55	0.066	3,107	0.51	0.679	3,108	3.59	**0.016**	3,87.4	0.47	0.706
	Soil	3,51	10.71	**<.001**	3,107	2.81	**0.043**	3,108	2.59	0.056	3,82.1	2.5	0.065
	Beetle × Soil	9,51	5.46	**<.001**	9,107	0.67	0.736	9,108	1.73	0.091	9,85	0.96	0.480
All	Soil	3,149	0.09	0.968	3,152	2.6	0.055	3,152	2.13	0.099	3,140	1.64	0.182

Table 3-2. Regression estimates for relationships between plant biomass and OTUs per pot and per species. Degrees of freedom (DF) are listed as numerator,denominator. P-values < 0.05 are shown in bold.

Variable	Parameter	Per pot	*Artemisia*	*Bouteloua*	*Linum*	*Nassella*	*Pascopyrum*	*Ratibida*	*Symph.*	*Vicia*
Total mass	Estimate	-0.306	-1.655	2.697	-1.242	0.739	-1.077	2.722	-0.399	0.08
	r^2	0.005	0.723	0.586	0.033	0.186	0.287	0.323	0.07	0.002
	DF	1,59	1,5	1,9	1,12	1,10	1,15	1,5	1,4	1,9
	F	0.321	13.05	12.72	0.4117	2.29	6.051	2.385	0.302	0.0167
	P	0.573	**0.015**	**0.006**	0.533	0.161	**0.027**	0.183	0.612	0.900
Root mass	Estimate	-0.208	-2.658	2.309	-3.187	0.641	-1.095	3.81	-0.776	0.154
	r^2	0.004	0.476	0.634	0.085	0.161	0.348	0.097	0.099	0.003
	DF	1,59	1,5	1,9	1,12	1,10	1,15	1,5	1,4	1,9
	F	0.2272	0.0862	15.618	1.121	1.915	8.015	0.537	0.4377	0.0306
	P	0.635	0.148	**0.003**	0.311	0.197	**0.013**	0.496	0.544	0.865
Root mass plant^{-1}	Estimate	-4.609	-5.21	15.48	-74.4	3.59	-3.9	2.227	-0.673	1.06
	r^2	0.051	0.159	0.709	0.268	0.277	0.207	0.009	0.014	0.002
	DF	1,59	1,5	1,9	1,12	1,10	1,15	1,5	1,4	1,9
	F	3.16	0.9448	21.92	4.401	3.831	3.912	0.0478	0.0559	0.0204
	P	0.081	0.376	**0.001**	0.058	0.079	0.067	0.836	0.825	0.890

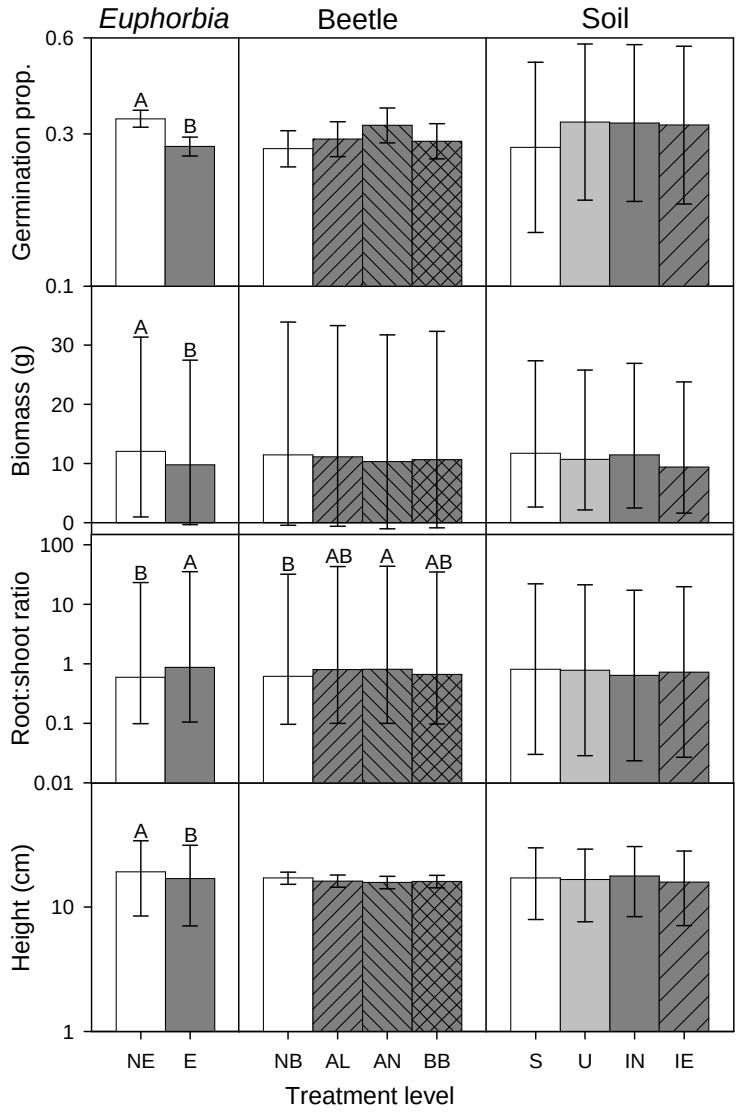


Figure 3-1. Per-pot treatment effects on germination proportion, biomass, root:shoot ratio, and height. Bar heights are least square means and error bars are 95% confidence intervals. Values were back-transformed to be on scale of original data; varying confidence interval magnitudes among treatments are caused by different data transformations prior to analysis. Germination proportion, root:shoot ratio, and height

are on the log scale. Treatment codes: NE = no *Euphorbia*, E = *Euphorbia*, NB = no beetles, AL = A. lacertosa, AN = A. nigriscutis, BB = both beetles, S = sterile soil, U = uninvaded soil, IN = invaded-native soil, IE = invaded-exotic soil.

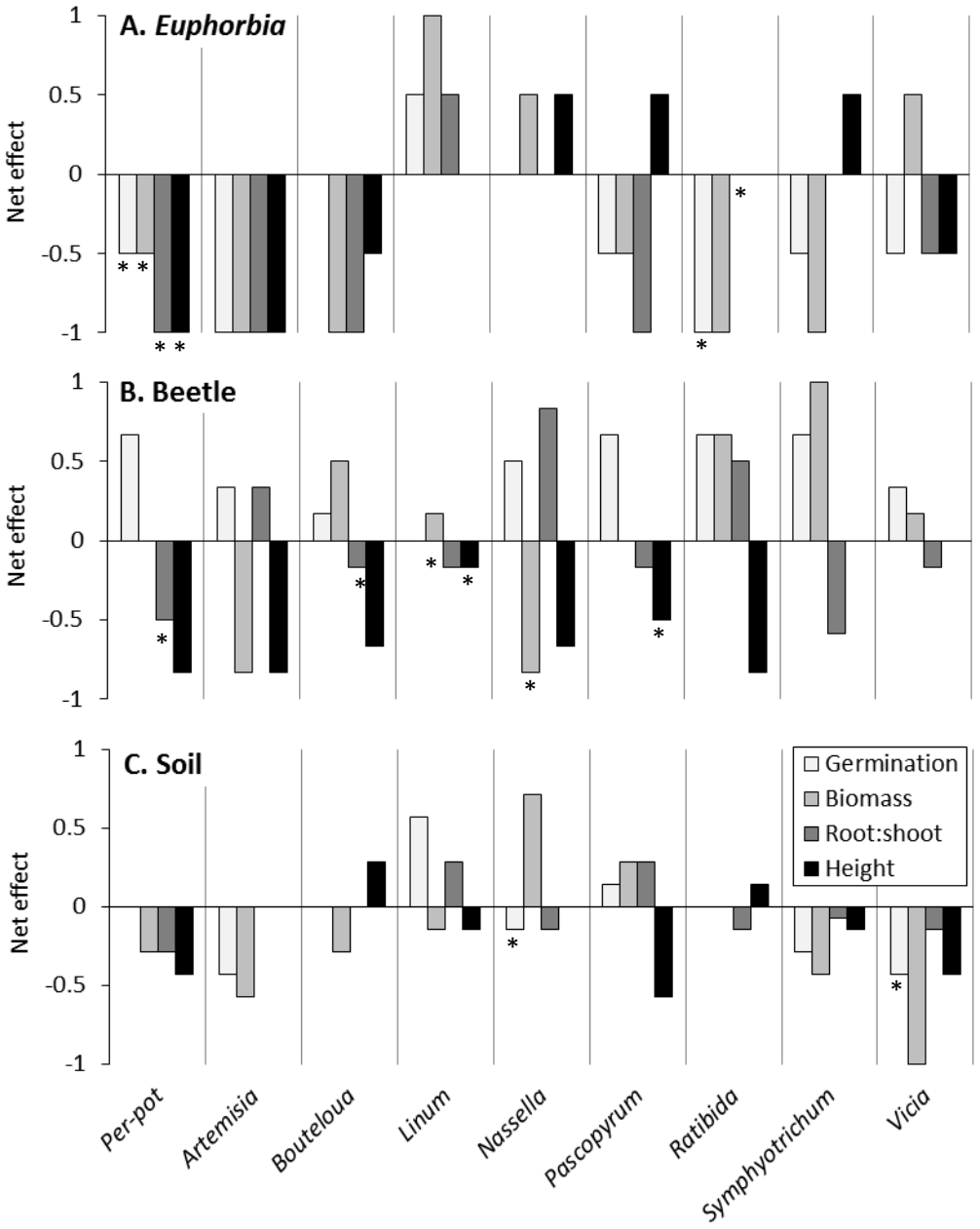


Figure 3-2. Summary of treatment effects by pot and species for germination, biomass, root:shoot ratio, and height. Net effects (the net proportion of estimate comparisons with positive or negative treatment effects) are shown for *Euphorbia* (A), beetle (B), and soil origin (C). Each asterisk indicates one significant type III test with either a positive or negative treatment effect (indicated by asterisk location above or below the

zero line, respectively). Note: missing bars indicate equal numbers of positive and negative estimate comparisons.

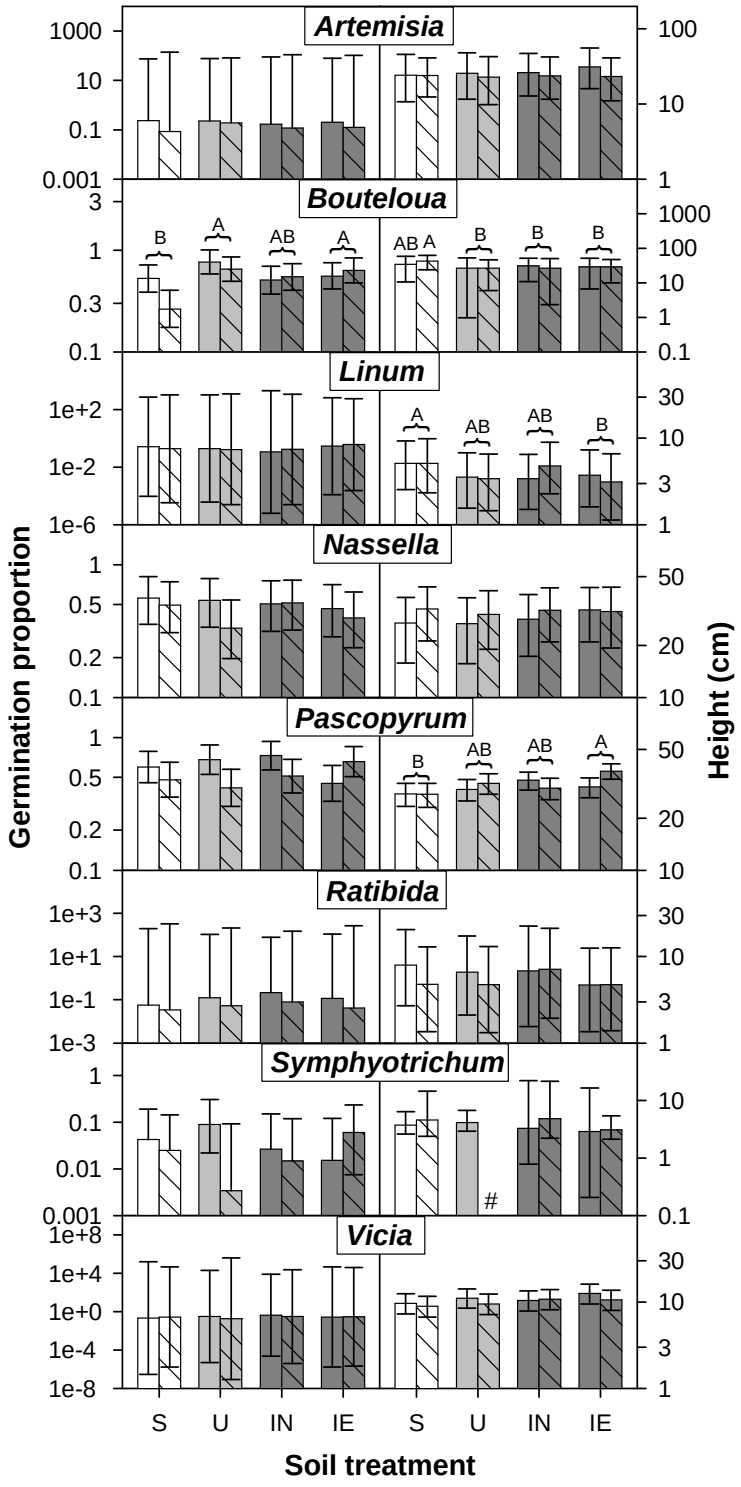

Artemisia
Bouteloua
Linum
Nassella
Pascopyrum
Ratibida
Symphyotrichum
Vicia
Germination proportion
Height (cm)
Soil treatment
S
U
IN
IE
S
U
IN
IE

Figure 3-3. Germination proportion and plant height by species for *Euphorbia* and soil treatments. Soil treatments are S = sterile soil (open bars), U = uninvaded soil (light gray), IN = invaded-native soil (dark gray), IE = invaded-exotic soil (dark gray), and *Euphorbia* treatments are no pattern = no *Euphorbia*, diagonal pattern = *Euphorbia*. Germination is on the log scale. Bar heights are least square means estimates and error bars are 95% confidence intervals. Values were back-transformed to be on scale of original data; varying confidence interval magnitudes among species and treatments are due partially to different data transformations prior to analysis. Significantly different estimates are indicated by different letters above bars. Non-estimable least square mean indicated by #.

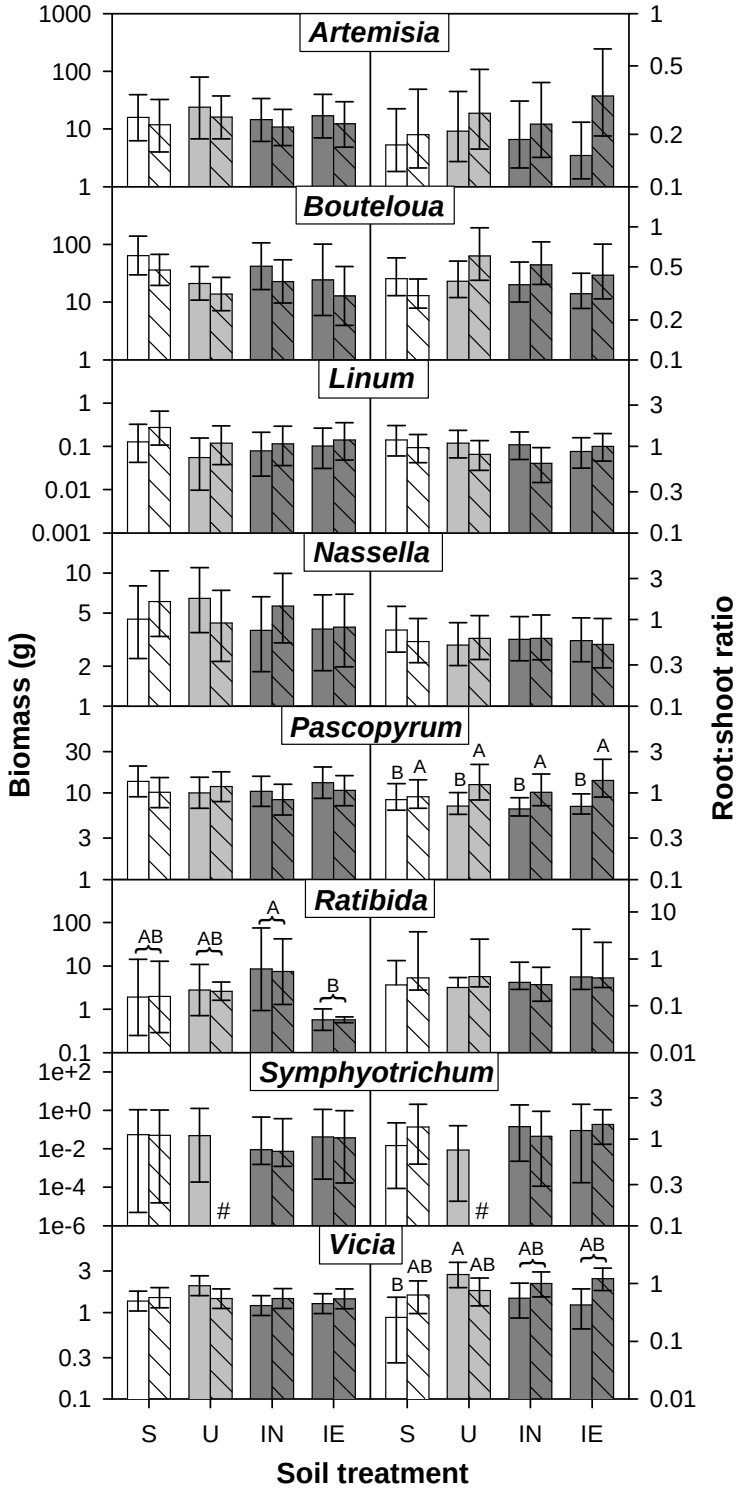

Artemisia
Bouteloua
Linum
Nassella
Pascopyrum
Ratibida
Symphyotrichum
Vicia
Biomass (g)
Root:shoot ratio
S
U
IN
IE
Soil treatment

Figure 3-4. Plant biomass and root:shoot ratios by species for *Euphorbia* and soil treatments. Soil treatments are S = sterile soil (open bars), U = uninvaded soil (light gray), IN = invaded-native soil (dark gray), IE = invaded-exotic soil (dark gray), and *Euphorbia* treatments are no pattern = no *Euphorbia*, diagonal pattern = *Euphorbia*. Biomass is on the log scale. Bar heights are least square means and error bars are 95% confidence intervals. Values were back-transformed to be on scale of original data; varying confidence interval magnitudes among species and treatments are due partially to different data transformations prior to analysis. Significantly different estimates are indicated by different letters above bars. Non-estimable least square means indicated by #.

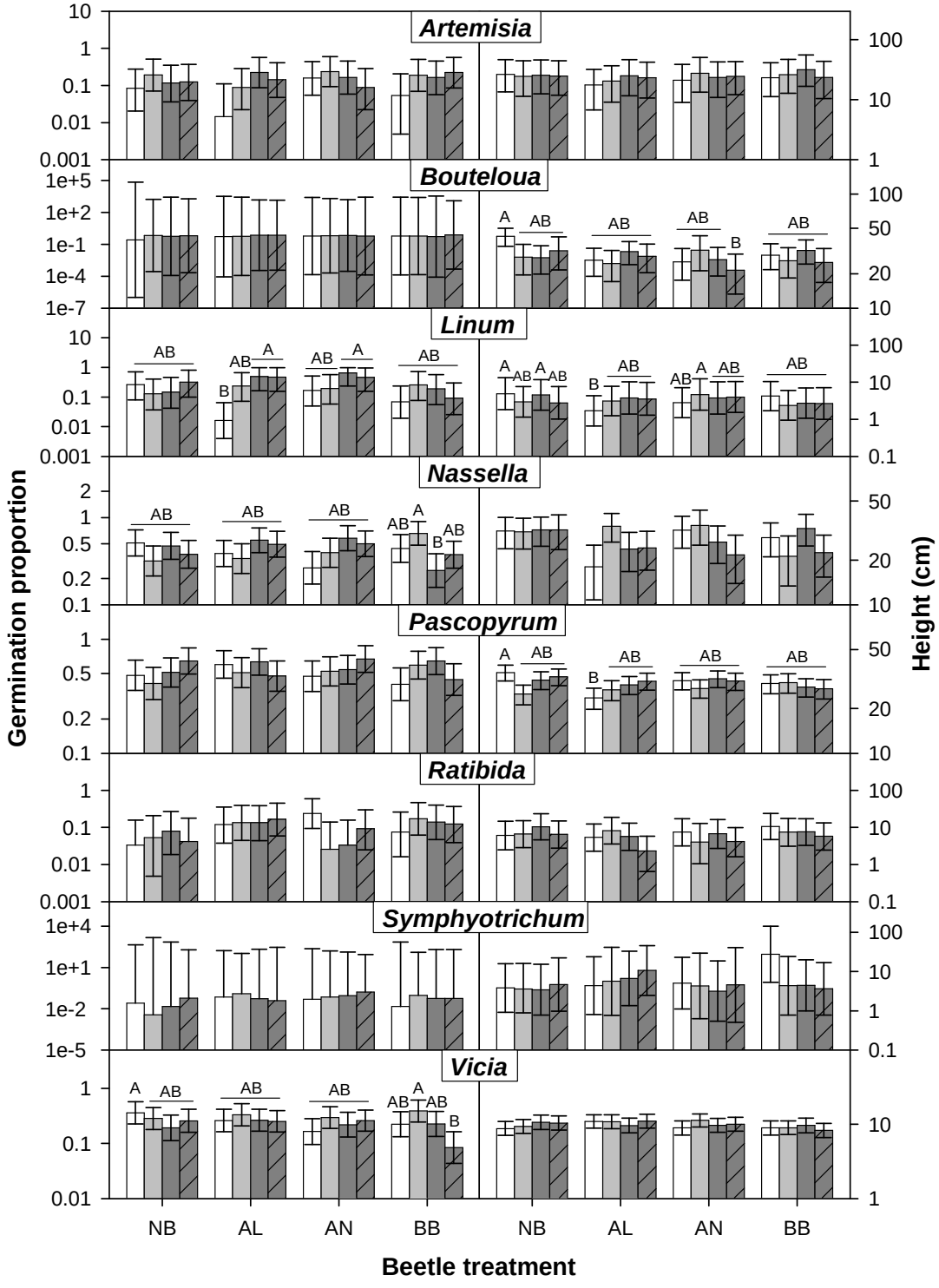

Artemisia
Bouteloua
Linum
Nassella
Pascopyrum
Ratibida
Symphyotrichum
Vicia
Germination proportion
Height (cm)
NB
AL
AN
BB
Beetle treatment

Figure 3-5. Germination proportion and height by species for beetle and soil treatments. Beetle treatments are NB = no beetles, AL = *A. lacertosa,* AN = *A. nigriscutis,* BB = both beetles. Soil treatments are open bar = sterile soil, light gray = uninvaded, dark gray = invaded-native, dark gray with diagonal pattern = invaded-exotic. Germination and height are on the log scale. Bar heights are least square mean estimates and error bars are 95% confidence intervals. Values were back-transformed to be on scale of original data; varying confidence interval magnitudes among species and treatments are due partially to different data transformations prior to analysis. Significantly different estimates are indicated by different letters above bars.

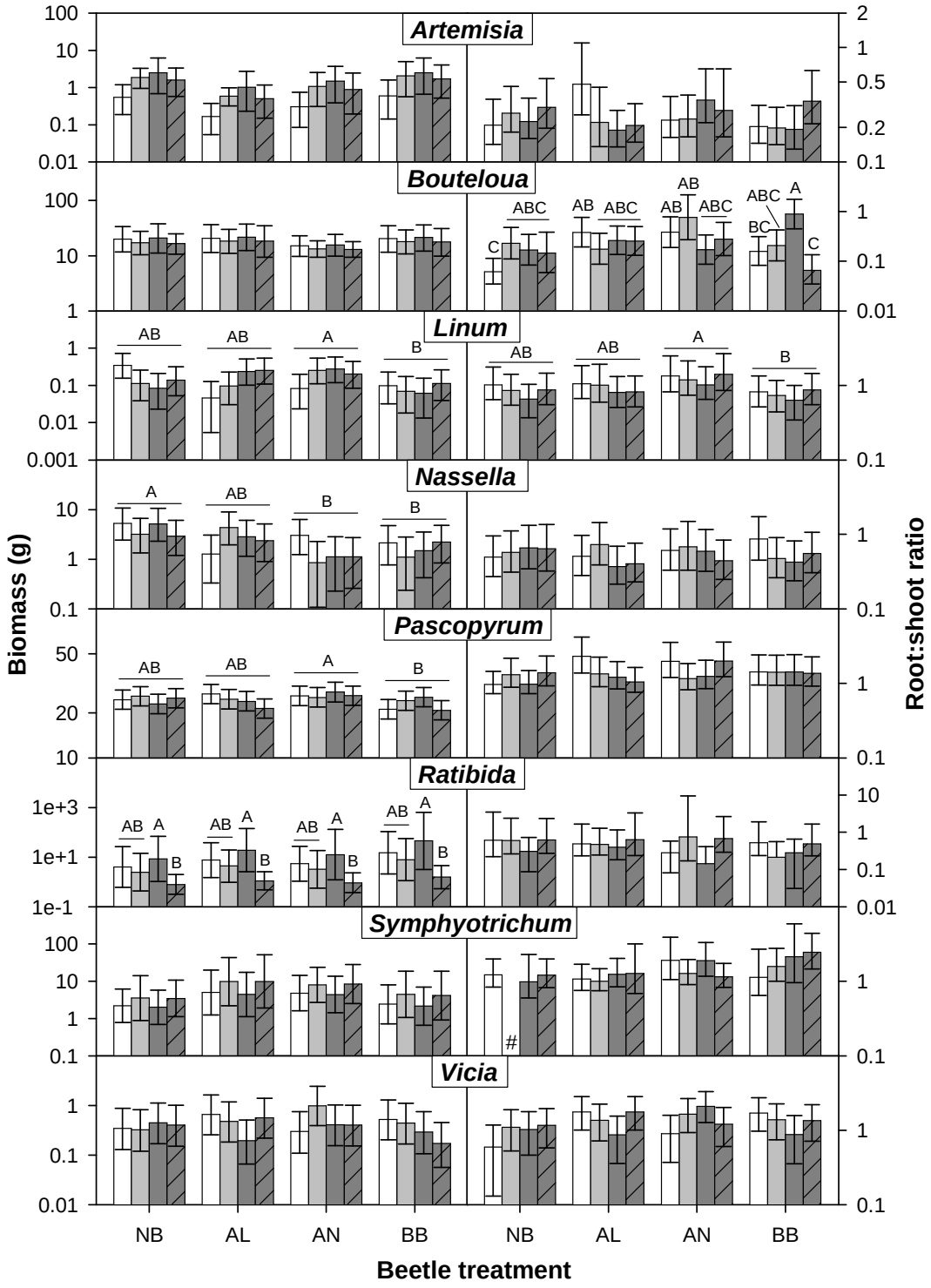


Figure 3-6. Plant biomass and root:shoot ratios by species for beetle and soil treatments.

Beetle treatments are NB = no beetles, AL = *A. lacertosa,* AN = *A. nigriscutis,* BB =

both beetles. Soil treatments are open bar = sterile soil, light gray = uninvaded, dark gray = invaded-native, dark gray with diagonal pattern = invaded-exotic. Biomass and root:shoot ratios are on the log scale. Bar heights are least square means and error bars are 95% confidence intervals. Values were back-transformed to be on scale of original data; varying confidence interval magnitudes among species and treatments are due partially to different data transformations prior to analysis. Significantly different estimates are indicated by different letters above bars. Non-estimable least square mean indicated by #.

Chapter 4:

Leafy spurge root exudates modify the effect of fungal pathogens on native plant germination and seedling growth

Plant-soil feedbacks can have strong beneficial or detrimental impacts on native plant communities. These associations can also be modified by invasive plants in ways that benefit the invader and harm native species. Native plant communities can have a slow recovery following control of leafy spurge (*Euphorbia esula*), an invasive non-native plant in the Great Plains. This plant may inhibit native plant recruitment via allelochemicals exuded from its roots, or through the accumulation of plant pathogens in soils adjacent to its roots. I investigated the singular and combined influence of leafy spurge root exudates and soilborne fungal plant pathogens on germination and seedling growth of plants native to the northern Great Plains. In a 15-day growth chamber germination assay, leafy spurge root exudates reduced the germination of 10 native plant species (*Artemisia frigida*, *Bouteloua gracilis*, *Echinacea angustifolia*, *Helianthus pauciflorus*, *Koeleria macrantha*, *Linum lewisii*, *Nassella viridula*, *Pascopyrum smithii*, *Symphyotrichum ericoides*, and *Vicia americana*), an effect which increased with increasing exudate concentration. In a 30-day factorial greenhouse experiment, seeds of five of these native species (*E. angustifolia*, *H. pauciflorus*, *K. macrantha*, *P. smithii*, and *V. americana*) were sown into soils inoculated with five different fungal pathogen

isolates (three *Rhizoctonia solani* isolates, and one isolate each of *Fusarium oxysporum* and *F. solani*) at two inoculum concentrations, and subjected to two leafy spurge root exudate concentrations. Germination rates were significantly increased by the combined presence of pathogens and root exudates when plant species were combined. Seedling biomass was significantly influenced by the treatments only for specific native plant-fungal isolate combinations. In some cases, biomass was increased only by increasing root exudate concentration. However, seedling biomass in other cases was different between the two inoculum concentrations, an effect which was frequently modified by root exudate presence. These findings indicate that there may be some temporary protection from pathogen attack via leafy spurge root exudates, which was opposite from what was expected under the pathogen accumulation scenario, but the root exudates alone appear to have allelopathic effects on native plant germination.

Introduction

Invasive exotic plants can impact native plant communities in well-documented ways, including via reductions in native plant growth, abundance, diversity, and fitness (Vilà et al. 2011). The mechanisms underlying these impacts have, in many cases, only recently come to light. One such mechanism involves plant-soil feedbacks, or interactions between the plant and the soil biotic communities that have reciprocal beneficial or deleterious effects on the plants (Bever 2003), including plant-pathogen interactions. Fungal, viral, or bacterial symbionts are transmitted within or between susceptible plant

host species, primarily via soil, water, air and insect vectors, and they can have negative, positive, or neutral effects on the reproduction, survival, and growth of the infected plant host. The direction and magnitude of these effects are variable, depending on the species involved and climatic conditions (Jarosz and Davelos 1995), and pathogens can be specialists or generalists with host ranges from a few to many plant species (Mitchell and Power 2006). Competition among plant species can be altered by plant-pathogen interactions by way of species' fitness differences, with the result being coexistence, competitive exclusion, or priority effects, where one species outcompetes another but the outcome depends on the initial starting conditions (Mordecai 2011).

Invasive plant success may be increased (Klironomos 2002, Callaway et al. 2004) or decreased (Turnbull et al. 2010) as a result of plant-pathogen interactions, but these dynamics have only recently been explored. Several competing hypotheses have attempted to explain the success of invasive plants in terms of their interactions with pathogens. The enemy release hypothesis (ERH), which has received much attention over the last decade, ascribes the success of invaders to significant reductions in pests and pathogens in their invaded as compared to their native ranges (Keane and Crawley 2002). Under this hypothesis, not only are the invader's specialist pathogens not present in the new habitat, but the invader is also not susceptible to local specialist pathogens due to lack of a recent coevolutionary history with the pathogens. The invasive plant therefore does not need to invest as much of its resources on inducible defense mechanisms (i.e., production of defense compounds as a response to pathogen attack) and thus has more resources to invest in competitive growth. Moreover, given enough time in the new

habitat, invasive plant defense traits may be lost if they do not offer substantial benefits to the plant, which may provide further fitness benefits to invasive species (Blossey and Notzold 1995, Leger and Rice 2003, Siemann and Rogers 2003). However, two additional, more recently proposed hypotheses provide alternative explanations for invasive plant success. The accumulation of local pathogens hypothesis (ALPH), which was first explored in simulation models by Eppinga et al. (2006), proposes that invader success can result from the invading species capacity to accumulate generalist pathogens. Under this scenario, the invasive plant can experience similar levels of negative plant-soil feedbacks in its invaded and native ranges, but the pathogens are more detrimental to the natives than to the invasive plant in the invasive range (Niemi et al. 2006). Support for the ALPH was found for *Chromolaena odorata* in India, where its root exudates increased the germination of soilborne fungal pathogens, leading to a decline in native plant growth (Mangla et al. 2008). Finally, the "novel weapons hypothesis" (NWH) suggests that invasive plants release chemicals that either have direct negative impacts on native plants, or that mediate plant-soil biota feedbacks in ways that are beneficial to invasives but not natives (Bais et al. 2003, Callaway and Ridenour 2004). In one example of the latter case, compounds released by the invasive plant may suppress plant pathogenic fungi in its rhizosphere, while native plant root exudates may not suppress pathogens. For example, *Solidago canadensis*, a destructive weed in China, produces root exudates that suppress the soilborne pathogens *Pythium ultimum* and *Rhizoctonia solani* more than root exudates from the co-occurring native plant *Kummerowia striata* (Zhang et al. 2009). While these hypotheses have been evaluated separately, they have only been

tested in very limited settings, and successful invasive plants may benefit from a combination of these mechanisms.

Leafy spurge (*Euphorbia esula* L.), a destructive invasive exotic in the Great Plains (USA), may benefit from several of these hypothesized mechanisms in its invaded range. There are few native species in North America, including large herbivores and insects, that consume *Euphorbia* (Scott and John 1993), which lends some support for the ERH. However, *Euphorbia* is susceptible to a number of indigenous fungal pathogens in its invaded range (Caesar 1994, 1996), so its escape from enemies is not complete. Several biocontrol insects from its native range have been introduced to control *Euphorbia* infestations with varying success (Lym 2005). However, use of these insects may lead to an increase in populations of soilborne fungal pathogens which may have negative consequences for both *Euphorbia* and native species. For example, *Euphorbia* spp. subjected to root-feeding insects in North America and Europe had larger rhizosphere soil populations of pathogenic *Fusarium* spp. compared to *Euphorbia* rhizosphere soils in the absence of insect attack (Caesar 2003). The capacity for herbivore-damaged *Euphorbia* to increase pathogen populations suggests some support for the ALPH. There is also evidence that *Euphorbia* can produce allelochemicals that are detrimental to neighboring plants, lending support to the NWH. Seedling root exudates and extractions from ground roots of *Euphorbia* inhibit the growth of *Arabidopsis* in laboratory settings (Qin et al. 2006), and leachates from *Euphorbia* litter reduce root lengths of two grasses (*Pascopyrum smithii* and *Pseudoroegneria spicata*) native to the Great Plains (Olson and Wallander 2002). However, the potential effects of *Euphorbia*

exudates on plant pathogens are unknown. Furthermore, there are no studies that examine the combined effects of pathogen accumulation and allelopathy, or how these mechanisms may contribute to the success of *Euphorbia* in North America.

My objective was to determine if *Euphorbia* root exudates and fungal pathogens work synergistically to negatively impact the establishment of native plants in the northern Great Plains where *Euphorbia* is successful as an invader, and to evaluate if the NWH or ALPH play a role in this system. To achieve these objectives, I investigated the effects of *Euphorbia* root exudates, soilborne plant pathogenic fungi, and their interactions, on seed germination and seedling growth of plants native to the Great Plains. If *Euphorbia* root exudates alone impact native plants, the NWH would be supported. If the combination of root exudates and pathogens produce strong negative effects on native plants, this would lend support to the ALPH. My findings will add to the sparse but growing body of knowledge regarding invasive plant interactions with pathogens, and the mechanisms behind invasive plant success in naïve habitats. It will also help guide *Euphorbia* management: if pathogen accumulation occurs in *Euphorbia*-occupied soils, management practices that reduce soil pathogen populations may improve native plant recruitment following *Euphorbia* control.

Methods

In growth chamber experiments, I investigated the impact of *Euphorbia* root exudates on seed germination of 12 plants native to northern Great Plains mixed grass

prairies. These were: *Artemisia frigida* Willd., *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths, *Echinacea angustifolia* DC., *Galium boreale* L., *Helianthus pauciflorus* Nutt., *Koeleria macrantha* (Ledeb.) Schult., *Linum lewisii* Pursh, *Nassella viridula* (Trin.) Barkworth, *Pascopyrum smithii* (Rydb.) Á. Löve, *Ratibida columnifera* (Nutt.) Woot. & Standl., *Symphyotrichum ericoides* (L.) G.L. Nesom, and *Vicia americana* Muhl. ex Willd (hereafter, these plants are referred to by their genera). These seeds were acquired from the Bismarck Plant Materials Center (Natural Resources Conservation Service, U.S. Department of Agriculture; Bismarck, ND, USA), Wind River Seed (Manderson, WY, USA), and from Prairie Moon Nursery (Winona, MN, USA).

In a glasshouse experiment, I conducted pathogenicity assays on these 12 native plant species and *Euphorbia* for 15 different plant pathogenic fungi. Pathogen isolates included *Rhizoctonia solani*, *Fusarium oxysporum*, and *F. solani*, which were all collected from soils and plants in the northern Great Plains (Table 4-1).

Lastly, I conducted a glasshouse growth assay using five of the above native plant species and five pathogen isolates to determine the impacts of *Euphorbia* root exudates, pathogens, and exudate-pathogen combinations on native seed germination and seedling growth.

Preparation of *Euphorbia* exudates

Euphorbia plants were reared in a glasshouse from seeds collected at the South Unit of Theodore Roosevelt National Park (North Dakota, USA) from 2006-2008. I used two cohorts of *Euphorbia* in producing root exudates to examine the effects of growth stage on native seed germination: mature (>2 years old) and immature (3 months old).

Root exudate quantity and composition can vary with plant growth stage (Granato et al. 1983, Aulakh et al. 2001), therefore it is important to know if exudates from immature and mature *Euphorbia* have similar influences on native plant seed germination. Mature plants were reared for 2 years in 20 cm pots in potting soil medium (Sunshine MVP, Sun Gro Horticulture Canada CM Ltd.) in a glasshouse at 20-35 °C with 12 hours supplemental illumination from September through March. All plants were watered three times weekly and fertilized with 50 ml full-strength Hoagland's solution (Hoagland and Arnon 1950) per pot per month. Two months prior to exudate collection, I transplanted six mature plants into each of 10, 16-liter polypropylene containers filled with perlite (Sunshine MVP). Seeds for the immature cohort were sown in 28 × 54 × 6 cm germination trays with potting soil medium (Sunshine MVP) three months prior to exudate collection. Two weeks after germination, I transplanted 20 seedlings into each of 10, six-liter polypropylene containers filled with perlite.

The immature and mature *Euphorbia* in perlite were grown in a glasshouse for two months with 12 hours supplemental illumination per day at 24-32 °C. All *Euphorbia* containers were watered three times weekly and fertilized every two weeks with 50 (immature plants) or 100 (mature plants) ml of Hoagland's solution per container. Two control containers containing only perlite were established for each container size. These containers were not fertilized, but they were watered using the same schedule as *Euphorbia*-planted container.

Forty-eight hours prior to exudate collection, I rinsed all of the *Euphorbia* containers twice with reverse osmosis (RO) water (0.75 and 2 liters per rinse per

container for immature and mature containers, respectively) to remove residual fertilizer. The rinsing was done by applying the RO water to a container and drawing off the water using an electric water pump and hose fitted with a 200 mesh (0.075 mm) plastic filter. I used RO water as it is free of ions which may alter seed germination when concentrated. *Euphorbia* containers were filled with RO water for 24 hours for exudate collection (Steinkellner et al. 2008, Weidenhamer et al. 2009). *Euphorbia* roots were soaked in different quantities of water between immature and mature plants (50 and 415 ml per plant, respectively) to compensate for greater adult biomass per plant. After 24 h, I removed the exudate solution by pumping as described above, and combined the exudate solution from all containers in each cohort. The solution was thoroughly mixed, divided into 240, 120-ml polypropylene containers of 100 ml each, frozen, and lyophilized for 7 days (Specimen freeze dryer Model 24DX48, Virtis Company, Gardiner, NY, USA). Lyophilized exudates were stored in the 120-ml containers with airtight lids at 2 °C for 3 to 35 days until used in the germination assay (described below).

To permit scaling of the exudate concentrations relative to *Euphorbia* biomass, I oven dried all of the plants following the exudate collection at 60°C for four days and weighed them.

Evaluating effects of exudates on native seed germination

I tested the effects of five *Euphorbia* exudate concentrations, from both immature and mature plants, on germination of each of the 12 native plant species. Concentrations were: 4×, 2×, 1×, 0.5×, and 0.25×, where × was the original concentration of the exudate solution. Two controls were also used for each species and *Euphorbia* cohort: perlite and

water. The perlite control used a 1× concentration of the lyophilized product from the perlite controls, and the water control used only DI water. All solutions were prepared immediately before initiation of the experiment.

One hundred seeds of each plant species were placed on germination paper (BB3.5 white blotter circles, Hoffman Manufacturing, Inc., Jefferson, OR, USA) inside 9 cm plastic petri dishes. Each dish was an experimental unit, and the experiment was replicated using 4 dishes per species per concentration per cohort (mature vs. immature *Euphorbia*). A one-time application of 7 ml of exudate or control solution occurred at the beginning of the experiment (day 0). This was enough liquid to saturate the germination paper and to provide a small liquid meniscus connection with the seeds, but not enough for standing liquid to be present. The exudate solutions were not sterilized prior to use to avoid inactivation of exudate compounds.

Following exudate application, lids were placed on the dishes, the dishes were arranged randomly on empty germination flats with clear lids, and the germination flats were arranged randomly in a growth chamber (LT-105HID Controlled Environment Chamber, Percival Scientific, Inc., Perry, IA, USA). The chamber was programmed for eight hours at 24°C and 16 hours at 10°C, with one hour of 66 μmol m^{-2} sec^{-1} illumination (24 mol m^{-2} day^{-1}) midway through the warm cycle (Baskin and Baskin 1998). I applied additional DI water to the dishes as needed to maintain adequate moisture for germination.

The number of germinating seeds within each petri dish was determined at five, 10, and 15 days after application of the exudate treatments, and the dish locations were re-randomized after each monitoring period.

I compared the *Euphorbia* root exudate concentrations in my study with theoretical field concentrations by examining both in terms of the number of *Euphorbia* plants (and the mass of *Euphorbia*) per m^2. In my study, I used the following formula:

$$\frac{\text{petri dish}}{0.00581\ \text{m}^2} \times \frac{7\ \text{ml exudate solution}}{\text{petri dish}} \times \frac{\text{Total \# } \textit{Euphorbia} \text{ plants}}{\text{Total exudate solution volume (ml)}}$$

This formula yielded the number of *Euphorbia* plants per m^2 at the 1× concentration. To obtain the ratio at each exudate concentration, this number was multiplied by its given concentration value (4, 2, 0.5, or 0.25). The ratio of *Euphorbia* mass per m^2 was calculated by replacing the total # *Euphorbia* plants in the formula above with the total *Euphorbia* mass (g). These conversions yielded 0.756 (for the 0.25× concentration) to 12.1 (for the 4× concentration) mature *Euphorbia* plants per m^2, while the ratios for immature *Euphorbia* ranged from 6.56 (0.25×) to 105 (4×). Ratios of g *Euphorbia* per m^2 ranged from 9.3 (0.25×) to 149 (4×) for mature *Euphorbia*, and from 2.36 (0.25×) to 37.8 (4×) for immature *Euphorbia*. These values are compared with field *Euphorbia* densities from published studies in the Discussion.

Evaluating effects of pathogens on native plants

Seedlings of *Euphorbia* and native plants were inoculated with pathogenic fungal isolates to determine the sensitivity of a diverse collection of native plants to a broad set of native fungal pathogens, and to select plant species and fungal isolates for use in

subsequent experiments (see below). All 12 native plant species used in the *Euphorbia* exudate germination experiment were evaluated for infection. Seedlings of each plant species were first grown in potting soil medium (Sunshine MVP, Sun Gro Horticulture Canada CM Ltd.) in a glasshouse at 24-32 °C with 12 hours supplemental illumination per day. All plants were watered three times weekly and grown for two weeks prior to the experiment.

Fifteen different fungal pathogen isolates (including multiple isolates of *Rhizoctonia solani*, *Fusarium oxysporum*, and *F. solani*) were used (Table 4-1). These fungi were isolated from soils and plants in the northern Great Plains (Carol Windels and Jim Kurle, personal communication) and are common pathogens found on many plant hosts worldwide. All of the *R. solani* isolates belonged to anastomosis group 4 (AG-4), which has previously been shown to be pathogenic on *Euphorbia* (Caesar et al. 1993, Caesar 1994). All isolates were cultured on full-strength potato dextrose agar (PDA) (Singleton et al. 1992) for 7-10 days at 24 °C prior to application to the plants. To prepare inoculum for plant application, I blended one PDA plate for a particular isolate with 100 ml sterilized, deionized water in an Oster blender (Sunbeam Products, Inc., Rye, NJ, USA) for 60 seconds.

Two-week old plant seedlings were inoculated and transplanted into pots for a one-month growth period. Seedlings were removed from the germination trays, their roots were cleaned of soil, ~0.5 cm was removed from the root tip with a razor, and the roots were soaked in an inoculum slurry for five minutes. Each seedling was then transplanted into individual pots (3.8 cm diameter conetainers) with twice-autoclaved

vermiculite medium (Sun Gro Horticulture Canada CM Ltd.). Each plant species was inoculated individually with each of the fungal isolates, with 10 pots per plant species-fungal isolate combination, and 10 non-inoculated control pots for each plant species. All plants were grown in a glasshouse using the same growing conditions as for rearing the seedlings. Plants were fertilized using 20 ml full-strength Hoagland's solution per pot two weeks after initiation of the experiment.

