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# Biochemical effects of Centaurea maculosa on soil nutrient cycles and plant communities

Andrea S. Thorpe
*The University of Montana*

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# BIOCHEMICAL EFFECTS OF *CENTAUREA MACULOSA* ON SOIL NUTRIENT CYCLES AND PLANT COMMUNITIES

by

Andrea S. Thorpe

M.S. San Diego State University, 2001

B.S. Oregon State University, 1998

presented in partial fulfillment of the requirements

for the degree of

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Approved by:

Chairperson

Dean, Graduate School

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ProQuest Information and Learning Company
300 North Zeeb Road
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The Biochemical Effects of *Centaurea maculosa* on soil nutrient cycles and plant communities

Chairperson: Ragan M. Callaway

The success of some invasive plants may be due to biochemcials that are novel to the invaded ecosystems. This is because plants, herbivores, and soil microbes native to invaded communities may lack a coevolutionary-based response to biochemical traits of the invader. In addition, these biochemicals may also alter abiotic components of soil nutrient cycles.

I measured the effects of *Centaurea maculosa* and the root exudate, (±)-catechin, on the soil nitrogen and phosphorus cycles and plant communities. Most importantly, I also compared the strength of many of these effects in the invaded range to those in the native range. My research provides evidence that *C. maculosa* has strong, biochemical effects on soil nutrient cycling, at least partially due to exudation of the polyphenol, (±)-catechin. Nitrification in soils from part of the invaded range of *C. maculosa* (Montana) was strongly reduced by both *C. maculosa* and application of (±)-catechin. In contrast, there were very weak effects on nitrification in soils from part of the native range of *C. maculosa* (Romania). A different study found that *C. maculosa* also has strong effects on the soil phosphorus cycle. In a greenhouse experiment and field study, I found that *Centaurea maculosa* was more phosphorus efficient than many native species, even when phosphorus was highly limiting. However, in the field study, soil phosphate was higher in areas dominated by *C. maculosa* than in areas where the invasive had been removed. I suggest that elevated soil phosphate and the resulting phosphorus-efficiency of *C. maculosa* are due to the exudation of (±)-catechin, a strong chelator for the metals that bind phosphorus in many of the soils invaded by *C. maculosa.* Finally, in a series of studies repeated over two years, I found that when tested *in situ*, (±)-catechin had strong allelopathic effects on the growth of several plant species in Montana, but very weak effects on species in Romania. Thus, *C. maculosa* appears to alter not just the aboveground plant community, but also fundamental ecosystem properties. These effects may persist long after the removal of the invasive species. Furthermore, biogeographic comparisons suggest that coevolution plays an important role in both plant community interactions and plant-soil interactions.

# ACKNOWLEDGEMENTS

For most of my life, I knew that I wanted to by a biologist. As early as I can remember, I would watch *Marty Stouffer's Wild America* and *National Geographic* specials, wanted to do "that," and from fairly early on, this dream included the graduate-school journey that is soon about to end. The achievement of this goal is due not only to my own hard-work, but the support and encouragement of so many people to whom I owe my gratitude. Before I go any further, I must thank my family, particularly my parents, MaryAlice and Royce Thorpe. Mom and Papa have never swayed in their love, encouragement, support, and interest in my endeavors. They also instilled in me a strong work ethic, sense of values, and stubbornness of character that I believe have contributed greatly to my success.

My eduction prior to coming to the University of Montana was shaped by many individuals. I must thank the many teachers (including those who insisted that a women had no role in science!) and professors (particularly Dr. Mark Wilson, OSU, and Dr. S. Hurlburt, SDSU) who educated and challenged me. My interest in interactions between plants and the soil ecosystem and applied ecology was fostered by my experiences as a student trainee with the USDA Natural Resources Conservation Service (thank-you Ken and Patrick!) and during a summer research experience with Dr. J.B. Kauffman. I am also very grateful for my Masters advisor, Jay Diffendorfer. His first year as a professor at San Diego State University, he (a small mammal biologist) took a chance on someone who wanted to study plants, offered me guidance and advice when I needed it, and added to my confidence as a scientist simply by believing in me.

I feel lucky that the last stop on this particular journey was at The University of Montana. My advisor, Ray Callaway believed in me and gave me freedom to explore my ideas; I am grateful for his support, generosity, and innovative thinking. I am also very grateful for Tom DeLuca, who has practially been an unofficial co-advisor. I am very appreciative of his advice, patience, and support. Anna Sala, John Maron, and Matthias Rillig provided many insightful comments and support throughout my Ph.D. The majority of the research in this dissertation would not have been possible without the generous help of Dr. Alecu Diaconu, with the Institute of Biological Research in Iaşi, Romania. Tomo Diaconu provided valuable assistance with field work in Romania and treated me as a member of his extended family. I also owe a big "mulțumeşc" to Marrius Grecu and Valentin Cosma not only the tireless translation and field assistance, but bringing a lot of fun to my trips to Romania. Many, many thanks to the Callaway lab members and hangers-on. My first year at UM, Brad, Kurt, Beth, and October served as stand-in advisors while Ray was on sabbatical – I wouldn't have gotten through that year without them. I count Giles, Courtney, Nick, Kendra, and José among my friends as well as co-workers, and they made the experience very enjoyable. I thank Caecelia and Tobey for their steadfast companionship these last several years. I am very grateful for my friends from Missoula, especially Clara, Randy, Anne, Kristina, Bruce, and Eric, for helping me maintain perspective, for the stress relief, for nights with *Sex and the City* or *Desperate Housewives*; Kettlehouse, Old Post, and Iron Horse nights; excursions into the woods; and long rides on Montana's back roads. Finally, I must thank Tom Kaye with the Institute for Applied Ecology for his support. It is fitting that I started the next chapter in my life as I was still finishing this one, particularly since it is so closely tied with my past.

## PREFACE

Recently, it has been suggested that novel biochemical constituents of invasive species may contribute to their success (Rabotnov 1982; Callaway & Aschehoug 2000; Czarnota *et al.* 2001; Bais *et al.* 2003; Ehrenfeld 2003; Vivanco et al. 2004; Cappoccino & Carpenter 2005; Carpenter & Cappoccino 2005). The Novel Weapons Hypothesis (Rabotnov 1982; Callaway & Aschehoug 2000; Mallik & Pellissier 2000; Baldwin 2003; Callaway & Ridenour 2004; Callaway & Hierro 2006) suggests that the success of some invasive species may be because native species lack a coevolutionary-based response to biochemical traits of the invasive species. It is also possible for novel biochemicals to have novel effects on the abiotic components of an ecosystem. This dissertation is an analysis of the biochemical effects of invasion on soil nutrient cycling and plant communities. Each chapter was written as a manuscript intended for publication in a peer reviewed publication.

In Chapter 1, I discuss interactions between invasive species and the soil ecosystem (pathogenic and nutrient-cycling components), the mechanisms for these interactions, and evidence that these interactions have very different effects on the survival of invasive versus native species. There is evidence that the success of some invasive plants may be due, at least in part, to different interactions with the soil microbial community compared to native plant species. If plant-soil feedbacks drive variation in fitness among individuals, then ultimately feedback interactions may be under selective pressure. I propose that the longevity of positive feedback interactions in evolutionary time may depend on which components of the soil ecosystem the plant interacts with most strongly. This chapter is in press as a chapter of the book, *Conceptual Ecology and Invasions Biology: Reciprocal Approaches to Nature* (Thorpe et al. 2006b).

Chapter 2 investigates the effects of the invasive forb, *Centaurea maculosa* Lam. (Asteraceae) on several aspects of the soil nitrogen cycle, and the role of a root exudate of *C. maculosa*, the polyphenol, (±)-catechin, on ammonification and nitrification. Most importantly, I also compared the strength of these effects in the invaded range to those in the native range. I found that *C. maculosa* and application of (±)-catechin had very strong inhibitory effects on nitrification in Montana, but very weak effects on nitrification in Romania. This is the first study to demonstrate that the effects of an invasive plant on soil ecosystem processes differ between the native and invaded ranges of the species.

In Chapter 3, I present the results of field and greenhouse studies that explored the P efficiency of *C. maculosa* and its effects on soil P levels. *Centaurea maculosa* was P efficient in a greenhouse experiment and field study, taking up at least twice the amount of P than native species, even when soil P was extremely limiting. However, soil P levels were elevated in sites invaded by *C. maculosa*, the opposite of what is expected if root or mycorrhizal uptake were responsible for the P efficiency of *C. maculosa*. It is likely that the increased levels of P in *C. maculosa* rhizospheres is due to the exudation of (±)-catechin, a known chelator. This chapter is in press in *Applied Soil Ecology* (Thorpe et al. 2006a).

In the final chapter, I present the results of two years of *in situ* experiments testing the effects of (±)-catechin in parts of both the invaded (Montana) and native (Romania) ranges of *C. maculosa*. I found substantial variability in the effects of (±)-catechin between years and among species, but (±)-catechin caused a large reduction in the growth of most native species in Montana and had very weak effects on species in Romania. This is the first *in situ* test of the Novel Weapons Hypothesis.

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# Chapter 1

# Interactions between invasive plants and soil ecosystems: positive feedbacks and their potential to persist

## Abstract

Complex interactions between plants and soil microbial communities play important roles in determining the relative abundance of plant populations, interactions among plant species, and the organization of plant communities. Plants directly affect soil microbial structure and activity, which has important implications for feedbacks due to the many functions of soil microbes, including pathogenicity, mutualism, herbivory, decomposition, and nutrient mineralization that affect plants. Feedbacks between plant and microbial communities have important effects on plant community structure and processes, including succession and the maintenance of diversity.

