

DISSERTATION

PLANT-SOIL FEEDBACKS: A POTENTIAL TOOL TO IMPROVE MANAGEMENT
OF INVASIVE KNAPWEEDS

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ABSTRACT

PLANT-SOIL FEEDBACKS: A POTENTIAL TOOL TO IMPROVE MANAGEMENT OF INVASIVE KNAPWEEDS

Invasive plants are globally recognized as a problem due to their negative impacts on biodiversity, ecosystem function, and agricultural production. The myriad of interactions between plants, soil microorganisms, and nutrients are well recognized, but little is known about how these plant-soil feedbacks affect vegetation dynamics and invasive species. The following PhD research studied the interactions between invasive plants and the soil to improve our understanding of complex systems and assist in the development of innovative management and control strategies. To augment our understanding of plant-soil feedbacks I studied decomposition, soil microbial diversity, and vegetation while manipulating soil microorganisms and plant competition in field and greenhouse experiments.

Chapter 1 provides a summary of the impacts of invasive plants on soil properties, nutrient cycling, and microbial communities. The chapter is currently in press for a CABI publication edited by Thomas Monaco and Roger Sheley (*Invasive Plant Ecology and Management: Linking Processes to Practice*). Chapter 2 uses soil conditioning by specific plant species and inoculation with the soil microbial communities of several plant communities to study the growth and competition of a native and invasive plant

species. The results of several greenhouse studies document how a native plant is only impacted by interspecific competition and avoids effects from soil microbes, while an invasive species avoids any negative impacts of exploitative competition, but is affected by soil microorganisms (interference competition). These results highlight the variability of competitive effects and how different types of competition may influence plant invasion. An improved understanding of the role of pathogens and interference competition in plant community dynamics could assist in invasive plant management practices based on manipulation of species' specific pathogen accumulation.

Field-based studies in three US states used inoculation with the soil microorganisms of adjacent native plant communities to study the influence of inoculation on the restoration of native plants in roto-tilled invasive knapweed infestations (Chapter 3). I compared alternative hypotheses that native plants would benefit from potential mutualisms with the native soil inoculation or that invasive plants would have reduced pathogen loading when inoculated with soil from a native plant community. Results highlight species and site specific responses of inoculation with whole soils from native plant communities. Spotted knapweed (*Centaurea stoebe*) was negatively impacted by native plant soil inoculation, while Russian knapweed (*Acroptilon repens*) responded positively. Inoculation was shown to impact plant growth, soil nutrients and microorganisms, although the variability of responses requires additional research before any applications in invasive plant management.

Litter decomposition experiments at three sites in Colorado utilized gradients between native and invasive knapweed vegetation to study the impacts of knapweed populations on decomposition of different litter types and the microbial communities of

litter and soil (Chapter 4). I hypothesized that invaded vegetation would decompose litter more rapidly and that a positive feedback would exist in the decomposition of knapweed litter in knapweed vegetation. The results showed increased amounts of litter decomposition inside of knapweed infestations and distinctly different soil and litter microbial communities between the sites, but not between vegetation types within a site. Understanding the interactions of invasive knapweed with soil, litter, and microbes could improve our ability to manage ecosystems through the manipulation of primary ecosystem processes, such as decomposition, litter inputs and quality, nutrient cycling, and microbial activity.

The research presented in this dissertation provides baseline information on the interactions and feedbacks between vegetation, microorganisms, and nutrients with a unique focus on invasive plant management. Results highlight the potential of inoculation as a tool to impact specific vegetation, although main challenges exist in the application as a management tool due to species' specific results and variability of impacts. The combination of competition and inoculation studies illustrates the responses of different species to exploitative (inter- or intraspecific competition) and interference (microbial interactions) competition and may help to elucidate why certain species are invasive. Through understanding when and why competition is most powerful, it may be possible to promote invasive plant management strategies that are based on maximizing competition. An overarching goal of this research is to promote management of invasive and native plant communities that develops sustainable and resilient systems through the manipulation of ecosystem processes, instead of top-down control strategies that are disruptive, expensive, and only applicable on small scales. Management of ecosystem

processes requires in-depth knowledge of specific systems and must take into account the variability of vegetation, soils, and microorganisms before use as a potential management tool. The utility of the research presented in this dissertation requires a paradigm shift away from our innate desire to manage for what humanity has known and instead offers creative approaches to managing the novel ecosystems that humans have created throughout the earth.

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Chapter 1 – Invasive plant impacts on soil properties, nutrient cycling, and microbial communities*

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INTRODUCTION

Before the 19th century biological invasions were probably infrequent, natural events that contributed to a diverse and ever evolving patchwork of species and ecosystems. The dramatic increase in the human population and our influence over the earth's biotic and abiotic systems has created a world that is dominated by the effects of humans (Vitousek et al. 1997). In general, humans have rapidly increased the movement of flora and fauna across what were once natural barriers to migration or survival and the consequences are now termed "biological invasions" (Elton 1958). All organisms influence and modify their environment, whether through competitive interactions that alter species composition, the addition or removal of resources, or the transformation of habitat and large-scale ecosystem processes (*sensu* Odum 1969). To successfully manage invasive species, it is critical that we understand how these species interact and modify their novel environment and use this knowledge to improve the efficiency and

effectiveness of management practices. This chapter will specifically address the impacts of invasive plants on the soil, including the physical and chemical composition of soil, litter decomposition and biogeochemical cycling of nutrients, and soil microbial communities. The primary emphasis will focus on the impacts of invasive species on these systems and utilizing this knowledge to apply ecological principles to the management of ecosystem processes.

An ecosystem is a representation of the biotic elements and abiotic variables that interact at a given place and time. Any dramatic change in the species composition, either above- or below- ground, will likely affect the composition and function of the whole. An understanding of the direct and indirect impacts of invasive species on a system is critical to delineate if we are to manage natural and utilitarian ecosystems effectively and with the goal of conserving ecosystem diversity and function. Invasion by exotic plants represents a fundamental change in a system and a unique management challenge for human society, both ethically and economically. From a Clementsian framework of successional dynamics, following invasion, a late-seral community of native species may no longer be a possible outcome of long-term ecosystem development, because the whole system has changed and its trajectory is different (Clements 1916). Within a Gleasonian individualistic perspective of plant dynamics, the key players have changed and they will influence the system in a unique and different manner (Gleason 1926). History is no longer a guide to the interaction of species and systems, therefore how they will interact and the resulting community composition is unknown. As the plant community composition changes due to the invaders, so will many of the ecological processes that link the above and below-ground elements of the

ecosystem (Wardle et al. 2004), including decomposition, nutrient cycling, rhizosphere exudation, microbial composition and function. It is likely that the change in plant community will influence the amount and type of animal herbivory, with cascading effects on soil properties, root growth responses from herbivory, and nutrient inputs from animal defecation. Lastly, a change in vegetation can alter the disturbance regime of a system, particularly fire cycles and intensity. Overall, any striking change in the plant community will have diverse effects throughout the whole realm of plant-soil-microbe interactions.

Invasive species tend to be highly successful through their rapid growth and reproduction, high fecundity, great dispersal abilities, and apparent predeposition to establish in disturbed areas. Many invasive plants have been removed from their evolutionarily constraints or have been released from enemies and are often found proliferating in disturbed areas that have available resources. These aggressive and opportunistic traits do not facilitate their dominance in the species' native range and this conundrum has been called the "invasive plant paradox" (Rout and Callaway 2009). It may be possible that invasive species dramatically modify the novel habitat to increase their fitness or negatively affect their neighbors and competitors. Most organisms attempt to do this in their struggle to reproduce and survive, but are usually constrained by resources or other species, especially in the long evolutionary time frame of an ecosystem's development. These interactions, both direct and indirect, may be part of a plant-soil feedback that facilitates invasion through either positive or negative interactions. Feedbacks may facilitate fecundity of the invader, negatively influence

competitors, or modify soil to the extent that the system is fundamentally different and less hospitable to certain organisms.

Much ecological research has focused on factors such as competition, dispersal, resource use efficiency, herbivory and predation to understand plant community composition, succession, and invasion. In-depth research has provided quantitative information about how plants, animals, soil, and the atmosphere interact and ecosystems are modified, but the focus on competition and resource efficiency has not completely addressed many questions about vegetation dynamics (Klironomos 2002), especially concerning the paradox of invasion. Competitive ability has been used to describe why one organism, species, or population displaces another or is more successful in the capture of a resource and the subsequent effect on its survival and fecundity. Obviously some species are better at acquiring a resource than another, but can the simplistic focus on one nutrient, growth habit or reproductive strategy explain the diversity of ecosystems or form a rationale for successful invasion by an exotic species? By focusing on a species' multi-scale direct and indirect interactions within an ecosystem and the subsequent impacts of a change in biodiversity, we may see the outcomes of competition are rooted in the interactions and feedbacks of a complex system. It may be possible to view these interactions as the mechanism that structures a community and facilitates invasion. This concept removes the idea that one species independently is a superior competitor and focuses on an organism or species' interactions with its environment as forming the mechanisms for the outcome of competitive scenarios. Theoretically, competition is removed from the attributes of an individual and placed in the realm of a complex and dynamic web of direct and indirect interactions of varying strengths that

include both resource and non-resource connections (Hierro and Callaway 2003). Given this systems-based approach or supposition, the focus of invasive species management should be on the system's interactions and the species' impacts and not just the inherent capabilities of the species, because the exotic entity cannot avoid being a constituent of a dynamic and inherently interconnected system.

Invasive species have dramatically impacted most ecosystems throughout the world and caused a rapid change to their diversity and function. Although plant invasions are a natural part of ecosystem development and succession, the rate and scale of exotic invasions have increased due to anthropogenic modification to the environment and movement of propagules in an increasingly globalized world (Mack et al. 2000). Restoration and control efforts on the most problematic invasive species are marginally successful at small scales, but not at landscape levels. The impacts of invasive species to agricultural production, recreation, and natural ecosystems are vast and costly, yet these are only the obvious outcomes of invasion and efficient management requires an understanding of how an exotic species successfully invades a system. To improve management or control of exotic species it is critical to understand the direct and indirect impacts of species on basic ecosystem processes and how these changes influence our ability to manage systems. If exotic species fundamentally modify nutrient cycling, litter decomposition, soil microbial communities and physical properties of soil, simple removal of undesirable species or revegetating areas by seeding with native species may be ineffective in restoring systems to pre-invasion states or former ecosystem functioning. By addressing the novel conditions and processes that have developed due to dominance by an exotic species we can develop a clearer understanding of how to

effectively manage systems, determine realistic restoration goals, and possibly prevent additional invasions. The purpose of this chapter is to review the processes and interactions by which exotic plant species alter the physical, chemical, and microbial characteristics of soil and provide a basis for applying these concepts to innovative soil management strategies that can reduce dominance of invasive plants and conserve diverse, functioning natural ecosystems.

IMPACTS OF INVASIVE SPECIES ON SOIL

The impacts of invasive species are pervasive. In many invasions, the whole plant community has changed from a diverse poly-culture, represented by many different functional traits, to a simplified system that is dominated by functionally similar organisms. Broadly speaking, the energy flow of the system increases as the biomass and net primary productivity of the invaded system are modified. The consequences are directly and indirectly proliferated throughout the ecosystem. Above-ground the changes in the plant community are obvious, but it is at the plant-soil interface and below-ground that scientific inquiry has begun to focus on questions concerning the effects of invasive plant litter on decomposition and nutrient cycling, and the subsequent changes in microbial community structure and function (Hawkes et al. 2005). Many of these activities are strongly regulated by microorganisms, which will also change as their primary habitat, the rhizosphere, is modified by changes in species specific root types, architecture, biomass, and physical and chemical characteristics of the soil. Dramatic changes in biochemistry of plant root exudates occur as functionally and biochemically diverse species are replaced by one dominant or functionally similar species. This

cascade of interactions begins with the invasion by an exotic species and in all likelihood most plant-soil-microbe interactions are impacted, although the degree and direction is variable. Wolfe & Klironomos (2005) propose three linkages that are directly impacted by invasive species: 1) plant community composition and ecosystem processes, 2) plant community composition and soil community composition, and 3) soil community composition and ecosystem processes (Figure 4.1). Exotic plant invasion directly affects these three primary linkages and potentially modifies the plant community, soil community, and ecosystem processes and function. In addition, numerous indirect interactions occur and dramatically increase the complexity of managing plant invasions based on ecological principles and successional theory (Sheley et al. 1996, Krueger-Mangold et al. 2006). Current research is attempting to understand the impacts of invasion on the soil and apply this information to the feedbacks between the complex systems that influence the invasion of exotic species (See Chapter 5).

Direct and Indirect Interactions Among Plants and Soil

The impacts of invasive species will influence both the potential vegetation and the ability to restore ecosystems to any semblance of their pre-invasion vegetation state and function. To comprehensively understand the impacts of an invasive species, it is critical to provide an overview of the direct and indirect interactions of an exotic species in its novel environment. Figure 4.2 attempts to illustrate a simplified scenario of the potential interactions between a plant and soil. Above-ground, the type and quantity of plant litter will likely change with invasion (Ehrenfeld 2003). Decomposition will be modified as biochemically different material is added to the system, often in larger

quantities than pre-invasion (Ehrenfeld 2003). Within the litter:soil interface, the microbial community will adapt to the new conditions and energy sources, producing different decomposition rates and fluxes of nutrients. Indirect effects of increased litter layers and reduced solar radiation could include modifications to soil moisture, temperature, and micro-sites for plant establishment. Disturbance regimes, such as the influence of fire on litter, soil nutrients, or nascent plants, are indirectly affected by invasion and could have long-term feedbacks that alter the successional trajectory and potential vegetation of the community. Change in the plant-community composition may also indirectly affect the amount or type of herbivory. Grazing can stimulate root growth, influence soil bulk density and nutrient inputs from fecal matter. Additionally, the feeding preferences of animals will likely change with a shift in vegetation. Below-ground, nutrient inputs from the litter and microbial activity are indirectly modified by invasion, as are the habitats for microorganisms due to the change in the physical and biochemical composition of roots. The rhizosphere will change as the root architecture becomes more homogenized by physically and functionally similar roots of the invading species. Biochemically, root exudates and decomposing root masses will modify the rhizosphere and energy sources for microorganisms, therefore influencing the diversity and function of soil microbes.

Understanding direct and indirect interactions develops a framework for the myriad of connections that drive a system and provides a conceptual basis to apply ecological principles to the management of natural systems. The complexity of ecological systems has long made it difficult to understand how an invasive plant or anthropomorphic management action modifies an ecosystem, but as our knowledge of an

ecosystem's above- and below-ground interconnectedness increases so does our ability to develop management programs based on ecological principles. Historically, much of vegetation management consisted of simplistic, one-dimensional approaches, such as removing an unwanted species or augmenting desirable species. This approach ignored each species influence on other aspects of the system (microsites, litter decomposition, soil nutrients, or microbial communities) and ultimately the system as a whole.

Ecosystems are collections of numerous interacting organisms and abiotic conditions that cannot easily be compartmentalized or isolated. Progressive, process-based management can utilize ecological principles to manipulate ecosystem processes and direct a system to a desired state, possibly with significantly less disturbance than historical eradication and revegetation programs. The following sections of this chapter will highlight specific examples related to direct and indirect impacts from invasive plants on litter decomposition, nutrient cycling and biogeochemistry, microbial community diversity and function, invertebrates, physical properties of soil, and allelopathy. Throughout this discussion on the impacts and interactions of invasive plants, the concept of utilizing ecological principles to develop innovative management practices will be highlighted. Management of ecological processes can include the manipulation of nutrients inputs (litter) or cycling rates, pathogens or plant-growth promoting microbes, invertebrate herbivory, and chemical interference (allelopathy). The consequences of process-based management of systems will probably not be immediate or obvious, but through the subtle manipulation of ecological processes it may be possible to restore diversity and resilience to ecosystems.

Invasive Plant Litter and Decomposition

A logical starting place to study the effects of an invasive species on the soil system is the plant litter being added to the environment. Every species has a unique biochemical composition (Ehrenfeld 2006) and varying amounts of potential litter-fall that will influence the system differently. The quantity and quality of plant litter will directly impact the potential nutrients, biogeochemical cycling and microbial diversity of a site. Litter decomposes at different rates based on the quality or type of litter and the environment, which will directly affect the amount and type of nutrients flowing into the soil. Invasive plants have been shown to generally have greater biomass or net primary productivity than adjacent native species (Ehrenfeld 2003), greater rates of decomposition (Ehrenfeld 2003, Ashton et al. 2005), and higher amounts of nitrogen (N) in litter, especially if the species is capable of symbiotic N fixation with bacteria. A review of numerous studies by Ehrenfeld (2003) found that 9 of 13 papers reported increased litter decomposition with invasion.

Decomposition occurs through three primary pathways: leaching of soluble materials, the comminution (the physical or mechanical breakdown) of biomass, and the conversion of fixed carbon to CO₂, H₂O, or energy via oxidation or catabolism (Seastedt 1984). These pathways reduce complex, organic structures into simpler compounds, which are used as an energy source for soil fauna and flora, affect the physical properties of the soil (i.e. soil organic matter), and ultimately provide nutrients for plants. Nitrogen is an essential nutrient for plants and is one of the most commonly limiting nutrients in the decomposition of plant matter, because it regulates both the growth and turnover of microbial communities (Heal et al. 1997). Nitrogen is often the focus of invasive plant

studies, because it is a dominant driver of plant growth and is essential to both plants and microbes. The influence of soil carbon:nitrogen ratios on decomposition and microbial diversity began to be acknowledged in the 1920s (Heal et al. 1997). Currently, lignin:nitrogen and polymer:nitrogen ratios are also used to analyze decomposition pathways. In general, leaf litter with higher N content decompose more rapidly due to preferential colonization by bacteria and fungi populations (Melillo et al. 1982), although C:N ratios are dynamic as decomposition progresses and microorganisms turnover. The microbial mineralization of N and subsequent death and decay of microbes is the primary source of N for most plants (Knops et al. 2002, Schmidt et al. 2007), although this traditional view of microbial control of N cycling has recently been challenged by the concept of plants being able to directly compete with microbes for organic N that has been depolymerized by the extracellular enzymes of microbes (Schimel and Bennett 2004, Chapman et al. 2006). An understanding of the decomposition of plant matter is necessary for the development of ecosystem management practices that acknowledge the complex interactions between plants, soil, and microbes; and ultimately how these processes influence plant communities and successional dynamics.

Plants directly affect nutrient cycling, edaphic characteristics, soil fauna, and microorganism communities of an ecosystem by their litter (quantity and quality)(Wardle et al. 2004, Georgieva et al. 2005, Chapman et al. 2006) and root exudates released to rhizosphere (Bais et al. 2006). Wardle et al. (2004) proposed the concept of plants as the integrator of above- and below-ground feedbacks, but emphasized the difficulties in understanding the mechanisms due to the complexity of organisms and environments involved. Due to the increased net primary productivity (NPP) of many invasive species

(Ehrenfeld 2003), litter quality and amounts represent a starting point for studies of plant invasions and their impacts. Different species have markedly divergent litter qualities and these physical characteristics modify their nutrient composition, decomposition rates, and potentially the biogeochemistry and microbial diversity of a system. Additionally, leaf litter and root exudates of invasive plants may contribute to indirect chemical interference or allelopathy (Bonner 1950, Muller 1966, Bais et al. 2003). In the context of invasive plants, changes in the quality and amount of litter within a system may represent a critical tipping point for an invasive species to modify many aspects of the system, including the rate of decomposition (*sensu* Ehrenfeld 2003), the flux of nutrients (Evans et al. 2001), and the microorganisms involved (Wardle et al. 2004).

The decomposition environment can be dramatically different between invaded and non-invaded regions of the same ecosystem. Studies have shown that in the eastern hardwood forests of the US, the litter of invasive species decomposes more rapidly than native plant litter and invaded ecosystems decompose litter faster regardless of the litter's origin (Ashton et al. 2005). A separate study did not determine differences in decomposition between sites dominated by either native or invasive species, but found that the loss rates of phosphorus, lignin, and trace elements from litterbags was reduced in invaded sites (Pritekel et al. 2006). The concept that an individual species can alter N cycling and create a self-perpetuating positive feedback system due to its litter quality and quantity has been thoroughly evaluated, although more recent research integrates the mechanisms facilitating the feedback processes by incorporating the analysis of soil microorganisms (Hawkes et al. 2005, Hawkes et al. 2006) and fauna (De Deyn et al. 2003), edaphic characteristics (Goslee et al. 2003, Grant et al. 2003), and the differences

between organic and inorganic N use by plants (Schimel and Bennett 2004, Chapman et al. 2006) into the complex systems.

Process-based management of natural systems requires understanding ecological processes and developing innovative techniques that utilize scientific principles to achieve land management objectives. The manipulation of litter quantities or qualities may be a possible management technique to reduce the flow of energy through an invaded system (Figure 4.2). The goal of manipulating litter inputs would be to stress the invasive species through limiting resource inputs and possibly increasing competition with species that are adapted to lower resource levels. This method is predicated on the assumption that reduced resources would add sufficient stress to the invasive species to influence vegetation dynamics. Management of litter inputs would require knowledge of the system and how invasive plants have modified decomposition, microbial communities, and nutrient cycling; otherwise it may be ineffective or have unintended consequences. Two potential strategies for litter management should be evaluated further: 1) removal of litter or 2) addition of low quality litter (i.e. high C:N ratio). The removal of litter in an invaded system could reduce nutrient inputs and potentially stress the invasive species because they have high productivity and subsequent nutrient demands. Experiments incorporating carbon amendments into soils of invaded systems or to reduce invasion in areas undergoing revegetation or restoration have had limited success in reducing N availability and invasive plants (Perry et al. 2010). Augmentation with low quality litter may achieve similar goals as soil carbon amendments, although the technique requires experimentation and outcomes could take years to be measurable. Another possible ecological consequence of litter manipulation would be the changes to

microsites for seed germination or establishment. The addition of litter could modify the microsites (temperature, sunlight, moisture) and make them less hospitable for germination by invasive species, although this requires in depth knowledge of an invasive species' autecology. The inherent connection between plant litter and plant-available nutrients emphasizes the importance of considering ecological principles related to decomposition as a potential method to manage ecosystem processes and direct a system towards a desirable vegetation state.

Nutrient Cycling and Biogeochemistry

In the mid-20th century a mechanistic understanding of decomposition led to the formation of many modern ecological theories and subsequently stressed the importance of nutrient transformations and cycling in the maintenance of ecosystem function (Heal et al. 1997). Plant productivity and diversity is inextricably linked to nutrients, although understanding this interaction has proven difficult. Historically, a majority of research on invasive species and biogeochemical cycling of nutrients has focused on N, since it is a primary limitation of productivity in terrestrial ecosystems (LeBauer and Treseder 2008). Many invasive species form symbiotic relationships with N fixing bacteria and this interaction is capable of dramatically increasing the amount of N in a system (Ehrenfeld 2003). While other invasive species can reduce the amount of N symbiotically fixed by native species (Wardle et al. 1994) and thus decrease the amount of plant available N in a soil. Changes in plant species composition due to invasion will likely modify a system's biogeochemistry as changes occur in the quantity and quality of litter inputs, root architecture and exudates, and microbial communities. Whether the plant or microbes

drive these changes in nutrients has long been debated, but will only be briefly discussed here as an introduction to the topic. At the center of this question are two competing yet likely co-occurring processes: 1) microbes control N cycling and plants can only access inorganic nutrients that remain after microbial turnover (Knops' et al. (2002) microbial N loop), and 2) plants are actively competing with microbes for organic N that is made available due to extra-cellular depolymerization by microbes (Chapman et al. 2006, Schimel and Bennett 2004). Knops' et al. (2002) theory infers that the type of litter and its quantities do not affect nutrient cycling, at least not as much as site or species specific impacts on N inputs and losses that are based on factors such as fire, leaching, atmospheric deposition, and symbiotic N fixation. Additionally, because the microbial N loop controls the flux of N, Knops' et al. (2002) theory places less importance on issues of litter quality and quantity, therefore invasion by an exotic would not greatly affect N levels, assuming that there is no loss or gain of species capable of symbiotic N fixation or modifying major disturbance cycles. An alternate theory posits that plants compete with microbes for organic N and that the consequences of a change in species due to invasion will have great ramifications on the system's biogeochemistry, both as the litter type and quantity change, but also as the net primary productivity (NPP) and nutrient requirements of the invasive species are modified. Given the later scenario, the subsequent impacts of an invasive plant species' dominance on an ecosystem will include all aspects of nutrient cycling, including effects on the richness, diversity, and functioning of the microbial community.

A review of the impacts of exotic plants on nutrient cycling by Ehrenfeld (2003) provides an excellent summary of our current knowledge. An overarching theme of this

review is that general trends are difficult to identify and both positive and negative impacts from invasion are found for C, N and water (Ehrenfeld 2003). This lack of uniformity should not be a surprise given the incredible variability of natural systems and the context dependency of ecological theory. Regardless of the variability highlighted in Ehrenfeld's (2003) review, the following list includes many important generalizations concerning the impacts of exotic species on soils:

1. Exotic plants often have greater above-ground biomass, net primary productivity, higher shoot to root ratios, and faster growth rates than co-occurring native species.
2. Exotic plant litter usually decomposes faster than co-occurring native species.
3. Exotic plant soils generally have more extractable inorganic N than soil from co-occurring native species.
4. Soils of exotic species frequently have increased rates of N mineralization and nitrification.
5. Exotic plants capable of symbiotic N fixation can dramatically affect N cycling.
6. Exotic species can affect symbiotic and non-symbiotic N fixing microorganisms that are associated with native plants.
7. Exotic species can influence the spatial distribution and temporal flux of nutrients, even if overall quantities of nutrients are not affected.
8. Both positive and negative changes in soil carbon, N, and water are associated with exotic species.

Adapted from Ehrenfeld 2003

When considering the effect of an invasive plant on nutrient dynamics, it is important to assess all aspects of nutrient cycling. The following examples will focus on N due to the breadth of research on this essential nutrient. The plant-soil N cycle receives additions from atmospheric fixation by bacteria or lightning, transformations by mineralization, immobilization in living tissues, and losses by denitrification and leaching. Each of these components can be impacted by a change in the dominant vegetation and different species will have unique impacts on N cycling, including indirect effects related to a species changing disturbance regimes that can dramatically affect N (i.e. fire). In general many invasive species increase the amount of plant available inorganic N and the rates of mineralization, nitrification, and atmospheric fixation (sensu Ehrenfeld 2003), although exceptions exist. The increased NPP of invaded areas may also require higher amounts of N to support larger standing crops, but also deposit greater amounts of litter. The cyclical nature of plant productivity, decomposition, nutrient availability, and plant nutrient requirements are important to consider when assessing how invasive species affect a system.

A study of wiener-leaf or saltlover (*Halogeton glomeratus*) invasion in the cold deserts of Utah USA documents distinct increases in nitrate and phosphorus concentrations in the invaded area compared to the adjacent ecotone and uninvaded areas (Duda et al. 2003). Interestingly, the ecotone had significant less ammonium than the adjacent invaded or uninvaded areas, which could signify higher mineralization and nitrification rates. Duda et al. (2003) also detected much higher salts (Na, Ca, K), organic matter, and bacterial functional diversity (BIOLOG substrate analysis) in the *H.*

glomeratus infestations. Due to a limited experimental design, the authors could not rule out the possibility that wiener-leaf preferentially invaded soils with specific nutrient and microbial characteristics, although they state that “the possibility of pre-existing gradients fails to explain the patterns in our data.” Cheatgrass (*Bromus tectorum*) is one of the most problematic and widespread noxious weeds in the western USA. A study in Canyonlands National Park of Utah USA found that cheatgrass invasion was associated with a decrease in the amount of plant available N and N mineralization, and increased N immobilization (Evans et al. 2001). The researchers found that the changes to N dynamics were linked to increased litter quantity, changes in the quality of litter and in the amount of soil organic matter directly modifying soil carbon to nitrogen ratios. Surprisingly, the impacts of cheatgrass on the N cycle occurred within 2 years of invasion. Additional research has documented higher nutrient levels in invaded habitats of Europe for several invasive species and prescribed the effect to increased NPP of the invasive species compared to native vegetation (Vanderhoeven et al. 2005, Dassonville et al. 2007). In a comparison of five exotic species (*Fallopia japonica*, *Heracleum mantegazzianum*, *Prunus serotina*, *Rosa rugosa*, and *Solidago gigantea*) in Belgium, Vanderhoeven et al. (2005) found significant increases in potassium and manganese in the invaded sites compared to the adjacent uninvaded areas. Another study in Belgium by Dassonville et al. (2007) concluded that *Fallopia japonica* increased nutrient cycling and topsoil fertility. These few examples illustrate the variability of invasive species impacts to the many different aspects of nutrient cycling and the capacity for invasive species to modify ecosystem function.

Novel nutrient acquisition strategies may aid invasion by an exotic species, especially if the system being invaded is limited in a specific nutrient or lacks a species capable of a unique strategy (i.e. actinorhizal nitrogen fixation). Due to the organism's inherent ability to obtain nutrients, an empty niche may be available for the exotic species to fill. Invasive plants that are capable of dinitrogen fixing symbioses with bacteria may have an advantage in the novel environment and the diazotrophic relationship may have large-scale and long-term impacts on nutrient availability, vegetation composition, and disturbance dynamics of the system. The invasion of the Hawaiian islands by *Myrica faya* (firetree) represents a pivotal point in our scientific understanding of how an invasive species can impact an ecosystem, modify nutrient dynamics, and alter successional processes. *Myrica faya* is a small tree from the Canary and Azores islands that invaded relatively young volcanic substrates in Hawaii beginning in the late 1800s. The invasive tree is capable of forming symbiotic relationships with N₂-fixing actinomycetes (*Frankia* spp.). No other species in this ecosystem develops actinorrhizal symbioses and this relationship dramatically changed the inputs and amount of biologically available N in these nitrogen limited systems (Vitousek et al. 1987, Vitousek and Walker 1989). N₂ fixation by *M. faya* increased the amount of N in the system and dramatically altered ecosystem development by increasing the amount of exotic plants following the decline of *M. faya* (Vitousek and Walker 1989, Adler et al. 1998) and increasing the potential for fire (Adler et al. 1998). Only by a thorough study of the impacts of invasion and the discovery of a novel source for N input to the system was it possible to understand the long-term impacts of *M. faya* on vegetation succession, ecosystem function, and large-scale disturbances (fire).

Russian olive (*Elaeagnus angustifolia*) is an intentionally introduced tree species that is currently getting a great amount of attention in the western USA. It invades riparian systems and modifies large scale biogeochemical cycling. The species also forms symbiotic associations with *Frankia* spp. and has leaf and litter N levels that are nearly double of the native cottonwoods (*Populus* spp.)(Katz and Shafroth 2003). Control or restoration of Russian olive invaded areas has proven difficult and has primarily focused on chemical and mechanical methods, although managing rivers to simulate historic flood regimes and promote recruitment of native cottonwoods is being tested (Lesica and Miles 2001). The significant amount of N added to invaded areas may have long term effects on revegetation success or promote invasion by exotic forbs or grasses. Similar issues have been noted with invasive N₂-fixing black locust (*Robinia pseudoacacia*) in Europe and parts of Asia (Weber 2003).

Strategic management of nutrients to reduce invasive plant populations is context specific and requires knowledge of how a target species or plant community will react to changes in nutrient cycling (Perry et al. 2010). Managing weeds by manipulating ecological processes attempts to utilize the inherent transformations and interactions of an ecosystem to achieve a management goal. Nutrient cycling is inexplicitly linked to litter inputs, decomposition, and microbial communities. Some species will probably not be affected by anthropogenic attempts to reduce nutrients and stress invasive species, while others may become less competitive and make it easier to establish desirable species. Overall, nutrients are only one component of the complex interactions that determine vegetation dynamics. Generally, when managing early seral, r-selected weedy species, reducing nutrient availability and increasing competition from aggressive native

species may be a successful approach. Conversely, some invasive species do not fit into the early seral concept and may not be affected by nutrient reductions (i.e. long-lived rhizomatous species such as *Acroptilon repens*). Hypothetically, adding nutrients and revegetating with aggressive early seral native species could assist in establishing species that can compete with the more K-selected invasive species or at least create an opening for establishment of competitive species. Applying Davis' *et al.* (2000) theory of fluctuating resources and the subsequent invasibility of an ecosystem to the manipulation of nutrients and revegetation (pseudo-invasion) with native species may provide effective methods to restore dominance of native plants in certain situations. Manipulation of a system's biogeochemistry to direct plant community dynamics must be based on a strong understanding of ecological principles that drive processes, otherwise unintended consequences may occur.