After one month, each plant was harvested and root systems were assessed for visible signs of pathogen infection or damage. I used a damage rating system modified from Caesar (2003) where: 0, no damage; 1, dead leaves (no wilting or chlorosis); 2, wilting; 3, chlorosis; 4, wilting and chlorosis; 5, moderate wilting, chlorosis and some tissue death; 6, persistent wilting, chlorosis, and death of one or more stems; 7, extensive wilting, chlorosis and tissue death or top dieback with regrowth; 8, mostly dead; 9, plant dead with no regrowth.

Based on these results, five pathogen isolates that elicited a range of damage ratings from the plants, and five plant species that showed infection signs from at least one of the five isolates were selected for further study. I selected both forbs and grasses, including the grass *Pascopyrum*, which is one of the most common plants encountered at Theodore Roosevelt National Park, North Dakota (see Chapter 2).

The fungal isolates selected were *Rhizoctonia solani* (isolates 87-18-2, 14-5-3, and E-0-20; hereafter *R. solani* 1, 2, and 3, respectively), *Fusarium oxysporum* f.sp. *medicaginis* (isolate 3/F3(#3)) and *F. solani* (isolate 910-4). The plant species were *Echinacea*, *Helianthus*, *Koeleria*, *Pascopyrum*, and *Vicia*.

Evaluating effects of pathogens and *Euphorbia* exudates on native plant germination and growth

The influences of soilborne fungal pathogens, leafy spurge root exudates, and their interactions, on native plant germination and growth were evaluated in glasshouse trials. Soils were inoculated with fungal pathogens, with or without *Euphorbia* root exudates, and five native plant species were grown in these soils in a factorial design. Seed germination was quantified and seedling biomass was determined after 30 days of growth.

Inoculum preparation

Inoculum suspensions were prepared and applied to the experimental soils in two densities for each of the fungal isolates. Suspensions were prepared so that soils in this experiment contained approximately 10 or 1000 cfu per gram soil for *R. solani* (low and high densities, respectively) (Caesar 1994, Aziz et al. 1997), and approximately 1000 or 1 $\times 10^5$ cfu per gram soil for *Fusarium* spp. (low and high densities, respectively) (Rodríguez-Molina et al. 2000, Ghini and Morandi 2006, Hao et al. 2009) after application. To prepare these inoculum suspensions, cultures of each isolate were first grown on half-strength potato dextrose agar (PDA) plates for 7 days at 23° C, and cfu per ml of PDA for each isolate was determined by serial dilution plating. High density inoculum suspensions for each isolate were prepared by blending the appropriate volume of inoculated PDA (ranging from 12 ml to 25 ml) with 4.6 liters of sterilized deionized water for 2 minutes in an Oster blender (Sunbeam Products, Inc., Rye, NJ, USA). The low density suspension was prepared by mixing a 45 ml subsample of high density

suspension with 4555 ml of sterile DI water. Twenty ml of the suspension volume was added per pot for both densities. I added the same quantity of suspension for the control, but used a non-inoculated PDA plate blended with 4.6 liters of sterilized deionized water to prepare the suspension.

Exudate preparation and experimental design

I prepared *Euphorbia* root exudates at two concentrations using mature *Euphorbia* grown as described previously for the exudate treatments, except that 10 mature *Euphorbia* were transplanted into 20 separate six liter containers. These plants were grown for two weeks under the same conditions as described previously. The same process of exudate collection (RO water rinsing, followed by 24 h root soaking) was subsequently used, with one liter of exudate solution collected from each container, yielding 20 liters of high-concentration exudate solution. The low concentration exudate was prepared by diluting one liter of high concentration exudate solution with 15 liters of deionized water (16 L total volume). I added 30 mL per pot for each concentration; for non-exudate controls, 30 mL of deionized water was used.

Potting soil (Sunshine MVP) was autoclaved on two consecutive days at 121° C for 2 hours prior to planting. Pots (3.8 cm diameter conetainers; Stuewe and Sons, Inc., Tangent, Oregon, USA) were filled with approximately 100 g of the autoclaved soil and four seeds of a single species (*Echinacea*, *Helianthus*, *Koeleria*, *Pascopyrum*, and *Vicia*) were sown in each pot. Each plant species/treatment combination was replicated in 10 pots, for a total of 1650 pots. I applied pathogen and exudate treatments to the pots at the time of planting. Seeds were covered with a one cm layer of twice-autoclaved

vermiculite after inoculum and exudate application to maintain soil moisture and to minimize spread of fungal pathogen inoculum among pots. At 14 days, I removed seedlings from pots to standardize plant density to one plant per pot. Pots were watered as needed to keep soils moist for seed germination and plant growth. The glasshouse was maintained at 25-30° C with 12 hours of daylight (7 a.m. – 7 p.m.) supplemented by artificial light during natural twilight. Plants were harvested after 30 days, presence or absence of pathogen infection symptoms (chlorosis, wilting, and necrosis) was determined for every plant, and plants were dried for three days at 60° C to determine above- and belowground dry biomass, and root:shoot ratios.

Analyses

Treatment effects on seed germination and plant biomass were analyzed using generalized linear mixed models and linear mixed models, respectively, using the GLIMMIX and MIXED procedures in SAS/STAT v. 9.3 (SAS Institute Inc., Cary, NC, 1989-2011). In the root exudate germination, and the pathogen-exudate interaction experiments, germination tray and conetainer rack, respectively, were used as random blocking effects. The germination count data were either Poisson or negative binomially distributed, and the log-link function was used for both distributions in the generalized models. Square root and Anscombe (Anscombe 1948) transformations were used in some instances of the germination data to equalize variances prior to analysis. Biomass data were normally or lognormally distributed; Johnson Sl or Su transformations (Johnson 1949, Chou et al. 1998) were used in a few cases to equalize variances prior to analysis. Tukey's HSD tests were used to determine differences among treatment combinations in

multiple comparisons. The Satterthwaite approximation method was used to calculate denominator degrees of freedom to account for different sample sizes among treatments in the second experiment (not all pots within given treatment combinations had seeds germinate or plants establish).

The pathogen damage ratings in the pathogen assay did not fit any known distribution; therefore I used nonparametric Wilcoxon chi-square tests and multiple pairwise comparisons to evaluate pathogen treatment effects on the native plants.

The values used in figures are estimates and 95% confidence intervals that were back-transformed into the scale of the original data. *P*-values less than 0.05 were deemed as significant in all analyses.

Results

Effects of exudates on native seed germination

Euphorbia root exudates reduced the germination of the majority (10 of 11) of the native plant species in both experiments, and half of the affected species showed persistent germination effects that lasted through day 15 of the experiment (Table 4-2 and Table 4-3). The 12th species, *Galium*, only had a few seeds germinate and was excluded from the analyses. All of the negative effects occurred at high exudate concentrations compared to low or no exudates, and *Bouteloua* and *Vicia* also exhibited significant negative effects at the low concentration (0.5×) compared to no exudates (Appendix 6). Some intermediate exudate concentrations also significantly enhanced germination compared to the controls, as exhibited on at least one day by *Echinacea*, *Nassella* and

Pascopyrum exposed to mature *Euphorbia* exudates (Appendix 6). There were also stronger negative effects from mature than immature *Euphorbia* exudates on germination rates, where germination rates were lower for the same concentration level when exposed to mature than immature exudates, for all species except *Helianthus* (Appendix 6).

Three patterns of exudate effects over time were apparent: persistent (significant treatment effects through days 10 and 15), temporary (significant effects on days five and/or 10, but not 15), and no effects (Figure 4-1 and Table 4-3). Four species (*Artemisia*, *Echinacea*, *Symphyotrichum*, and *Vicia*) showed persistent negative effects from *Euphorbia* exudates, so that germination was substantially delayed. A majority of the species, meanwhile, showed at least temporary negative effects from *Euphorbia* exudates, whereby germination was delayed by exposure to exudates, though seeds germinated eventually. Only one species (*Ratibida*) was not significantly affected by exudates at any date (Figure 4-1 A and B, respectively). Exudates from mature *Euphorbia* plants had more persistent impacts than exudates from immature plants (Table 4-3). Specifically, among the species impacted, none had persistent impacts from exudates of immature *Euphorbia*, while all four species with persistent impacts were due to exudates of mature *Euphorbia*. Four species (*Nassella*, *Pascopyrum*, *Ratibida* and *Vicia*) were not influenced by exudates from immature *Euphorbia*.

Effects of pathogens on native plants

The 15 fungal isolates varied in their impacts on *Euphorbia* and the 12 native plant species, with some isolates having a strong negative impact on multiple plant species, while other isolates had only moderately negative effects on a few plant species

(Figure 4-2). Combining the damage ratings across all plant species, *R. solani* 1 had stronger negative impacts on plants than two other *R. solani* (7 and 9), and *F. oxysporum* 3 (Appendix 7 A). With the exception of *Symphyotrichum*, all plant species varied significantly in their sensitivity to the 15 isolates, or their sensitivity to the isolates was only marginally non-significant, including *Euphorbia* ($P = 0.0627$) (Appendix 8). This indicates significant specificity among these pathogen isolates. There was also a large range of damage exhibited by the plant species when all pathogen isolates were pooled (Appendix 7 B). *Euphorbia* exhibited a relatively high damage rating, while all of the grasses except for *Nassella* exhibited low ratings.

Plant species with higher damage ratings than the control for a large number of fungal isolates were selected for further study, including *Echinacea, Helianthus, Koeleria* and *Vicia*. In addition, *Pascopyrum* was selected due to its status as a dominant, widespread grass in the Great Plains. Fungal isolates that had high damage ratings on the most plant species were also selected for further study, including *R. solani* 1, 2, 3, and *F. solani*. The fifth isolate selected for further study, *F. oxysporum* 3 (hereafter, *F. oxysporum*), was selected because it is a common soilborne pathogen, and it had the strongest (but non-significant) negative effects among the *F. oxysporum*. Pairwise comparisons of damage to the selected plant species among the selected fungal isolates are summarized in Appendix 9.

Effects of pathogens and *Euphorbia* exudates on native plant germination and growth

In the absence of *Euphorbia* root exudates, pathogens tended to decrease native plant germination with increasing inoculum density (Figure 4-3). There were decreased plant germination rates in high compared to low inoculum densities for *R. solani* 1 (t_{266} = -27.9, $P < 0.001$), *R. solani* 2 (t_{266} = -6.12, $P < 0.001$), and *F. solani* (t_{266} = -4.14, $P < 0.001$). However, germination rates were greater with high compared to low inoculum densities for *R. solani* 3 (t_{266} = 14.1, $P < 0.001$).

When fungal pathogens were absent, plant germination rates decreased with increasing exudate concentration (Figure 4-3). There were lower germination rates of seeds exposed to high compared to low concentration exudates and no exudates (t_{119} = -∞, $P < 0.001$, and t_{119} = 6.79, $P < 0.001$, respectively), and lower germination rates of seeds exposed to low concentration exudates than no exudates (t_{119} = 6.15, $P < 0.001$).

In contrast to the absence of pathogens, there were *increases* in native plant germination when seeds were exposed to *Euphorbia* exudates and pathogens (Figure 4-3). For example, germination rates were greater when high exudate concentrations were present compared to no exudates for both low and high *R. solani* 3 inoculum densities (t_{266} = -40.8, $P < 0.001$ and t_{266} = -11.5, $P < 0.001$, respectively) and for the low *F. oxysporum* density (t_{266} = -16.8, $P < 0.001$). Similar non-significant trends include both pathogen densities of *R. solani* 2 and *F. solani*. Germination rates were also greater when seeds were exposed to high compared to low concentration exudates except for the non-inoculated control. These differences were significant for seeds exposed to *R. solani* 1

low density inoculum ($t_{266} = 4.08$, $P < 0.001$), and low and high density inocula for *R. solani* 3 ($t_{266} = \infty$, $P < 0.001$ for both) and *F. oxysporum* ($t_{266} = \infty$, $P < 0.001$ for both). Furthermore, there were no cases (within a given inoculum density) where germination rates were significantly higher in the absence of exudates than when high exudate concentrations were applied (Figure 4-3 and Appendix 10).

There were also significant interactions of exudates and pathogens in two cases, where the effect of pathogens on germination depended on *Euphorbia* exudates (Table 4-4 and Figure 4-3). For *R. solani* 3, more seeds germinated in high than low density inoculum in the absence of *Euphorbia* exudates, and in low concentration exudates (Figure 4-3). However, seed germination was greatest in high concentration exudates and did not vary between inoculum densities. For *F. oxysporum*, more native plant seeds germinated in high versus low inoculum density in the absence of exudates, but fewer seeds germinated in high versus low inoculum density for both exudate concentrations (Figure 4-3). Additionally, germination in the low *F. oxysporum* inoculum density was greatest for the high exudate concentration, but there was no difference in germination between high exudate concentration and no exudates for the high inoculum density.

Despite significant effects of exudates and pathogens on germination after combining all plant hosts, only one plant species exhibited significant reductions in germination in response to a specific pathogen when considered individually. Specifically, *Echinacea* germination, when exposed to *R. solani* 1, was lower in high compared to low inoculum densities ($t_{54} = -4.57$, $P < 0.001$) (Appendix 11).

Native plant biomass was generally unaffected by pathogens in the absence of *Euphorbia* root exudates, or by exudates in the absence of pathogens, but there were some exceptions (Figure 4-4). In the absence of exudates, *Echinacea* biomass increased with increasing *F. oxysporum* inoculum density ($t_{36.5}$ = -3.11, P = 0.039), while the opposite was found for *Vicia* biomass grown with *R. solani* 1 present ($t_{29.2}$ = -3.08, P = 0.046). Meanwhile, in the absence of pathogens, *Helianthus* biomass was greater with low than with high concentration exudates or with no exudates ($t_5 = -6 \times 10^4$, P <0.001 and t_5 = -5920, P <0.001, respectively), and greater with high concentration exudates than with no exudates ($t_5 = -5.4 \times 10^4$, P <0.001).

Plant biomass was consistently larger when exposed to *Euphorbia* root exudates than in their absence, but mostly when plants were also exposed to pathogens (Figure 4-4). For example, plant biomass was greater in low concentration exudates than in the absence of exudates for *Echinacea* with *R. solani* 3 (t_{46} = -2.48, P = 0.044), and for *Koeleria* with *R. solani* 2 and *F. solani* (t_{43} = -2.74, P = 0.024 and $t_{33.8}$ = -2.99, P = 0.014, respectively). Likewise, plant biomass was greater under high concentration exudates than with no exudates for *Echinacea* and *Vicia* exposed to *R. solani* 2 (t_{27} = -2.48, P = 0.05 and t_{44} = -2.3, P = 0.027, respectively). A similar pattern was found for *Echinacea* exposed only to low density *F. oxysporum* inocula, whose biomass was greater in high exudate concentration than in the absence of exudates ($t_{34.7}$ = -3.15, P = 0.036). This pattern was reversed, however, in one case. The biomass of *Echinacea* was lower in high compared to low exudate concentrations in low *R. solani* 1 inoculum densities ($t_{22.1}$ = -3.72, P = 0.013).

In two out of the four significant interactions of pathogen and exudate treatments, individual plant species biomass was larger when grown in higher concentration exudates, further supporting the previously described biomass enhancement via exudates (Figure 4-4 and Appendix 13). *Koeleria* biomass was greater with high concentration *Euphorbia* exudates than in their absence when exposed to low density inoculum of *R. solani* 1 ($F_{2,43}$ = 3.53, P = 0.038), and *Vicia* biomass was greater in high than in low exudate concentration when exposed to low density *F. oxysporum* inoculum ($t_{49.4}$ = 3.11, P = 0.034). However, there was not a consistent pattern for the two cases where biomass was dependent upon inoculum density. *Echinacea* biomass was greater in low than high *R. solani* 1 inoculum density, but only with low concentration exudates ($t_{20.8}$ = -3.42, P = 0.027). Meanwhile, *Echinacea* biomass was greater in high than in low *F. oxysporum* inoculum density only in the absence of exudates ($t_{36.5}$ = -3.11, P = 0.039).

Plant root:shoot ratios were positively affected by pathogen inoculum density when *Euphorbia* root exudates were present. For example, root:shoot ratios (usually smaller in plants that have adequate access to soil nutrients or moisture compared to plants that lack moisture or nutrient access) were significantly smaller in high than in low inoculum densities in four cases (Appendix 13). These cases include *Echinacea* and *Vicia* when exposed to *R. solani* 1 ($t_{10.2}$ = -2.86, P = 0.017 and $t_{30.8}$ = -3.34, P = 0.002, respectively), and *Koeleria* when exposed to *R. solani* 1 and 2 (t_{37} = -2.09, P = 0.044 and t_{47} = -2.28, P = 0.027, respectively). However, there were inconsistent effects of exudate concentrations on root:shoot ratios. *Pascopyrum* root:shoot ratios were larger in control than in low exudates when exposed to *R. solani* 2 (t_{53} = 2.94, P = 0.013), while *Vicia*

root:shoot ratios exposed to *R. solani* 1 were larger in high than in low exudates (t_{31} = 2.72, P = 0.028).

Discussion

Euphorbia root exudates reduced the germination of the majority of the native plant species in my study in both experiments, which lends support to the novel weapons hypothesis (NWH). This is consistent with a 14-year study on native plant recovery following the control of *Euphorbia* by flea beetles in Montana, where four species (*Artemisia*, *Bouteloua*, *Pascopyrum*, and *Vicia*) did not increase in frequency over time, and *Koeleria* displayed idiosyncratic effects (increasing, decreasing, or no change) in different study plots (Lesica and Hanna 2009). In my study, germination of three of these species (*Artemisia*, *Bouteloua*, and *Koeleria*) were reduced temporarily by exudates, while reductions of the other two (*Pascopyrum* and *Vicia*) were persistent. Both temporary and persistent effects may have important long-term impacts on native plant recruitment, especially if the timing of germination is important for seedling survival, or if interspecific competition leads to reduced seedling recruitment. These kinds of priority effects can be especially beneficial to invasive plants that begin growing in the spring before natives (Dickson et al. 2012). *Euphorbia*, for example, is among the first plants to emerge in the spring (Messersmith et al. 1985), so it may be a successful competitor against the natives on two fronts: through its early emergence, and through exudate-linked native germination delay.

Evidence for allelopathic effects from *Euphoria* was first evaluated 40 years ago, when extracts from dead roots were found to inhibit the germination of *Euphorbia* seeds and seeds from three other exotic species, crested wheatgrass (*Agropyron cristatum*), smooth brome (*Bromus inermis*) and charlock mustard (*Sinapis arvensis*) (Selleck 1972). In subsequent research, the germination of the invasive spotted knapweed (*Centaurea maculosa*) and the native bluebunch wheatgrass (*Pseudoroegneria spicata)* were lower when sown in soils occupied by *Euphorbia* compared to non-occupied soils (Olson and Wallander 2002). Extracts from living *Euphorbia* roots and roots harvested from field specimens also reduced *Arabidopsis thaliana* biomass, an effect which was enhanced at higher extract concentrations (Qin et al. 2006). The living extract solution in the Qin et al. study was created by soaking six-week-old *Euphorbia* plant roots for 3 days, which resulted in significant negative effects at 0.0625 and 0.125 *Euphorbia* plants per ml. The former concentration is slightly less than my 4× immature *Euphorbia* exudate concentration (0.087 *Euphorbia* per ml), a level at which I also saw significant negative effects (Appendix 6). Despite the assay species being different, measureable negative effects were observed at very similar concentrations in both studies.

The stronger negative impact from mature than immature *Euphorbia* exudates on native seed germination indicates that there may be qualitative differences in exudates that are linked to *Euphorbia* growth stages (Table 4-3). Changes in root exudates over time have been observed in other species. For example, the proportion of non-polar compounds in soybean root exudates increased with plant age (Granato et al. 1983). If similar qualitative changes occur in *Euphorbia* exudates, compounds that inhibit

germination may increase with plant age and produce the effects I observed. While quantitative differences in exudates released by mature and immature *Euphorbia* may also be important, this was not measured in my study.

Euphorbia field densities can range from 12.1 to 27.1 mature stems and 7.9–15.7 seedlings m^{-2} at Theodore Roosevelt National Park (TRNP), North Dakota (Larson and Grace 2004), 1–104 stems m^{-2} in North Dakota grasslands (Richardson et al. 2008), and ~75–225 stems m^{-2} in Montana grasslands (Lesica and Hanna 2004). My density of immature *Euphorbia* (6.55–105 per m^2) was well within these ranges, but my 4× treatment exceeded TRNP field seedling densities by a factor of 6.6. However, the densities of mature *Euphorbia* in my study (0.756–12.1 per m^2) were on the lower range of field densities, which were up to ~20 times my 4× treatment. *Euphorbia* biomass in the field may also exceed the density used in my experiments. Above-ground dry *Euphorbia* biomass ranged from 2.9–49.7 g/m^2 in Idaho riparian areas (Progar et al. 2010), 41.6–75.2 g/m^2 in various vegetation types at TRNP (Larson and Grace 2004), and ~45–250 g/m^2 in Montana rangelands (Rinella and Hileman 2009). My mature *Euphorbia* treatments (9.3–149 g *Euphorbia* per m^2) were well within the range of field populations, but they may be up to 1.67 times my 4× treatment (for Montana rangelands). However, these field *Euphorbia* biomass estimates were above-ground only, while ours was total plant biomass, meaning that field mass per area could be even higher if root mass were included.

The *Euphorbia* root exudate concentrations I used appear to be well within realistic field concentrations, but there are three important caveats to this assertion. First,

Euphorbia root exudates are likely exuded into a larger volume of soil than the volume occupied by the majority of the seed bank. Most seeds in Great Plains mixed grassland seed banks were found within the top 5 cm of soil (Perez et al. 1998). However, a majority of *Euphorbia* roots (56.2%) were found within the top 15 cm of soil in Saskatchewan, Canada prairies (Coupland and Alex 1954), and the roots can penetrate up to 4.8 meters deep (Bakke 1936). Therefore, the exudates that seed banks are exposed to may be only a fraction of the total exuded by *Euphorbia* plants in the field. Second, soil microbes use root exudates as a carbon source (Curl and Truelove 1986), and the potential allelopathic activity of root exudates can be modified by soil organisms (Inderjit 2001, Kaur et al. 2009), neither of which were accounted for in my study. Lastly, however, field *Euphorbia* densities can greatly exceed those used in my research, as explained above. This may partially mitigate the prior two caveats, but additional research is required to determine if seeds in the seed bank are exposed to *Euphorbia* root exudate concentrations similar to those used in my study, and if reductions in native seed germination occur in response to exudates in field settings.

Most studies of invasive plant allelopathic effects on native plant germination have used non-living invasive plant material, which is appropriate in contexts where dead and decaying plant tissues have an impact, including the influence of invasive plant litter on native communities (Sheley et al. 2009, Eppinga et al. 2011). However, this approach is not appropriate to assess the effects of living invasive plants on native plant communities, and studies using only dead tissues may have limited application in

ongoing plant invasions. My study, along with others that use exudates from living plants, provides a way of addressing these concerns.

All of the plant species in my study varied with respect to their susceptibility to the 15 fungal isolates, but *Euphorbia* exhibited a larger amount of damage than most of the native species, indicating a high susceptibility to fungal pathogen infection (Figure 4-2 and Appendix 7 B). Other research also found multiple *Rhizoctonia* and *Fusarium* spp. from the Great Plains that cause significant damage, and death, to *Euphorbia* (Caesar 1994, 1996, Caesar et al. 1999). In my study, four native plant species (*Helianthus*, *Linum*, *Nassella* and *Vicia*) exhibited damage equal to or greater than that shown by *Euphorbia*. In previous research on 11 *Fusarium* spp. strains from the Great Plains that are pathogenic to *Euphorbia*, the dry weight of three native grass species were also significantly reduced by some of these strains: Idaho fescue (*Festuca idahoensis*), big bluestem (*Andropogon gerardi*), and big bluegrass (*Poa ampla*) (Caesar et al. 1999). If the response of a group of native plants to a particular pathogen is negative, and if that pathogen is accumulating in the soils due to *Euphorbia*, then those native species may also decrease in abundance.

Despite the negative effects of exudates alone on plant germination, there were consistent benefits to native plant germination and growth when both *Euphorbia* root exudates and fungal pathogens were present (Figure 4-3 and Figure 4-4). There are two reasons why the addition of pathogens to soils with exudates may have had a positive effect on the plants. First, the fungi may be using carbon compounds in the exudates that are easily metabolized (Parmeter 1971, Hunt et al. 1987, Pollierer et al. 2012), leading to

reduced pathogen attack on the seeds and seedlings, and if exudates are being broken down, they would have no negative effects on native plants. This effect may be temporary: if my study continued beyond 30 days, I may have observed greater impacts from the pathogens if exudate-derived carbon sources diminished over time. However, this would only be the case if *Euphorbia* plants were no longer present (e.g., if *Euphorbia* presence was removed via a control program). Second, *Euphorbia* exudates may inhibit some fungal pathogens. Root exudate inhibition of soilborne pathogens has been observed in agricultural (Park et al. 2004, Steinkellner et al. 2005, Hao et al. 2010, Kumar et al. 2010, Yuan et al. 2012) and invasive plants (Prithiviraj et al. 2007, Zhang et al. 2009). If *Euphorbia* exudates suppress soilborne fungal pathogens, then the plants growing in soils with these exudates would likely be under reduced pathogen attack, resulting in higher native plant germination rates and larger plant biomass, which are two things that I observed in some treatment combinations. This would lend support for the novel weapons hypothesis (NWH), especially if localized pathogen inhibition induced by *Euphorbia* exudates does not provide strong benefits for neighboring native plants in field settings. Further research on this topic, especially through longer-term field studies, would help identify if exudate/pathogen interactions benefit plant recruitment and growth in the wild, as I found, or if these effects are temporary.

Benefits from exudate/pathogen interactions were not ubiquitous among all the isolates. In particular, *R. solani* 1 tended to reduce rates of germination and plant growth, especially in high inoculum densities. This pattern is consistent with the accumulation of local pathogens hypothesis (ALPH); however, without quantification of fungal

populations in the soil, the mechanisms behind this pattern, and the previously described enhancement of native plants, remain uncertain. Additional work quantifying fungal populations in the soil would further elucidate these mechanisms.

My findings add to the growing body of evidence that *Euphorbia* interacts with soil microbiota to create soil microbial "legacies" that can affect native plant communities. Previous studies indicate that impacts from *Euphorbia* are largely negative towards natives (Jordan et al. 2008, Jordan et al. 2011). These studies focused on mutualistic arbuscular mycorrhizal fungi and did not explicitly involve fungal pathogens, which can have different impacts on plant communities than the mutualists. What these studies and ours have in common, however, is that soil microbial legacy effects can be species-specific, where some native species are negatively affected, some are unaffected, and some benefit from the *Euphorbia*-soil microbiota feedbacks. Additionally, feedback effects are not only specific to the plant species, but are also specific to the fungal pathogens involved, illustrated in particular by the opposing effects I observed from *R. solani* 1 compared to the other isolates.

My research on *Euphorbia* root exudates indicates that the abundance of *Euphorbia* commonly found in field settings may be high enough to cause reductions in native plant germination. I further suggest that observed reductions in native plant recruitment and species richness in areas occupied by *Euphorbia* in the Great Plains (Lesica and Hanna 2009, Butler and Wacker 2010) may result, in part, from the direct action of *Euphorbia* root exudates. While I cannot propose that *Euphorbia* abundances greater than a given level are problematic, the fact that I found strong reductions in native

seed germination for many species at densities greater than 6.05 *Euphorbia* per m^2 is suggestive of negative impacts occurring at even low *Euphorbia* densities.

The enhancement of native plant germination and biomass of most species in my study when both *Euphorbia* root exudates and fungal pathogens were present indicates that native plant establishment may not be negatively impacted by exudate/pathogen interactions, at least in the short-term. However, my study involved a single application of *Euphorbia* root exudates and only lasted one month. Moreover, such effects are likely to be highly localized as a function of the exudate diffusion dynamic within the *Euphorbia* rhizosphere or in the bulk soil. Additionally, the combined presence of *Euphorbia* root exudates and *R. solani* 1 reduced the biomass of *Echinacea* and *Vicia* seedlings, indicating that some pathogens may have negative impacts on the establishment of particular native species when *Euphorbia* plants are present. If the continuous interaction between *Euphorbia* and pathogens eventually results in pathogen accumulation, native plant populations may be negatively impacted. For example, if *Euphorbia* exudates serve as a carbon source for soilborne pathogens, this may eventually increase soil pathogen populations, or *Euphorbia* exudates may enhance native plant host infection in the long run. Biocontrol organisms may also lead to additional pathogen accumulation, as was observed around *Euphorbia* roots when root-feeding herbivorous insects were present (Caesar 2003). If pathogen accumulation occurs through the application of biocontrol organisms such as flea beetles, alternative control methods will be required in settings where successful native plant recruitment is crucial to management goals.

Table 4-1. Supplementary information for fungal pathogens. All 15 isolates were used in the pathogen assay, and isolates denoted with an asterisk in the code column were used for the greenhouse experiment (Fo3 below is Fo in the greenhouse experiment). Carol Windels (University of Minnesota, Crookston) supplied the isolates Rs1 through Rs10, and Jim Kurle (University of Minnesota, Twin Cities) supplied Rs11 and the four *Fusarium* spp.

Code	Fungal pathogen	Isolate	Origin
Rs1*	*Rhizoctonia solani*	87-18-2	Baited from soil, Walsh County, ND
Rs2*	*Rhizoctonia solani*	14-5-3	Baited from soil, Marshall County, MN
Rs3*	*Rhizoctonia solani*	E-0-20	Isolated from wheat, Roseau County, MN
Rs4	*Rhizoctonia solani*	95-3-6	Isolated from sugar beet, NW MN
Rs5	*Rhizoctonia solani*	86-17-17	Isolated from sugar beet, Polk County, MN
Rs6	*Rhizoctonia solani*	95-16-6A	Isolated from sugar beet, Pembina County, ND
Rs7	*Rhizoctonia solani*	86-34-8	Isolated from sugar beet, Traill County, ND
Rs8	*Rhizoctonia solani*	88-11-3	Baited from soil, Pembina County, ND
Rs9	*Rhizoctonia solani*	88-12-2	Baited from soil, Polk County, MN
Rs10	*Rhizoctonia solani*	86-4-1	Isolated from sugar beet, Southern MN
Rs11	*Rhizoctonia solani*	5/7 Windels 4-58-035	Baited from soil, MN
Fo1	*Fusarium oxysporum*	452-8A	Baited from soil, MN
Fo2	*Fusarium oxysporum* f.sp. *medicaginis*	7F3 (#9)	Baited from soil, MN
Fo3*	*Fusarium oxysporum* f.sp. *medicaginis*	3/F3 (#3)	Baited from soil, MN
Fs*	*Fusarium solani*	910-4	Baited from soil, MN

Table 4-2. Exudate treatment effects on native plant germination for adult and immature *Euphorbia* root exudates. Degrees of freedom (DF) are displayed as numerator/denominator, and *P*-values less than 0.05 are in bold.

		Immature			Mature		
Species	Day	DF	*F*	*P*	DF	*F*	*P*
Artemisia	5	6,12	5.09	**0.008**	6,12	8.99	**0.001**
	10	6,12	1.92	0.158	6,12	5.52	**0.006**
	15	6,12	1.26	0.343	6,12	2.93	0.054
Bouteloua	5	6,13	16.2	**<.001**	6,12	44	**<.001**
	10	6,13	1.45	0.268	6,12	0.69	0.665
	15	6,13	0.88	0.535	6,12	0.09	0.996
Echinacea	5	6,13	1.81	0.175	3,12	3.03	0.071
	10	6,13	3.93	**0.019**	6,12	32.8	**<.001**
	15	6,13	1.59	0.227	6,12	48.6	**<.001**
Helianthus	5	6,13	5.87	**0.004**	6,12	3.9	**0.021**
	10	6,13	0.42	0.851	6,12	1.15	0.394
	15	6,13	0.61	0.718	6,12	1.49	0.261
Koeleria	5	6,13	4.76	**0.009**	6,13	4.35	**0.013**
	10	6,13	1.43	0.276	6,13	1.94	0.149
	15	6,13	0.98	0.478	6,13	0.84	0.56
Linum	5	6,13	3.83	**0.02**	6,12	13.7	**<.001**
	10	6,13	0.17	0.98	6,12	0.35	0.897
	15	6,13	0.27	0.942	6,12	0.36	0.889
Nassella	5	5,10	0.63	0.68	4,7	37.3	**<.001**
	10	5,10	1.95	0.173	4,7	15.2	**0.002**
	15	5,10	2.49	0.103	4,7	0.87	0.527
Pascopyrum	5	5,10	0.67	0.654	4,7	10.7	**0.004**
	10	5,10	0.46	0.799	4,7	2.91	0.103
	15	5,10	0.23	0.943	4,7	0.39	0.81
Ratibida	5	6,14	1.62	0.215	6,13	0.58	0.742
	10	6,14	1.12	0.401	6,13	0.35	0.898
	15	6,14	0.89	0.53	6,13	0.33	0.907
Symphyotrichum	5	6,13	5.33	**0.006**	6,12	9.66	**0.001**
	10	6,13	0.61	0.718	6,12	9.23	**0.001**
	15	6,13	0.33	0.907	6,12	5.18	**0.008**
Vicia	5	5,11	0.55	0.734	6,12	17	**<.001**
	10	5,11	1.1	0.414	6,12	8.97	**0.001**
	15	5,11	0.97	0.477	6,12	4.1	**0.018**

Table 4-3. Summary of effects from immature (I) and mature (M) *Euphorbia* exudates on native plant seed germination. Temporary = significant treatment effects at five or 10 days, but not 15; Persistent = significant treatment effects by days 10 and 15. The asterisk indicates marginally non-significant effects on day 15 ($P = 0.054$).

Species	Temporary	Persistent
Artemisia	I	M*
Bouteloua	I, M	-
Echinacea	I	M
Helianthus	I, M	-
Koeleria	I, M	-
Linum	I, M	-
Nassella	M	-
Pascopyrum	M	-
Ratibida	-	-
Symphyotrichum	I	M
Vicia	-	M

Table 4-4. Inoculum density and exudate concentration effects on native plant germination for each fungal isolate. Germination data are combined across all species for each fungal isolate. *P*-values less than 0.05 are in bold.

Isolate	Effect	DF	*F*	*P*
R. solani 1	Density	1,266	777	**<.0001**
	Exudate	2,266	8.89	**0.0002**
	Density × Exudate	2,266	1.88	0.172
R. solani 2	Density	1,266	37.48	**<.0001**
	Exudate	2,266	2.89	0.0574
	Density × Exudate	2,266	0.3	0.739
R. solani 3	Density	1,266	200	**<.0001**
	Exudate	2,266	318.5	**<.0001**
	Density × Exudate	2,266	∞	**<.0001**
F. oxysporum	Density	1,266	0.68	0.4101
	Exudate	2,266	15.36	**<.0001**
	Density × Exudate	2,266	∞	**<.0001**
F. solani	Density	1,266	17.13	**<.0001**
	Exudate	2,266	3.21	**0.0418**
	Density × Exudate	2,266	1.43	0.2401
Control	Exudate	2,119	37.82	**<.0001**

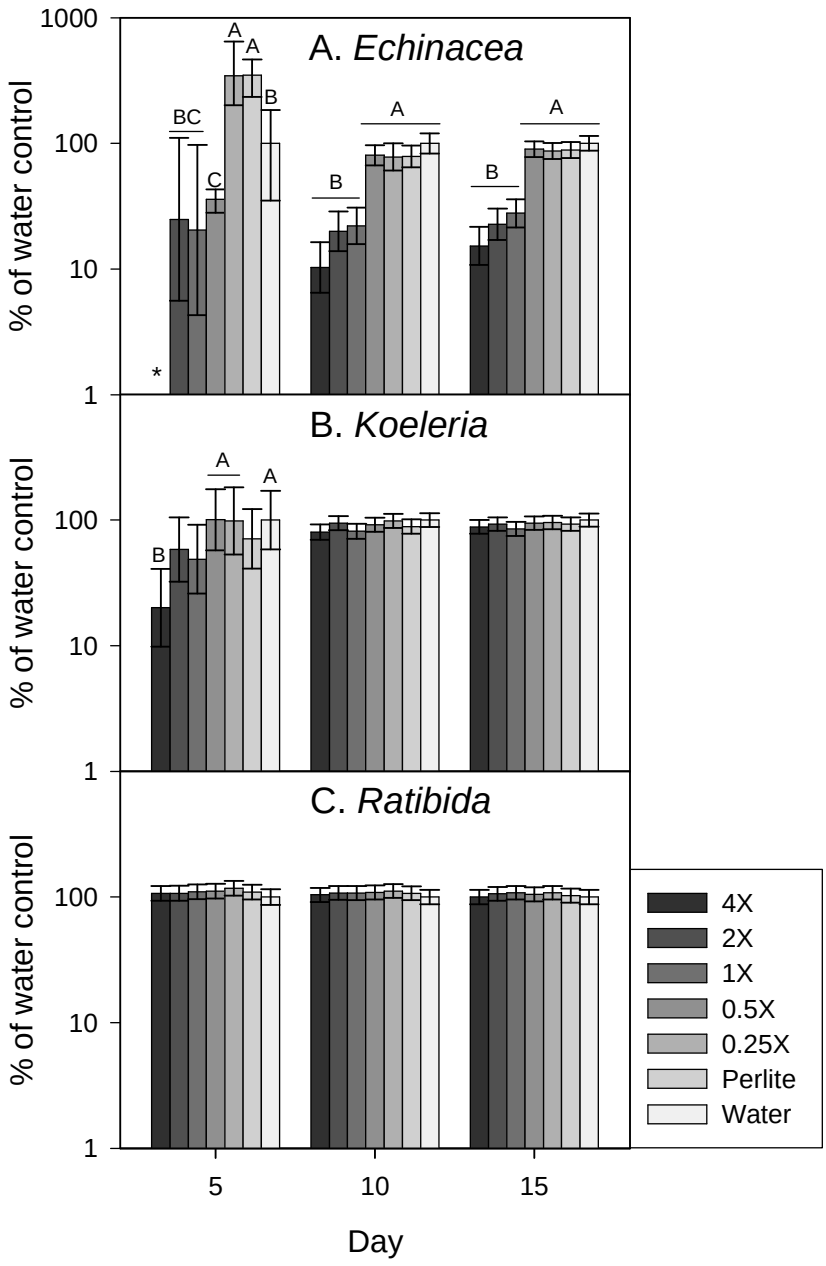


Figure 4-1. Examples of *Euphorbia* root exudate effects on germination of *Echinacea* (A), *Koeleria* (B), and *Ratibida* (C) over 15 days. Germination is represented as the percent difference in germination compared to the water control [(treatment ÷ water) × 100], and only the effects from mature *Euphorbia* are shown above. These three graphs

represent the common patterns of exudate effects: persistent effects throughout the study (A), temporary effects at the beginning (B) and no effects at any stage (C). Bar heights are least square mean estimates and error bars are 95% confidence intervals. Different letters above bars represent different estimates via Tukey HSD tests; separate analyses conducted within each day. Note: the asterisk for the 4× exudate concentration at day five for *Echinacea* indicates zero seeds germinated. Full output of all graphs is shown in Appendix 6.

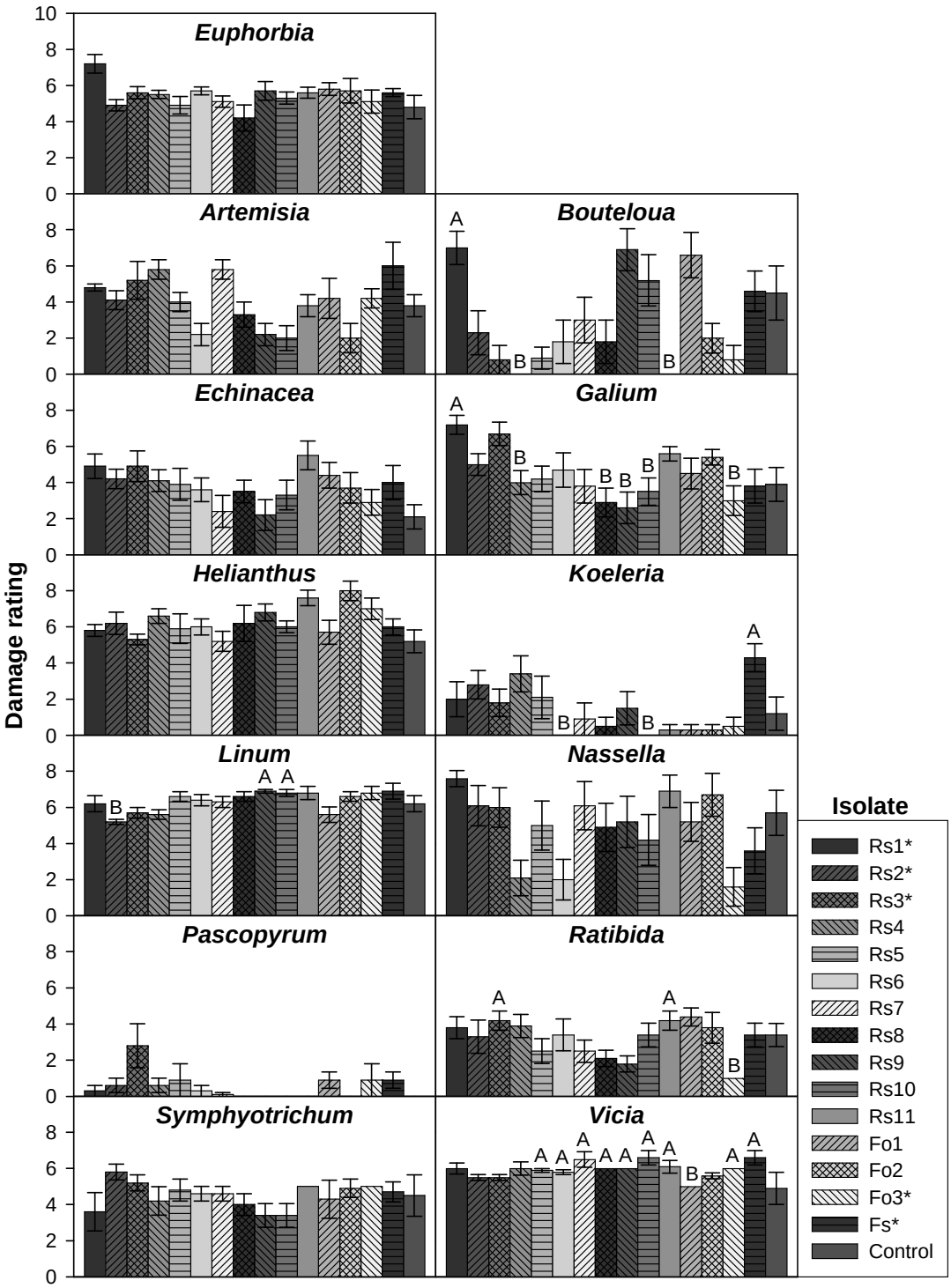

Euphorbia
Artemisia
Bouteloua
Echinacea
Galium
Helianthus
Koeleria
Linum
Nassella
Pascopyrum
Ratibida
Symphyotrichum
Vicia
Damage rating
Isolate
Rs1*
Rs2*
Rs3*
Rs4
Rs5
Rs6
Rs7
Rs8
Rs9
Rs10
Rs11
Fo1
Fo2
Fo3*
Fs*
Control

Figure 4-2. Pathogen damage ratings to *Euphorbia* and twelve native plant species from 15 fungal isolates. Bar heights are average damage ratings for each isolate, and error bars are ± one standard error. Isolates that caused significantly different damage to a given native plant species, as determined via Steel-Dwass nonparametric pairwise comparisons, are indicated with different letters. Isolates without letters did not have significantly different damage ratings from other isolates or the non-inoculated control. Isolates indicated with an asterisk were used in the pathogen/exudate glasshouse experiment.

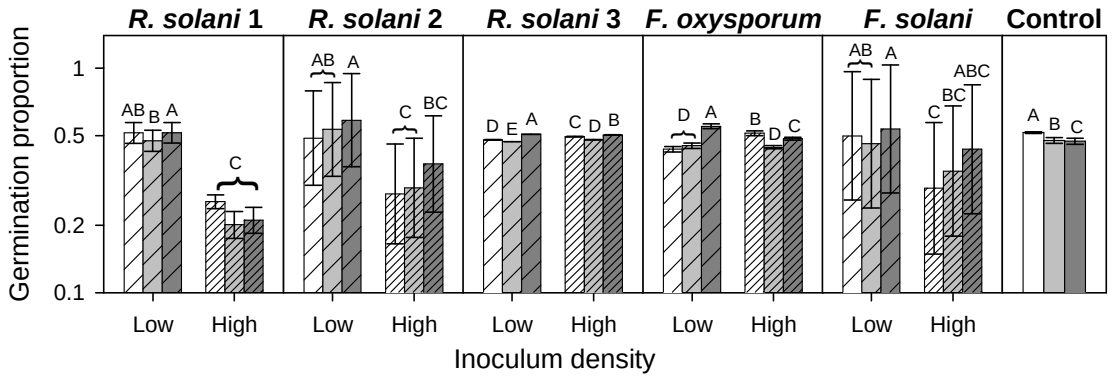


Figure 4-3. Germination proportion of all species combined under pathogen and *Euphorbia* root exudate treatments for the five pathogens and the non-inoculated control. Exudate treatments are no exudate (open bars), and low (light gray) and high (dark gray) exudate concentrations, and pathogen treatments are no inoculum (no pattern), and low (coarse diagonal lines) and high (fine diagonal lines) inoculum densities. Bar heights are estimates of least square means, and error bars are 95% confidence intervals, determined via generalized linear mixed models. Different letters above bars represent different estimates via Tukey HSD tests; germination for each pathogen and the non-inoculated control was analyzed separately. Note: different magnitudes in confidence levels among pathogens results partially from different distributions and link functions used for the pathogens, and some error bars are not visible due to the vertical axis scale.