There is evidence that the success of some invasive plants may be due, at least in part, to different interactions with the soil microbial community compared to native plant species. For example, several invasive plant species benefit from positive feedbacks; for example, they take advantage of local mycorrhizal fungi, but are not significantly affected by soil pathogens where they invade. If plant-soil feedbacks drive variation in fitness differences among individuals, then ultimately feedback interactions may be under selective pressure. In general, positive feedbacks are likely to lead the plant community to shift towards a monoculture of the invasive species. However, I propose that the longevity of positive feedback interactions in evolutionary time may depend on which components of the soil ecosystem the plant interacts with most strongly. Through evolution, pathogens may respond relatively rapidly to the invasion of non-native plants, which may ultimately lead to greater coexistence among species. In contrast, feedbacks between invasive plants and nutrient cycles may be much more likely to persist. When the biotic component of the soil nutrient cycles is involved, it is expected that there would be a shift in the microbial community to populations that are better adapted to the new nutrient status of the ecosystem. In the case of alterations to abiotic components of the soil ecosystem, there is no direct selective pressure to stimulate adaptation.

Here, I discuss interactions between invasive species and the soil ecosystem (pathogenic and nutrient-cycling components), the mechanisms for these interactions, and evidence that these interactions have very different effects on the survival of invasive versus native species.

Keywords: positive feedbacks, invasive plants, evolution, soil microbial community, soil ecosystem

## Introduction

Plants indirectly affect their neighbors in many ways, but one of the most important is by altering the biotic, physical, and chemical characteristics of soils (Hobbie 1992; Angers and Caron 1998; Berendse 1998; Binkley and Giardina 1998; Northrup et al. 1998; Schlesinger and Pilmanis 1998; Van Breemen 1993; Wardle et al. 1998; Chen and Stark 2000; Eaton and Farrell 2004). These general effects have been understood for decades, but only recently have experiments demonstrated that complex interactions between plants and soil microbial communities can have strong effects on plant populations (Bever et al. 1997; Clay and Van der Putten 1999; Packer and Clay 2000), interactions among plant species (West 1996), and the organization of plant communities (Grime et al. 1987; Van der Putten et al. 1993; Bever 1994; Van der Putten 1997; van der Heijden et al. 1998; Hooper et al. 2000; Klironomos 2002). Soil communities alter competitive outcomes among plants through their pathogenic effects (Van der Putten and Peters 1997), by favoring obligate mycorrhizal species over non-mycorrhizal or facultative mycorrhizal species (Hetrick et al. 1989; Hartnett et al. 1993), and by transferring resources and fixed carbon between species (Chiarello et al. 1982; Francis and Read 1984; Grime et al. 1987; Moora and Zobel 1996; Watkins et al. 1996; Simard et al. 1997; Marler et al. 1999; but see Robinson and Fitter 1999). By altering the biotic and abiotic characteristics of soils, plants can drive positive or negative feedbacks (box 1), and these feedbacks can profoundly affect plant populations and communities.

Jim Bever (1994; Bever et al. 1997) proposed the feedback model, in which a plant or population of plants alters the composition of the soil community. This change then feeds back to affect the growth and survival of the plant or plant population. These models described how two different types of feedback could be established; positive feedback, which should occur when the negative effects of soil pathogens are outweighed by the beneficial effects of mutualistic mycorrhizae, and negative feedback, which is expected to occur either when the effects of pathogens outweigh the effects of mycorrhizae or when the soil community enhances the growth of competing plants more than that of the associated plant (Bever 1994; Bever et al. 1997). By favoring the local replacement of species, negative feedback is expected to maintain species diversity, whereas positive feedback is expected to lead to species dominance and a decrease in local species diversity (Bever 1994; Bever et al. 1997; Watkinson 1998). A growing body of research has demonstrated that plant-soil feedbacks can have important ecological consequences (Bever et al. 1996; Wardle and Nicholson 1996; Westover et al. 1997; Klironomos 2002). For example, in a study comparing feedback and relative abundance of 61 co-existing old field species from southern Ontario, Canada, Klironomos (2002) found a strong positive relationship between a plant's feedback with the soil community and its relative abundance (Fig. 1). Plant species that were found in low abundance in the field consistently displayed negative feedback interactions, whereas plants with high abundance either had low negative or positive feedback interactions (Klironomos 2002).

Plant invasions provide an exceptional opportunity for understanding how plants affect soils and drive feedback processes. Numerous studies have documented the effects of invasive species on the composition of soil biota (Belnap and Phillips 2001; Kourtev et al. 2002) and soil microbial function (Kourtev et al. 2002; Ehrenfeld 2003). However, much less is known about the role of feedbacks in the success of exotic plants. Feedbacks may also affect evolution – potentially most apparent in the rapid evolutionary changes that can accompany exotic invasion (Rice and Emory 2003).

If plant-soil feedbacks drive variation in fitness differences among individuals, then ultimately feedback interactions may be under selective pressure (Van der Putten 1997; Van Breeman and Finzi 1998). Based on theory developed by Bever et al. (1997) and Klironomos (2002), we hypothesize that positive feedbacks in general are likely to lead the plant community to shift towards a monoculture of the invasive species.

However, we propose that the longevity of positive feedback interactions in evolutionary time may depend on whether the invasive plant is having its strongest effects on the pathogenic or the nutrient-cycling components of the soil ecosystem (Fig. 2). Microbes have a short generation time and thus can respond to evolutionary pressures quickly. Therefore pathogens may respond relatively rapidly to the invasion of non-native plants. This may ultimately lead to greater coexistence among species, where, although the invasive may still be present, it will not be the over-whelming community dominant. In contrast, feedbacks between invasive plants and nutrient cycles may be much more likely to persist. When the biotic component of the soil nutrient cycles is involved, it is expected that there would be a shift in the microbial community to populations that are better adapted to the new nutrient status of the ecosystem (Fig. 2; Atlas and Bartha 1998; Schimel and Bennett. 2004). In the case of alterations to abiotic components of the soil ecosystem, there is no direct selective pressure to stimulate adaptation (Fig. 2).

Here we discuss interactions between invasive species and the soil ecosystem (pathogenic and nutrient-cycling components), the mechanisms for these interactions, and evidence that these interactions have very different effects on the survival of invasive versus native species.

## II. Invasive plants and soil pathogens

One of the leading hypotheses for the remarkable success of some exotic species is that they have escaped the specialist enemies that control them in their native ranges (Keane and Crawley 2002). Embedded within this hypothesis is the idea that if microbial pathogens limit the growth of invasive plants in their native range, there will be negative feedbacks between the soil microbial community and the plant in the native range due to the accumulation of species-specific soil pathogens (Klironomos 2002; Mitchell and Power 2003; Callaway et al. 2004). In contrast, positive feedbacks may occur in the invaded range where exotic species are largely free from species-specific soil pathogens but can still interact with less host-specific mutualists such as mycorrhizal fungi and bacteria that drive nutrient cycles.

In this section, first we will describe the evidence that invasive species have escaped pressure from soil pathogens and the potential feedback effects on the fitness of invasive species. Second, we will discuss the implications of this type of feedback for ecosystem stability.

### *1. Do invasive species benefit from escaping soil pathogens?*

In a review of 473 species that were naturalized in the United States, Mitchell and Power (2003) found that 84% fewer fungi and 24% fewer virus species infected the plant species in their invaded ranges compared to their native ranges. Furthermore, they reported that species that experienced greater release from microbial pathogens were more invasive (Mitchell and Power 2003). However, the relationships were relatively weak, particularly for invaders of natural areas, soil pathogens were not distinguished from other pathogens, and the presence or abundance of pathogens does not necessarily correlate with the strength of their effects.

More recently, experiments using soils from native and invaded ranges have suggested that some invasive species have escaped from soil pathogens. If invasive species suffer from the effects of soil pathogens in their native soils, then sterilization of native soils should result in an increase in the growth of the invasive. In contrast, in invaded soils, the invasive should be relatively free from pathogens but may benefit from less host-specific mutualistic microbes. If so, sterilization of invaded soils should result in a neutral to negative effect on the invasive species. These interactions have been explored for *Prunus serotina* (black cherry), *Centaurea maculosa* (spotted knapweed) and two *Acer* (maple) species.

Reinhart et al. (2003) compared the effects of soil microbes on the growth of *Prunus serotina* in both its native and invaded ranges. In its native, North American range, the soil microbial community occurring near *P. serotina* strongly inhibited the establishment of neighboring conspecifics and reduced seedling performance in the greenhouse. In contrast, in its non-native European range, *P. serotina* readily establishes in close proximity to conspecifics, and soil microbial communities enhance the growth of seedlings. Previous research in the native range of *P. serotina* demonstrated that soil-borne *Pythium* species (Oomycota) inhibit the survival, growth, and abundance of *P. serotina* (Packer and Clay 2000, 2002). Although the genus *Pythium* is found around the world, genotypes are often host-specific (Deacon and Donaldson 1993; Mills and Bever 1998). Thus, in the native range, *P. serotina* experiences negative plant-soil feedbacks interactions, likely due to the negative effects of *Pythium*. In contrast, in the invasive region, *P. serotina* experiences positive feedbacks due to escape from its main natural enemy (Reinhart et al. 2003).

*Centaurea maculosa* is one of western North America's worst invasive weeds. In several experiments, Callaway et al. (2004) have compared the effects of soil microbes from the native range in Europe to the effects of soil microbes collected from invasive populations in the northwestern United States. European soil biota had much stronger inhibitory effects on *C. maculosa* than North American soil biota. Sterilization of European soils caused, on average, a 166% increase in the total biomass of *C. maculosa*, suggesting a release from pathogenic microbes. In contrast, sterilizing invaded North American soils led at most to a slight increase in total biomass of 24%. For most North American soils, sterilization led to a decrease in growth of 20-30%, suggesting that *C. maculosa* had benefited from mutualistic soil microbes. These results support Mitchell and Power's (2003) conclusion that invasive species should suffer much higher fungal and viral infection in their home ranges compared to invaded ranges. They also suggest that in some cases, mutualisms may be more beneficial in non-native ranges because the negative effect of natural enemies do not attenuate the positive effect of mutualists.