Microbial Communities

The soil is often represented as a black box in ecological experiments and studies of plant invasion (Kardol et al. 2006, Kulmatiski and Beard 2011). The diversity of prokaryotic species (bacteria and archaea) is potentially in the millions, while only approximately 4,500 species have been identified (Torsvik et al. 2002). Approximately 170,000 soil organisms have been identified and fungi have the most described species of the general taxonomic groups (Wall and Virginia 1997, Wall and Moore 1999). The advent of DNA sequencing and metagenomic methods will facilitate the detection and identification of many more species or taxonomic groups, especially bacteria. Our limited understanding of soil organisms and their interactions with plants and the

environment has made it difficult to incorporate these complex systems into ecological theory, much less management of plant invasions. Considering the role of microorganisms in the mineralization of nutrients, decomposition, N fixation, soil aggregation and aeration, and their positive or negative growth effects on plants, it is essential to assess the impacts of invasive species on microbial communities. Field and greenhouse experiments have documented different soil microbial communities in the soils of different plant species (Kourtev et al. 2002) and the influence of unique plant species on the differentiation of the soil microbial community (Westover et al. 1997). Recent research has documented how the soil microbial community differs between native and invasive plant species (Hawkes et al. 2005, Hawkes et al. 2006, Klein et al. 2006) and that these changes in composition and function can affect nutrient cycling and availability. It is broadly acknowledged that soil microorganisms mediate or regulate nutrient cycling in the soil and our recent understanding of the indirect effects of plant invasion on microbial composition highlights the importance of understanding the cascade of effects invasion will cause on soil microbe composition, nutrient cycling, and potentially pathogen accumulation (Figure 4.2). These effects can influence the dominance of invasive species through feedback cycles, but will also affect our ability to target specific ecosystem processes to achieve management goals in an efficient and timely manner. If the impacts of invasion fundamentally alter the composition of microorganisms and ultimately the biogeochemical functioning of ecosystems, it becomes critical to recognize these changes and adapt management practices to the novel conditions created by the invasive species. The following examples highlight how plant

invasion can change the soil microbial community and indirectly affect ecosystem processes.

The impacts of invasive species on soil microorganisms are often determined by microbially mediated changes to the system's biogeochemistry and research has frequently focused on N due to plants' heavy reliance on this essential macro-nutrient. A recent study by Hawkes et al. (2005) documented increased amounts of nitrifying bacteria and unique DNA signatures (restriction length patterns based on polymerase chain reaction (PCR) methods) in experimentally grown monocultures of exotic grasses compared to monocultures of a native grass, forb, or poly-cultural mixtures of exotic and native species. The authors related these changes in the microbial community to different plant compositions and linked the impacts of the exotic grass(es) and modified soil microbial community to functional changes in the system's nutrient cycling. A field based study in the forests of the northeastern USA documented different microbial community composition and function in the soils of two invasive and one native understory species (Kourtev et al. 2002). The modification of soil communities was strongest in the rhizosphere soils, but surprisingly the impact was also documented in nearby bulk soil. Using canonical correlation analysis the researchers found that changes in the function of the soils were correlated to changes in the microbial composition and structure. A greenhouse experiment by the same researchers and with the same plant species replicated the field results and also identified increased nitrification rates and pH in the soil of one exotic species (Kourtev et al. 2003).

The below-ground diversity of arbuscular mycorrhizal fungi (AMF) has been directly related to the functioning and stability of plant communities. At low AMF

diversity plant community composition has been shown to fluctuate greatly (lack of stability), while high AMF diversity promoted greater nutrient capture and productivity (van der Heijden et al. 1998). We expect exotic, invasive species to have different AMF communities than neighboring native species due to inherent physiological and phenological differences between plant species, but invasive species have also been shown to cause changes in the fungal diversity of co-occurring native species following invasion (Hawkes et al. 2006). The consequences of change to the AMF community of an invaded area are unknown, but are potentially an important mechanism in successful plant invasion (Callaway et al. 2004) depending on the role of fungi in plant nutrient uptake, nutrient immobilization and turnover of fungal hyphae, and plant root responses to fungal infection (carbon exudation). Busby *et al.* (In press) have suggested that the recovery of AMF communities after invasion by exotic cheatgrass may be dependent on the species identity of native plant used for restoration. Wolfe and Klironomos (2005) provide an excellent overview of specific invasive species and their documented effects on the structure and function of native soil communities. Based on the examples provided, invasive species generally decreased AMF, fungi abundance, or diversity, although the results varied between species (Wolfe and Klironomos 2005).

An interesting feedback system was recently documented in India in which an exotic, invasive plant (Jack in the Bush, *Chromolaena odorata*) promotes the growth of a native, generalist soil pathogen (*Fusarium* sp.) and subsequently creates a negative effect on native plant species (Mangla et al. 2008). The root exudates of *C. odorata* were shown to promote *Fusarium* growth in non-invaded soils and activated carbon (AC) reduced the promotion of the fungi by the root exudates. This unique feedback pathway

illustrates the complexity and variability in the reaction between plants and microorganisms, regardless of their origin (i.e. home versus foreign). Although not a direct impact of an invasive species on the soil, several experiments have documented exotic species experiencing less negative impact from microbial pathogens (Mitchell and Power 2003) or accumulating pathogens at a slower rate than native species (Klironomos 2002, Eppinga et al. 2006) and have hypothesized that this release from enemies contributes to the invaders success.

Microorganisms represent an incredible breadth of diversity, although our understanding of species and functional groups is limited. The regulation of decomposition and nutrient cycling by microbes requires in-depth research and elucidation if we intend to manage ecosystems through the manipulation of ecological processes. Molecular techniques (Polymerase Chain Reaction (PCR) and DNA community profiling techniques and sequencing), fatty acid analysis (Phospholipid Fatty Acids, Fatty Acid Methyl Esters), and carbon substrate utilization (i.e. Biolog plates and Substrate Induced Respiratory responses (SIR)) methods are beginning to classify and describe the functional traits of microorganisms. As this knowledge base expands and the methods become more consistent and less expensive, the classification of a plant community's microbially regulated nutrient cycling will make it possible to incorporate microbial communities in land management practices. When we understand how an invasive species modifies the microbial community, and subsequently decomposition and biogeochemistry, it will be realistic to attempt to modify microorganisms in ways that enhance desirable plant communities and suppress unwanted species. This will probably focus on managing N fluxes and availability or utilizing species' specific pathogens.

Ecological principles guided by system and species specific knowledge can lead innovative management practices that manipulate processes to achieve land management goals. Although, because microbes are the smallest and most numerous, their interactions may be the most complex to grasp for management purposes. This novel type of management will require a massive increase in our understanding of plant-microbe interactions and the potential feedbacks that could ensue when we begin to tinker with complex systems.

Soil Invertebrates

Soil fauna play an important role in many ecological processes, including: decomposition of biomass via comminution, root herbivory, movement of nutrients, and modification of bacterial and fungal communities through feeding activities. These activities can influence plant succession directly and indirectly, although the impacts of soil fauna and how to incorporate these effects into ecological management remain controversial or unknown. Early studies utilized litterbags with differing mesh sizes to exclude certain soil biota (Seastedt 1984, Huhta 2006), while others have applied chemicals (i.e. naphthalene) or X-rays to eliminate arthropods (Newell et al. 1987). Both biocide methods have been shown to have non-target effects on soil fungi (Newell et al. 1987), although the results of litterbag studies have overwhelmingly shown that soil fauna increase the decomposition of plant litter (Huhta 2006) and can have variable effects on nutrient cycling. A well replicated field study in Ohio used electroshocking of soil to reduce earthworm populations without non-target effects on microarthropods, nematodes, or microorganisms (Bohlen et al. 1995). In the context of invasive plant

research and management, the role of soil invertebrates is relevant due to their influence on decomposition rates and nutrient availability. Comminution affects the size and surface area of litter, which consequently modifies many factors that regulate invertebrates and microorganisms, including: micro-sites, predator-prey relationships due to size limitations, and access to water or nutrients. Although bacteria and fungi are the primary decomposers of organic matter, soil fauna are intricately involved in the physical processing and movement of plant biomass (fecal excretion)(Davidson and Grieve 2006), and the direct or indirect regulation of soil microorganism communities by their feeding habits (Seastedt 1984). Due to difficulties identifying and describing soil fauna, the organisms are frequently grouped into the following functional groups based on Brussard et al. (1997). Macro-fauna consist of root herbivore insects, termites, ants and earthworms. Meso-fauna include mites, collembola, and enchytraeids. Micro-fauna are protozoas, ciliates, and nematodes. The macro- and meso-fauna generally decrease particle size of plant litter and indirectly increase surface area (habitat for microorganisms) and mobilization of nutrients. Micro-fauna and some meso-fauna (mites and collembola) graze on fungal spores and bacteria. Soil fauna can directly impact plants by root herbivory, spreading of pathogens, and modification of soil microorganism populations through predation. Invasive plants will directly change the inputs to the soil (litter, roots and exudates) and therefore the habitat and food sources for soil fauna are likely to be modified with invasion. To adequately understand the impacts of invasive plants, the interactions of soil invertebrates with microorganisms, the rhizosphere, and the physical properties of soil must be incorporated into ecological studies. Previous studies and several review papers (Seastedt 1984, Huhta 2006) provide

a general framework for incorporating the effects of soil fauna into plant-soil interactions and plant successional dynamics. Many studies are based upon litter quality (i.e. labile or resistant) and initial soil N levels as important factors in the interactions of micro-flora and –fauna. Huhta (2006) summarized the role of soil fauna in N limited systems as ‘...generally enhance(ing) decomposition and mineralisation, whereas in the presence of excess nitrogen they have little effect.’

Soil fauna may alter succession due to selective predatory effects upon the dominant species, therefore releasing the sub-dominant plant species from competition and facilitating the succession and development of a more diverse and heterogeneous vegetation community (i.e. – less dominance by any species based upon Simpson’s evenness index)(De Deyn et al. 2003). In the context of invasive species, Mayer et al. (2005) documented increases in decomposition when macro-detritivores were allowed access to litterbags and the amount of decomposition correlated positively with increasing cover of an invasive grass (*Festuca arundinacea*). In general, our understanding of the interactions between soil invertebrates and invasive species is too limited to base management practices upon. The addition or removal of soil fauna are possible, although the practice must be based on ecological principles and a sound understanding of how the management will affect the system and target species. The primary manners in which soil fauna can influence vegetation are through root herbivory, comminution of litter (increased decomposition and possibly nutrients), and predation of microorganisms (pathogens, N fixers, decomposers) (Figure 4.2). The utilization of soil fauna to influence any of these ecological processes will require thorough knowledge of the specific plant-soil system to successfully achieve management goals.

Soil Physical Properties

The physical properties of soil are the results of long-term interaction between a region's geology, climate, and biota. Hans Jenny (1941) first described the formation of soil with a simple function:

$$\text{soil} = f(\text{climate, organisms, topography, parent material, and time})$$

Each of these factors will affect the composition of soil and subsequent plant-soil feedbacks. The incredible variability in soil development and interactions with plants will make it difficult to identify consistent impacts on the soil due to plant invasion, but an understanding of the soils, plants, and microbial species involved can identify novel ecological interactions and improve our management practices minimizing invasive species or restoring native flora. As the medium for plants, microbes, and nutrients to interact, the soil is often represented as a black box due to its complexity and poorly understood systems. Yet it is also the arena in which many of the impacts from a change in biodiversity or dominance by an invasive species will be manifested. Impacts of invasion on the soil's physical properties are highly variable and probably species and site specific, but can include the following characteristics: soil moisture content, salinity, pH, organic matter content, soil aggregation and microclimate effects.

Invasive plants frequently have greater biomass than surrounding native vegetation and therefore probably require more nutrients and water, but also increase litter inputs to the system. Consequently, these dramatic changes to the productivity,

water usage, nutrient cycling, and inputs to the system will affect many aspects of the soil, including pH. In New Zealand, studies have shown that mouse-eared hawkweed (*Hieracium pilosella*) decreased soil pH by approximately 0.5 units (McIntosh et al. 1995). Conversely, Kourtev et al. (2003) documented increased soil pH in greenhouse incubations of the exotic Japanese stiltgrass (*Microstegium vimineum*) compared to a native blueberry. Ehrenfeld's (2003) review highlights the variability of soil pH following occupation by an exotic species. A continental scale study of soil microbial composition in North and South America found that differences in plant species richness and diversity were largely explained by soil pH and plant community (Fierer and Jackson 2006), although the paper did not directly address invasive species.

Water use by invasive plants may alter evapotranspiration and overall water usage rates, which can cause changes in soil moisture content, water table levels, and salinity. The impacts of saltcedar (*Tamarix* spp.) in the southwestern USA are hotly debated, since the major river systems it infests supply water to millions of people and has numerous contractual and international obligations. The prolific invasive species was thought to use more water than native species and targeted for eradication by states and municipalities in order to salvage water for anthropocentric uses. Early measurements of saltcedar water usage (>200 gallons per tree per day) may have been inaccurate and overestimated the economic benefits of control methods (Owens and Moore 2007). Water salvage experiments that focused on saltcedar control have not been as successful as expected (Shafroth et al. 2005). The impacts of saltcedar on riverine and ground water are difficult to measure consistently due to issues of scale. Plant water use measurements are conducted at the leaf, stem, plant, or ecosystem scale and comparisons across scales

can be inconsistent, although a general trend for greater water use by invasive species in drier, hotter climates and at larger scales have been documented (Cavaleri and Sack 2010). The impacts of saltcedar on water resources remain unclear, but the species has significant effects on soil salinity (Nagler et al. 2008) and riparian forest structure.

Soil aggregation can be used as a segway to generically describe soil quality, because more stable aggregates are less prone to erosion and hold greater amounts of water, nutrients, and carbon (Batten et al. 2005). Soil structure or aggregation is frequently studied through the quantification of glomalin, a glyco-protein that is produced by arbuscular mycorrhizal fungi (AMF) and is positively correlated with the stability of soil aggregates (Lutgen and Rillig 2004). Because many invasive plants affect soil microbial communities, it is of interest to determine if the impacts cascade to soil aggregate stability or soil quality. A study of chemically and mechanically controlled spotted knapweed (*Centaurea stoebe*) infestations found that total glomalin levels and AMF hyphal lengths were negatively correlated with percent cover of the invasive plant, but did not detect a reduction in aggregate stability (Lutgen and Rillig 2004). The authors stated that soil aggregate water stability was initially high at the study sites and that “spotted knapweed may exert a deleterious effect on soil structure” in areas with lower initial stability. Preliminary evidence of the invasive Jack in the Bush (*Chromolaena odorata*) improving soil structure through the promotion of earthworm activity was documented in eucalyptus plantations in the Congo (Mboukou-Kimbatsa et al. 2007). The variability of responses in soil aggregate stability following invasion exemplifies the species specificity of impacts and the importance of considering initial

soil conditions and the wide variety of ecosystems when assessing an exotic species impact.

Allelopathy and Invasive Plants

Although the concept of allelopathy or chemical interference between plants has long been postulated, many difficulties have been encountered in the detection and quantification of this elusive interaction. The success of many invasive plant species has been attributed to allelopathy, primarily through the soil matrix, and therefore it is important to consider the potential impacts of allelopathic invasive species on soil and potential management or restoration. Allelopathy specifically describes the release of a chemical into the environment by a plant or microorganism, via exudation, volatilization or transformation of biomass, that has a direct or indirect positive or negative effect on another species (Rice 1984). Currently, the discussion of allelopathy focuses on negative or inhibitory effects on a plant due to the release of a chemical by another species and usually ignores potential stimulatory effects. If chemical interference can be determined to affect plant growth and the availability of water or nutrients within natural plant communities, the consequences of allelopathic induced changes in vegetation composition or succession within ecosystems should be considered in addition to the traditional view of ecological theory that is based primarily upon the competition for resources (Bonner 1950, Muller 1966). A major point of contention concerning allelopathy has assumed that the effects are direct and therefore measureable. We emphasize the importance of understanding that the impacts of potentially allelopathic root exudates are most likely weak, indirect and will occur over long time frames and

involve multiple scales, including interactions with microbes and subsequent changes to soil chemistry. For these reasons, we promote the concept of soil chemical ecology over allelopathy, because it highlights the complexity of interactions in a plant-soil system and removes much of the historical controversy surrounding allelopathy (Inderjit and Weiner 2001).

Potential allelopathic interactions in chick pea (*Cicer arietinum*) was first noted by Theophrastus around 300 B.C. (Rice 1984). Although many years have passed, there is still much confusion about both the definition of allelopathy and its detection in natural systems. Agricultural problems related to 'soil sickness' brought the issue of chemical interference between plants into the scientific realm in the early 1800's. In 1832, a system of crop rotation was developed by the botanist A.P. DeCandolle based upon his research into the interspecific inhibitory effects of certain agricultural species upon others (Bonner 1950). The early theories on chemical interference or allelopathy, were dismissed by many researchers as information concerning the depletion of soil nutrients and competition for these minerals and water formed the prevailing theory in plant interactions (Bonner 1950). Additional research in the early to mid-1900's detected toxic substances in or around many plant species, including: the leaves of a desert shrub (*Encelia farinosa*), the leaves and roots of black walnut (*Juglans nigra* L.), the roots of smooth brome (*Bromus inermis*), and the soils of peach and rubber tree plantations (Bonner 1950). The major problem in determining that chemical inhibition was influencing the vegetation composition and plant community succession related to the lack of evidence connecting the known phytotoxins to its release from the plant, accumulation in the soil, and the mechanism that negatively affects the surrounding

vegetation. Many of the early experiments could detect phytotoxic chemicals, but they could only be correlated with the inhibition of neighboring plants. Direct evidence regarding the release of the chemical and how it interacts with the soil and neighboring plants are still proving difficult to document. The fact that most allelopathic effects are weak relative to other factors suggests that any impacts on neighboring plants would play out on longer time scales than are typically considered in ecological studies. Slow and chronic antagonistic effects would be difficult to document against a backdrop of other competitive processes. The field of allelopathy was thus heavily criticized in the mid-1900's due to the correlative nature of the studies and the vast amount of research supporting competition for resources as the primary driver in plant interactions and successional dynamics.

The subject of allelopathy came into the forefront of science again in the 1960's, primarily due to the work of C.H. Muller on the bare zones surrounding the aromatic shrubs of coastal California. Muller's (1966) well-known paper indicated that certain shrubs produce phytotoxic substances that could inhibit the establishment of seedlings (intra- and interspecific) and therefore are important factors in the diversity and successional changes in a plant community. Although Muller had considered the effects of herbivory and granivory on the bare zones, his work and allelopathy as a whole came under intense scrutiny with the publication of Bartholomew's (1970) paper concerning the role of animals in the bare zones between the shrub and grassland communities.

To this day, the separation of chemical interference and resource competition is still the largest methodological hurdle in the drive to detect and understand allelopathy (Muller 1966, Weidenhamer 1996, Wardle et al. 1998, Romeo 2000, Ridenour and

Callaway 2001, Inderjit and Callaway 2003). The importance of conducting appropriately controlled laboratory experiments (Inderjit and Dakshini 1995)(i.e. realistic toxin concentrations) and similar field based experiments (Inderjit et al. 2001) are essential to isolate chemical interference from resource competition. Experiments on allelopathy should include density-dependent factors (competitors and/or chemicals) and methodologies that attempt to separate resource competition from chemical interference, such as activated carbon or resource addition and removal (Romeo 2000, Inderjit and Callaway 2003). Methodologically, allelopathy research needs dramatic improvement in field based studies and more in-depth knowledge of the chemical agents and their potential multi-functionality over the ecologically meaningful time spans that weak interactions are likely to play out.

SELECT METHODS TO MANAGE SOIL PROCESSES FOR INVASIVE SPECIES CONTROL

Traditional management of invasive species has focused on eradication with chemical, mechanical, or biological control methods and occasional follow up with revegetation with native plants or restoration of a specific ecosystem process (i.e. hydrology). Although these control techniques have proven effective in some situations, they are also cost prohibitive and frequently have non-target or unintended effects. Novel ecosystem management practices attempt to utilize our understanding of ecosystem interactions and processes to manipulate plant communities towards a desired outcome. The manipulation of soil nutrients, microorganisms, invertebrates, and soil chemistry may provide low impact methods to achieve resource management objectives, although

many contingencies exist when working with specific species and ecosystems. The immediate results of these novel practices may not be as dramatic as eradication methods, but by attempting to work within an existing system we reduce the amount of disturbance and may promote more resilient and stable ecosystems in the long term. Here we offer a few illustrative examples.

Carbon Addition and Fertilization

High N availability has been shown to facilitate invasion by exotics (*sensu* Perry *et al.*, 2010), primarily because N is a major limiting resource in most communities (LeBauer and Treseder 2008) and promotes the rapid growth of early-seral species, often to the detriment of late-seral native species. Nitrogen levels have been dramatically increased worldwide due to anthropogenic fixation and increased agricultural growth of N fixing legumes (soybeans and alfalfa)(Vitousek *et al.* 1997). Long-term research in the shortgrass steppe of Colorado USA has shown that fertilization with N increased the abundance of annual grasses and forbs compared to perennial plant growth in control and carbon addition treatments (Paschke *et al.* 2000). Control methods that extend the focus beyond eradication and directly address the causes of invasion (*i.e.* high N availability) are more likely to prevent reinvasion and achieve long-term management objectives (Perry *et al.* 2010). Many methods exist to reduce N in an ecosystem, including: carbon addition, burning, grazing, biomass and top soil removal. Carbon addition causes N to be temporarily immobilized in microorganisms, while the other methods remove N from the system. A reasonably large body of evidence has shown that carbon addition reduces or prevents plant invasion, primarily by reducing the growth of invasive species that have

high nutrient demands and therefore increasing the competitiveness of native species, or disrupting feedbacks between exotic plants and nutrient cycling (Perry et al. 2010).

Carbon is usually added in the form of sugar, sawdust, or wood chips and sequesters N by increasing microbial growth and activity, thus causing more N to be immobilized in microbes. Alpert (2010) provides an excellent overview of the results of carbon addition in different ecosystems, amounts of carbon required, and a timeline of impacts. The monetary cost and application of carbon to a system can be expensive and disruptive, especially on a large scale, although the method may be practical for the management of important, high value sites.

Conceptually similar to carbon addition, removal of litter or biomass may be a potential tool to reduce invasive plant populations, especially if litter and nutrient cycling in the invaded habitat are enhanced or accelerated by the exotic species' presence. The removal of plant material will reduce nutrient inputs to the system and reduce the competitiveness or growth of species that have high nutrient demands. Another potential technique may be the addition of low quality litter to a system. The decomposition of low nutrient biomass will affect nutrient cycling, microbial communities, and could have beneficial management outcomes. If applied in conjunction, these two methods may be a low impact, cost effective method to slowly reduce populations of invasive plants. The challenge however is to concomitantly establish desired native species in the site with lowered nutrient availability.

Activated Carbon as a Tool for Management

In recent years, activated carbon (AC) has had a resurgence as an experimental treatment to study allelopathic interactions between plants and potentially as a tool to minimize the impacts of some invasive plants. Activated carbon is known to sorb large, organic molecules indiscriminately and therefore is a ‘blunt’ tool for studying the highly complex chemical interactions in the plant-soil interface. It is produced from charcoal, wood, or nutshells and has an incredibly high surface to volume ratio. The large surface area and pore volume gives the compound the ability to sequester organic molecules, including: phyto-toxic root exudates and organic nutrients. Many suspected allelopathic compounds are secondary metabolites (Muller 1966), such as sesquiterpene lactone or polyacetylenes, and presumably would be bound by AC. Experimentally, AC has been shown to be effective in modifying competitive outcomes between native and invasive plants (Mahall and Callaway 1992, Callaway and Aschehoug 2000, Abhilasha et al. 2008), although many methodological hurdles exist in our understanding and utilization of AC. In addition to AC’s effects on chemical interference, recent research on the impacts of AC to microbial communities support the compound’s role in aiding the restoration of native plant communities beyond a treatment consisting of only seeding with native species (Kulmatiski 2011).

Due to the physical and chemical properties of AC, additional ‘non-target’ effects are known to occur and can confound experimental results (Lau et al. 2008). A study by Ridenour and Callaway (2001) found that AC decreased the rate of water loss in soil. It has been hypothesized that AC could decrease microbial activity due to the sequestering of organic compounds and a subsequent reduction in bacterial transformations of N

(Kulmatiski and Beard 2006). Kulmatiski and Beard (2006) found that organic N and C were decreased in the presence of AC, but inorganic nitrate increased, possibly due to a decrease in microbial activity. A separate study found an alteration of the microbial community composition with the addition of AC and an increase in carbon sequestration compared to other organic adsorbing compounds included in the experiment (Pietikainen et al. 2000).

A recent review of AC by Lau et al. (2008) documented experimental artifacts in the use of AC and variable species-specific responses to the amendment. The study found an increase of plant biomass with AC addition in most of the species studied, increased potting soil pH, and positive or negative changes in the amounts of specific nutrients. Plant-available N was significantly increased in the presence of AC even though the amount of N in the AC was relatively small (0.549% to 0.637% depending upon the source of AC). Methodologically, the confounding effects of AC may be minimized or ‘controlled’ by an understanding of the treatment’s effect on individual species and in interspecific competitive scenarios with and without the addition of AC and fertilizer (Lau et al. 2008). Due to the indiscriminate nature of AC, its value as an experimental or more importantly a management tool depends heavily on the experimental design utilized and an evaluation of the interactions with the species being studied. An important factor limiting the use of AC in management settings is the need to incorporate it into soils to be effective. Regardless of the problems and contingencies associated with AC use, it is one of few tools available to potentially minimize the impacts of invasive plants in the soil or alter the microbial community (Kulmatiski 2011) and additional research may yield practical applications for the compound.

Manipulation of Soil Microbial and Invertebrate Populations

The impacts of invasive plants may be managed by progressive methods that directly or indirectly modify soil microbial or invertebrate communities. Although research in this area is limited and many contingencies exist, the manipulation of soil microbial or invertebrate communities is a potential method to minimize or modify the negative consequences of invasion or assist in the restoration of invaded communities (Boyetchko 1996). Inoculation with N fixing bacteria has long been used to promote plant growth or higher N levels in the soil and it may be possible to add soil micro-fauna or flora that will promote native species or have a detrimental effect on invasive plants, potentially via pathogen accumulation. Similar to biological control with insects, the addition of pathogens from an invasive species' native habitat may help to suppress the species. Another method is to reduce or remove microbes or invertebrates with x-rays or chemicals, possibly to reduce pathogen accumulation on native or rare which have been shown to accumulate pathogens more rapidly than exotic plants (Klironomos 2002, Mitchell and Power 2003). This action must be specifically tailored to a known ecological interaction with the invasive species. De Deyn et al. (2003) showed how invertebrates enhanced secondary succession of European grassland vegetation by suppressing dominant early seral species. Increasing an invaded communities' succession towards later seral vegetation may promote diversity and slowly diminish the dominance of invasive species without physically disturbing the system. It is possible to manage earthworm populations using electroshocking without non-target effects (Bohlen et al. 1995) and indirectly affect microbial activity (Binet et al. 1998) and potentially

nutrient cycling. The impacts of earthworms, native and invasive, have been overlooked in many ecosystems and may provide novel management strategies. Naturally any application of these practices must have a well-defined goal and understanding of the plant-soil-microbial interactions.

A novel concept promoted by Harris (2009) utilizes fungal:bacterial ratios across dominant vegetation groups as a measure of a system's successional development and suggests that the manipulation of microbial communities may be used to enhance the restoration of degraded systems towards a specific late seral plant community. The use of microbes as a "restoration shortcut" probably depends upon whether microorganisms facilitate vegetation dynamics or are followers of the change (Harris 2009). In all likelihood, this will be species and system dependent, but the concept of microbial manipulation is worthy of additional research and management applications especially if known pathogens or beneficial symbioses can be appropriately applied. Few studies have researched the impacts of microbial inoculation on invasive plants or restoration projects and the outcomes have been mixed (Dean Stacy et al. 2005, Rowe et al. 2007, Abhilasha et al. 2008, Rowe et al. 2009). Another approach may be to manage the species composition of plant communities either thru plant species additions or removals in order to exact desired changes in soil microbial communities (Boyetchko 1996, Busby et al. In press). Most of these novel management practices are based on the Natural Enemies theory (Elton 1958), Enemy Release Hypothesis (Mitchell and Power 2003, Levine et al. 2006, Mitchell et al. 2006), or an understanding of how a species accumulates pathogens (Eppinga et al. 2006) and will require extensive knowledge of the system and proper experimentation prior to use as a management tool.

An important premise of this type of resource management is a relatively clear understanding of the organisms and interactions involved, otherwise unintended outcomes will occur. Clear examples of non-target effects by management have occurred in biological control with insects and below-ground manipulations must strive to avoid these mistakes (Louda et al. 1997, Pemberton 2000, Pemberton and Cordo 2001). It is critical to avoid introducing potentially harmful or invasive microorganisms into systems in which our comprehension is limited (van der Putten et al. 2007).

CONCLUSION

Invasive plant species impact all aspects of soil, including litter decomposition, nutrient cycling, soil fauna and flora, and the physical characteristics of soil. The complex interactions between these organisms and entities requires in-depth research to clarify our understanding of plant-soil feedbacks, yet recent research is beginning to provide information that can be used to manage basic ecological processes for the control of invasive species. Historically, invasive plants were managed by in a top-down, command and control approach based on removing the species or propagules, often repeatedly. Although this approach can be effective in the short term, it creates disturbance and frequently promotes re-invasion. Additionally, chemical and mechanical control methods are expensive, energy intensive, and may have undesirable effects on the environment and perpetuate disturbance cycles.

As our understanding of dynamic biological systems increase, we have the knowledge and skills to manage invasive species through the manipulation of basic ecosystem function or processes. The modification of nutrient availability due to

microbial function or inputs from decomposition can influence plant community dynamics, although it will not be immediate and will rarely cause the complete removal of an unwanted species. Similarly, a change in microbial pathogens or levels of root herbivory will be unlikely to create a dramatic difference in vegetation, at least in the short-term. Soil amendments or fertilization can alter microorganisms and plant dynamics leading to different successional trajectories and plant communities. Vegetation, soils, and microbial systems are inter-connected and their linkages are beginning to be understood. Potential management methods are based on tinkering in the incredibly complex feedbacks between plants, soil, and microbes in order to find a balance or stability wherein higher species diversity is reached, while preventing a non-native species from becoming dominant. Few invasive species form monocultures in their native ranges. Managing ecological processes and successional trends may require a paradigm shift away from the simplistic terms of native and exotic, as it promotes a style of management based on supporting resilient and diverse ecosystems through an understanding of the ecological interactions that drive systems. It does not mean that chemical and mechanic control of invasive species have no place in management, only that we need to approach ecosystem management with long term goals and understand how our actions will impact the entire system as we attempt to support and restore natural and resilience biotic complexes.