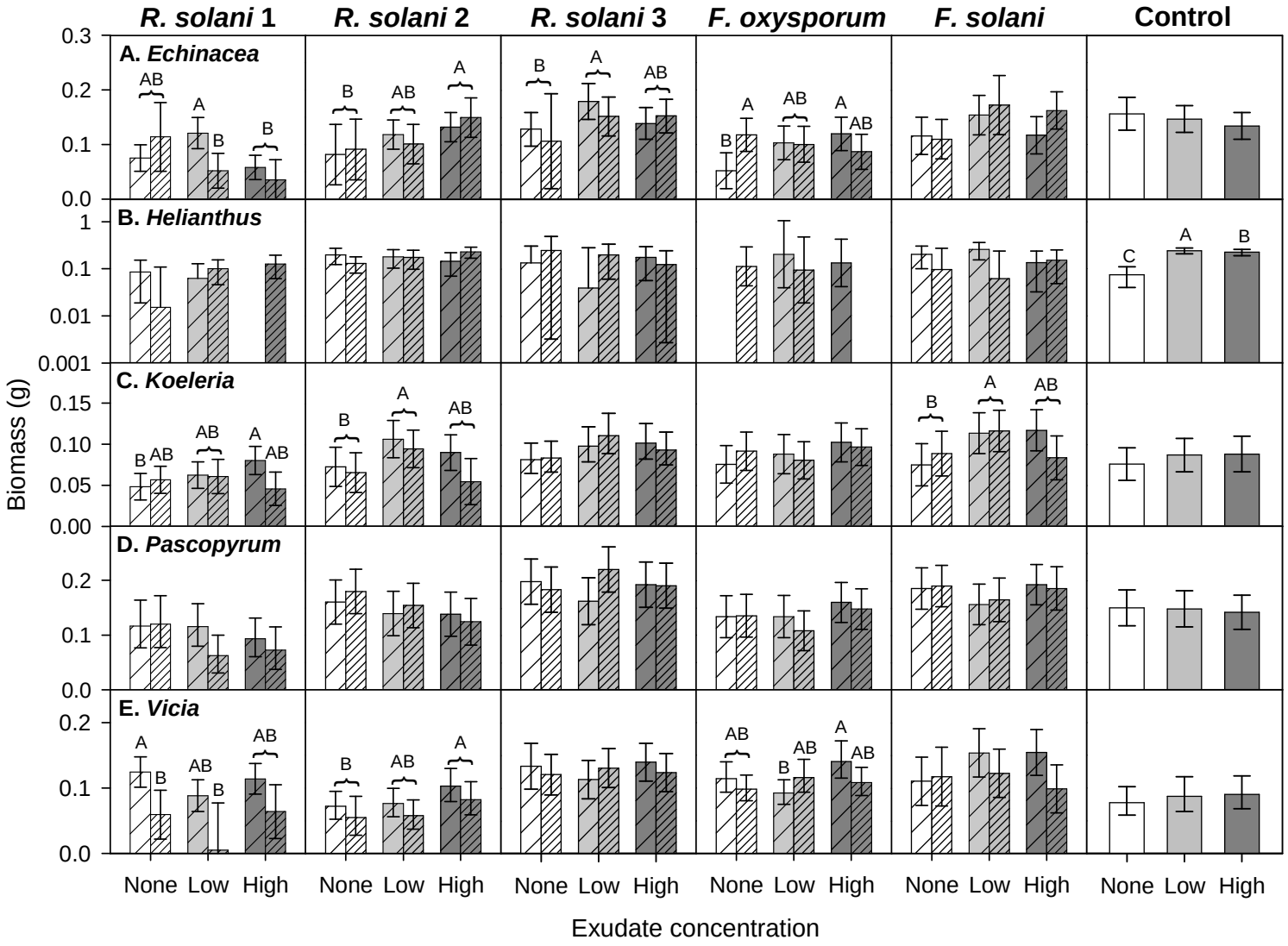

R. solani 1
R. solani 2
R. solani 3
F. oxysporum
F. solani
Control
A. Echinacea
B. Helianthus
C. Koeleria
D. Pascopyrum
E. Vicia
Biomass (g)
0.3
0.2
0.1
0.0
1
0.1
0.01
0.001
0.15
0.10
0.05
0.00
None Low High
Exudate concentration

Figure 4-4. Per plant biomass of *Echinacea* (A), *Helianthus* (B), *Koeleria* (C), *Pascopyrum* (D) and *Vicia* (E) under pathogen and *Euphorbia* root exudate treatments for the five pathogens and the non-inoculated control. Exudate treatments are no exudate (open bars), and low (light gray bars) and high (dark gray bars) exudate concentrations, and pathogen treatments are no inoculum (no pattern), and low (coarse diagonal lines) and high (fine diagonal lines) inoculum densities. Bar heights are least square mean estimates, and error bars are 95% confidence intervals. Different letters above bars represent significantly different least square means; biomass for each pathogen was analyzed separately. Note: the differences shown between treatment combinations in the *R. solani* 1 and 2 inoculations for *Koeleria* were marginally insignificant via Tukey's HSD tests (P = 0.051 and 0.052, respectively).

Chapter 5:

Exotic and native prairie plant root exudates vary in their effects on soilborne fungal pathogen growth

Invasive plants often have a selective advantage over native plants in their invaded range, which may result from plant-microbe interactions that are simultaneously beneficial for the invasive and detrimental for native species. Slow native plant recovery has occurred in areas previously dominated by leafy spurge (*Euphorbia esula*), a widespread invasive plant in the northern Great Plains, which may be caused by an accumulation of fungal pathogens in soils of *Euphorbia* spp. when exposed to root-feeding herbivores. I investigated the relative effects of leafy spurge and eight native plant root exudates on *in vitro* growth of five fungal plant pathogens (*Rhizoctonia* and *Fusarium* spp.) from soils of the Great Plains. The effects of root exudate concentration and polar vs. nonpolar exudates on fungal growth were also evaluated. Leafy spurge root exudates inhibited fungal growth more than exudates from several native plant species, indicating that pathogen accumulation from leafy spurge may not occur for the fungal isolates studied. Grass species enhanced fungal pathogen growth more than forbs, and fungal growth varied significantly among plant species. Root exudate concentration influenced fungal growth, with some plant species increasing and other species decreasing growth with increasing exudate concentration. Concentration effects also varied between the fungal genera, with *Rhizoctonia* growth enhanced and *Fusarium*

growth reduced with increasing exudate concentration. Polar and nonpolar exudates had varying effects on pathogen growth among plant species, and between fungal genera. While leafy spurge root exudates did not enhance growth of the fungal isolates in my study, the variety of responses observed suggests that a broader sample of pathogens may yield different results. The potential influence of herbivory on pathogen accumulation also should be addressed. Additional research into these topics will more conclusively determine if pathogens are responsible for reduced native plant recovery in areas formerly occupied by leafy spurge.

Introduction

Plant pathogenic fungi often play a critical role in determining plant community composition (Bruehl 1987, Mordecai 2011), and these pathogens can, in turn, be strongly influenced by plant root exudates (Schroth and Hildebrand 1964, Curl and Truelove 1986). Root exudates can both stimulate (Nelson 1990, Mandeel 2006) and inhibit (Park et al. 2004, Yokose et al. 2004, Hao et al. 2010) germination and growth of different pathogenic fungi, and they can influence diversity of soilborne pathogens (Broeckling et al. 2008). Variation in fungal responses to root exudates can depend on the concentration (Lagrange et al. 2001) and composition (Nelson 1990) of exudate compounds, which vary with plant species, plant growth stage and environmental conditions (Grayston et al. 1997, Neumann and Römheld 2001). Carbon-rich simple sugars and amino acids (polar molecules) can be a nutrient source supporting

saprotrophic growth of fungi (Parmeter 1971), while phenols (nonpolar) and other secondary metabolites can have the opposite effect, often being antibiotic in nature (Stevenson et al. 1995, Bais et al. 2005, Hao et al. 2010, Kumar et al. 2010). These antibiotics do not affect all fungal pathogens, which can vary in their ability to produce detoxifying enzymes or metabolize antibiotics (Bouarab et al. 2002). Despite the importance of these plant-pathogen relationships, the influences of root exudates and the polarity of their components on fungal pathogens are infrequently studied, and not well-known.

Leafy spurge (*Euphorbia esula*), an invasive exotic plant in North America, often dominates areas to the exclusion of native plants. Its success as an invader, particularly in the northern Great Plains, may partly reflect competitive advantages the plant obtains through native enemy escape (Messersmith et al. 1985). One of the major hypotheses explaining the success of exotic plants in areas they invade is the enemy release hypothesis (ERH), which posits that exotics are under reduced attack from pests and pathogens in the introduced range compared to their native range (Keane and Crawley 2002). However, experimental support for the ERH is mixed (Colautti et al. 2004, Blumenthal et al. 2009), and invasive plants can actually *accumulate* pathogens in their invaded ranges instead of escape from them. This type of pathogen accumulation has been implicated in the success of some invasive plant species: there may be stronger negative effects of generalist pathogens, which infect a broad range of hosts, on natives than invasives (Eppinga et al. 2006, Mangla et al. 2008). One mechanism for this accumulation could be carbon-rich root exudates. For example, root exudates from

Chromolaena odorata, an invasive plant in the Old World tropics, increased the density of fungal pathogen (*Fusarium* spp.) spores in field soils, leading to reduced native plant growth (Mangla et al. 2008). In a study on *Euphorbia esula*/*virgata* and *E. stepposa* in the US, Europe and Asia, Caesar (2003) found an increased population of *Fusarium* spp. in soil adjacent to *Euphorbia* roots when root-feeding insects were present vs. when they were absent, presumably in response to root exudates.

Euphorbia infestations in North America are frequently controlled using biocontrol insects such as flea beetles (*Aphthona* spp.), whose larvae are root herbivores. However, native plant recovery following *Euphorbia* control by flea beetles has been slow and incomplete, with native species diversity and abundance rarely returning to pre-invasion levels (Lesica and Hanna 2009, Butler and Wacker 2010). Herbivory has been demonstrated to stimulate root exudation in other species (Holland et al. 1996), which can subsequently influence the soil microbial community (Denton et al. 1999, Currie et al. 2006). This may contribute to the potential for *Euphorbia* root exudates to alter soil microbial communities. If *Euphorbia* root exudates stimulate fungal pathogens, high soil pathogen populations may result, leading to subsequent reduced native plant recruitment. It is unknown, however, if fungal pathogens respond differently to *Euphorbia* compared to native plant root exudates.

I hypothesize that *Euphorbia* root exudates enhance the growth of soilborne fungal pathogens, and that the growth response of fungal pathogens is stronger when subjected to *Euphorbia* root exudates than to native plant root exudates. To address this hypothesis, I compared the responses of five fungal pathogens to multiple

concentrations of root exudates from leafy spurge and eight native plant species from the northern Great Plains. I had three objectives: 1) to examine leafy spurge and native plant root exudate effects on fungal pathogen growth; 2) to determine the relationship between root exudate concentration and fungal growth; and 3) to quantify how fungal growth varied with the polarity of exudate components.

Methods

Root exudate preparation

Root exudates of *Euphorbia* (leafy spurge) and eight native species, including the grasses *Bouteloua gracilis* (H.B.K.) Lag. ex Griffiths, *Koeleria macrantha* (Ledeb.) Schult., *Nassella viridula* (Trin.) Barkworth, and *Pascopyrum smithii* (Rydb.) Á. Löve, and the perennial forbs *Artemisia frigida* Wiild., *Echinacea angustifolia* DC, *Helianthus pauciflorus* Nutt., and *Linum lewisii* Pursh, were studied here. These species are native to the northern Great Plains and occur in areas where *Euphorbia* is a frequent invader. Seeds of the native species were acquired from the Bismarck Plant Materials Center (Natural Resources Conservation Service, U.S. Department of Agriculture; Bismarck, ND, USA), Wind River Seed (Manderson, WY, USA), and from Prairie Moon Nursery (Winona, MN, USA). Native plants were reared from seed in polypropylene containers (6 L volume) filled with perlite in a greenhouse with 12 h/day supplemental light for a period of 6 months. *Euphorbia* plants were reared for one year prior to the experiment in 20 cm pots with potting soil medium; mature *Euphorbia* plants were used because

Euphorbia-invaded areas are dominated by mature plants. Hoagland's solution was applied at 100 ml/container/month for to all plant species. At the conclusion of the growth period, plants were harvested and roots were gently cleaned of most of the perlite or soil mix and rinsed with deionized water. At least 10 g fresh weight per species was used for exudate collection, but plant numbers and mass used for exudate collection were not equal among species due to varying germination rates and plant size. For each species, all plants (N = 3 (*Euphorbia*) to 293 (*Bouteloua*)) were loosely bundled, their roots were soaked in one liter of sterile deionized water for 24 hours, and the liquid (hereafter, exudate solution) was removed. Subsequently, dry root and shoot mass were weighed after oven drying (50°C for 4 days).

Ethyl acetate extractions were performed on exudate solutions immediately after collection in order to separate polar (e.g., carbohydrates such as glucose) from nonpolar (e.g., phenols and polyphenols) components. Exudate solutions were added to an equal volume of ethyl acetate, mixed, and the ethyl acetate was separated from the aqueous layer in a separation funnel. Extractions were performed three times for each exudate solution. The ethyl acetate (~3 liters) and aqueous (~1 liter) portions were dried in a vacuum using a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Schwabach Germany). The resulting dried exudates were dissolved into methanol or deionized water for the ethyl acetate (nonpolar) and the aqueous (polar) phases, respectively, and the resulting solutions were placed in 30 ml glass vials and dried down in a centrifugal evaporator (Eppendorf, Hamburg, Germany). Ethyl acetate extractions yielded an

average of 6.86 (±1.34 SE) mg dried product per original liter of plant exudate solution, while aqueous extractions yielded an average of 119 (±17.8 SE) mg.

Disc and plate preparation

The paper disc diffusion method was used to determine the effects of root exudates on fungal growth. This method has been used to examine the effects of plant extracts on *Rhizoctonia solani* (Sehajpal et al. 2009) and bacteria (Shanab et al. 2010). Filter paper discs (grade 113, wet strengthened, 30 µm thick, 7.2 mm diameter; Whatman, Little Chalfont, UK) were sterilized by autoclaving and placed on a sterile plastic petri dish under a laminar flow hood for root exudate application. Dried ethyl acetate and aqueous extractions were put back into solution using methanol and deionized water, respectively, to yield the following exudate/solvent ratios: 20 mg/ml and 1 mg/ml for aqueous and ethyl acetate fractions, respectively. Resulting solutions were filter sterilized (0.2 µm) and applied to the discs. The aqueous extractions yielded an average of 18.6 times the mass of the ethyl acetate extractions, so a broader range of concentrations was explored for aqueous than for ethyl acetate extractions. For the aqueous extractions, three concentrations for all species were applied to discs: 0.01, 0.1, and 1 mg of dried exudate product per disc (aka, low, medium, and high concentrations). For the ethyl acetate extractions, exudate concentrations per disc depended on the mass of exudate extract available for each species. For smaller yields (*Bouteloua*, *Echinacea*, *Koeleria*, *Nassella*, and *Pascopyrum*), 0.01, 0.025, and 0.05 mg dried product per disc were used, while for the remaining species (*Artemisia*, *Euphorbia*, *Helianthus*, and *Linum*), 0.01, 0.05, and 0.1 mg dried product per disc were

used (low, medium, and high concentrations, respectively). Volumes of 0.005 to 0.01 ml of exudate solution were applied to each disc, which were then air dried and applications were repeated as necessary to achieve the desired exudate concentration per disc. Exudate concentrations were scaled to total plant biomass for each species using the following conversion: plant mass (g) per disc = exudate mass (mg) per disc × [plant mass (g) / total exudate residue mass from extraction (mg)]. Water (polar) and ethyl acetate (nonpolar) extractions were analyzed separately.

Five plant pathogenic fungi were used in this study: *Rhizoctonia solani* (isolates 87-18-2, 14-5-3, and E-0-20; hereafter *R. solani* 1, 2, and 3, respectively) one *Fusarium oxysporum* f.sp. *medicaginis* (isolate 3/F3(#3)) and one *F. solani* (isolate 910-4) (Table 5-1). These fungi were isolated from soils and plants in the northern Great Plains and are common pathogens found on many plant hosts worldwide (Carling and Sumner 1992, Windels 1992). All of these fungi were used in a previous greenhouse experiment (see Chapter 4). Inoculum for each fungus was prepared by blending one water agar plate containing 14 days of fungal growth with 80 ml of sterilized deionized water in an Oster blender for 60 seconds (Sunbeam Products, Inc., Rye, NJ, USA). This slurry was mixed with molten but cooled water agar solution at a ratio of 20.8 ml inoculant slurry per 500 ml water agar solution for all fungi but one. *Fusarium oxysporum*, which yielded higher colony forming unit (cfu) counts than the other isolates, was mixed at a rate of 10.4 ml slurry with 500 ml water agar. Five ml of inoculated water agar was poured onto the surface of previously prepared and solidified water agar in petri plates

(~20 ml per plate) and allowed to cool – these plates were used in the final experimental setup (described below).

There were a total of 65 disc treatments in this experiment: exudates from nine plant species, with three concentrations from each of the two extractions (ethyl acetate & aqueous) for each plant species, and 11 control treatments. Four types of controls were used in this experiment: deionized water, methanol, dextrose, and blank (nothing added to discs). The deionized water and methanol were each applied to discs using the same volumes of water and methanol applied to discs for the aqueous (0.01, 0.1, and 1 ml/disc; three aqueous controls) and ethyl acetate (0.01, 0.025, 0.05, and 0.1 ml/disc; four methanol controls) extractions, respectively. The dextrose control was composed of dextrose solution (1 g dextrose/100 ml deionized water) approximating the range of carbon in carbohydrates expected to occur in root exudates (Jalali and Suryanarayana 1971, Aulakh et al. 2001), and applied at 0.01, 0.1, and 1 mg dextrose per disc. The deionized water, methanol, and dextrose solutions were filter sterilized (0.2 μm) before application to discs. Finally, a no-addition, disc-only control was also evaluated. The water and methanol controls could not be compared directly to plant root exudate treatments, which were on a mass basis, but were rather used to determine if volume or type of solvent applied had an effect on fungal growth.

To evaluate pathogen responses to plant exudates, exudate-infused discs were placed on the previously-described inoculated water agar plates. Each plate received six discs, which were placed uniformly on each plate using a template. In the root exudate treatments, all six treatments (three concentrations of each of the two extractions) for an

individual plant species were placed on the same plate. The deionized water and dextrose controls were placed on the same plate, and methanol and blank controls were placed on a single plate. Each treatment combination was replicated on six different plates for each of the five fungal isolates. Plates were incubated at approximately 24 °C for 28 days. Mycelial growth was detected on the plates within 3 days, but enhanced growth surrounding the discs was not observed until ~7-10 days after inoculation, and growth of the fungi around the discs continued slowly until ~21-28 days after inoculation.

Measurement and analysis

Agar plates were photographed after 28 days of incubation using a black matte foam core background and fluorescent back lighting. Fungi grew immediately adjacent to every disc, perhaps caused by fungal growth on the cellulose in the paper discs, which is known to occur in *R. solani* (Garett 1962), *F. oxysporum* (Johnston 1962), and *F. solani* (Wood and Phillips 1969). Fungal growth diameters were measured on the photographs using ImageJ v. 1.46 (Rasband 1997-2012). Specifically, two perpendicular diameters were determined for each disc, from the edge of the disc to the edge of the enhanced fungal growth. All zone diameters were averaged per disc (replicate) prior to analysis. These diameters were used to evaluate the relative inhibition or enhancement of fungal growth among the root exudate treatments, with inhibition referring to smaller zone diameters, and enhancement referring to larger zone diameters, than other treatments.

The effects of root exudate concentrations on fungal growth could be analyzed as categorical variables (i.e., low, medium, and high exudate concentrations) or continuous variables (exudate from a given plant mass). Both approaches were used in this study, as using only one approach may lead to different conclusions about treatment impacts compared to the other approach. This may not normally be the case when comparing the output these types of analyses, especially when linear models are used for both approaches, but that was not the case with my data. Re-scaling the root exudates to a plant mass basis shifted species with larger plant mass:exudate ratios to higher ranges on the exudate axis, and species with smaller ratios to lower ranges. In turn, this changed species intercepts and slopes, which affected the probability of the regression lines being similar.

Differences in fungal colony diameters among root exudate concentrations (low, medium, and high), among plant species, and between polar and non-polar root exudates were analyzed using generalized linear mixed models (GLMM). A full factorial model, with plant species, exudate concentration, and polarity as fixed effects, was used to determine how fungal growth varied for each fungal isolate. Reduced models were used to examine interactions of plant species with exudate concentration, and plant species with polarity, in cases where three-way interactions were non-significant.

The influence of plant species on the linear relationship between root exudate concentration and fungal growth was analyzed using analyses of covariance (ANCOVA). A full-factorial model similar to that used in the GLMM was used to

examine how fungal growth varied with the three fixed effects, except that exudate concentration was a continuous variable. To determine how fungal growth varied with exudate species and concentration, separate analyses were performed on each fungal isolate/polarity combination. Lastly, to determine how fungal growth varied with exudate concentration and polarity, analyses were performed on each fungal isolate/plant species combination. Multiple comparisons for this final analysis were created using preplanned contrasts to determine significance of differences among plant species in exudate concentration/fungal isolate relationships. All ANCOVA models had Pearson chi-square/degrees of freedom ratios of less than one and were therefore considered to fit the linear models.

The GLMMs and ANCOVAs were run using the GLIMMIX procedure in SAS/STAT v. 9.3 (SAS Institute Inc., Cary, NC, 1989-2011), with code modified from Littell et al. (2006). Petri plates were used as a random blocking effect. Most of the data were non-normal; normality was achieved using the lognormal conditional probability distribution (using a log link function), or using Johnson Sl or Su transformations (Johnson 1949, Chou et al. 1998) and subsequently analyzed using the normal probability distribution. Since exudates were applied in exponential (or close to exponential) concentrations, plant masses per disc were log-transformed prior to ANCOVAs. The Satterthwaite approximation method was used to calculate denominator degrees of freedom to account for missing values; this was chiefly a concern for *F. solani*, where there was little fungal growth on approximately 1/3 of the petri plates. Multiple least square means comparisons were adjusted using Tukey's HSD

tests for the GLMMs, and using the Bonferroni method for the ANCOVA. The values used in figures are estimates and 95% confidence intervals that were back-transformed into the scale of the original data, and *P*-values less than 0.05 were deemed as significant.

Results

Euphorbia vs. native plant root exudate effects on fungal growth

Euphorbia root exudates did not significantly enhance the growth of any of the fungal isolates in my study relative to native plant species. On the contrary, *Euphorbia* root exudates significantly *inhibited* fungal growth more than the exudates from some native plant species (Figure 5-1). In general, grass species tended to enhance fungal growth compared to forb species, while *Euphorbia* and the other forbs tended to inhibit fungal growth (Figure 5-1). Specifically, *Bouteloua*, *Nassella*, and *Koeleria* enhanced growth of more fungi than they inhibited, but *Echinacea*, *Euphorbia*, *Artemisia* and *Helianthus* inhibited more fungi than they enhanced. The remaining two plant species, *Linum* and *Pascopyrum*, inhibited and enhanced equal numbers of fungal isolates (two and one isolates, respectively). However, the total plant biomass from which root exudates were collected was not correlated with fungal response to root exudates for any plant species, eliminating plant mass as a potential confounding variable. Fungal growth was also not significantly impacted by dextrose mass, or by water or methanol

volume, applied to control discs. The disc-only control means and standard errors are displayed for comparative purposes on the GLMM figures.

Plant species varied significantly in their effects on fungal growth, which is consistent with previous research (Table 5-2). For example, *Nassella* exudates induced more growth (greater increases in fungal diameters) than did *Echinacea*, *Euphorbia*, or *Helianthus* in *R. solani* 1 (t_{178} = -3.99, P = 0.003; t_{178} = -4, P = 0.003; and t_{178} = -6.04, P < 0.001, respectively), and *Bouteloua* exudates induced more fungal growth than exudates from all other plant species except *Koeleria* in *R. solani* 3 (t_{214} > 3.4, P < 0.02 for all comparisons) (Figure 5-2). Similarly, fungal isolates varied in their sensitivities to specific exudate compounds. For example, *Nassella* exudates stimulated the growth of *R. solani* 1 more than other plant exudates, but did not stimulate the growth of *R. solani* 3 more than other plant species (Figure 5-2).

Concentration had a significant effect on fungal growth for exudates from some plant species but not others (Figure 5-2 and Figure 5-3). For example, the growth of *F. oxysporum* among concentrations was dependent on the root exudate species. Its growth did not vary among concentrations for *Artemisia*, *Echinacea*, *Helianthus* and *Nassella* root exudates, but high concentration *Euphorbia*, *Koeleria*, *Linum* and *Pascopyrum* root exudates inhibited growth compared to low concentrations (Figure 5-2). Species-specific root exudate concentration effects on fungal growth in the ANCOVA are most easily observed in *R. solani* 3, *F. oxysporum* and *F. solani* (Figure 5-3 and Figure 5-4). For example, *Artemisia* polar root exudates enhanced the growth of *R. solani* 3 with

increasing concentration more than *Euphorbia* (t_{97} = 3.53, P = 0.023) and *Pascopyrum* (t_{97} = 3.55, P = 0.022) polar exudates (Figure 5-3).

There was also a trend (mostly non-significant) of opposing fungal growth effects between *Koeleria* and *Echinacea* root exudates (as their concentration increased) in the ANCOVA. For example, *Koeleria* exudates caused fungal growth to decrease with increasing concentration, while *Echinacea* exudates caused a positive growth response, for polar exudates applied to both *Fusarium* isolates, and for nonpolar exudates applied to *R. solani* 3 and *F. oxysporum* (Figure 5-3 and Figure 5-4).

Root exudate concentration and polarity effects on fungal growth

Root exudate concentration had a significant effect on the growth of all isolates except *F. solani* (Table 5-2). This effect varied among the fungal isolates and between exudate polarities. For example, the two pathogen genera had distinct growth responses to root exudate concentration. *Rhizoctonia* isolates grew more with increasing exudate concentration, while *Fusarium* isolates grew less with increasing exudate concentration (Figure 5-2). This pattern was not consistent between polarities for *Rhizoctonia*, as the positive relationship between exudate concentration and growth was more often found for polar than nonpolar exudates (Figure 5-6). The exudates of four plant species caused significantly less growth of *F. oxysporum* at high than at low concentrations: *Euphorbia* (t_{197} = 6.41, P < 0.001), *Koeleria* (t_{197} = 9.57, P < 0.001), *Linum* (t_{197} = 5.42, P < 0.001), and *Pascopyrum* (t_{197} = 5.29, P < 0.001) (Figure 5-2). Therefore, root exudates of these four species inhibited *F. oxysporum* growth with increasing concentration.

Polar and nonpolar root exudates also had varying effects on fungal growth for all isolates in the GLMM, and for three isolates in the ANCOVA (Table 5-2). The polarity effect varied among the fungal isolates: all *Rhizoctonia* isolates and *F. solani* grew more when exposed to polar than nonpolar exudates, but exposure to nonpolar exudates caused *F. oxysporum* to grow more than exposure to polar exudates (Figure 5-5). In nine of 44 fungal isolate-plant species combinations there was a significantly different fungal growth response between polar and nonpolar exudates, but the differences were not consistent among species or fungi (Figure 5-5). For three fungal isolates (*R. solani* 2 and 3, and *F. oxysporum*), polar and nonpolar exudates differed significantly in their effects on fungal growth for certain species, leading to significant plant species by polarity interactions. *R. solani* 2 exhibited contrasting polarity effects among species, shown best by comparing *Koeleria*, which stimulated greater fungal growth via polar than nonpolar exudates (t_{224} = -4.4, P = 0.002), with *Pascopyrum*, which exhibited the opposite trend (t_{224} = 5.75, P < 0.001) (Figure 5-5). *R. solani* 3 and *F. oxysporum* growth differed between polar and nonpolar exudates for some plant species (*Artemisia*, *Bouteloua*, *Helianthus* and *Linum* for *R. solani* 3; *Echinacea*, *Euphorbia*, *Koeleria*, and *Pascopyrum* for *F. oxysporum*), while fungal growth did not vary between exudate polarities for any other plant species in these isolates (Figure 5-5).

Polar and nonpolar root exudates also vary in their effects on *Fusarium* spp. growth with plant species and exudate concentration (i.e., three-way interactions; Table 5-2 and Figure 5-4). For example, while root exudates of *Koeleria* inhibited *F.*

oxysporum growth more than exudates of other species for both polar and nonpolar exudates with increasing concentration, only nonpolar *Echinacea* exudates significantly enhanced fungal growth with increasing concentration (Figure 5-4 and Appendix 14). Therefore, plant species identity, and concentration and polarity of root exudates, simultaneously have significant influence on the growth of some fungal isolates.

Discussion

Effect of *Euphorbia* vs. native plant exudates

Euphorbia exudates did not enhance pathogen growth: instead, they inhibited fungal growth more than some native plant species. This suggests it is unlikely that fungal pathogens selectively accumulate in soils surrounding *Euphorbia* roots if exudates alone determine fungal dynamics. However, only a small subset of fungal pathogens with which *Euphorbia* and native plants may interact were considered in this study, and more research is needed to determine if there are broader generalizable patterns.

My results are consistent with previous work showing that the effects of root exudates on fungal pathogens vary among plant species (Nelson 1990). For example, grasses tended to encourage growth of large fungal colonies more often than forbs. While other comparisons of grass and forb effects on soilborne fungal pathogens are generally unavailable, van Elsas et al. (2002) hypothesized that grasses caused stronger microbial suppression in grassland compared to arable land soils towards the potato

pathogen *Rhizoctonia solani* AG3. Pathogen suppression, which is usually a function of pathogen-inhibiting (antibiotic-producing) microbes in soil, was not addressed in my research. Both fungal pathogen growth and antibiotic-producing bacterial populations could be enhanced, or inhibited, by plant root exudates, and *in vivo* studies would be necessary to determine the positive or negative feedbacks to plants. Additional research is needed to determine if soil pathogen populations differ in areas dominated by distinct plant functional groups, and the extent to which pathogens are influenced by plant root exudates directly, and indirectly via antibiotic-producing microbes.

Among the native prairie plant species considered here, *Koeleria* and *Echinacea* exhibited the greatest difference in exudate concentration effects on fungal growth. With increasing root exudate concentration, *Koeleria* decreased while *Echinacea* increased fungal growth, an effect which was observed in three pathogens. In field soils, dense stands of *Koeleria* might therefore reduce some soilborne fungal pathogen populations via root exudates, while dense *Echinacea* stands may increase pathogen densities. However, implications for these differences in the field will depend on a complex array of factors including plant resistance to infection, plant competitive ability, and soil nutrient status. Moreover, this work does not consider the potential effects of *Koeleria* exudates on other soilborne organisms, including beneficial bacteria and mycorrhizal fungi. In this case, the benefits of inhibiting pathogenic fungi may be mitigated by a reduced presence of beneficial organisms. In a field experiment on native prairie plant responses to mutualistic mycorrhizal presence, *Koeleria* seedling emergence was reduced in plots with suppressed mycorrhizal activity (accomplished via

treatment with the fungicide benomyl) compared to control plots (Hartnett et al. 1994), suggesting fitness costs to *Koeleria* when beneficial soil microorganisms are suppressed.

In general, native plants are hypothesized to establish beneficial mutualisms and suppress pathogens in ways that increase the plant species fitness in the long term. Additionally, the community-wide effects of plant root exudation will depend on the relative sensitivity of different plant hosts to the negative impacts of pathogens versus the positive impacts of beneficial soil organisms. For example, in Great Plains prairies, experimental plant growth responses to mycorrhizal root colonization varied significantly with plant life history (annual vs. perennial), taxonomic group (grasses, forbs, legumes, and nonlegumes) and phenological guild (cool-season vs. warm-season) (Wilson and Hartnett 1998).

Meanwhile, if *Echinacea* root exudates enhance fungal pathogen growth in soils, the low densities of *Echinacea* found in field settings (personal observation) may result from increased seedling survival with increasing distance from enhanced soilborne pathogen populations near the adults. While this general pattern of seedling survival was first described by Janzen and Connell (Connell 1970, Janzen 1970) and demonstrated experimentally in grasslands (Petermann et al. 2008), temperate forests (Packer and Clay 2000), and tropical forests (Mangan et al. 2010), further information on the potential mechanistic links between root exudates, pathogens, and beneficial symbionts will improve our understanding of the drivers of plant diversity. Further investigation of the effects of *Euphorbia* on suppression or enhancement of fungal plant

pathogens may improve our understanding of plant community dynamics in *Euphorbia*-occupied areas.

Effects of exudate concentrations and polarity

Growth of all of the fungal isolates except *F. solani* were significantly influenced by root exudate concentration, but the exact relationship of these experimental concentrations with real-world exudate concentrations in the rhizosphere is uncertain. Many studies have measured root exudation in sterile lab conditions (Jones et al. 2004), but root exudation is notoriously difficult to quantify in the field. Measurements of field exudation have usually used trees (Phillips et al. 2008), while most other studies have estimated the relative proportion of carbon allocated to root exudation (Uren 2007). Converting root exudates to a plant mass basis (the units used in the ANCOVA) allows for comparisons between my study and published field biomass values. For example, field estimates of *Bouteloua* at a mixed grass prairie site in Montana ranged from 50–1132 g per m^2 above-ground biomass (Karl et al. 1999). Polar *Bouteloua* exudates had a comparable range of 84.2–8420 g per m^2 total plant biomass (from lowest concentration to highest) when adjusted for diffusion disc area. Using similar comparisons, the exudate concentrations of some of the forbs in my study would likely be much higher than those found in the field unless plant populations occur at high densities. However, my study provides a starting point for examining root exudate effects on pathogens.

The two pathogen genera had distinct responses to changes in root exudate concentration. For example, all three *R. solani* isolates responded similarly to root

exudate concentration, with positive exudate-growth relationships to most plant species. In contrast, the *Fusarium* spp. tended to respond negatively to exudates. These results are consistent with research demonstrating the stimulation of Fusarium spores (conidia) by root exudates (Odunfa 1978, Nelson 1990, Steinkellner et al. 2008), including following the application of root exudates from the exotic invasive *Chromolaena odorata* in India (Mangla et al. 2008). Future studies should consider a broader array of fungal pathogens, and separate root exudate effects on mycelial growth from effects on spore germination.

Polar and nonpolar exudates varied significantly in their effects on growth of fungi. Polar exudates, which typically contain sugars and amino acids that fungi can use for growth (Parmeter 1971), elicited more growth than nonpolar exudates for the *Rhizoctonia* isolates and *F. solani*, but not for *F. oxysporum*, where the opposite pattern was found. Phenols, which tend to be nonpolar, can inhibit the germination and sporulation of pathogens, including *F. oxysporum* (Hao et al. 2010, Wu et al. 2010a, Wu et al. 2010b). However, in my study, the enhancement of *F. oxysporum* growth by nonpolar exudates may indicate that there are other signaling molecules that stimulate the isolate's growth. Some non-polar flavonoids (Andersen and Markham 2006), for example, can stimulate *F. solani* spore germination (Ruan et al. 1995). Compounds in the nonpolar fraction could also be better sources of readily-metabolized carbon than compounds in the polar fraction. Fungi vary in their abilities to break down antibiotic compounds (Bouarab et al. 2002) (many of which are nonpolar), and metabolize different carbon sources (usually polar) in root exudates (Nelson 1990). Identifying the

specific compounds that elicit fungal pathogen responses and quantifying the concentrations of those compounds exuded by various plant species would further our understanding of these plant-pathogen interactions.

Management implications

While the present study shows little support for a root exudate-mediated "accumulation of local pathogens" hypothesis (Eppinga et al. 2006), there are several factors that were not explored. Root exudates can differ between seedling and mature plants (Aulakh et al. 2001), so seedling *Euphorbia* root exudates may be associated with different fungal effects than those from mature plants. However, most *Euphorbia*-dominated areas are composed of mature plants rather than seedlings (personal observation), so this may have limited application to my study system. Root exudate volume (Holland et al. 1996) and chemical composition (Metlen et al. 2009) can also change following herbivory. This may be very important, because flea beetles are widely used to control *Euphorbia* in the Great Plains (Hodur et al. 2006), and local pathogen accumulation may be likely with *Euphorbia* in the presence of flea beetles. This is highlighted by two lines of evidence. First, fungal pathogen populations were found to be higher in soils adjacent to two *Euphorbia* spp. (including *E. esula*) when root herbivores were present compared to when the herbivores were absent (Caesar 2003). And second, native plants do not always return to pre-invasion diversity or abundance in areas where *Euphorbia* has been treated with biocontrol herbivores, as observed in long-term studies (Lesica and Hanna 2009, Butler and Wacker 2010).

Scientists and managers looking for ways to reduce the competitive advantages of invasive species over native plants have recently been investigating the use of activated carbon (Kulmatiski and Beard 2006). Organic compounds released by plants, including allelochemicals and organic molecules that are used by soil microbes for growth or signaling (Bais et al. 2004), can be adsorbed by activated carbon (Inderjit and Callaway 2003), thereby reducing their concentration in soil. Activated carbon can also be modified to adsorb compounds of various polarities (Bansal and Goyal 2005). If *Euphorbia* root exudates contained a particular polar or nonpolar compound responsible for pathogen accumulation, activated carbon may be tailored for limiting this accumulation. However, activated carbon can have a wide range of effects on soil biota (Lehmann et al. 2011) and undesired effects such as stimulating the growth of exotic plants (Kulmatiski and Beard 2006). Its utility in exotic plant management and habitat restoration is therefore a topic of ongoing research (Beesley et al. 2011, Kulmatiski 2011, Mitchell and Bakker 2011).

Further research is necessary to address the root exudate-driven local pathogen accumulation hypothesis, including the influence of herbivory on exudate quantity and composition, how these altered exudates influence pathogens, and if there is comprehensive evidence for pathogen accumulation in field settings. If pathogen accumulation is occurring as a result of current management practices (i.e., if flea beetle root herbivory is unintentionally resulting in increased soil pathogen populations), then managers should consider alternative practices. For example, flea beetle species are not all alike in their damage to *Euphorbia* (Gassmann et al. 1996), and may induce varying

exudation responses to flea beetle species. A biocontrol species that is both effective at *Euphorbia* control, and which does not encourage pathogen accumulation, is critical for areas where native plant recovery is required for successful land management.

Table 5-1. Origin of experimental isolates.

Fungal pathogen	**Isolate**	**Origin**	**Source**
Rhizoctonia solani	87-18-2	Baited from soil, Walsh County, ND	Carol Windels, U. of Minnesota, Crookston
Rhizoctonia solani	14-5-3	Baited from soil, Marshall County, MN	Carol Windels, U. of Minnesota, Crookston
Rhizoctonia solani	E-0-20	Isolated from wheat, Roseau County, MN	Carol Windels, U. of Minnesota, Crookston
Fusarium oxysporum f.sp. *medicaginis*	31F3 (#3)	Baited from soil, MN	Jim Kurle, U. of Minnesota, Twin Cities
Fusarium solani	910-4	Baited from soil, MN	Jim Kurle, U. of Minnesota, Twin Cities

Table 5-2. GLMMs and ANCOVA with full factorial effects of plant species, polarity, and exudate concentration on colony diameters. Degrees of freedom (DF) are displayed as "numerator,denominator" and *P*-values less than 0.05 are in bold. Isolate codes: Rs = *R. solani*, Fo = *F. oxysporum*, Fs = *F. solani*.

		GLMM			ANCOVA		
Isolate	Effect	DF	*F*	*P*	DF	*F*	*P*
Rs1	Species	8,178	7.3	**<.001**	8,196	5.4	**<.001**
	Polarity	1,178	14.5	**<.001**	1,196	0.7	0.409
	Concentration	2,178	5.6	**0.004**	1,196	1.3	0.264
	Species × Polarity	8,178	1.8	0.081	8,196	1.6	0.14
	Concentration × Species	16,178	1.3	0.225	8,196	2	0.053
	Concentration × Polarity	2,178	1.7	0.195	1,196	3.2	0.073
	Concentration × Species × Polarity	16,178	0.8	0.715	8,196	0.8	0.64
Rs2	Species	8,224	5.1	**<.001**	8,242	4.7	**<.001**
	Polarity	1,224	5	**0.027**	1,242	7	**0.009**
	Concentration	2,224	17	**<.001**	1,242	19.4	**<.001**
	Species × Polarity	8,224	11.8	**<.001**	8,242	5.3	**<.001**
	Concentration × Species	16,224	1.8	**0.03**	8,242	2.3	**0.02**
	Concentration × Polarity	2,224	0.4	0.692	1,242	0	0.833
	Concentration × Species × Polarity	16,224	1.2	0.306	8,242	1.2	0.288
Rs3	Species	8,214	5.4	**<.001**	8,232	2.7	**0.008**
	Polarity	1,214	125.8	**<.001**	1,232	22.5	**<.001**
	Concentration	2,214	31.5	**<.001**	1,232	33.8	**<.001**
	Species × Polarity	8,214	2.5	**0.012**	8,232	2	**0.046**
	Concentration × Species	16,214	1.6	0.067	8,232	3.4	**0.001**
	Concentration × Polarity	2,214	15.3	**<.001**	1,232	7.8	**0.006**
	Concentration × Species × Polarity	16,214	1.4	0.157	8,232	1.8	0.087
Fo	Species	7,197	14.6	**<.001**	7,213	8.6	**<.001**
	Polarity	1,197	38.8	**<.001**	1,213	24.2	**<.001**
	Concentration	2,197	70.6	**<.001**	1,213	54.9	**<.001**
	Species × Polarity	7,197	3	**0.005**	7,213	3.5	**<.001**
	Concentration × Species	14,197	7.8	**<.001**	7,213	9.4	**<.001**
	Concentration × Polarity	2,197	2.7	0.071	1,213	2.5	0.115
	Concentration × Species × Polarity	14,197	2.1	**0.013**	7,213	3.4	**0.002**
Fs	Species	8,35	4.2	**0.001**	8,46	1.4	0.211
	Polarity	1,35	23.5	**<.001**	1,46	0.4	0.532
	Concentration	2,35	2.3	0.116	1,46	1.9	0.179
	Species × Polarity	8,35	1.1	0.392	8,46	1.1	0.397
	Concentration × Species	15,35	4.4	**<.001**	8,46	2.7	**0.015**
	Concentration × Polarity	2,35	1.2	0.318	1,46	0	0.94
	Concentration × Species × Polarity	11,35	3.8	**0.001**	8,46	1.7	0.121

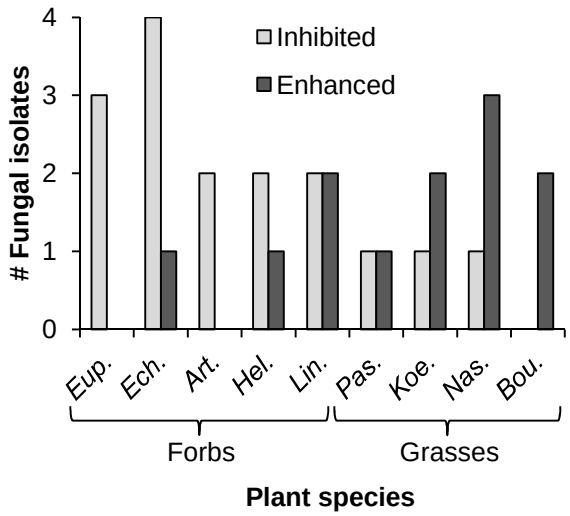


Figure 5-1. Number of fungal isolates exhibiting inhibition or enhancement for each plant species. A fungal isolate was included only if its growth was significantly less (inhibited) or greater (enhanced) than when exposed to other plant species exudates (determined via Tukey HSD tests within the GLMM). Bars represent the total number of fungi enhanced or inhibited.