Mutualists have also been found to play an important role in the plant-soil feedback interactions of two *Acer* species (Reinhart and Callaway, *in press*). In the field, distances between *Acer* conspecifics were 56-77% less in their invaded ranges than in their native ranges. In a greenhouse experiment, the effect of soil microbial communities also differed between native and invaded ranges. Relative to sterilized controls, soil associated with both conspecifics and heterospecifics from the native range decreased the total biomass of *Acer* seedlings by 35% suggesting inhibition by pathogenic microbes. In the invaded range, soil associated with conspecifics decreased the biomass of *Acer* seedlings by an even greater magnitude, 112%. However, soil associated with heterospecifics in the non-native ranges *increased* biomass of *Acer* seedlings by 13%. Thus, while *Acers* accumulate pathogens in their invaded range, the surrounding soil is relatively free from inhibitory microbes, potentially enhancing invasion by these trees.

Thus, there is evidence that, not only do invasive species escape the negative effects of soil pathogens in their invaded ranges, but that potentially due to the effects of mutualists, feedback effects in invaded ranges are often positive. Next, we will explore the potential for these feedback effects to affect community stability.

### *2. Will escape from negative feedbacks from soil pathogens persist?*

The experiments described above indicate that invasive species are likely to experience positive feedback in their invaded habitat because they escape specialist soil pathogens at home but can utilize generalist mutualists where they invade. What remains to be determined is how this feedback affects community dynamics. Plants participating in strong positive feedbacks with soil biota are more likely to become community dominants than those that do not. The most complete study of these interactions was done by Klironomos (2002), who explored feedback interactions among plant species and soil microbial communities in grasslands in eastern North America. In experiments using only the mycorrhizal fraction of the microbial community, he found that the origin of the filtrate (from soils in which the same species or a different species had previously been grown) did not alter the response (either positive or neutral) to mycorrhizal fungi. In contrast, in experiments using only the pathogenic/saprobic fractions, the rare native species experienced negative feedbacks when the fractions were from soils that had previously grown the same species. However, the origin of the pathogenic/saprobic fraction had no effect on the growth of invasive species. Overall, relatively rare native species consistently exhibited negative feedback interactions with the soil microbial community (a relative decrease in growth on 'home' soil in which conspecifics had previously been grown), whereas invasive species consistently exhibited positive feedback interactions with the soil community. Similarly Agrawal et al. (*in review*) found that introduced plants were subject to half the negative soil feedback as congeneric species. How long, in terms of evolutionary time scales, such positive interactions will be maintained remains an unknown.

The basic nature of microbes suggests that they will be able to respond relatively quickly to pressures exerted by invasion by exotic plants. As discussed above, soil-borne pathogens can be relatively host specific (Neergaard 1977; Kirkpatrick and Bazzaz 1979; Harman 1982; Agarwal and Sinclair 1997; Mills and Bever 1998). However, many soil-borne pathogens are generalists (Dix and Webster 1995). For example, Blaney and Kotanen (2001) found that seed germination of 15 congeneric pairs of invasive and native plant species from western Ontario displayed a similar positive response to the application of fungicide, suggesting non-species specificity of fungal seed pathogens in their system. Furthermore, microbes have a short generation time and thus can respond to evolutionary pressures within a short timeframe. Thus, pathogenic microbes may be able to rapidly switch to a new invasive host. *Sclerotinia sclerotiorum*, a fungus native to intermountain prairies invaded by *C. maculosa*, has been found to damage *C. maculosa* when applied to the rhizospheres at high concentrations (Jacobs et al. 1996, Ridenour and Callaway 2003). As described above, Reinhart and Callaway (*in press*) found that while the soil community associated with other tree species had a positive effect on the growth of invasive *Acers*, soils associated with conspecifics had a negative effect. This suggests that the pathogenic soil microbial community may have been able to adapt to this new host, and accumulation of soil pathogens eventually suppresses the offspring of *Acer* recruits.

In summary, some invasive species appear to have escaped pressure from soil pathogens and thus benefit from positive feedback interactions with the soil biota where they invade. Although not yet explicitly addressed in the literature, microbial communities may change over time and thus break down positive plant-soil microbial feedbacks. If this occurs, the abundance of the invasive species should decrease, and the community should move to a point where negative feedback interactions restrict the invasive's dominance. This was originally suggested by Klironomos (2002) who observed that plant-specific pathogen loads are maximized under high population densities, particularly monocultures such as those created by some invasive species, and will eventually result in negative feedback on abundant plants. Next we consider how feedbacks driven by microbes in nutrient cycles might respond differently over evolutionary time than feedbacks driven by soil pathogens.

## III. Invasive plants and soil nutrient cycling

In general, plant-soil feedbacks are thought to be determined by the direct effects of pathogens and mutualists (Bever 1994; Mills and Bever 1998; Packer and Clay 2000; Bever 2002; Klironomos 2002), but other components of the soil ecosystem may participate in feedbacks. In particular, individual plant characteristics, such as phenology, nutrient uptake, litter-fall, tissue chemical composition, and association with symbiotic microbes, can have significant effects on soil nutrient cycles (Hobbie 1992; Angers and Caron 1998; Berendse 1998; Binkley and Giardina 1998; Northrup et al.

1998; Schlesinger and Pilmanis 1998; Van Breemen 1993; Wardle et al. 1998; Chen and Stark 2000; Eaton and Farrell 2004) which may, in turn, alter the growth and survival of the species that drive these effects. Because they are novel, may have different biochemical constituents (Bais et al. 2003, Vivanco et al. 2004), and are often dominant components of plant communities, invasive plants can have unusually strong effects on soil nutrient cycles (Vitousek 1986; Vitousek et al. 1987; Vitousek 1990; D'Antonio and Vitousek 1992; Ehrenfeld et al. 2001; Ehrenfeld and Scott 2001; Ehrenfeld 2003).

In the previous section we showed that many invasive species exhibit positive feedbacks after escaping soil pathogens, and then speculated that these feedbacks may eventually become neutral or negative as generalist pathogens switch to the host or specialists adapt. In contrast to this scenario in which invasive dominance may fade, positive feedbacks between invasive plants and soil nutrient cycles may lead to much longer time periods of invasive dominance. Nutritional constraints may lead to significant shifts in microbial communities, resulting in long-term changes in nutrient pools and cycling rates. These changes may also occur due to the introduction of novel plant-microbe interactions, such as symbiotic nitrogen-fixation. Similarly, the direct effects of invasive plants on soil nutrients may be particularly long-lived because there is no mediation by another organism with the potential to evolve.

In this section, we briefly review the mechanisms by which invasive species may alter soil nutrient cycles and illustrate the potential for long-lived positive feedback interactions by describing the interactions of *Bromus tectorum* (cheatgrass, downy brome) and *Myrica faya* (fire tree) with soil nutrient cycles in invaded communities in the western United States and Hawaii.

### *1. The effects of invasive species on nutrient cycles*

There are many mechanisms by which invasive species may alter soil nutrient cycles (see review by Ehrenfeld 2003). Through changes in litter production and quality, invasive plants may increase (Ehrenfeld et al. 2001; Mack et al. 2001) or decrease (Saggar et al. 1999; Ehrenfeld et al. 2001; Evans et al. 2001) microbially-mediated decomposition and/or mineralization rates. For example, litter of *Microstegium vimineum*, an exotic $C_4$ grass that has invaded Eastern deciduous forests, has a higher C:N ratio, decomposes slower, and immobilizes more N than litter from uninvaded forests (Ehrenfeld et al. 2001).

Invasive species may also alter the input of nitrogen by nitrogen-fixing bacteria. Nearly 10% of the invasive species listed by the U.S. Department of Agriculture are in the Fabaceae family (Ehrenfeld 2003), and changes in ecosystem nitrogen availability due to association of invasive plants with symbiotic nitrogen-fixing bacteria have been documented in several ecosystems (Versfeld and van Wilgren 1986; Vitousek et al. 1987; Stock et al. 1995; Yelenik et al. 2004). Furthermore, changes in litter quality from non-nitrogen fixing invaders may alter the abundance and activity of non-symbiotic nitrogen-fixing bacteria, as found in Hawaiian forests invaded by African grasses (Ley and D'Antonio 1998).

Invasive species may affect soil nutrient cycles through the production of secondary chemicals. Roots of *Centaurea maculosa* exude the polyphenol, (±)-catechin. (+)-Catechin displays strong antimicrobial properties for at least some groups of bacteria (Bais et al. 2002, 2003) and appears to affect at least some aspects of the soil nitrogen cycle (A. Thorpe, *unpublished data*). Furthermore, by chelating metal-phosphorus complexes, catechin may increase phosphorus availability in phosphorus-limited soils (Thorpe et al. *unpublished manuscript*; Stevenson and Cole 1999). An allelochemical produced by *Centaurea diffusa*, 8-hydroxyquinoline, may also alter nutrient cycling through antimicrobial (Vivanco et al. *in press*) and chelation (The Merck Index 1996) properties. The dry mass of leaves of *Melaleuca* spp. (paperbark), which has invaded large areas of the coastal southeast United States, particularly the Everglades, is up to 7% monoterpenes (Boon and Johnstone 1997). These compounds inhibit microbial colonization and decomposition of leaf litter in both the native and invaded ranges of *Melaleuca* spp. (Boone and Johnstone 1997). It has also been suggested that allelopathic chemicals released by some invasive species may alter nitrogen-fixation in neighboring plants (Wardle et al. 1994, 1995). Many other invasive species produce chemicals with antimicrobial activity (Rice 1964; Ehrenfeld 2003), however, the role of these chemicals in the plants' invasive success is generally unknown.