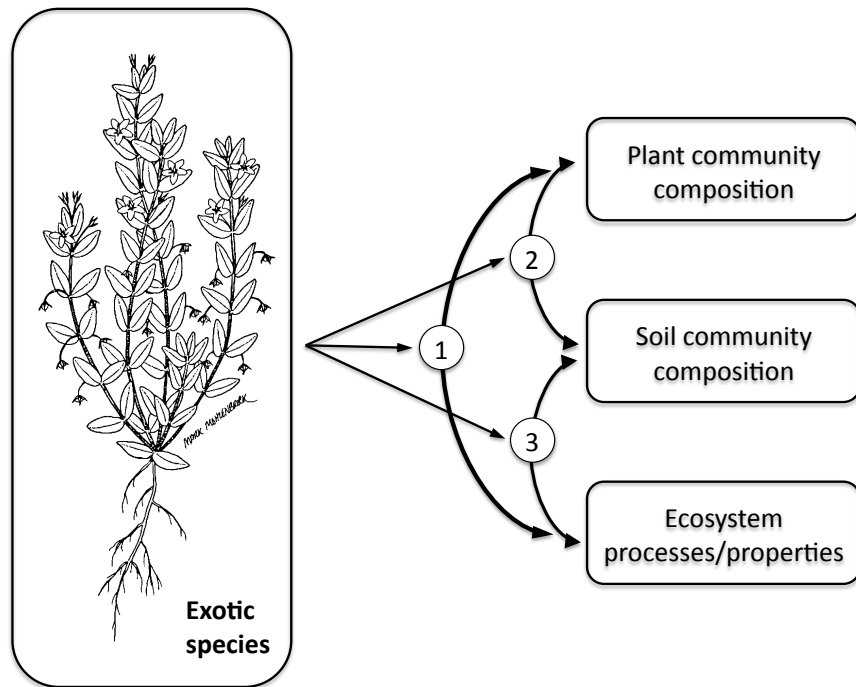


Figure 1.1 – Impacts of an invasive plant on the direct interactions and linkages between the plant community, soil community and ecosystem processes. Adapted/redrawn from Wolfe & Klironomos (2005). *Anagallis arvensis* L. (scarlet pimpernel) illustration by Robert H. Mohlenbrock (Robert H. Mohlenbrock @ USDA-NRCS PLANTS Database / USDA SCS. 1991. *Southern wetland flora: Field office guide to plant species*. South National Technical Center, Fort Worth.)

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Chapter 2 – Effects of soil conditioning and inoculation on biomass allocation and competition in *Solidago canadensis* and *Acroptilon repens*

INTRODUCTION

Competitive interactions have been a primary focus of ecological research for decades and are critical forces in the structuring of communities and evolution of species. The results of competition experiments have provided the fundamental basis for vegetation succession theory, invasion biology, and the conceptual basis for numerous population models. Given the fundamental importance of competition experiments in ecology it is unfortunate that misconceptions concerning competition (Birch 1957) and experimental methods to measure interactions (Underwood 1986) are pervasive. The negative consequences of competitive interactions may be variable in time and space (Connell 1983), and our perceptions of current competition could be relics of previous competition (Bazzaz 1996). An understanding of the interactions that determine when competition occurs and the scale of impacts is critical to our management, conservation and restoration of natural ecosystems, especially to successfully manage invasive plants based upon ecological principles (Sheley et al. 1996, Krueger-Mangold et al. 2006). Studies of competition have generally focused on two primary types of competition: 1) indirect interaction of organisms for a shared resource (exploitative) or 2) direct interaction via chemicals or territory (interference) (Connell 1990, Morin 1999). Historically most competition experiments focused on plant production or soil

macronutrients (exploitative) and ignored the direct interactions between organisms of different trophic levels and the complex above- and below-ground connections of plant-soil feedbacks (Wardle et al. 2004, Bardgett and Wardle 2010).

Interspecific competition represents an interaction between two or more species for a resource and by definition produces a negative effect on the fitness of both species (Morin 1999). Fitness can be represented by many measures, including an organism's growth, productivity, fecundity, or survival. Exploitative competition only occurs if an essential resource is limited and probably occurs transiently, while interference competition may be more prevalent due to the constant interaction between microbial pathogens and plant roots or chemical interference (allelopathy) between plants. Most studies of competition focus on interspecific exploitative competition, yet avoid the foundational nature of the intraspecific interactions that may precede any interspecific competition that could affect community structure or function, especially in the context of an exotic plant invasion. Intraspecific competition is generally thought to be stronger than interspecific competition because an individual competes against another individual with nearly identical physiological and morphological capacities (Darwin 1859), therefore when the overlap of each organisms' realized niche is maximized the effects of competition are expected to be strongest. Morphologically and physiologically similar plants will likely prefer similar habitats and we would also expect the competition between neighbors to be intense (Bazzaz 1991). Invasive plants frequently form monocultures that persist regardless of the extreme intraspecific competition that traditional ecological theory predicts. Connell (1983) surveyed 527 field experiments and when inter- and intraspecific competition could be separated, intraspecific

competition was “as strong or stronger than interspecific in three-quarters of the experiments (page 682)”. Successful invasion and dominance may be based on a species’ avoiding or responding to intraspecific competition less negatively than interspecific competition, at least relative to its neighbors. To prevent additional plant invasions and manage existing noxious plants more effectively, it is critical to broaden our focus of competitive interactions and include all aspects of the plant-soil system, including interactions between plants and soil microorganisms.

Current research in plant-soil feedbacks (PSF) is beginning to explore the complex relationships between plants and soil microorganisms. Little research has integrated competitive studies and manipulations of the soil community (Kardol et al. 2006), yet ultimately the rationale for studying plant-soil feedbacks is to improve our ability to manage natural ecosystems, especially in the context of invasive species and biodiversity loss. Plant-soil feedbacks connect the chemical, biogeochemical, physical, and biological properties and processes of the soil with all living organisms (Ehrenfeld et al. 2005). In the context of plant invasion, several hypotheses guide research in plant-soil feedbacks: non-native or invasive species accumulate pathogens slower than common, native species (Klironomos 2002, Mitchell and Power 2003), exotic species increase the accumulation of local pathogens with subsequent negative impacts on native species (Eppinga et al. 2006, Mangla et al. 2008), and non-native species are released from soil pathogens (i.e. natural enemies theory or enemy release hypothesis on a microbial scale)(Darwin 1859, Elton 1958, Mitchell and Power 2003). Plant-soil feedback adds complexity to traditional theories based on competition for resources through the inclusion of direct and indirect interactions between biotic and abiotic entities. A

primary focus of PSF has been the incorporation of soil flora and fauna dynamics with litter quality and quantity, decomposition, and biogeochemical cycling to improve our understanding of plant invasion (van der Putten et al. 2007), succession (van der Putten et al. 1993), and coexistence between strong competitors (Bever 2003). Plant-soil feedback integrates the above- and below-ground parts of an ecosystem and provides a framework for improving our understanding of complex systems (Wardle et al. 2004).

We present two greenhouse experiments using *Acroptilon repens* (L.) D.C. and *Solidago canadensis* L. to summarize the effects of soil conditioning (legacies) and inoculation on plant productivity and competition (inter- and intraspecific). Using plant material from North America, we explored the differences between the invasive and native genotypes of *A. repens* and *S. canadensis* respectively. In experiment 1, we examined the effects of conspecific and heterospecific conditioning of the soil on each species. Based on plant-soil feedback theory (Klironomos 2002, Mitchell and Power 2003, Eppinga et al. 2006), we hypothesized that plants would have greater biomass in heterospecific conditions because there would be less pathogen loading or accumulation in the soils conditioned by a different species. The second experiment utilized varying densities and frequencies of *A. repens* and *S. canadensis* to investigate competition and the effect of inoculation with wild collected soils from each species or a control. We hypothesized that plants inoculated with the soil microbial community from the other species (novel inoculation treatment) would have increased biomass because they may be released from their existing pathogens and species receiving the novel inoculation in interspecific competitive scenarios would out-compete the other species. Additionally, we postulated that *A. repens* (invasive genotype) would be less impacted by inoculation

than the native species, possibly due to slower accumulation of pathogens than the native species (Klironomos 2002). Our goal is to highlight the inherent linkages between plants and soil to improve our understanding of the mechanisms that facilitate exotic plant invasion and subsequent loss of biodiversity.

MATERIALS AND METHODS

Acroptilon repens (Russian knapweed) is a member of the Asteraceae family and is a listed noxious weed in at least 18 states within the USA (USDA-NRCS 2011).

Native to the overlapping regions of the European and Asian continents, the species is originally known from Russia, Iran, Kazakhstan, and Mongolia (Beck 2008). Russian knapweed forms near monocultures and is often found in pastures, rangelands, or degraded croplands in the United States. Its primary mode of reproduction is through aggressive rhizomatous spread and stands are known to live 75 years or longer.

Acroptilon repens produces relatively large seeds (2-4 mm length (FNA 2011)) and in small quantities, only 50-500 seeds per shoot (Beck 2008). Russian knapweed is believed to be allelopathic (Stevens 1986, Stermitz et al. 2003), although determination of specific allelopathic compounds from field samples has lacked repeatability (Quintana et al. 2008). Additional studies of elemental allelopathy from zinc accumulation in the upper soils of *A. repens* infestations did not document negative impacts on native species or Russian knapweed (Morris et al. 2006). Management and control of the species is difficult and requires a long-term commitment and utilization of multiple strategies, including herbicides, mowing, and inter-seeding with aggressive graminoids (Beck 2008).

Solidago canadensis (Canada goldenrod, Asteraceae) is native to the vast majority of the USA and Canada (USDA-NRCS 2011), although it is considered weedy in parts of North America and invasive in much of Europe and Asia (Abhilasha et al. 2008, Zhang et al. 2009). A relatively large body of evidence supports *S. canadensis*' allelopathy as contributing to the species' invasiveness and modification of soil microbial communities, including pathogens (Butcko and Jensen 2002, Sun et al. 2006, Yang et al. 2007, Abhilasha et al. 2008, Zhang et al. 2009). *Solidago canadensis* produces large amounts of relatively small seeds (1-1.5 mm length (FNA 2011), ~2,000,000 per kg (USDA-NRCS 2011)) with physical mechanisms (pappus) to aid in dispersal. The two study species (*A. repens* and *S. canadensis*) are functionally very similar, as they belong to the same family, form dense monospecific stands, are long-lived perennial forbs with aggressive rhizomatous growth, and probably produce allelopathic compounds or chemical interference that affects surrounding vegetation and microorganisms. The species differ markedly in the size and number of seeds produced. *Solidago canadensis* produces large numbers of relatively small seeds, while *A. repens* produces few, large seeds.

Soil Conditioning Greenhouse Experiment (Experiment 1)

In experiment 1 *S. canadensis* and *A. repens* were individually grown over two separate stages and a portion of soil from the first growth stage was utilized as a treatment in the second stage of growth. During the first stage, the twice-autoclaved soils (20 minutes at 121 °C and 17 psi) were trained or conditioned by the growth of either *A. repens* or *S. canadensis* and the subsequent development of a microbial community based

on interactions with one of the plant species. Soils of each species from stage one were used to grow each of the two species during stage two; therefore, four treatments were developed in the second stage of growth through the use of the soils conditioned in stage one (*A. repens* grown in *A. repens* soil, *A. repens* grown in *S. canadensis* soil, *S. canadensis* grown in *A. repens* soil, or *S. canadensis* grown in *S. canadensis* soil). A plant grown in soils conditioned by the same species is referred to as a conspecific treatment and soil conditioned by a different species is referred to as heterospecific. Throughout the experiment, samples in stages one and two were linked to allow comparisons of biomass and the effect of the soil treatment between stages.

Forty-nine plants of each species were grown individually in twice-autoclaved soils for 84 days following transplanting from a 3 month germination and establishment period. Plant material was harvested to determine the shoot and root dry weights for stage one (n=96). Approximately 75 ml of the soil from each container (164 ml) was collected and stored at 4 °C for less than 1 week, until being applied to the soil media of the second stage of growth. Each of the stage one soils (75 ml) were mixed with 150 ml of a twice-autoclaved soil mixture and used to replant the containers with one seedling. Approximately 1/3 of the soil medium in stage two was conditioned during the previous growth stage. Species selection for the replanting of stage two was random and a total of 96 containers were planted. Each of the four treatments in stage two had 24 or 25 replicates and were grown in the greenhouse for 107 days before harvesting to determine shoot and root biomass. All biomass was oven-dried at 60 °C until constant mass was reached, approximately 7 to 10 days.

Acroptilon repens seed was collected from established wild populations of the invasive species near Waverly, CO USA. *Solidago canadensis* seed was collected from populations in the managed natural areas of Chicago Botanic Gardens, Chicago, IL USA. The Soil Conditioning (Exp. 1) and Competition/Inoculation (Exp. 2) experiments were initiated at the same time and used the same plant material, soils, greenhouse procedures and conditions. Seed was germinated and grown in a 1:1:1 by volume soil mixture of Quikrete play sand, Schultz clay conditioner, and Fafard 4P soil mix (45% peat moss, 30% bark, 15% vermiculite, 10% perlite). The soil mixture was autoclaved twice (20 minutes at 121 °C and 17 psi) before germination and transplanting into Stuewe and Sons' Ray Leach conetainers (SC10 supercells – 164 ml, 3.81 x 21.59 cm, UV stabilized plastic). Seeds for both greenhouse experiments were sown in flats on May 16, 2009, thinned on June 9, 2009, and planted in conetainers over several days in mid-August 2009. Plant material for the second stage of growth in the Soil Conditioning experiment were sown on September 9, 2009, transplanted to conetainers on November 12-13, 2009, and harvested on February 27-28, 2010. The length of stage one and two growth in the soil conditioning experiment was 84 and 107 days, respectively.

Plants were germinated and grown in greenhouses on the Colorado State University campus (Fort Collins, CO USA) with a photoperiod of 16 hours daylight. Ambient light and photoperiod were augmented with 430 watt HID lights. The Soil Conditioning experiment was moved between two different greenhouses and had temperatures between 18-24 °C. Conetainers were watered twice daily and no fertilizers were applied during the experiments. The root biomass of 22 samples were lost during harvesting, leaving only 74 root biomass samples from stage two. Statistical analysis

used one-way Analysis of Variance (Anova) in JMP (version 8.0.2, SAS Institute Inc., Cary, NC USA) to test for differences between species' biomass in stage one or soil conditioning in stage two. Data were tested for normality before analysis and transformed by the square root or natural logarithm when necessary.

Competition and Soil Inoculation Greenhouse Experiment (Experiment 2)

All possible combinations of *A. repens* and *S. canadensis* were grown in one or two plant mixtures based on Underwood's (1986) competitive asymmetry experimental design. This experimental approach varies planting density (one or two plants per pot) and species frequency to explore the effects of competition and density. The competitive scenarios include growth of *A. repens* alone (monospecific), *S. canadensis* alone (monospecific), two plants of *A. repens* grown together (intraspecific), two plants of *S. canadensis* grown together (intraspecific), or one plant of each species grown together (interspecific). The five competitive scenarios were developed through the transplanting of seedlings of a similar size and age into 164 ml conetainer style pots (Stuewe & Sons' Ray Leach SC10 Supercells). The competitive scenarios explored intra- and interspecific competition between the two species, while providing baseline information on the species' growth without plant competition. Conetainers were inoculated with a soil slurry using soil collected from wild populations of *A. repens*, *Solidago* spp., or a control (no soil). All combinations of competition scenarios (5) and inoculations (3) were replicated ten times (n=150 conetainers, 240 individual plants).

Experiment 2 differs from classical additive and substitutive experimental designs because all possible combinations of the two species in densities of 1-2 plants per

container are included. Additive experimental designs increase density while holding the frequencies of species constant. They have been extensively used by agronomists to determine reductions in crop yield at varying weed densities, but are criticized in ecological studies for not allowing both species' densities to vary and therefore not providing information about the long term outcomes of competition (Law and Watkinson 1987). Conversely, substitutive designs vary the frequency of species while holding the overall density constant. Law and Watkinson (1987) state that this experimental design can only provide qualitative information about competition and the results will be based on the densities used in the experiment. We utilized Underwood's (1986) Competitive Asymmetry experimental design to incorporate the dynamics of both additive and substitutive designs through the inclusion of all possible combinations of species frequency and density in a two species and one to two plants per container greenhouse experiment. Additionally, we utilized this unique aspect of the experimental design to develop Relative Interaction Indices (RII) (Armas et al. 2004) to standardized comparisons of inter- and intraspecific competition for the two species and determine if interactions are asymmetrical (Morin 1999).

Following a germination and seedling establishment period of 3 months, the plants were transplanted to containers and grown in the Colorado State University greenhouses. Plants were harvested following 3 months of growth in the competitive scenarios and shoot and root biomass were individually determined for each plant. Experiment 2 used the same plant material, seeding and transplanting dates as Experiment 1, but was harvested November 16-20, 2009. Greenhouse temperatures ranged between 18-21 °C for the Competition and Soil Inoculation experiment. Roots

were washed under tap water to remove most soil or inert materials and all biomass was oven-dried for 11 days at 60°C. In two plant competitive scenarios (inter- or intraspecific), the roots were detangled as best as was possible and material that could not be directly designated to a specific plant were appropriated to each plant based on the proportional relationship of the two plant's known root biomass. Conetainers were inoculated monthly with a soil slurry made from fresh, wild collected soils of *A. repens* or *Solidago* spp. populations near Fort Collins, CO USA. The inoculation treatments consisted of 80 g fresh soil mechanically shaken with 975 ml of distilled, deionized water for 1 hour. Following shaking, the soil slurries were immediately applied to the conetainers and care was taken to keep the mixtures oxygenated and avoid anoxic conditions. Each of the 164 ml conetainers received 40 ml of inoculum monthly and the flats were rotated within the growing space three times throughout the growth period. The control treatment consisted of 40 ml distilled, deionized water.

The variable planting densities and species' frequencies in the five competitive scenarios made it possible to develop a comparative index of the interaction strength of a species in inter- and intraspecific competition, while standardizing the species' growth with and without competitors. Using the Relative Interaction Index (RII) developed by Armas et al. (2004), we calculated measures of interaction strength in the inter- and intraspecific competitive scenarios for both *A. repens* and *S. canadensis* that are symmetrical around zero, have bounded limits (between 1 and -1), and can be used in statistical analysis because of their linear nature and approximately normal distribution (Armas et al. 2004). The RII's unique properties make it ideal for the analysis of plant interactions

that are focused on competition (negative RII's) or facilitation (positive RII's). The RII is calculated as follows:

$$RII = \Delta B_{fc} / (\Delta B_{fc} + 2B_o)$$

$$\text{Where: } \Delta B_{fc} = B_w - B_o$$

B_o = mass of target plant growing without other plants

B_w = mass of target plant growing with other plants

The B_o values were determined from the mean biomass of a species growing alone (monospecific) within each inoculation treatment. Relative Interaction Index values were calculated for root biomass, shoot biomass, and root to shoot ratios. The experimental design creates two response or dependent variables for each competitive interaction (inter- or intraspecific) and therefore a reduced sample size ($n=60$) because the monospecific scenarios are used to create the index, but are not included as samples in the analyses. Additionally, each plant from the two plant interspecific competitive scenarios becomes a response variable for each species and the two individuals from an intraspecific scenario are averaged to form the response variable. A Multivariate Analysis of Variance (MANOVA) using an identity response design was used to statistically analyze the indices against the competition scenarios (2 levels; inter- or intraspecific), inoculation treatments (3 levels) and scenario*treatment interaction. Univariate Analysis of Variance (ANOVA) and Tukey's Honest Significance Difference (HSD) post-hoc tests were also used to determine treatment differences in plant biomass and root to shoot ratios. All statistical analysis was conducted in JMP (version 8.0.2,

SAS Institute Inc., Cary, NC USA). Data were tested for normality before analysis and transformed by the square root or natural logarithm when necessary. One outlier was removed from the univariate analysis due to its impact on normality, although its removal did not affect univariate test results.

RESULTS

Soil Conditioning Experiment 1

Plant biomass from the first stage of growth was greater than the amount of biomass production from stage two (Figure 2.1). During stage one, *S. canadensis* had greater shoot ($F_{1,97}=81.1746$, $P<0.0001$) and root ($F_{1,97}=46.5185$, $P<0.0001$) biomass than *A. repens*, although it had lower root:shoot ratios ($F_{1,97}=9.0046$, $P=0.0034$). Shoot biomass from stage two growth was different between species and soil conditioning treatments (Figure 2.1) ($F_{3,97}=31.3294$, $P<0.0001$). *Solidago canadensis* had the greatest shoot biomass when grown in soil conditioned by a conspecific, while *A. repens* had the lowest in conspecific soils. The two species' responded to the conspecific or heterospecific soil conditioning with opposite growth trends in shoot biomass. During stage two, soil conditioning did not affect root biomass within a species, although *A. repens* grown in conspecific trained soils had lower biomass than *S. canadensis* in either soils treatment (Figure 2.1) ($F_{3,75}=8.4448$, $P<0.0001$). Root:shoot ratios in stage two growth varied between species with *A. repens* having greater root:shoot ratios, although within a species no effect of soil conditioning was detected (Figure 2.1) ($F_{3,75}=16.8194$, $P<0.0001$). In general, *A. repens* had greater root:shoot ratios than *S. canadensis*, while the *Solidago* species produced more biomass, especially shoot biomass. Biomass

production was greatly reduced when the plants were grown in conspecific or heterospecific trained soils, even though the conditioned and potentially nutrient depleted soils from stage one were less than 1/3 of the total volume of soil in the second stage of growth.

Competition and Soil Inoculation Experiment 2

Total biomass represents the sum of all roots and shoots within a container for each of five competitive scenarios and three inoculation treatments ($F_{14,149}=2.2381$, $P=0.0092$)(Table 2.1 and Figure 2.2). Competitive scenarios were statistically different ($P=0.0002$), but the inoculation treatment ($P=0.2158$) and interaction ($P=0.8393$) of the two factors were not. The total biomass of *A. repens* grown alone was less than all other competitive scenarios, except the total biomass of two *S. canadensis* plants grown together in intraspecific competition.

Based on a three-way Anova, competitive scenario was significant for shoot biomass, root biomass, and root:shoot ratios, although inoculation had no effect (Table 2.1). Differences in shoot biomass ($F_{17,179}=9.7125$, $P<0.0001$) were related to species and competition, but not inoculation. Individual plants of each species grown without competition (monospecific) had greater shoot biomass than individual plants from any of the two plant competitive scenario (Figure 2.3). Additionally, monospecific *S. canadensis* had greater shoot biomass than monospecific *A. repens*. Root biomass varied between competitive scenarios, but not by species, inoculation, or interactions ($F_{17,179}=4.7909$, $P<0.0001$). Individual plants grown without competitors always produced more root biomass than individuals in inter- or intraspecific competition

(Figure 2.3). Root to shoot ratios varied considerably between species and in the competitive scenarios of *S. canadensis* ($F_{17,178}=3.5226$, $P<0.0001$). The intra- and monospecific scenarios of *S. canadensis* root:shoot ratios were lower than interspecific *S. canadensis* and some of the *A. repens* competitive scenarios. *Solidago canadensis* modified its root to shoot allocations between competitive scenarios and promoted root over shoot growth when interspecific competition was present. The statistically significant differences between monospecific and competitive scenarios documents a reduction of root or shoot biomass in competitive situations and the occurrence of competition.

Based on Relative Interaction Indices (RII), competitive scenarios produced negative effects (competition) on plant biomass, regardless of the species or type of competition (inter- or intraspecific) (Figure 2.4). The root:shoot ratios of the each species responded differently to competition. *Solidago canadensis* root:shoot ratios increased in the presence of competition (positive RII's) and therefore root growth was stimulated over shoot growth, especially in interspecific competition with *A. repens*. Root:shoot ratios of *A. repens* decreased in competitive scenarios (negative RII's) and the interaction index was stronger in the interspecific competitive scenarios of each species. Overall, the absolute strength of the RII was lower for each species in the intraspecific scenario compared to interspecific competition (i.e. less negative for *A. repens* and less positive for *S. canadensis*). Based on Manova analysis the RII's for shoot or root biomass did not differ, although the root:shoot ratios were significant for competitive scenarios and inoculation treatments (Table 2.2). When RII root:shoot ratios are grouped by inoculation treatment all *S. canadensis* values are positive (increased root:shoot ratios)

and all *A. repens* values are negative (reduced root:shoot ratios), except *A. repens* inoculated with a soil slurry from *Solidago* spp (Table 2.3).

DISCUSSION

Our greenhouse studies illustrate how two weedy species react differently to microbial legacies or inoculation of the soil and varying competitive situations. The opposite responses of the two species in the two experiments highlights potential trade-offs between exploitative competition (limited resources) and interference competition, potentially due to pathogen accumulation or a lack of mutualisms. Although *A. repens* and *S. canadensis* are functionally and physiologically similar, the species responded to soil legacies (Exp. 1) and plant competition (Exp. 2) in opposite manners and this may represent fundamental differences between how an invasive species and a weedy, native species avoid or respond to competition. Each species is weedy in its native habitat and invasive in its novel range, although we only tested genotypes of North American *S. canadensis* (native) and *A. repens* (invasive). Invasive Russian knapweed reacted to soil legacies from the conditioning treatment by increasing root:shoot ratios and decreasing shoot biomass in conspecific conditioned soils (Figure 2.1), but was relatively unresponsive to inter- or intraspecific competition (Figures 2.2 and 2.3). Additionally, *A. repens* distinctly increased RII values of root:shoot ratios when inoculated with *Solidago* spp. compared to the *A. repens* or control inocula (Table 2.3). Conversely, *S. canadensis* increased shoot biomass in conspecifically conditioned soils, but did not modify its root:shoot allocation of biomass in the presence of microbial legacies (Figure 2.1). The native *S. canadensis* was strongly impacted by competitive scenarios and reacted by

dramatically increasing its root:shoot allocation in the presence of interspecific competition (Figures 2.2, 2.3, 2.4)(Exp. 2). These opposing responses to soil conditioning and competition may be an important aspect of invasion and vegetation dynamics. An invasive species may be able to avoid competition, inter- or intraspecific, only to modify its growth patterns based on interactions with soil microbes, potentially pathogens or a lack of mutualisms. Whereas, a native and functionally similar plant may be heavily impacted by competition, especially interspecific, while not being affected by soil microorganisms. To fully test this theory it is necessary to conduct additional experiments with *A. repens* from its native range (Europe or Asia) and *S. canadensis* from its novel or invasive range (Europe or Asia). This concept addresses the mechanisms that facilitate invasion and how different species and geographically different genotypes of a species are affected and respond to their native or novel habitat. The variable responses of species to microbial communities or plant competition may identify an important difference between aggressive species that are native or exotic, and signify a trade-off between the relative impacts of plant-microbe or plant-plant interactions. Knowledge of the interactions that most strongly affect a species could be used to design control programs that focus on the most vulnerable aspect of an invasive species. For example, *A. repens* is unresponsive to competition, and therefore seeding with aggressive native species alone may be ineffective in reducing its population densities. However, increasing pathogen accumulation or reducing potential mutualisms in addition to seeding with desirable species could increase stress on the unwanted species. The manipulation of soil microbial communities or pathogens as a control treatment for invasive species may be conceptually similar to herbicide applications,

although the pathogens may increase over time while a herbicide will generally decrease. Additionally, the manipulation of exotic soil microbes may encounter issues related to the use of biological control agents and requires additional research to better understand and responsibly apply this potential management approach (van der Putten et al. 2007).

The effects of soil conditioning reduced both root and shoot biomass in stage two compared to stage one growth, although within a species only shoot biomass was impacted by the different soil conditioning treatments (Figure 2.1). Presumably, the soil conditioning created a legacy of microbes, potentially pathogens or a lack of mutualists that negatively impacted plant production in stage two. Conspecific conditioned soils negatively impacted *A. repens* shoot biomass, but increased *S. canadensis* shoot biomass compared to heterospecific soils. Pathogen accumulation and the species-specific rate of accumulation have been postulated as primary drivers of vegetation succession (van der Putten et al. 1993) and invasiveness (Klironomos 2002). Different species may accumulate pathogens at different rates and this can drive vegetation dynamics in a system through the extirpation or domination of a species. The Natural Enemies and Enemy Release hypotheses are developed on the concept that exotic species have fewer enemies in novel habitats and this facilitates invasion, possibly through the allocation of resources from defense to increased production or fecundity (EICA hypothesis)(Blossey and Notzold 1995). Mitchell and Power (2003) found a large reduction of fungi and viral species in plant species' naturalized ranges and documented support for the Enemy Release Hypothesis. Our experiments show the variable strategies that plants exhibit in response to soil microorganisms and how these responses may influence the species' aggressiveness or invasive potential. The reduced biomass production during stage two

may be due to nutrient depletion of the soil from stage one, although only 1/3 of the total soil volume in stage two was from the initial growth stage and the soil matrix was not root bound, therefore a lack of available nutrients is unlikely to explain the large reduction in growth between the two stages.

The Competitive and Inoculation Experiment (#2) documents the presence of competition based on the reduction of biomass between one and two plant scenarios (Figure 2.3). This study also highlights the different responses of the two species to competition. Contrary to classical ecological theory (Darwin 1859, Connell 1983), the strength of intraspecific interactions was equal to or lower than interspecific competition in the biomass and root:shoot ratios of each species (Table 2.2, Figure 2.4). The greatest differences in RII values occurred between competition scenarios in the root:shoot ratios. Based on RII values, intraspecific interactions for root:shoot ratios were less negative for *A. repens* and less positive for *S. canadensis* compared to interspecific values (Figure 2.4). Overall, interspecific interactions for root:shoot ratios of the two species were stronger, but the species responded in opposite directions. Our results document reduced interaction strengths in the intraspecific competition of our two weedy species, compared to interspecific interactions. These greenhouse based results oppose traditional ecological theory (Darwin 1859, Elton 1958) and findings based on a broad survey of field experiments (Connell 1983). Based upon our study species we propose that weedy, aggressive species may avoid the negative effects of intraspecific competition and the lack of this negative interaction could facilitate their invasiveness. Additionally, *A. repens* RII of root:shoot ratios was dramatically greater in the *Solidago* spp. inoculation (Table 2.3), possibly signaling that the species increased root production relative to

shoots when inoculated with a novel microbial community. These findings support the concept of increased invasion by *A. repens* when released from enemies (microbial pathogens) or assisted by increased mutualisms, since the species primarily spreads by creeping roots and subsequent rhizomes.

The two species may respond differently to manipulation of soil microbial communities from soil conditioning or inoculation based on their life history traits, especially reproductive strategies. Although the species are very similar morphologically and physiologically, their above-ground growth rates and seed production differs. *Solidago canadensis* is a prolific producer of small seeds and may avoid pathogen loading through increased shoot growth (Figure 2.1) and presumably increased seed production. Conversely, *A. repens* produces small amounts of large seeds and may respond to pathogen loading by increasing root:shoot ratios and focusing resources on escaping soil pathogens instead of producing shoots and seeds. *Acroptilon repens* had increased root:shoot ratios when grown in conditioned soils compared to the sterile soils of the first growth stage, although the source of soil conditioning was not important. Understanding how an invasive species responds to different microbial communities may assist in the development of innovative, low impact control strategies guided by ecological principles, such as introducing pathogens or root herbivores to control *A. repens*, or preventing seed maturation in *S. canadensis*.

Soil conditioning and inoculation affected plant growth and resource allocation differently, even though each treatment attempted to manipulate the soil microbial communities based on the presence of specific plant species. Soil conditioning, or the experimental creation of soil legacies, appeared to have a greater impact on plant growth

than monthly inoculation with soil slurry from specific wild plant species. This is surprising because the soil inoculation treatments were from natural soils that have interacted with plants for long periods of time, while the soils conditioned in the greenhouse had only 3 months to modify the microbial community, although the soils were sterile and of horticultural quality. Presumably, the natural soils that were used for inoculation would have accumulated large amounts of microbes from the rhizosphere of each species that it was collected from (Kulmatiski and Beard 2008) and based on pathogen accumulation theory these could have powerful conspecific treatment effects in the greenhouse experiment. Our results indicate that plants can rapidly create soil legacies and that subsequent impacts are primarily documented in the shoot biomass and root:shoot ratios for our two study species. The monthly inoculation with a soil slurry may not have been frequent enough to establish the desired microbial communities and impact the plant community or the preparation of soil slurries may have been detrimental to the inoculum. Future research should quantify soil pathogens, plant growth promoting rhizobacteria (PGPR), potential mutualists such as AM fungi or symbiotic nitrogen fixing bacteria, although the two studies species represented here do not form diazotrophic relationships.