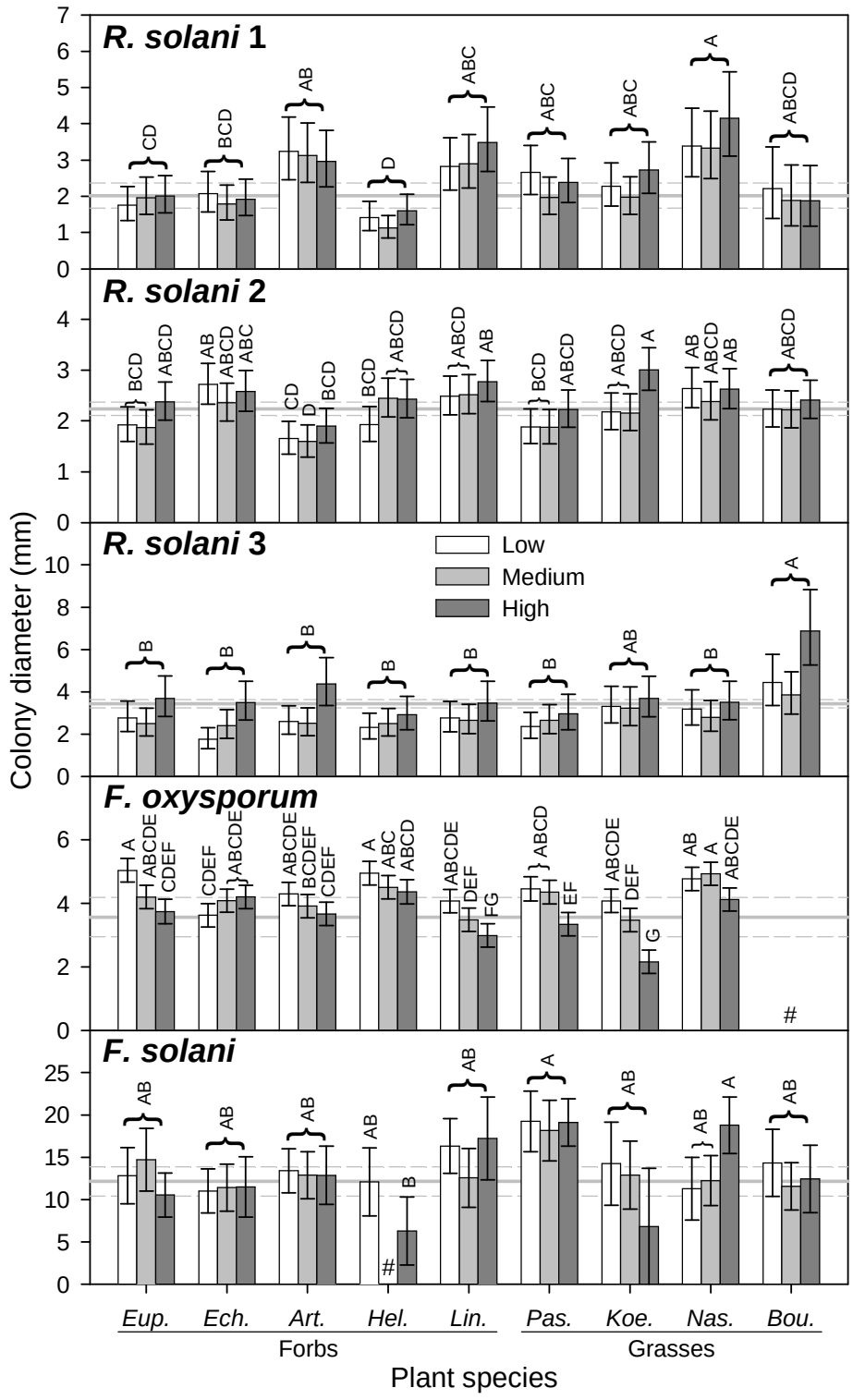

R. solani 1
R. solani 2
R. solani 3
F. oxysporum
F. solani
Low
Medium
High
Colony diameter (mm)
Eup.
Ech.
Art.
Hel.
Lin.
Pas.
Koe.
Nas.
Bou.
Forbs
Grasses
Plant species

Figure 5-2. Fungal colony diameters following exposure to varying plant species root exudate concentrations (low, medium and high exudate mass) by fungal isolate. Bars are least square mean estimates with confidence intervals ($\alpha = 0.05$), determined via GLMMs. Different letters above bars represent significantly different colony diameters via Tukey HSD tests. Control means ± one standard error represented by gray solid and dashed lines, respectively. Unavailable least square mean estimates are denoted by #.

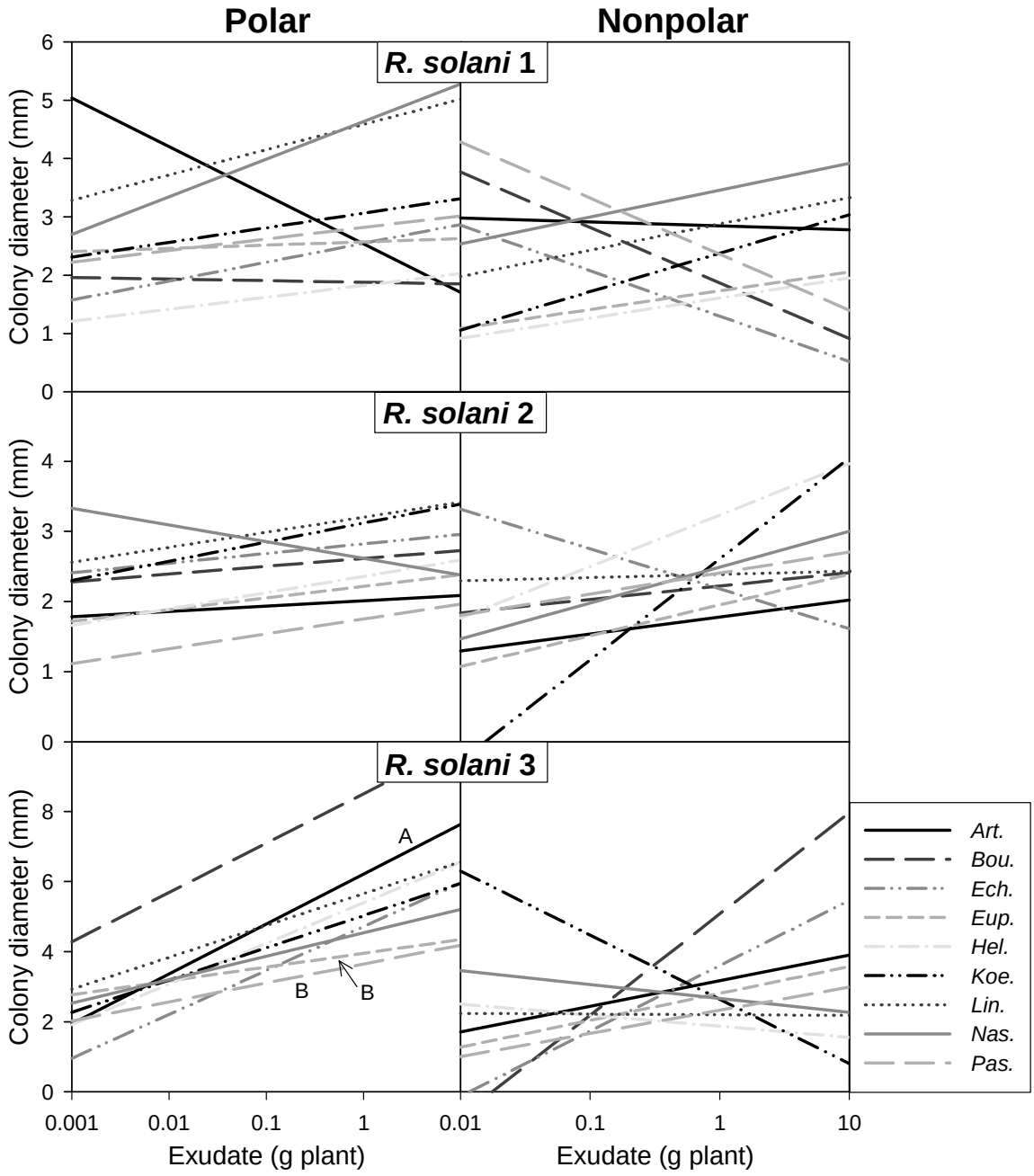


Figure 5-3. Linear influence of root exudate concentration and species identity on *R. solani* colony diameters by exudate polarity. Linear regressions are shown for each species (data points removed for clarification of lines), and the species with different letters have significantly different slopes, determined via Bonferroni corrected ANCOVA estimate comparisons. Horizontal and vertical axes are on same scale for

stacked plots. Horizontal axis represents the amount of exudate from a given plant mass on the log scale.

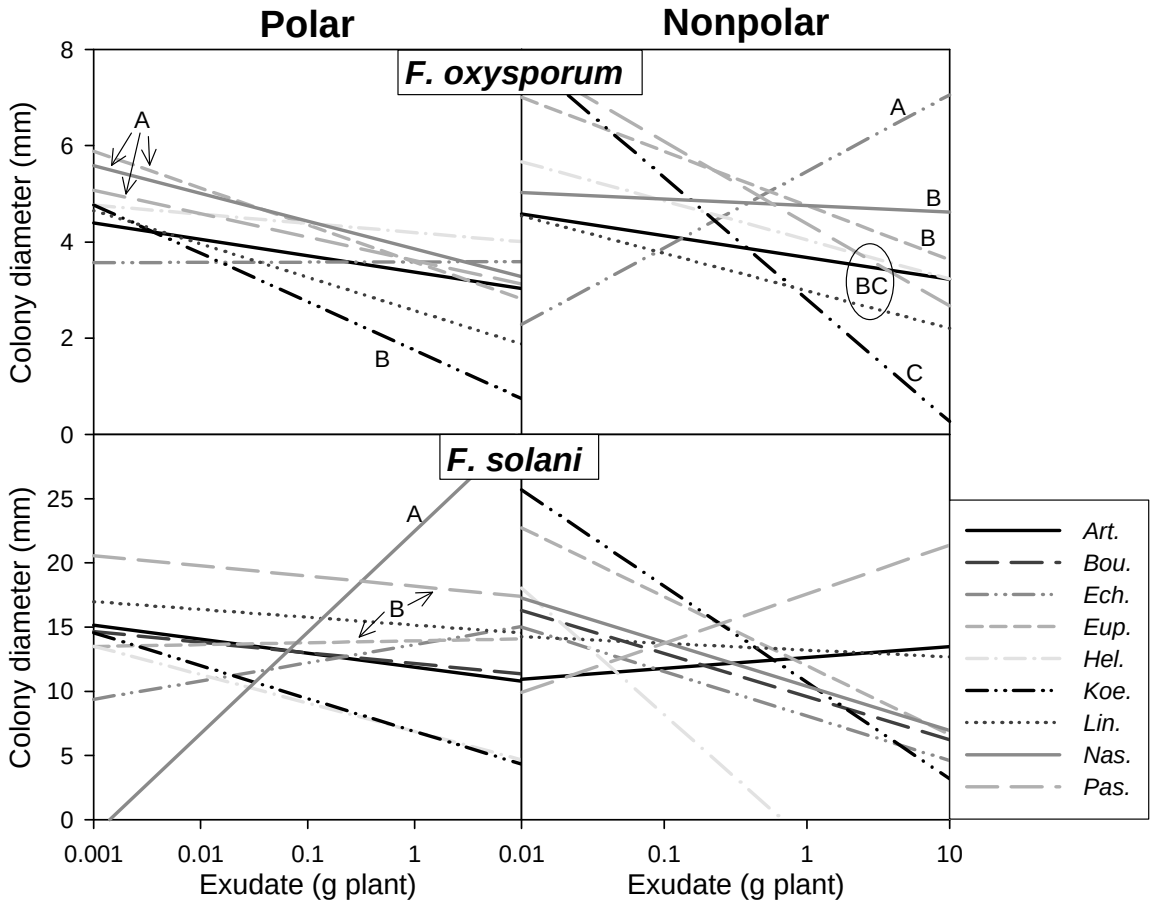


Figure 5-4. Linear influence of root exudate concentration and species identity on *Fusarium* spp. colony diameters by exudate polarity. Linear regression lines are shown for each species (data points removed for clarification of lines), and the species with different letters have significantly different slopes, determined via Bonferroni corrected ANCOVA estimate comparisons. Horizontal and vertical axes are on same scale for stacked plots. Horizontal axis represents the amount of exudate from a given plant mass on the log scale.

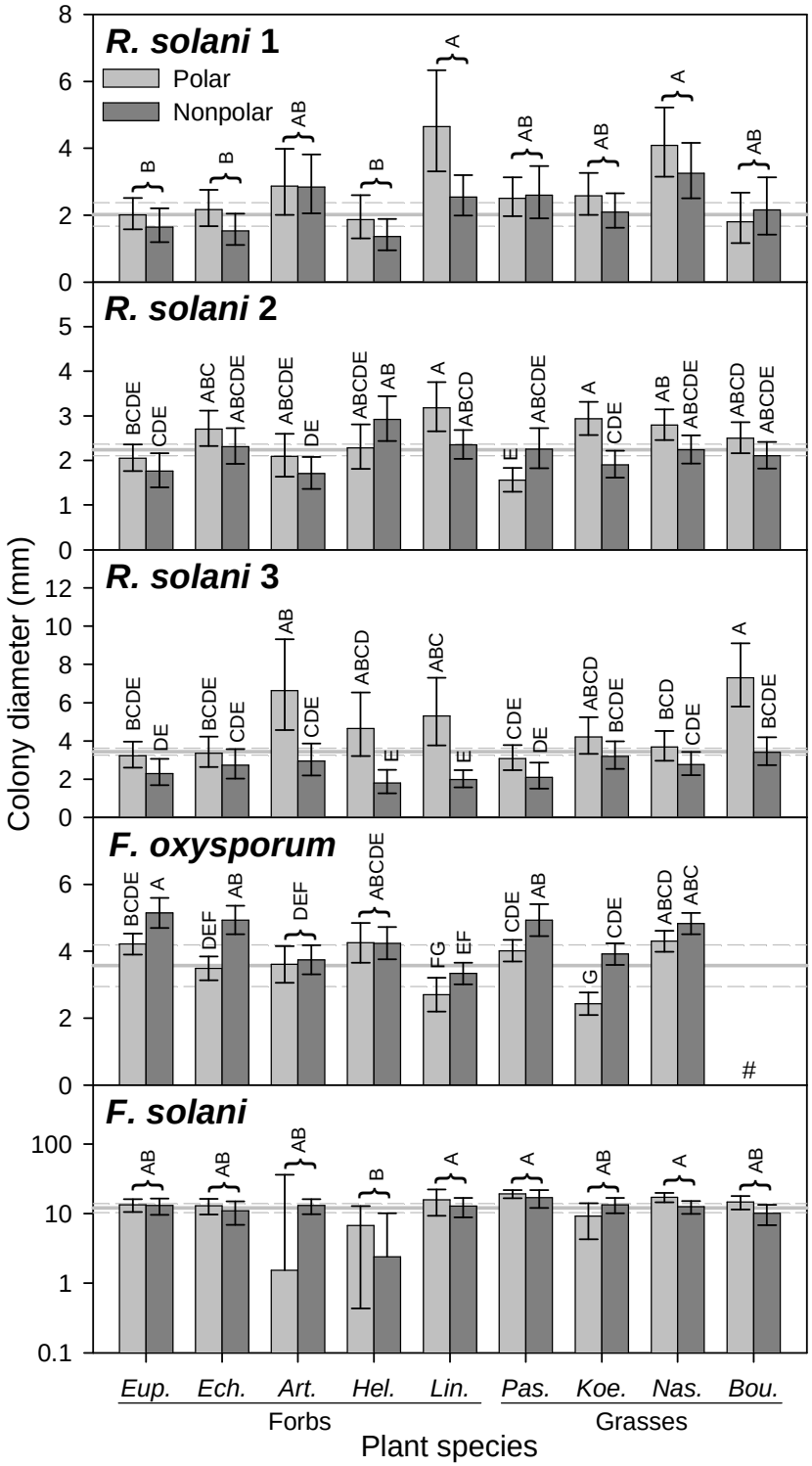


Figure 5-5. Fungal colony diameters following exposure to polar and nonpolar fractions of plant species root exudates by fungal isolate. Bars are least square mean estimates

with confidence intervals ($\alpha = 0.05$), determined via GLMMs. Different letters above bars represent significantly different colony diameters via Tukey HSD tests. Control means ± one standard error represented by gray solid and dashed lines, respectively. Unavailable least square mean estimates are denoted by #.

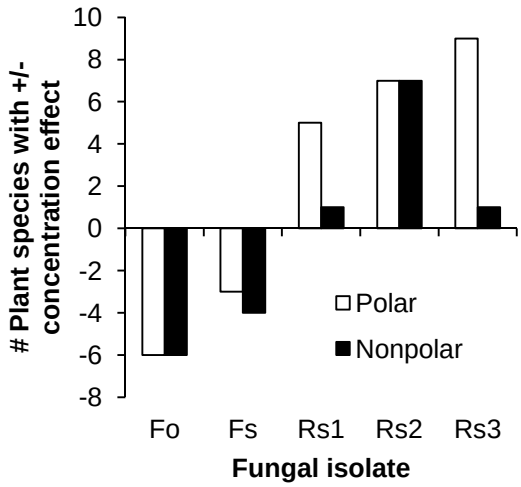


Figure 5-6. Number of plant species with negative or positive root exudate concentration effects on fungal growth by fungal isolate and exudate polarity. All species were included regardless of the significance of their exudate concentration-fungal growth relationships (determined via ANCOVA), and bars represent the number of species with a positive relationship minus the number with a negative relationship. Isolate codes: Rs = *R. solani*, Fo = *F. oxysporum*, Fs = *F. solani*.

Chapter 6:

Summary and Synthesis

I investigated the potential causes of reduced native plant recruitment in leafy spurge (*Euphorbia esula*) occupied areas of the northern Great Plains. Below my studies are summarized, and a synthesis of my findings is presented.

Euphorbia impacts on native seed banks

Native plant communities may have problems recovering in areas previously invaded by *Euphorbia* if the invasion reduces the presence of native seeds in the soil seed banks. To address this, I investigated how soil seed banks and standing vegetation at Theodore Roosevelt National Park vary among areas that had never been invaded by *Euphorbia*, previously invaded areas with subsequent occupation by native plants, and previously invaded areas with subsequent occupation by other exotic plants. The native seed banks were largely intact following *Euphorbia* invasion, while the standing vegetation was more impacted by invasion than the seed banks. This was the case in terms of abundance (seed bank density and vegetation cover), species richness, and species similarity among *Euphorbia* invasion histories. There were some detectable differences in seed banks among invasion histories in terms of community composition, but these differences were mostly due to the differential presence of a few species and did not indicate a depauperate native seed bank in previously invaded areas.

These findings support previous research on seed banks in the northern Great Plains which found desirable native plant seed banks in areas previously dominated by *Euphorbia* (Laufmann 2006, Cline et al. 2008, Setter and Lym 2013). This indicates that the slow recovery of native plant cover following *Euphorbia* invasion and control (Lesica and Hanna 2009, Butler and Wacker 2010) is probably not due to modified seed banks. However, areas previously invaded by *Euphorbia* are often occupied by other exotic plants, especially the grasses *Bromus inermis* and *Poa pratensis* (Larson and Larson 2010). Future research and management in areas where *Euphorbia* has been controlled should investigate restoration methods that can simultaneously improve the return of native plant species, and reduce secondary invasions of these exotic grasses.

Euphorbia legacy and flea beetle impacts on native plants

Native plant recovery after *Euphorbia* invasion and biocontrol application may also be hindered by changes in soil abiotic or biotic characteristics via *Euphorbia* soil occupation, or through nontarget effects from biocontrol insects. I evaluated the effects of historical and recent *Euphorbia* soil conditioning, and of flea beetle (*Aphthona* spp.) biocontrol presence, on subsequent native plant germination and growth. Native plant seed germination and growth were negatively affected by recent *Euphorbia* soil conditioning. These effects were predominantly not observed in individual species, but rather on the scale of the entire experiment when species were combined. There were also species-specific negative effects on native plant germination and growth in field soils previously invaded by *Euphorbia*, and these effects tended to be most negative in soils that had secondary invasions of exotic grasses. *Euphorbia* treated by flea beetles

had a positive, but non-significant, influence on native plant seed germination for most of the native species. Meanwhile, flea beetle presence on *Euphorbia* lead to detrimental effects on native plant biomass, root:shoot ratio, and height, with biomass and height impacts being species-specific. The two flea beetle species were not equivocal in their effects, however: *A. nigriscutis* was associated with more negative effects on plant species than *A. lacertosa*.

My research indicates that *Euphorbia* soil conditioning alone can have negative impacts on native plant establishment, and that flea beetles may have negative nontarget effects on native plants as well, which are both suggestive of changes in soil abiotic or biotic characteristics. This lends support to field observations of slow native plant recovery in areas where flea beetles were used to control *Euphorbia* (Lesica and Hanna 2009, Butler and Wacker 2010). The different severity of impacts between the two flea beetle species is compelling, especially since the most effective species at controlling *Euphorbia* (*A. lacertosa*) produced less negative nontarget effects on native plants. Equally compelling is my finding that soils from sites with secondary invasions of exotic grasses had the strongest negative impacts on native plants. These findings imply that native plant recruitment will be most successful when *A. lacertosa* biocontrol is used, and when exotic grasses can be prevented from occupying sites following *Euphorbia* control.

Pathogen and *Euphorbia* root exudate effects on native plants

Invasive plants may reduce the recruitment of co-occurring native plants by exuding compounds through their roots that are inhibitory to native plants (aka, novel

weapons) (Callaway and Ridenour 2004), or by stimulating pathogen accumulation in the soils they occupy (Eppinga et al. 2006). To determine if *Euphorbia* can exhibit these effects, I evaluated the relative impacts of *Euphorbia* root exudates, soilborne fungal plant pathogens, and exudates applied to the pathogens, on native plant germination and seedling growth. Germination rates were significantly reduced by *Euphorbia* root exudates for a majority of the native species tested. However, the combined influence of fungal pathogens and *Euphorbia* root exudates on native seed germination and seedling growth varied with fungal isolate and plant species, with two emergent patterns. One, germination rates were mostly increased by the application of both exudates and pathogens, but high inoculation densities and high exudate concentrations, when applied singly, generally reduced germination rates. This pattern was only found when all data were combined, not for individual species. Two, seedling biomass was either enhanced or reduced by a combination of exudates and pathogens, with the pattern frequently reversing between low and high density pathogens. This pattern depended on the plant species - fungal isolate combination.

My findings revealed that while *Euphorbia* root exudates may directly reduce native seed germination, the combined influence of exudates and fungal pathogens on native plants is more complicated. The enhancement of germination from exposure to both exudates and pathogens is opposite from my expectation, and may indicate that the fungi were growing saprotrophically on *Euphorbia* root exudates (Hunt et al. 1987) instead of attacking seeds. The combined effects of exudates and pathogens on seedling biomass, meanwhile, illustrates that there may be a high degree of specificity in

Euphorbia/pathogen interactive effects on native plant species. I did find some plant species whose biomass was negatively impacted by combined exposure to *Euphorbia* root exudates and particular fungal isolates. While this was not a direct test of the hypothesis that exotic plants can accumulate local pathogens in their rhizospheres (Eppinga et al. 2006), it does indicate that *Euphorbia* root exudates may exacerbate pathogen impacts on native plants.

Euphorbia vs. native plant root exudate effects on pathogens

Euphorbia root exudates can exacerbate the negative effects of soilborne fungal plant pathogens on native plant growth, as described in the previous section. However, do *Euphorbia* and native plant root exudates vary in their effects on fungal pathogen growth, and does pathogen growth vary with root exudate concentration? To answer these questions, I measured fungal pathogen growth in petri dishes when exposed to *Euphorbia* and native plant root exudates of varying concentrations. I found that *Euphorbia* root exudates did not stimulate fungal pathogen growth more than other species – in fact, they tended to inhibit fungal growth more than some native plant species. Plant species, in fact, was a strong determinant of fungal growth response to root exudates, and root exudates from grass species tended to produce larger fungal colonies than forb root exudates. Root exudate concentration was also strongly related to fungal growth, but this effect varied with fungal isolate – *Rhizoctonia* spp. growth increased with exudate concentration, while *Fusarium oxysporum* growth decreased with increasing exudates.

The inhibiting effect of *Euphorbia* root exudates on fungal pathogen growth compared to root exudates from some native plant species suggests that the negative interactive effect of *Euphorbia* exudates/fungal pathogens on native seedling biomass in my previous research was not due to pathogen growth enhancement. However, if *Euphorbia* root exudates are inhibitory to some fungal pathogens, this may provide a competitive advantage to *Euphorbia* in the habitats it invades. The strong influence of exudate concentration on fungal growth, meanwhile, indicates that the effects of plants on soil pathogen populations may be dependent on plant biomass or density.

Synthesis

While there are many factors that can potentially affect native plant recruitment in the northern Great Plains during and after *Euphorbia* occupation, my research has revealed some illuminating patterns. I found that, while there was not a reduction in native plant seed banks following *Euphorbia* invasion, these native seeds may experience conditions that suppress their germination and persist after *Euphorbia* removal. Reduced native seed germination was observed in three separate experiments either by *Euphorbia* conditioning or treatment with root exudates, lending strong support to the direct role of *Euphorbia* in reduced native plant recruitment. Field soils with a *Euphorbia* legacy also negatively impacted native plant germination and growth, but soils that were occupied by other invasives were particularly suppressive to select native plant species. This pattern is perhaps best supported by the seed bank and vegetation surveys, where I found no differences in germinable native seed banks

among invasion histories, yet the invaded-exotic sites had lower native vegetation richness than uninvaded sites.

Native plant responses to *Euphorbia* get considerably more interesting, and complicated, when fungal pathogens are present. The combination of *Euphorbia* root exudates and fungal pathogens, for example, actually *enhanced* native seed germination for most of the isolates. However, native seedling biomass did not follow the same pattern, having a broad range of responses that depended on isolate and plant species identity. A potential explanation is revealed by comparing seedling responses to exudates and fungi, against the fungal responses to *Euphorbia* exudates. The isolate *Rhizoctonia solani* 1, for example, exhibited more growth with increasing concentrations of *Euphorbia* and *Echinacea* root exudates (for both polar and nonpolar components). The biomass of *Echinacea* seedlings exposed to *R. solani* 1, meanwhile, decreased with increasing *Euphorbia* root exudates. This indicates that fungal pathogen growth can be stimulated by root exudates, and that this enhanced fungal presence can lead to reduced native plant seedling growth. The existence of this mechanism is further supported by native plants when exposed to *Fusarium oxysporum*. *Euphorbia* root exudates have a negative effect on *F. oxysporum* growth with increasing exudate concentrations, and increasing *Euphorbia* exudates were associated with higher rates of native seed germination, and larger *Echinacea* and *Vicia* seedling biomass, but only at the low pathogen density. In this case, *Euphorbia* exudates may have suppressed the growth of *F. oxysporum*, which allowed more growth in *Echinacea* seedlings. While these correlations between experiments did not always support the hypothesized

mechanism, the research I have conducted provides a baseline for more in-depth studies.

Future directions

Several lines of inquiry are needed to more fully understand the lack of native plant recruitment in landscapes previously occupied by *Euphorbia*. First, field experiments would help determine if native plant recruitment limitation in areas previously dominated by *Euphorbia* is a result of seed limitation. The fact that native seed banks in previously invaded areas are relatively intact does not necessarily mean that these habitats are not seed limited: many habitats are seed limited, including grasslands (Turnbull et al. 2000, Myers and Harms 2009). If these areas are seed limited, then seed addition may be an effective native plant restoration technique, as well as simultaneously reducing secondary invasions of exotic grasses. If seed limitation is not detected, then establishment limitation may be indicated instead, which would be informative towards other lines of inquiry (such as those below). This research could be accomplished by conducting a combination of seed rain, seed bank, and seed addition experiments in field settings with a variety of *Euphorbia* histories and standing vegetation types (similar to the seed bank study above).

Second, additional work is required to determine if *Euphorbia* is a successful invader due, in part, to novel weapons (for example, allelopathy), whether or not biocontrol organisms enhance this effect, and whether or not these weapons impact communities after *Euphorbia* has been controlled. I found that *Euphorbia* root exudates reduce native seed germination, but the mechanism behind this finding is unknown. To

determine the identity of a putative allelochemical, the various components of *Euphorbia* root exudates could be separated and identified via high-performance liquid chromatography, and the effect of the individual components could be tested on native seed germination and growth. A similar technique could also be used to determine if any components of dead *Euphorbia* tissue (or "cane") has an allelopathic effect on native plant germination and growth. Previous research on *Centaurea maculosa*, an invasive plant in North America, found that insect herbivory can enhance its production of the allelochemical (±)-catechin (Thelen et al. 2005). The potential effect of flea beetle herbivory on the production of allelochemicals could be examined by subjecting *Euphorbia* to flea beetles before and during the collection of root exudates. *Euphorbia* plants could also be subjected to artificial herbivory via removal of leaf tissue (simulating beetle herbivory) and puncturing roots (simulating larval herbivory) as a comparison against living beetle herbivory. However, allelopathic compounds may be broken down quickly by microbial action (Kaur et al. 2009), or microbes may be the target of allelochemicals, with indirect effects on native plants (Cipollini et al. 2012). Therefore, the persistence of potential allelochemicals should be examined when subjected to native soil microbial communities over time, and the effects of these compounds on the soil microbiota should be monitored along with their effects on native plants, as suggested by Bais et al. (2006).

Third, the accumulation of local pathogens hypothesis should be examined in more detail. *Euphorbia* rhizospheres can be occupied by enhanced fungal pathogen populations, and this effect appears to be greater when root-feeding herbivores are

present (Caesar 2003). However, the prevalence of this pattern, its mechanistic connection with herbivores, and whether or not this has a negative impact on native plant communities, is unclear. Field surveys of soilborne pathogen populations in areas with and without *Euphorbia* and flea beetles could be used to evaluate the first question, and whether or not it is linked to herbivory. If there are strong signatures of *Euphorbia*-pathogen connections in field settings, the mechanisms behind these interactions, and the effect they have on native plant communities, could be explored in more detail using mesocosms and lab analyses. For example, an outdoor mesocosm with *Euphorbia* planted into sterile and non-sterile field soils could be subjected to various flea beetle and soilborne fungal pathogen treatments, with native seeds sown into adjacent soils. Testing the accumulation of local pathogens hypothesis would require monitoring native plant germination, growth and signs of infection, using Koch's postulates to confirm pathogen identity in infected plants, and assaying soils for change in pathogen populations.

Fourth, the association of *Euphorbia* with arbuscular mycorrhizal fungi (AMF), and the potential for AMF-derived protection from fungal pathogens (Vierheilig et al. 2008) could also be an interesting area for future research. Despite the low proportions of AMF associations with plants in my research, other studies indicate that *Euphorbia* is a good host for AMF, and that interactions of native and exotic plants with AMF are very important in *Euphorbia*-invaded areas (Jordan et al. 2011, Jordan et al. 2012). *Euphorbia* is also easily infected with a range of native fungal plant pathogens (Caesar 1994, 1996), some of which are also harmful to native plants (Caesar et al. 1999). If

Euphorbia hosts AMF that provides protection from fungal pathogens, then it may have a competitive advantage against native species that do not have this protection. To test this hypothesis, soils of potted *Euphorbia* and native plants could be inoculated with several AMF species, and subsequently inoculated with fungal pathogens. If *Euphorbia* plants show fewer signs of pathogen infection and have reduced rates of mortality and biomass loss, then the hypothesis would be supported.

Conclusion

My research has not only revealed some potential explanations for reduced native plant recovery following the control of *Euphorbia*, but it has also provided some interesting insights into invasive plant ecology. Future research should further explore the interactions of *Euphorbia* and the soil biotic community, including the potential direct and indirect effects of biocontrol agents on soil organisms, and how these factors influence subsequent native plant recruitment. In addition, placing the research in a restoration context will help improve management of affected wildlands in the Great Plains.

Bibliography

Abella, S. R., L. P. Chiquoine, and D. M. Backer. 2012. Ecological characteristics of stes invaded by buffelgrass (*Pennisetum ciliare*). Invasive Plant Science and Management **5**:443-453.

Aerts, R., A. Huiszoon, J. H. A. VanOostrum, C. VandeVijver, and J. H. Willems. 1995. The potential for heathland restoration on formerly arable land at a site in Drenthe, The Netherlands. Journal of Applied Ecology **32**:827-835.

Aldrich-Wolfe, L. 2007. Distinct mycorrhizal communities on new and established hosts in a trasitional tropical plant community. Ecology **88**:559-566.

Andersen, Ø. M. and K. R. Markham, editors. 2006. Flavonoids: Chemistry, biochemistry and applications. Taylor & Francis, New York, NY.

Anderson, G. L. 1995. Using remote sensing and Geographic Information Systems for mapping noxious weed infestations within North Dakota. Pages 28-32 *in* Proceedings of the Alien Plant Invasions: Increasing Deterioration of Rangeland Ecosystem Health Symposium. Bureau of Land Management, Phoenix, AZ.

Anderson, G. L. 1998. South Unit - 1993 leafy spurge inventory (trsuspgp).*in* USDA-ARS, editor., Weslaco, Texas.

Anderson, G. L., J. H. Everitt, D. E. Escobar, N. R. Spencer, and R. J. Andrascik. 1996. Mapping leafy spurge (*Euphorbia esula*) infestations using aerial photography and geographic information systems. Geocarto International **11**:81-89.

Anderson, G. L., R. F. Kokaly, S. N. Hager, and R. R. Root. 2004. Evaluating leafy spurge change over a ten-year period in Theodore Roosevelt National Park using multiple aerial imaging platforms. TEAM Leafy Spurge Symposium, Society for Range Management 57th Annual Meeting. TEAM Leafy Spurge, Agricultural Research Service, U.S. Department of Agriculture, Salt Lake City, Utah.

Andrascik, R. J. 1994. A post workshop review: discussion, process, and highlights for developing a leafy spurge strategic management plan within Theodore Roosevelt National Park. Pages 109-117 Leafy Spurge Strategic Planning Workshop, Dickinson, North Dakota.

Anscombe, F. J. 1948. The transformation of Poisson, binomial and negative-binomial data. Biometrika **35**:246-254.

Aulakh, M. S., R. Wassmann, C. Bueno, J. Kreuzwieser, and H. Rennenberg. 2001. Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. Plant Biology **3**:139-148.

Aziz, N. H., M. Z. ElFouly, A. A. ElEssawy, and M. A. Khalaf. 1997. Influence of bean seedling root exudates on the rhizosphere colonization by Trichoderma lignorum for the control of *Rhizoctonia solani*. Botanical Bulletin of Academia Sinica **38**:33-39.

Baer, S. G., S. L. Collins, J. M. Blair, A. K. Knapp, and A. K. Fiedler. 2005. Soil heterogeneity effects on tallgrass prairie community heterogeneity: An

application of ecological theory to restoration ecology. Restoration Ecology **13**:413-424.
Bai, W.-J., J. Mitchley, and J.-Y. Jiao. 2010. Soil seed bank and standing vegetation of abandoned croplands on Chinese Loess Plateau: implications for restoration. Arid Land Research and Management **24**:98-116.
Bais, H. P., S.-W. Park, T. L. Weir, R. M. Callaway, and J. M. Vivanco. 2004. How plants communicate using the underground information superhighway. Trends in Plant Science **9**:26-32.
Bais, H. P., B. Prithiviraj, A. K. Jha, F. M. Ausubel, and J. M. Vivanco. 2005. Mediation of pathogen resistance by exudation of antimicrobials from roots. Nature **434**:217-221.
Bais, H. P., R. Vepachedu, S. Gilroy, R. M. Callaway, and J. M. Vivanco. 2003. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. Science **301**:1377-1380.
Bais, H. P., T. L. Weir, L. G. Perry, S. Gilroy, and J. M. Vivanco. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology **57**:233-266.
Bakke, A. L. 1936. Leafy spurge, *Euphorbia esula* L. Iowa Agricultural Research Bulletin **198**:209-245.
Bakker, J., P. Poschlod, R. Strykstra, R. Bekker, and K. Thompson. 1996. Seed banks and seed dispersal: important topics in restoration ecology. Acta Botanica Neerlandica **45**:461-490.
Bakker, J. P. and F. Berendse. 1999. Constraints in the restoration of ecological diversity in grassland and heathland communities. Trends in Ecology & Evolution **14**:63-68.
Bansal, R. C. and M. Goyal. 2005. Activated carbon adsorption. Taylor & Francis, Boca Raton.
Bartelt-Ryser, J., J. Joshi, B. Schmid, H. Brandl, and T. Balser. 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. Perspectives in Plant Ecology Evolution and Systematics **7**:27-49.
Barto, E., P. Antunes, K. Stinson, A. Koch, J. Klironomos, and D. Cipollini. 2011. Differences in arbuscular mycorrhizal fungal communities associated with sugar maple seedlings in and outside of invaded garlic mustard forest patches. Biological Invasions **13**:2755-2762.
Bartuszevige, A. M. and B. A. Endress. 2008. Do ungulates facilitate native and exotic plant spread?: Seed dispersal by cattle, elk and deer in northeastern Oregon. Journal of Arid Environments **72**:904-913.
Baskin, C. C. and J. M. Baskin. 1998. Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego.
Beesley, L., E. Moreno-Jiménez, J. L. Gomez-Eyles, E. Harris, B. Robinson, and T. Sizmur. 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environmental Pollution **159**:3269-3282.

Bekker, R. M., G. L. Verweij, R. E. N. Smith, R. Reine, J. P. Bakker, and S. Schneider. 1997. Soil seed banks in European grasslands: Does land use affect regeneration perspectives? Journal of Applied Ecology **34**:1293-1310.

Belcher, J. W. and S. D. Wilson. 1989. Leafy spurge and the species composition of a mixed-grass prairie. Journal of Range Management **42**:172-175.

Benvenuti, S. 2003. Soil texture involvement in germination and emergence of buried weed seeds. Agron. J. **95**:191-198.

Bever, J. D. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytologist **157**:465-473.

Blossey, B. and R. Notzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. Journal of Ecology **83**:887-889.

Blumenthal, D., C. E. Mitchell, P. Pyšek, and V. Jarošík. 2009. Synergy between pathogen release and resource availability in plant invasion. Proceedings of the National Academy of Sciences **106**:7899-7904.

Blumenthal, D. M., N. R. Jordan, and E. L. Svenson. 2003. Weed control as a rationale for restoration: The example of tallgrass prairie. Conservation Ecology **7**:-.

Bossuyt, B. and M. Hermy. 2003. The potential of soil seedbanks in the ecological restoration of grassland and heathland communities. Belgian Journal of Botany **136**:23-34.

Bossuyt, B. and O. Honnay. 2008. Can the seed bank be used for ecological restoration? An overview of seed bank characteristics in European communities. Journal of Vegetation Science **19**:875-884.

Bouarab, K., R. Melton, J. Peart, D. Baulcombe, and A. Osbourn. 2002. A saponin-detoxifying enzyme mediates suppression of plant defences. Nature **418**:889-892.

Bowes, G. G. and A. G. Thomas. 1978. Longevity of leafy spurge seeds in the soil following various control programs. Journal of Range Management **31**:137-140.

Bray, J. R. and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs **27**:326-349.

Broeckling, C. D., A. K. Broz, J. Bergelson, D. K. Manter, and J. M. Vivanco. 2008. Root exudates regulate soil fungal community composition and diversity. Appl. Environ. Microbiol. **74**:738-744.

Bruehl, G. W. 1987. Soilborne plant pathogens. Macmillan Pub. Co., New York.

Butler, J. L. and D. R. Cogan. 2004. Leafy spurge effects on patterns of plant species richness. Journal of Range Management **57**:305-311.

Butler, J. L., M. S. Parker, and J. T. Murphy. 2006. Efficacy of flea beetle control of leafy spurge in Montana and South Dakota. Rangeland Ecology & Management **59**:453-461.

Butler, J. L. and S. D. Wacker. 2010. Lack of native vegetation recovery following biological control of leafy spurge. Rangeland Ecology & Management **63**:553-563.

Caesar, A. J. 1994. Comparative virulence of strains of *Rhizoctonia* spp. on leafy spurge (*Euphorbia esula*) and disease reactions of cultivated plants in the greenhouse. Plant Disease **78**:183-186.

Caesar, A. J. 1996. Identity, pathogenicity, and comparative virulence of *Fusarium* spp. related to stand declines of leafy spurge (*Euphorbia esula*) in the northern plains. Plant Disease **80**:1395-1398.

Caesar, A. J. 2003. Synergistic interaction of soilborne plant pathogens and root-attacking insects in classical biological control of an exotic rangeland weed. Biological Control **28**:144-153.

Caesar, A. J., G. Campobasso, and G. Terragitti. 1999. Effects of European and U.S. strains of *Fusarium* spp. pathogenic to leafy spurge on North American grasses and cultivated species. Biological Control **15**:130-136.

Caesar, A. J., N. E. Rees, N. R. Spencer, and P. C. Quimby. 1993. Characterization of *Rhizoctonia* spp. causing disease of leafy spurge in the Northern Plains. Plant Disease **77**:681-684.

Callaway, R. M., T. H. DeLuca, and W. M. Belliveau. 1999. Biological-control herbivores may increase competitive ability of the noxious weed *Centaurea maculosa*. Ecology **80**:1196-1201.

Callaway, R. M. and W. M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment **2**:436-443.

Callaway, R. M., G. C. Thelen, A. Rodriguez, and W. E. Holben. 2004. Soil biota and exotic plant invasion. Nature **427**:731-733.

Cantor, A., A. Hale, J. Aaron, M. Traw, and S. Kalisz. 2011. Low allelochemical concentrations detected in garlic mustard-invaded forest soils inhibit fungal growth and AMF spore germination. Biological Invasions **13**:3015-3025.

Carling, D. E. and D. R. Sumner. 1992. *Rhizoctonia*. Pages 157-165 *in* L. L. Singleton, J. D. Mihail, and C. M. Rush, editors. Methods for research on soilborne phytopathogenic fungi. APS Press, St. Paul, Minn.

Chancellor, R. J. 1966. The identification of weed seedlings of farm and garden. Blackwell Scientific, Oxford.

Chou, Y.-M., A. M. Polansky, and R. L. Mason. 1998. Transforming non-normal data to normality in statistical process control. Journal of Quality Technology **30**:133.

Cione, N. K., P. E. Padgett, and E. B. Allen. 2002. Restoration of a native shrubland impacted by exotic grasses, frequent fire, and nitrogen deposition in southern California. Restoration Ecology **10**:376-384.

Cipollini, D., C. Rigsby, and E. Barto. 2012. Microbes as Targets and Mediators of Allelopathy in Plants. Journal of Chemical Ecology **38**:714-727.

Clewley, G. D., R. Eschen, R. H. Shaw, and D. J. Wright. 2012. The effectiveness of classical biological control of invasive plants. Journal of Applied Ecology **49**:1287-1295.

Cline, D., C. Juricek, R. G. Lym, and D. R. Kirby. 2008. Leafy spurge (*Euphorbia esula*) control with *Aphthona* spp. affects seedbank composition and native grass reestablishment. Invasive Plant Science and Management **1**:120-132.

Colautti, R. I., A. Ricciardi, I. A. Grigorovich, and H. J. MacIsaac. 2004. Is invasion success explained by the enemy release hypothesis? Ecology Letters **7**:721-733.

Connell, J. H. 1970. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Pages 298-312 *in* P. J. den Boer and G. R. Gradwell, editors. Dynamics of populations. Center for Agricultural Publication and Documentation, Wageningen, The Netherlands.

Corbin, J. D. and C. M. D'Antonio. 2004. Effects of exotic species on soil nitrogen cycling: Implications for restoration. Weed Technology **18**:1464-1467.

Cornett, M. W., P. J. Bauman, and D. D. Breyfogle. 2006. Can we control leafy spurge? Adaptive management and the recovery of native vegetation. Ecological Restoration **24**:145-150.

Coupland, R. T. and J. F. Alex. 1954. Distribution of the underground parts of leafy spurge (*Euphorbia esula* L.). Canadian Journal of Agricultural Science **34**:161-176.

Cox, R. and E. Allen. 2008. Composition of soil seed banks in southern California coastal sage scrub and adjacent exotic grassland. Plant Ecology **198**:37-46.

Crimmins, T. M. and G. R. McPherson. 2008. Vegetation and seedbank response to *Eragrostis lehmanniana* removal in semi-desert communities. Weed Research **48**:542-551.

Cuevas, Y. A. and S. M. Zalba. 2010. Recovery of native grasslands after removing invasive pines. Restoration Ecology **18**:711-719.

Curl, E. A. and B. Truelove. 1986. The rhizosphere. Springer-Verlag, Berlin ; New York.

Currie, A. F., P. J. Murray, and A. C. Gange. 2006. Root herbivory by *Tipula paludosa* larvae increases colonization of *Agrostis capillaris* by arbuscular mycorrhizal fungi. Soil Biology & Biochemistry **38**:1994-1997.

Denton, C. S., R. D. Bardgett, R. Cook, and P. J. Hobbs. 1999. Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. Soil Biology & Biochemistry **31**:155-165.

Dickson, T., J. Hopwood, and B. Wilsey. 2012. Do priority effects benefit invasive plants more than native plants? An experiment with six grassland species. Biological Invasions **14**:2617-2624.

DiTomaso, J. M. and E. A. Healy. 2007. Weeds of California and other western states. 1st edition. University of California, Agriculture and Natural Resources, Oakland, CA.

Drake, D. R. 1998. Relationships among the seed rain, seed bank and vegetation of a Hawaiian forest. Journal of Vegetation Science **9**:103-112.

Dremann, C. C. and M. Shaw. 2002. Releasing the native seedbank: An innovative approach to restoring a coastal California ecosystem. Ecological Restoration **20**:103-107.

Duncan, C. A., J. J. Jachetta, M. L. Brown, V. F. Carrithers, J. K. Clark, J. M. DiTomaso, R. G. Lym, K. C. McDaniel, M. J. Renz, and P. M. Rice. 2004. Assessing the economic, environmental, and societal losses from invasive plants on rangeland and wildlands. Weed Technology **18**:1411-1416.

Dunn, P. H. 1985. Origins of leafy spurge in North America. Pages 7-13 *in* A. K. Watson, editor. Leafy spurge. Weed Science Society of America, Champaign, IL.

Dutoit, T. and D. Alard. 1995. Permanent seed banks in chalk grassland under various management regimes: their role in the restoration of species-rich plant communities. Biodiversity and Conservation **4**:939-950.

Elgersma, K., J. Ehrenfeld, S. Yu, and T. Vor. 2011. Legacy effects overwhelm the short-term effects of exotic plant invasion and restoration on soil microbial community structure, enzyme activities, and nitrogen cycling. Oecologia **167**:733-745.

Elton, C. S. 1958. The ecology of invasions by animals and plants. Chapman and Hall, New York.

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.

Eppinga, M. B., M. A. Kaproth, A. R. Collins, and J. Molofsky. 2011. Litter feedbacks, evolutionary change and exotic plant invasion. Journal of Ecology **99**:503-514.

Eppinga, M. B., M. Rietkerk, S. C. Dekker, P. C. De Ruiter, and W. H. Van der Putten. 2006. Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. Oikos **114**:168-176.