In sum, there is good evidence that by introducing a novel characteristic (e.g. a higher C:N ratio, association with nitrogen-fixing bacteria, or exudation of an anti-microbial chemical), invasive species can alter soil nutrient cycles in invaded communities. Although explicitly studies of the ramifications of such alteration of nutrient cycles are rare, these effects may ultimately feed back to the plants that cause them and affect the organization of plant communities. Two different species, *Bromus tectorum* and *Myrica faya* provide excellent examples of (1) how invasive plants may affect the soil, and (2) how soil changes affect the survival of invasive and native species.

These studies also illustrate how positive feedbacks between invasive plants and soil nutrient cycles may persist in an ecosystem.

## *2. The effects of* Bromus tectorum *on soil nutrient cycles*

*Bromus tectorum* is an annual (occasionally biennial) Eurasian grass that has invaded over 40.5 million ha in the Intermountain West of North America (Ypsilantis 2003). The effects of *B. tectorum* on nutrient cycles differ in fire-prone and non-fire-prone systems.

*Bromus tectorum* tends to germinate and complete its life cycle earlier than most native species in the systems it invades, and its dead, dry stems create an unusually large fuel load in the summer (Harris 1967; Mack 1981; Upadhyaya et al. 1986; Ypsilantis 2003). In fire-prone sagebrush-grassland ecosystems, fire recurrence intervals decrease from 20-100 years to 3-5 years (Mack 1981; Upadhyaya et al. 1986; Ypsilantis 2003). Since *B. tectorum* germinates earlier and grows faster than most native species (Harris 1967; Mack 1981; Upadhyaya et al. 1986; Ypsilantis 2003), this invader appears to take better advantage of the post-fire flush of nitrogen than native species (Lowe et al. 2003). Early nitrogen uptake by *B. tectorum* reduces total soil nitrogen and creates higher soil carbon to nitrogen ratios than native vegetation (Blank et al. 1994; Halvorson et al. 1997). *Bromus tectorum* may also limit nitrogen availability by shading biological soil crusts that fix nitrogen (Ypsilantis 2003).

In ecosystems that lack fire, there are very different interactions between *B. tectorum* and the soil ecosystem. Grassland communities in Utah invaded by *B. tectorum* have higher levels of exchangeable potassium and ratios of potassium or phosphorus to calcium carbonate and magnesium or iron oxides than uninvaded soils (Belnap and Phillips 2001; Belnap et al. 2003). Although it is unknown whether these nutrient differences are due to *B. tectorum* invasion or if *B. tectorum* preferentially invades sites with these characteristics, it is clear that *B. tectorum* can dramatically alter phosphorus cycling in invaded soils. Although there is no net change in total soil phosphorus pools, *B. tectorum* appears to access forms of P that are recalcitrant and unavailable to natives, which increases levels of labile phosphorus (R.L. Sanford, *personal communication*).

By altering the biotic and abiotic components of nutrient cycles, *B. tectorum* alters nutrient availability in ways that ultimately feedback to increase its own survival relative to native species (Belnap and Phillips 2001; Evans et al. 2001). By altering fundamental ecosystem characteristics, these effects may significantly alter plant community structure and dynamics.

### *2. The effects of* Myrica faya *on soil nutrient cycles*

Invasion of Hawaiian ecosystems by the nitrogen fixing tree *Myrica faya* (Gerrish and Mueller-Dombois 1980; Vitousek 1986; Vitousek and Walker 1989; Aplet 1990; Hughes et al. 1991) provides an example of how ecosystem scale changes may result in “invasional meltdown” (box 2; Simberloff and van Holle 1999). The volcanic soils of Hawaii are usually nitrogen-limited (Vitousek 1986; Vitousek and Walker 1989). There are no native nitrogen-fixing plants that colonize early successional habitats in these systems (Vitousek 1986; Vitousek and Walker 1989), and invasion by *M. faya* introduces a novel ecosystem process that results in substantial increases in soil nitrogen (Vitousek 1986; Vitousek and Walker 1989). Most successful invasions in Hawaii have occurred on soils that are relatively fertile (Gerrish and Mueller-Dombois 1980) and the nitrogen fertilization that results from invasion by *M. faya* may facilitate invasion by other species with higher nitrogen requirements. For example, another invasive tree, *Psidium cattleianum* (strawberry guava) grew much larger when grown in soil from under *M faya* than from soil collected under the native *Metrosideros polymorpha* (`Ohi`a lehua; Hughes et al. 1991). *Myrica faya* also enhances populations of exotic earthworms, which increase nitrogen burial and further alter nutrient cycling (Aplet 1990). Thus, invasion by *M. faya* results in positive feedbacks that not only enhance the invasion of this species, but promote invasion by other invasive species and lead to fundamental ecosystem changes.

### *3. Will positive feedbacks between invasive plants and soil nutrient cycles persist?*

As described above, since invasive plants often differ from native species in characteristics such as phenology, nutrient uptake, litter-fall, tissue chemical composition, and association with symbiotic microbes, they can have significant effects on soil nutrient cycles. Similar effects are often seen during succession (Berendse 1998; Schimel and Bennett 2004). We speculate that, in contrast to our predictions for evolutionary change for interactions between invasive plants and soil pathogens, positive feedbacks between invasive plants and soil nutrient cycles are more likely to result in long term shifts in community composition that resemble succession. These changes may provide examples of invasional meltdown (Simberloff and Von Holle 1999).

We know of no examples where, during succession, characteristics of the soil ecosystem revert back those resembling the initial characteristics after disturbance. What little is known about the biotic components of the soil ecosystem during succession indicates that change in the microbial community is towards populations that are better adapted to the new nutrient conditions of the later successional stages (Schimel and Bennett 2004). These successional shifts appear to be ecological in nature, not evolutionary, and driven by shifts in the species composition of communities over time. In contrast, in the case of alterations to abiotic components of the soil ecosystem, there is no direct selective pressure to stimulate adaptation. Ultimately, these types of changes require that the soil and plant communities may have to respond adaptively to new nutrient conditions.

When invader-soil ecosystems feedbacks occur through effects on nutrient cycles, we suggest that long term effects will be similar to those that occur during succession, the development of a new community composed of species that are better adapted to the specific nutrient conditions of the soil (e.g. Berendse 1998). This has been observed in systems invaded by both native (Maron and Jefferies 1999) and invasive (Hughes et al. 1991) nitrogen-fixing species. In California, Maron and Connors (1996) found that nitrogen-rich patches that were left by death of *Lupinus arboreous* (bush lupine) were invaded by exotic annual grasses. Similarly, as described in the preceding section, soils that have been enriched by the exotic nitrogen-fixer *M. faya* are more prone to invasion by other exotic plants, including *Psidium cattleianum* (strawberry guava; Hughes et al. 1991). In addition, although only a few native species have been found to "monopolize" sites by creating positive feedbacks (van der Putten 1997), invasive species may be particularly capable of this, especially if they introduce a novel ecosystem process to which native species are not adapted. For example, although *B. tectorum* first became established in fire-prone communities in the Great Basin over 100 years ago (Mack 1981), no other species, either native or invasive, have been able to establish in substantial numbers. These examples show how ecosystem changes involving exotic species can result in unpredictable successional trajectories.

## IV. Conclusion

Exotic plant invasions can often involve positive feedbacks between the invader and the native soil community. Positive feedbacks have the potential to lead to long-lasting dominance in communities. However, these feedbacks can be caused by a number of different reasons, and different drivers of positive feedbacks may result in different predictions of how long invaders may dominate a community. Typically, positive feedbacks are thought to be caused by the absence of soil pathogens but the presence of soil mutualists in invaded soils. Such pathogen-driven positive feedbacks may result in long term invasive dominance, but evolutionary changes in native pathogens might ultimately lead to suppression of the invader and a return to native coexistence. Positive feedbacks can also be caused by the effects of invasive plants on the soil biota that drive nutrient cycles or on the abiotic components of the nutrient cycles themselves. We hypothesize that positive feedbacks caused by the effects of the invader on the soil biota involved in nutrient cycles are likely to lead to community shifts resembling succession and perhaps invasional meltdown. Dominance by invaders that drive positive feedbacks through abiotic components of nutrient cycles may persist for much longer than dominance occurring through positive feedbacks involving the microbial community due to the lack of mediation by an organism with the potential to adapt. However, understanding potential evolutionary changes among invaders and soil ecosystems will help us to accurately predict the long-term effects of biological invasions.

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**Box 1.**

Positive feedbacks occur when plant species accumulate microbes that have beneficial effects on the plants that cultivate them, such as mycorrhizal fungi and nitrogen fixers. Positive feedbacks promote species dominance and are thought to lead to a loss of local community diversity (Bever et al. 1997, Bever 2002). Negative feedbacks occur when plant species accumulate pathogenic microbes in their rhizospheres and these interactions outweigh the benefits received from mutualistic interactions. Negative feedbacks create conditions that are increasingly hostile to the plants that cultivate the pathogens and are thought to promote community diversity (Van der Putten et al. 1993, Bever 1994, Klironomos 2002).

**Box 2.**

In 1999, Simberloff and van Holle introduced the concept of "invasional meltdown." During this process, invasion by one exotic species promotes invasion by other exotic species. These species interact synergistically, causing ecosystem scale changes (such as more frequent fire cycles or more rapid nitrogen cycling through increased nitrogen fixation) that ultimately result in the collapse of native ecosystems.