Invasion biology and ecology have generally focused on a species' competitive ability or resource use efficiency to explain the success of an exotic species in a novel habitat. The release of exotic species from natural enemies, herbivores or microbes, has scientific support for contributing to the success of invasive plants. We propose that the relative importance of plant competition or soil microbial community interactions for a species and its genotype (native or invasive) can be determined by how a species

responds or reacts to the different types of competition (exploitative or interference). Our study species modified their root:shoot biomass allocation based on different factors; soil conditioning for *A. repens* and interspecific plant competition for *S. canadensis*. This concept assumes that a species reacts or responds to an interaction that affects it most strongly and this may present potential targets for invasive plant management based on ecological principles. This does not refute theories of invasion based on enemy release, but highlights how plant species may respond and that competition or pathogen accumulation can be avoided or ignored when another stressor is more prevalent or detrimental. By determining what causes a plant species to modify its resource allocation, we can identify the source of the impact and use this knowledge to improve our understanding of the mechanisms of invasion or efficiency of control techniques.

When invading novel habitats, aggressive exotic species may be unresponsive to competition with other plants, especially if resources are not limited or the species can persist at lower resource requirements than its neighbors, but the invaders may be responsive to pathogens or soil legacies. Essentially, the classical negative effects of competition could be irrelevant to an exotic species because the competition is not intense enough to force the species to react, but the numerous effects of pathogens may force the species to adapt its allocation of resources and provide opportunities for targeted management. Trade-offs between responses to competition or pathogen accumulation highlight the importance of considering the potential plant-soil-microbe feedbacks in the management of invasive species. Based on our limited greenhouse study, the interaction of invasive species with soil microbes may be more important than competition and this could lead to novel management practices that utilize soil

inoculation to introduce pathogens or herbivores, or amendments with activated carbon to modify the microbial community (Kulmatiski and Beard 2006, Kulmatiski 2011).

Research with additional species and utilization of genotypes from each species' native and exotic range is necessary to untangle the web of plant-soil feedbacks and determine the mechanisms of invasion and promote innovative, low impact management practices that utilize ecological principles to achieve land management goals (Krueger-Mangold et al. 2006).

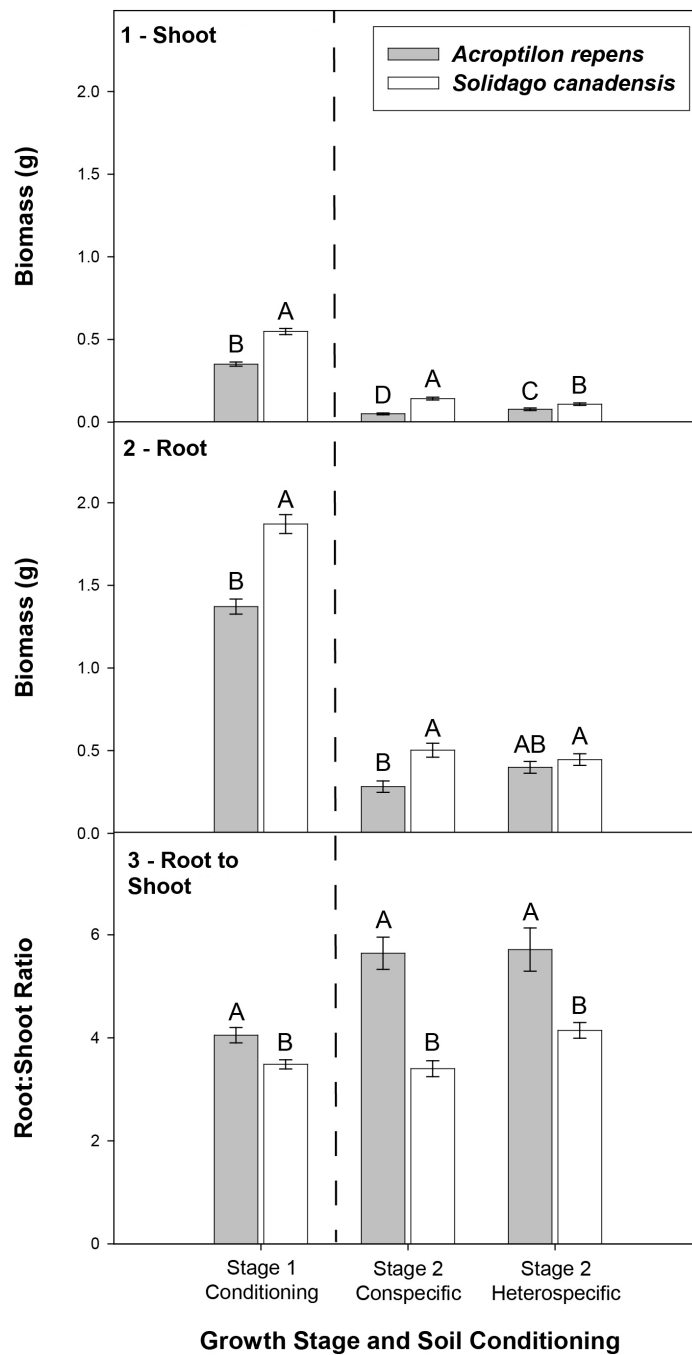


Figure 2.1 – Plant biomass and root:shoot ratios for *Acroptilon repens* and *Solidago canadensis* grown over two stages in the Soil Conditioning Experiment (Exp. 1). The experiment used the species specific conditioned soils from stage one as a treatment in stage two to develop conspecific and heterospecific soil treatments. Different letters indicate treatment means differed significantly ($\alpha = 0.05$) between species in stage one or between species and soil conditioning treatments in stage two.

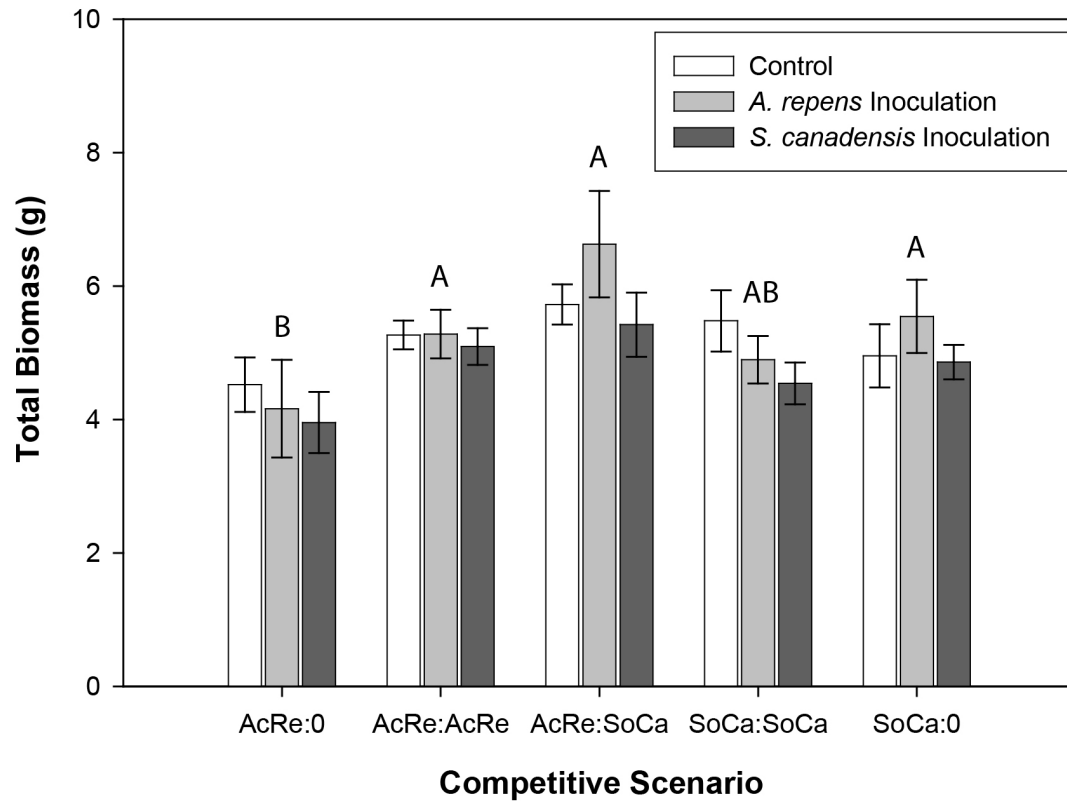


Figure 2.2 – Total biomass (g) of all roots and shoots in containers of varying density, species, and inoculation treatments for Experiment 2 (Competition and Soil Inoculation). Competition scenarios are represented by species abbreviations for *Acroptilon repens* (AcRe) and *Solidago canadensis* (SoCa), and vary between one or two plants per container and all possible combinations of the two species. Inoculation consisted of a monthly addition of a slurry made from fresh soils collected from natural populations of *A. repens* or *Solidago* spp. Different letters indicate treatment means differed significantly ($\alpha = 0.05$) between competitive scenarios based a post-hoc Tukey test of Honest Significant Difference. Inoculation treatment was not significant and results are not shown.

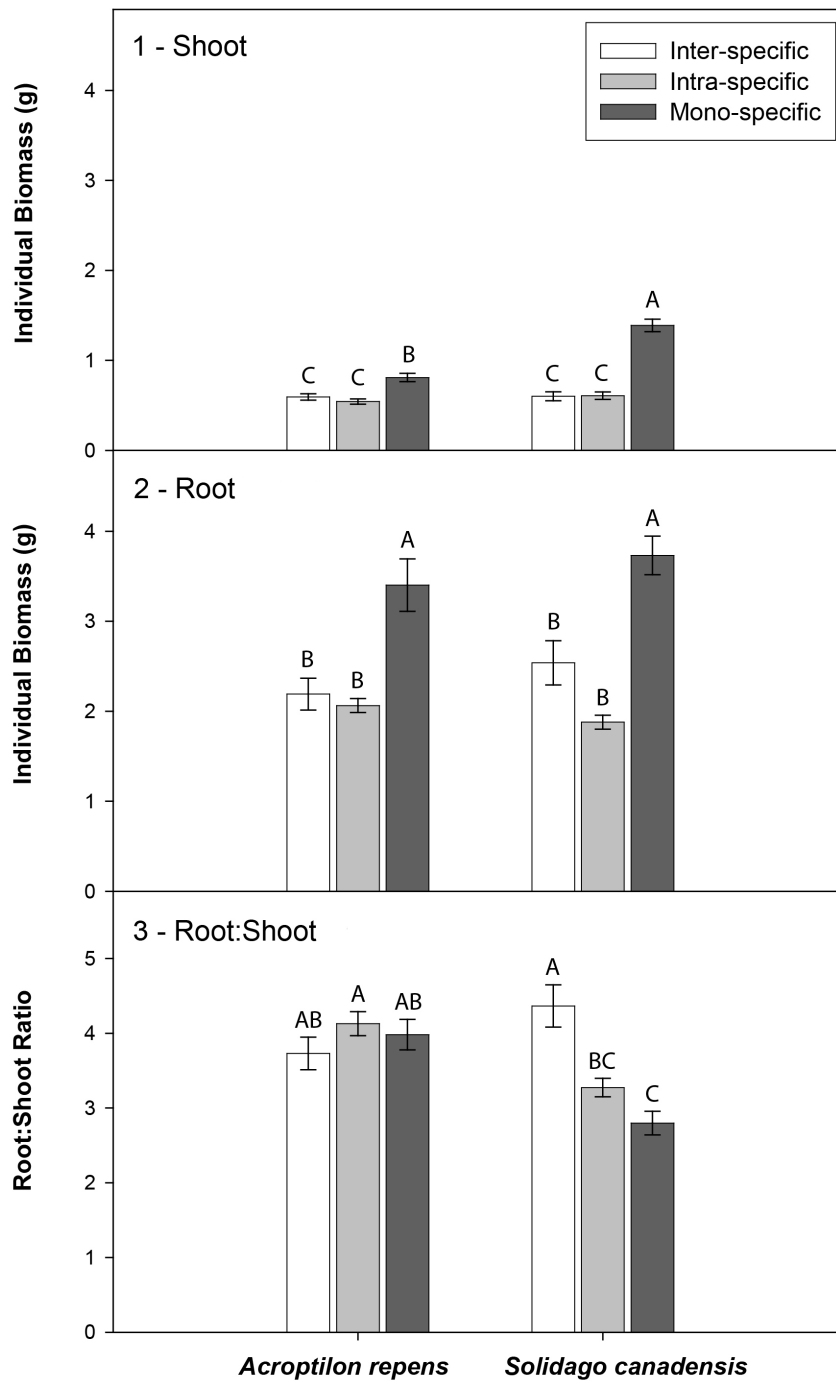


Figure 2.3 - Individual plant root or shoot biomass (g) or root:shoot ratio for *Acroptilon repens* and *Solidago canadensis* individuals grown in three competitive scenarios (Interspecific, Intraspecific, or Monospecific (one plant grown without competition)). Different letters indicate treatment means differed significantly ($\alpha = 0.05$) in the species and competitive scenarios interaction of a three-way ANOVA.

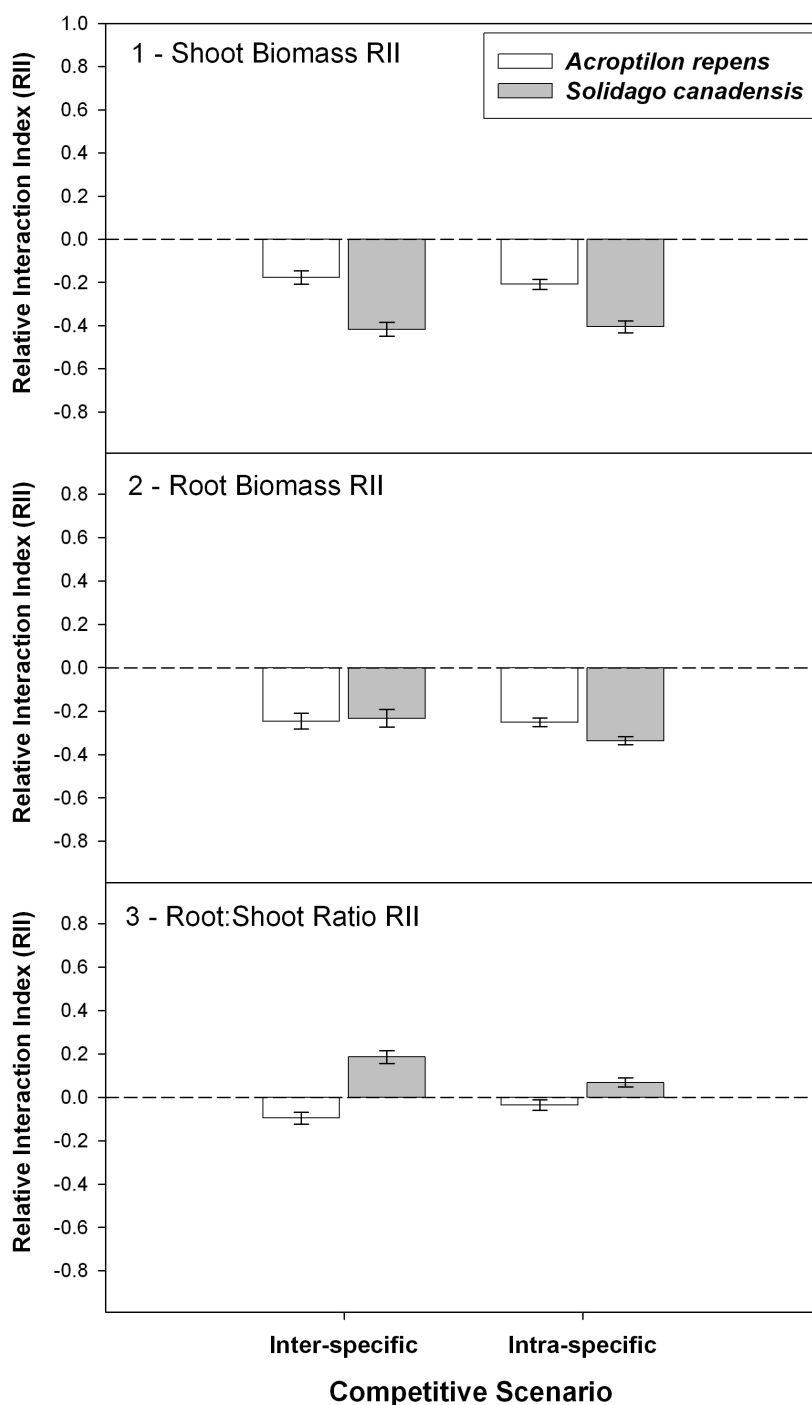


Figure 2.4 – Relative Interaction Index (RII) for shoot biomass, root biomass, or root to shoot ratios of *Acroptilon repens* and *Solidago canadensis* grown in interspecific and intraspecific competition. RII determines an index of facilitation (>0) or competition (<0) by standardizing for a species' potential growth without competitors. The index is symmetrical, bound between 1 and -1, and approximates a normal distribution. See Table 2.2 for Multivariate Anova results.

Table 2.1 - Statistical results of Anova analysis for individual plant shoot or root biomass, total conetainer biomass, or root to shoot ratios from the Competition and Inoculation experiment (Exp. 2). Analysis compared species (*Acroptilon repens*, *Solidago canadensis*), competition (interspecific, intraspecific, monospecific), and inoculation (control, *A. repens*, *S. canadensis*). Inoculation represents the addition of a soil slurry from wild collected soils of each species or a control (3 levels).

Analysis of Variance Table					
	Source	DF	Sum of Squares	F Ratio	Prob > F
Individual Plant Shoot Biomass	C. Total (Main Test)	179	7.5716	9.7125	<.0001
	Inoculation	2	0.0023	0.0507	0.9506
	Species	1	0.3524	15.2251	0.0001
	Competition	2	2.8140	60.7864	<.0001
	Inoculation*Species	2	0.0247	0.5332	0.5877
	Inoculation*Competition	4	0.0626	0.6757	0.6097
	Species*Competition	2	0.5182	11.1929	<.0001
	Species*Competition*Inoculation	4	0.0476	0.5146	0.7251
Individual Plant Root Biomass	C. Total (Main Test)	179	6.7543	4.7907	<.0001
	Inoculation	2	0.0647	1.1653	0.3144
	Species	1	0.0278	1.0026	0.3182
	Competition	2	1.9312	34.8027	<.0001
	Inoculation*Species	2	0.0182	0.3273	0.7214
	Inoculation*Competition	4	0.0108	0.0973	0.9832
	Species*Competition	2	0.1017	1.833	0.1632
	Species*Competition*Inoculation	4	0.1053	0.9484	0.4376
Total Conetainer Biomass (1 or 2 plants)	C. Total (Main Test)	149	16.8008	2.2381	0.0092
	Inoculation	2	0.3133	1.5508	0.2158
	Competition	4	2.4309	6.0166	0.0002
	Inoculation*Competition	8	0.4207	0.5207	0.8393
Individual Plant Root:Shoot Ratio	C. Total (Main Test)	178	3.4271	3.5226	<.0001
	Inoculation	2	0.0453	1.4593	0.2355
	Species	1	0.1828	11.7805	0.0008
	Competition	2	0.1907	6.1471	0.0027
	Inoculation*Species	2	0.0021	0.0693	0.9331
	Inoculation*Competition	4	0.0683	1.0999	0.3585
	Species*Competition	2	0.3745	12.0702	<.0001
	Species*Competition*Inoculation	4	0.0595	0.9582	0.4322

Table 2.2 - Multivariate Analysis of Variance (MANOVA) for Relative Interaction Indices (RII) from the Competition and Soil Inoculation experiment (Exp. 2). Table contains P values from a MANOVA analysis (Identity response design) for Relative Interaction Indices (RII) of *Acroptilon repens* and *Solidago canadensis* biomasses and root to shoot ratios. Based on Underwood's (1986) Competitive Asymmetry experimental design, the experiment varied the density of plants (1-2) and frequency of the two species to develop a relative measure of each species' interaction strength (RII, Armas et al. 2004) in inter- and intraspecific competitive scenarios (2 levels), while standardizing for each species grown without competition. Inoculation represents the addition of a soil slurry from wild collected soils of each species or a control (3 levels).

MANOVA Results of RII				
RII Measurement	Main Test - Wilks' Lambda (N)	Inoculation - Wilks' Lambda	Competitive Scenario - F test	Inoculation*Competition - Wilks' Lambda
Shoot Biomass	0.4174 (60)	0.1119	0.6989	0.7448
Root Biomass	0.2454 (60)	0.1686	0.0813	0.9118
Root:Shoot Ratio	0.0018 (60)	0.0161	0.0023	0.4281

Table 2.3 – Mean Relative Interaction Index (RII) of root:shoot ratios (± 1 SE) for *Acroptilon repens* and *Solidago canadensis* in the Competition and Soil Inoculation Experiment (#2). Means are grouped by the three inoculation treatments. Inoculation represents the addition of soil slurry made from wild collected soils of each species or a control without soil.

Inoculation	RII Root:Shoot Ratios	
	<i>Acroptilon repens</i>	<i>Solidago canadensis</i>
<i>Acroptilon repens</i>	-0.113 (0.032)	0.130 (0.037)
Control (no soil)	-0.104 (0.030)	0.101 (0.036)
<i>Solidago canadensis</i>	0.019 (0.027)	0.150 (0.029)

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Chapter 3 – Soil inoculation as a potential tool to manipulate plant-soil feedbacks and improve management of exotic knapweed invasions

INTRODUCTION

The interactions of plants with soil fauna and flora has recently received a great deal of attention due to the potential for plant-soil feedbacks to influence plant community structure and function, successional dynamics (Kardol et al. 2006), and the spread of invasive species (Callaway et al. 2004, Wolfe and Klironomos 2005). To augment our understanding of plant-soil feedbacks, I used whole soil from native plant communities to study the impacts of inoculation on two invasive knapweed species in a field based restoration experiment and subsequent greenhouse experiments. Many inoculation experiments have been conducted in the laboratory or greenhouse, or are based on conceptual models (Eppinga et al. 2006). Few studies have included multiple years of data, paired field and greenhouse experiments, or a quantitative assessment of the soil microbial community and soil biogeochemistry. My goal was to determine the effect of whole soil inoculation as a potential tool to aid in the management of exotic knapweed invasions, improve the restoration of native vegetation, and to increase our understanding of plant-soil feedbacks that can be utilized for ecological management (Krueger-Mangold et al. 2006, Sheley et al. 1996).

Plant-soil interactions are complex and obscured from ready observation. Abiotic factors directly affect plant community composition at large scales due to spatial and temporal variations in climate and soils, but at an ecosystem level many factors can influence the species richness, diversity, and successional dynamics of a plant community. In-depth ecological analysis has provided quantitative information about how plants, animals, soil, and the atmosphere interact and ecosystems are modified, but we still lack a mechanistic understanding of ecosystem dynamics. The effects of soil organisms on plant population dynamics has become a topic of interest, especially since many of the primary interactions (competition, biogeochemistry, resource limitations) have been thoroughly studied, yet many questions remain concerning vegetation dynamics (Klironomos 2002). Many scientists believe that factors such as competition, dispersal, resource use, and predation have not resolved this essential issue of ecology (sensu Klironomos 2002). Current research has begun to investigate how soil, microorganisms, and plants interact to form feedback systems that can positively or negatively influence species (van der Putten et al. 1993, Bever 1994, Bever et al. 1997, Klironomos 2002, Bever 2003, Reynolds et al. 2003, Kardol et al. 2006, Kardol et al. 2007) and directly alter the richness or diversity (De Deyn et al. 2003), abundance (Klironomos 2002), primary succession (van der Putten et al. 1993) or secondary succession (De Deyn et al. 2003) of a plant community.

In order for a plant-soil feedback to occur, a plant species must modify the soil community or physical properties of the soil and then the plant-induced changes to soil organisms or the soil itself need to affect the plant's performance (Bever 1994, Kardol et al. 2007). Kardol et al. (2006) concisely describes plant-soil feedbacks as the interactions

between plants and their biotic and abiotic soil environment. The feedbacks between plants and soil microbes can be negative or positive. Many feedbacks are thought to be negative and may augment successional change in the community through the accumulation of pathogens (van der Putten et al. 1993) or facilitate the coexistence of strong competitors (Bever 2003). Positive feedbacks may occur when a species enhances the quality of the habitat (directly or indirectly) and subsequently enhances its own growth. The inherent complexity of the soil system has made it very difficult to understand these interconnections and has provided an incredible challenge of detangling the web of soil interactions to determine the importance of feedbacks in plant community dynamics and succession.

Many studies have highlighted the importance of plant-soil interactions and how these feedback systems can affect plant population dynamics (Bever et al. 1997, Bever 2003, Reynolds et al. 2003, Kardol et al. 2006, Kardol et al. 2007), including the invasion by exotic species (van der Putten et al. 1993, Callaway and Aschehoug 2000, Reinhart et al. 2003, Wolfe and Klironomos 2005, Reinhart and Callaway 2006, Eppstein and Molofsky 2007, van der Putten et al. 2007, Mangla et al. 2008). The aggressive and opportunistic traits of a species that is invasive in its novel range do not facilitate their dominance in the species' native range and this conundrum has been called the "invasive plant paradox" (Rout and Callaway 2009). Invasive species may dramatically modify their novel habitats and subsequently increase their fitness or negatively affect their neighbors and competitors. Invasive plant species that modify plant-soil feedbacks which facilitates their establishment may have a competitive advantage over other species. As our understanding of invasive species and plant-soil feedbacks improve, it may become

possible to manipulate or direct plant-soil feedbacks as a management tool. The concept of ecologically-based management is founded on understanding the species, systems, and interactions to manipulate the biology or ecosystem processes and direct a plant community towards a desirable goal (Sheley et al. 1996).

The use of inoculum is a potential tool to affect plant-soil feedbacks through the manipulation of microbial communities, pathogen accumulation, and nutrient cycling. The technique could be the primary method to implement ecological, processed based management that includes manipulation of the soil microbial community. Soil inoculation has been used experimentally in agriculture to increase crop yields and is especially common with leguminous species (Date 2001). Topsoil storage and reuse is frequently incorporated into mineland reclamation, although it is probably focused on the physical and nutrient properties of the soil and not the biological composition or function. Soil inoculation differs from topsoil storage in mine reclamation, because it utilizes small amounts of soil and attempts to add a microbial community to the ecosystem. Inoculation use in ecosystem restoration is relatively new, especially in field-based experiments. Commercially available arbuscular mycorrhizal fungi (AMF) have been promoted to increase plant establishment and growth in revegetation for decades, but improvements need to be made in the successful delivery of products to plant roots in the field (Date 2001) and root infection rates based on Mycorrhizal Infection Potential (MIP) can be very low (Rowe et al. 2007). The use of field soil or whole soil inoculum to improve the establishment of native species is controversial, since soil from native plant communities may harbor pathogens for native plant species or invasive species may facilitate the growth of native pathogens with subsequent detrimental consequences on

native plant species (i.e. the Accumulation of Local Pathogens Hypothesis) (Eppinga et al. 2006, Mangla et al. 2008). Conversely, field soils from native plant communities could assist in the development of mutualistic relationships with native species that have coevolved over millennia. A potential downfall of whole soil inoculation is that the microbial constituents are unknown, whereas microbial isolates or commercial inoculum contain specific microorganisms. Overall, many of the theories and concepts in plant-soil feedbacks are based on the natural enemies or enemy release hypotheses (Darwin 1859, Elton 1958), or the rate at which common versus weedy species acquire pathogens and subsequent changes in vegetation due to pathogen loading (Klironomos 2002). Whole soil inoculation has the potential to facilitate low cost and effective methods to ecologically manage vegetation, although a greater understanding of plant-soil feedbacks is necessary to implement large-scale management practices. The utility and low-cost of revegetating degraded areas in Australia with inoculated *Acacia* seed (specific nitrogen fixing bacteria strains) have been documented (Thrall et al. 2005), although inoculation with soil microbial communities represent additional ecological and economic challenges.

If inoculation has the potential to modify PSF, the change could be negative or positive to vegetation, nutrients, and soil microorganisms. Originally, I hypothesized that inoculation with the whole soil of a native plant community would decrease invasive species dominance and increase native species survival and growth, because native plants would re-establish mutualistic interactions with microbes and out compete exotics. Following in-depth reading on the subject of PSF, I modified my hypothesis based on a microbial perspective of the enemy release hypothesis and general theories of pathogen

accumulation (van der Putten et al. 1993, Klironomos 2002). Alternate hypotheses were compared based on the concepts of invasive plant species being released from pathogens in naïve soil communities or native plant species developing beneficial mutualisms in native soil communities. No specific *a priori* hypotheses were developed for soil microbial communities and biogeochemistry, except that inoculation would modify the study sites compared to controls.

METHODS

Research Sites and Invasive Species

Soil inoculation experiments were implemented at four sites in the United States that were infested with exotic, invasive knapweeds. The first field experiment was initiated in 2005 to study *Acroptilon repens* (L.) DC (Russian knapweed) in Washington and *Centaurea stoebe* L. (spotted knapweed) in Wisconsin. A second field experiment was initiated in 2008 to study *A. repens* at two study sites in Colorado. The first experiment was installed at two Department of Defense (DOD) military bases: Fort McCoy (FMC) in west-central Wisconsin and Yakima Training Center (YTC) in central Washington. The predominant vegetation at FMC consists of oak forests, savanna and brushlands that historically had frequent fires that probably maintained oak forests by limiting natural succession to white pine (*Pinus strobus* L.) (Army 2005). The research site at FMC was infested with two invasive species: *C. stoebe* (spotted knapweed) and *Euphorbia esula* L. (leafy spurge). The vegetation at YTC is dominated by sagebrush, bitterbrush, and bunch grasses. The primary invasive species at the YTC study site was *A. repens*, although *Bromus tectorum* L. (cheatgrass) was also prevalent. Both knapweed

species are perennial, but differ greatly in root architecture, reproductive strategies, and longevity. The second experiment, Weld County and Waverly, has study sites in north central Colorado and the vegetation is typical of a short-grass steppe ecosystem. This plant community is considered a late seral system and is dominated by several native grasses: *Bouteloua gracilis* (Willd. Ex Kunth) Lag. Ex Griffiths (blue grama) and *Bouteloua dactyloides* (Nutt.) J.T. Columbus (buffalo grass). The Waverly site has a history of disturbance from cattle or horse grazing and much of the vegetation is dominated by the native *Ericameria nauseosa* (Pall. Ex Pursh) G.L Nesom & Baird (rubber rabbitbrush) and exotic *Agropyron cristatum* (L.) Gaertn. (crested wheatgrass). The Weld County site has relatively intact short-grass steppe plant communities outside of the *A. repens* infestations.

Acroptilon repens is a member of the Asteraceae family and is a listed noxious weed in at least 18 states within the USA (USDA-NRCS 2011). It is the dominant species at the Washington and Colorado study sites. Native to the overlapping regions of the European and Asian continents, the species is originally known from Russia, Iran, Kazakhstan, and Mongolia (Beck 2008b). *Acroptilon repens* forms near monocultures and is often found in pastures, rangelands, or degraded croplands in the United States. Its primary mode of reproduction is through aggressive rhizomatous spread and stands are known to live 75 years or longer. *Acroptilon repens* produces relatively large seeds (2-4 mm length (FNA 2011)) and in small quantities, only 50-500 seeds per shoot (Beck 2008b). Russian knapweed is believed to be allelopathic (Stevens 1986, Stermitz et al. 2003), although determination of specific allelopathic compounds from field samples has lacked repeatability (Alford et al. 2007, Quintana et al. 2008). Management and control

of the species is difficult and requires a long-term commitment and utilization of multiple strategies, including herbicides, mowing, and inter-seeding with aggressive graminoids (Beck 2008b).

Centaurea stoebe (Asteraceae) is native to southeastern Europe, but is currently found throughout Europe and most of North America (FNA 2011). The species is regulated as a noxious or invasive plant in at least 15 US states (USDA-NRCS 2011). Spotted knapweed is a biennial or short-lived perennial that reproduces by seed and develops new shoots each year (Beck 2008a). Management of *C. stoebe* using herbicides has been successful and several biological control agents are currently available in Colorado (Beck 2008a). *Centaurea stoebe* has long been thought to be allelopathic (Stevens 1982, 1986, Perry et al. 2005), although some studies refute the supposed chemical agent (+/- catechin) based on laboratory methodologies (Blair et al. 2005).