Evans, R. A. and J. A. Young. 1975. Enhancing germination of dormant seeds of downy brome. Weed Science **23**:354-357.

Fagan, K., R. Pywell, J. Bullock, and R. Marrs. 2010. The seed banks of English lowland calcareous grasslands along a restoration chronosequence. Plant Ecology **208**:199-211.

Fahnestock, J. T., D. L. Larson, G. E. Plumb, and J. K. Detling. 2003. Effects of ungulates and prairie dogs on seed banks and vegetation in a North American mixed-grass prairie. Plant Ecology **167**:255-268.

Fenner, M. 1985. Seed ecology. Chapman and Hall, London ; New York.

Fenner, M. 1995. Ecology of seed banks. Pages 507-523 *in* J. Kigel and G. Galili, editors. Seed development and germination. M. Dekker, New York.

Fisher, J. L., W. A. Loneragan, K. Dixon, and E. J. Veneklaas. 2009. Soil seed bank compositional change constrains biodiversity in an invaded species-rich woodland. Biological Conservation **142**:256-269.

Folmar, L. C., H. O. Sanders, and A. M. Julin. 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Archives of Environmental Contamination and Toxicology **8**:269-278.

Fornasari, L. 1993. Life-history of the flea beetle, *Aphthona abdominalis* Duftschmid, on *Euphorbia esula* L (leafy spurge) in Italy. Biological Control **3**:161.

Garett, S. D. 1962. Decomposition of cellulose in soil by *Rhizoctonia solani* Kühn. Transactions of the British Mycological Society **45**:115-120.

Gassmann, A., D. Schroeder, E. Maw, and G. Sommer. 1996. Biology, ecology, and host specificity of European *Aphthona* spp (Coleoptera, Chrysomelidae) used as

biocontrol agents for leafy spurge, *Euphorbia esula* (Euphorbiaceae), in North America. Biological Control **6**:105-113.
Ghini, R. and M. A. B. Morandi. 2006. Biotic and abiotic factors associated with soil suppressiveness to *Rhizoctonia solani*. Scientia Agricola **63**:153-160.
Giantomasi, A., P. A. Tecco, G. Funes, D. E. Gurvich, and M. Cabido. 2008. Canopy effects of the invasive shrub *Pyracantha angustifolia* on seed bank composition, richness and density in a montane shrubland (Córdoba, Argentina). Austral Ecology **33**:68-77.
Gioria, M. and B. Osborne. 2010. Similarities in the impact of three large invasive plant species on soil seed bank communities. Biological Invasions **12**:1671-1683.
Granato, T. C., W. L. Banwart, P. M. Porter, and J. J. Hassett. 1983. Effect of variety and stage of growth on potential allelochemic compounds in soybean roots. Journal of Chemical Ecology **9**:1281-1292.
Grayston, S. J., D. Vaughan, and D. Jones. 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology **5**:29-56.
Grilz, P. L. and J. T. Romo. 1995. Management considerations for controlling smooth brome on fescue prairie. Natural Areas Journal **15**:148-156.
Grime, J. P. 1981. The role of seed dormancy in vegetation dynamics. Annals of Applied Biology **98**:555-558.
Hager, S. 1998. South Unit biocontrol sites.*in* Theodore Roosevelt National Park, editor., Medora, North Dakota.
Hao, J. J., M. E. Yang, and R. M. Davis. 2009. Effect of soil inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* Race 4 on disease development in cotton. Plant Disease **93**:1324-1328.
Hao, W.-y., L.-x. Ren, W. Ran, and Q.-r. Shen. 2010. Allelopathic effects of root exudates from watermelon and rice plants on *Fusarium oxysporum* f.sp. *niveum*. Plant and Soil **336**:485-497.
Harbour, J. D. 1992. Vesicular-arbuscular mycorrhizae and leafy spurge association. University of Wyoming, Laramie, Laramie, Wyoming.
Hartnett, D. C., R. J. Samenus, L. E. Fischer, and B. A. D. Hetrick. 1994. Plant demographic responses to mycorrhizal symbiosis in tallgrass prairie. Oecologia **99**:21-26.
Henderson, D. C. and M. A. Naeth. 2005. Multi-scale impacts of crested wheatgrass invasion in mixed-grass prairie. Biological Invasions **7**:639-650.
Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Page 32 *in* U. o. C. The College of Agriculture, Berkeley, editor. California Agricultural Experiment Station.
Hodur, N. M., F. L. Leistritz, and D. A. Bangsund. 2006. Biological control of leafy spurge: utilization and implementation. Rangeland Ecology & Management **59**:445-452.
Holl, K. D., H. N. Steele, M. H. Fusari, and L. R. Fox. 2000. Seed banks of maritime chaparral and abandoned roads: potential for vegetation recovery. Journal of the Torrey Botanical Society **127**:207-220.

Holland, J. N., W. X. Cheng, and D. A. Crossley. 1996. Herbivore-induced changes in plant carbon allocation: Assessment of below-ground C fluxes using carbon-14. Oecologia **107**:87-94.

Holmes, P. M. 2002. Depth distribution and composition of seed-banks in alien-invaded and uninvaded fynbos vegetation. Austral Ecology **27**:110-120.

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.

Hutchings, M. J. and K. D. Booth. 1996. Studies on the feasibility of re-creating chalk grassland vegetation on ex-arable land .1. The potential roles of the seed bank and the seed rain. Journal of Applied Ecology **33**:1171-1181.

Inderjit. 2001. Soil: Environmental effects on allelochemical activity. Agron. J. **93**:79-84.

Inderjit and R. M. Callaway. 2003. Experimental designs for the study of allelopathy. Plant and Soil **256**:1-11.

Inderjit and W. H. van der Putten. 2010. Impacts of soil microbial communities on exotic plant invasions. Trends in Ecology & Evolution **25**:512-519.

Jalali, B. and D. Suryanarayana. 1971. Shift in the carbohydrate spectrum of root exudates of wheat in relation to its root-rot disease. Plant and Soil **34**:261-267.

Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. The American Naturalist **104**:501-528.

Jarosz, A. M. and A. L. Davelos. 1995. Tansley Review No. 81. Effects of Disease in Wild Plant Populations and the Evolution of Pathogen Aggressiveness. New Phytologist **129**:371-387.

Johnson, D., R. E. Booth, A. S. Whiteley, M. J. Bailey, D. J. Read, J. P. Grime, and J. R. Leake. 2003. Plant community composition affects the biomass, activity and diversity of microorganisms in limestone grassland soil. European Journal Of Soil Science **54**:671-678.

Johnson, N. L. 1949. Systems of frequency curves generated by methods of translation. Biometrika **36**:149-176.

Johnston, H. H. 1962. The decomposition of cellulose by soil fungi. The Ohio Journal of Science **62**:108-112.

Jones, D. L., A. Hodge, and Y. Kuzyakov. 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist **163**:459-480.

Jonsen, I. D., R. S. Bourchier, and J. Roland. 2001. The influence of matrix habitat on *Aphthona* flea beetle immigration to leafy spurge patches. Oecologia **127**:287-294.

Jordan, N., D. Larson, and S. Huerd. 2008. Soil modification by invasive plants: effects on native and invasive species of mixed-grass prairies. Biological Invasions **10**:177-190.

Jordan, N. R., L. Aldrich-Wolfe, S. C. Huerd, D. L. Larson, and G. Muehlbauer. 2012. Soil–occupancy effects of invasive and native grassland plant species on composition and diversity of mycorrhizal associations. Invasive Plant Science and Management **5**:494-505.

Jordan, N. R., D. L. Larson, and S. C. Huerd. 2011. Evidence of qualitative differences between soil-occupancy effects of invasive vs. native grassland plant species. Invasive Plant Science and Management **4**:11-21.

Joshi, A. 2008. Integrating flea beetles (*Aphthona* spp.) with herbicide and grasses for leafy spurge (*Euphorbia esula*) management. Weed Technology **22**:523-529.

Kalamees, R., K. Püssa, K. Zobel, and M. Zobel. 2012. Restoration potential of the persistent soil seed bank in successional calcareous (alvar) grasslands in Estonia. Applied Vegetation Science **15**:208-218.

Kalischuk, A. R., R. S. Bourchier, and A. S. McClay. 2004. Post hoc assessment of an operational biocontrol program: efficacy of the flea beetle *Aphthona lacertosa* Rosenhauer (Chrysomelidae : Coleoptera), an introduced biocontrol agent for leafy spurge. Biological Control **29**:418-426.

Karl, M. G., R. K. Heitschmidt, and M. R. Haferkamp. 1999. Vegetation biomass dynamics and patterns of sexual reproduction in a northern mixed-grass prairie. The American Midland Naturalist **141**:227-237.

Kaur, H., R. Kaur, S. Kaur, I. T. Baldwin, and Inderjit. 2009. Taking ecological function seriously: soil microbial communities can obviate allelopathic effects of released metabolites. PLoS ONE **4**:6.

Keane, R. M. and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution **17**:164-170.

Keddy, P. 1992. Assembly and response rules: two goals for predictive community ecology. Journal of Vegetation Science **3**:157-164.

Kenward, M. G. and J. H. Roger. 1997. Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood. Biometrics **53**:983-997.

Kettenring, K. M. and C. R. Adams. 2011. Lessons learned from invasive plant control experiments: a systematic review and meta-analysis. Journal of Applied Ecology **48**:970-979.

Kirby, D. R., R. G. Lym, J. J. Sterling, and C. H. Sieg. 2003. Observation: Leafy spurge control in western prairie fringed orchid habitat. Journal of Range Management **56**:466-473.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature **6884**:67-69.

Kokaly, R. F., G. L. Anderson, R. R. Root, K. E. Brown, C. S. Mladinich, S. N. Hager, and K. B. Dudek. 2004. Mapping leafy spurge by identifying signatures of vegetation field spectra in Compact Airborne Spectrographic Imager (CASI) data. TEAM Leafy Spurge Symposium, Society for Range Management 57th Annual Meeting, Salt Lake City, Utah.

Kulmatiski, A. 2011. Changing Soils to Manage Plant Communities: Activated Carbon as a Restoration Tool in Ex-arable Fields. Restoration Ecology **19**:102-110.

Kulmatiski, A. and K. H. Beard. 2006. Activated carbon as a restoration tool: potential for control of invasive plants in abandoned agricultural fields. Restoration Ecology **14**:251-257.

Kumar, L., R. G. Chaudhary, N. Shukla, and R. K. Prajapati. 2010. Isolation of sorghum allelochemicals and their efficacy against crops pathogens. Allelopathy Journal **25**:369-381.

Kummer, A. P. 1951. Weed seedlings. University of Chicago Press, Chicago, Illinois.

Lagrange, H., C. Jay-Allgmand, and F. Lapeyrie. 2001. Rutin, the phenolglycoside from eucalyptus root exudates, stimulates *Pisolithus* hyphal growth at picomolar concentrations. New Phytologist **149**:349-355.

Lambert, D. 1992. Zero-Inflated Poisson Regression, with an Application to Defects in Manufacturing. Technometrics **34**:1-14.

Larson, D. L. and J. B. Grace. 2004. Temporal dynamics of leafy spurge (*Euphorbia esula*) and two species of flea beetles (*Aphthona* spp.) used as biological control agents. Biological Control **29**:207-214.

Larson, D. L., J. B. Grace, and J. L. Larson. 2008. Long-term dynamics of leafy spurge (*Euphorbia esula*) and its biocontrol agent, flea beetles in the genus *Aphthona*. Biological Control **47**:250-256.

Larson, D. L., J. B. Grace, P. A. Rabie, and P. Andersen. 2007. Short-term disruption of a leafy spurge (*Euphorbia esula*) biocontrol program following herbicide application. Biological Control **40**:1-8.

Larson, D. L. and J. L. Larson. 2010. Control of one invasive plant species allows exotic grasses to become dominant in northern Great Plains grasslands. Biological Conservation **143**:1901-1910.

Larson, D. L., R. A. Royer, and M. R. Royer. 2006. Insect visitation and pollen deposition in an invaded prairie plant community. Biological Conservation **130**:148-159.

Laufmann, J. A. 2006. Biological control affects native vegetation and recovery potential in Theodore Roosevelt National Park, North Dakota. Colorado State University, Fort Collins, CO, USA.

Leger, E. A. and K. J. Rice. 2003. Invasive California poppies (Eschscholzia californica Cham.) grow larger than native individuals under reduced competition. Ecology Letters **6**:257-264.

Lehmann, J., M. C. Rillig, J. Thies, C. A. Masiello, W. C. Hockaday, and D. Crowley. 2011. Biochar effects on soil biota – A review. Soil Biology and Biochemistry **43**:1812-1836.

Leistritz, F. L., D. A. Bangsund, and N. M. Hodur. 2004. Assessing the economic impact of invasive weeds: The case of leafy spurge (*Euphorbia esula*). Weed Technology **18**:1392-1395.

Lesica, P. and D. Hanna. 2004. Indirect effects of biological control on plant diversity vary across sites in Montana grasslands. Conservation Biology **18**:444-454.

Lesica, P. and D. Hanna. 2009. Effect of biological control on leafy spurge (*Euphorbia esula*) and diversity of associated grasslands over 14 years. Invasive Plant Science and Management **2**:151-157.

Levang-Brilz, N. and M. E. Biondini. 2003. Growth rate, root development and nutrient uptake of 55 plant species from the Great Plains Grasslands, USA. Plant Ecology **165**:117-144.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for mixed models. Safari Tech Books Online, Boston, MA.
Loh, R. and C. Daehler. 2008. Influence of woody invader control methods and seed availability on native and invasive species establishment in a Hawaiian forest. Biological Invasions **10**:805-819.
Louda, S. M., R. W. Pemberton, M. T. Johnson, and P. A. Follett. 2003. Nontarget effects - The Achilles' Heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annual Review of Entomology **48**:365-396.
Lym, R. G. 2005. Integration of biological control agents with other weed management technologies: Successes from the leafy spurge (*Euphorbia esula*) IPM program. Biological Control **35**:366-375.
Mandeel, Q. A. 2006. Influence of plant root exudates, germ tube orientation and passive conidia transport on biological control of fusarium wilt by strains of nonpathogenic *Fusarium oxysporum*. Mycopathologia **161**:173-182.
Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. Mack, M. C. Valencia, E. I. Sanchez, and J. D. Bever. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature **466**:752-755.
Mangla, S., Inderjit, and R. M. Callaway. 2008. Exotic invasive plant accumulates native soil pathogens which inhibit native plants. Journal of Ecology **96**:58-67.
Manitoba Agriculture Food and Rural Initiatives. 2004. Weed seedling identification guide. Manitoba Agriculture, Food and Rural Initiatives Publications, Winnipeg, Manitoba, Canada.
Marchante, H., H. Freitas, and J. H. Hoffmann. 2011. The potential role of seed banks in the recovery of dune ecosystems after removal of invasive plant species. Applied Vegetation Science **14**:107-119.
Martin, L. M. and B. J. Wilsey. 2006. Assessing grassland restoration success: relative roles of seed additions and native ungulate activities. Journal of Applied Ecology **43**:1098-1109.
Martin, L. M. and B. J. Wilsey. 2012. Assembly history alters alpha and beta diversity, exotic–native proportions and functioning of restored prairie plant communities. Journal of Applied Ecology **49**:1436-1445.
Mason, T. J., K. French, and K. G. Russell. 2007. Moderate impacts of plant invasion and management regimes in coastal hind dune seed banks. Biological Conservation **134**:428-439.
McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, Oregon.
McGregor, R. L. and T. M. Barkley. 1986. Flora of the Great Plains. University Press of Kansas, Lawrence, Kan.
McKinney, M. L. 2004. Measuring floristic homogenization by non-native plants in North America. Global Ecology and Biogeography **13**:47-53.
McLaughlin, S. P. and J. E. Bowers. 2007. Effects of exotic grasses on soil seed banks in southeastern Arizona grasslands. Western North American Naturalist **67**:206-218.

McLerran, D. 2008. Re: Is it true that a zero-inflated Gamma model is not possible in either GENMOD or NLMIXED? SAS-L listserv archive, The University of Georgia, Athens, Georgia, USA.
Messersmith, C. G., R. G. Lym, and D. S. Galitz. 1985. Biology of leafy spurge. Pages 42-56 *in* A. K. Watson, editor. Leafy spurge.
Metlen, K. L., E. T. Aschehoug, and R. M. Callaway. 2009. Plant behavioural ecology: dynamic plasticity in secondary metabolites. Plant, Cell & Environment **32**:641-653.
Mitchell, C. E. and A. G. Power. 2006. Disease dynamics in plant communities. Pages 58-72 *in* S. K. Collinge and C. Ray, editors. Disease ecology : community structure and pathogen dynamics. Oxford University Press, Oxford ; New York.
Mitchell, R. M. and J. D. Bakker. 2011. Carbon addition as a technique for controlling exotic species in Pacific Northwest prairies. Northwest Science **85**:247-254.
Mladinich, C. S., M. R. Bustos, S. Stitt, R. Root, K. Brown, G. L. Anderson, and S. Hager. 2006. The use of Landsat 7 enhanced thematic mapper plus for mapping leafy spurge. Rangeland Ecology & Management **59**:500-506.
Mokany, K., R. J. Raison, and A. S. Prokushkin. 2006. Critical analysis of root : shoot ratios in terrestrial biomes. Global Change Biology **12**:84-96.
Mordecai, E. A. 2011. Pathogen impacts on plant communities: unifying theory, concepts, and empirical work. Ecological Monographs **81**:429-441.
Murphy, R. K. and T. A. Grant. 2005. Land management history and floristics in mixed-grass prairie, North Dakota, USA. Natural Areas Journal **25**:351-358.
Myers, J. A. and K. E. Harms. 2009. Seed arrival, ecological filters, and plant species richness: a meta-analysis. Ecology Letters **12**:1250-1260.
Nannipieri, P., J. Ascher, M. T. Ceccherini, L. Landi, G. Pietramellara, G. Renella, and F. Valori. 2008. Effects of root exudates in microbial diversity and activity in rhizosphere soils. Pages 339-365 *in* C. S. Nautiyal and P. Dion, editors. Molecular mechanisms of plant and microbe coexistence. Springer, Berlin Heidelberg.
National Park Service. 2005. Northern Great Plains Exotic Plant Management Plan and Environmental Assessment. National Park Service, U.S. Department of the Interior, Northern Great Plains.
NCDC. 2012. National Climatic Data Center. U.S. Department of Commerce, Asheville, NC.
Nelson, E. B. 1990. Exudate molecules initiating fungal responses to seeds and roots. Plant and Soil **129**:61-73.
Neumann, G. and V. Römheld. 2001. The release of root exudates as affected by the plant's physiological status. Pages 41-93 *in* R. Pinton, Z. Varanini, and P. Nannipieri, editors. The rhizosphere : biochemistry and organic substances at the soil-plant interface. Marcel Dekker, New York.
Newingham, B. A. and R. M. Callaway. 2006. Shoot herbivory on the invasive plant, *Centaurea maculosa*, does not reduce its competitive effects on conspecifics and natives. Oikos **114**:397-406.

Niemi, L., A. Wennström, J. Hjältén, P. Waldmann, and L. Ericson. 2006. Spatial variation in resistance and virulence in the host–pathogen system Salix triandra–Melampsora amygdalinae. Journal of Ecology **94**:915-921.

Norton, A., A. Blair, J. Hardin, S. Nissen, and G. Brunk. 2008. Herbivory and novel weapons: no evidence for enhanced competitive ability or allelopathy induction of *Centaurea diffusa* by biological controls. Biological Invasions **10**:79-88.

Nunez-Palenius, H. G., D. J. Cantliffe, H. H. Klee, N. Ochoa-Alejo, R. Ramirez-Malagon, and E. Perez-Molphe. 2006. Methods in plant tissue culture. Pages 553-593 *in* K. Shetty, G. Paliyath, A. Pometto, and R. E. Levin, editors. Food Biotechnology. CRC Press, Boca Raton.

O'Neill, M., S. L. Ustin, S. Hager, and R. Root. 2000. Mapping the distribution of leafy spurge at Theodore Roosevelt National Park using AVIRIS. AVIRIS Airbone Geoscience Workshop. Jet Propulsion Laboratory.

Odunfa, V. S. A. 1978. Root exudation in cowpea and sorghum and the effect on spore germination and growth of some soil *Fusaria*. New Phytologist **80**:607-612.

Ogden, J. A. E. and M. Rejmánek. 2005. Recovery of native plant communities after the control of a dominant invasive plant species, *Foeniculum vulgare*: Implications for management Biological Conservation **125**:427-439.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2011. vegan: Community Ecology Package.

Olson, B. E. and R. T. Wallander. 2002. Effects of invasive forb litter on seed germination, seedling growth and survival. Basic and Applied Ecology **3**:309-317.

Otfinowski, R., N. C. Kenkel, and R. C. Van Acker. 2008. Reconciling seed dispersal and seed bank observations to predict smooth brome (*Bromus inermis*) invasions of a northern prairie. Invasive Plant Science and Management **1**:279-286.

Packer, A. and K. Clay. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature **404**:278-281.

Pahel, B. T., J. S. Preisser, S. C. Stearns, and R. G. Rozier. 2011. Multiple imputation of dental caries data using a zero-inflated Poisson regression model. Journal of Public Health Dentistry **71**:71-78.

Park, S., Y. Takano, H. Matsuura, and T. Yoshihara. 2004. Antifungal compounds from the root and root exudate of *Zea mays*. Bioscience Biotechnology and Biochemistry **68**:1366-1368.

Parmeter, J. R. 1971. *Rhizoctonia solani*: Biology and pathology. University of California Press.

Pearson, D. E. and R. M. Callaway. 2008. Weed-biocontrol insects reduce native-plant recruitment through second-order apparent competition. Ecological Applications **18**:1489-1500.

Perez, C. J., S. S. Waller, L. W. Moser, J. L. Stubbendieck, and A. A. Steuter. 1998. Seedbank characteristics of a Nebraska sandhills prairie. Journal of Range Management **51**:55-62.

Perkins, L. and R. Nowak. 2012. Soil conditioning and plant–soil feedbacks affect competitive relationships between native and invasive grasses. Plant Ecology:1-8.

Petermann, J. S., A. J. F. Fergus, L. A. Turnbull, and B. Schmid. 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. Ecology **89**:2399-2406.

Phillips, R. P., Y. Erlitz, R. Bier, and E. S. Bernhardt. 2008. New approach for capturing soluble root exudates in forest soils. Functional Ecology **22**:990-999.

Pimentel, D. 2002. Biological invasions : economic and environmental costs of alien plant, animal, and microbe species. CRC Press, Boca Raton.

Plassmann, K., N. Brown, M. L. M. Jones, and G. Edwards-Jones. 2009. Can soil seed banks contribute to the restoration of dune slacks under conservation management? Applied Vegetation Science **12**:199-210.

Pollierer, M. M., J. Dyckmans, S. Scheu, and D. Haubert. 2012. Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific 13C fatty acid analysis. Functional Ecology **26**:978-990.

Pritekel, C., A. Whittemore-Olson, N. Snow, and J. C. Moore. 2006. Impacts from invasive plant species and their control on the plant community and belowground ecosystem at Rocky Mountain National Park, USA. Applied Soil Ecology **32**:132-141.

Prithiviraj, B., L. G. Perry, D. V. Badri, and J. M. Vivanco. 2007. Chemical facilitation and induced pathogen resistance mediated by a root-secreted phytotoxin. New Phytologist **173**:852-860.

Progar, R. A., G. Markin, J. Milan, T. Barbouletos, and M. J. Rinella. 2010. Inundative release of *Aphthona* spp. flea beetles (Coleoptera: Chrysomelidae) as a biological “herbicide” on leafy spurge in riparian areas. Journal of Economic Entomology **103**:242-248.

Prosser, C. W. 2004. TEAM Leafy Spurge and Theodore Roosevelt National Park: A partnership for the management and control of leafy spurge. Park Science **2**:5.

Qin, B., L. G. Perry, C. D. Broeckling, J. Du, F. R. Stermitz, M. W. Paschke, and J. M. Vivanco. 2006. Phytotoxic allelochemicals from roots and root exudates of leafy spurge (*Euphorbia esula* L.). Plant Signaling & Behavior **1**:323-327.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rasband, W. S. 1997-2012. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA.

Reid, A. M., L. Morin, P. O. Downey, K. French, and J. G. Virtue. 2009. Does invasive plant management aid the restoration of natural ecosystems? Biological Conservation **142**:2342-2349.

Reid, F. A. 2006. Mammals of North America. Fourth Edition edition. Houghton Mifflin, Boston.

Renker, C., J. Heinrichs, M. Kaldorf, and F. Buscot. 2003. Combining nested PCR and restriction digest of the internal transcribed spacer region to characterize arbuscular mycorrhizal fungi on roots from the field. Mycorrhiza **13**:191-198.

Richardson, L. A., C. J. Juricek, R. G. Lym, D. R. Kirby, and D. A. Tober. 2008. Integrated leafy spurge (*Euphorbia esula*) control using Imazapic, Aphthona spp. biological control agents, and seeded native grasses. Invasive Plant Science and Management **1**:255-264.

Richter, R. and J. C. Stromberg. 2005. Soil seed banks of two montane riparian areas: implications for restoration. Biodiversity and Conservation **14**:993-1016.

Ridenour, W. L. and R. M. Callaway. 2003. Root herbivores, pathogenic fungi, and competition between *Centaurea maculosa* and *Festuca idahoensis*. Plant Ecology **169**:161-170.

Rinella, M. J. and B. J. Hileman. 2009. Efficacy of prescribed grazing depends on timing intensity and frequency. Journal of Applied Ecology **46**:796-803.

Rinella, M. J., B. D. Maxwell, P. K. Fay, T. Weaver, and R. L. Sheley. 2009. Control effort exacerbates invasive-species problem. Ecological Applications **19**:155-162.

Roberts, H. A. 1981. Seed banks in soils. Advances in Applied Biology **6**:1-55.

Robertson, S. and K. Hickman. 2012. Aboveground plant community and seed bank composition along an invasion gradient. Plant Ecology **213**:1461-1475.

Rodríguez-Molina, M. C., J. C. Tello-Marquina, L. M. Torres-Vila, and P. Bielza-Lino. 2000. Micro-scale systematic sampling of soil: heterogeneity in populations of *Fusarium oxysporum*, *F. solani*, *F. roseum* and *F. moniliforme*. Journal of Phytopathology **148**:609-614.

Rosas, C. A., D. M. Engle, J. H. Shaw, and M. W. Palmer. 2008. Seed dispersal by *Bison bison* in a tallgrass prairie. Journal of Vegetation Science **19**:769-778.

Rosburg, T. R., T. W. Jurik, and D. C. Glenn-Lewin. 1994. Seed banks of communities in the Iowa Loess Hills: ecology and potential contribution to restoration of native grassland. Pages 221-237 *in* R. G. Wickett, P. D. Lewis, A. Woodliffe, and P. Pratt, editors. Proceedings of the Thirteenth North American Prairie Conference: Spirit of the Land, Our Prairie Legacy. Department of Parks and Recreation, Windsor, ON, Canada, Windsor, ON, Canada.

Rosef, L. 2008. Germinable soil seed banks in abandoned grasslands in central and western Norway and their significance for restoration. Applied Vegetation Science **11**:223-230.

Royer, F. and R. Dickinson. 1999. Weeds of Canada and the Northern United States. Lone Pine Publishing & University of Alberta Press, Edmonton, Alberta, Canada.

Ruan, Y., V. Kotraiah, and D. C. Straney. 1995. Flavonoids stimulate spore germination in *Fusarium solani* pathogenic on legumes in a manner sensitive to inhibitors of cAMP-dependent protein kinase. Molecular Plant-Microbe Interactions **8**:929-938.

Samuel, L. W. and R. G. Lym. 2008. Aminopyralid effects on Canada thistle (*Cirsium arvense*) and native plant species. Invasive Plant Science and Management **1**:265-278.

SAS Institute Inc. 2011. SAS/STAT 9.3 User's Guide. SAS Institute, Inc., Cary, NC.

Schroth, M. N. and D. C. Hildebrand. 1964. Influence of plant exudates on root-infecting fungi. Annual Review of Phytopathology **2**:101-132.
Scott, L. K. and W. W. John. 1993. Ruminal metabolism of leafy spurge in sheep and goats: A potential explanation for differential foraging on spurge by sheep, goats, and cattle. Journal of Chemical Ecology **V19**:2007-2017.
Seabloom, E. W., E. T. Borer, V. L. Boucher, R. S. Burton, K. L. Cottingham, L. Goldwasser, W. K. Gram, B. E. Kendall, and F. Micheli. 2003. Competition, seed limitation, disturbance, and reestablishment of California native annual forbs. Ecological Applications **13**:575-592.
Sehajpal, A., S. Arora, and P. Kaur. 2009. Evaluation of plant extracts against *Rhizoctonia solani* causing sheath blight of rice. The Journal of Plant Protection Sciences **1**:25-30.
Selleck, G. W. 1972. The antibiotic effects of plants in laboratory and field. Weed Science **20**:189-194.
Setter, C. M. and R. G. Lym. 2013. Change in leafy spurge (*Euphorbia esula*) density and soil seedbank composition 10 years following release of *Aphthona* spp. biological control agents. Invasive Plant Science and Management **6**:147-160.
Shanab, S. M. M., E. A. Shalaby, D. A. Lightfoot, and H. A. El-Shemy. 2010. Allelopathic effects of water hyacinth [*Eichhornia crassipes*]. PLoS ONE **5**:e13200.
Sheley, R., E. Vasquez, and C. Hoopes. 2009. Functional group responses to reciprocal plant litter exchanges between native and invasive plant dominated grasslands. Invasive Plant Science and Management **2**:158-165.
Siemann, E. and W. E. Rogers. 2003. Increased competitive ability of an invasive tree may be limited by an invasive beetle. Ecological Applications **13**:1503-1507.
Singleton, L. L., J. D. Mihail, and C. M. Rush, editors. 1992. Methods for research on soilborne phytopathogenic fungi. APS Press, St. Paul, Minn.
Steel, R. G. D. 1960. A Rank Sum Test for Comparing All Pairs of Treatments. Technometrics **2**:197-207.
Steenhagen, D. A. and R. L. Zimdahl. 1979. Allelopathy of leafy spurge (*Euphorbia esula*). Weed Science **27**:1-3.
Steinkellner, S., R. Mammerler, and H. Vierheilig. 2005. Microconidia germination of the tomato pathogen *Fusarium oxysporum* in the presence of root exudates. Journal of Plant Interactions **1**:23 - 30.
Steinkellner, S., R. Mammerler, and H. Vierheilig. 2008. Germination of *Fusarium oxysporum* in root exudates from tomato plants challenged with different *Fusarium oxysporum* strains. European Journal of Plant Pathology **122**:395-401.
Stevenson, P. C., D. E. Padgham, and M. P. Haware. 1995. Root exudates associated with the resistance of four chickpea cultivars (Cicer arietinum) to two races of Fusarium oxysporum f.sp. ciceri. Plant Pathology **44**:686-694.
Stitt, S., R. Root, K. Brown, S. Hager, C. Mladinich, G. L. Anderson, K. Dudek, M. R. Bustos, and R. Kokaly. 2006. Classification of leafy spurge with Earth Observing-1 Advanced Land Imager. Rangeland Ecology & Management **59**:507-511.

Stucky, J. M., T. J. Monaco, and A. D. Worsham. 1981. Identifying seedling and mature weeds common in the southeastern United States. The North Carolina Agricultural Research Service and The North Carolina Agricultural Extension Service, North Carolina State University, Raleigh, Raleigh, North Carolina.

Symstad, A. J., C. L. Wienk, and A. D. Thorstenson. 2008. Precision, Repeatability, and Efficiency of Two Canopy-Cover Estimate Methods in Northern Great Plains Vegetation. Rangeland Ecology & Management **61**:419-429.

te Beest, M., N. Stevens, H. Olff, and W. van der Putten, H. 2009. Plant-soil feedback induces shifts in biomass allocation in the invasive plant *Chromolaena odorata*. Journal of Ecology **97**:1281-1290.

Thelen, G. C., J. M. Vivanco, B. Newingham, W. Good, H. P. Bais, P. Landres, A. Caesar, and R. M. Callaway. 2005. Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. Ecology Letters **8**:209-217.

Thompson, K. and J. P. Grime. 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology **67**:893-921.

Tracy, B. F. and M. A. Sanderson. 2000. Seedbank Diversity in Grazing Lands of the Northeast United States. Journal of Range Management **53**:114-118.

Trammell, M. A. and J. L. Butler. 1995. Effects of exotic plants on native ungulate use of habitat. Journal of Wildlife Management **59**:808-816.

Turnbull, L. A., M. J. Crawley, and M. Rees. 2000. Are plant populations seed-limited? A review of seed sowing experiments. Oikos **88**:225-238.

Turnbull, L. A., J. M. Levine, A. J. F. Fergus, and J. S. Petermann. 2010. Species diversity reduces invasion success in pathogen-regulated communities. Oikos **119**:1040-1046.

Uren, N. C. 2007. Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. Pages 1-21 *in* R. Pinton, Z. Varanini, and P. Nannipieri, editors. The rhizosphere : biochemistry and organic substances at the soil-plant interface. CRC, Boca Raton, Fla.

USDA NRCS. 2012. The PLANTS Database (http://plants.usda.gov, 1 February 2012). National Plant Data Center, Baton Rouge, LA.

Vaillancourt, G., editor. 1996. Weed seedling guide. Alberta Agriculture, Food and Rural Development, Edmonton, Alberta, Canada.

van der Valk, A. G. and R. L. Pederson. 1989. Seed banks and the management and restoration of natural vegetation. Pages 329-346 *in* M. A. Leck, V. T. Parker, and R. L. Simpson, editors. Ecology of soil seed banks. Academic Press, San Diego.

Van Driesche, R. G., R. I. Carruthers, T. Center, M. S. Hoddle, J. Hough-Goldstein, L. Morin, L. Smith, D. L. Wagner, B. Blossey, V. Brancatini, R. Casagrande, C. E. Causton, J. A. Coetzee, J. Cuda, J. Ding, S. V. Fowler, J. H. Frank, R. Fuester, J. Goolsby, M. Grodowitz, T. A. Heard, M. P. Hill, J. H. Hoffmann, J. Huber, M. Julien, M. T. K. Kairo, M. Kenis, P. Mason, J. Medal, R. Messing, R. Miller, A. Moore, P. Neuenschwander, R. Newman, H. Norambuena, W. A. Palmer, R. Pemberton, A. Perez Panduro, P. D. Pratt, M. Rayamajhi, S. Salom, D. Sands, S.

Schooler, A. Sheppard, R. Shaw, M. Schwarzländer, P. W. Tipping, and R. D. van Klinken. 2010. Classical biological control for the protection of natural ecosystems. Biological Control **54**:S2-S33.

van Elsas, J., P. Garbeva, and J. Salles. 2002. Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. Biodegradation **13**:29-40.

Van Riper, L. and D. Larson. 2009. Role of invasive *Melilotus officinalis* in two native plant communities. Plant Ecology **200**:129-139.

Vécrin, M. P., F. Grévilliot, and S. Muller. 2007. The contribution of persistent soil seed banks and flooding to the restoration of alluvial meadows. Journal for Nature Conservation **15**:59-69.

Vierheilig, H., S. Steinkellner, T. Khaosaad, and J. M. Garcia-Garrido. 2008. The biocontrol effect of mycorrhization on soilborne fungal pathogens and the autoregulation of the AM symbiosis: one mechanism, two effects? Pages 307-320 Mycorrhiza.

Vilà, M., J. L. Espinar, M. Hejda, P. E. Hulme, V. Jarošík, J. L. Maron, J. Pergl, U. Schaffner, Y. Sun, and P. Pyšek. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters **14**:702-708.

Vilà, M. and I. Gimeno. 2009. Does invasion by an alien plant species affect the soil seed bank? Journal of Vegetation Science **18**:423-430.

Vinton, M. A. and E. M. Goergen. 2006. Plant-soil feedbacks contribute to the persistence of *Bromus inermis* in tallgrass prairie. Ecosystems **9**:967-976.

Von Loh, J., D. Cogan, J. Butler, D. Faber-Langendoen, D. Crawford, and M. J. Pucherelli. 2000. USGS-NPS Vegetation Mapping Program: Theodore Roosevelt National Park.*in* B. o. R. U.S. Department of the Interior, editor. Tech. Service Center, Denver, Colorado.

Wang, N., J.-Y. Jiao, Y.-F. Jia, W.-J. Bai, and Z.-G. Zhang. 2010. Germinable soil seed banks and the restoration potential of abandoned cropland on the Chinese hilly-gullied Loess Plateau. Environmental Management **46**:367-377.

Weidenhamer, J., P. Boes, and D. Wilcox. 2009. Solid-phase root zone extraction (SPRE): a new methodology for measurement of allelochemical dynamics in soil. Plant and Soil **322**:177-186.

Wellstein, C., A. Otte, and R. Waldhardt. 2007. Seed bank diversity in mesic grasslands in relation to vegetation type, management and site conditions. Journal of Vegetation Science **18**:153-162.

Wetzel, P. R., A. G. van der Valk, and L. A. Toth. 2001. Restoration of wetland vegetation on the Kissimmee River floodplain: Potential role of seed banks. Wetlands **21**:189-198.

Wheeler, G. C. and E. W. Wheeler. 1944. The ants of North Dakota, University of North Dakota.

Whitson, T. D., editor. 2002. Weeds of the west. 9th edition. Western Society of Weed Science in cooperation with the Western United States Land Grant Universities Cooperative Extension Services, Newark, CA.

Wilson, G. W. T. and D. C. Hartnett. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. American Journal of Botany **85**:1732-1738.

Wilson, S. D. and M. Partel. 2003. Extirpation or coexistence? Management of a persistent introduced grass in a prairie restoration. Restoration Ecology **11**:410-416.

Windels, C. E. 1992. *Fusarium*. Pages 115-128 *in* L. L. Singleton, J. D. Mihail, and C. M. Rush, editors. Methods for research on soilborne phytopathogenic fungi. APS Press, St. Paul, Minn.

Wood, T. M. and D. R. Phillips. 1969. Another source of cellulase. Nature **222**:986-987.

Wu, H. S., Y. D. Liu, X. I. Yang, X. Q. Chen, Z. H. Wang, X. Y. Kong, X. X. Liu, and S. A. Yan. 2010a. Growth responses of in vitro *Fusarium oxysporum* f. sp *niveum* to external supply of tannic acid. Journal of Environmental Biology **31**:1017-1022.

Wu, H. S., J. Luo, W. Raza, Y. X. Liu, M. A. Gu, G. Chen, X. F. Hu, J. H. Wang, Z. S. Mao, and Q. R. Shen. 2010b. Effect of exogenously added ferulic acid on in vitro *Fusarium oxysporum* f. sp *niveum*. Scientia Horticulturae **124**:448-453.

Yokose, T., K. Katamoto, S. Park, H. Matsuura, and T. Yoshihara. 2004. Anti-fungal sesquiterpenoid from the root exudate of *Solanum abutiloides*. Bioscience, Biotechnology, and Biochemistry **68**:2640-2642.

Young, J. A. and C. G. Young. 1986. Collecting, processing and germinating seeds of wildland plants. Timber Press, Portland, Oregon.

Yuan, J. D., J. S. Qi, B. Zhang, Y. L. Zhang, K. Feng, Z. C. Qu, and C. S. Li. 2012. Allelopathic effects of garlic root exudates on germination and seedling growth of some crops and to control *Pythium* species. Allelopathy Journal **29**:241-249.

Zhan, X., L. Li, and W. Cheng. 2007. Restoration of *Stipa kryloviisteppes* in Inner Mongolia of China: Assesment of seed banks and vegetation composition. Journal of Arid Environments **68**:298-307.

Zhang, S., Y. Jin, J. Tang, and X. Chen. 2009. The invasive plant *Solidago canadensis* L. suppresses local soil pathogens through allelopathy. Applied Soil Ecology **41**:215-222.

Zuur, A. F., E. N. leno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Zero-truncated and zero-inflated models for count data. Pages 1-33 *in* A. F. Zuur, E. N. leno, N. J. Walker, A. Saveliev, and G. M. Smith, editors. Mixed effects models and extensions in ecology with R. Springer.

Appendix 1. Statistical analysis details.

Assessment of normality and transformations applied. To determine the appropriate distributions and transformations for all data sets, I examined distributions using histograms and box-cox plots and, before and after transformations, normality was assessed using goodness-of-fit tests (Pearson's chi-square test or Shapiro-Wilk W test); normality and equal variance were further assessed by examining residuals. I compared model fits using likelihood ratio tests and Akaike information criterion values corrected for small sample sizes (AICc). Square root and Anscombe (Anscombe 1948) transformations helped in correcting unequal variance in count data, and log and Johnson Sl, Sb and Su power transformations (Johnson 1949, Chou et al. 1998) were used for improving normality and variance in continuous data. In the linear mixed models and generalized liner mixed models, I used the Tukey-Kramer multiple comparison procedure to examine differences among treatment levels, and degrees of freedom were approximated using the Kenward-Roger method (Kenward and Roger 1997) to account for the unbalanced experimental design. *P*-values lower than 0.05 are considered significant for all statistical tests in this study.

Appendix 1 (continued).

Statistical models

Zero-inflated Poisson nonlinear mixed model (for seed bank density)

$$y_{ijk} \sim \begin{cases} 0, & \text{with probability } \pi_{ijk} \\ \text{Poisson}(\mu_{ijk}), & \text{with probability } (1-\pi_{ijk}) \end{cases}$$
$$\text{logit}\,(\pi_{ijk}) = \beta_{z\_jk} + b_{z\_i}$$
$$\log(\mu_{ijk}) = \beta_{p\_jk} + b_{p\_i}$$
$$E(y_{ijk}) = \mu_{ijk} \times (1-\pi_{ijk})$$

Generalized linear mixed model (for native seed density, species diversity, and soil comparisons among invasion histories)

$$y_{ijk} = g^{-1}(\mu + \beta_{jk} + b_i)$$

Linear mixed model (for vegetation cover)

$$y_{ijk} = \mu + \beta_{jk} + b_i + e_{ijk}$$

Zero-inflated gamma nonlinear mixed model (for seed bank and seed bank–vegetation Sørensen values)

$$y_{ijk} \sim \begin{cases} 0, & \text{with probability } \pi_{ijk} \\ \text{Gamma}(k,\theta), & \text{with probability } (1-\pi_{ijk}) \end{cases}$$
$$\text{logit}\,(\pi_{ijk}) = \beta_{z\_jk} + b_{z\_i}$$
$$\mu_{ijk} = k \times \theta$$
$$\log(\mu_{ijk}) = \beta_{p\_jk} + b_{p\_i}$$
$$E(y_{ijk}) = \mu_{ijk} \times (1-\pi_{ijk})$$

Generalized linear mixed model (for correlations of soils with seed banks and vegetation)

$$y_{ijk} = g^{-1}(\mu + \alpha_k + (\alpha\beta)_{jk} + b_i)$$

Model variables

i = 1, 2, 3 = sites
j = 1, 2, 3 = invasion histories
k = 1, 2, 3, … 94 = plots
y_{ijk} is the response variable for invasion history j, within site i plot k
π_{ijk} is the probability of the response variable being zero for invasion history j, within site i plot k
μ_{ijk} is the mean response variable for invasion history j within site i plot k
β_{jk} is the fixed effect of invasion history j on the response variable in plot k

b_i is the random effect of site *i* on the response variable

$E(y_{ijk})$ is the expected value of the response variable for invasion history *j* within site *i* plot *k*

g is the link function

μ is the mean response variable value

e_{ijk} is the random error for invasion history *j* in site *i* plot *k*.

k is the shape parameter for the gamma distribution of the response variable

θ is the scale parameter for the gamma distribution of the response variable

α_k is the linear combination of soil characteristic fixed effects on the response variable in plot *k*

$(\alpha\beta)_{jk}$ is the linear combination of each soil characteristic's interaction with invasion history

In the subscripts for these models, *z* indicates zero values and *p* indicates positive non-zero values.

Appendix 1 (continued).

Species similarity calculation method.

The *vegdist* function of the 'vegan' package (Oksanen et al. 2011) in R v. 2.14.1 (R Development Core Team 2011) was used to obtain a matrix of Bray-Curtis dissimilarity (BC) values (Bray and Curtis 1957) for all possible plot-plot comparisons, calculated as $BC_{ij} = 2C_{ij}/(S_i + S_j)$, where C_{ij} is the number of species common between plots *i* and *j*, and S_i and S_j are the total number of species at the two plots. Sørensen similarity index (QS) values were then obtained as follows: $QS_{ij} = 1 - BC_{ij}$. The *vegdist* function was used on three data sets: seed bank data only (for comparisons within the seed bank), vegetation data only (for comparisons within the vegetation), and combined seed bank and vegetation data (for comparisons between the seed bank and vegetation). Each of the three resulting matrices of Sørensen values were limited to values derived by comparing plots within the same invasion histories.

Appendix 2. Standardized canonical coefficients for seed bank and vegetation species. Bold values indicate relatively large coefficients for the given canonical functions (Can1 and Can2), determined as greater than one standard deviation from the mean, and dashes indicate that the species was not encountered in the given year. The second canonical functions for seed banks are not shown due to non-significant canonical correlation tests.