**Figure 1**. The relationship between relative plant abundance in an old-field site and soil feedback response. Numbers represent different plant species. 1, *Carex garberi*; 2, *Carex aurea*; 3, *Carex granularis*; 4, *Daucus carota*; 5, *Agrostis gigantea*; 6, *Solidago graminifolia*; 7, *Solidago nemoralis*; 8, *Aster simplex*; 9, *Aster vimineus*; 10, *Aster novaeangliae*; 11, *Circium vulgare*; 12, *Chenopodium ambrosioides*; 13, *Oenothera biennis*; 14, *Carex flava*; 15, *Juncus dudleyi* ; 16, *Solidago canadensis*; 17, *Linaria vulgaris*; 18, *Cichorium intybus*; 19, *Circium arvense*; 20, *Solidago rugosa*; 21, *Geum aleppicum*; 22, *Satureja vulgaris*; 23, *Potentilla recta*; 24, *Coronilla varia*; 25, *Asclepias syriaca*; 26, *Achillea millefolium*; 27, *Apocynum cannabinum*; 28, *Hypericum perforatum*; 29, *Agrostis scabra*; 30, *Phleum pratense*; 31, *Poa compressa*; 32, *Echium vulgare*; 33, *Centaurea jacea*; 34, *Rudbeckia serotina*; 35, *Poa pratensis*; 36, *Dactylis glomerata*; 37, *Cerastium vulgatum*; 38, *Galium palustre*; 39, *Oenothera perennis*; 40, *Prunella vulgaris*; 41, *Trifolium pratense*; 42, *Convolvulus arvensis*; 43, *Silene cucubalus*; 44, *Erigeron strigosus*; 45, *Asparagus officinalis*; 46, *Hieracium auranticum*; 47, *Erigeron philadelphicus*; 48, *Veronica officinalis*; 49, *Plantago lanceolata*; 50, *Galium mollugo*; 51, *Hieracium pilosella*; 52, *Vicia cracca*; 53, *Hieracium pratense*; 54, *Medicago lupulina*; 55, *Ranunculus acris*; 56, *Taraxacum officinale*; 57, *Fragaria virginiana*; 58, *Chrysanthemum leucanthemum*; 59, *Tragopogon pratensis*; 60, *Bromus inermis*; 61, *Panicum lanuginosum.* Reprinted from Klironomos 2002.

**Figure 2**. Potential feedback interactions between invasive species and (A) soil pathogens, (B) microbes responsible for soil nutrient cycles, and (C) soil nutrients.

Fig. 1

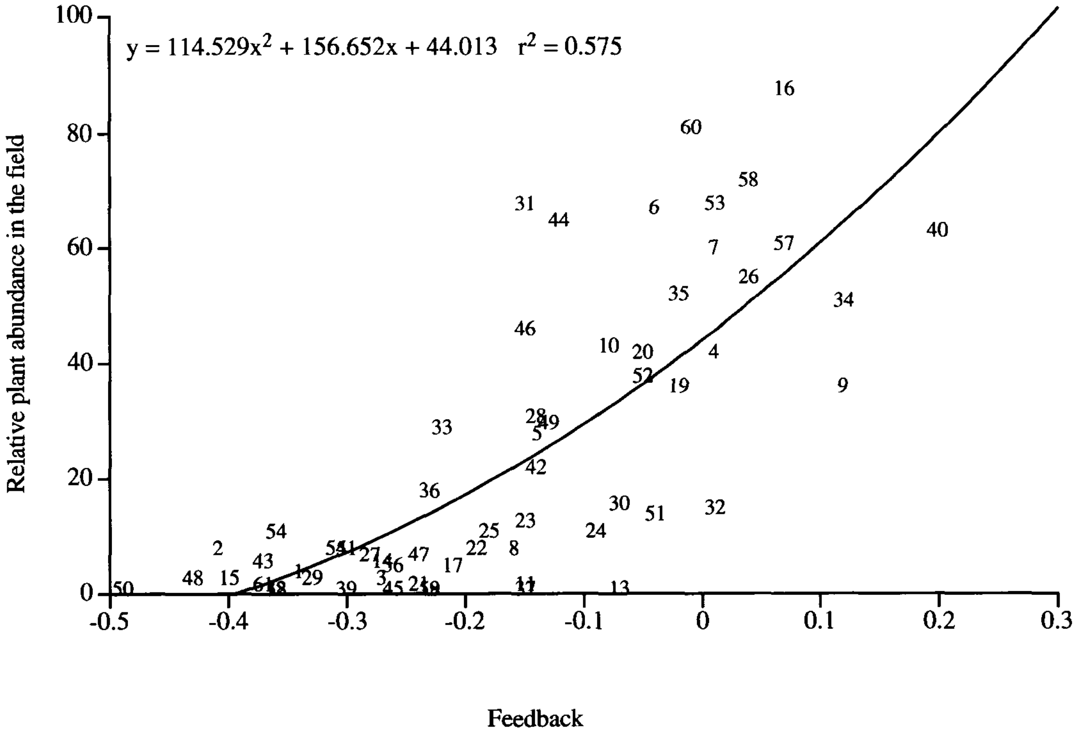
y = 114.529x$^2$ + 156.652x + 44.013 r$^2$ = 0.575
Relative plant abundance in the field
Feedback
0
20
40
60
80
100
-0.5
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
16
60
58
31
44
6
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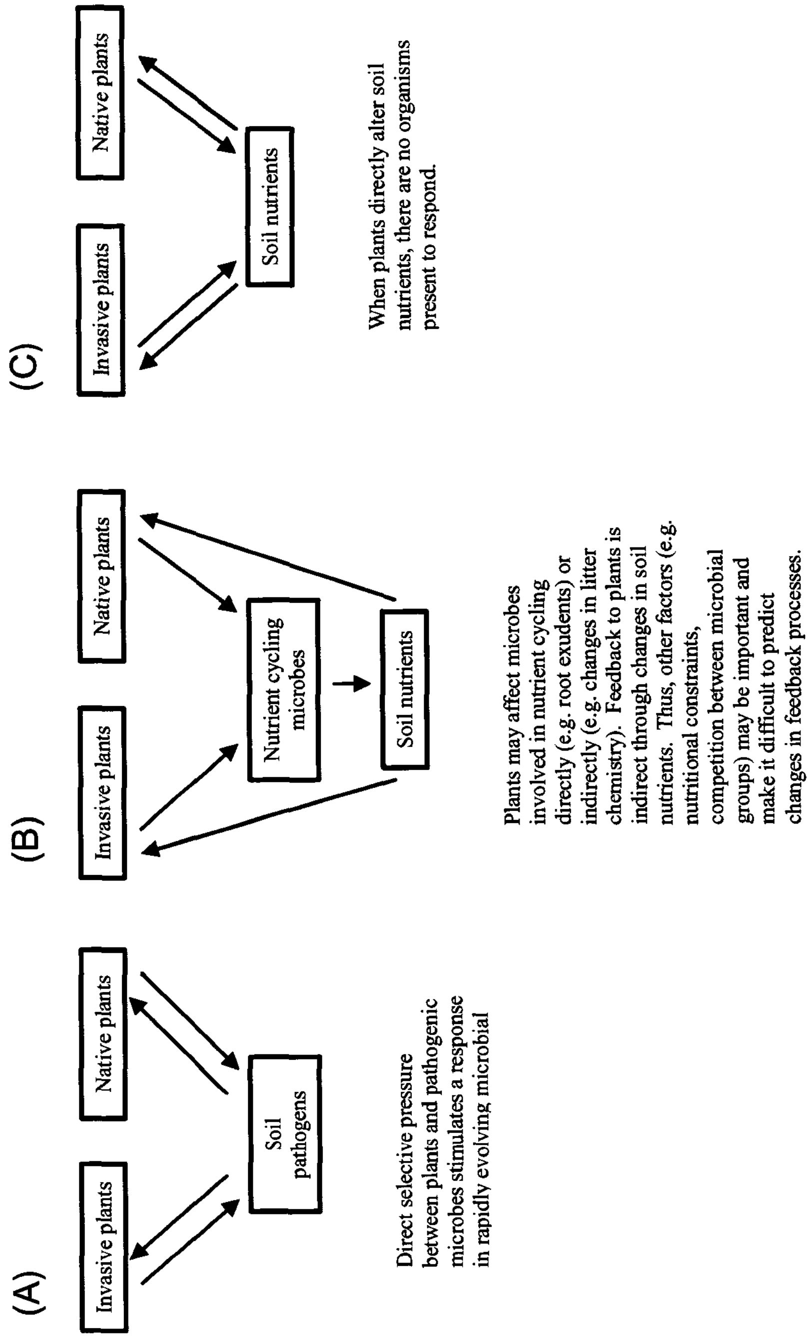
Fig. 2



(A) Direct selective pressure between plants and pathogenic microbes stimulates a response in rapidly evolving microbial

(B) Plants may affect microbes involved in nutrient cycling directly (e.g. root exudents) or indirectly (e.g. changes in litter chemistry). Feedback to plants is indirect through changes in soil nutrients. Thus, other factors (e.g. nutritional constraints, competition between microbial groups) may be important and make it difficult to predict changes in feedback processes.

(C) When plants directly alter soil nutrients, there are no organisms present to respond.

# Chapter 2

## Biogeographic differences in the effects of *Centaurea maculosa* on the soil nitrogen cycle.

*Abstract.* As novel and often dominant components of ecosystems, invasve plants have the potential to change ecosystem processes such as nutrient cycling. Invasive plants can affect nutrient cycles by altering nutrient uptake, organic matter inputs, forming unique symbiotic associations with microbes, exuding novel biochemicals that alter the activity of soil microbes, or a combination of these mechanisms. I tested for effects of the invasive forb, *Centaurea maculosa,* on several aspects of the soil nitrogen cycle, and the role of a root exudate, the polyphenol, (±)-catechin, on ammonification and nitrification. Importantly, I also compared the strength of these effects in the invaded range to those in the native range.