The Washington and Wisconsin sites were rototilled, inoculated, and seeded with five native plants species in the fall of 2005. Two inoculation treatments were applied to these sites and consisted of recently collected soil from an adjacent native plant community or a sterilized portion of the same soil (native or sterile treatments, respectively). Freshly collected soil was refrigerated (4 °C) overnight or sterilized overnight using an electric soil sterilizer (Pro-Grow model SS-5) and applied the next day. Inoculation consisted of 40 grams (g) of soil being sown in each 2 x 2 m plot during seeding in the fall of 2005 and inoculation was repeated the following spring. The FMC site was inoculated a third time in the spring of 2007. Each inoculation treatment was replicated five times at each site. Each site was broadcast seeded with five native species (two graminoids and three forbs) at a seeding rate of 538 pure live seed (PLS) per m².

Following broadcast seeding the soil surface was lightly raked by hand. Appendix A lists the native plant species seeded at each site and general life history information.

Soil inoculation experiments at the two Colorado study sites were installed in the spring of 2008 and had different methodologies than the WA and WI experiments. In addition to the native and sterilized inoculation treatments, the Colorado sites had a control treatment (no soil inoculation) and used more inoculum, native plant species, and seed than the previous experiments. Following rototilling, each 2 x 2 m plot had 150 g of soil inoculum sown in conjunction with a seed mixture of 16 native species (861 pls m⁻²) and an additional 150 g of inoculum sown on the soil surface following a light raking. Each treatment was replicated five times at each site. Appendix A lists the native plant species seeded at each site and general life history information for each species.

Vegetation Sampling Methods

Plots were monitored annually near the end of the growing season between 2006 and 2009 for the Washington and Wisconsin sites, and 2008 through 2009 for the two Colorado sites. Density of selected invasive species and cover of all biotic and abiotic entities were determined annually. Rosette and flowering stem (bolts) density of *C. stoebe* were counted separately. Composite soil samples and biomass of individual plant species were collected from all plots in 2009. Within each 2 x 2 m plot, all sampling took place within four permanently marked 0.25 x 0.75 m subplots (0.1875 m²) and the data from all subplots within a plot were combined for analysis. To standardize the optical point cover measurements, each subplot was sampled using an elevated PVC frame with 48 grid cells (4x12) and a long wooden dowel (~0.5 cm diameter) as a pointer for each

cell. A total of 192 cover points were recorded for each plot. Species richness was determined by the presence or absence of species within the subplots and included records from the cover and biomass data.

Soil Sampling Methods

Soil sampling used electric drills and a 1.9 cm wide auger drill bit to extract six soil cores from each subplot (24 cores per plot). Samples were composited for each plot and stored on ice until return from the field (1-2 days). Upon return from the field, two subsamples of each soil sample were taken for microbial analysis. One subsample was extracted using K_2SO_4 for determination of microbial biomass carbon and nitrogen, and stored at $-20^\circ C$ until analysis. The second subsample sample was immediately frozen ($-20^\circ C$) until quantification of soil microbial community composition using Fatty Acid Methyl Esters (FAME). The remaining soil was air-dried for biogeochemical analysis at AgSource Harris Laboratories (Lincoln, NE). The following soil nutrients and electro-chemical variables were quantified (units are ppm unless otherwise noted): calcium, CEC (Cation Exchange Capacity), hydrogen (%), manganese, nitrogen (NO_3), organic matter (%), pH (log scale), phosphorus (1 Bray method), potassium, sodium, soluble salts (conductivity), sulfur, and zinc. Soil nutrient levels were determined by the following methods: cadmium reduction (nitrate), ammonium acetate on (potassium, magnesium, and calcium), DTPA (Zinc), and monocalcium phosphate (Sulphur). Nutrient levels were measured on an inductively coupled plasma spectrometer (ICP). Organic matter was determined by the loss on ignition method.

Greenhouse Inoculation Experiments

Acroptilon repens seed and inoculation (soil slurry) was collected from established wild populations of the invasive species near Waverly, CO USA. Seeds were hand scarified with sandpaper and sown on January 28 2008, transplanted to Stuewe and Sons' Ray Leach conetainers (SC10 supercells – 164 ml, 3.81 x 21.59 cm, UV stabilized plastic) on March 31 2008, inoculated three times (March 31 2008, April 21 2008, May 21 2008), fertilized twice (March 21 2008 – 25 ppm N Scott's Champion and July 1 2008 – 200 ppm N Scott's Champion), and harvested August 30 – September 1 2008.

Acroptilon repens plants were inoculated with a soil slurry made from soils collected 5 m inside of the *A. repens* infestation (invaded), the ecotone between knapweed dominance (ecotone) and native vegetation 5 m outside of the infestation (native). Inoculation consisted of 64 g of freshly collected soil mechanically shaken with 800 ml distilled, deionized water (DI) for 90 minutes and 10 ml was immediately applied to each plant.

Centaurea stoebe seed and inoculation was collected from Fort McCoy, WI.

Centaurea stoebe seed was sown on May 20 2010, transplanted to conetainers on June 15-17 2010, inoculated on July 2 2010 and August 10 2010, fertilized with a 200 ppm N solution of Scott's Champion on August 6 2010, and harvested on September 9 2010.

Inoculation of the *C. stoebe* experiment followed the same methods as *A. repens*, but included a control with no soil in the DI water and the native vegetation soil was collected from the closest high quality grassland savanna, approximately 3 - 4 miles away. All shoots and roots were harvested separately for each conetainer and roots were hand washed under tap water to remove most soil and inert materials. All biomass was oven-dried for 7 to 10 days at 60 °C.

Seed was germinated and grown in a 1:1:1 by volume soil mixture of Quikrete play sand, Schultz clay conditioner, and Fafard 4P soil mix (45% peat moss, 30% bark, 15% vermiculite, 10% perlite). The soil mixture was autoclaved twice (20 minutes at 121 °C and 17 psi) before germination and transplanting into containers. Seeds were germinated and grown in greenhouses on the Colorado State University campus (Fort Collins, CO USA) with a photoperiod of 16 hours daylight. Ambient light and photoperiod were augmented with 430 watt HID lights. Greenhouse temperatures ranged between 18-24 °C and plants were watered twice daily.

Soil Microbial Communities

Community composition and relative abundances of microbes in soil were determined by the ester-linked extraction method of analyzing fatty acid methyl esters (FAME) and followed the methods of Schutter and Dick (2000). FAME methods are similar to the phospholipid fatty acid (PLFA) method to extract microbial fatty acids from soil, but are simpler, requires less time, and may not extract free fatty acids from the soil (Schutter and Dick 2000). Individual fatty acids were categorized into general microbial functional groups based on the current literature (Vestal and White 1989, Ringelberg et al. 1997, Zogg et al. 1997, Drenovsky et al. 2004, Denef et al. 2009). Fungal to bacterial ratios were developed from the FAME functional groups. Common diversity indices (richness, Shannon's, Simpson's, evenness) were developed from the individual fatty acid data using PC-ord software (PC-ord 5.0 Gleneden Beach OR).

FAMES were extracted from 3 g of soil through a mild alkaline methanolysis process in 0.2M KOH and methanol. Samples were vortexed every 10 minutes while being incubated for 1 hour at 37°C. The alkalinity was adjusted by the addition of 1M acetic acid and FAMES were isolated into an organic phase by the addition of hexane and centrifuging. Following transfer to clean test tubes, an internal carbon standard (19:0) was added to each sample so that gas chromatography (GC) peak areas could be converted to nmoles. Hexane was evaporated from the test tubes using nitrogen gas. Samples were analyzed on a gas chromatograph with a flame ionization detector (FID) at the University of Delaware and the identity of FAMES were characterized using the Sherlock Eukary program by MIDI software (Microbial ID, Inc., Newark, DE). At the University of Delaware FAMES were dissolved in 0.5ml of 1:1 hexane:methyl-*tert* butyl ester and analyzed on an Agilent model 6890 gas chromatograph (Wilmington, Delaware USA) using a Hewlett Packard column (#19091B-102 Ultra 2) of 25 meters length and at a flow rate of 0.8 ml/min.

Microbial Biomass Carbon and Nitrogen

The quantity of microbial biomass carbon and nitrogen in the soil was estimated using the Chloroform Fumigation Extraction (CFE) technique and followed the methods of Horwarth and Paul (1994) and Voroney et al. (2008). The CFE method is based on the difference in C or N between the chloroform fumigated and non-fumigated samples. Following field collection and sieving (2 mm) in the laboratory, soil samples were stored at 4° C for 7-12 days until fumigation and/or extraction was completed. Microbial C and N were extracted from an equivalent of 8 grams dry weight soil with 0.5M K₂SO₄ in a 5:1

(K₂SO₄:soil) ratio and shaken for 1 hour. Solution was filtered using a #1 Whatman filter paper and the extract was stored at -20° C until analysis on a Shimadzu Total Organic Carbon & Total Nitrogen analyzer (model TOC-v cpn with a TNM-1, Columbia, Maryland USA). No correction (Kec) was applied to the final data. Two samples were excluded from the analysis of microbial biomass due to errors in the Total Organic Carbon analysis that produced negative values.

Statistical Analysis

Treatment effects were estimated using one-way analysis of variance (Anova) in JMP statistical software (JMP 8.0.2, Cary, NC). All vegetation data were tested for normality and homogeneity of variance using a Shapiro-Wilks W-test or Levene's F-test, respectively. Non-normal data were transformed using a natural logarithm, square root, or power transformation. Data that could not meet the assumptions of normality and homogeneity were analyzed using a non-parametric Kruskal-Wallis test. When the Levene's F-test documented unequal variance, a Welch's Anova was used to determine treatment effects. All post-hoc comparisons used a Tukey-Kramer Honestly Significant Difference (HSD) test. Where 4 consecutive years of data existed a repeated measures Anova was used to test for differences in treatments (between subjects) and time (within subjects). When fewer than 4 years of data existed, paired T-tests were utilized to compare between years. Data represented in figures and tables are not transformed. PC-ord software was used to calculate plant and soil microbial FAME diversity indices (evenness, Simpson' index of diversity, and Shannon diversity).

Stochastic Gradient Boosting Machines (GBM), also called Generalized Boosting Models, were utilized to determine the multivariate interactions between vegetation (cover, biomass, and density), soil biogeochemistry, soil microbial communities (FAMEs), microbial biomass, and soil inoculation treatments. Stochastic GBMs use least squared regression equations and are based on non-parametric Classification and Regression Trees (CART), but incorporate iterative subsampling and machine learning to train models and select the optimal decision trees. The method was primarily developed by Friedman and is quite robust to “less than clean data” (Friedman 2001, 2002). Preliminary evidence is showing that GBMs perform equally or better than Generalized Linear Models (GLM) and Generalized Additive Models (GAM), and avoids over-fitting complex models (Abeare 2009). Once a GBM has been developed it can be used to make predictions based on new datasets, although I only used GBM to determine the relative influence (%) of multiple explanatory (independent) variables upon a response variable (dependent). Data from the four field locations were combined for the GBM analysis (N=50) and the following model specifications were adjusted to address the small sample size: fraction of data used to train the model (train.fraction) was increased to 0.75, minimum number of samples within a node (n.minobsinnode) was decreased to five, and the number of cross-validations was decreased to three. Gradient Boosting Models were run in R statistical software (The R Foundation for Statistical Computing, R version 2.11.1) and used the GBM package. Six models were created to determine the relative influence of vegetation on total soil FAMEs or soil N, FAME groups’ influence on % cover of native or invasive plants, and soil biogeochemical characteristics’ effect on % cover of native or invasive plants. Diversity indices for individual plant species and fatty

acids (FAMES) were included as explanatory variables in GBM models. Appendix B lists the categories and explanatory variables used in the GBM analysis.

RESULTS

Greenhouse Inoculation Experiments

Greenhouse experiments using a soil slurry to inoculate knapweed species with the soil microbial community from different vegetation types (knapweed invaded, adjacent native vegetation, or ecotone) indicated distinct differences in *C. stoebe* growth between the control and inoculations treatments, although the origin of the inoculum was not significant (Figure 3.1). In the control treatment *C. stoebe* had increased shoot biomass ($F_{3,171}=8.1092$, $P<0.0001$), decreased root biomass ($F_{3,171}=4.6444$, $P=0.0038$), and reduced root:shoot ratios ($F_{3,171}=14.1417$, $P<0.0001$) compared to the inoculation treatments. The *A. repens* greenhouse experiment identified greater root biomass ($F_{2,23}=5.6909$, $P=0.0106$) in the native vegetation inoculum compared to the ecotone or invaded inoculations (Figure 3.1), but did not include a control treatment.

Field Inoculation Experiments – Vegetation Cover

Centaurea stoebe cover at FMC WI was reduced in the native inoculum treatment based on a repeated measures Anova (2006-2009, Treatment $P = 0.0487$, Time $P = 0.0039$) (Figure 3.2 panel 1), but no differences were found for the secondary invasive species (*Euphorbia esula*) at the FMC site (2006-2009, Treatment $P = 0.5469$, Time $P = 0.1201$). At YTC WA the percent cover of *A. repens* (2006-2009, Treatment $P = 0.6739$, Time $P = 0.0567$) and *B. tectorum* (2006-2009, Treatment $P = 0.4018$, Time $P < 0.001$)

did not vary by inoculation treatment, although knapweed cover decreased over time while *B. tectorum* cover increased. The inoculation treatment did not affect *A. repens* cover at the Waverly CO site in 2008 or 2009 ($F_{2,14}=0.1641$, $P=0.8505$ and $F_{2,14}=0.3571$, $P=0.7069$ respectively) (Figure 3.2 panel 3). Russian knapweed cover at Weld County CO was also unaffected by soil inoculation treatments in 2008 and 2009 ($F_{2,14}=2.3138$, $P=0.1413$ and $F_{2,14}=1.8456$, $P=0.2001$ respectively) (Figure 3.2 panel 4).

Repeated measures Anova were used to determine soil inoculation treatment and time effects on the percent native species cover at FMC WI and YTC WA sites (2006-2009). At FMC WI neither treatment (between subjects $F_{1,8}=1.3097$, $P=0.2855$) or time (within subjects $F_{3,6}=1.0349$, $P=0.4419$) were significant (Figure 3.3 panel 1). Native species cover changed over time at YTC WA (within subjects $F_{3,6}=7.2068$, $P=0.0205$), but inoculation had no effect (between subjects $F_{1,8}=0.4841$, $P=0.5063$) (Figure 3.3 panel 2). No differences among the three soil inoculation treatments were found at either of the Colorado sites for 2008 or 2009 (Figure 3.3 panels 3 and 4). Total native species cover did increase from 2008 to 2009 at the Weld County CO site (paired t-test: $t=8.35$, $DF=14$, $P<0.0001$).

Field Inoculation Experiments – Invasive Species Density

Both t-tests and repeated measures Anovas were utilized for analysis of FMC WI and YTC WA density measurements. *Centaurea stoebe* produces vegetative rosettes and flowering stems (bolts) that are distinct, therefore the two were documented separately during density measurements. Fewer rosettes of *C. stoebe* existed in the native soil inoculation, but only in 2008 ($F_{1,9}=3.67$, $P=0.0917$) (Table 3.1). Additionally, the

repeated measures Anova of *C. stoebe* rosettes (2006-2009) was marginally significant for treatment (between subjects $F_{1,8}=4.2509$, $P=0.0732$) and highly significant for time (within subjects $F_{3,6}=29.8280$, $P=0.0005$). Fewer bolting stems of *C. stoebe* were found in the native soil inoculation treatment in 2007 (Welch's Anova $F_{1,5.9509}=6.6.031$, $P=0.0427$) and 2008 (Welch's Anova $F_{1,7.193}=7.4094$, $P=0.0289$). A repeated measures Anova for 2006-2009 period was significant for both treatment (between subjects $F_{1,8}=12.6631$, $P=0.0074$) and time (within subjects $F_{3,6}=28.1397$, $P=0.0006$). Inoculation treatments did not affect *A. repens* density at the YTC WA site based on annual t-tests or a repeated measures Anova (Table 3.1), although stem density rose over time based on the repeated measures Anova (within subjects $F_{3,6}=7.4020$, $P=0.0193$). Density of *A. repens* at the Waverly CO site did not differ statistically in 2008 or 2009 based on soil inoculation treatments (Table 3.1). Inoculation treatment was marginally significant for *A. repens* density in 2009 at the Weld County CO site ($F_{2,14}=3.3715$, $P=0.0549$) with the control showing lower density than the sterile inoculation treatment, but not the native treatment.

Soil Microbial Community (FAME) Results

The four sites varied greatly in the total quantity (nmoles/g dry soil) of microbial fatty acids detected by the FAME extraction. Waverly CO consistently had the most fatty acids of the four sites and FMC WI the least (Figure 3.4). General trends between inoculation treatments within a site were consistent across most microbial groups. The major differences in treatments occurred between the control and soil addition inocula, few large differences were seen between the native or sterile inoculations. At FMC WI

(*C. stoebe* dominated) the sterile inoculation had more microorganisms than the native treatment in all microbial groups, except for saprophytic fungi. Conversely, YTC WA (*A. repens* dominated) had more microbes in the native treatment for all groups except AM fungi. At Waverly CO the control treatment usually had fewer microbes than the native or sterile inoculation treatments, especially in the total FAMEs, cyanobacteria and actinobacteria, Firmicutes, and saprophytic fungi groups. The native inoculation treatment was lower than the control and sterile treatments for most of the microbial functional groups at the Weld County CO site. Low amounts of fungi and protozoa were documented in all of the sites, except Waverly CO. Generally, there were relatively large amounts of AM fungi, saprophytic fungi, and Firmicutes compared to the other microbial groups.

Stochastic Gradient Boosting Model (GBM) results

In general the soil inoculation treatments were weak predictors of vegetation cover, soil N, or the relative abundance of microbial groups (FAMEs) (Figure 3.5). Rarely did an inoculation treatment have greater than 5% of the total influence upon a response variable. Panel 1 of Figure 3.5 illustrates the strong and positive effects of native plant biomass and exotic species cover on the total amount of soil fatty acids (FAMEs). Subsequently, the Shannon diversity index for vegetation was also a good predictor of FAMEs and it is probably positively influenced by the greater values of native species biomass and exotic species cover. The total amount of plant biomass was the overwhelming predictor of soil N and the origin or nativity of plant species was irrelevant (panel 2). Arbuscular mycorrhizal fungi (AMF) and gram-negative bacteria

were powerful predictors of knapweed cover (panel 3), while no one microbial group was an overwhelming predictor of native species cover, although actinomycetes were the strongest (panel 4). Soil N and C:N ratios were by far the strongest predictors of knapweed cover (panel 5), although the two variables were weak indicators of native plant cover (panel 6). Native cover was best predicted by Mg and K. When soil variables were used to predict knapweed or native species cover (panels 5 and 6), very different variables were good predictors between the two types of vegetation, suggesting that the inherent differences based on plant origin or invasiveness also respond to different nutrients. Additionally, the vegetation variables that were good predictors of FAMEs were not good predictors of soil N and vice versa. This potentially highlights the specificity of microbes that are directly involved in the processing and availability of nitrogen, and that the total amount of FAMEs is not directly relevant to addressing the connection between microorganisms and soil nitrogen.

Microbial Biomass Carbon and Nitrogen

Microbial biomass carbon (C) varied significantly at three of the four study sites (Figure 3.6). At the *C. stoebe* infestation in Fort McCoy WI ($F_{1,9}=3.6439$, $P=0.0927$) and *A. repens* infestation in Weld County CO ($F_{2,14}=3.2424$, $P=0.0749$) the soil microbial biomass C was greater when inoculated with a sterilized portion of soil from an adjacent native plant community. The second *A. repens* site in CO (Waverly) had an opposite trend in microbial biomass C with the sterilized inoculation treatment significantly less than the native or control treatments ($F_{2,13}=6.7540$, $P=0.0122$). Yakima Training Center WA is also an *A. repens* site, but no statistical differences were found between

inoculation treatments. Inoculation affected microbial biomass nitrogen (N) at the Fort McCoy WI ($F_{1,9}=6.2843$, $P=0.0366$), Yakima Training Center WA ($F_{1,9}=6.4125$, $P=0.0351$), and Weld County CO ($F_{2,14}=3.8898$, $P=0.0499$) sites (Figure 3.6). The Washington and Weld County CO sites responded to the inoculation treatments in an opposite manner. No statistical differences were found in the analysis of microbial biomass C:N ratios at any of the study sites (Figure 3.6).

Soil Biogeochemistry

Table 3.1 lists mean values for selected soil nutrients and chemical characteristics for each of the four study sites. The soils from the three *A. repens* study sites (WA and CO) had few or no statistical differences in nutrient or chemical characteristics between the inoculation treatments. Zinc levels (ppm) varied significantly between inoculation treatments at the WA and Weld County CO sites, although the two sites had opposing responses (native inoculation treatment had lower zinc in CO and higher zinc in WA). Soil inoculations did not appear to modify soil nutrients, and subsequently plant growth, since the native and sterile treatments rarely differed from the controls at the CO sites. At the *C. stoebe* site in Wisconsin, seven of the nine soil characteristics measured varied statistically between inoculation treatments and in each of the significant tests the sterile treatment had higher nutrient or chemical values than the native plant inoculation.

DISCUSSION

Ecosystems are dynamic complexes with a myriad of nearly undetectable interconnections between plants, microorganisms, and soil biogeochemistry. The

incredible diversity and variability of scales in these systems has made understanding the connections difficult and the soil medium is often considered a “black box” in research and management endeavors (Kulmatiski 2011). A primary goal of research on plant-soil feedbacks is to begin untangling these interconnections to improve our understanding of successional dynamics in a plant community, elucidate the “Invasive Plant Paradox” and find methods to manage ecosystems that incorporate ecological principles and plant-soil feedbacks. This requires the integration of above and below ground organisms and abiotic cycles (Wardle et al. 2004). The complexity of plant-soil feedbacks makes it difficult to utilize traditional concepts of ecological management (Sheley et al. 1996, Krueger-Mangold et al. 2006) to manage invasive plant communities through the manipulation of soil and microorganisms. As additional research develops and microbial techniques improve it may be possible to incorporate soil microorganism systems into management practices, especially in the restoration of native plant communities. The primary goal of this study was to determine the effects of soil inoculation on the restoration of native plants in areas dominated by invasive knapweeds. Through the incorporation of multiple vegetation measurements, soil biogeochemical characteristics, and quantification of microbial community function and diversity, I attempted to untangle a complex system and begin to elucidate ecologically based management methods that are focused on the interconnectedness of these ecosystems.

The results of previous inoculation experiments have had mixed results. Frequently, field and greenhouse based inoculation treatments have been shown to have little or no effect on the system or are overshadowed by other treatments (such as mowing) (Antonsen and Olsson 2005, Russo 2006). Conversely, some experiments with

invasive plants and soil inoculations have had positive results. Rowe et al. (2007) found that native, whole soil inocula improved growth of late seral native species, while decreasing the growth of early seral species. A study by Meiman et al. (2006) found that *Centaurea diffusa* responded negatively to native soil communities when grown in a greenhouse with field-collected soils. The origin of inoculum may be critical, as shown by Williams et al. (2011) in their study documenting the positive effects of forest AM fungi on a native forest species, compared to the negative impact of AM fungi from an agricultural field. The forest AM fungi also improved the native tree's growth when competing with an invasive grass. Mycorrhizal fungi are often highlighted in inoculation experiments, but any whole soil inoculum experiment must consider a much broader suite of microorganisms, including pathogens, Plant Growth Promoting Rhizobacteria (PGPR), and mutualistic species. Revegetation of *Acacia* spp. in dry-land Australian ecosystems had improved survivorship and growth when inoculated with specific bacterial isolates (Thrall et al. 2005). The origin of soil inocula or the selection of specific microbial species will be critical to using inoculation to modify targeted positive or negative interactions with plant species, although this also depends upon detailed known of plant-microbe interactions. Additionally, it is important to acknowledge that not all inoculations have beneficial effects and that the potential to introduce invasive and potentially damaging microorganisms must be considered (Schwartz et al. 2006).

These experiments show that soil inoculation can affect the vegetation, soil nutrients, and microbial communities, although the results of inoculation were variable and highly species and site specific. In a greenhouse experiment, all soil inoculation treatments suppressed shoot growth and increased root biomass of *C. stoebe* compared to

the control (Figure 3.1). *Acroptilon repens* increased root biomass when inoculated with native soil compared to inoculum from the ecotone or *A. repens* community (Figure 3.1). These results highlight that inoculation is affecting plant growth, but also that the origin (vegetation type) of the inoculum was only important for *A. repens* while *C. stoebe* was impacted by the presence or absence of microbial inoculation. The strongest effects of inoculation in my field experiments were seen on the vegetation and soil nutrients of the one *C. stoebe* site in Wisconsin (Figures 3.2 and 3.3). The three *A. repens* sites had weaker and more variable results. In the context of restoring knapweed-infested communities to native plants, the inoculation treatments primarily influenced the knapweeds and not the native species that were sown in conjunction with the inoculum (Figures 3.2 and 3.3). Even though all study sites were rototilled prior to seeding and inoculation, the existing seed banks (primarily *C. stoebe*) and numerous rhizome fragments (exclusively *A. repens*) present a greater amount of active biomass than the germinating seedlings of native species for the interaction with introduced microbes, especially if germination of native or invasive plants were negatively affected by the knapweeds' allelopathic compounds (Perry et al. 2005).

Significantly greater soil nutrient levels within the sterilized soil inoculation treatment were documented at the *C. stoebe* site in Wisconsin (Table 3.2), as were significant treatment differences in Microbial Biomass Carbon (Figure 3.6). The sterilization process (heat) may alter soil nutrients or availability, although few differences between treatments were seen in the biogeochemistry of the three remaining sites. Additionally, the control treatments in the Colorado sites rarely differed from the native and sterile inoculation treatments. This diminishes the possibility that the soil

inoculum is adding nutrients and may support the concept that the sterilization process modifies the chemical or physical structure of the soil inoculum and this could alter subsequent microbial activity. Additional research should be conducted on the effects of sterilization on soil organic matter, nutrients, physical structure, and microbial recolonization.

When inoculation treatments are considered within a broader perspective through the inclusion of numerous soil, microbial, or vegetation characteristics, the impact of inoculation treatments was minimal (usually < 7% of total relative influence based on stochastic GBMs). Figure 3.5 illustrates the limited influence of treatments when addressed in a multivariate framework. The GBM analysis also highlights the potential use of inoculation treatments to target specific microbial functional groups that are highly related to invasive or native plants, although additional research is required due to the variability of responses and relatively small sample sizes. The targeted use of specific microbes for use as pathogens or PGPR may provide better results, because it removes the confusion surrounding the microbial contents of a whole soil inoculum. This method also raises costs and demands a very specific understanding of the plant-soil feedbacks of specific plant and microbe species. Successful revegetation using specific inoculants (Thrall et al. 2005) often includes symbiotic nitrogen fixing bacteria and leguminous species because the interactions are well understood. Additional broad scale research with soil inoculation should be used to determine the microbial and biogeochemical drivers of specific desirable or unwanted plant species, followed by fine scale experimentation to develop inoculations that utilize specific microbes to target specific ecological processes. The practicality of this type of vegetation management depends

heavily on effectiveness of potential treatments and the costs associated with developing and implementing microbial based ecosystem management.

GBM models illustrated broad differences in the soil nutrients and microbial functional groups that most strongly affected knapweeds or native species (Figure 3.5 panels 3-6). Although the experiments are based on the limited sample size, reducing the amounts of AM fungi and Proteobacteria could decrease the cover of invasive knapweeds (Figure 3.5 panel 3), while increasing the amount of Actinobacteria may promote native plant cover (panel 4). The diversity and density of AM fungi may be important to consider in management practices since research has linked the richness of plant and AM fungal communities (van der Heijden et al. 1998). In respect to AM fungi, my results differ from Klein et al. (2006) conclusions that show an inverse relationship between filamentous fungi and invasive plant biomass, although knapweed species differed between the studies. An additional study links the diversity of AM Fungi to the stability and maintenance of plant biodiversity (van der Heijden et al. 1998). My research did not specifically consider the diversity of AM fungi, but it may be important to separate quantity of a microbial group from diversity in future studies. From the soil nutrient perspective of my studies, decreasing soil N while adding Mg and K may reduce invasives and increase natives, respectively (panels 5 and 6). Assuming that the two knapweeds are not late seral species, these results support existing research concerning higher N levels correlating with the extended presence of earlier seral, weedy species compared to perennial plants that occur in later successional dynamics of short-grass steppe systems (Paschke et al. 2000). The GBM analysis also shows that increasing total plant biomass increases soil N and amounts of fatty acids (FAMES). Although additional

research is required to develop management programs, these initial results show that specific manipulations of the microbial community through inoculation, reduction of soil N, increasing soil salts, and decreasing the amount and diversity of vegetation may increase native plant cover in knapweed infestations undergoing restoration. This type of information is critical to developing low cost and ecologically-based management practices that minimize disturbance and can be implemented on larger scales, although the effectiveness and costs of inoculation at larger scales are still unknown.

Management programs that are based on ecological principles and the interconnectedness of the above and below ground components of ecosystems hold great potential to revolutionize restoration of native plant communities through the subtle manipulation of multiple ecosystem processes. However, this type of management also demands a paradigm shift, as it assumes that we will never eradicate an invasive species and we will need to accept a limited role of exotic species in managed natural areas. If this type of microbial-based management is shown to be feasible, both in application and economics, it may reduce the use of traditional top-down approaches of weed control that are based on eradicating unwanted species and replacing them with desirable plants. Eradicated of invasive species from a novel region has shown to be difficult and the process creates additional disturbances that can promote invasive by additional unwanted species. In a globalized world with enormous vectors for propagule movement and a changing climatic envelope, the management of an exotic species as a component of a diverse and resilient semi-natural system may be the most sustainable management option.

Ecosystems are complex and multi-disciplinary studies are needed to understand plant-soil feedbacks and develop management practices that can exploit these interactions. My unique study incorporates vegetation, soil biogeochemistry, and microbial diversity within experimental soil inoculation treatments. The results provide initial evidence for potential ecological management that incorporates inoculation, nutrients, and biomass modification into resource management, but the applicability of the results are limited due to the small sample sizes and limited replication. These preliminary studies show that soil inoculation can affect many attributes of a system, including vegetation, nutrients, and microbes, although additional research is necessary to understand the species and site specific interactions and apply these methods at larger scales.