		Seed Bank		Vegetation			
		2006	2007	2007		2008	
Functional group	Species	Can1	Can1	Can1	Can2	Can1	Can2
exotic cool-season grass	*Agropyron* sp.	–	–	–	–	0.03	-0.15
	Bromus inermis	**0.39**	**0.46**	-0.2	**-0.44**	**1.03**	-0.11
	Bromus japonicus	–	–	0.27	**0.41**	-0.03	0.21
	Poa compressa	–	–	-0.1	0.13	–	–
	Poa pratensis	–	**0.61**	0.22	-0.13	**0.39**	-0.07
exotic forb	*Alyssum desertorum*	–	–	**-1.19**	**0.34**	0.14	0.15
	Brassica sp.	–	–	**-0.7**	-0.2	–	–
	Descurania sophia	–	0.36	**-0.38**	0.15	–	–
	Euphorbia esula	**-0.36**	0.32	**-0.58**	0.01	**0.35**	0.2
	Medicago sativa	–	–	**0.66**	-0.24	–	–
	Melilotus officinalis	–	–	**0.54**	-0.02	–	–
	Portulaca oleracea	-0.02	–	–	–	–	–
	Taraxacum officinale	**-0.5**	**-0.26**	0.14	0.09	-0.15	0.26
	Tragopogon dubius	–	–	0.23	0.2	0.05	0.12
native cool-season grass	*Hesperostipa comata*	–	–	**-0.47**	**0.32**	**0.53**	0.2
	Hesperostipa spartea	–	–	0.07	**0.48**	-0.06	0.02
	Koeleria macrantha	–	–	–	–	-0.11	-0.14
	Nassella viridula	-0.12	**-0.15**	**0.65**	**-0.71**	**-0.32**	**0.72**
	Pascopyron smithii	-0.1	–	0.12	-0.38	0.02	-0.1
	Vulpia octoflora	–	–	0.44	-0.3	–	–
native warm-season grass	*Aristida purpurea*	–	–	–	–	**-0.32**	-0.07
	Bouteloua gracilis	–	–	0.28	**0.34**	-0.2	**-0.35**
	Calamovilfa longifolia	–	–	-0.1	-0.01	0.01	0.15

		Seed Bank		Vegetation			
		2006	2007	2007		2008	
Functional group	Species	Can1	Can1	Can1	Can2	Can1	Can2
	Echinochloa muricata	–	-0.04	–	–	–	–
	Eragrostis pectinacea	**-0.19**	0.3	–	–	–	–
	Muhlenbergia cuspidata	–	–	–	–	-0.18	**0.57**
	Schizachyrium scoparium	–	–	–	–	0.09	0.3
	Sporobolus cryptandrus	-0.16	–	–	–	–	–
native sedge	*Carex* sp.	**0.41**	**0.57**	0.31	**-0.47**	**0.38**	0.29
	Carex filifolia	–	–	**-0.6**	**-0.39**	0.27	**0.8**
native forb	*Achillea millefolium*	–	-0.07	**0.47**	-0.21	-0.22	0.31
	Allium textile	–	–	0.24	-0.28	–	–
	Ambrosia psilostachya	–	–	0.05	0.01	–	–
	Androsace occidentalis	–	0.15	–	–	–	–
	Artemisia campestris	–	–	-0.23	**-0.41**	–	–
	Artemisia frigida	–	-0.04	0.31	0.23	**-0.26**	**-0.26**
	Asclepias sp.	–	–	**-0.38**	0.19	–	–
	Asclepias verticillata	–	–	0.01	0.06	-0.08	**0.65**
	Astragalus sp.	–	–	0.16	0.04	–	–
	Chamaesyce sp.	–	–	–	–	-0.24	**-0.28**
	Chenopodium album	–	-0.04	–	–	0.12	-0.06
	Conyza canadensis	–	-0.04	–	–	**0.52**	0.15
	Draba sp.	–	–	0.43	**0.47**	–	–
	Draba nemorosa	–	0.04	–	–	–	–
	Draba reptans	**0.49**	0.3	–	–	–	–
	Echinacea angustifolia	–	–	-0.09	-0.07	0.04	-0.18
	E. spathulata or *Chamaesyce* sp.	–	–	0.43	0.25	–	–
	Euphorbia spathulata	–	0.1	-0.06	-0.31	–	–
	Gaura coccinea	–	–	0.4	**0.57**	–	–
	Hedeoma hispida	–	0.15	-0.32	0	–	–
	Helianthus sp.	–	–	0.19	-0.14	–	–

		Seed Bank		Vegetation			
		2006	2007	2007		2008	
Functional group	Species	Can1	Can1	Can1	Can2	Can1	Can2
	Helianthus pauciflorus	–	–	–	–	0.16	0.12
	Heterotheca villosa	0.07	–	-0.15	-0.28	–	–
	Lactuca oblongifolia	-0.29	–	0.27	-0.23	0.19	-0.09
	Lappula occidentalis	0.02	–	**0.84**	0.07	–	–
	Liatris sp.	–	–	0.2	**-0.42**	–	–
	Linum lewisii	–	**-0.17**	-0.05	**1**	0.09	-0.18
	Lygodesmia juncea	–	–	–	–	-0.05	0.03
	Packera plattensis	–	–	-0.04	**0.45**	–	–
	Pediomelum esculentum	–	–	0.13	-0.1	–	–
	Phlox hoodii	–	–	-0.17	-0.06	-0.23	**-0.44**
	Plantago patagonica	–	0.26	–	–	–	–
	Polygala alba	–	–	-0.13	**-0.51**	0.2	-0.12
	Potentilla sp.	**0.38**	**0.41**	–	–	–	–
	Ratibida columnifera	–	–	-0.01	0.27	–	–
	Senecio sp.	–	–	**0.56**	0.17	–	–
	Solidago sp.	–	–	**-0.45**	-0.33	–	–
	Sphaeralcea coccinea	–	–	0.43	-0.31	**-0.38**	-0.06
	Symphyotrichum ericoides	–	–	0.41	0.01	0.06	-0.05
	Tradescantia occidentalis	–	–	**-0.53**	-0.04	–	–
	Verbena bracteata	**0.48**	0.32	–	–	–	–
	Vicia americana	–	–	**-0.65**	0.08	–	–
	Viola nuttallii	–	–	**0.54**	**-0.95**	–	–
native shrub	*Artemisia cana*	–	–	0	0.06	0.04	0.29
	Atriplex gardneri	–	–	–	–	0.03	**-0.23**
	Escobaria sp.	–	–	0.33	0.05	-0.17	**0.41**
	Krascheninnikovia lanata	–	–	0.21	**-0.65**	**-0.32**	**-0.35**
	Opuntia polyacantha	–	–	-0.03	-0.03	–	–
	Symphoricarpos occidentalis	–	–	-0.29	-0.21	0.16	0.16

Appendix 3. Structure coefficients for seed bank and vegetation species. Dashes indicate that the species was not encountered in the given year. The second canonical functions for seed banks are not shown due to non-significant canonical correlation tests.

		Seed bank		Vegetation			
		2006	2007	2007		2008	
Functional group	Species	Can1	Can1	Can1	Can2	Can1	Can2
exotic cool-season grass	*Agropyron* sp.	–	–	–	–	0.14	0.14
	Bromus inermis	0.47	0.24	-0.47	0.03	0.47	-0.03
	Bromus japonicus	–	–	0.16	0.22	-0.09	0.1
	Poa compressa	–	–	0	0.29	–	–
	Poa pratensis	–	0.55	-0.5	0.18	0.44	0.08
exotic forb	*Alyssum desertorum*	–	–	-0.33	0.1	0.28	0.05
	Brassica sp.	–	–	-0.37	-0.18	–	–
	Descurania sophia	–	0.34	0.35	0.03	–	–
	Euphorbia esula	-0.23	0.44	-0.32	-0.03	0.41	0.03
	Medicago sativa	–	–	0.003	-0.01	–	–
	Melilotus officinalis	–	–	-0.3	0.1	–	–
	Portulaca oleracea	-0.11	–	–	–	–	–
	Taraxacum officinale	-0.5	-0.17	0.19	-0.41	0.11	0.16
	Tragopogon dubius	–	–	0.44	0.33	-0.33	-0.08
native cool-season grass	*Hesperostipa comata*	–	–	-0.24	0.12	0.13	0.14
	Hesperostipa spartea	–	–	-0.02	0.07	-0.03	-0.03
	Koeleria macrantha	–	–	–	–	0.03	-0.06
	Nassella viridula	-0.21	-0.21	0.7	-0.25	-0.69	0.17
	Pascopyron smithii	-0.18	–	0.27	-0.31	-0.41	-0.13
	Vulpia octoflora	–	–	0.19	-0.08	–	–
native warm-season grass	*Aristida purpurea*	–	–	–	–	-0.09	0.29
	Bouteloua gracilis	–	–	0.47	-0.2	-0.38	-0.25
	Calamovilfa longifolia	–	–	-0.2	0.16	0.5	0.16
	Echinochloa muricata	–	-0.09	–	–	–	–
	Eragrostis pectinacea	-0.1	0.13	–	–	–	–

		Seed bank		Vegetation			
		2006	2007	2007		2008	
Functional group	Species	Can1	Can1	Can1	Can2	Can1	Can2
	Muhlenbergia cuspidata	–	–	–	–	-0.36	0.33
	Schizachyrium scoparium	–	–	–	–	0.1	0.14
	Sporobolus cryptandrus	-0.08	–	–	–	–	–
native sedge	*Carex* sp.	0.17	0.27	0.31	0.1	0.35	-0.03
	Carex filifolia	–	–	-0.29	0.05	-0.22	0.39
native forb	*Achillea millefolium*	–	-0.09	0.34	-0.1	-0.25	0.09
	Allium textile	–	–	0.39	-0.07	–	–
	Ambrosia psilostachya	–	–	-0.29	0.09	–	–
	Androsace occidentalis	–	0.42	–	–	–	–
	Artemisia campestris	–	–	-0.05	0.03	–	–
	Artemisia frigida	–	-0.13	0.33	0.14	-0.28	0.14
	Asclepias sp.	–	–	-0.14	0.06	–	–
	Asclepias verticillata	–	–	-0.24	0.07	-0.09	0.39
	Astragalus sp.	–	–	0.18	0.15	–	–
	Chamaesyce sp.	–	–	–	–	-0.1	-0.3
	Chenopodium album	–	-0.19	–	–	0.2	-0.03
	Conyza canadensis	–	-0.04	–	–	0.34	0.06
	Draba sp.	–	–	0.49	0.53	–	–
	Draba nemorosa	–	0.12	–	–	–	–
	Draba reptans	0.35	0.33	–	–	–	–
	Echinacea angustifolia	–	–	0.3	0.29	0.15	0.05
	E. spathulata or *Chamaesyce* sp.	–	–	0.36	0.27	–	–
	Euphorbia spathulata	–	-0.03	0.14	-0.16	–	–
	Gaura coccinea	–	–	0.29	0.37	–	–
	Hedeoma hispida	–	0.02	0.29	0.24	–	–
	Helianthus sp.	–	–	0.04	-0.27	–	–
	Helianthus pauciflorus	–	–	–	–	0.2	-0.01

		Seed bank		Vegetation			
		2006	2007	2007		2008	
Functional group	Species	Can1	Can1	Can1	Can2	Can1	Can2
	Heterotheca villosa	-0.02	–	-0.04	-0.1	–	–
	Lactuca oblongifolia	-0.3	–	-0.08	0.12	0.3	0.06
	Lappula occidentalis	0.09	–	0.42	-0.01	–	–
	Liatris sp.	–	–	0.13	-0.28	–	–
	Linum lewisii	–	-0.14	0.31	0.15	0.07	-0.06
	Lygodesmia juncea	–	–	–	–	0.17	0.21
	Packera plattensis	–	–	0.32	0.33	–	–
	Pediomelum esculentum	–	–	-0.13	0.17	–	–
	Phlox hoodii	–	–	0.32	-0.08	-0.33	0.09
	Plantago patagonica	–	0.18	–	–	–	–
	Polygala alba	–	–	0.16	-0.06	0.05	-0.08
	Potentilla sp.	0.31	0.32	–	–	–	–
	Ratibida columnifera	–	–	0.28	-0.04	–	–
	Senecio sp.	–	–	0.21	0.11	–	–
	Solidago sp.	–	–	-0.06	-0.1	–	–
	Sphaeralcea coccinea	–	–	0.49	-0.23	-0.58	-0.02
	Symphyotrichum ericoides	–	–	0.31	0.4	0.14	-0.01
	Tradescantia occidentalis	–	–	-0.21	-0.01	–	–
	Verbena bracteata	0.54	0.14	–	–	–	–
	Vicia americana	–	–	-0.42	0.13	–	–
	Viola nuttallii	–	–	0.35	-0.64	–	–
native shrub	*Artemisia cana*	–	–	-0.11	0.04	0.41	0.05
	Atriplex gardneri	–	–	–	–	-0.17	-0.19
	Escobaria sp.	–	–	0.18	0.07	-0.06	0.2
	Krascheninnikovia lanata	–	–	0.49	-0.39	-0.57	-0.14
	Opuntia polyacantha	–	–	-0.18	-0.02	–	–
	Symphoricarpos occidentalis	–	–	-0.29	0.13	0.32	0.24

Appendix 4. Statistical models.

The generalized linear mixed model used for the effect of *Euphorbia* & soil treatments on seed germination is

$$y_{ijk} = g^{-1}(\mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + s_k)$$

where

y_{ijk} is the germination within pots that received *Euphorbia* treatment *i*, soil treatment *j*, and species combination *k*
g is the link function
μ is the intercept
α_i is the fixed effect of the i^{th} level of *Euphorbia*
β_j is the fixed effect of the j^{th} level of Soil
$(\alpha\beta)_{ij}$ is the ij^{th} *Euphorbia**Soil interaction fixed effect, and
s_k is the random effect of the k^{th} combination of Species per pot.

A log link function was used in this model for both Poisson and negative binomial distributions. The model for the beetle & soil treatment combination is identical to the above model, with the following differences in variable identities:

α_i is the fixed effect of the i^{th} level of Beetle
β_j is the fixed effect of the j^{th} level of Soil
$(\alpha\beta)_{ij}$ is the ij^{th} Beetle*Soil interaction fixed effect.

These substitutions were also made for the beetle & soil treatment combination models for biomass and height analyses (below).

The linear mixed model used for the effect of *Euphorbia* & soil treatments on plant biomass is

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + s_k$$

where

y_{ijk} is the total biomass within pots that received *Euphorbia* treatment *i*, soil treatment *j*, and species combination *k*
μ is the intercept
α_i is the fixed effect of the i^{th} level of *Euphorbia*
β_j is the fixed effect of the j^{th} level of Soil
$(\alpha\beta)_{ij}$ is the ij^{th} *Euphorbia**Soil interaction fixed effect, and
s_k is the random effect of the k^{th} combination of Species per pot.

The nonlinear zero-inflated mixed model used for species with unusually high numbers of zero values is

$$y_{ijk} = 0 \begin{cases} p(y_{ijk} = 0) = 1/(1 + e^{-\eta_{z\_ijk}}) \\ \eta_{z\_ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + s_k \end{cases}$$

$$y_{ijk} > 0 \begin{cases} \mu_{p\_ijk} = e^{\eta_{p\_ijk}} \\ \eta_{p\_ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + s_k \end{cases}$$

$$E(Y_{ijk}) = \mu_{p\_ijk} \times \left(1 - p(y_{ijk} = 0)\right)$$

where

y_{ijk} is the total biomass within pots that received *Euphorbia* treatment *i*, soil treatment *j*, and species combination *k*

$p(y_{ijk} = 0)$ is the probability that the biomass of the *ijk*th sample is zero

η_{z_ijk} is the linear combination of zero-inflation fixed-effect and random-effect parameters

μ is the intercept

α_i is the fixed effect of the *i*th level of *Euphorbia*

β_j is the fixed effect of the *j*th level of Soil

$(\alpha\beta)_{ij}$ is the *ij*th *Euphorbia**Soil interaction fixed effect

s_k is the random effect of the *k*th combination of Species per pot

μ_{p_ijk} is the mean for the positive biomass

η_{p_ijk} is the linear combination of positive biomass fixed-effect and random-effect parameters

$E(Y_{ijk})$ is the mean biomass for *Euphorbia* treatment *i*, soil treatment *j*, species combination *k*.

The linear mixed model used for the effect of *Euphorbia* & soil treatments on plant height is

$$y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + s_k + p_l$$

where

y_{ijk} is the total biomass within pots that received *Euphorbia* treatment *i*, soil treatment *j*, species combination *k*, pot *l*

μ is the intercept

α_i is the fixed effect of the *i*th level of *Euphorbia*

β_j is the fixed effect of the *j*th level of Soil

$(\alpha\beta)_{ij}$ is the *ij*th *Euphorbia**Soil interaction fixed effect

s_k is the random effect of the *k*th combination of Species per pot, and

p_l is the random effect of the *l*th pot.

In this model, height was used as a random blocking effect as there were multiple height measurements per species per pot, and pots were randomly arranged on glasshouse benches. Pot was not used as a random effect in the other models because there was only one value per pot within each individual analysis, and variance cannot be determined using one value.

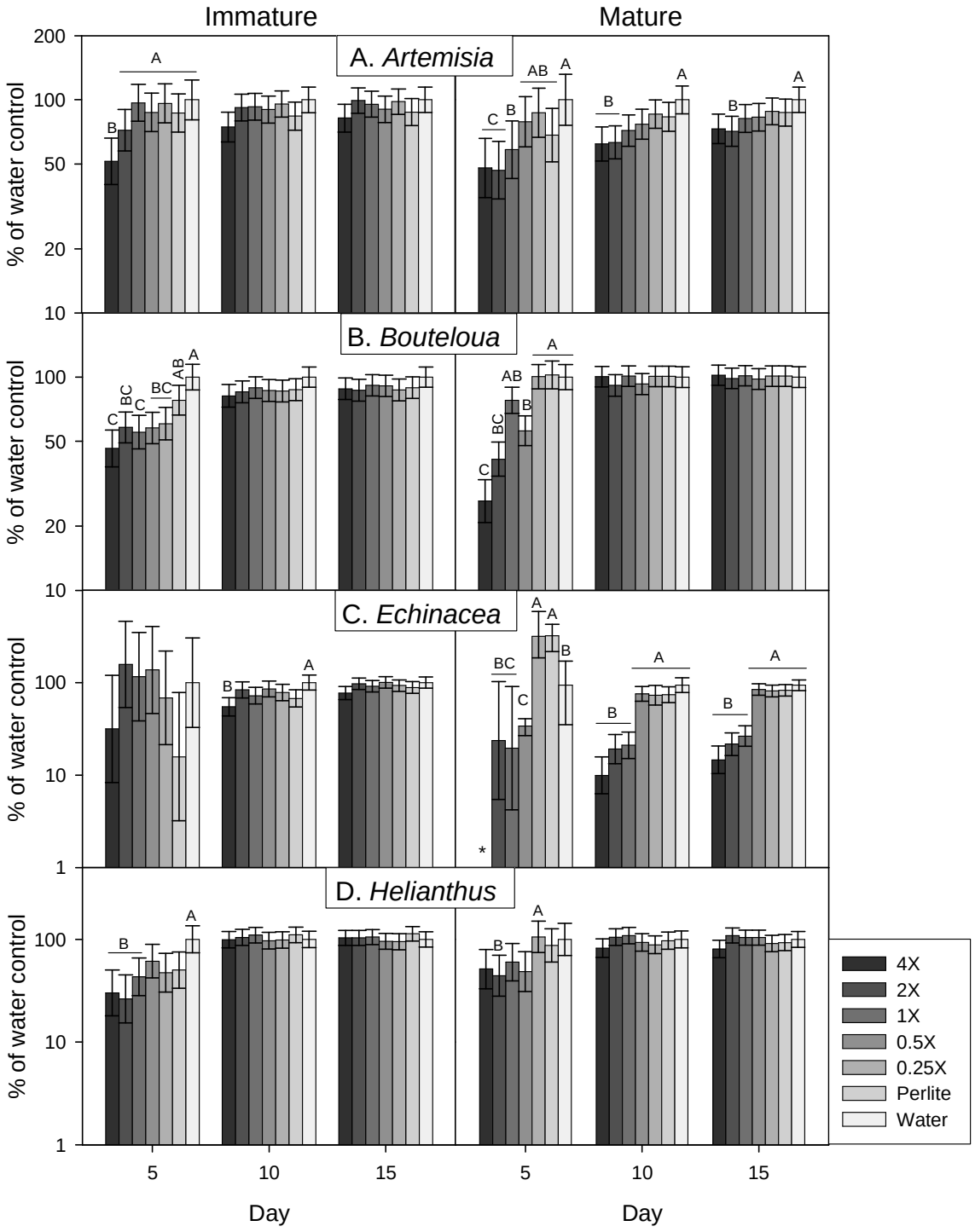
Appendix 5. Type III tests of fixed effects by species for germination, biomass, root:shoot and height. Degrees of freedom (DF) are listed as numerator,denominator. *P*-values < 0.05 are shown in bold, and values that are significant after Bonferroni corrections for multiple comparisons among all species for a given test ($P < 0.00625$) are followed by an asterisk (*). The genus *Symphyotrichum* is abbreviated as *Symph*.

				Species							
Variable	Pots	Treatment	Stats	*Artemisia*	*Bouteloua*	*Linum*	*Nassella*	*Pascopyrum*	*Ratibida*	*Symph.*	*Vicia*
Germination	No beetle	*Euphorbia*	DF	1,1	1,24	1,1	1,19.7	1,24	1,1	1,24	1,1
			F	1.89	2.38	0.01	1.4	3.11	3.37	0.58	0.04
			P	0.400	0.136	0.941	0.251	0.090	0.317	0.455	0.872
		Soil	DF	3,1	3,24	3,1	3,22.7	3,24	3,1	3,24	3,1
			F	0.28	5.8	0.58	0.52	0.47	0.98	0.1	0.08
			P	0.847	**0.004***	0.719	0.675	0.708	0.614	0.960	0.960
		Euphorbia × Soil	DF	3,1	3,24	3,1	3,19.2	3,24	3,1	3,24	3,1
			F	0.26	2.57	0.13	0.43	3.86	0.07	1.25	0.06
			P	0.856	0.078	0.934	0.734	**0.022**	0.970	0.315	0.973
	Euphorbia	Beetle	DF	3,48	3,1	3,43.6	3,48	3,48	3,48	3,1	3,48
			F	0.7	0.08	1.67	0.12	0.42	2.34	1.9	1.36
			P	0.558	0.961	0.188	0.946	0.741	0.085	0.480	0.266
		Soil	DF	3,48	3,1	3,41.6	3,48	3,48	3,48	3,1	3,48
			F	2.23	0.18	4.3	0.38	1.33	0.09	0.52	3.25
			P	0.097	0.899	**0.010**	0.771	0.274	0.966	0.739	**0.030**
		Beetle × Soil	DF	9,48	9,1	9,41	9,48	9,48	9,48	9,1	9,48
			F	0.87	0.08	2.2	3.65	1.73	1.09	0.68	1.75
			P	0.554	0.994	**0.042**	**0.002***	0.108	0.390	0.744	0.103
	All	Soil	DF	3,1	3,1	3,76	3,76	3,76	3,76	3,1	3,1
			F	1.3	0.16	1.54	0.03	1.63	0.16	0.71	0.23
			P	0.555	0.915	0.212	0.994	0.190	0.922	0.680	0.874
Biomass	No beetle	*Euphorbia*	DF	1,19	1,19	1,24	1,12.4	1,22.5	1,19	1,21	1,20.2
			F	0.39	0.02	2.57	0.2	1.02	0	0	0.04
			P	0.540	0.888	0.122	0.663	0.324	0.978	0.959	0.850
		Soil	DF	3,19	3,19	3,24	3,17.2	3,20.2	3,19	3,21	3,18.7
			F	0.2	1.96	1.17	0.55	0.69	3.88	0.14	1.89
			P	0.894	0.154	0.341	0.655	0.571	**0.025**	0.937	0.166
		Euphorbia × Soil	DF	3,19	3,19	3,24	3,13.2	3,19.6	2,19	3,21	3,20.9
			F	0.06	0.53	0.13	1.11	0.61	0.65	0.01	2.05

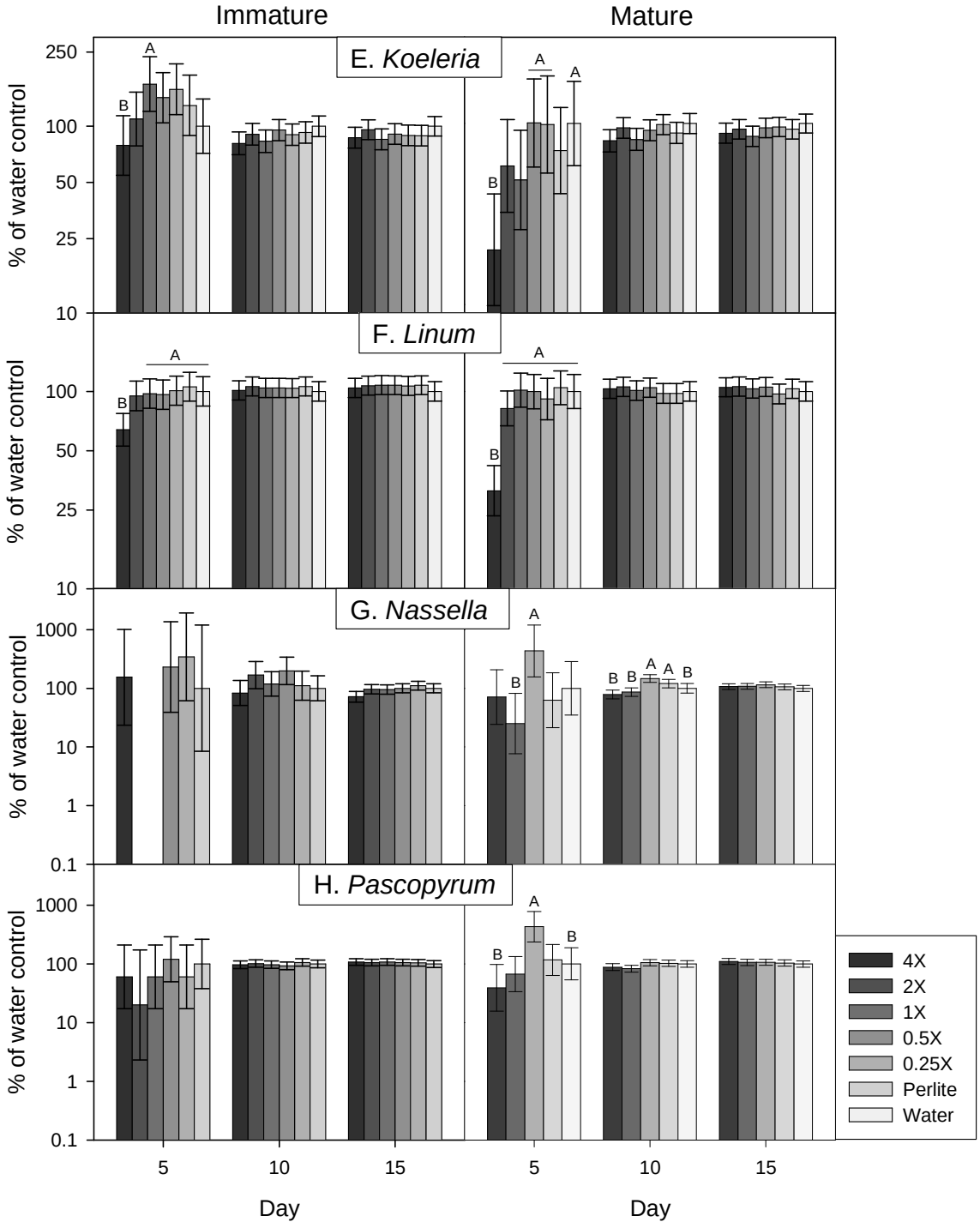
Variable	Pots	Treatment	Stats	Species							
				Artemisia	*Bouteloua*	*Linum*	*Nassella*	*Pascopyrum*	*Ratibida*	*Symph.*	*Vicia*
			P	0.979	0.668	0.939	0.380	0.616	0.533	0.999	0.137
	Euphorbia	Beetle	DF	3,29	3,29	3,42.4	3,32.3	3,41.9	3,30	3,29	3,48
			F	1.49	0.69	2.94	7.04	2.84	0.6	0.98	0.44
			P	0.239	0.564	**0.044**	**<.001***	**0.050**	0.620	0.414	0.727
		Soil	DF	3,29	3,29	3,39.6	3,31.7	3,40.1	3,30	3,29	3,48
			F	2.84	0.36	0.97	0.55	0.97	3.07	0.6	0.79
			P	0.055	0.782	0.415	0.653	0.415	**0.043**	0.619	0.506
		Beetle × Soil	DF	3,29	3,29	9,40.1	9,32.2	9,40.9	3,30	3,29	9,48
			F	0.3	0.14	2.74	1.96	1.24	0.5	0.31	0.99
			P	0.825	0.935	**0.014**	0.078	0.297	0.687	0.818	0.463
	All	Soil	DF	3,33	3,32	3,31	3,73	3,74.1	3,31	3,32	3,75.6
			F	2.09	0.46	0.25	0.82	0.23	3.94	0.6	2.18
			P	0.121	0.714	0.864	0.486	0.877	**0.017**	0.617	0.098
Root:shoot	No beetle	*Euphorbia*	DF	1,22.6	1,19	1,16.9	1,14.3	1,19.9	1,14	1,6.08	1,17.6
			F	4.21	2.28	2.75	0.06	14.93	0.93	0.53	4.13
			P	0.052	0.147	0.115	0.806	**0.001***	0.351	0.493	0.058
		Soil	DF	3,18.6	3,19	3,15.1	3,20	3,18.1	3,14	3,3.38	3,7.37
			F	0.35	1.58	0.71	0.15	0.44	0.42	2.8	4.93
			P	0.786	0.228	0.560	0.927	0.724	0.744	0.194	**0.035**
		Euphorbia × Soil	DF	3,21.6	3,19	3,14.6	3,16.4	3,18.6	3,14	2,3.69	3,7.09
			F	0.84	2.28	0.97	0.27	1.27	0.86	1.25	4.43
			P	0.485	0.112	0.431	0.844	0.315	0.487	0.385	**0.047**
	Euphorbia	Beetle	DF	3,39.8	3,24.8	3,47	3,34.1	3,41.3	3,25.6	3,22.2	3,44.9
			F	0.49	4.37	3.03	0.39	1.62	0.81	2.2	1.9
			P	0.692	**0.013**	**0.039**	0.760	0.199	0.501	0.117	0.144
		Soil	DF	3,39.6	3,23.4	3,47	3,35.2	3,35.6	3,26.5	3,23.3	3,44.2
			F	0.64	2.59	1.27	0.54	1.12	2.45	0.55	0.43
			P	0.596	0.077	0.295	0.657	0.356	0.085	0.654	0.731
		Beetle × Soil	DF	9,39.8	9,26.3	9,47	9,34.7	9,40.6	9,24.6	8,22.8	9,44.4
			F	1.54	4.91	0.14	0.76	1.54	.66	0.83	1.76
			P	0.169	**0.001***	0.998	0.652	0.166	0.733	0.588	0.103
	All	Soil	DF	3,69	3,67	3,73.5	3,72.8	3,72.2	3,50.4	3,43	3,76
			F	0.12	1.02	0.79	0.4	1.22	1.93	1.49	0.76
			P	0.948	0.390	0.501	0.756	0.308	0.137	0.230	0.521

							Species				
Variable	Pots	Treatment	Stats	*Artemisia*	*Bouteloua*	*Linum*	*Nassella*	*Pascopyrum*	*Ratibida*	*Symph.*	*Vicia*
Height	No beetle	*Euphorbia*	DF	1,13.7	1,11.9	1,22.1	1,20.3	1,22.5	1,10.9	1,27	1,22.3
			F	3.29	0.33	0.09	0.94	1.29	0.94	0.32	0.99
			P	0.092	0.5748	0.763	0.344	0.2679	0.354	0.5778	0.3294
		Soil	DF	3,13.8	3,12.2	3,22.2	3,20	3,22.3	3,9.76	3,27	3,22.2
			F	0.5	14.72	3.57	0.18	3.45	0.77	0.31	0.95
			P	0.688	**0.0002***	**0.03**	0.906	**0.0336**	0.538	0.8211	0.4356
		Euphorbia × Soil	DF	3,13.7	3,12	3,22	3,20	3,22.3	3,10.1	2,27	3,22.2
			F	0.7	3.83	1.14	0.17	2.62	0.47	0.04	0.25
			P	0.571	**0.0389**	0.356	0.917	0.0757	0.712	0.963	0.8589
	Euphorbia	Beetle	DF	3,33.2	3,30.2	3,45.5	3,38.8	3,38.6	3,28.3	3,25.8	3,45.7
			F	1.25	1.57	2.4	2.5	2.58	2.47	2.19	1.65
			P	0.307	0.2169	0.08	0.074	0.0675	0.083	0.1134	0.1914
		Soil	DF	3,33.5	3,29.6	3,46	3,39	3,37.6	3,27.9	3,24.9	3,45.5
			F	1.14	0.96	0.63	1.26	2.02	2.83	1.14	0.35
			P	0.347	0.4242	0.601	0.301	0.128	0.057	0.3505	0.7858
		Beetle × Soil	DF	9,32.5	9,32	9,44.9	9,38.6	9,38.4	9,27.3	8,22.3	9,45.4
			F	0.65	1.54	3.19	1.98	2.26	1.04	1.42	0.61
			P	0.75	0.1775	**0.005***	0.068	**0.0389**	0.438	0.2439	0.7786
	All	Soil	DF	3,52.3	3,52.1	3,65.2	3,67	3,74.4	3,42.7	3,42.4	3,68.7
			F	0.84	1.85	0.58	0.64	0.91	3.16	0.47	0.65
			P	0.481	0.1489	0.628	0.595	0.4427	**0.034**	0.7022	0.5846

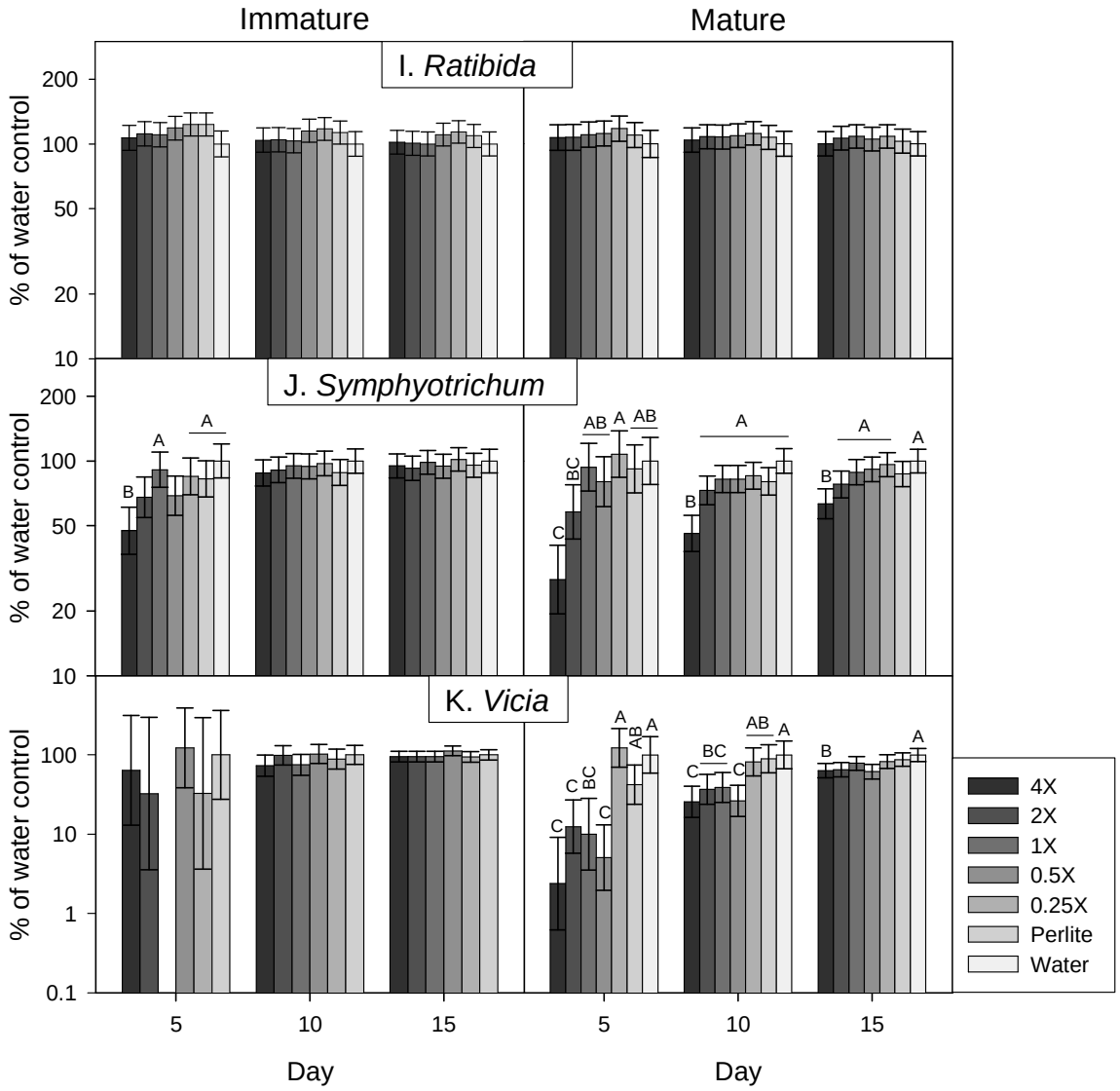
Appendix 6. Graphs for *Euphorbia* exudate effects on germination for immature and mature exudates and all plant species. Germination is represented as the percent difference in germination compared to the water control [(treatment ÷ water) × 100]. Different letters above bars represent different estimates for each day, determined via Tukey HSD tests. Absence of letters indicates no difference among means. Missing bars either represent non-calculable estimates, or zero germination for the given day/treatment (the latter identified by *).

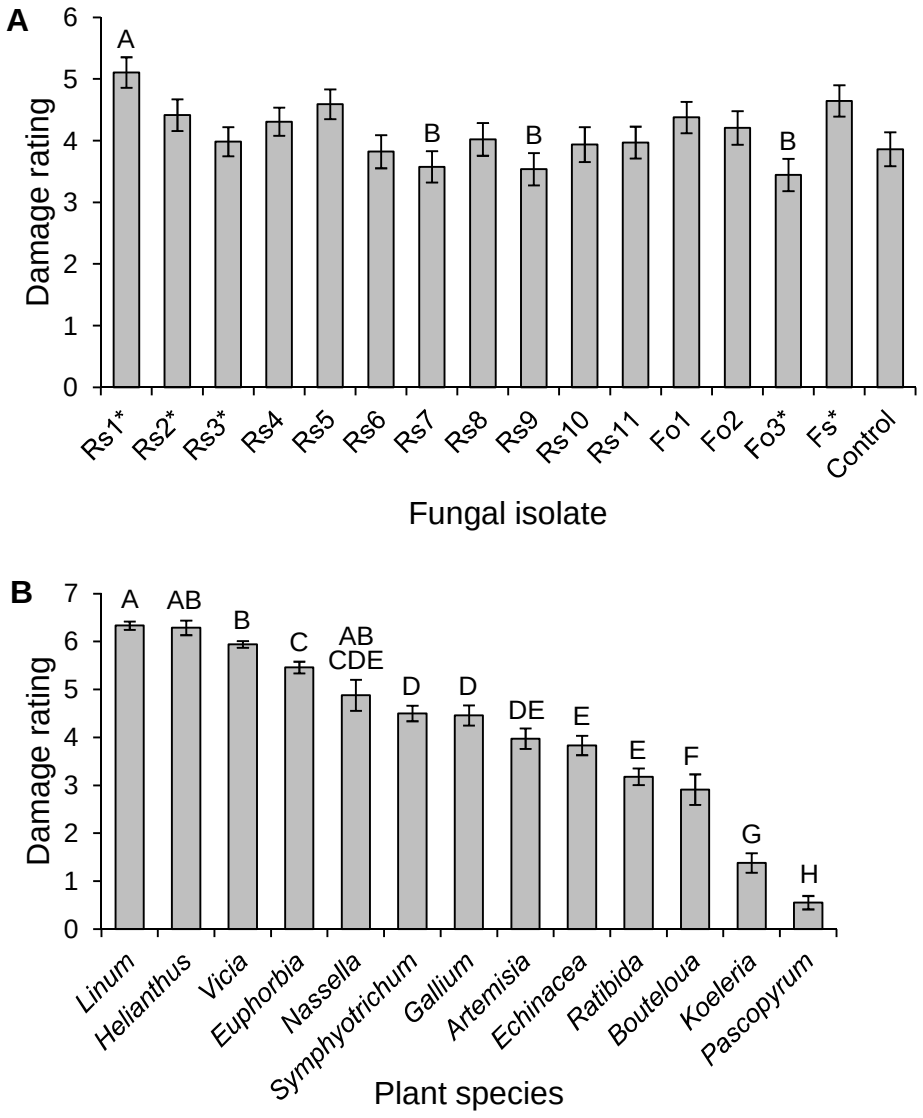

Immature
Mature
A. Artemisia
B. Bouteloua
C. Echinacea
D. Helianthus
% of water control
Day
4X
2X
1X
0.5X
0.25X
Perlite
Water

Appendix 6 (continued)



Appendix 6 (continued)





Appendix 7. Mean damage ratings by fungal isolate (A) and by plant species (B). Bars represent means, and error bars are ± one standard error. Different letters represent different means, determined through Steel-Dwass adjustments of Wilcoxon nonparametric pairwise comparisons; bars without letters indicate no difference in damage ratings. Isolates marked with an asterisk were used in the greenhouse experiment; isolate code explanations are found in Table 4-1.

Appendix 8. Effects of fungal isolate identity on damage ratings by plant species in pathogen assay, determined via Wilcoxon nonparametric tests. *P*-values < 0.05 are in bold. Species marked with an asterisk were used in the greenhouse experiment. DF = degrees of freedom.

Species	χ^2	DF	*P*
Artemisia	38.79	15	**0.0007**
Bouteloua	57.57	15	**<.0001**
*Echinacea**	24.7	15	0.0542
Euphorbia	24.14	15	0.0627
Gallium	44.52	15	**<.0001**
*Helianthus**	30.33	15	**0.0108**
*Koeleria**	45.65	15	**<.0001**
Linum	41.04	15	**0.0003**
Nassella	30.03	15	**0.0118**
*Pascopyrum**	24.01	15	0.0649
Ratibida	33.28	15	**0.0043**
Symphyotrichum	14.15	15	0.5139
*Vicia**	51.35	15	**<.0001**

Appendix 9. Multiple comparisons of 15 fungal isolate effects on damage ratings for *Euphorbia* and all 12 native plant species. C is the non-inoculated control, and codes for the isolates are found in Table 4-1. Comparisons performed via Steel-Dwass nonparametric tests. *P*-values less than 0.05 are in bold.