In the non-native range in western Montana, soil nitrate ($NO_3^-$) was lower in invaded grasslands than in uninvaded grasslands. However, over two years of sampling, I found no difference in the C:N ratio of *C. maculosa* litter in invaded communities and that of litter from native plants in uninvaded communities. The N:P ratio of native litter was higher than litter of *C. maculosa.* When applied to soils collected from uninvaded sites in Montana, (±)-catechin significantly reduced resin extractable N, the maximum rate of nitrification, and gross nitrification. Thus, lower $NO_3^-$ concentrations in invaded communities appears to be at least partially driven by biochemical effects of *C. maculosa* on the the activity of nitrifying bacteria.

The effects of *C. maculosa* on N-related processes were different in Romanian grasslands, where *C. maculosa* is native. In Romanian soil, $NO_3^-$ concentrations were not lower in *C. maculosa* patches. However, ammonium ($NH_4^+$) concentrations were lower in *C. maculosa* patches. Furthermore, in Romanian soil *Centaurea maculosa* had no effect on resin extractable N, the maximum rate of nitrification, or gross nitrification. In a field experiment, a higher concentration of (±)-catechin than used in Montana reduced the maximum rate of nitrification, but substantially less than in Montana, and there was no effect of (±)-catechin on gross nitrification measured in Romanian soils in the laboratory. It appears that populations of nitrifying bacteria in Romania may have adapted to the effects of *C. maculosa*, and are more resistant to (±)-catechin than "naïve" nitrifying bacteria populations in Montana.

This is the first study to demonstrate that the effects of an invasive plant on soil ecosystem processes differ between the native and invaded ranges of the species. This type of biogreographical comparison is crucial to understanding the impact of exotic plant invasions, and can shed light on how invasives succeed. Furthermore, this research supports a growing body of evidence suggesting that invasive species can have significant impacts on belowground processes, which are effects that may persist long after the invasive has been removed from a community.

*Key words*: Nitrification, *Centaurea maculosa*, biogeography, ammonification, nitrogen, decomposition

## INTRODUCTION

Plants indirectly affect their neighbors in many ways, including altering the biotic, physical, and chemical characteristics of soils (Hobbie 1992, Angers and Caron 1998, Berendse 1998, Binkley and Giardina 1998, Northrup et al. 1998, Schlesinger and Pilmanis 1998, Van Breemen 1993, Wardle et al. 1998, Chen and Stark 2000, Eaton and Farrell 2004). These general effects have been understood for decades, but only recently have experiments demonstrated that complex interactions between plants and soil microbial communities can have strong effects on plant populations (Bever et al. 1997, Clay and Van der Putten 1999, Packer and Clay 2000), interactions among plant species (West 1996), and the organization of plant communities (Grime et al. 1987, Van der Putten et al. 1993, Bever 1994, Van der Putten 1997, van der Heijden et al. 1998, Hooper et al. 2000, Klironomos 2002). By altering the biotic and abiotic characteristics of soils, plants can drive positive or negative feedbacks, and these feedbacks can profoundly affect plant populations and communities.

In general, plant-soil feedbacks are often thought to be determined by the direct effects of pathogens and mutualists (Bever 1994, Mills and Bever 1998, Packer and Clay 2000, Bever 2002, Klironomos 2002), but other components of the soil ecosystem may participate in feedbacks. In particular, individual plant characteristics, such as phenology, nutrient uptake, litter-fall, tissue chemical composition, association with symbiotic microbes, and root exudates, can have significant effects on soil nutrient cycles (Hobbie 1992, Northup et al.1995, Angers and Caron 1998, Berendse 1998, Binkley and Giardina 1998, Schlesinger and Pilmanis 1998, Van Breemen 1993, Wardle et al. 1998, Chen and Stark 2000, Eaton and Farrell 2004) which may, in turn, alter the growth and survival of the species that drive these effects. Because exotic plants, and particularly exotic plants that become invasive, may have biochemical constituents that are novel in the communities they invade (Bais et al. 2003, Czarnota et al. 2003, Vivanco et al. 2004, Cappuccino and Carpenter 2005, Carpenter and Cappuccino 2005), and because invaders are often locally abundant, invasive plants can have unusually strong effects on soil nutrient cycles (Vitousek 1986, Vitousek et al. 1987, Vitousek 1990, D'Antonio and Vitousek 1992, Ehrenfeld et al. 2001, Ehrenfeld and Scott 2001, Ehrenfeld 2003). These strong effects can drive feedbacks affecting the invasive plant itself or natives (Thorpe and Callaway 2006).

*Centaurea maculosa* was introduced to the United States from Eurasia in the late 1800's and by the late 1990's covered over three million hectares in Washington, Idaho, Montana, and Wyoming (Rice et al. 1997, Sheley et al. 1998). *Centaurea maculosa* invades both disturbed habitats (Watson and Renney 1974, Maddox 1979, Lacey et al. 1992) and native grasslands (Tyser and Key 1988, Ridenour and Callaway 2001). Despite the introduction of 13 biocontrol insects, extenseive weed management plants and structure herbicide programs, *C. maculosa* appears to be expanding its range in North America (http://www.fs.fed.us/database/feis/plants/forb/cenmac/all.html). A growing body of evidence suggests that the success of *C. maculosa* is partially due to the exudation of chemicals, particularly the polyphenol (±)-catechin, that are toxic to other plants (Bais et al. 2002, 2003, Callaway and Ridenour 2004, Perry et al. 2005a,b, Thelen et al. 2005), herbivores (Thelen et al. 2005), and soil microbes (Bais et al. 2002, 2003, Callaway et al. 2005). There is also evidence that a large part of this weed's impact on native ecosystems is due to complex interactions with the soil ecosystem, including soil microbes and nitrogen (N) and phosphorous (P) cycling (Marler et al. 1999, LeJeune and Seastedt 2001, Newingham 2002, Callaway et al. 2004a, 2004b, Hook et al. 2004, Thorpe et al. 2006).

Here, I focus on the effects of *C. maculosa* on soil N. In experiments with N fertilization, the competitive ability of *C. maculosa* is highest in high N treatments (Story et al. 1989, Sheley and Jacobs 1997, Jacobs and Sheley 1999, Jacobs et al. 2000, Herron 2001, Olson and Blicker 2002). Interestingly however, *C. maculosa* appears to occur more frequently in field conditions in low N soils. For example, in a sagebrush-steppe community in southeast Washington, Meiman et al. (*in press*) found that soil $NO_3^-$ was lower in the central core of *C. maculosa* invasions compared to adjacent native areas. In a survey of several sites in western Montana, Hook et al. (2004) found reduced levels of N in soils from some, but not all communities invaded by *C. maculosa*. However, these results are difficult to interpret as the authors measured *in situ* N availability at only a subset of the sites and did not distinguish between soil $NH_4^+$ and $NO_3^-$. As plants may have different affinities for these two forms of N and different groups of soil microbes are responsible for making these nutrients available (Stevenson and Cole 1999), it is important to consider each form separately.

I explored the mechanisms by which *C. maculosa* reduces these available forms of soil nitrogen. Furthermore, I compared the effects of *C. maculosa* and (±)-catechin on inorganic soil N in Montana, the invaded range of *C. maculosa* where it often forms near monocultures, and Romania, the native range of *C. maculosa* where it is relatively rare (A. Thorpe, *personal observation*). Biogeographic comparisons are the most rigorous way to determine if particular mechanisms contribute to the invasive success of invasive organisms (Hierro et al. 2005). Through a series of field, greenhouse, and lab experiments, I tested the hypotheses that (1) *Centaurea maculosa* reduces soil $NH_4^+$ and $NO_3^-$ availability, (2) these reductions are caused in part by exudation of (±)-catechin, and (3) due to a coevolutionary history, "experienced" populations of ammonifying and nitrifying bacteria from the home range of *C. maculosa* would be more resistant to (±)-catechin than relatively naïve populations from the invaded range.

## METHODS AND MATERIALS

Field, greenhouse, and laboratory experiments were used to test for differences in inorganic nitrogen, decomposition, ammonification, and nitrification in soils from sites in Romania and Montana (Table 1). In all invaded sites in Montana, *C. maculosa* was the dominant species, comprising >60% of the total cover of each area (in many areas forming near monocultures). On the large community scale, cover of *C. maculosa* in all sites in Romania varied from less than 2% (e.g. Valea David) to *c.* 5%. However, I sampled the rhizospheres of individual *C. maculosa* in the densest local patches (*c.* 1 $m^2$) I could find (*c.* 30% - 60%). Areas classified as free from *C. maculosa* had no *C. maculosa* individuals within at least 25m of the sample point.

### *Field nitrogen levels*

I used ionic resin capsules to to determine if soil $NH_4^+$ and $NO_3^-$ were affected by the presence of *C. maculosa* in the invaded and native ranges of *C. maculosa.*

*Invaded, Montana.* – In May 2002, ionic resin capsules (to measure inorganic nitrogen levels) were placed in the soil of paired patches of un-invaded vegetation and patches invaded by *C. maculosa* at three different sites: Mt. Sentinel, the National Bison Range, and the Bandy Ranch; all in Montana, U.S.A. Soil characteristics of these sites are given in Table 1. In each of these six patches, ionic resin capsules were buried in the rhizospheres of five replicates each of *Pseudoroegneria spicata* (Pursh) Á. Löve (Poaceae), *Festuca idahoensis* Elmer (Poaceae), *Lupinus sericeus* Pursh (Fabaceae), and *C. maculosa* (*C. maculosa* patches only). In each patch, five 0.25 $m^2$ plots were randomly selected, then one individual of each species was half-hazardly selected from within the plot. If a species was missing from inside the plot, the nearest plant of that species was selected. Resins were collected September 2002 and frozen until analyzed.