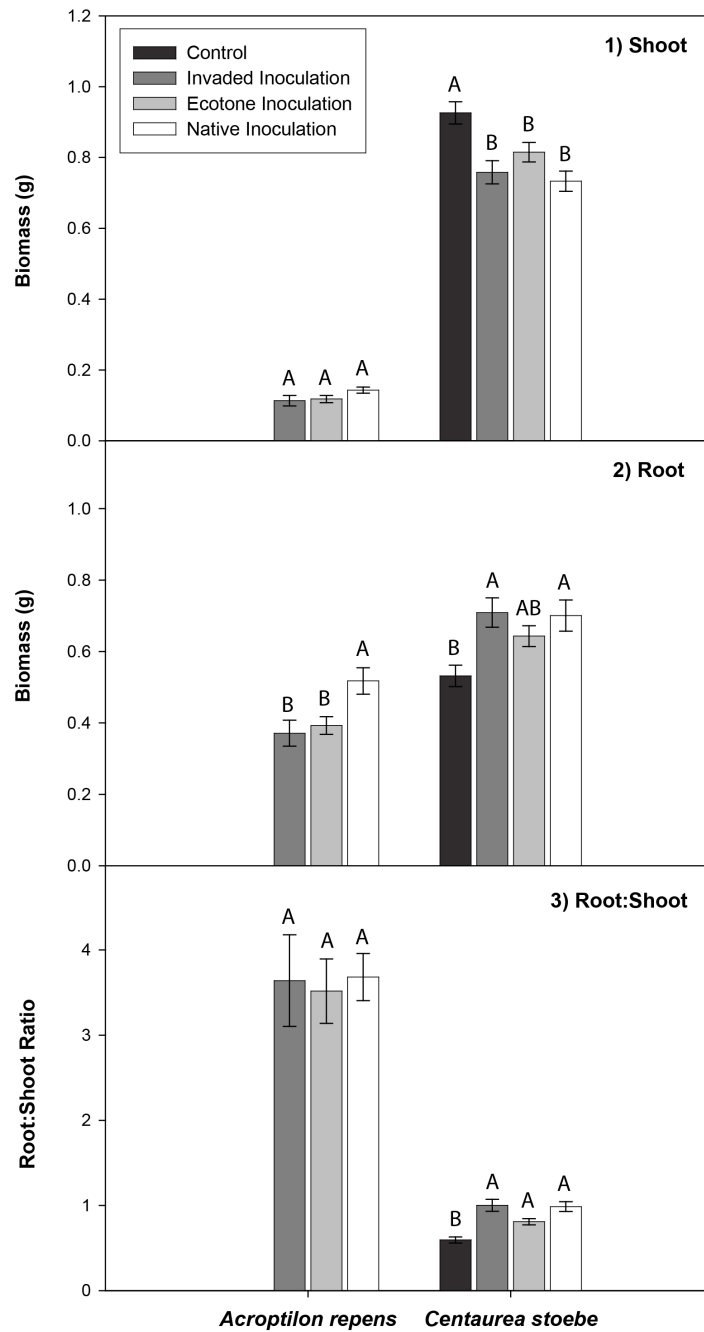


Figure 3.1 – Two separate greenhouse experiments studied the effect of inoculating *Acroptilon repens* (n=24) or *Centaurea stoebe* (n=172) with a soil slurry from different vegetation communities (knapweed infestation, adjacent native plant community, ecotone between the two plant communities, or a control). Different letters within a species denotes that treatment means differed at $\alpha = 0.05$. Errors bars represent ± 1 standard error. Only the *C. stoebe* experiment included a control treatment.

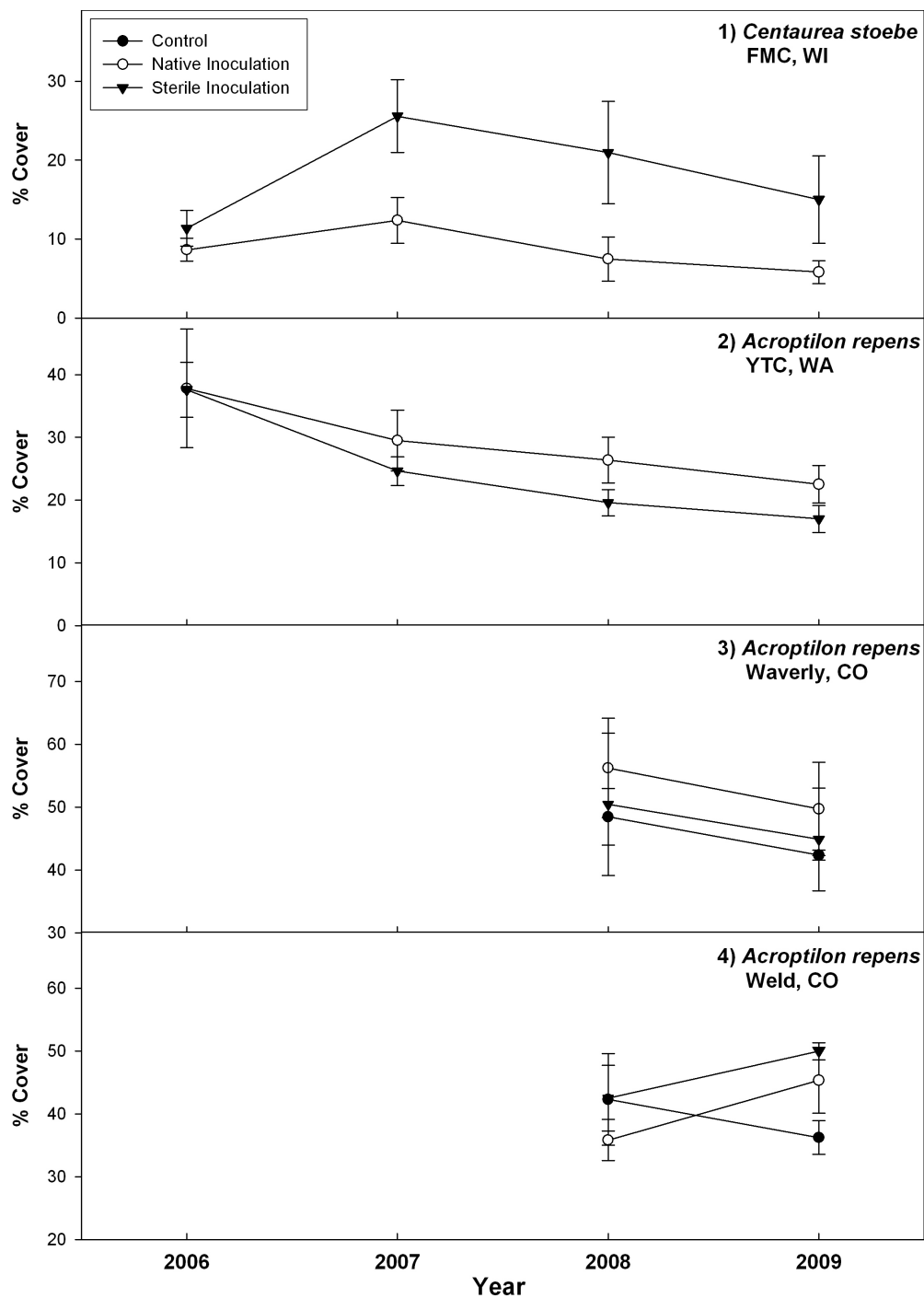


Figure 3.2 – Percent invasive knapweed cover at each of four sites (± 1 SE). Treatments consisted of inoculation with fresh, wild collected soil from an adjacent native plant community, a sterilized portion of the native soil, or a control treatment at the Colorado sites. Colorado sites only have data from 2008 to 2009. Note different % cover scales for each site.

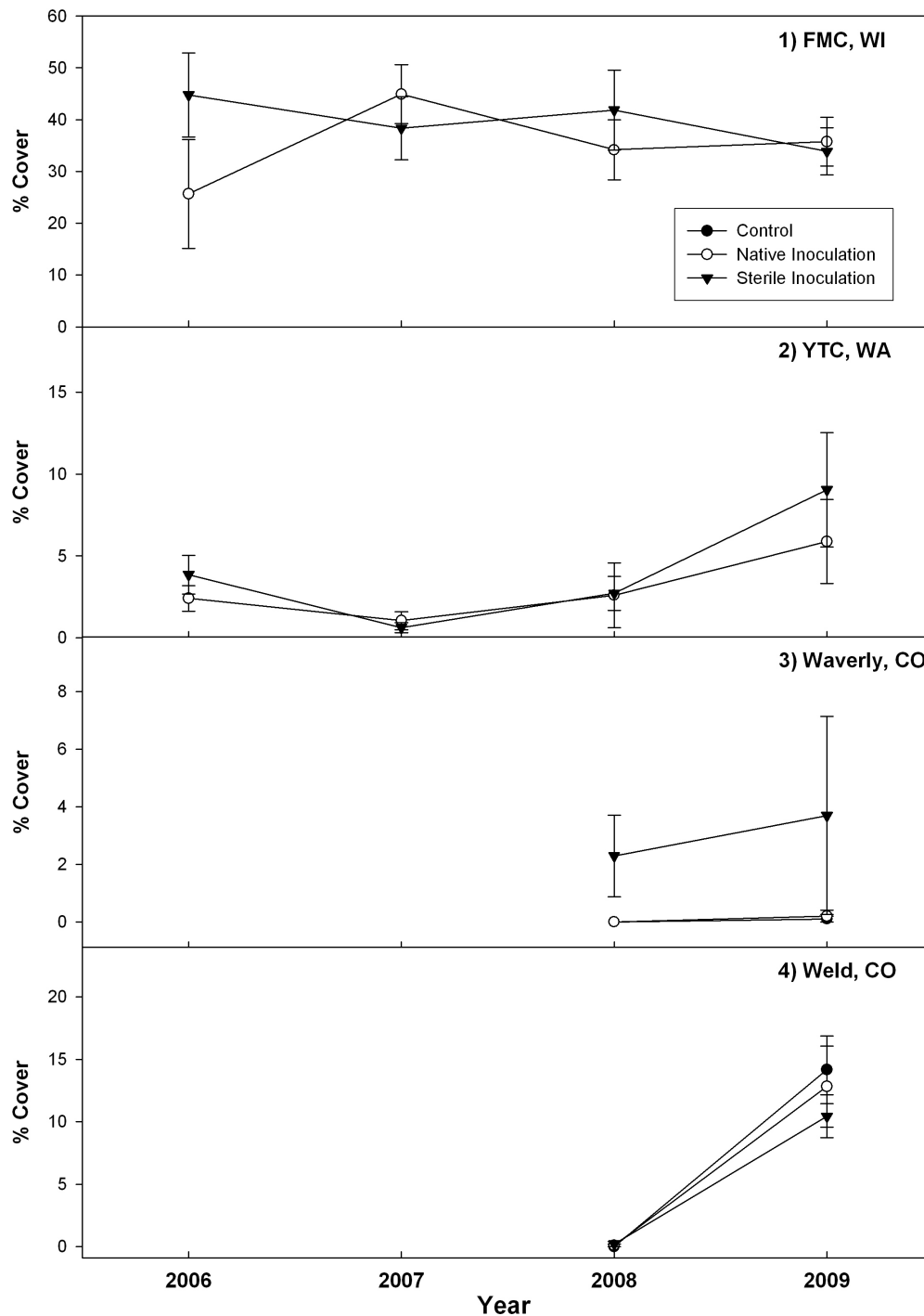


Figure 3.3 – Percent native species cover at each of four sites from 2006 to 2009 (± 1 SE). Treatments consisted of inoculation with fresh, wild collected soil from an adjacent native plant community, or a sterilized portion of the native soil, or a control treatment at the Colorado sites. Colorado sites only have data from 2008 to 2009. Note different % cover scales for each site.

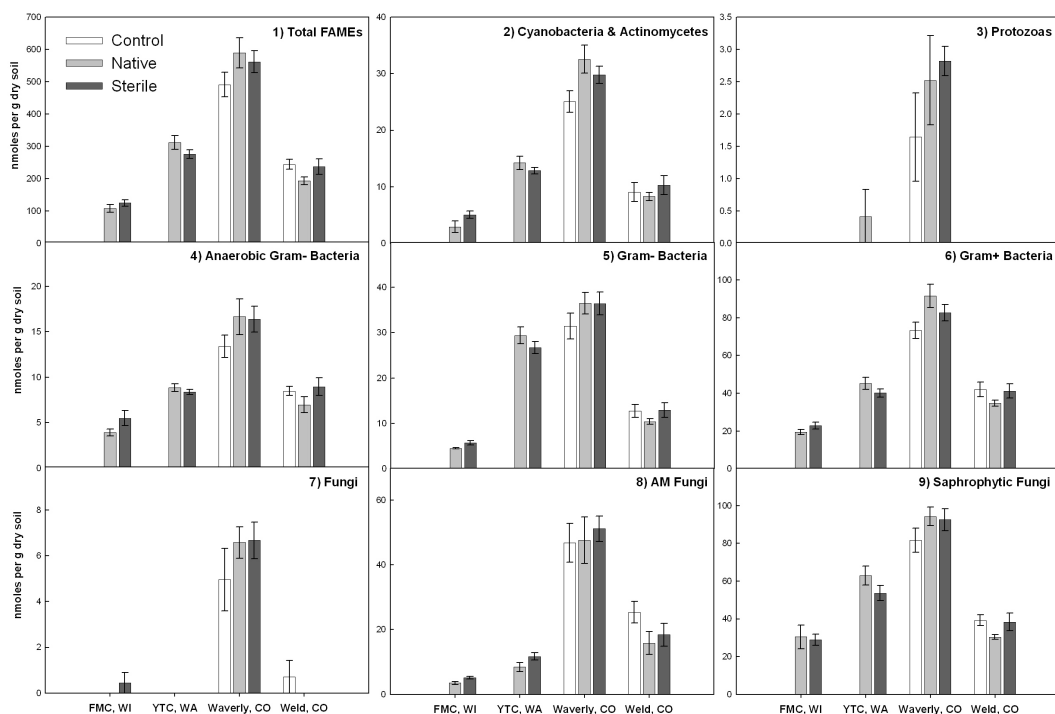


Figure 3.4 – Soil microbial classification based on microorganism functional groups (Fatty Acid Methyl Ester (FAME) extraction - nmoles g^{-1} dry soil) from composite soil samples of four study sites (Fort McCoy WI (n=10), Yakima Training Center WA (n=10), Waverly CO (n=15), and Weld County CO (n=15)). The soil inoculation treatments are represented by different color histogram bars (control, native vegetation inoculum, sterilized portion of native vegetation inoculum). Error bars represent ± 1 standard error. Note different scale for each graph.

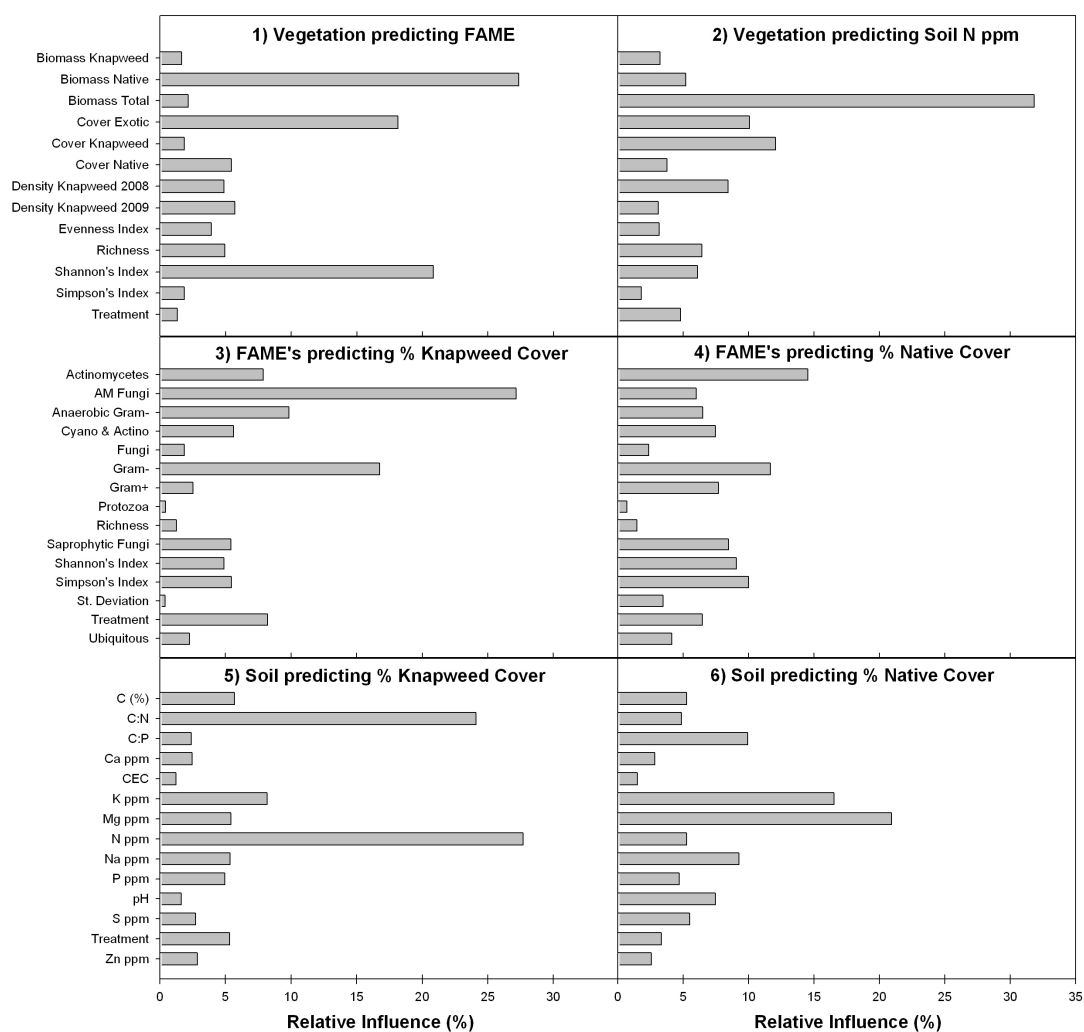


Figure 3.5 – The relative influence (%) of explanatory variables upon a response variable (native or invasive plant cover, soil nitrogen (N), or soil microbial community (total quantity of FAMES)) were determined using Stochastic Gradient Boosting Machines (GBM). Stochastic GBM's utilized least square regression and iterative machine learning regression tree models to predict the influence of multiple variables on a response. Soil inoculation treatments (control, native inoculum, or sterilized inoculum) are included in each model.

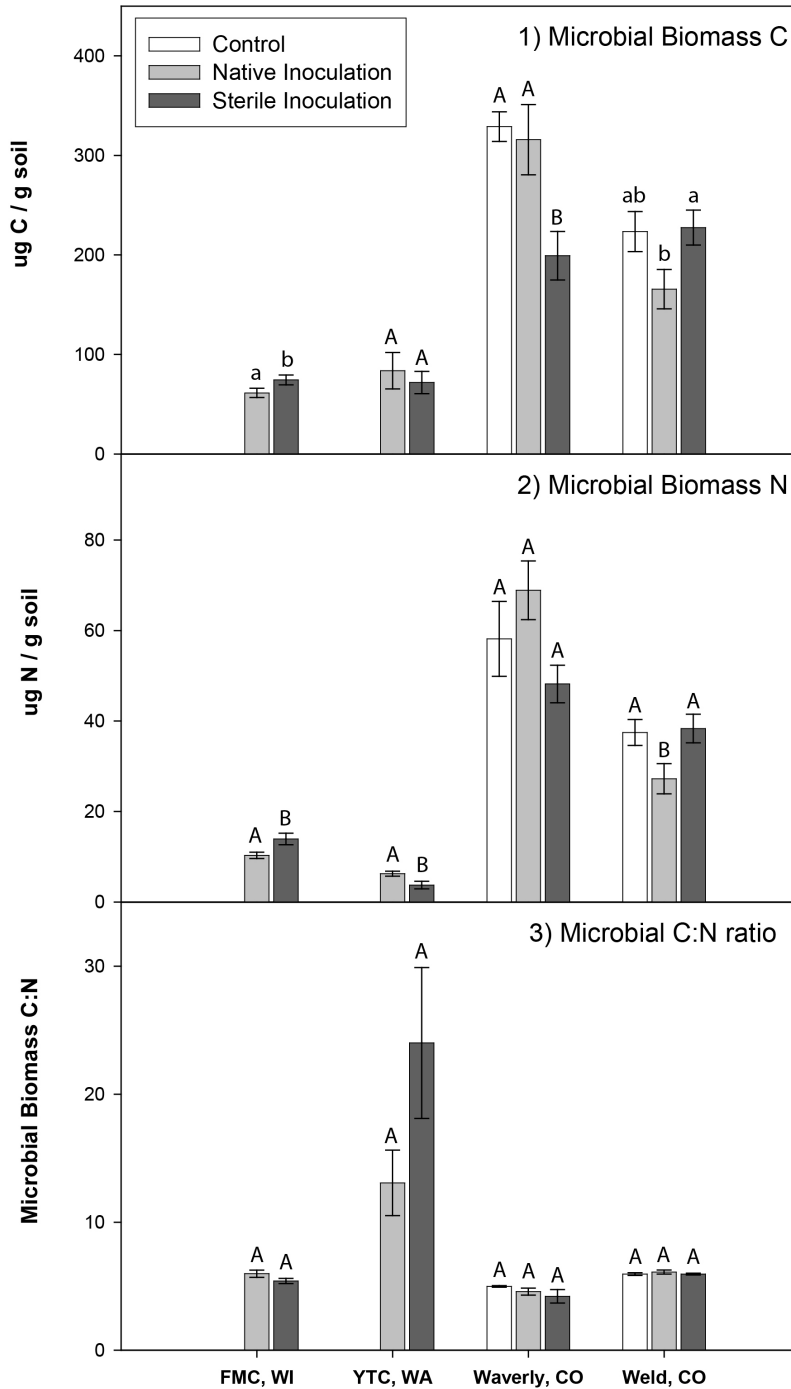


Figure 3.6 – Microbial biomass of composite soil samples from four study sites (Fort McCoy WI (n=10), Yakima Training Center WA (n=10), Waverly CO (n=15), and Weld County CO (n=15)) and three soil inoculation treatments (control, native vegetation inoculum, sterilized portion of native vegetation inoculum). Different letters signify that inoculation treatment means varied statistically within a site (upper case letters $\alpha < 0.05$, lower case letter $\alpha < 0.1$). Error bars represent ± 1 standard error.

Table 3.1 – Mean (± 1 SE) flowering stem (bolts) or rosette density (per m²) of invasive knapweeds at each of four study sites. Statistical significance between soil inoculation treatments within a year were determined using pooled t-tests and treatment means that differed significantly are marked as follows: * ($\alpha \leq 0.1$) or ** ($\alpha \leq 0.05$).

Site	Species	Plant Type	Year			
			2006	2007	2008	2009
FMC, WI	<i>C. stoebe</i>	Rosettes	30.27 (5.10)	4.40 (0.80)	6.4 (1.21)*	7.87 (2.44)
FMC, WI	<i>C. stoebe</i>	Bolts	2.27 (0.69)	26.0 (4.28)**	18.53 (3.39)**	10.8 (3.11)
YTC, WA	<i>A. repens</i>	Bolts	23.07 (3.70)	29.20 (5.20)	27.47 (3.58)	26.13 (4.97)
Waverly, CO	<i>A. repens</i>	Bolts	-	-	99.64 (10.40)	66.13 (7.98)
Weld, CO	<i>A. repens</i>	Bolts	-	-	84.44 (5.98)	48.09 (6.01)*

Table 3.2 – Mean (± 1 SE) of select soil characteristics for field based soil inoculation experiments at Fort McCoy WI (n=10), Yakima Training Center WA (n=10), Waverly CO (n=15) and Weld County CO (n=15). Studies sites were inoculated with soils from an adjacent native plant community, sterilized soil from adjacent native plant community, or a control (CO sites only). Type of statistical test and level of significance are as follows when means differed significantly between inoculation treatments: parametric one-way Anova (* $\alpha < 0.1$, ** $\alpha < 0.05$, *** $\alpha < 0.01$) or non-parametric Kruskal Wallis one-way Anova ($\delta \alpha < 0.1$, $\delta\delta \alpha < 0.05$, $\delta\delta\delta \alpha < 0.01$).

	Sites			
	FMC, WI	YTC, WA	Waverly, CO	Weld, CO
N ppm	10.70 (0.75)*	2.10 (0.18)	31.60 (4.30)	57.13 (6.83)
P ppm	43.18 (1.97)	46.51 (2.61)	6.93 (0.76)	49.78 (3.75)
%OM	1.81 (0.08)**	2.86 (0.16)	3.47 (0.07)	2.97 (0.70)
pH	5.19 (0.07)	6.69 (0.05)	7.69 (0.03)	6.54 (0.12)
CEC	5.06 (0.28)*	16.07 (0.54)	31.36 (0.30)	12.65 (0.32)
Zn ppm	2.71 (0.20)*	0.63 (0.10)**	0.30 (0.04)	0.45 (0.04) δ
K ppm	48.80 (3.65)*	681.30 (35.91)	955.40 (28.41)	592.60 (18.71)
Mg ppm	73.00 (2.55)*	352.00 (18.47)***	548.73 (29.96)	225.13 (4.90)
Ca ppm	388.80 (21.95)**	2213.20 (92.79)	4849.47 (59.79)	1691.27 (62.77)

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Chapter 4 – Unexpected plant-soil feedbacks: Invasive plant (*Acroptilon repens*) impacts on soil and litter microbial communities and decomposition

INTRODUCTION

Plant-soil interactions are complex and obscured from ready observation, yet they may be important in the numerous direct and indirect interactions that affect ecosystem dynamics and invasion. Abiotic factors directly affect plant community composition at large scales due to spatial and temporal variations in climate and soils, but at an ecosystem level many factors can influence the species richness, diversity and successional dynamics of a plant community. In-depth analysis of ecological interactions has provided quantitative information about how plants, animals, soils and the atmosphere interact and ecosystems are modified, but factors such as competition, dispersal and migration, resource use, and predation do not completely explain the diversity and succession of plant communities (sensu Klironomos 2002). Recent experimentation has begun to investigate how soil, microorganisms and plants interact to form feedback systems that can positively or negatively influence species (van der Putten et al. 1993, Bever 1994, Bever et al. 1997, Klironomos 2002, Bever 2003, Reynolds et al. 2003, Kardol et al. 2006, Kardol et al. 2007) and directly alter the richness or diversity (De Deyn et al. 2003), abundance (Klironomos 2002), primary succession (van der Putten et al. 1993) or secondary succession (De Deyn et al. 2003) of a plant community. Several

studies have highlighted the importance of plant-soil interactions on the invasion by exotic species (van der Putten et al. 1993, Callaway and Aschehoug 2000, Reinhart et al. 2003, Wolfe and Klironomos 2005, Reinhart and Callaway 2006, Eppstein and Molofsky 2007, van der Putten et al. 2007, Mangla et al. 2008) and a greater understanding of these feedback systems may aid in the development of management and control strategies. The complexity of the soil system has made it difficult to understand the seemingly infinite feedbacks or determine the importance of these interactions in plant community dynamics and ultimately our ability to successfully manage semi-natural and natural ecosystems.

Plants directly affect nutrient cycling, edaphic characteristics, soil fauna, and microorganism communities of an ecosystem by the quantity and quality of litter they release (Wardle et al. 2004, Georgieva et al. 2005, Chapman et al. 2006). Wardle et al. (2004) proposed the concept of plants as the integrator of above- and below-ground feedbacks, but emphasized the difficulties in understanding the mechanisms due to the complexity of organisms and environments involved. Based on the increased net primary productivity (NPP) of invasive species (Ehrenfeld 2003), litter quality and quantity represent a logical starting point for studies of plant-soil feedback (PSF) and plant invasions. Different species have markedly divergent litter quality and these physical characteristics modify their nutrient composition, decomposition rates, and potentially the biogeochemistry and microbial diversity of a system. In the context of invasive plants and potential plant-soil feedbacks, changes in the quality and quantity of litter within a system may represent a critical tipping point for an invasive species to create a positive feedback system that promotes its dominance or cause a reduction in negative feedbacks

that results from strong competition for resources. The decomposition environment can be dramatically different between invaded and non-invaded systems. Studies have shown in the eastern hardwood forests of the US that the litter of invasive species decomposes more rapidly than native species litter and invaded ecosystems decompose litter faster regardless of the litter's origin (Ashton et al. 2005). Another study did not determine differences in decomposition between native and invaded sites, but found that the loss rates of phosphorus, lignin and trace elements from litterbags was reduced in invaded sites (Pritekel et al. 2006) and therefore the nutrients remained in the invaded system longer.

Additional research has documented higher nutrient levels in invaded habitats of Europe, presumably due to increased NPP of the invasive species (Vanderhoeven et al. 2005, Dassonville et al. 2007). If invaded ecosystems generally have higher NPPs, consist of litter commonly with higher N content (Vitousek et al. 1987) which decomposes more rapidly (Melillo et al. 1982), most of the requirements necessary to establish a positive feedback system that promotes the “Fertile, Productive Ecosystems” of Wardle et al. (2004) or the “Fast Cycle” of Moore et al. (2007) are in place and may partially explain invasive species' ability to dominate an ecosystem. The Moore et al. (2007) model addresses energy channels in a soil food web and how changes in the energy flow reduce stability of the system. Pathways dominated by labile, high quality litter promote a “fast cycle” that is dominated by bacteria, bacterivores, reduced C:N ratios, and increased NPP and nitrogen mineralization. Conversely, the recalcitrant, low quality litter pathway is slow and dominated by fungi, fungivores, higher C:N ratios, and decreased NPP and N mineralization. The concept that an individual species can alter N

cycling and create a self-perpetuating positive feedback system due to its litter quality and quantity has been addressed, although more recent research aids our attempts to understand the mechanisms facilitating the feedback processes by incorporating the analysis of soil microorganisms (Hawkes et al. 2005, Hawkes et al. 2006), fauna (De Deyn et al. 2003), and edaphic characteristics (Goslee et al. 2003, Grant et al. 2003) into the complex system of plant-soil feedbacks.

An idealized and theoretical example of invasive species affecting vegetation dynamics is the invasion of a native, late seral plant community associated with recalcitrant litter and a fungal dominated microbial community (Moore et al. (2005 and 2007) slow energy cycle) by an invasive plant with labile litter and greater quantities of litter and root exudates. Invasion could change the soil community away from fungal/fungivore dominance, increase NPP and N-mineralization; therefore promoting species with higher N requirements and greater productivity (i.e. near monoculture of invasive species or species with early seral traits). This shift from the “slow cycle” to “fast cycle” could be predicated upon the change in litter quality by the invading species and be amplified by modifications to the soil microbial and faunal communities. This theoretical example is also based on Harris’ (2009) concept of vegetation succession towards late seral plant communities having higher fungal:bacterial ratios (F:B). Based on this example, invasive species would represent early seral plant communities consisting of r-selected species with low F:B ratios and bacterial dominance of the soil foodweb. The classification of invasive species as r- or k-selected species may oversimplify the complexity of ecosystem dynamics, especially when considering a

species like *Acroptilon repens* (L.) DC (Russian knapweed) which behaves as both an r- and k-selected species.

To study the effects of the invasive *Acroptilon repens* on decomposition and the microbial community, I quantified the decomposition of plant litter from three species along a gradient from the inside of an *A. repens* population to the surrounding native vegetation. The three litter types (wood, knapweed, alfalfa) represent a range of litter qualities along a C:N gradient (wood>knapweed>alfalfa, high to low C:N respectively), where the litter with the lowest C:N ratio or highest quality (Alfalfa) is expected to have greater decomposition and wood (highest C:N) would have the lowest amount of decomposition (Melillo 1982). The different litter qualities and locations along the vegetation gradient provides a framework to understand differences in decomposition due to invasion by Russian knapweed and subsequent modifications in the microbial communities of both the litter and soil. To investigate the effects of Russian knapweed invasion on litter decomposition and potential plant-soil feedbacks caused by an invasive species, I hypothesized that decomposition rates would be greater inside or on the edge of the noxious weed infestation compared to the adjacent native plant community. To further our understanding of how an invasive species modifies plant-soil feedbacks, I predicted that an interaction between litter type and vegetation community would be manifested through an increase in the rate of *A. repens* litter decomposition inside *A. repens* populations above the increase of the other litter types along the vegetation gradient. Within the litter and soil microbial communities I expected to see higher fungal:bacterial ratios in the native vegetation, since they represent an intact late seral

plant community. I also hypothesized that microbial diversity indices would be greatest in the ecotone, or interface, between the native and invaded plant communities.

Lastly, I attempted to understand the effects of invasion by Russian knapweed on the plant-soil feedbacks of a system in the context of Moore et al. (2005, 2007) with fast and slow energy channels and expand this model to include vegetation dynamics and a bottom-up approach to ecosystem interactions (Moore et al. 2003). By combining Harris' (2009) and Moore's et al. (2003, 2005, 2007) concepts I have a framework to address the interactions of litter quality and quantity, microbial function, biogeochemistry, and plant invasion. I hypothesized that Russian knapweed dominance would facilitate a shift from a slow, fungal dominated energy cycle to a faster, bacterial dominated cycle through changes in the litter quality, decomposition rates, and microbial cycling of energy. This hypothesis is predicated on the idea that the native short-grass steppe plant community is in a late seral stage of succession and functions under Moore's "slow cycle" and Harris' fungal domination with low microbial biomass. Conversely, the invading Russian knapweed infestations represent an early seral community dominated by bacteria and fast energy channels. By expanding the energy channels concept to include decomposition and ultimately the effects on vegetation, the different modes of energy flows to the mechanisms of plant invasion are interconnected. My analysis focuses on differences in litter decomposition, nutrients and the microbial communities of soil and decomposing litter along a gradient between invaded and native vegetation to address how an invasive species can affect plant-soil feedbacks through the cycling of energy.