	Artemisia		*Bouteloua*		*Echinacea*		*Euphorbia*		*Galium*		*Helianthus*		*Koeleria*	
Comparison	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*
C vs. Rs1	-1.19	0.998	-0.56	1	-2.68	0.342	-2.48	0.487	-3.01	0.163	-0.94	1	-0.84	1
C vs. Rs2	-0.09	1	1.05	1	-2.49	0.479	0.85	1	-1.42	0.99	-0.89	1	-1.64	0.962
C vs. Rs3	-1.03	1	2.07	0.782	-2.38	0.561	-0.61	1	-2.12	0.751	0	1	-0.84	1
C vs. Rs4	-2.14	0.737	2.47	0.497	-2.24	0.667	0.04	1	-0.05	1	-1.95	0.854	-1.72	0.941
C vs. Rs5	-0.09	1	1.75	0.934	-1.39	0.992	0.85	1	-0.56	1	-0.5	1	-0.5	1
C vs. Rs6	1.7	0.947	1.33	0.995	-1.66	0.956	-0.55	1	-0.38	1	-1.03	1	1.38	0.993
C vs. Rs7	-2.14	0.737	1.01	1	-0.32	1	0.77	1	0.21	1	-0.22	1	0.49	1
C vs. Rs8	0.54	1	1.33	0.995	-1.21	0.998	1.17	0.999	0.89	1	-1.16	0.999	0.55	1
C vs. Rs9	1.7	0.947	-0.58	1	-0.37	1	-0.43	1	0.75	1	-2.01	0.819	-0.4	1
C vs. Rs10	1.81	0.914	0	1	-1.43	0.989	0.28	1	0.27	1	-1.26	0.997	1.38	0.993
C vs. Rs11	0	1	2.47	0.497	-2.63	0.377	-0.04	1	-1.69	0.95	-2.76	0.295	0.61	1
C vs. Fo1	0.35	1	-1.05	1	-1.86	0.893	-0.83	1	-0.57	1	0.32	1	0.61	1
C vs. Fo2	1.98	0.837	1.22	0.998	-1.61	0.967	-1.54	0.978	-1.16	0.999	-2.75	0.297	0.61	1
C vs. Fo3	-0.45	1	2.07	0.782	-1.11	0.999	0.12	1	0.66	1	-1.88	0.884	0.55	1
C vs. Fs	-1.39	0.992	0.16	1	-1.68	0.953	-0.21	1	0.2	1	-1.03	1	-2.44	0.515
Fs vs. Rs1	1.23	0.998	-1.53	0.98	-0.91	1	-2.35	0.588	-2.86	0.237	0.13	1	1.94	0.857
Fs vs. Rs2	1.43	0.989	1.31	0.996	-0.46	1	1.5	0.983	-1.42	0.99	0	1	1.39	0.992
Fs vs. Rs3	0.73	1	2.45	0.513	-0.75	1	-0.12	1	-2.17	0.717	1.21	0.998	2.1	0.768
Fs vs. Rs4	0.62	1	3.08	0.138	-0.29	1	0.34	1	-0.38	1	-1.09	1	0.69	1
Fs vs. Rs7	0.62	1	0.92	1	1.38	0.992	1.34	0.995	0	1	0.78	1	2.59	0.406
Fs vs. Rs8	1.58	0.973	1.63	0.964	0.47	1	1.5	0.983	0.56	1	-0.68	1	3.01	0.164
Fs vs. Rs9	1.71	0.944	-1.57	0.974	1.5	0.983	0.12	1	0.6	1	-1.23	0.998	2.31	0.613
Fs vs. Rs10	1.77	0.927	-0.51	1	0.41	1	0.9	1	0	1	-0.17	1	3.46	**0.044**
Fs vs. Rs11	1.39	0.992	3.08	0.138	-1.09	0.999	0.3	1	-1.74	0.935	-2.26	0.656	3.27	0.081
Fs vs. Fo1	0.92	1	-1.48	0.984	-0.19	1	-0.36	1	-0.7	1	0.98	1	3.27	0.081
Fs vs. Fo3	1.33	0.995	2.45	0.513	0.73	1	0.5	1	0.37	1	-1.19	0.999	3.01	0.164
Fo3 vs. Rs7	-1.9	0.879	-1.44	0.988	0.7	1	0.45	1	-0.38	1	1.84	0.902	0	1
Fo3 vs. Rs8	0.99	1	-0.67	1	-0.04	1	0.85	1	0.22	1	0.28	1	0	1
Fo3 vs. Rs9	2.15	0.735	-3.1	0.129	0.69	1	-0.12	1	0.13	1	0.2	1	-0.97	1
Fo3 vs. Rs10	2.17	0.715	-2.37	0.572	-0.38	1	0.29	1	-0.41	1	1.18	0.999	0.9	1

	Artemisia		*Bouteloua*		*Echinacea*		*Euphorbia*		*Galium*		*Helianthus*		*Koeleria*	
Comparison	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*
Fo3 vs. Fo1	0.75	1	-3.18	0.103	-0.8	1	-0.62	1	-1.27	0.997	1.68	0.952	0	1
Fo2 vs. Rs1	-2.48	0.483	-3.03	0.155	-1.29	0.996	-1.17	0.999	-2.78	0.281	2.7	0.33	-1.54	0.977
Fo2 vs. Rs2	-1.74	0.936	0.04	1	-0.82	1	2.06	0.789	-0.56	1	1.96	0.847	-2.42	0.531
Fo2 vs. Rs3	-2.1	0.766	1.25	0.997	-0.97	1	0.98	1	-1.51	0.982	3.05	0.149	-1.59	0.97
Fo2 vs. Rs4	-2.91	0.21	2.12	0.749	-0.65	1	1.44	0.989	1.34	0.994	2.01	0.817	-2.46	0.498
Fo2 vs. Rs7	-2.91	0.21	-0.47	1	1.35	0.994	1.85	0.897	1.45	0.987	2.89	0.22	0	1
Fo2 vs. Rs8	-1.53	0.979	0.52	1	0.32	1	2.14	0.737	2.27	0.644	1.37	0.993	0	1
Fo2 vs. Rs9	-1.24	0.998	-2.81	0.262	1.47	0.986	0.74	1	1.92	0.867	1.7	0.946	-1.08	1
Fo2 vs. Rs10	-1.24	0.998	-1.75	0.933	0.21	1	1.46	0.986	1.68	0.952	2.56	0.424	0.9	1
Fo2 vs. Rs11	-1.98	0.837	2.12	0.749	-1.3	0.996	1.07	1	-0.7	1	0.84	1	0	1
Fo2 vs. Fo1	-1.23	0.998	-2.53	0.451	-0.35	1	0.52	1	0.37	1	2.45	0.513	0	1
Fo2 vs. Fo3	-2.21	0.687	1.25	0.997	0.53	1	1.17	0.999	2.01	0.822	1.22	0.998	0	1
Fo2 vs. Fs	-2.36	0.581	-1.68	0.951	-0.12	1	1.27	0.997	1.38	0.993	2.4	0.551	-3.27	0.081
Rs11 vs. Rs7	-2.14	0.737	-2.11	0.759	2.38	0.561	0.89	1	1.93	0.865	2.89	0.22	0	1
Rs11 vs. Rs8	0.54	1	-1.38	0.992	1.77	0.926	1.2	0.998	2.74	0.302	0.76	1	0	1
Rs11 vs. Rs9	1.7	0.947	-3.41	0.053	2.52	0.455	0.12	1	2.3	0.623	1.19	0.998	-1.08	1
Rs11 vs. Rs10	1.81	0.914	-2.77	0.288	1.55	0.977	0.69	1	2.34	0.596	2.43	0.525	0.9	1
Rs11 vs. Fo1	0.35	1	-3.46	**0.044**	1.06	1	-0.38	1	0.83	1	2.05	0.795	0	1
Rs11 vs. Fo3	-0.45	1	-0.9	1	1.8	0.917	0.27	1	2.54	0.44	0.69	1	0	1
Rs10 vs. Rs7	-3.08	0.136	1.27	0.997	1.04	1	0.13	1	0	1	1.03	1	-0.9	1
Rs10 vs. Rs8	-1.06	1	1.52	0.981	0.12	1	0.64	1	0.7	1	-0.75	1	-0.9	1
Rs10 vs. Rs9	-0.3	1	-0.69	1	1.12	0.999	-0.35	1	0.52	1	-1.22	0.998	-1.76	0.929
Rs10 vs. Fo1	-1.54	0.978	-1.14	0.999	-0.59	1	-0.97	1	-0.96	1	0.95	1	-0.9	1
Rs9 vs. Rs7	-3.19	0.103	2.16	0.728	0	1	0.6	1	-0.58	1	1.97	0.841	0.92	1
Rs9 vs. Rs8	-0.94	1	2.2	0.699	-1.22	0.998	1.09	1	0	1	0	1	0.97	1
Rs9 vs. Fo1	-1.13	0.999	-0.52	1	-1.97	0.841	-0.39	1	-1.32	0.995	1.49	0.984	1.08	1
Rs8 vs. Rs7	-2.38	0.56	-0.61	1	1.09	1	-0.42	1	-0.62	1	1.23	0.998	0	1
Rs8 vs. Fo1	-0.04	1	-2.43	0.524	-0.67	1	-1.62	0.966	-1.45	0.988	0.82	1	0	1
Rs7 vs. Fo1	1.79	0.922	-2.27	0.65	-1.78	0.922	-1.25	0.997	-0.76	1	0.27	1	0	1
Rs6 vs. Rs1	-2.89	0.218	-2.38	0.565	-1.49	0.984	-2.17	0.72	-2.42	0.534	0.13	1	-2.11	0.759
Rs6 vs. Rs2	-1.9	0.874	-0.35	1	-0.89	1	1.78	0.924	-0.81	1	0	1	-2.78	0.28
Rs6 vs. Rs3	-1.76	0.93	0.67	1	-1	1	0.04	1	-1.63	0.964	1.21	0.998	-2.12	0.756
Rs6 vs. Rs4	-3.19	0.103	1.38	0.992	-0.75	1	0.71	1	0.31	1	-1.09	1	-2.78	0.28
Rs6 vs. Rs5	-1.96	0.847	0.16	1	0.08	1	1.64	0.961	-0.04	1	0.12	1	-1.76	0.929
Rs6 vs. Rs7	-3.19	0.103	-0.61	1	1.32	0.995	1.64	0.961	0.61	1	0.78	1	-0.9	1

	Artemisia		*Bouteloua*		*Echinacea*		*Euphorbia*		*Galium*		*Helianthus*		*Koeleria*	
Comparison	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*
Rs6 vs. Rs8	-0.94	1	0	1	0.57	1	1.78	0.924	1.27	0.997	-0.68	1	-0.9	1
Rs6 vs. Rs9	0	1	-2.2	0.699	1.37	0.993	0.28	1	1.18	0.999	-1.23	0.998	-1.76	0.929
Rs6 vs. Rs10	0.3	1	-1.52	0.981	0.12	1	1.13	0.999	0.69	1	-0.17	1	.	.
Rs6 vs. Rs11	-1.7	0.947	1.38	0.992	-1.34	0.995	0.59	1	-1.11	0.999	-2.26	0.656	-0.9	1
Rs6 vs. Fo1	-1.13	0.999	-2.43	0.524	-0.12	1	-0.16	1	-0.08	1	0.98	1	-0.9	1
Rs6 vs. Fo2	1.24	0.998	-0.52	1	0	1	-1.11	0.999	-0.64	1	-2.4	0.551	-0.9	1
Rs6 vs. Fo3	-2.15	0.735	0.67	1	0.5	1	0.74	1	1.05	1	-1.19	0.999	-0.9	1
Rs6 vs. Fs	-1.71	0.944	-1.63	0.964	-0.16	1	0.33	1	0.63	1	0	1	-3.46	**0.044**
Rs5 vs. Rs1	-1.14	0.999	-3.39	0.055	-1.29	0.996	-2.97	0.184	-3.19	0.103	0	1	-0.22	1
Rs5 vs. Rs2	0	1	-0.7	1	-0.77	1	0.25	1	-1.05	1	-0.25	1	-0.92	1
Rs5 vs. Rs3	-1.03	1	0.43	1	-1.05	1	-1.08	1	-1.96	0.848	0.58	1	-0.18	1
Rs5 vs. Rs4	-2.14	0.74	1.38	0.993	-0.65	1	-1.03	1	0.7	1	-0.71	1	-1.05	1
Rs5 vs. Rs7	-2.14	0.74	-1.21	0.998	1.18	0.999	0	1	0.79	1	0.45	1	0.98	1
Rs5 vs. Rs8	0.67	1	-0.16	1	0.15	1	0.42	1	1.56	0.976	-0.43	1	1.08	1
Rs5 vs. Rs9	1.96	0.847	-3.06	0.144	1.34	0.995	-0.6	1	1.29	0.996	-0.87	1	0.09	1
Rs5 vs. Rs10	2.1	0.768	-2.19	0.706	0.2	1	-0.13	1	1.03	1	-0.16	1	1.76	0.929
Rs5 vs. Rs11	0.09	1	1.38	0.993	-1.43	0.989	-0.89	1	-1.12	0.999	-1.58	0.972	1.14	0.999
Rs5 vs. Fo1	0.55	1	-2.94	0.196	-0.5	1	-1.25	0.997	-0.12	1	0.62	1	1.14	0.999
Rs5 vs. Fo2	1.98	0.838	-1.08	1	-0.04	1	-1.85	0.897	-0.49	1	-1.93	0.863	1.14	0.999
Rs5 vs. Fo3	-0.35	1	0.43	1	0.47	1	-0.45	1	1.35	0.994	-1	1	1.08	1
Rs5 vs. Fs	-1.35	0.994	-2.46	0.498	-0.19	1	-1.34	0.995	0.68	1	-0.12	1	-1.66	0.956
Rs4 vs. Rs1	1.64	0.961	-3.71	**0.019**	-0.87	1	-2.54	0.442	-3.64	**0.025**	1.41	0.991	1.11	0.999
Rs4 vs. Rs2	2.14	0.74	-1.76	0.929	-0.05	1	1.23	0.998	-1.83	0.907	0.86	1	0.42	1
Rs4 vs. Rs3	0.22	1	-0.9	1	-0.4	1	-0.36	1	-2.45	0.506	2.26	0.651	1.17	0.999
Rs4 vs. Rs7	0	1	-2.11	0.759	1.79	0.922	1.03	1	0.4	1	1.88	0.886	1.97	0.841
Rs4 vs. Rs8	2.38	0.56	-1.38	0.992	1.41	0.99	1.23	0.998	1.22	0.998	-0.2	1	2.26	0.655
Rs4 vs. Rs9	3.19	0.103	-3.41	0.053	1.8	0.919	0	1	0.92	1	-0.25	1	1.5	0.982
Rs4 vs. Rs10	3.08	0.136	-2.77	0.288	0.69	1	0.67	1	0.45	1	1.03	1	2.78	0.28
Rs4 vs. Rs11	2.14	0.737	.	.	-0.77	1	0	1	-2.14	0.74	-1.59	0.97	2.46	0.498
Rs4 vs. Fo1	1.79	0.922	-3.46	**0.044**	0.6	1	-0.57	1	-0.64	1	1.35	0.994	2.46	0.498
Rs4 vs. Fo3	1.9	0.879	-0.9	1	1.17	0.999	0.26	1	0.9	1	-0.44	1	2.26	0.655
Rs3 vs. Rs1	0.65	1	-3.3	0.074	-0.22	1	-2.01	0.822	-0.8	1	-1.13	0.999	0	1
Rs3 vs. Rs2	1.11	0.999	-1.08	1	0.31	1	1.38	0.993	1.26	0.997	-1.03	1	-0.88	1
Rs3 vs. Rs7	-0.22	1	-1.44	0.988	2.1	0.763	1.08	1	2.25	0.662	-0.31	1	1.25	0.998
Rs3 vs. Rs8	1.29	0.996	-0.67	1	1.43	0.989	1.45	0.987	2.88	0.227	-1.32	0.995	1.4	0.991

	Artemisia		*Bouteloua*		*Echinacea*		*Euphorbia*		*Galium*		*Helianthus*		*Koeleria*	
Comparison	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*
Rs3 vs. Rs9	1.76	0.93	-3.1	0.129	2.22	0.684	0.04	1	2.7	0.33	-2.32	0.609	0.49	1
Rs3 vs. Rs10	1.75	0.934	-2.37	0.572	1.18	0.999	0.67	1	2.6	0.397	-1.49	0.984	2.12	0.756
Rs3 vs. Rs11	1.03	1	0.9	1	-0.28	1	0.12	1	1.15	0.999	-3.13	0.12	1.59	0.97
Rs3 vs. Fo1	0.94	1	-3.18	0.103	0.72	1	-0.35	1	1.64	0.961	0.36	1	1.59	0.97
Rs3 vs. Fo3	0.85	1	0	1	1.48	0.985	0.36	1	2.76	0.294	-2.13	0.746	1.4	0.991
Rs2 vs. Rs1	-1.08	1	-2.23	0.679	-0.81	1	-3.01	0.164	-2.56	0.429	0.04	1	0.9	1
Rs2 vs. Rs7	-2.14	0.74	-0.27	1	2.09	0.769	-0.29	1	1.58	0.971	0.67	1	1.93	0.864
Rs2 vs. Rs8	0.62	1	0.35	1	1.5	0.983	0.16	1	2.37	0.573	-0.37	1	2.17	0.72
Rs2 vs. Rs9	1.9	0.874	-2.07	0.783	2.17	0.715	-0.97	1	2.06	0.791	-0.99	1	1.38	0.992
Rs2 vs. Rs10	2.02	0.813	-1.3	0.996	0.92	1	-0.56	1	2.02	0.815	-0.17	1	2.78	0.28
Rs2 vs. Rs11	0.09	1	1.76	0.929	-0.73	1	-1.2	0.998	0	1	-1.77	0.926	2.42	0.531
Rs2 vs. Fo1	0.67	1	-2.21	0.688	0.64	1	-1.58	0.973	0.68	1	1.19	0.999	2.42	0.531
Rs2 vs. Fo3	-0.35	1	1.08	1	1.4	0.991	-0.77	1	2.22	0.685	-0.98	1	2.17	0.72
Rs1 vs. Rs7	-1.64	0.961	2.16	0.724	2.21	0.688	2.97	0.184	3.03	0.157	0.66	1	1.29	0.996
Rs1 vs. Rs8	1.64	0.96	2.38	0.565	2.1	0.767	3.01	0.164	3.64	**0.024**	-0.92	1	1.39	0.992
Rs1 vs. Rs9	2.89	0.218	-0.32	1	2.22	0.684	1.82	0.909	3.46	**0.045**	-1.55	0.977	0.45	1
Rs1 vs. Rs10	2.85	0.24	0.52	1	1.34	0.995	2.56	0.425	3.63	**0.026**	-0.39	1	2.11	0.759
Rs1 vs. Rs11	1.19	0.998	3.71	**0.019**	-0.04	1	2.33	0.599	2.56	0.43	-2.65	0.364	1.54	0.977
Rs1 vs. Fo1	1.37	0.993	-0.62	1	1.33	0.995	1.75	0.934	2.61	0.393	0.83	1	1.54	0.977
Rs1 vs. Fo3	0.67	1	3.3	0.074	1.78	0.922	2.34	0.59	3.63	**0.025**	-1.44	0.988	1.39	0.992

Appendix 9, continued.

	Linum		*Nassella*		*Pascopyrum*		*Ratibida*		*Symphyotrichum*		*Vicia*	
Comparison	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*
C vs. Rs1	0	1	-0.9	1	-0.9	1	-0.69	1	0.78	1	-0.73	1
C vs. Rs2	1.74	0.936	-0.4	1	-1.38	0.992	0.08	1	-0.85	1	0.17	1
C vs. Rs3	0.72	1	-0.12	1	-2.11	0.758	-1.2	0.998	-0.37	1	0.17	1
C vs. Rs4	0.88	1	2.07	0.784	-1.38	0.992	-0.88	1	0.2	1	-0.59	1
C vs. Rs5	-0.92	1	0.04	1	-0.9	1	0.73	1	-0.12	1	-1.03	1
C vs. Rs6	-0.52	1	1.7	0.948	-0.9	1	0	1	-0.13	1	-0.67	1
C vs. Rs7	-0.34	1	-1.09	1	-0.9	1	0.85	1	-0.13	1	-1.46	0.987
C vs. Rs8	-0.92	1	0.34	1	.	.	1.62	0.965	0.33	1	-1.44	0.988
C vs. Rs9	-1.57	0.974	-0.44	1	.	.	1.69	0.949	0.53	1	-1.44	0.988
C vs. Rs10	-1.36	0.994	0.41	1	.	.	-0.21	1	0.53	1	-1.82	0.908
C vs. Rs11	-1.1	0.999	-0.77	1	.	.	-1.2	0.998	-0.4	1	-0.9	1
C vs. Fo1	1.17	0.999	0.28	1	-1.77	0.927	-1.29	0.996	0.38	1	1.68	0.952
C vs. Fo2	-0.92	1	-1.47	0.986	.	.	-0.41	1	-0.12	1	-0.04	1
C vs. Fo3	-1.1	0.999	2.19	0.701	-0.9	1	2.54	0.441	-0.4	1	-1.44	0.988
C vs. Fs	-1.1	0.999	0.88	1	-1.77	0.927	-0.21	1	-0.08	1	-1.82	0.908
Fs vs. Rs1	1.1	0.999	-1.92	0.866	1.04	1	-0.41	1	0.69	1	1.2	0.998
Fs vs. Rs2	2.98	0.177	-1.26	0.997	0.45	1	0.29	1	-1.34	0.994	2.65	0.367
Fs vs. Rs3	1.98	0.835	-1.14	0.999	-0.93	1	-0.9	1	0.25	1	2.65	0.367
Fs vs. Rs4	2.2	0.696	0.83	1	0.45	1	-0.66	1	0.98	1	1.54	0.977
Fs vs. Rs7	0.92	1	-1.36	0.993	1.19	0.998	1.07	1	0.43	1	0.35	1
Fs vs. Rs8	0.31	1	-0.48	1	1.77	0.927	1.77	0.928	1.19	0.998	1.38	0.992
Fs vs. Rs9	-0.14	1	-0.85	1	1.77	0.927	1.74	0.936	1.45	0.987	1.38	0.992
Fs vs. Rs10	0	1	-0.2	1	1.77	0.927	0	1	1.45	0.987	0	1
Fs vs. Rs11	0	1	-1.64	0.961	1.77	0.927	-0.9	1	0	1	1.25	0.998
Fs vs. Fo1	2.22	0.684	-0.82	1	0	1	-0.89	1	0.2	1	4.15	**0.003**
Fs vs. Fo3	0	1	1.29	0.996	0.87	1	2.81	0.265	0	1	1.38	0.992
Fo3 vs. Rs7	1.03	1	-2.47	0.491	0	1	-2.12	0.756	0.9	1	-0.55	1
Fo3 vs. Rs8	0.35	1	-1.8	0.918	0.9	1	-2.12	0.756	1.76	0.929	.	.
Fo3 vs. Rs9	-0.11	1	-2.02	0.812	0.9	1	-1.76	0.929	2.12	0.749	.	.
Fo3 vs. Rs10	0	1	-1.48	0.985	0.9	1	-2.81	0.265	2.12	0.749	-1.38	0.992
Fo3 vs. Fo1	2.22	0.681	-2.24	0.672	-0.87	1	-3.31	0.071	0.37	1	4.32	**0.002**
Fo2 vs. Rs1	0.92	1	0.76	1	-0.9	1	-0.09	1	0.4	1	-0.89	1

	Linum		*Nassella*		*Pascopyrum*		*Ratibida*		*Symphyotrichum*		*Vicia*	
Comparison	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*
Fo2 vs. Rs2	3.15	0.115	0.88	1	-1.38	0.992	0.49	1	-1.93	0.865	0.39	1
Fo2 vs. Rs3	2.02	0.813	1.15	0.999	-2.11	0.758	-0.5	1	-0.82	1	0.39	1
Fo2 vs. Rs4	2.31	0.619	2.62	0.387	-1.38	0.992	-0.3	1	0.37	1	-0.66	1
Fo2 vs. Rs7	0.8	1	0.38	1	-0.9	1	1.17	0.999	-0.75	1	-1.84	0.903
Fo2 vs. Rs8	0	1	1.67	0.955	.	.	1.75	0.932	0.13	1	-2.12	0.749
Fo2 vs. Rs9	-0.67	1	0.86	1	.	.	1.77	0.928	0.87	1	-2.12	0.749
Fo2 vs. Rs10	-0.55	1	1.61	0.968	.	.	0.22	1	0.87	1	-2.39	0.554
Fo2 vs. Rs11	-0.35	1	0.68	1	.	.	-0.5	1	-1.44	0.988	-1.13	0.999
Fo2 vs. Fo1	2.17	0.715	1.34	0.994	-1.77	0.927	-0.49	1	0.12	1	2.81	0.265
Fo2 vs. Fo3	-0.35	1	2.83	0.249	-0.9	1	2.78	0.28	-1.44	0.988	-2.12	0.749
Fo2 vs. Fs	-0.31	1	1.76	0.93	-1.77	0.927	0.22	1	-0.93	1	-2.39	0.554
Rs11 vs. Rs7	1.03	1	-0.28	1	-0.9	1	2.02	0.813	0.9	1	-0.75	1
Rs11 vs. Rs8	0.35	1	1.12	0.999	.	.	2.88	0.227	1.76	0.929	-0.55	1
Rs11 vs. Rs9	-0.11	1	0.28	1	.	.	2.72	0.321	2.12	0.749	-0.55	1
Rs11 vs. Rs10	0	1	1.18	0.999	.	.	0.9	1	2.12	0.749	-1.25	0.998
Rs11 vs. Fo1	2.22	0.681	1.13	0.999	-1.77	0.927	0	1	0.37	1	3.46	**0.044**
Rs11 vs. Fo3	0	1	2.86	0.235	-0.9	1	3.51	**0.038**	.	.	-0.55	1
Rs10 vs. Rs7	1.4	0.991	-1.21	0.998	-0.9	1	1.07	1	-1.46	0.987	0.35	1
Rs10 vs. Rs8	0.55	1	-0.08	1	.	.	1.77	0.928	-0.4	1	1.38	0.992
Rs10 vs. Rs9	0	1	-0.69	1	.	.	1.74	0.936	0	1	1.38	0.992
Rs10 vs. Fo1	2.56	0.428	-0.43	1	-1.77	0.927	-0.89	1	-0.35	1	4.15	**0.003**
Rs9 vs. Rs7	1.59	0.97	-0.45	1	-0.9	1	-0.71	1	-1.46	0.987	-0.55	1
Rs9 vs. Rs8	0.67	1	0.69	1	.	.	-0.49	1	-0.4	1	.	.
Rs9 vs. Fo1	2.85	0.24	0.27	1	-1.77	0.927	-2.8	0.268	-0.35	1	4.32	**0.002**
Rs8 vs. Rs7	0.8	1	-1.28	0.997	-0.9	1	-0.52	1	-1.03	1	-0.55	1
Rs8 vs. Fo1	2.17	0.715	-0.12	1	-1.77	0.927	-3.02	0.159	-0.2	1	4.32	**0.002**
Rs7 vs. Fo1	1.74	0.936	0.9	1	-1.19	0.998	-2.02	0.815	0.16	1	3.78	**0.015**
Rs6 vs. Rs1	0.52	1	-2.81	0.263	0	1	-0.52	1	0.67	1	-0.22	1
Rs6 vs. Rs2	2.68	0.346	-2.12	0.753	-0.55	1	0.16	1	-1.95	0.854	1.33	0.995
Rs6 vs. Rs3	1.54	0.979	-2.04	0.802	-1.64	0.961	-0.92	1	0	1	1.33	0.995
Rs6 vs. Rs4	1.8	0.918	-0.18	1	-0.55	1	-0.67	1	0.89	1	0.05	1
Rs6 vs. Rs5	-0.45	1	-1.38	0.992	0	1	0.75	1	0.4	1	-0.55	1
Rs6 vs. Rs7	0.27	1	-2.01	0.819	0	1	0.72	1	0	1	-1.25	0.998
Rs6 vs. Rs8	-0.45	1	-1.3	0.996	0.9	1	1.21	0.998	1.03	1	-1.38	0.992
Rs6 vs. Rs9	-1.19	0.998	-1.55	0.977	0.9	1	1.29	0.996	1.46	0.987	-1.38	0.992

	Linum		*Nassella*		*Pascopyrum*		*Ratibida*		*Symphyotrichum*		*Vicia*	
Comparison	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*
Rs6 vs. Rs10	-1.04	1	-0.99	1	0.9	1	-0.13	1	1.46	0.987	-1.9	0.879
Rs6 vs. Rs11	-0.75	1	-2.49	0.478	0.9	1	-0.92	1	-0.9	1	-0.35	1
Rs6 vs. Fo1	1.81	0.914	-1.91	0.871	-1.04	1	-0.9	1	0.16	1	3.51	**0.038**
Rs6 vs. Fo2	-0.45	1	-2.44	0.52	0.9	1	-0.33	1	0.75	1	0.9	1
Rs6 vs. Fo3	-0.75	1	0.45	1	0	1	2.45	0.509	-0.9	1	-1.38	0.992
Rs6 vs. Fs	-0.69	1	-0.9	1	-1.04	1	-0.13	1	-0.43	1	-1.9	0.879
Rs5 vs. Rs1	0.92	1	-0.94	1	0	1	-1.38	0.993	0.57	1	0.05	1
Rs5 vs. Rs2	3.15	0.115	-0.51	1	-0.43	1	-0.49	1	-1.69	0.95	1.85	0.896
Rs5 vs. Rs3	2.02	0.813	-0.28	1	-1.35	0.994	-1.8	0.918	-0.45	1	1.85	0.896
Rs5 vs. Rs4	2.31	0.619	1.59	0.97	-0.43	1	-1.51	0.982	0.54	1	0.5	1
Rs5 vs. Rs7	0.8	1	-1	1	0	1	-0.04	1	-0.4	1	-0.92	1
Rs5 vs. Rs8	0	1	0.21	1	0.9	1	0.37	1	0.44	1	-0.9	1
Rs5 vs. Rs9	-0.67	1	-0.44	1	0.9	1	0.43	1	0.99	1	-0.9	1
Rs5 vs. Rs10	-0.55	1	0.29	1	0.9	1	-0.98	1	0.99	1	-1.64	0.961
Rs5 vs. Rs11	-0.35	1	-0.83	1	0.9	1	-1.8	0.918	-1.03	1	0	1
Rs5 vs. Fo1	2.17	0.715	0	1	-0.87	1	-1.72	0.941	0.23	1	3.9	**0.01**
Rs5 vs. Fo2	0	1	-1.4	0.991	0.9	1	-1.13	0.999	0.25	1	1.46	0.987
Rs5 vs. Fo3	-0.35	1	1.87	0.891	0	1	1.45	0.988	-1.03	1	-0.9	1
Rs5 vs. Fs	-0.31	1	0.6	1	-0.87	1	-0.98	1	-0.61	1	-1.64	0.961
Rs4 vs. Rs1	-0.88	1	-3.33	0.067	0.55	1	0.27	1	0.24	1	-0.17	1
Rs4 vs. Rs2	1.08	1	-2.36	0.58	0	1	0.79	1	-1.92	0.866	1.03	1
Rs4 vs. Rs3	-0.13	1	-2.33	0.605	-1.26	0.997	-0.05	1	-0.98	1	1.03	1
Rs4 vs. Rs7	-1.6	0.968	-2.2	0.696	0.67	1	1.63	0.964	-0.89	1	-1.07	1
Rs4 vs. Rs8	-2.31	0.619	-1.56	0.975	1.38	0.992	2.3	0.627	-0.04	1	-1.03	1
Rs4 vs. Rs9	-3.16	0.111	-1.67	0.955	1.38	0.992	2.24	0.671	0.38	1	-1.03	1
Rs4 vs. Rs10	-2.85	0.243	-1.08	1	1.38	0.992	0.66	1	0.38	1	-1.54	0.977
Rs4 vs. Rs11	-2.34	0.592	-2.85	0.242	1.38	0.992	-0.05	1	-1.44	0.988	-0.36	1
Rs4 vs. Fo1	0.61	1	-2	0.825	-0.45	1	-0.05	1	-0.08	1	3.12	0.124
Rs4 vs. Fo3	-2.34	0.592	0.61	1	0.43	1	3.12	0.124	-1.44	0.988	-1.03	1
Rs3 vs. Rs1	-0.72	1	-0.77	1	1.64	0.961	0.45	1	0.75	1	-1.22	0.998
Rs3 vs. Rs2	1.17	0.999	-0.2	1	1.26	0.997	1	1	-1.45	0.988	0	1
Rs3 vs. Rs7	-1.34	0.995	-0.75	1	1.64	0.961	2.02	0.813	0	1	-2.13	0.748
Rs3 vs. Rs8	-2.02	0.813	0.52	1	2.11	0.758	2.88	0.227	0.93	1	-2.47	0.497
Rs3 vs. Rs9	-2.82	0.256	-0.12	1	2.11	0.758	2.72	0.321	1.47	0.986	-2.47	0.497
Rs3 vs. Rs10	-2.54	0.439	0.67	1	2.11	0.758	0.9	1	1.47	0.986	-2.65	0.367

	Linum		*Nassella*		*Pascopyrum*		*Ratibida*		*Symphyotrichum*		*Vicia*	
Comparison	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*	*Z*	*P*
Rs3 vs. Rs11	-2.1	0.768	-0.59	1	2.11	0.758	0	1	-0.55	1	-1.49	0.984
Rs3 vs. Fo1	0.66	1	0.51	1	0.93	1	0	1	0.43	1	2.47	0.497
Rs3 vs. Fo3	-2.1	0.768	2.42	0.531	1.35	0.994	3.51	**0.038**	-0.55	1	-2.47	0.497
Rs2 vs. Rs1	-1.74	0.936	-0.44	1	0.55	1	-0.64	1	1.5	0.983	-1.22	0.998
Rs2 vs. Rs7	-2.57	0.42	-0.48	1	0.67	1	0.46	1	1.95	0.854	-2.13	0.748
Rs2 vs. Rs8	-3.15	0.115	0.76	1	1.38	0.992	0.93	1	2.33	0.6	-2.47	0.497
Rs2 vs. Rs9	-3.99	**0.007**	0.04	1	1.38	0.992	0.96	1	2.54	0.44	-2.47	0.497
Rs2 vs. Rs10	-3.63	**0.025**	0.87	1	1.38	0.992	-0.29	1	2.54	0.44	-2.65	0.367
Rs2 vs. Rs11	-3.11	0.128	-0.32	1	1.38	0.992	-1	1	1.76	0.929	-1.49	0.984
Rs2 vs. Fo1	-0.16	1	0.66	1	-0.45	1	-0.94	1	0.99	1	2.47	0.497
Rs2 vs. Fo3	-3.11	0.128	2.49	0.479	0.43	1	1.83	0.907	1.76	0.929	-2.47	0.497
Rs1 vs. Rs7	-0.34	1	-0.36	1	0	1	1.54	0.979	-0.67	1	-0.81	1
Rs1 vs. Rs8	-0.92	1	1.29	0.996	0.9	1	2.32	0.611	-0.13	1	-0.45	1
Rs1 vs. Rs9	-1.57	0.974	0.28	1	0.9	1	2.22	0.681	-0.12	1	-0.45	1
Rs1 vs. Rs10	-1.36	0.994	1.32	0.995	0.9	1	0.41	1	-0.12	1	-1.2	0.998
Rs1 vs. Rs11	-1.1	0.999	0.04	1	0.9	1	-0.45	1	-0.89	1	-0.09	1
Rs1 vs. Fo1	1.17	0.999	1.46	0.986	-1.04	1	-0.45	1	-0.35	1	3.09	0.133
Rs1 vs. Fo3	-1.1	0.999	3.2	0.1	0	1	3.15	0.113	-0.89	1	-0.45	1

Appendix 10. Multiple comparisons for analysis of pathogen and exudate treatment effects on germination by fungal isolate. These comparisons were used for determining differences among treatment levels in Figure 4-3. In the comparison column, treatment combinations are displayed as "inoculum-exudate". *P*-values <0.05 are in bold.

Isolate	Comparison	DF	*t*	*P*
R. solani 1	Low-Control vs. Low-Low	266	∞	**<.0001**
	Low-Control vs. Low-High	266	-0.01	1
	Low-High vs. Low-Low	266	4.08	**0.0008**
	High-Control vs. Low-Control	266	-8.2	**<.0001**
	High-Control vs. Low-Low	266	-7.2	**<.0001**
	High-Control vs. Low-High	266	-8.21	**<.0001**
	High-Control vs. High-Low	266	2.37	0.1721
	High-Control vs. High-High	266	1.92	0.3941
	High-Low vs. Low-Control	266	-∞	**<.0001**
	High-Low vs. Low-Low	266	-∞	**<.0001**
	High-Low vs. Low-High	266	-43.2	**<.0001**
	High-High vs. Low-Control	266	-41.3	**<.0001**
	High-High vs. Low-Low	266	-37.2	**<.0001**
	High-High vs. Low-High	266	-∞	**<.0001**
	High-High vs. High-Low	266	1.85	0.4345
R. solani 2	Low-Control vs. Low-Low	266	-0.66	0.986
	Low-Control vs. Low-High	266	-1.4	0.7265
	Low-High vs. Low-Low	266	0.75	0.9758
	High-Control vs. Low-Control	266	-3.57	**0.0057**
	High-Control vs. Low-Low	266	-4.18	**0.0006**
	High-Control vs. Low-High	266	-4.87	**<.0001**
	High-Control vs. High-Low	266	-0.35	0.9993
	High-Control vs. High-High	266	-1.83	0.4485
	High-Low vs. Low-Control	266	-3.23	**0.0172**
	High-Low vs. Low-Low	266	-3.86	**0.002**
	High-Low vs. Low-High	266	-4.56	**0.0001**
	High-High vs. Low-Control	266	-1.81	0.463
	High-High vs. Low-Low	266	-2.46	0.1414
	High-High vs. Low-High	266	-3.18	**0.02**
	High-High vs. High-Low	266	1.48	0.6766
R. solani 3	Low-Control vs. Low-Low	266	12.09	**<.0001**
	Low-Control vs. Low-High	266	-40.8	**<.0001**
	Low-High vs. Low-Low	266	∞	**<.0001**
	High-Control vs. Low-Control	266	33.36	**<.0001**
	High-Control vs. Low-Low	266	35.25	**<.0001**
	High-Control vs. Low-High	266	-17	**<.0001**
	High-Control vs. High-Low	266	23.6	**<.0001**
	High-Control vs. High-High	266	-11.5	**<.0001**
	High-Low vs. Low-Control	266	-0.01	1
	High-Low vs. Low-Low	266	17.08	**<.0001**
	High-Low vs. Low-High	266	-57.7	**<.0001**
	High-High vs. Low-Control	266	24.97	**<.0001**
	High-High vs. Low-Low	266	66.74	**<.0001**
	High-High vs. Low-High	266	-8.01	**<.0001**
	High-High vs. High-Low	266	∞	**<.0001**
F. oxysporum	Low-Control vs. Low-Low	266	-2.53	0.1198

Isolate	Comparison	DF	*t*	*P*
	Low-Control vs. Low-High	266	-16.9	**<.0001**
	Low-High vs. Low-Low	266	∞	**<.0001**
	High-Control vs. Low-Control	266	20.39	**<.0001**
	High-Control vs. Low-Low	266	10.18	**<.0001**
	High-Control vs. Low-High	266	-5.63	**<.0001**
	High-Control vs. High-Low	266	10.36	**<.0001**
	High-Control vs. High-High	266	4.08	**0.0008**
	High-Low vs. Low-Control	266	1.02	0.9108
	High-Low vs. Low-Low	266	-1.98	0.3551
	High-Low vs. Low-High	266	-26.9	**<.0001**
	High-High vs. Low-Control	266	5.65	**<.0001**
	High-High vs. Low-Low	266	8.93	**<.0001**
	High-High vs. Low-High	266	-15.9	**<.0001**
	High-High vs. High-Low	266	∞	**<.0001**
F. solani	Low-Control vs. Low-Low	266	0.61	0.9904
	Low-Control vs. Low-High	266	-0.55	0.9939
	Low-High vs. Low-Low	266	1.16	0.8545
	High-Control vs. Low-Control	266	-3.56	**0.0059**
	High-Control vs. Low-Low	266	-2.97	**0.038**
	High-Control vs. Low-High	266	-4.07	**0.0009**
	High-Control vs. High-Low	266	-1.08	0.8879
	High-Control vs. High-High	266	-2.58	0.1067
	High-Low vs. Low-Control	266	-2.52	0.1217
	High-Low vs. Low-Low	266	-1.93	0.3884
	High-Low vs. Low-High	266	-3.06	**0.0291**
	High-High vs. Low-Control	266	-1.01	0.9133
	High-High vs. Low-Low	266	-0.41	0.9985
	High-High vs. Low-High	266	-1.57	0.621
	High-High vs. High-Low	266	1.52	0.6494
Control	Control vs. Low	119	6.15	**<.0001**
	Control vs. High	119	6.79	**<.0001**
	High vs. Low	119	-∞	**<.0001**

Appendix 11. Effects of inoculum density and exudate concentration on germination proportion by plant species and isolate. Degrees of freedom (DF) are displayed as numerator/ denominator, and P-values <0.05 are in bold.

Species	Isolate	Effect	DF	*F*	*P*
Echinacea	Rs1	Inoculum density	1/54	20.86	**<.001**
		Exudate concentration	2/54	0.44	0.647
		Inoculum × Exudate	2/54	0.03	0.971
	Rs2	Inoculum density	1/1	10.05	0.195
		Exudate concentration	2/1	4.72	0.31
		Inoculum × Exudate	2/1	1.84	0.462
	Rs3	Inoculum density	1/1	1.39	0.448
		Exudate concentration	2/1	0.21	0.837
		Inoculum × Exudate	2/1	0.43	0.735
	Fo	Inoculum density	1/1	0.67	0.564
		Exudate concentration	2/1	0.37	0.756
		Inoculum × Exudate	2/1	0.46	0.721
	Fs	Inoculum density	1/1	9.78	0.197
		Exudate concentration	2/1	1.45	0.507
		Inoculum × Exudate	2/1	1.74	0.472
	Control	Exudate concentration	2/1	0.34	0.769
Helianthus	Rs1	Inoculum density	1/54	0.49	0.489
		Exudate concentration	2/54	0.33	0.723
		Inoculum × Exudate	2/54	1.37	0.263
	Rs2	Inoculum density	1/1	0.3	0.682
		Exudate concentration	2/1	1.41	0.511
		Inoculum × Exudate	2/1	0.24	0.821
	Rs3	Inoculum density	1/1	0.02	0.902
		Exudate concentration	2/1	0.78	0.626
		Inoculum × Exudate	2/1	0	0.998
	Fo	Inoculum density	1/1	0	0.96
		Exudate concentration	2/1	0.01	0.988
		Inoculum × Exudate	2/1	1.15	0.55
	Fs	Inoculum density	1/1	2.58	0.354
		Exudate concentration	2/1	0.4	0.745
		Inoculum × Exudate	2/1	1.73	0.474
	Control	Exudate concentration	2/27	0.55	0.585
Koeleria	Rs1	Inoculum density	1/1	11.35	0.184
		Exudate concentration	2/1	0.2	0.844
		Inoculum × Exudate	2/1	1.11	0.557
	Rs2	Inoculum density	1/1	18.36	0.146
		Exudate concentration	2/1	0.13	0.892
		Inoculum × Exudate	2/1	0.25	0.819
	Rs3	Inoculum density	1/1	0	0.957
		Exudate concentration	2/1	0.02	0.982
		Inoculum × Exudate	2/1	0.14	0.883
	Fo	Inoculum density	1/1	0.23	0.714
		Exudate concentration	2/1	0	0.996
		Inoculum × Exudate	2/1	0.66	0.656
	Fs	Inoculum density	1/1	6.86	0.232
		Exudate concentration	2/1	0.22	0.831
		Inoculum × Exudate	2/1	0.07	0.935

Species	Isolate	Effect	DF	*F*	*P*
	Control	Exudate concentration	2/1	0.29	0.797
Pascopyrum	Rs1	Inoculum density	1/1	1.2	0.471
		Exudate concentration	2/1	0.09	0.921
		Inoculum × Exudate	2/1	0.38	0.754
	Rs2	Inoculum density	1/1	1.01	0.499
		Exudate concentration	2/1	0.55	0.69
		Inoculum × Exudate	2/1	0.07	0.933
	Rs3	Inoculum density	1/1	0.01	0.948
		Exudate concentration	2/1	0.11	0.907
		Inoculum × Exudate	2/1	1.45	0.507
	Fo	Inoculum density	1/1	0.32	0.672
		Exudate concentration	2/1	1.07	0.564
		Inoculum × Exudate	2/1	1.12	0.556
	Fs	Inoculum density	1/1	0.1	0.808
		Exudate concentration	2/1	0.45	0.725
		Inoculum × Exudate	2/1	0.3	0.792
	Control	Exudate concentration	2/27	0.21	0.811
Vicia	Rs1	Inoculum density	1/1	19.2	0.143
		Exudate concentration	2/1	0.93	0.592
		Inoculum × Exudate	2/1	0.39	0.748
	Rs2	Inoculum density	1/1	7.54	0.222
		Exudate concentration	2/1	1.32	0.524
		Inoculum × Exudate	2/1	0.75	0.633
	Rs3	Inoculum density	1/1	1.08	0.487
		Exudate concentration	2/1	0.12	0.895
		Inoculum × Exudate	2/1	0.22	0.832
	Fo	Inoculum density	1/1	7.54	0.222
		Exudate concentration	2/1	1.32	0.524
		Inoculum × Exudate	2/1	0.75	0.633
	Fs	Inoculum density	1/1	3.86	0.3
		Exudate concentration	2/1	4.4	0.319
		Inoculum × Exudate	2/1	0.99	0.58
	Control	Exudate concentration	2/1	0.67	0.655

Appendix 12. Multiple comparisons of inoculum density and exudate concentration effects on per plant biomass. These comparisons were used for determining differences among treatment levels in Figure 4-3. In the comparison column, treatment combinations are displayed as "inoculum-exudate". *P*-values <0.05 are in bold.