*Native, Romania.* – In May 2003, ionic resin capsules were placed in the soil of patches of vegetation with *C. maculosa* and patches without *C. maculosa* at three sites in Romania. At each site, ionic resin capsules were buried in the rhizospheres of five replicates each of *Agropyron repens* (L.) Beauv. (Poaceae), *Festuca valesiaca* Schleich. ex Gaud. s.l. (Poaceae), *Medicago falcata* L. (Fabaceae) and *Centaurea maculosa* (*C. maculosa* sites only). These species represented an effort at phylogenetic control of target species as *Pseudoroegneria* and *Agropyron* are closely related genera, and *Lupinus* and *Medicago* are both in the Fabaceae. Plants were selected as in Montana. Resins were collected September 2003 and frozen until analyzed. At one of the sites in Romania, all resin capsules in the *C. maculosa* patch were removed by vandals. Thus, n=3 sites, without *C. maculosa* and n=2 sites with *C. maculosa*, with multiple replicates nested with each site,

Ionic resins were extracted by three successive 10 ml rinses of 2 M KCl, each shaken for 30 min (Kjønass 1999, Morse et al. 2000, MacKenzie et al. *in press*). Extracts from the three successive rinses were mixed and centrifuged at 3000 rpm for 20 min to remove soil particles. The extracts were analyzed for $NH_4^+$-N and $NO_3^-$-N on a segmented flow Auto Analyzer 3 (Bran and Luebbe, Chicago, IL). $NH_4^+$ was analyzed using the salicilate-nitroprusside method and the cadmium reduction method for the determination of $NO_3^-$ (Keeney and Nelson 1982). Resin capsules provide an integrated measure of inorganic N availability over time because N flows or diffuses into capsules and sorbs to resin surfaces from soil solution making it only sparingly available for uptake (Binkley and Matson 1983, MacKenzie et al., *in press*) as a result, it is difficult to determine exactly what portion of the available N pool was sampled, making this measurement something between gross and net N mineralization. It is also impossible to estimate the volume of soil sampled by these small diameter capsules, therefore the values are reported as µg N per capsule. The benefits of using resin capsules include 'real time' measurements of N availability, instead of 'point in time' measurements, and minimal disturbance to the soil environment during installation and removal (Kjønass 1999, MacKenzie et al. *in press*)

Independent ANOVAs (SPSS 2004) were used to test for the effects of site, species, and community (with or without *C. maculosa*) on $NH_4^+$ and $NO_3^-$ levels in Romania and Montana. Data were log-transformed to meet assumptions of normality.

### *Litter decomposition*

I used a litter decomposition experiment to determine (1) if differences in nutrient composition between native and *C. maculosa* tissue resulted in different decomposition rates (comparing native litter bags in native patches vs. *C. maculosa* litter bags placed in invaded patches) and (2) if differences in community structure altered decomposition rates (comparing native litter bags in native patches vs. native litter bags in invaded patches). Litter was collected in September 2002 from the paired sites with and without *C. maculosa* located at Mt. Sentinel, the National Bison Range, and the Bandy Ranch in Montana, U.S.A., creating two litter types, "*C. maculosa*" (predominately *C. maculosa* tissue with <5% native tissue) and "native" (approximately 80% native grasses and 20% native forbs). For each community x site combination, standing dead leaves and stems were collected from all plants within five 0.25-$m^2$ randomly placed plots and then combined. Litter was dried for three days at 60°C. Six grams of oven-dry litter were placed in 1-mm mesh fiberglass-screen litterbags. At each of the native sites, 36 bags of native litter were placed on the soil surface. At each invaded site, 36 bags each of *C. maculosa* and native litter were placed on the soil surface. Litter of *C. maculosa* was not placed at non-*C. maculosa* sites due to potential of introducing *C. maculosa* seeds to pristine sites. Bags were placed in the field in September 2002 and six bags of each litter type collected May 2003, September 2003, May 2004, and September 2004. Litter from each sampling period (including September 2002) was ground using a Wiley Mill and passed through a 40 mm mesh sieve, then analyzed for %C, N, and P by the Colorado State University Soil, Water, and Plant Testing Laboratory and the Oregon State University Central Analytical Lab. Data were analyzed using an ANOVA (SPSS 2004) to test for the effects of litter source and community on % C, N, and P, C:N ratio, and N:P ratio..

### *Greenhouse experiment*

In order to determine if (1) *C. maculosa* alters the activity of ammonifying and/or nitrifying bacteria and (2) if (+)-catechin is responsible for these effects, *P. spicata*, *F. idahoensis*, and *L. sericeus* were grown alone, in competition with *C. maculosa*, or treated with the (+) isomer of catechin in soil collected from an uninvaded community at the Bandy Ranch, Montana. Although (+)-catechin has antimicrobial properties, it has weak allelopathic properties (Perry et al. 2005b); thus, any differences in soil nitrogen in the soils treated with (+)-catechin would be due primarily to (+)-catechin's effects on soil microbes, and not to any possible indirect effects on soil nitrogen through allelopathic effects of (-)-catechin on the plants. Ten replicates of each species x treatment combination were grown in a 2:1 sand:soil mixture in 1.6 L pots. At the initiation of the experiment, ionic resin capsules were placed approximately 5 cm below the soil surface. Four weeks after seeding (week 4), native species were thinned to one individual per pot and *C. maculosa* was sown into 1/3 of the pots. At week six, *C. maculosa* pots were thinned to one seedling per pot and (+)-catechin was applied to the pots assigned that treatment. Using a micropipette, I applied 0.20 $\mu g\ \mu L^{-1}$ (+)-catechin dissolved in methanol and injected two doses of 400 µL into each pot (800 µL total per pot). This is similar to levels used in other experiments and is assumed to be consistent with the lower range of concentrations measured in soils invaded by *C. maculosa* (Bais et al. 2002, 2003, Thelen et al. 2005, but see Blair et al. 2005). Resins were collected three months after the application of (+)-catechin and analyzed as described above.

An ANOVA (SPSS 2004) was used to test for the effects of species and treatment on soil $NH_4^+$ and $NO_3^-$ levels. A Bonferonni test for multiple comparisons was used to test for differences between species and treatments.

### *(±)-Catechin field experiment*

I used a field experiment to o determine if there was an effect of (±)-catechin on nitrification in uninvaded soils in Montana and if I could induced an effect in Romanian soils.

*Montana.* – In an experiment designed to isolate the effects of (±)-catechin on nitrification, I applied (±)-catechin, to the rhizospheres of *P. spicata*, *F. idahoensis*, and *L. sericeus* in May 2003. This experiment was conducted in a native (not invaded by *C. maculosa* or other exotic species) grassland, on Moccasin Ridge (see Table 1). For each species, pairs (n = 10 pairs for each species) of nearby individuals were selected for similar sizes and one of two treatments was randomly assigned to each individual of the pair. Using a micropipette, I applied either a methanol control or 0.0625 $\mu g\ \mu L^{-1}$ (±)-catechin (dissolved in methanol). For each target individual, I injected 800 µL of solution into the rhizosperhere. The anti-microbially active (+)-catechin would have been present at one half this concentration. Plant-plant allelopathic interactions identified in these experiments are described in Thelen et al. (2005). An ionic resin capsule was placed in the rhizosphere of each plant. After two weeks, resin capsules and mineral soil samples from the 0-10 cm depth were collected from each plant. Resin capsules were frozen until analyzed for $NH_4^+$ and $NO_3^-$ as described above. Soil samples were collected with a standard, 2.5 cm diameter soil sampling probe and at stored at 5°C for less than 24 hours prior analysis for the maximum potential rate of nitrification.

The aerated nitrifier slurry assay (Hart et al. 1994a, 1994b) was used to examine the maximum potential rate of nitrification ($V_m$). Moist soil samples (15 g dry equivalent) were placed into 250 ml of a buffered solution containing 1.5 mM $NH_4^+$ and 1.0 mM $PO_4^{-3}$ (pH 7.2). The suspensions were placed on an orbital shaker on low and allowed to shake for 24 h. Subsamples of 10 ml were removed from the slurries using a modified pipette after 1, 2, 23, and 24 h and vacuum filtered into 50 ml centrifuge tubes. Ten milliliters of 2 M KCl was then added and extracts were refrigerated until analyzed (within 12 hours) for $NO_3^-$ as described above. The slope of the line generated by plotting $NO_3^-$ concentration versus time is reported as the nitrification rate in μg $NO_3^-$ $h^{-1}$.

Data was log-transformed to meet assumptions of normality. An ANOVA (SPSS 2004) was used to test for the effects of species and treatment on $NH_4^+$, $NO_3^-$, and nitrification rates and a Bonferonni test for multiple comparisons was used to test for differences between species and treatments.

*Romania.* – In May 2004, using a micropipette, I applied either a methanol control or 0.125 mg $mL^{-1}$ (±)-catechin (dissolved in methanol), approximately double the concentration used in Montana to the rhizospheres of ten individuals each of *A. repens*, *F. valesiaca*, and *Achillea setacea* in an uninvaded community near Iaşi, Romania. Plants were selected as in Montana. In addition, samples were taken from the rhizospheres of 10 randomly selected *C. maculosa* individuals. An ionic resin capsule was placed in the rhizosphere of each plant. Two weeks later, resin capsules and soil samples from the 0-10 cm depth were collected from each plant. Resin capsules were frozen until analyzed for $NH_4^+$-N and $NO_3^-$- N as described above. Soil samples were collected with a standard, 2.5 cm diameter soil sampling probe and stored at 5°C for approximately 24 hours until analysis for the maximum potential rate of nitrification using the aerobic nitrifier slurry assay as described above.

An ANOVA (SPSS 2004) was used to test for the effects of species and treatment on soil $NH_4^+$ (log transformed to meet assumptions of normality) and $NO_3^-$ levels and a Bonferonni test for multiple comparisons was used to test for differences between species and treatments.