METHODS

Study Species and Study Sites

Acroptilon repens is a member of the Asteraceae family and is a state listed noxious weed in at least 18 US states (USDA-NRCS 2011). Native to the overlapping regions of the European and Asian continents, the species is originally known from Russia, Iran, Kazakhstan, and Mongolia (Beck 2008). Russian knapweed forms near monocultures and is often found in pastures, rangelands, or degraded croplands in the United States. Its primary mode of reproduction is through aggressive rhizomatous spread and stands are known to live 75 years or longer. Russian knapweed is believed to be allelopathic (Stevens 1986, Stermitz et al. 2003, Morris et al. 2006), although determination of specific allelopathic compounds from field samples has lacked repeatability (Quintana et al. 2008). Management and control of the species is difficult and requires a long-term commitment and utilization of multiple strategies, including: herbicides, mowing, and inter-seeding with aggressive graminoids (Beck 2008).

I used three study sites (Kesa, Long's Peak Dairy (LPD) and Waverly) representing nine populations of Russian knapweed that are typical of a short-grass steppe ecosystem in north-central Colorado to investigate plant-soil feedbacks. The short-grass steppe plant community is considered a late seral system and is dominated by several native grasses: *Bouteloua gracilis* (Willd. Ex Kunth) Lag. Ex Griffiths (blue grama) and *Bouteloua dactyloides* (Nutt.) J.T. Columbus (buffalo grass). The Waverly site has a history of disturbance from cultivation and livestock grazing and much of the vegetation is dominated by the native shrub *Ericameria nauseosa* (Pall. Ex Pursh) G.L. Nesom & Baird (rubber rabbitbrush) and exotic *Agropyron cristatum* (L.) Gaertn.

(crested wheatgrass). Kesa and LPD have intact short-grass steppe plant communities outside of the *A. repens* invasions and appear to have little recent disturbance or overgrazing. The Kesa site has periodic grazing by horses and LPD was grazed by dairy cows several decades ago. The clonal growth of *A. repens* facilitated the use of a blocking design upon each roughly circular population of the knapweed. A decomposition experiment and soil sampling protocol utilized each population or block of Russian knapweed as the framework to study a vegetation gradient between the invaded and native plant communities. Each site has the following number of blocks: Waverly (3), LPD (3), Kesa (1).

Decomposition Litterbags

243 litterbags of nylon mesh (10 x 10 cm and 1 mm² pore size) were installed on the soil surface at three sites in the short-grass steppe ecosystem of north-central Colorado, USA. Each litterbag was individually tagged and contained a known quantity (~ 5 g) of one of three plant litter materials (*Acroptilon repens* (Russian knapweed), *Medicago sativa* (Alfalfa), or commercial craft sticks made of processed birch wood (*Betula* spp.)). Litterbags of each type of litter were installed along a transect determined by a random compass bearing from the center of an *A. repens* population and placed at the edge of the population (ecotone) and 5 m inside (invaded) and outside of the edge (native). Each litter type and litterbag placement in relation to the *A. repens* population was replicated nine times at each of the three sites.

Litter material was hand clipped (*A. repens*, *M. sativa*) or mechanically chipped (*Betula* sp.) to a length of approximately 2.5 cm and air-dried before enclosure in the

litterbags. Fresh, green *M. sativa* biomass was air-dried and used in litterbags, but the wild collected *A. repens* was relatively dry and brown prior to decomposition. The C:N ratio of each litter type was determined from three samples of each litter analyzed at the Colorado State University Soil, Water, and Plant Testing Laboratory. The moisture content of the litter before installation in the field was determined from the mean of 23 samples oven-dried at 65°C for 72 hours. Litterbags were individually stored in mailing envelopes during transport to the field sites and any material lost from the litterbags was adjusted to dry weight and removed from the original biomass weight (Barlocher 2005). Litterbags were installed during the late spring or early summer of 2008, although each site was installed and removed from the field at different times and therefore analyzed independently. Field decomposition occurred for 531 days at LPD, 552 days at Waverly, and 716 days at Kesa. Litterbags were covered with a protective wire mesh, but 17 litterbags (7% of total) were removed from analysis due to damage by wildlife or invertebrates, or they could not be relocated.

Following collection of the litterbags, a wet weight of all litter and inorganic material was determined and a sub-sample of 1 g was removed from each of the LPD and Waverly litterbags for extraction of Fatty Acid Methyl Esters (FAME) and quantification of the microbial community structure and diversity. FAME analysis was not conducted on the Kesa litterbags. Litter and inorganic content was oven-dried at 65°C until constant mass was reached, weighed, and then incinerated at 550°C using a muffle furnace to determine the amount of soil or inorganic material that had entered the litterbags. Following incineration, the weight of inert material within each sample was removed from the post-decomposition litter dry weights and the percentage of litter decomposition

was calculated by a comparison of the original and post-decomposition dry weights of a sample. Based on the determined moisture content of the samples following decomposition in the field, the material removed for FAME analysis was adjusted for moisture and inert content, and then interpolated into the litter dry weight data.

Soil Sampling

At each study site composite soil samples were collected at random at each location along the vegetation gradient (invaded, ecotone, native) for a total of three replicate samples per block and vegetation type (total n=81). Samples were collected in June of 2009. Soil was collected using a 5 cm diameter bulb planter to an approximate depth of 5 cm and each composite sample consisted of three cores from within 1 m². Samples were stored on ice and processed on the day collected (sieved - 2 mm). Upon return from the field, two subsamples of each soil sample were taken for microbial analysis. One subsample was extracted using K₂SO₄ for determination of microbial biomass carbon and nitrogen, and stored at -20° C until analysis. The second subsample was immediately frozen (-20° C) until quantification of soil microbial composition using Fatty Acid Methyl Esters (FAME). The remaining soil was air-dried for biogeochemical analysis at AgSource Harris Laboratories (Lincoln, NE). The following soil nutrients and electro-chemical variables were quantified (units are ppm unless otherwise noted): calcium, CEC (Cation Exchange Capacity), hydrogen (%), manganese, nitrogen (NO₃), organic matter (%), pH (log scale), phosphorus (1 Bray method), potassium, sodium, soluble salts (conductivity), sulfur, and zinc. Soil nutrient levels were determined by the following methods: cadmium reduction (nitrate), ammonium acetate on (potassium,

magnesium, and calcium), DTPA (Zinc), and monocalcium phosphate (Sulphur).

Nutrient levels were measured on an inductively coupled plasma spectrometer (ICP).

Organic matter was determined by the loss on ignition method.

Microbial Community and Functional Analysis

Community composition and functional diversity of microbes in soil and partially decomposed plant litter were determined by the ester-linked extraction method of analyzing fatty acid methyl esters (FAME) and followed the methods of Schutter and Dick (2000). FAME methods are similar to the phospholipid fatty acid (PLFA) method to extract microbial fatty acids from soil, but are simpler, requires less time, and may not extract free fatty acids from the soil (Schutter and Dick 2000). Only litter samples from LPD and Waverly were extracted for microbial analysis and samples from the same block, vegetation type, and litter type were composited and sub-sampled for FAME analysis (N=54). Individual fatty acids were categorized into general microbial functional groups based on the current literature (Vestal and White 1989, Ringelberg et al. 1997, Zogg et al. 1997, Drenovsky et al. 2004, Denef et al. 2009). Fungal to bacterial ratios were developed from the FAME functional groups for both the soil and plant litter samples. Common diversity indices (richness, Shannon's, Simpson's) were calculated in PC-ord software (PC-ord 5.0 Gleneden Beach OR) from the individual fatty acid data for the litter and soil samples.

FAMEs were extracted from 1 g of plant litter or 3 g of soil through a mild alkaline methanolysis process in 0.2 M KOH and methanol. Samples were vortexed every 10 minutes while being incubated for 1 hour at 37 °C. The alkalinity was adjusted

by the addition of 1 M acetic acid and FAMES were isolated into an organic phase by the addition of hexane and centrifuging. Following transfer to clean test tubes, an internal carbon standard (19:0) was added to each sample so that gas chromatography (GC) peak areas could be converted to nmoles. Hexane was evaporated from the test tubes using nitrogen gas. Samples were analyzed on a GC with a flame ionization detector (FID) at the University of Delaware. The FAMES were characterized using the Sherlock Eukary program by MIDI software (Microbial ID, Inc., Newark, DE). At the University of Delaware FAMES were dissolved in 0.5 ml of 1:1 hexane:methyl-*tert* butyl ester and analyzed on an Agilent model 6890 gas chromatograph (Wilmington, Delaware USA) using a Hewlett Packard column (#19091B-102 Ultra 2) of 25 m length and at a flow rate of 0.8 ml/min.

Microbial Biomass Carbon and Nitrogen

The quantity of microbial biomass carbon and nitrogen in the soil was estimated using the Chloroform Fumigation Extraction (CFE) technique and followed the methods of Horwarth and Paul (1994) and Voroney et al. (2008). The CFE method is based on the difference in C or N between the chloroform fumigated and non-fumigated samples. Following field collection and sieving (2 mm) in the laboratory, soil samples were stored at 4 ° C for 7-12 days until fumigation and/or extraction was completed. Microbial C and N were extracted from an equivalent of 8 grams dry weight soil with 0.5 M K₂SO₄ in a 5:1 (K₂SO₄:soil) ratio and shaken for 1 hour. Solution was filtered using a #1 Whatman filter paper and the extract was stored at -20 ° C until analysis on a Shimadzu Total

Organic Carbon & Total Nitrogen analyzer (model TOC-v cpn with a TNM-1, Columbia, Maryland USA). No correction (Kec) was applied to the final data.

Statistical Analysis

Effects on decomposition, litter and soil microbial communities were compared between vegetation types (invaded, ecotone, native) for each site independently using one-way Analysis of Variance (Anova) in JMP statistical software (JMP 8.0.2, Cary, NC). All response data were tested for normality using a Shapiro-Wilks W-test. Non-normal data were transformed using a natural logarithm, square root, or power transformation. Data that could not meet the assumptions of normality were analyzed using a non-parametric Kruskal-Wallis one-way Anova. All post-hoc tests were made with the Tukey-Kramer Honestly Significant Difference (HSD) test.

Additional ordination based statistical methods were used to investigate the interactions between microbial communities from the partially decomposed litter or soil sampled along the vegetation gradient from *A. repens* monocultures to native vegetation. Principal Components Analysis (PCA) was conducted in PC-ord software to ordinate nmoles of fatty acids per gram dry material of individual fatty acid markers (FAMES). Rare fatty acids (those present in fewer than five samples) were removed from the analysis and all data were relativized by the maximum value for each fatty acid. Ordination using PCA attempts to find patterns in complex multivariate data by reducing numerous variables (individual fatty acids) into a smaller set of composite variables or Principal Components (McCune et al. 2002). Principal Components Analysis is based on eigenvector solutions and is best suited to data with approximately linear relationships

between variables. PCA was run using a correlation cross products matrix and a randomization test (999 runs). PC-ord software was used to calculate microbial diversity indices (evenness, richness, Simpson' index of diversity, and Shannon diversity).

A Multiple Response Permutation Procedure (MRPP) or blocked MRPP test was used to identify differences in the litter and soil's microbial communities based on pairwise comparisons between samples' locations along the vegetation gradient. MRPP is a non-parametric, permutation based (1000 Monte Carlo iterations) procedure to test for treatment effects between pre-defined groups (vegetation or sites) and avoids the stringent requirements of multivariate normality. Our experimental design was amenable to blocking for the comparison of vegetation's effect on microbial communities, since each population of *A. repens* represents a unique unit comprised of all treatments, but is also implicitly different than another population of *A. repens*. Blocking helps control for variance between populations of Russian knapweed. A Bonferroni Correction was applied to adjust P values for the multiple comparisons of the MRPP pairwise comparisons (three comparisons between all possible combinations of sample locations: invaded vs. ecotone, invaded vs. native, ecotone vs. native).

RESULTS

Litter Decomposition

The three species of litter followed expected C:N ratios with *Betula* spp. having the highest C:N ratio (279.2 ± 21.5), *A. repens* intermediary (34.7 ± 1.1), and *M. sativa* the lowest (14.8 ± 0.6). The amount of decomposition varied greatly between litter types and generally followed C:N ratios with the lower C:N litters having greater

decomposition. Statistical differences in the amount of decomposition were documented in two litter types, but not at all sites (Figure 4.1). At LPD, decomposition in the invaded vegetation were greater than within the native vegetation for *A. repens* and *M. sativa* litter. The Waverly location had a different trend for the *A. repens* litter with decomposition in the native and invaded vegetations having greater decomposition than the ecotone. None of the litter types at Kesa had significant differences in decomposition between the vegetation types, although this site had fewer populations of *A. repens* sampled, but the same number of samples.

Litter Microbial Analysis

Principal Components Analysis (PCA) of the microbial community in *A. repens* litter distinctly separated between the two study sites, but did not identify clear differences between the types of vegetation in which the litter was decomposing (Figure 4.2a). Site differences were driven by differences in gram-negative and gram-positive bacteria in principal component 1 (PC1), which explained 52% of the ordination's variation. The second principal component explained 19% of the variation and was driven by fungi, protozoa, and fatty acids that are not linked to a known microbial group (unknowns). PCA of the *Betula* spp. litter displays similarities in the microbial community of samples from the ecotone vegetation of each site (Figure 4.2b). *Betula* spp. litter that decomposed in LPD's native vegetation is distinctly separated from LPD's other vegetation types, especially along principal component 1 (PC1) which explained 72% of the variation. Principal Component 1 was dominated by gram-negative bacteria, gram-positive bacteria, and saprophytic fungi, while PC2 explained only 10% of the

variation and was driven by cyanobacteria, actinomycetes, and unknown fatty acids. PCA ordination of *Medicago sativa* litter exhibited a large amount of variability along PC1, especially in the LPD samples (Figure 4.2c). PC1 explained 56% of the variation and was driven by gram-positive bacteria, actinomycetes, cyanobacteria, and unknowns. The *M. sativa* litter's microbial community from Waverly's native vegetation was distinctly separated along PC1 from the invaded and ecotone decomposition sites and was most similar to the microbial community from LPD's native vegetation. Additionally, LPD's *M. sativa* litter that decomposed in the invaded vegetation was separated from all other samples along the PC2, which explained 17% of the variation and was dominated by anaerobic gram-negative bacteria, gram-positive bacteria, arbuscular mycorrhizal fungi (AMF), and unknowns.

The composition and diversity of microbial groups varied greatly between litter types. In general *M. sativa* litter had the highest quantity of fatty acids (nmoles g⁻¹ dry litter), greatest richness, and diversity; while the *Betula* spp. litter had the lowest for each of these measurements (Table 4.1). Conversely, *Betula* spp. litter had higher fungal:bacterial ratios than the other litter types. Results of one-way Anovas (parametric and non-parametric) tested for statistical differences between the vegetation types (invaded, ecotone, or native vegetation) in which the litter was decomposing for each microbial group (nmoles/g dry litter) and diversity index. The following microbial functional groups differed statistically between vegetation types: total FAMES, AM fungi, gram-negative bacteria, gram-positive bacteria, and the cyanobacteria and actinomycetes group (Table 4.1). When the same litter type was statistically significant at both study sites for a microbial functional group, the trend or pattern in the significant

pairwise comparisons between vegetations types were opposite or different. This occurred for the following microbial functional groups: AM fungi, gram-positive bacteria, and the Cyanobacteria/Actinomycetes group. Whether the litter was decomposing in native, invaded, or ecotone vegetation affected the amounts of these microbial functional groups present in the litter, although the trends were not consistent between functional groups or sites. Blocked Multiple Response Permutation Procedures (MRPP) run for each litter type independently found no statistical differences in the microbial communities of litter that decomposed in different vegetation, although a MRPP comparing all litter types between the Waverly and LPD sites was significant at $\alpha=0.1$ (Table 4.3).

Soil Microbial Community Analysis

Principal Components Analysis (PCA) of the soil microbial community using FAME analysis clearly separated between the three study sites, but differences based on the invasive to native vegetation gradient were not distinct (Figure 4.3). A MRPP test comparing the soil microbial communities of the three sites, regardless of the vegetation gradient within each site, reinforces these differences between sites (MRPP main test: $n=81$, $A=0.149$, $P<0.0001$). Based on pairwise comparisons between sites, both Kesa and LPD are statistically different from Waverly, but not from each other (Table 4.3). The results from the PCA ordination and MRPP statistical test may represent the inherent differences between the sites that are dominated by native, late seral short-grass steppe (Kesa and LPD) and Waverly, which has a history of disturbance due to overgrazing and cultivation.

Significant differences based on one-way Anova between vegetation types in soil microbial functional groups, diversity indices, nutrients or microbial biomass were infrequent and not consistent across sites (Table 4.2). At Kesa only the C:N ratio of microbial biomass was significant between vegetation types, while both AM fungi and pH were significant at Waverly. Relative abundance of AM fungal FAME biomarker was greatest in the invaded vegetation and lowest in the native plant community. The following soil measurements varied significantly among vegetation types at LPD: Cyanobacteria and Actinomycetes, Fungal:Bacterial ratio, Simpson's and Shannon's diversity indices, and pH. Only pH was significant for more than one site and the trend in pH across the vegetation gradient differed between the two study sites. At Waverly the invaded vegetation had the lowest pH and differed from both the ecotone and native vegetation. Conversely, at LPD the invaded vegetation had the highest pH and was significant from the lowest pH in the ecotone plant community.

A blocked MRPP test comparing the soil microbial communities between the three vegetation types for all sites was significant for the main test, but not for any of the pairwise comparisons between vegetation types (blocked MRPP main test: $n=81$, $A=0.0341$, $P=0.0164$) (Table 3). Principal Component 1 (PC1) of Figure 4.3 described 59% of the variation in microbial communities and was dominated by gram-positive bacteria. PC2 explained nearly 12% of the ordination's variance, although none of the fatty acids driving this principal component could be identified into a microbial functional group. Blocked MRPP tests for each site separately identified weak differences between microbial communities from different vegetation types. Separation between the microbial communities of Waverly's invaded and native vegetation types

along PC1 may represent a difference in the amount of gram-positive bacteria present in the different plant communities, although the blocked MRPP results are slightly different since they are influenced by both principal components. The soil microbial communities at Waverly are significantly different ($n=45$, $A=0.0597$, $P=0.0016$) and the pairwise comparison between the ecotone and invaded vegetation types were significantly different from each other at $\alpha=0.1$ ($n=45$, $A=0.0745$, $P=0.081$ after Bonferroni correction for multiple comparisons) (Table 4.3). The main test at LPD also had significant differences at $\alpha=0.1$ between soil microbial communities from the different vegetation types ($n=27$, $A=0.0524$, $P=0.0659$), but no pairwise comparisons between vegetation types were significant.

DISCUSSION

The development of human society and subsequent globalization of resource use and transportation vectors has had an unprecedented effect on the flora and fauna of the world. Many ecosystems are degraded and monopolized by weedy, cosmopolitan species. Understanding the interactions between invasive plants and the soil is critical to our ability to manage weedy species and collectively decide upon the future of our remaining natural ecosystems. At a global scale the invasion of exotic species is irreversible, yet at regional and local scales our ability to understand the mechanisms of invasion can directly influence our management practices and assist in preventing the domination by problematic or unwanted exotics. An understanding of the mechanisms of plant invasion may facilitate management that reduces or prevents the loss of biodiversity and possibly assist in the identification of a niche or functional role where upon the non-

native species will be a minor component of a functioning and diverse ecosystem. This concept is based on a paradigm shift that accepts the transient nature of species and focuses on preventing the loss of diversity and ecosystem resilience, even if it is at the cost of accepting non-native species into a functioning ‘natural’ ecosystem. By understanding the mechanisms by which an invasive species invades, land managers can use this knowledge to manipulate the interactions or feedbacks in a system and possibly create a functional role for non-native species in resilient and diverse ecosystems.

These results document differences in decomposition of plant litter based upon their location along the gradient from Russian knapweed infestations to native vegetation (Figure 4.1). Although the results vary between sites, as a whole the type or quality of plant litter and the dominant vegetation influence the decomposition of litter and affect microbial communities of both litter and soil (Tables 4.1 and 4.2 respectively). I hypothesized that the differences in decomposition due to invasion would modify microbial systems and increase the flow of energy through a system (Moore et al. (2007) energy channels) and that this switch from a slow, fungal energy channel to a fast, bacterial channel could be the mechanism for Russian knapweed’s successful invasion and domination. Some aspects of this conceptual change in energy channels can be seen at the LPD site where the invaded vegetation has faster decomposition and higher soil nitrate than the native vegetation. From a successional view of plant dynamics, LPD does not fit into this concept of energy shifts, since the invasive vegetation is presumably an early seral species yet has a significantly higher fungal:bacterial ratio and higher amounts of AM fungi than the late seral short-grass steppe plant community. This finding questions the idea that Russian knapweed is an early seral invasive species or that

some species cannot adapt to different conditions and energy channels as an ecosystem matures and the energy channels shift toward fungal dominance (Harris 2009) or Odum's (1969) attributes of a mature ecosystem, specifically: closed nutrient systems, a large portion of nutrients retained in detritus, and long life cycles. Arguable, a mature Russian knapweed population has many of the traits that Odum (1969) prescribes to a mature successional ecosystem, but it also begs the question about how the species can bridge the gap between invasion and long-term stability, while remaining dominant throughout. Modification of soil and litter microbial communities by Russian knapweed may affect PSF in ways that promote the long-term stability of the invasive species from the early seral stages of invasion (r-selection) through the later seral development of a mature (Odum 1969), slow energy channel (Moore et al. 2007) system.

Interestingly, some important differences also occur between the edge and inside locations of *A. repens* that may require deeper consideration of how a dominant species interacts with its environment between its own advancing front of rhizomatous growth and areas of long established monocultures. Areas of active invasion by Russian knapweed probably have very different energy channels than interior portions of the monoculture. Simple classification of a species as early or late seral may overlook the complexity of a species and its interactions, especially in the context of an invasive species in a new environment (i.e. – rhizomatous expansion or a novel continent).

Differences in the microbial communities were greater between sites than vegetation types for both the soil and litter microbial communities (Figures 4.2 and 4.3). Our limited study of three sites consisting of nine populations of *A. repens* highlights the microbial diversity between sites and the similarity of microbial communities of adjacent

vegetation communities. The microbial communities of *A. repens* litter showed the greatest amount of separation between study sites along PC1 (Figure 4.2a) and the differences were driven by gram-negative and gram-positive bacteria. Most sites were distinctly segregated along PC2, especially when considering the large amount of variation (large standard errors) along PC1's. Although PC2's generally have significantly less explanatory power than the primary principal component (PC1), they may provide valuable information. Principal component 1 was commonly dominated by bacteria and occasionally fungi, while PC2 had more variability of microbial taxonomic groups (Figures 4.2 and 4.3). This may signify that some microbial taxonomic groups are common across diverse vegetation types and geographic scales (i.e. gram-negative and gram-positive bacteria in PC1's), and that the less common but more functionally specific groups (AM fungi, fungi, protozoa, actinomycetes, cyanobacteria) that drive the secondary principal components of the ordinations may differentiate between sites and potential vegetation types.

Acroptilon repens litter decomposition at Waverly was quite different than the other sites. Decomposition was either suppressed at the ecotone or accelerated in the native vegetation (Figure 4.1). This may be due to a history of disturbance and the native vegetation being degraded from an intact short-grass steppe community. Waverly's microbial communities have an opposite trend across vegetation types than LPD in one soil parameter (pH) and three litter parameters (AM fungi, gram-positive bacteria, and Cyanobacteria/ Actinomycetes). Distinct differences in soil and litter microbial communities (Figures 4.2 and 4.3, Table 4.3) highlight the importance of considering the history or legacy of sites and the potential variability in microbial systems. The degraded

native vegetation at Waverly decomposes Russian knapweed litter as quickly as the invaded areas and this highlights the possibility for degraded natural vegetation to enhance the impacts and spread of invasive species through altered plant-soil feedbacks. If degraded, semi-natural plant communities have feedbacks similar to invaded systems or can contribute to the quicker decomposition of invasive species litter and availability of nutrients, it could support feedback systems that benefit invasive species or make restoration of native plant communities more difficult.

LPD and Kesa had relatively intact native plant communities surrounding the *A. repens* infestations and the least amount of disturbance or degradation, while Waverly has a history of grazing and potentially seeding with exotic grasses. LPD had the most variation in microbial measurements along the vegetation gradient in the *Betula* spp. litter (lowest quality litter)(Tables 4.1 and 4.2). This may be related to the ecotone representing a major shift in energy channels, while the intact native community was relatively stable and remains in a slow energy cycle. Within the *Betula* spp. litter, the ecotone was significantly different from the native vegetation for the total amount of fatty acids, gram-negative bacteria, and gram-positive bacteria (Table 4.1). The native vegetation at Waverly was degraded and may have some similarities to the fast energy channels in the ecotone and invaded vegetation. The majority of microbial measurements that varied along the vegetation gradient occurred in the *M. sativa* litter, which was labile and high quality. This type of litter was likely to promote fast energy cycling in systems that have low C:N ratios, are bacterial dominated, and due to overall degradation of the site may be less responsive to different vegetation types.

Although this project was limited due to only three study sites and nine populations, the condition and quality of the native vegetation influenced decomposition and microbial communities. Knapweed populations that were surrounded by intact late seral short-grass steppe communities had greater decomposition of the labile, high quality litter inside the invaded areas, whereas sites with disturbed native vegetation surrounding Russian knapweed decomposed knapweed litter fastest in both the invaded and native vegetation. The sites with the least disturbance to the native plant community had the most variation along the vegetation gradient in the lowest quality litter (Birch wood). Conversely, the sites with the greatest disturbance to the native plant community impacted the highest quality litter (alfalfa). Soil condition and history may affect the stability of a system and may push degraded systems towards fast, bacterial impacts to labile, high N litter sources, while intact systems remain in slow, fungal dominated cycles. Gradients between different dominant plant communities (native or invaded) may produce unexpected discontinuities in the system's energy flows, including decomposition, nutrient cycling, and microbial function. Overall, invasive vegetation affects decomposition and microbial communities, but a site's history and amount of disturbance may have profound impacts on how an invasive species modifies energy flows and plant-soil feedbacks. Additional sites are required to determine the effects of site history and vegetation condition on plant-soil feedbacks.

Our current understanding of plant-soil interactions, especially concerning invasive species, is basic. Without studying the complex interactions that occur between plants, microorganisms, and the physical and biogeochemical elements of soil, society cannot address the mechanisms of plant invasion or develop novel management methods.

The results of this study provide initial information for understanding how a site's history or legacy can affect these complex interactions and how the discontinuities in the vegetation gradient may affect energy cycling. This research is not focused on the eradication of Russian knapweed, but on a more complete understanding of how it becomes invasive through the modification of plant-soil feedbacks and ultimately applying this understanding to creative management methods that are not resource or disturbance intensive. If litter from Russian knapweed is a driving factor in the species' eventual domination of an area, the removal of litter during inter-seeding with aggressive native plants could be unique and low-impact method. Additionally, the manipulation of fungi:bacteria (via fungicides) or carbon:nitrogen ratios by carbon amendments of the soil could force the energy channels in a desirable direction for restoration with native species. Other methods may include the manipulation of soil nutrients through fertilization or microbial inoculation, the promotion of pathogens, but these concepts have not been directly addressed by this research.

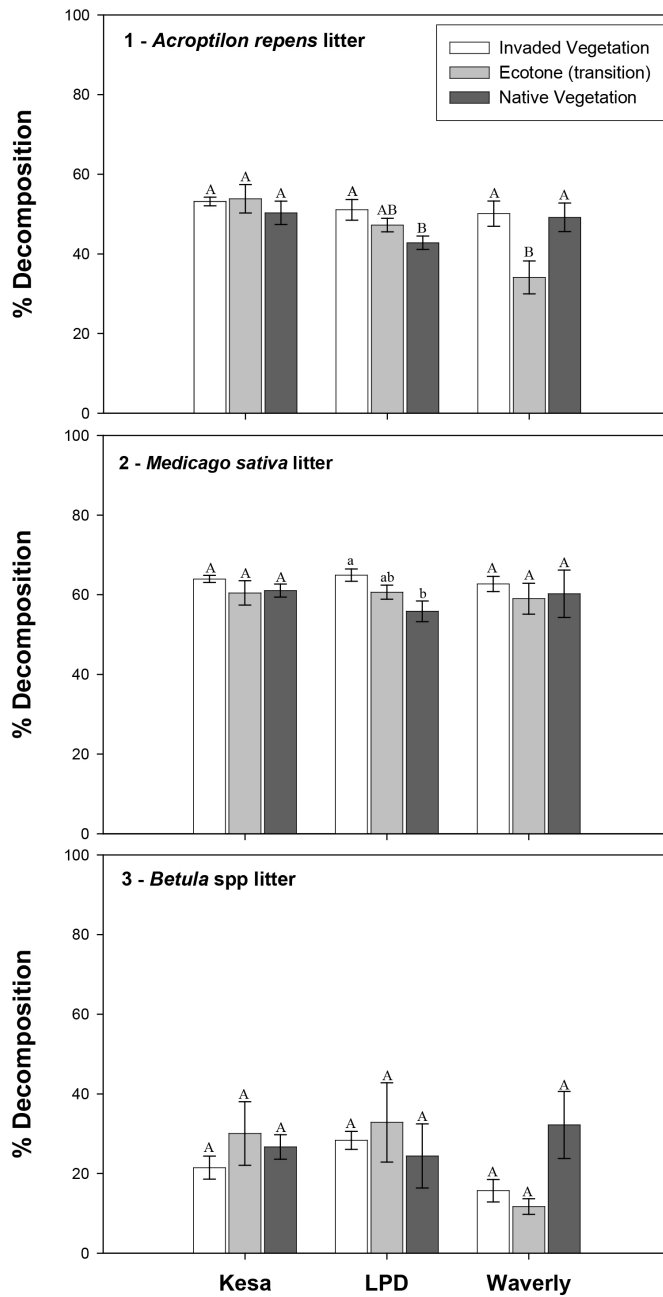
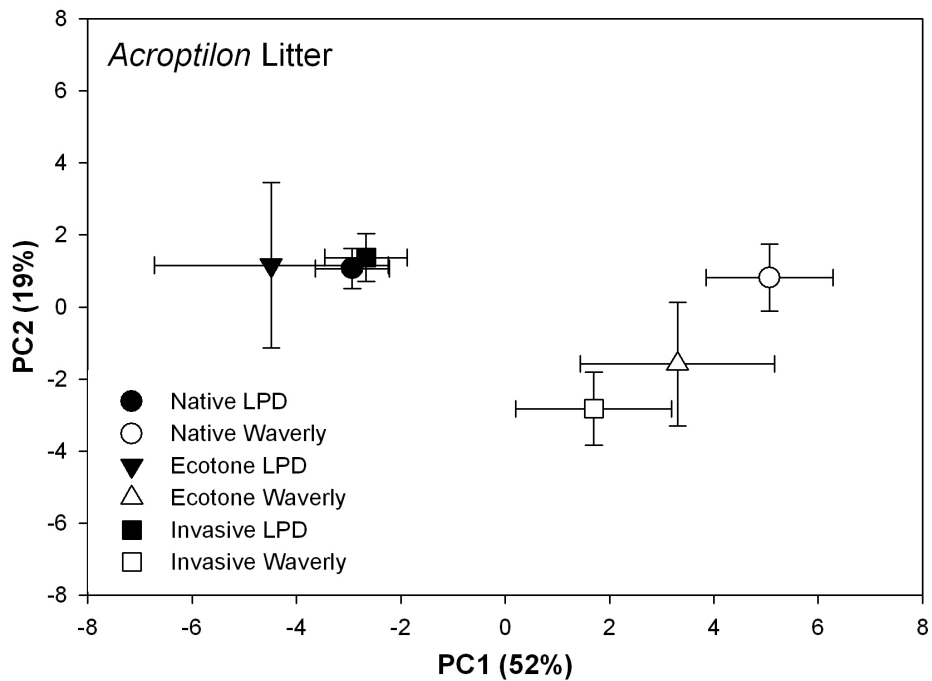


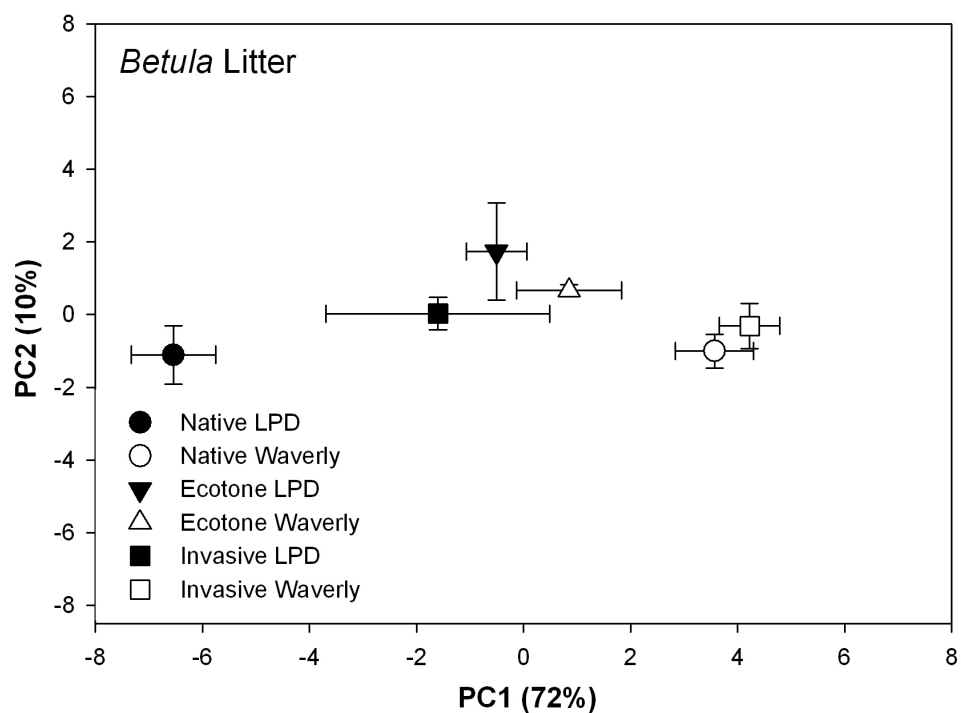
Figure 4.1 – Mean percent decomposition of three plant species litter along a vegetation gradient (Invaded to Native). Each panel represents one species of plant litter (1 – *Acroptilon repens*, 2 – *Medicago sativa*, 3 – *Betula* spp.) and three study sites (Kesa, LPD, Waverly). Different letters within a site indicate statistical significance based on a non-parametric Kruskal-Wallis one-way anova (Capital letters $\alpha < 0.05$, lower case letters $\alpha < 0.1$) (error bars ± 1 standard error).