Species	Isolate	Comparison	DF	*t*	*P*
Echinacea	*R. solani* 1	Low-Control vs. Low-Low	22.1	-2.58	0.145
		Low-Control vs. Low-High	23	1.11	0.873
		Low-High vs. Low-Low	22.1	-3.72	**0.013**
		High-Control vs. Low-Control	16.6	1.23	0.815
		High-Control vs. Low-Low	19.5	-0.21	1
		High-Control vs. Low-High	17.2	1.79	0.494
		High-Control vs. High-Low	18.9	1.84	0.464
		High-Control vs. High-High	19.7	2.26	0.257
		High-Low vs. Low-Control	22.7	-1.2	0.831
		High-Low vs. Low-Low	20.8	-3.42	**0.027**
		High-Low vs. Low-High	14.2	-0.34	0.999
		High-High vs. Low-Control	22.9	-1.88	0.44
		High-High vs. Low-Low	22.6	-3.82	**0.01**
		High-High vs. Low-High	19	-1.14	0.861
		High-High vs. High-Low	18.5	-0.74	0.975
	R. solani 2	Low-Control vs. Low-Low	17	-1.29	0.784
		Low-Control vs. Low-High	26.9	-1.68	0.554
		Low-High vs. Low-Low	21.5	0.75	0.973
		High-Control vs. Low-Control	27	0.25	1
		High-Control vs. Low-Low	26	-0.91	0.941
		High-Control vs. Low-High	26.7	-1.38	0.741
		High-Control vs. High-Low	26.8	-0.3	1
		High-Control vs. High-High	26.7	-1.81	0.478
		High-Low vs. Low-Control	26.9	0.6	0.99
		High-Low vs. Low-Low	25.8	-0.81	0.962
		High-Low vs. Low-High	23.3	-1.48	0.678
		High-High vs. Low-Control	26.9	2.12	0.31
		High-High vs. Low-Low	25.8	1.46	0.692
		High-High vs. Low-High	27	0.82	0.962
		High-High vs. High-Low	26.2	2.01	0.361
	R. solani 3	Low-Control vs. Low-Low	46	-1.54	0.641
		Low-Control vs. Low-High	46	-0.05	1
		Low-High vs. Low-Low	46	-1.49	0.673
		High-Control vs. Low-Control	46	-0.25	1
		High-Control vs. Low-Low	46	-1.75	0.509
		High-Control vs. Low-High	46	-0.3	1
		High-Control vs. High-Low	46	-1.94	0.39
		High-Control vs. High-High	46	-2.13	0.289
		High-Low vs. Low-Control	46	1.78	0.489
		High-Low vs. Low-Low	46	0.57	0.992
		High-Low vs. Low-High	46	1.74	0.514
		High-High vs. Low-Control	46	1.93	0.397
		High-High vs. Low-Low	46	0.34	0.999
		High-High vs. Low-High	46	1.88	0.427
		High-High vs. High-Low	46	-0.32	1
	F. oxysporum	Low-Control vs. Low-Low	39.3	-2.34	0.204

Species	Isolate	Comparison	DF	*t*	*P*
		Low-Control vs. Low-High	34.7	-3.15	**0.036**
		Low-High vs. Low-Low	39.9	0.77	0.97
		High-Control vs. Low-Control	36.5	3.11	**0.039**
		High-Control vs. Low-Low	41	0.67	0.984
		High-Control vs. Low-High	36.9	-0.1	1
		High-Control vs. High-Low	39.5	0.8	0.966
		High-Control vs. High-High	41	1.46	0.692
		High-Low vs. Low-Control	38.2	2.14	0.288
		High-Low vs. Low-Low	34.7	-0.14	1
		High-Low vs. Low-High	39.3	-0.88	0.949
		High-High vs. Low-Control	38	1.59	0.612
		High-High vs. Low-Low	40.5	-0.78	0.97
		High-High vs. Low-High	39.3	-1.55	0.633
		High-High vs. High-Low	41	-0.61	0.99
	F. solani	Low-Control vs. Low-Low	33	-2.3	0.222
		Low-Control vs. Low-High	33	-0.51	0.996
		Low-High vs. Low-Low	33	-1.88	0.434
		High-Control vs. Low-Control	33	-0.48	0.997
		High-Control vs. Low-Low	33	-1.59	0.609
		High-Control vs. Low-High	33	-0.72	0.978
		High-Control vs. High-Low	33	-0.98	0.922
		High-Control vs. High-High	33	-1.01	0.91
		High-Low vs. Low-Control	33	1.01	0.912
		High-Low vs. Low-Low	33	-1.16	0.851
		High-Low vs. Low-High	33	0.57	0.993
		High-High vs. Low-Control	33	1.13	0.866
		High-High vs. Low-Low	33	-1.21	0.828
		High-High vs. Low-High	33	0.66	0.986
		High-High vs. High-Low	33	0.04	1
	Control	Control vs. Low	21	0.53	0.86
		Control vs. High	21	1.19	0.473
		High vs. Low	21	-0.74	0.743
Helianthus	*R. solani* 1	Low-Control vs. Low-Low	5	0.62	0.965
		High-Control vs. Low-Control	5	-1.56	0.571
		High-Control vs. Low-Low	5	-1.06	0.822
		High-Control vs. High-Low	5	-2.02	0.373
		High-Control vs. High-High	5	-2.51	0.225
		High-Low vs. Low-Control	5	0.45	0.989
		High-Low vs. Low-Low	5	1.14	0.784
		High-High vs. Low-Control	5	1.16	0.773
		High-High vs. Low-Low	5	1.78	0.469
		High-High vs. High-Low	5	0.82	0.915
	R. solani 2	Low-Control vs. Low-Low	16.6	0.38	0.999
		Low-Control vs. Low-High	16.7	1.05	0.893
		Low-High vs. Low-Low	16.2	-0.68	0.982
		High-Control vs. Low-Control	19	-1.56	0.634
		High-Control vs. Low-Low	17.6	-1.14	0.858
		High-Control vs. Low-High	18.9	-0.34	0.999
		High-Control vs. High-Low	17.9	-1.02	0.907
		High-Control vs. High-High	18.9	-2.79	0.103
		High-Low vs. Low-Control	19	-0.47	0.997
		High-Low vs. Low-Low	18.9	-0.1	1

Species	Isolate	Comparison	DF	*t*	*P*
		High-Low vs. Low-High	15.8	0.57	0.991
		High-High vs. Low-Control	17.1	0.68	0.982
		High-High vs. Low-Low	17.9	1.1	0.875
		High-High vs. Low-High	17.6	1.85	0.462
		High-High vs. High-Low	17.6	1.22	0.824
	R. solani 3	Low-Control vs. Low-Low	9	0.73	0.973
		Low-Control vs. Low-High	8.76	-0.46	0.996
		Low-High vs. Low-Low	8.98	1.15	0.848
		High-Control vs. Low-Control	9	0.85	0.95
		High-Control vs. Low-Low	8.93	1.36	0.747
		High-Control vs. Low-High	8.98	0.57	0.991
		High-Control vs. High-Low	8.98	0.37	0.999
		High-Control vs. High-High	8.98	1.02	0.899
		High-Low vs. Low-Control	4.41	0.7	0.972
		High-Low vs. Low-Low	8.98	1.3	0.78
		High-Low vs. Low-High	8.96	0.28	1
		High-High vs. Low-Control	8.76	-0.13	1
		High-High vs. Low-Low	8.98	0.7	0.977
		High-High vs. Low-High	6.5	-0.74	0.969
		High-High vs. High-Low	8.96	-0.94	0.926
	F. oxysporum	Low-High vs. Low-Low	3	-0.67	0.901
		High-Control vs. Low-Low	3	-1	0.76
		High-Control vs. Low-High	3	-0.37	0.98
		High-Control vs. High-Low	3	0.32	0.987
		High-Low vs. Low-Low	3	-1.07	0.728
		High-Low vs. Low-High	3	-0.56	0.938
	F. solani	Low-Control vs. Low-Low	8	-0.86	0.947
		Low-Control vs. Low-High	8	1.07	0.879
		Low-High vs. Low-Low	8	-1.93	0.448
		High-Control vs. Low-Control	8	-1.19	0.829
		High-Control vs. Low-Low	8	-1.8	0.514
		High-Control vs. Low-High	8	-0.43	0.997
		High-Control vs. High-Low	8	0.32	0.999
		High-Control vs. High-High	8	-0.62	0.986
		High-Low vs. Low-Control	8	-1.58	0.628
		High-Low vs. Low-Low	8	-2.19	0.335
		High-Low vs. Low-High	8	-0.82	0.954
		High-High vs. Low-Control	8	-0.81	0.957
		High-High vs. Low-Low	8	-1.67	0.581
		High-High vs. Low-High	8	0.26	1
		High-High vs. High-Low	8	1.01	0.903
	Control	Control vs. Low	5	-60211	**<.001**
		Control vs. High	5	-54291	**<.001**
		High vs. Low	5	-5920	**<.001**
Koeleria	*R. solani* 1	Low-Control vs. Low-Low	45.9	-1.3	0.785
		Low-Control vs. Low-High	42.5	-2.97	**0.051**
		Low-High vs. Low-Low	44.6	1.62	0.587
		High-Control vs. Low-Control	46.2	0.76	0.972
		High-Control vs. Low-Low	46.4	-0.53	0.995
		High-Control vs. Low-High	46.4	-2.09	0.311
		High-Control vs. High-Low	46.3	-0.32	1
		High-Control vs. High-High	47	0.87	0.952

Species	Isolate	Comparison	DF	*t*	*P*
		High-Low vs. Low-Control	45	0.98	0.923
		High-Low vs. Low-Low	41.7	-0.14	1
		High-Low vs. Low-High	40.8	-1.57	0.621
		High-High vs. Low-Control	43.9	-0.24	1
		High-High vs. Low-Low	46.1	-1.37	0.744
		High-High vs. Low-High	44.1	-2.79	0.078
		High-High vs. High-Low	36.6	-1.19	0.84
	R. solani 2	Low-Control vs. Low-Low	38.6	-2.12	0.298
		Low-Control vs. Low-High	43.1	-1.1	0.879
		Low-High vs. Low-Low	38	-1.09	0.881
		High-Control vs. Low-Control	39.8	-0.42	0.998
		High-Control vs. Low-Low	42	-2.52	0.14
		High-Control vs. Low-High	43	-1.54	0.641
		High-Control vs. High-Low	44	-1.77	0.498
		High-Control vs. High-High	43.6	0.61	0.99
		High-Low vs. Low-Control	41.5	1.37	0.746
		High-Low vs. Low-Low	42.6	-0.76	0.973
		High-Low vs. Low-High	43.7	0.3	1
		High-High vs. Low-Control	43.9	-0.99	0.918
		High-High vs. Low-Low	41.2	-2.97	**0.052**
		High-High vs. Low-High	38.8	-2.09	0.314
		High-High vs. High-Low	44	-2.25	0.238
	R. solani 3	Low-Control vs. Low-Low	51	-1.24	0.814
		Low-Control vs. Low-High	49.3	-1.52	0.654
		Low-High vs. Low-Low	49.7	0.25	1
		High-Control vs. Low-Control	48.7	0.16	1
		High-Control vs. Low-Low	48.1	-1.11	0.876
		High-Control vs. Low-High	47.3	-1.37	0.743
		High-Control vs. High-Low	47.8	-1.95	0.388
		High-Control vs. High-High	44.8	-0.77	0.971
		High-Low vs. Low-Control	52	1.99	0.362
		High-Low vs. Low-Low	49.1	0.85	0.955
		High-Low vs. Low-High	50.6	0.59	0.991
		High-High vs. Low-Control	50.6	0.9	0.944
		High-High vs. Low-Low	45.4	-0.37	0.999
		High-High vs. Low-High	49.3	-0.61	0.99
		High-High vs. High-Low	47.2	-1.23	0.822
	F. oxysporum	Low-Control vs. Low-Low	53.9	-0.77	0.971
		Low-Control vs. Low-High	51.3	-1.72	0.528
		Low-High vs. Low-Low	53.6	0.89	0.947
		High-Control vs. Low-Control	47.6	1.05	0.899
		High-Control vs. Low-Low	52.7	0.23	1
		High-Control vs. Low-High	52.8	-0.68	0.983
		High-Control vs. High-Low	53.9	0.69	0.983
		High-Control vs. High-High	53.5	-0.32	1
		High-Low vs. Low-Control	48.8	0.32	1
		High-Low vs. Low-Low	51.5	-0.47	0.997
		High-Low vs. Low-High	52.2	-1.4	0.729
		High-High vs. Low-Control	51.6	1.36	0.75
		High-High vs. Low-Low	52.9	0.56	0.993
		High-High vs. Low-High	53.6	-0.36	0.999
		High-High vs. High-Low	51.1	1.05	0.899

Species	Isolate	Comparison	DF	*t*	*P*
	F. solani	Low-Control vs. Low-Low	37.8	-2.44	0.167
		Low-Control vs. Low-High	45.7	-2.47	0.156
		Low-High vs. Low-Low	41.3	0.21	1
		High-Control vs. Low-Control	40	0.8	0.965
		High-Control vs. Low-Low	42	-1.44	0.702
		High-Control vs. Low-High	42.9	-1.66	0.566
		High-Control vs. High-Low	36.3	-1.68	0.553
		High-Control vs. High-High	46.1	0.29	1
		High-Low vs. Low-Control	35	2.62	0.119
		High-Low vs. Low-Low	42.9	0.16	1
		High-Low vs. Low-High	46.7	-0.05	1
		High-High vs. Low-Control	47	0.48	0.997
		High-High vs. Low-Low	45	-1.8	0.475
		High-High vs. Low-High	47.3	-1.94	0.389
		High-High vs. High-Low	47.6	-1.86	0.44
	Control	Control vs. Low	23	-0.83	0.688
		Control vs. High	23.3	-0.9	0.645
		High vs. Low	25.8	0.09	0.996
Pascopyrum	*R. solani* 1	Low-Control vs. Low-Low	44	0.03	1
		Low-Control vs. Low-High	44	0.85	0.956
		Low-High vs. Low-Low	44	-0.87	0.952
		High-Control vs. Low-Control	44	0.11	1
		High-Control vs. Low-Low	44	0.15	1
		High-Control vs. Low-High	44	0.94	0.934
		High-Control vs. High-Low	44	2.04	0.339
		High-Control vs. High-High	44	1.59	0.612
		High-Low vs. Low-Control	44	-1.99	0.364
		High-Low vs. Low-Low	44	-2.07	0.324
		High-Low vs. Low-High	44	-1.25	0.811
		High-High vs. Low-Control	44	-1.52	0.652
		High-High vs. Low-Low	44	-1.57	0.623
		High-High vs. Low-High	44	-0.78	0.969
		High-High vs. High-Low	44	0.4	0.999
	R. solani 2	Low-Control vs. Low-Low	53	0.73	0.977
		Low-Control vs. Low-High	53	0.78	0.97
		Low-High vs. Low-Low	53	-0.04	1
		High-Control vs. Low-Control	53	0.67	0.984
		High-Control vs. Low-Low	53	1.4	0.724
		High-Control vs. Low-High	53	1.45	0.697
		High-Control vs. High-Low	53	0.89	0.948
		High-Control vs. High-High	53	1.88	0.424
		High-Low vs. Low-Control	53	-0.22	1
		High-Low vs. Low-Low	53	0.52	0.995
		High-Low vs. Low-High	53	0.56	0.993
		High-High vs. Low-Control	53	-1.23	0.822
		High-High vs. Low-Low	53	-0.52	0.995
		High-High vs. Low-High	53	-0.47	0.997
		High-High vs. High-Low	53	-1.02	0.91
	R. solani 3	Low-Control vs. Low-Low	47	1.26	0.804
		Low-Control vs. Low-High	43.7	0.21	1
		Low-High vs. Low-Low	44.9	1.07	0.89
		High-Control vs. Low-Control	46.3	-0.52	0.995

Species	Isolate	Comparison	DF	*t*	*P*
		High-Control vs. Low-Low	44.4	0.76	0.973
		High-Control vs. Low-High	48.4	-0.31	1
		High-Control vs. High-Low	46.1	-1.32	0.772
		High-Control vs. High-High	41.2	-0.25	1
		High-Low vs. Low-Control	47	0.79	0.967
		High-Low vs. Low-Low	48.1	2.02	0.345
		High-Low vs. Low-High	47.8	1	0.917
		High-High vs. Low-Control	45.6	-0.27	1
		High-High vs. Low-Low	41.5	1.01	0.91
		High-High vs. Low-High	44.8	-0.07	1
		High-High vs. High-Low	41.5	-1.09	0.882
	F. oxysporum	Low-Control vs. Low-Low	44.2	-0.01	1
		Low-Control vs. Low-High	47.8	-1.03	0.905
		Low-High vs. Low-Low	46.8	1.03	0.904
		High-Control vs. Low-Control	42.2	0.07	1
		High-Control vs. Low-Low	49	0.06	1
		High-Control vs. Low-High	45.5	-0.98	0.921
		High-Control vs. High-Low	44.3	1.12	0.872
		High-Control vs. High-High	47.4	-0.47	0.997
		High-Low vs. Low-Control	43.5	-1.06	0.896
		High-Low vs. Low-Low	47.5	-1.03	0.904
		High-Low vs. Low-High	43.8	-2.15	0.28
		High-High vs. Low-Control	47.1	0.54	0.994
		High-High vs. Low-Low	44.2	0.54	0.994
		High-High vs. Low-High	36	-0.53	0.995
		High-High vs. High-Low	38.8	1.68	0.556
	F. solani	Low-Control vs. Low-Low	47	1.15	0.857
		Low-Control vs. Low-High	40.8	-0.29	1
		Low-High vs. Low-Low	42.6	1.48	0.678
		High-Control vs. Low-Control	39.9	0.19	1
		High-Control vs. Low-Low	43	1.37	0.742
		High-Control vs. Low-High	47.8	-0.09	1
		High-Control vs. High-Low	49.6	0.97	0.926
		High-Control vs. High-High	52.7	0.16	1
		High-Low vs. Low-Control	47.5	-0.81	0.965
		High-Low vs. Low-Low	43.8	0.33	1
		High-Low vs. Low-High	43.3	-1.11	0.875
		High-High vs. Low-Control	53	0.01	1
		High-High vs. Low-Low	44.7	1.19	0.84
		High-High vs. Low-High	51.6	-0.26	1
		High-High vs. High-Low	51.7	0.79	0.968
	Control	Control vs. Low	25	0.08	0.996
		Control vs. High	25	0.36	0.932
		High vs. Low	25	-0.28	0.959
Vicia	*R. solani* 1	Low-Control vs. Low-Low	27.8	2.29	0.231
		Low-Control vs. Low-High	29.5	0.67	0.984
		Low-High vs. Low-Low	30.7	1.58	0.615
		High-Control vs. Low-Control	29.2	-3.08	**0.047**
		High-Control vs. Low-Low	29.8	-1.35	0.755
		High-Control vs. Low-High	25.8	-2.55	0.146
		High-Control vs. High-Low	28.7	1.36	0.747
		High-Control vs. High-High	30.3	-0.17	1

Species	Isolate	Comparison	DF	*t*	*P*
		High-Low vs. Low-Control	29.4	-3.22	**0.034**
		High-Low vs. Low-Low	29.5	-2.23	0.254
		High-Low vs. Low-High	29.3	-2.93	0.064
		High-High vs. Low-Control	29.6	-2.65	0.118
		High-High vs. Low-Low	27.3	-1.06	0.893
		High-High vs. Low-High	30.2	-2.18	0.274
		High-High vs. High-Low	30.3	1.45	0.699
	R. solani 2	Low-Control vs. Low-Low	39.6	-0.28	1
		Low-Control vs. Low-High	43.7	-1.92	0.403
		Low-High vs. Low-Low	43.5	1.67	0.56
		High-Control vs. Low-Control	44	-0.96	0.929
		High-Control vs. Low-Low	43.5	-1.16	0.853
		High-Control vs. Low-High	44	-2.42	0.172
		High-Control vs. High-Low	42	-0.18	1
		High-Control vs. High-High	43.2	-1.41	0.723
		High-Low vs. Low-Control	43.1	-0.95	0.931
		High-Low vs. Low-Low	41.6	-1.2	0.835
		High-Low vs. Low-High	43	-2.75	0.086
		High-High vs. Low-Control	43.4	0.64	0.987
		High-High vs. Low-Low	44	0.37	0.999
		High-High vs. Low-High	42.7	-1.18	0.846
		High-High vs. High-Low	43.8	1.5	0.664
	R. solani 3	Low-Control vs. Low-Low	39.1	1.01	0.913
		Low-Control vs. Low-High	41.7	-0.3	1
		Low-High vs. Low-Low	42.4	1.45	0.698
		High-Control vs. Low-Control	40.1	-0.63	0.988
		High-Control vs. Low-Low	36.6	0.43	0.998
		High-Control vs. Low-High	44.3	-0.99	0.918
		High-Control vs. High-Low	42.7	-0.53	0.995
		High-Control vs. High-High	46.2	-0.15	1
		High-Low vs. Low-Control	41.4	-0.13	1
		High-Low vs. Low-Low	38.4	0.99	0.92
		High-Low vs. Low-High	41.5	-0.49	0.996
		High-High vs. Low-Control	39	-0.5	0.996
		High-High vs. Low-Low	42.3	0.57	0.992
		High-High vs. Low-High	43.7	-0.87	0.951
		High-High vs. High-Low	41	-0.39	0.999
	F. oxysporum	Low-Control vs. Low-Low	51.1	1.58	0.613
		Low-Control vs. Low-High	52.1	-1.48	0.676
		Low-High vs. Low-Low	49.4	3.11	**0.034**
		High-Control vs. Low-Control	50.5	-1.13	0.869
		High-Control vs. Low-Low	46.1	0.48	0.997
		High-Control vs. Low-High	48.5	-2.66	0.101
		High-Control vs. High-Low	53	-1.15	0.856
		High-Control vs. High-High	49.3	-0.71	0.98
		High-Low vs. Low-Control	52.3	0.08	1
		High-Low vs. Low-Low	53	1.59	0.609
		High-Low vs. Low-High	52.6	-1.36	0.753
		High-High vs. Low-Control	49.6	-0.43	0.998
		High-High vs. Low-Low	48.3	1.18	0.842
		High-High vs. Low-High	50.5	-1.93	0.398
		High-High vs. High-Low	51.9	-0.5	0.996

Species	Isolate	Comparison	DF	*t*	*P*
	F. solani	Low-Control vs. Low-Low	46	-1.67	0.557
		Low-Control vs. Low-High	46	-1.74	0.51
		Low-High vs. Low-Low	46	0.03	1
		High-Control vs. Low-Control	46	0.25	1
		High-Control vs. Low-Low	46	-1.25	0.81
		High-Control vs. Low-High	46	-1.3	0.783
		High-Control vs. High-Low	46	-0.17	1
		High-Control vs. High-High	46	0.65	0.986
		High-Low vs. Low-Control	46	0.46	0.997
		High-Low vs. Low-Low	46	-1.21	0.83
		High-Low vs. Low-High	46	-1.27	0.799
		High-High vs. Low-Control	46	-0.45	0.998
		High-High vs. Low-Low	46	-2.12	0.293
		High-High vs. Low-High	46	-2.21	0.254
		High-High vs. High-Low	46	-0.91	0.941
	Control	Control vs. Low	20.2	-0.62	0.811
		Control vs. High	21.8	-0.83	0.692
		High vs. Low	26	0.18	0.983

Appendix 13. Effects of inoculum density and exudate concentration on per plant biomass and root:shoot ratios by plant species and isolate. Degrees of freedom (DF) are displayed as numerator/ denominator, and *P*-values <0.05 are in bold.

			Biomass			Root:shoot		
Species	Isolate	Effect	DF	*F*	*P*	DF	*F*	*P*
Echinacea	Rs1	Inoculum density	1/18	1.55	0.23	1/10.2	8.16	**0.017**
		Exudate concentration	2/21.2	5.2	**0.015**	2/11.9	0.76	0.488
		Inoculum × Exudate	2/18.5	4.38	**0.028**	2/8.7	0.34	0.72
	Rs2	Inoculum density	1/26.6	0.04	0.846	1/27	1.28	0.267
		Exudate concentration	2/25.8	3.86	**0.034**	2/27	2.91	0.072
		Inoculum × Exudate	2/23.8	0.7	0.509	2/27	1.16	0.328
	Rs3	Inoculum density	1/46	1.48	0.23	1/44.5	0.41	0.528
		Exudate concentration	2/46	3.22	**0.049**	2/44.1	0.43	0.654
		Inoculum × Exudate	2/46	1.11	0.337	2/41.2	0.01	0.993
	Fo	Inoculum density	1/35.5	0.66	0.423	1/35.6	0.59	0.447
		Exudate concentration	2/39.3	0.89	0.418	2/38.8	2.49	0.096
		Inoculum × Exudate	2/37.3	5.65	**0.007**	2/37.7	3.21	0.052
	Fs	Inoculum density	1/33	0.42	0.521	1/33	0.75	0.393
		Exudate concentration	2/33	1.96	0.156	2/33	2.98	0.065
		Inoculum × Exudate	2/33	0.92	0.408	2/33	0.23	0.797
	Control	Exudate concentration	2/21	0.73	0.493	2/17.5	1.08	0.36
Helianthus	Rs1	Inoculum density	1/5	0.33	0.59	1/5	1.34	0.3
		Exudate concentration	2/5	2.39	0.187	2/5	0.12	0.888
		Inoculum × Exudate	1/5	3.74	0.111	1/5	0.68	0.447
	Rs2	Inoculum density	1/16.8	0.02	0.896	1/19	0.02	0.902
		Exudate concentration	2/17.1	0.28	0.76	2/19	0.57	0.576
		Inoculum × Exudate	2/18.5	3.05	0.072	2/19	1.08	0.358
	Rs3	Inoculum density	1/8.38	1.28	0.289	1/9	0.77	0.404
		Exudate concentration	2/8.67	0.32	0.735	2/9	2.13	0.174
		Inoculum × Exudate	2/8.36	1.42	0.294	2/9	2.02	0.189
	Fo	Inoculum density	1/3	1.15	0.362	1/3	1.89	0.263
		Exudate concentration	2/3	0.28	0.775	2/3	1.32	0.388
		Inoculum × Exudate	.	.	.	.	.	.
	Fs	Inoculum density	1/8	4.1	0.078	1/8	0.02	0.901
		Exudate concentration	2/8	0.04	0.958	2/8	0.06	0.943
		Inoculum × Exudate	2/8	2.01	0.196	2/8	0.2	0.826
	Control	Exudate concentration	2/1	2×10^{9}	**<.001**	2/5	2.23	0.203
Koeleria	Rs1	Inoculum density	1/44.4	1.83	0.183	1/47	5.18	**0.027**
		Exudate concentration	2/45.4	0.91	0.411	2/47	0.11	0.893
		Inoculum × Exudate	2/43	3.53	**0.038**	2/47	2.82	0.069
	Rs2	Inoculum density	1/39.4	3.68	0.062	1/37	4.37	**0.044**
		Exudate concentration	2/43	4.63	**0.015**	2/41.8	1.22	0.306
		Inoculum × Exudate	2/40.7	0.83	0.444	2/38.8	1.02	0.369
	Rs3	Inoculum density	1/50.9	0.05	0.819	1/51.3	0.19	0.662
		Exudate concentration	2/48.7	2.54	0.089	2/48.3	1.3	0.282
		Inoculum × Exudate	2/47.8	0.54	0.589	2/47.1	1	0.375
	Fo	Inoculum density	1/48.1	0.01	0.913	1/46.8	1.56	0.218
		Exudate concentration	2/53	1.35	0.269	2/52.7	0.18	0.838
		Inoculum × Exudate	2/52.5	0.71	0.496	2/51.3	1.93	0.155
	Fs	Inoculum density	1/44.5	0.33	0.57	1/49	0.18	0.675
		Exudate concentration	2/41.6	4.46	**0.018**	2/49	0.09	0.916
		Inoculum × Exudate	2/43.1	2	0.147	2/49	2.66	0.08

			Biomass			Root:shoot		
Species	Isolate	Effect	DF	*F*	*P*	DF	*F*	*P*
	Control	Exudate concentration	2/24	0.52	0.603	2/24.9	1.38	0.27
Pascopyrum	Rs1	Inoculum density	1/44	2.32	0.135	1/42.8	0.71	0.405
		Exudate concentration	2/44	1.77	0.182	2/41.4	0.75	0.48
		Inoculum × Exudate	2/44	1.12	0.337	2/42.1	0.24	0.789
	Rs2	Inoculum density	1/53	0.16	0.689	1/53	0.05	0.827
		Exudate concentration	2/53	1.82	0.173	2/53	4.5	**0.016**
		Inoculum × Exudate	2/53	0.38	0.685	2/53	0.46	0.631
	Rs3	Inoculum density	1/49.2	0.71	0.402	1/50.3	0.21	0.647
		Exudate concentration	2/43.5	0	1	2/43.5	1.18	0.317
		Inoculum × Exudate	2/44.1	1.85	0.169	2/44.2	0.37	0.691
	Fo	Inoculum density	1/44	0.73	0.399	1/45.7	0.65	0.425
		Exudate concentration	2/45.7	1.77	0.181	2/46.1	0.11	0.898
		Inoculum × Exudate	2/41.8	0.32	0.728	2/43	0.08	0.924
	Fs	Inoculum density	1/44.1	0.02	0.888	1/53	0.18	0.674
		Exudate concentration	2/48.6	1.64	0.204	2/53	0.66	0.52
		Inoculum × Exudate	2/45.3	0.09	0.912	2/53	0.15	0.859
	Control	Exudate concentration	2/25	0.07	0.931	2/25	0.1	0.903
Vicia	Rs1	Inoculum density	1/30.5	16.7	**<.001**	1/30.8	11.17	**0.002**
		Exudate concentration	2/30.4	2.4	0.108	2/30.4	3.74	**0.035**
		Inoculum × Exudate	2/30.2	0.31	0.739	2/30.7	3.06	0.061
	Rs2	Inoculum density	1/43.2	3.58	0.065	1/42.4	0.22	0.641
		Exudate concentration	2/44	3.58	**0.036**	2/44	0.97	0.388
		Inoculum × Exudate	2/42.8	0	1	2/42.1	0.43	0.653
	Rs3	Inoculum density	1/37.9	0.12	0.726	1/45.3	1.68	0.202
		Exudate concentration	2/42.3	0.28	0.759	2/49.4	0.83	0.443
		Inoculum × Exudate	2/41.7	1.05	0.358	2/48.5	0.22	0.805
	Fo	Inoculum density	1/48.9	0.63	0.433	1/53	0	0.947
		Exudate concentration	2/49.4	1.98	0.15	2/53	0.41	0.666
		Inoculum × Exudate	2/52.4	3.32	**0.044**	2/53	0.85	0.433
	Fs	Inoculum density	1/46	2.98	0.091	1/46	0.35	0.555
		Exudate concentration	2/46	0.77	0.469	2/46	0.05	0.951
		Inoculum × Exudate	2/46	1.34	0.271	2/46	0.01	0.986
	Control	Exudate concentration	2/22.9	0.38	0.687	2/19.7	1.59	0.229

Appendix 14. ANCOVA multiple comparisons of species' relationships between exudate concentration and colony diameter for polar and nonpolar exudates. Species are abbreviated using first three letters of genus. Estimates (Est.) indicate sign and magnitude of differences between slopes of the given species. *P*-values are Bonferroni adjusted, and values less than 0.05 are in bold. Rs = *R. solani*, Fo = *F. oxysporum*, Fs = *F. solani*.

		Polar				Nonpolar			
Isolate	Label	Est.	DF	*t*	*P*	Est.	DF	*t*	*P*
Rs1	*Art. vs. Bou.*	-0.44	81	-0.61	1	0.39	78	0.5	1
	Art. vs. Ech.	-0.81	81	-1.25	1	0.89	78	1.15	1
	Art. vs. Eup.	-0.68	81	-1.21	1	-0.34	78	-0.63	1
	Art. vs. Hel.	-1.60	81	-2.04	1	-0.46	78	-0.57	1
	Art. vs. Koe.	-0.99	81	-1.61	1	-0.60	78	-0.97	1
	Art. vs. Lin.	-1.35	81	-1.83	1	-0.63	78	-1	1
	Art. vs. Nas.	-1.05	81	-1.77	1	-0.37	78	-0.6	1
	Art. vs. Pas.	-0.72	81	-1.27	1	0.20	78	0.37	1
	Bou. vs. Ech.	-0.37	81	-0.61	1	0.50	78	0.62	1
	Bou. vs. Eup.	-0.24	81	-0.47	1	-0.73	78	-1.25	1
	Bou. vs. Hel.	-1.15	81	-1.54	1	-0.86	78	-1.01	1
	Bou. vs. Koe.	-0.55	81	-0.97	1	-0.99	78	-1.52	1
	Bou. vs. Lin.	-0.90	81	-1.29	1	-1.02	78	-1.53	1
	Bou. vs. Nas.	-0.61	81	-1.12	1	-0.76	78	-1.16	1
	Bou. vs. Pas.	-0.27	81	-0.53	1	-0.19	78	-0.32	1
	Ech. vs. Eup.	0.13	81	0.34	1	-1.23	78	-2.12	1
	Ech. vs. Hel.	-0.79	81	-1.19	1	-1.36	78	-1.61	1
	Ech. vs. Koe.	-0.18	81	-0.41	1	-1.49	78	-2.3	0.871
	Ech. vs. Lin.	-0.54	81	-0.89	1	-1.52	78	-2.29	0.894
	Ech. vs. Nas.	-0.24	81	-0.58	1	-1.26	78	-1.94	1
	Ech. vs. Pas.	0.09	81	0.25	1	-0.69	78	-1.16	1
	Eup. vs. Hel.	-0.91	81	-1.57	1	-0.12	78	-0.2	1
	Eup. vs. Koe.	-0.31	81	-0.97	1	-0.26	78	-0.77	1
	Eup. vs. Lin.	-0.66	81	-1.29	1	-0.29	78	-0.79	1
	Eup. vs. Nas.	-0.37	81	-1.32	1	-0.03	78	-0.09	1
	Eup. vs. Pas.	-0.03	81	-0.16	1	0.54	78	2.63	0.366
	Hel. vs. Koe.	0.61	81	0.96	1	-0.13	78	-0.19	1
	Hel. vs. Lin.	0.25	81	0.34	1	-0.16	78	-0.23	1
	Hel. vs. Nas.	0.54	81	0.89	1	0.09	78	0.13	1
	Hel. vs. Pas.	0.88	81	1.52	1	0.67	78	1.04	1
	Koe. vs. Lin.	-0.35	81	-0.62	1	-0.03	78	-0.07	1
	Koe. vs. Nas.	-0.06	81	-0.17	1	0.23	78	0.51	1
	Koe. vs. Pas.	0.28	81	0.88	1	0.80	78	2.27	0.927
	Lin. vs. Nas.	0.29	81	0.53	1	0.26	78	0.55	1
	Lin. vs. Pas.	0.63	81	1.23	1	0.83	78	2.17	1
	Nas. vs. Pas.	0.34	81	1.22	1	0.57	78	1.59	1
Rs2	*Art. vs. Bou.*	1.05	98	0.89	1	1.04	99	0.58	1
	Art. vs. Ech.	0.47	98	0.38	1	3.83	99	1.74	1
	Art. vs. Eup.	1.02	98	0.95	1	0.98	99	0.65	1
	Art. vs. Hel.	-0.44	98	-0.3	1	-1.41	99	-0.65	1

		Polar				Nonpolar			
Isolate	Label	Est.	DF	*t*	*P*	Est.	DF	*t*	*P*
	Art. vs. Koe.	0.04	98	0.03	1	-1.62	99	-0.95	1
	Art. vs. Lin.	-0.30	98	-0.21	1	1.17	99	0.65	1
	Art. vs. Nas.	1.80	98	1.61	1	0.49	99	0.28	1
	Art. vs. Pas.	0.85	98	0.79	1	1.14	99	0.73	1
	Bou. vs. Ech.	-0.58	98	-0.68	1	2.79	99	1.45	1
	Bou. vs. Eup.	-0.03	98	-0.05	1	-0.06	99	-0.05	1
	Bou. vs. Hel.	-1.50	98	-1.27	1	-2.45	99	-1.29	1
	Bou. vs. Koe.	-1.01	98	-1.3	1	-2.66	99	-2.01	1
	Bou. vs. Lin.	-1.35	98	-1.21	1	0.13	99	0.09	1
	Bou. vs. Nas.	0.75	98	1.07	1	-0.55	99	-0.41	1
	Bou. vs. Pas.	-0.20	98	-0.33	1	0.10	99	0.08	1
	Ech. vs. Eup.	0.56	98	0.77	1	-2.84	99	-1.71	1
	Ech. vs. Hel.	-0.91	98	-0.74	1	-5.24	99	-2.28	0.883
	Ech. vs. Koe.	-0.43	98	-0.5	1	-5.45	99	-2.96	0.14
	Ech. vs. Lin.	-0.77	98	-0.66	1	-2.65	99	-1.37	1
	Ech. vs. Nas.	1.33	98	1.69	1	-3.34	99	-1.8	1
	Ech. vs. Pas.	0.38	98	0.54	1	-2.69	99	-1.57	1
	Eup. vs. Hel.	-1.47	98	-1.37	1	-2.39	99	-1.45	1
	Eup. vs. Koe.	-0.98	98	-1.59	1	-2.61	99	-2.81	0.216
	Eup. vs. Lin.	-1.32	98	-1.32	1	0.19	99	0.17	1
	Eup. vs. Nas.	0.78	98	1.53	1	-0.50	99	-0.52	1
	Eup. vs. Pas.	-0.17	98	-0.45	1	0.15	99	0.25	1
	Hel. vs. Koe.	0.48	98	0.41	1	-0.21	99	-0.12	1
	Hel. vs. Lin.	0.14	98	0.1	1	2.59	99	1.34	1
	Hel. vs. Nas.	2.25	98	2.01	1	1.90	99	1.03	1
	Hel. vs. Pas.	1.30	98	1.21	1	2.55	99	1.51	1
	Koe. vs. Lin.	-0.34	98	-0.3	1	2.80	99	2.06	1
	Koe. vs. Nas.	1.76	98	2.52	0.479	2.11	99	1.7	1
	Koe. vs. Pas.	0.81	98	1.33	1	2.76	99	2.75	0.252
	Lin. vs. Nas.	2.10	98	1.99	1	-0.69	99	-0.5	1
	Lin. vs. Pas.	1.15	98	1.15	1	-0.04	99	-0.03	1
	Nas. vs. Pas.	-0.95	98	-1.89	1	0.65	99	0.63	1
Rs3	*Art. vs. Bou.*	3.10	97	2.29	0.87	0.03	90	0.04	1
	Art. vs. Ech.	2.46	97	1.77	1	-0.64	90	-0.58	1
	Art. vs. Eup.	4.36	97	3.53	**0.023**	1.12	90	1.58	1
	Art. vs. Hel.	1.37	97	0.81	1	1.84	90	1.74	1
	Art. vs. Koe.	3.41	97	2.52	0.485	1.86	90	2.32	0.813
	Art. vs. Lin.	2.58	97	1.58	1	1.45	90	1.68	1
	Art. vs. Nas.	4.10	97	3.17	0.073	1.37	90	1.7	1
	Art. vs. Pas.	4.39	97	3.55	**0.022**	1.22	90	1.65	1
	Bou. vs. Ech.	-0.64	97	-0.68	1	-0.67	90	-0.68	1
	Bou. vs. Eup.	1.26	97	1.78	1	1.09	90	2.09	1
	Bou. vs. Hel.	-1.73	97	-1.28	1	1.81	90	1.93	1
	Bou. vs. Koe.	0.31	97	0.35	1	1.83	90	2.86	0.19
	Bou. vs. Lin.	-0.52	97	-0.4	1	1.42	90	1.98	1
	Bou. vs. Nas.	1.00	97	1.24	1	1.34	90	2.07	1

		Polar				Nonpolar			
Isolate	Label	Est.	DF	*t*	*P*	Est.	DF	*t*	*P*
	Bou. vs. Pas.	1.29	97	1.8	1	1.18	90	2.13	1
	Ech. vs. Eup.	1.91	97	2.48	0.54	1.75	90	2.03	1
	Ech. vs. Hel.	-1.09	97	-0.78	1	2.47	90	2.12	1
	Ech. vs. Koe.	0.96	97	1.01	1	2.49	90	2.65	0.343
	Ech. vs. Lin.	0.13	97	0.1	1	2.08	90	2.1	1
	Ech. vs. Nas.	1.64	97	1.91	1	2.01	90	2.12	1
	Ech. vs. Pas.	1.94	97	2.5	0.51	1.85	90	2.09	1
	Eup. vs. Hel.	-2.99	97	-2.43	0.613	0.72	90	0.89	1
	Eup. vs. Koe.	-0.95	97	-1.32	1	0.74	90	1.7	1
	Eup. vs. Lin.	-1.78	97	-1.54	1	0.33	90	0.61	1
	Eup. vs. Nas.	-0.27	97	-0.45	1	0.25	90	0.57	1
	Eup. vs. Pas.	0.03	97	0.06	1	0.10	90	0.33	1
	Hel. vs. Koe.	2.04	97	1.51	1	0.02	90	0.02	1
	Hel. vs. Lin.	1.21	97	0.74	1	-0.39	90	-0.41	1
	Hel. vs. Nas.	2.73	97	2.12	1	-0.47	90	-0.52	1
	Hel. vs. Pas.	3.02	97	2.45	0.586	-0.62	90	-0.75	1
	Koe. vs. Lin.	-0.83	97	-0.65	1	-0.41	90	-0.62	1
	Koe. vs. Nas.	0.68	97	0.84	1	-0.49	90	-0.84	1
	Koe. vs. Pas.	0.98	97	1.35	1	-0.64	90	-1.35	1
	Lin. vs. Nas.	1.52	97	1.25	1	-0.08	90	-0.12	1
	Lin. vs. Pas.	1.81	97	1.56	1	-0.23	90	-0.41	1
	Nas. vs. Pas.	0.29	97	0.5	1	-0.15	90	-0.32	1
Fo	*Art. vs. Ech.*	-0.57	85	-0.5	1	-6.12	88	-4.22	**0.002**
	Art. vs. Eup.	-0.01	85	-0.01	1	-1.04	88	-1.04	1
	Art. vs. Hel.	-0.14	85	-0.1	1	0.91	88	0.63	1
	Art. vs. Koe.	2.15	85	1.93	1	1.59	88	1.41	1
	Art. vs. Lin.	2.58	85	1.92	1	-0.10	88	-0.08	1
	Art. vs. Nas.	0.51	85	0.48	1	-1.55	88	-1.36	1
	Art. vs. Pas.	-0.23	85	-0.22	1	-0.51	88	-0.5	1
	Ech. vs. Eup.	0.55	85	0.86	1	5.09	88	4.63	**<.001**
	Ech. vs. Hel.	0.43	85	0.36	1	7.03	88	4.65	**<.001**
	Ech. vs. Koe.	2.72	85	3.49	**0.022**	7.72	88	6.34	**<.001**
	Ech. vs. Lin.	3.15	85	2.91	0.129	6.02	88	4.71	**<.001**
	Ech. vs. Nas.	1.08	85	1.52	1	4.58	88	3.74	**0.009**
	Ech. vs. Pas.	0.34	85	0.54	1	5.61	88	4.98	**<.001**
	Eup. vs. Hel.	-0.13	85	-0.12	1	1.95	88	1.79	1
	Eup. vs. Koe.	2.17	85	3.65	**0.013**	2.63	88	4.3	**0.001**
	Eup. vs. Lin.	2.59	85	2.71	0.226	0.94	88	1.28	1
	Eup. vs. Nas.	0.52	85	1.06	1	-0.51	88	-0.81	1
	Eup. vs. Pas.	-0.21	85	-0.56	1	0.53	88	1.3	1
	Hel. vs. Koe.	2.29	85	1.95	1	0.68	88	0.57	1
	Hel. vs. Lin.	2.72	85	1.95	1	-1.01	88	-0.79	1
	Hel. vs. Nas.	0.65	85	0.57	1	-2.46	88	-2.02	1
	Hel. vs. Pas.	-0.09	85	-0.08	1	-1.42	88	-1.27	1
	Koe. vs. Lin.	0.43	85	0.41	1	-1.69	88	-1.89	1
	Koe. vs. Nas.	-1.64	85	-2.48	0.423	-3.14	88	-3.85	**0.006**

		Polar				Nonpolar			
Isolate	Label	Est.	DF	*t*	*P*	Est.	DF	*t*	*P*
	Koe. vs. Pas.	-2.38	85	-4.08	**0.003**	-2.10	88	-3.18	0.057
	Lin. vs. Nas.	-2.07	85	-2.07	1	-1.45	88	-1.59	1
	Lin. vs. Pas.	-2.81	85	-2.96	0.112	-0.41	88	-0.53	1
	Nas. vs. Pas.	-0.74	85	-1.53	1	1.04	88	1.53	1
Fs	*Art. vs. Bou.*	-22.19	14	-0.46	1	8.08	12	0.52	1
	Art. vs. Ech.	-22.77	14	-0.47	1	7.26	12	0.73	1
	Art. vs. Eup.	-19.64	14	-0.41	1	8.15	12	1.38	1
	Art. vs. Hel.	-3.70	14	-0.07	1	39.14	12	2.29	1
	Art. vs. Koe.	-9.54	14	-0.19	1	17.15	12	1.01	1
	Art. vs. Lin.	-15.63	14	-0.32	1	1.54	12	0.15	1
	Art. vs. Nas.	-37.48	14	-0.78	1	6.17	12	0.81	1
	Art. vs. Pas.	-17.59	14	-0.36	1	2.03	12	0.32	1
	Bou. vs. Ech.	-0.58	14	-0.1	1	-0.82	12	-0.05	1
	Bou. vs. Eup.	2.56	14	0.61	1	0.07	12	0.01	1
	Bou. vs. Hel.	18.49	14	1.6	1	31.06	12	1.44	1
	Bou. vs. Koe.	12.65	14	1.4	1	9.07	12	0.42	1
	Bou. vs. Lin.	6.56	14	0.67	1	-6.54	12	-0.39	1
	Bou. vs. Nas.	-15.29	14	-3.13	0.268	-1.92	12	-0.13	1
	Bou. vs. Pas.	4.60	14	1.1	1	-6.05	12	-0.41	1
	Ech. vs. Eup.	3.13	14	0.63	1	0.89	12	0.11	1
	Ech. vs. Hel.	19.07	14	1.61	1	31.88	12	1.77	1
	Ech. vs. Koe.	13.23	14	1.41	1	9.89	12	0.55	1
	Ech. vs. Lin.	7.14	14	0.71	1	-5.72	12	-0.47	1
	Ech. vs. Nas.	-14.71	14	-2.66	0.678	-1.10	12	-0.12	1
	Ech. vs. Pas.	5.18	14	1.07	1	-5.24	12	-0.59	1
	Eup. vs. Hel.	15.93	14	1.44	1	30.99	12	1.92	1
	Eup. vs. Koe.	10.10	14	1.2	1	9.00	12	0.56	1
	Eup. vs. Lin.	4.00	14	0.44	1	-6.62	12	-0.74	1
	Eup. vs. Nas.	-17.85	14	-5.01	**0.007**	-1.99	12	-0.39	1
	Eup. vs. Pas.	2.04	14	0.85	1	-6.13	12	-2.01	1
	Hel. vs. Koe.	-5.83	14	-0.43	1	-21.99	12	-0.97	1
	Hel. vs. Lin.	-11.93	14	-0.84	1	-37.60	12	-2.05	1
	Hel. vs. Nas.	-33.78	14	-2.98	0.357	-32.98	12	-1.96	1
	Hel. vs. Pas.	-13.89	14	-1.26	1	-37.11	12	-2.28	1
	Koe. vs. Lin.	-6.10	14	-0.5	1	-15.61	12	-0.85	1
	Koe. vs. Nas.	-27.95	14	-3.19	0.235	-10.98	12	-0.66	1
	Koe. vs. Pas.	-8.05	14	-0.97	1	-15.12	12	-0.93	1
	Lin. vs. Nas.	-21.85	14	-2.29	1	4.63	12	0.46	1
	Lin. vs. Pas.	-1.96	14	-0.21	1	0.49	12	0.05	1
	Nas. vs. Pas.	19.89	14	5.72	**0.002**	-4.14	12	-0.72	1