***Acroptilon repens* litter Principal Component Weights by Fatty Acid**

PC1			PC2		
Fatty Acid	Functional Group	Weight	Fatty Acid	Functional Group	Weight
16:1 w7c	Gram- bacteria	-0.9582	18:0	Unknown	-0.8899
16:0 ISO	Gram+ bacteria	-0.9558	15:1 w6c	Unknown	-0.7663
15:0 ANT	Gram+ bacteria	-0.9436	18:3 w6c	Fungi	-0.7637
17:0 ANT	Gram+ bacteria	-0.9382	16:0	Ubiquitous	-0.7486
15:1 ISO	Unknown	-0.9346	20:3 w6c	Protozoa	-0.7234

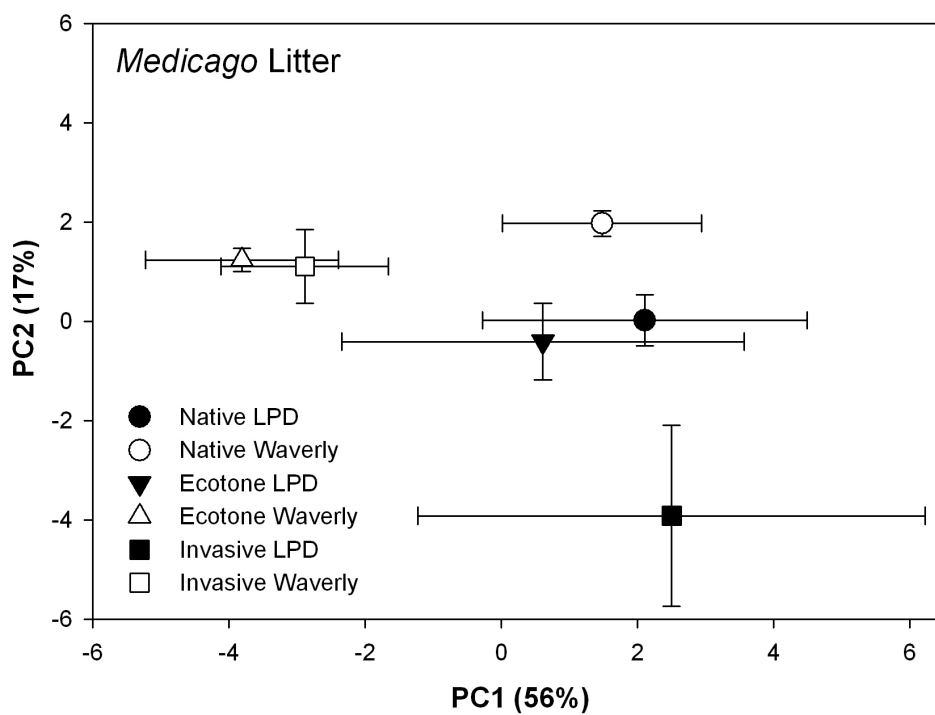
Figure 4.2 (a,b, and c) – Principal Components Analysis (PCA) ordination of individual Fatty Acid Methyl Esters (FAME, n=40) found in the plant biomass of litter decomposition bags (n=54). Each graph displays one type of plant litter (2a - *Acroptilon repens*, 2b - *Betula* spp., 2c - *Medicago sativa*) and a table of the weighting of the strongest fatty acids for each axis (principal component). Marker shape represents the vegetation where decomposition occurred (squares = Invaded, triangles = Ecotone, circles = Native) and marker shading indicates the site (black = LPD, white = Waverly). Principal components (PC) or axes are labeled with the variance explained in parentheses. The five fatty acids with the strongest weighting (or factor loading) on each axis are displayed along with their microorganism functional classification.



***Betula* spp. litter Principal Component Weights by Fatty Acid**

PC1			PC2		
Fatty Acid	Functional Group	Weight	Fatty Acid	Functional Group	Weight
15:0 ISO	Gram+ bacteria	-0.978	15:0	Cyanobacteria, Actinomycetes	0.7914
16:1 w7c	Gram- bacteria	-0.969	19:0 3OH	Unknown	0.6446
18:2 w6c	Saprophytic fungi	-0.9606	18:0	Unknown	0.5573
16:0 ISO	Gram+ bacteria	-0.9555	17:0	Cyanobacteria, Actinomycetes	0.3875
14:0	Unknown	-0.9435	C9 Di-carboxylic Acid	Unknown	0.331

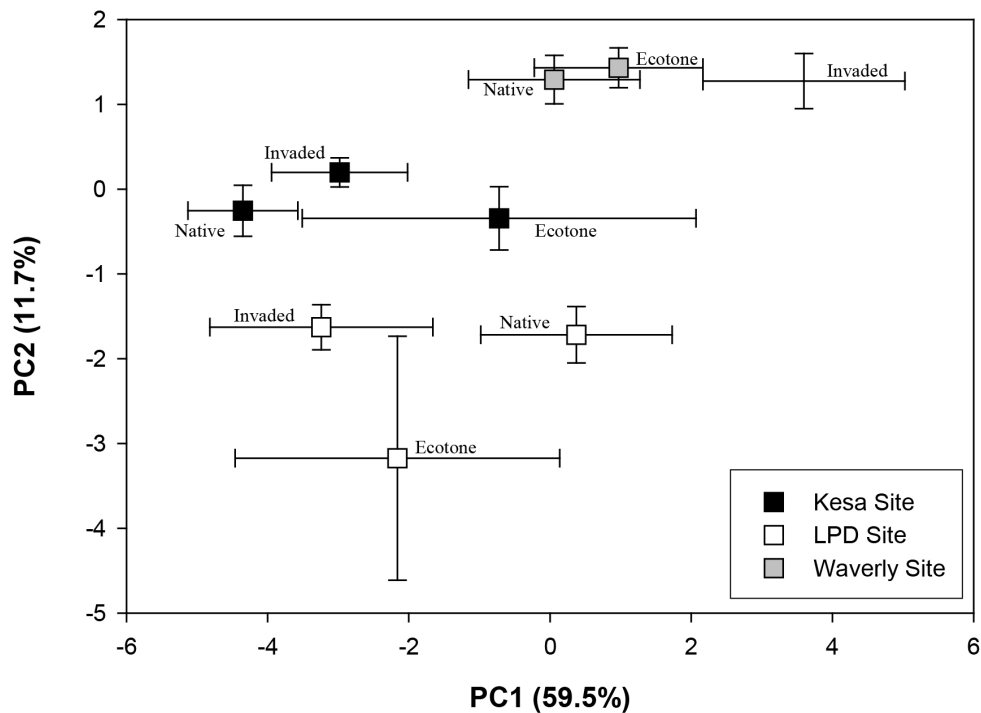
Figure 4.2b – PCA *Betula* spp. litter and Principal Components Weights



***Medicago sativa* litter Principal Component Weights by Fatty Acid**

PC1			PC2		
Fatty Acid	Functional Group	Weight	Fatty Acid	Functional Group	Weight
16:0	Ubiquitous	0.959	12:0	Unknown	0.6757
16:0 ISO	Gram+ bacteria	0.9581	17:0 CYC	Anaerobic Gram-bacteria	-0.6643
14:00	Unknown	0.9238	16:1 ISO	Gram+ bacteria	0.6576
15:1 ISO	Unknown	0.9166	16:1 w5c	AM Fungi	-0.6505
17:0	Cyanobacteria, Actinomycetes	0.9067	C15 N Alcohol	Unknown	0.6151

Figure 4.2c – PCA *Medicago sativa* litter and Principal Components Weights



PC1			PC2		
Fatty Acid	Functional Group	Weight	Fatty Acid	Functional Group	Weight
16:0 ISO	Gram+ bacteria	0.9853	10:0	Unknown	-0.7015
15:0 ISO	Gram+ bacteria	0.9821	11:0 ISO	Unknown	-0.7015
16:0	Ubiquitous	0.9819	C14 N Alcohol	Unknown	-0.7015
17:0 ANT	Gram+ bacteria	0.9794	C20 N Alcohol	Unknown	-0.6852
15:0 ANT	Gram+ bacteria	0.9738	C9 Di-carboxylic Acid	Unknown	-0.6458

Figure 4.3 – Principal Components Analysis (PCA) ordination of soil microbial community based on FAME analysis (nmoles g^{-1} dry soil)(N=81). Ordination displays three study sites differentiated by marker shading and with the vegetation community (Invaded, Ecotone, Native) labeled next to the marker. Vertical and horizontal error bars represent ± 1 standard error with nine replicates per site and vegetation community. Each axis represents a principal component (PC) and the amount of variation explained by the axis is in parentheses. The table below the ordination lists the five fatty acids with the strongest weighting (or factor loading) on each axis and the microbial functional classification for each fatty acid.

Table 4.1 - Litterbag Microbial Diversity along a Vegetation Gradient between Invasive and Native Species Dominance

Summary of microbial community diversity in partially decomposed plant litter using Fatty Acid Methyl Ester (FAME) analysis to quantify microbial functional groups and develop diversity indices. Data are the mean values (n=9) with ± 1 standard error in parentheses for the LPD and Waverly study sites and three species of plant litter (*Acroptilon repens*, *Medicago sativa*, *Betula* spp). Statistical differences in microbial measurements are based on comparisons of decomposition sites along a vegetation gradient (Native, Ecotone, Invaded). Level of statistical significance are as follows based on a parametric one-way Anova: * $\alpha < 0.1$, ** $\alpha < 0.05$, *** $\alpha < 0.01$. Microbial functional groups are in units of nmoles g⁻¹ dry plant litter.

	LPD			Waverly		
	<i>A. repens</i>	<i>M. sativa</i>	<i>Betula</i> spp.	<i>A. repens</i>	<i>M. sativa</i>	<i>Betula</i> spp.
Total FAME	4131 (242)	5108 (329)	2124 (170)*	3351 (213)	4769 (217)*	1183 (97)
AM Fungi	43.8 (4)	69 (4.3)***	12.5 (2.7)	25.5 (2.8)	52.8 (3.4)**	4.1 (1.4)
Fungi	4.4 (2.5)	4.8 (2.4)	0 (0)	14 (2.2)	2 (2)	0 (0)
Saprophytic Fungi	1679 (158)	1945 (167)	1024 (95)	1410 (91)	1973 (95)	513 (44)
Gram - Bacteria	263 (13)	334 (18)	99 (12)*	159 (14)	299 (16)**	44 (6)
Gram + Bacteria	333 (16)	433 (27)	105 (15)*	190 (15)	385 (18)*	43 (7)*
Cyanobacteria & Actinomycetes	153.9 (5.6)	163.2 (11.1)	63.4 (4.9)**	118.3 (9.1)	137.9 (6.1)*	32.5 (2.1)**
Protozoa	21.1 (3.3)	23.6 (4.1)	1.6 (1.6)	7.2 (2.5)	8.3 (3.1)	0 (0)
Fungal:Bacterial Ratio	2.3 (0.2)	2.2 (0.1)	4.0 (0.2)	3.2 (0.1)	2.5 (0.1)	4.5 (0.2)
Fatty Acid Richness	28.8 (0.9)	30.8 (1)	21.7 (1.1)	28 (1.6)	29.1 (0.4)	15.8 (0.5)
Fatty Acid Simpson's Diversity	0.84 (0.007)	0.85 (0.004)	0.8 (0.005)	0.82 (0.005)	0.84 (0.003)	0.8 (0.004)
Fatty Acid Shannon's Diversity	2.37 (0.04)	2.44 (0.03)	2.07 (0.03)	2.2 (0.03)	2.35 (0.01)	1.94 (0.02)

Table 4.2 - Summary of Soil Characteristics, Microbial Biomass, and Microbial Functional Diversity

Summary of soil nutrients, microbial biomass, and microbial functional diversity using Fatty Acid Methyl Ester (FAME). Data are the mean values with ± 1 standard error in parentheses for the Kesa, LPD, and Waverly study sites. Statistical significance between the vegetation types in which the soil was collected (Native, Ecotone, Invaded) are bolded and represent the following types of tests and levels of significance: parametric one-way Anova (* $\alpha < 0.1$, ** $\alpha < 0.05$, *** $\alpha < 0.01$) or non-parametric Kruskal Wallis one-way Anova (δ $\alpha < 0.1$, $\delta\delta$ $\alpha < 0.05$, $\delta\delta\delta$ $\alpha < 0.01$). Microbial functional groups are in units of nmoles g⁻¹ dry soil.

	SITE		
	Kesa (n=9)	LPD (n=27)	Waverly (n=45)
Total FAME	293 (36)	281 (33)	500 (30)
AM Fungi	24.3 (3.6)	10.1 (1.1)	75.3 (5.9)*
Fungi	0 (0)	2.9 (0.6)	3.7 (0.5)
Saprophytic Fungi	52 (5.7)	42.7 (4.5)	73.8 (3.7)
Gram - Bacteria	25 (3.4)	23.5 (2.7)	43.7 (2.5)
Gram + Bacteria	44.6 (5.3)	49.1 (5.5)	64.7 (4)
Cyanobacteria & Actinomycetes	13.2 (2.6)	16.4 (2.2)*	22 (1.7)
Protozoa	0.27 (0.27)	0.78 (0.33)	2.21 (0.36)
Fungal:Bacterial Ratio	0.96 (0.06)	0.67 (0.03)*	1.32 (0.1)
Fatty Acid Richness	24.9 (1.6)	27.7 (1.4)	29.1 (1)
Fatty Acid Simpson's Diversity	0.93 (0.002)	0.92 (0.003)$\delta\delta$	0.92 (0.003)
Fatty Acid Shannon's Diversity	2.86 (0.04)	2.91 (0.04)δ	2.86 (0.03)
Microbial Biomass Carbon	430.9 (46.3)	304.3 (24.7)	364.8 (22.7)
Microbial Biomass Nitrogen	84.5 (9.2)	54 (4.9)	73.7 (4.9)
Microbial Biomass C:N ratio	5.1 (0.04)**	5.84 (0.19)	5.1 (0.14)
NO ₃ ppm	11.6 (1.1)	27.3 (2.7)**	56.8 (5.7)
P ppm	8.4 (1.5)	60.5 (5.5)	12.1 (1.6)
pH	7.8 (0.05)	6.4 (0.17)*	7.5 (0.04)***
% Organic Matter	3.57 (0.25)	4.13 (0.42)	3.81 (0.22)

Table 4.3 - MRPP Results for Litter and Soil Microbial Communities

P values for Blocked MRPP or MRPP analysis of litter or soil microbial communities (FAMEs nmoles/g dry material). Table lists P-values for main tests and pairwise comparisons between vegetation types or sites. Sample size (N) are listed in parentheses following the main test results. P-values in pairwise comparisons are corrected for multiple comparisons and occasionally exceed a value of 1.0 due to correction.

Blocked MRPPs			Pairwise Comparisons - Vegetation Types		
FAME Type	Factor	Main Test (N)	Invaded vs. Ecotone	Invaded vs. Native	Ecotone vs. Native
Soil	All Sites	0.0164 (81)	0.226	>1.0	0.107
	Kesa	0.547 (9)	>1.0	>1.0	>1.0
	LPD	0.066 (27)	0.322	>1.0	0.327
	Waverly	0.0016 (45)	0.082	0.286	0.194
Litter	All Litter Types	0.438 (54)	>1.0	0.385	>1.0
	<i>A. repens</i>	0.205 (18)	0.591	0.985	>1.0
	<i>Betula</i> spp.	0.170 (18)	0.831	0.087	0.828
	<i>M. sativa</i>	0.181 (18)	0.687	0.55	0.38
MRPPs			Pairwise Comparisons - Sites		
FAME Type	Factor	Main Test (N)	Kesa vs. LPD	Kesa vs. Waverly	LPD vs. Waverly
Soil	Between Sites	0.0001 (81)	0.1403	0.0018	0.0001
Litter	Between Sites	0.077 (54)	-	-	0.077

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Chapter 5 – Research Synthesis

Plant-soil feedbacks (PSF) are the culmination of extensive research in numerous fields, including botany, microbiology, and soil science. Feedbacks that ultimately affect plant growth and community dynamics epitomizes the science of ecology, because the focus of PSF is on understanding the results of interactions between biotic and abiotic elements. The enormous number of direct and indirect interactions between plants, soil, and microorganisms cannot be ignored if we intend to understand the mechanistic underpinnings of competition, vegetation succession, and plant invasion. Feedbacks occur on the smallest of scales (single-celled prokaryotic organisms interacting with individual root hairs), but have impacts that scale up to the landscape levels and potentially beyond. The simplistic idea of considering these nearly infinite interactions into a theory of plant-soil feedbacks is daunting, especially given the context dependency of most ecological research. Given these challenges, the multi-disciplinary nature of research in PSF is beginning to establish baseline theories that can guide management and microbial methodologies that shine light within the “black box” of soil and microorganisms.

Early research on allelopathy, or chemical interference, in agriculture and natural plant communities may represent the first systems approach to the interactions between plants and soil. Potential allelopathic interactions in chick pea (*Cicer arietinum*) was first noted by Theophrastus around 300 B.C. (Rice 1984) and agricultural problems related to

‘soil sickness’ brought the issue of chemical interference between plants into the scientific realm in the early 1800’s. In 1832, a system of crop rotation was developed by the botanist A.P. DeCandolle based upon his research into the interspecific inhibitory effects of certain agricultural species upon others (Bonner 1950). These concepts laid the groundwork for the inclusion of nutrients and microorganisms in the web of plant-soil feedbacks. The role of microorganisms in decomposition and nutrient dynamics is broadly accepted, but the idea that these minute organisms can influence large-scale vegetation dynamics has only recently become a focus of research. Conversely, the impact of invasive vegetation altering soil nutrient dynamics, vegetation succession, and subsequent disturbance cycles was nearly inconceivable until a few decades ago. These top-down and bottom-up examples are exemplified by Vitousek and Walker’s (1989) and Adler’s et al. (1998) research on actinorhizal *Myrica faya* invasions in Hawaii and the subsequent change in large scale ecosystem function (increased nitrogen), fire dynamics (more frequent fire), and plant invasions. Plant-soil feedback attempts to integrate the top-down and bottom-up nature of interactions, develop theories based on understanding mechanisms, and ultimately apply this knowledge to novel management methods directed at ecosystems processes.

The research presented in this dissertation utilizes two primary pathways to improve our understanding of PSF: soil microbial communities and litter decomposition. Experiments manipulating soil microorganisms (Chapters 2 and 3) attempted to use a bottom-up approach to determine how the microbial community affects vegetation, primarily invasive knapweed species. Chapter 4 studies the interactions of different plant litter and plant communities on decomposition and the microbial communities of soil and

plant litter. These complimentary approaches attempted to dissect the interactions between plants, soil, and microorganisms in ecosystems invaded by exotic knapweeds (*Acroptilon repens* and *Centaurea stoebe*).

Chapter one provides an in-depth literature review of the impacts of invasive plant species on soil, microorganisms, and decomposition. Chapter two documents the opposite responses of two species in two greenhouse experiments and highlights the potential trade-offs between exploitative competition (limited resources) and interference competition (pathogen accumulation or a lack of mutualisms). Although *A. repens* and *Solidago canadensis* are functionally and physiologically similar, the species responded to soil legacies (Exp. 1) and plant competition (Exp. 2) in opposite manners and this may represent fundamental differences between how an invasive species and a weedy, native species avoid or respond to competition. The variable responses of species to microbial communities or plant competition could identify an important difference between aggressive species that are native or invasive, and signify a trade-off between the relative impacts of plant-microbe or plant-plant interactions. Knowledge of the interactions that most strongly affect a species could be used to design control programs that focus on the most vulnerable aspect of an invasive species. The experiments also document reduced interaction strengths in the intraspecific competition of the two weedy species, compared to interspecific interactions. These results oppose traditional ecological theory (Darwin 1859, Elton 1958) and findings based on a broad survey of field experiments (Connell 1983). I propose that weedy, aggressive species may avoid the negative effects of exploitative, intraspecific competition and the reduction of this negative interaction at high plant densities could facilitate their invasiveness.

Chapter three shows that soil inoculation in field-based experiments can affect the vegetation, soil nutrients, and microbial communities during ecological restoration of invasive knapweeds infestations, although the results of inoculation were variable and highly species and site specific. Inoculating *C. stoebe* infestations with whole soil from adjacent native plant communities decreased the cover and density of invasive knapweeds, although it had no discernible effect on seeded native species. *Acroptilon repens* was less responsive to soil inoculation in field studies, but in greenhouse studies the species increased root growth when inoculated with soil from a native plant community. This may support the concept of *A. repens* escaping pathogen accumulation in its own soils and provides a potential mechanism for the rhizomatous species' invasiveness. Multivariate modeling of vegetation, soil nutrients, and microbial functional groups identified several interesting relationships that may drive exotic knapweed invasions. Knapweed cover was heavily influenced by their interaction with AM fungi and gram-negative bacteria, while the cover from native species was promoted by actinomycetes. Additionally, soil nitrogen was strongly correlated with invasive species. These results partially support previous research on the interactions of invasive plants and soil. Managing ecological processes that impact fungi, bacteria or soil nitrogen could direct plant communities towards a reasonable combination of native and exotic species, although additional research is required to determine how plant species and ecosystems will respond to the manipulation of plant soil feedbacks with targeted inoculations.

Chapter four documents differences in decomposition of plant litter based upon the location along a gradient from *A. repens* infestations to native vegetation. Although

the results varied between sites, as a whole we see that the type or quality of plant litter and the dominant vegetation influence the decomposition of litter and affect microbial communities of both litter and soil. I hypothesized that the differences in litter decomposition due to *A. repens* invasion would modify microbial energy systems and increase the flow of energy through a system (i.e. applying Moore et al.'s (2007) energy channels concepts to whole ecosystems) and that this switch from a slow, fungal energy channel to a fast, bacterial channel could be a mechanism for *A. repens*' successful invasion and domination. Some aspects of this conceptual change in energy channels can be seen at the LPD site where the invaded vegetation had faster decomposition and higher nitrate than the native vegetation, although a great deal of variation existed among sites. Although the results are limited by a small number of sites, degradation of the native vegetation may affect the stability of the system and push degraded systems towards fast, bacterial impacts to labile, high N litter sources, while intact native plant communities remain in slow, fungal dominated cycles. Surprisingly, little differences in the soil or litter microbial communities could be identified between vegetation types, although sites appeared to have distinct microorganism communities.

Many challenges still exist before management prescriptions and invasive species control will be based on manipulating ecosystem processes. As our methods of microbial analysis and quantification improve, our ability to understand the impacts of specific pathogens, plant growth promoting rhizobacteria (PGPR), and mycorrhizal fungi on plant communities and subsequent feedbacks increases. Eventually this may lead to targeting invasive species with specific pathogens, assisting native species with PGPR, and potentially influencing decomposition or manipulating soil nutrient availability via

microbes. Additionally, the impact of specific plants on the microbial community and nutrient cycling can also be incorporated into management practices, primarily based on the quantity and quality of their litter and rhizosphere exudates. This type of management alters basic ecosystem processes to guide dynamic and complex systems to a desirable outcome, although it can only work when a thorough understanding of the system exists. The multi-disciplinary nature of research in PSF demands that plant ecologists, microbiologists, entomologists, and soil scientists collaborate and continue to experiment in the complex world of plant-soil feedbacks.

For PSF to elucidate ecological mechanisms and innovative management techniques, we must synthesize concepts and methods from different fields. My research utilized inoculation to manipulate the soil microbial communities and indirectly affect nutrients and vegetation. Additionally, I studied ecological gradients to understand the interactions that litter quality and vegetation type have on microorganisms and subsequent nutrient dynamics. The synthesis of research in soil inoculation and decomposition are complimentary and together address the greatest challenge of studies in PSF, our lack of knowledge about what is really going on belowground. Most research in PSF has dealt with the soil and microorganisms as a black box or used a few specific bacterial or fungal isolates. I attempted to address the full cycle of a feedback system by incorporating data on soil biogeochemistry and the richness and diversity of vegetation and microbial systems. To make sense of the complex systems, I utilized ordination and stochastic gradient boosting machines (regression trees and machine learning) to bring order and pattern to the chaos. These diverse datasets and novel techniques revealed

some interesting results that will further research in PSF and management practices that address ecological processes to achieve goals.

Overall, my field and greenhouse experiments show that soil inoculation can modify all aspects of PSF systems, native and invasive species respond to different types of competition (exploitative versus interference), and that plant species may contribute to modification of a system's energy channels due to their litter inputs. Lastly, the strengths of inter- and intraspecific competition varied and my results show that an invasive species may avoid most negative effects of plant competition, but not microbial interactions. My research would have benefited from a higher degree of resolution of microbial communities, especially the quantification of pathogens, and additional sites for field experiments. Some of my conclusions have limited applicability, since I had few sites or species in the experiments. Nonetheless, the results provide valuable information about PSF and how the manipulation of microbial communities might be utilized to impact feedbacks and manage vegetation. Plant-soil feedback is a relatively new discipline and requires extensive research and experimentation to establish basic guidelines that can be applied to management problems. As our foundation of knowledge increases, it may be possible to manage ecosystems by directly affecting specific ecological processes. This approach will dramatically change how we interact with ecosystems and our current paradigm of native versus invasive species.

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APPENDIX A (Chapter 3) – Native plant seed mix for the Soil Inoculation Experiments. Table 1 lists the seed mixture applied at the Fort McCoy WI (FMC) and Yakima Training Center WA (YTC) sites in 2005 and at a pure live seed (PLS) rate of 538 pls/m². Table 2 lists the seed mixture applied at the Waverly and Weld County CO sites in 2008 and at a PLS of 861 pls/m². Each table includes the scientific name (% of total seed mix), plant family, and plant functional group classification.

Table 1 – FMC WI and YTC WA seeding mix

Species	Family	Functional Group
<i>Coreopsis tinctoria</i> (24%)	Asteraceae	annual forb
<i>Oenothera pallida</i> (23%)	Onagraceae	perennial forb
<i>Pascopyrum smithii</i> (15%)	Poaceae	perennial graminoid
<i>Pseudoroegneria spicata</i> ssp. <i>spicata</i> (15%)	Poaceae	perennial graminoid
<i>Sphaeralcea munroana</i> (23%)	Malvaceae	perennial forb

Table 2 - Waverly and Weld County CO seeding mix

Species	Family	Functional Group
<i>Amaranthus retroflexus</i> (5%)	Amaranthaceae	annual forb
<i>Bouteloua curtipendula</i> (5%)	Poaceae	perennial graminoid
<i>Bouteloua gracilis</i> (12.5%)	Poaceae	perennial graminoid
<i>Buchloe dactyloides</i> (10%)	Poaceae	perennial graminoid
<i>Cleome serrulata</i> (5%)	Capparaceae	annual forb
<i>Coreopsis tinctoria</i> (5%)	Asteraceae	annual forb
<i>Gaillardia aristata</i> (5%)	Asteraceae	perennial forb
<i>Helianthus annuus</i> (5%)	Asteraceae	annual forb
<i>Koeleria macrantha</i> (5%)	Poaceae	perennial graminoid
<i>Lupinus argenteus</i> ssp. <i>rubicaulis</i> (10%)	Fabaceae	perennial forb
<i>Pascopyrum smithii</i> (5%)	Poaceae	perennial graminoid
<i>Rudbeckia hirta</i> (5%)	Asteraceae	perennial forb
<i>Schizachyrium scoparium</i> (5%)	Poaceae	perennial graminoid
<i>Solidago canadensis</i> (5%)	Asteraceae	perennial forb
<i>Sphaeralcea coccinea</i> (7.5%)	Malvaceae	perennial forb
<i>Sporobolus cryptandrus</i> (5%)	Poaceae	perennial graminoid

APPENDIX B (Chapter 3) – Explanatory (Independent) variables included in Generalized Boosting Machine (GBM) analysis of Soil Inoculation Experiments in Colorado, Washington, and Wisconsin. Variables are organized alphabetically within general categories.

Soil Microorganism Fatty Acid Methyl Esters (FAME) Functional Diversity

Variables (all units are nmoles per gram dry soil, except ratios):

Actinobacteria, Arbuscular Mycorrhizal Fungi (AMF), anaerobic proteobacteria, cyanobacteria & actinobacteria, fungi, proteobacteria, firmicutes, protozoa, richness (based on individual fatty acid markers), saprophytic fungi, Shannon's index of diversity, Simpson's diversity index, standard deviation, ubiquitous FAMEs.

Microbial Biomass Variables (Chloroform Fumigation Extraction method, all units are ug per g dry soil, except ratios):

microbial biomass carbon, microbial biomass nitrogen, microbial biomass carbon:microbial biomass nitrogen ratio.

Soil Biogeochemical Variables:

calcium (ppm), carbon (%), carbon:nitrogen ratio, carbon:phosphorus ratio, CEC, magnesium (ppm), nitrogen (ppm), pH, phosphorus (Bray 1P ppm), potassium (ppm), sodium (ppm), sulfur (ppm), zinc (ppm).

Vegetation variables (2009 data unless otherwise noted):

biomass knapweeds (g/m^2), biomass native species (g/m^2), biomass total vegetation (g/m^2), cover exotic species (%), cover knapweed (%), cover native species (%), density knapweed 2008 and 2009 (per m^2), evenness index, richness, Shannon's index of diversity, Simpson's diversity index.