### *$^{15}N$ pool dilution*

I used $^{15}$N pool dilution to determine if there was a biogeographically-based difference in the effects of *C. maculosa* and (±)-catechin on nitrification and ammonification (a process not studied in the field experiment). A laboratory incubation was necessary due to the logistics of conducting this experiment in both Montana and Romania.

In May 2004, soils were collected from five sites in Montana (the Bandy Ranch, Calf Creek, Moccasin Ridge, Mt. Jumbo, and Thompson Falls) and Romania (Breazu, Şorogari, Ştenga, Suceava, and Timişesti, see Table 1). At each site, samples were taken from patches of vegetation either with or without *C. maculosa*. At sites with *C. maculosa*, 5 cm x 10 cm soil cores were collected from the rhizospheres of ten half-hazardly selected mature *C. maculosa* plants. In sites free from *C. maculosa*, 20 replicate samples were collected from the half-hazardly selected rhizospheres of the native grass *P. spicata* in Montana and *Agropyron repens* in Romania. Soil cores were air dried and stored intact until the initiation of the experiment.

In June 2005, soils from each site were sifted through a 40 mm mesh screen. For each country x site x treatment combination, five 80 g soil samples were placed into 120 ml plastic specimen cups. All soils were brought up to 80% water holding capacity at the initiation and approximately every three days of the experiment. Half of the grass soils were injected with (±)-catechin, giving a total of three soil treatments, *C. maculosa*, native grass, or (±)-catechin. At time = 0, (±)-catechin was added to each cup assigned (±)-catechin treatment at a concentration of 0.4 mg $g^{-1}$ soil. Three weeks later (time = 3), an additional 1 mg (±)-catechin per g soil was added to each (±)-catechin-cup. Cups from each 3-way combination were collected 5, 9, and 12 weeks after the initial injection with (±)-catechin. Thus, the experiment consisted of two places of origin (Montana or Romania) by three soil treatments (grass, grass + (±)-catechin, *C. maculosa*) by five replicate sites by three sampling periods.

Every three days throughout the experiment, deionized (DI) water was added to each cup to maintain soil water at 60% water-holding capacity (WHC). Water-holding capacity was determined by saturating approximately 50 g of sieved soil contained in a funnel with DI water, allowing soil to saturate for 30 min., then allowing soil to drain by gravity for 30 min. The drained soil was transferred to pre-weighed soil tins and dried in an oven at 40°C for 24 hours. The water lost upon drying was the WHC of the soil sample.

At each sampling period, one cup from each country x site x treatment combination was split into subsamples to be analyzed for inorganic N ($NH_4^+$, $NO_3^-$) and gross rates of ammonification and nitrification. A solution (4.8 ml) containing either $(^{15}NH_4)_2SO_4$ or $K^{15}NO_3$ (99 atom% $^{15}N$) was added to soil subsamples with a needle and syringe. Subsamples were harvested within 1 h (time 0) and 24 h after injection. Soils were extracted for inorganic N analysis by shaking 20 g soil in 40 ml of 2 M KCl for 30 min and filtered with Whatman 42 filter papers, büchner funnels and a vacuum manifold. The extracts were analyzed for $NH_4^+$-N and $NO_3^-$-N on a segmented flow Auto Analyzer 3 (Bran and Luebbe, Chicago, IL). Gross rates of ammonification were determined by $^{15}NH_4^+$ isotope dilution, and gross rates of nitrification were determined by $^{15}NO_3^-$ isotope dilution (Hart et al., 1994b).

To measure $^{15}N$, soil N from the extracts and digests was concentrated by diffusion onto paper disks (Brooks et al., 1989; Herman et al., 1995; Stark and Hart, 1996). Disks were measured for N and $^{15}N$ enrichment using continuous-flow direct combustion and mass spectrometry using a Europa Scientific SL-2020 system by the Utah State University Stable Isotope Laboratory.

At the end of the experiment, soil pH was measured in a 1:1 suspension of a soil sample from each country x site x treatment combination using an AccuFet field effect transistor (FET) pH electrode (Fisher Scientific).

An ANOVA (SPSS 2004) was used for each country separately to test for the effect of treatment on gross ammonification and nitrification. Country of origin was not included in these analyses as the hypothesis being tested was not if ammonification and nitrification differed between countries, but rather, how the treatments altered soil processes within each country. Since differences in soil pH could affect ammonification and nitrification, an ANOVA (SPSS 2004) was used to test for the effects of country and treatment on soil pH.

## RESULTS

### *Field nitrogen levels*

In Montana, communities invaded by *C. maculosa* had similar levels of soil ammonium to adjacent uninvaded communities (*P=0.277*, Fig. 2). However, this pattern varied between sites and species ($F_{\text{site x patch x species}}$=2.334, df=4,150, *P=0.058).* In contrast, soil nitrate summed across all sites and species was 60% lower in patches invaded by *C. maculosa* than in uninvaded patches (Fig. 2; $F_{\text{patch}}$=19.340, df=1,149, *P<0.0005*). There was some variability in this pattern between sites ($F_{\text{site x patch}}$=1.352, df=2,149, *P=0.002*), with significantly less nitrate in invaded communities at two sites (*P<0.0005*) and a non-significant trend towards lower soil nitrate in invaded patches at the third site.

In Romania, soil ammonium differed between sites ($F_{\text{site}}$=3.129, df=1,72, *P=0.02*), but across all sites, ammonium was lower in patches with *C. maculosa* than in patches without *C. maculosa* (Fig. 2; $F_{\text{patch}}$=5.685, df=1,72, P = 0.02). There was significant variation in soil nitrate levels between species, patch, and sites ($F_{\text{site*patch*species}}$=4.508, df=1,86, *P=0.037*). However, there was no over-all difference in soil nitrate levels between patches where *C. maculosa* was present versus where *C. maculosa* was absent (Fig. 2; $F_{\text{patch}}$=2.592, df=1,86, *P=0.111*).

### *Litter decomposition*

It is unlikely that differences in decomposition due to different levels of tissue nitrogen caused the observed reduction in inorganic nitrogen in invaded communities. The C:N ratio of all litter decreased over the period of study until the final sampling periods in spring 2005, when C:N ratios increased (Table 2, Fig. 3). Initially, native litter tended to have a lower C:N ratio than *C. maculosa* litter, but this difference did not become significant until one year after placement in the field. At the fall 2003 sampling, the C:N ratio of native litter placed in invaded communities tended to be lower than either native litter in uninvaded communities or *C. maculosa* litter in invaded communities (One-way ANOVA, $F_{treatment}$= 2.760; df=2,42; P=0.075). This difference was even greater in the final, fall 2004 sampling (One-way ANOVA, $F_{treatment}$=10.575; df=2,40; P<0.0005).

During all but one sampling period, the N:P ratio of *C. maculosa* litter was <80% that of the native litter (Table 2). The N:P ratio of native litter increased approximately 70%, reaching a maximum of approximately 17:1 in spring 2004. The N:P ratio of *C. maculosa* litter increased to a maximum of approximately 15:1 in fall 2003, then decreased to approximately 11:1 at the end of the experiment. Over the two years of the decomposition experiment, there was no difference in the N:P ratio of native litter placed in communities with *C. maculosa* versus communities without *C. maculosa.*

### *Greenhouse experiment*

In the greenhouse experiment, the presence of *C. maculosa* and the addition of (±)-catechin affected the amount of $NO_3^-$ captured on ionic resin capsules. Species and treatment interacted to affect the amount of N available to native species (Fig. 4; $F_{species*treatment}$=24.354, df=4.65, *P<0.0005*). Neither the presence of *C. maculosa* nor application of (±)-catechin affected $NO_3^-$ levels in pots with the nitrogen fixing *L. sericeus*. For pots planted with *F. idahoensis*, treatment with (±)-catechin resulted in significantly less $NO_3^-$ than either the presence of *C. maculosa* or the control (*P<0.036*). Surprisingly, when *P. spicata* was grown with *C. maculosa*, $NO_3^-$ was significantly higher than that of the control or treatment with (±)-catechin (*P<0.0005).*

### *(±)-Catechin field experiment*

(±)-Catechin reduced the maximum rate of nitrification in soils in an uninvaded grassland in Montana. Application of 0.0625 mg (±)-catechin $mL^{-1}$ reduced the nitrification rate 41% (Fig. 5; $F_{treatment}=4.558$, df=1,39, *P=0.039*), potentially indicating inhibition of nitrifying bacteria. However, analysis of resin capsules showed no effect of (±)-catechin on $NH_4^+$ or $NO_3^-$ (Table 1). In contrast to the significant effect of (±)-catechin, there was no effect of different species on $NH_4^+$, $NO_3^-$, or nitrification rates in rhizosphere soils.

In Romania, neither $NO_3^-$ nor nitrification rates differed between *C. maculosa* and other plant species (Table 1). Application of 0.125 mg (±)-catechin $ml^{-1}$ (double the rate used in Montana) reduced the nitrification rate 25% (Fig. 5; $F_{treatment}=0.132$, df=1,40, *P=0.030*).

### *$^{15}N$ pool dilution*

In Montana soils, gross nitrification, measured by $^{15}N$ pool dilution, was reduced by both *C. maculosa* and (±)-catechin, but this reduction did not occur in Romanian soils (Table 3). At the first sampling period, (week 5), nitrification in soils from *C. maculosa* rhizospheres was only 21% that of native soils. Gross nitrification in soils treated with (±)-catechin was almost zero (Fig. 6; $F_{treatment}=1033.407$, df=2,11, *P=0.047*). This effect disappeared in weeks 9 and 12 (Table 2). There were no treatment effects on nitrification in the Romanian soils during any of the sampling periods.

While there were biogeographic differences in gross ammonification, they were opposite that demonstrated for nitrification. There were no treatment effects on ammonification in soils from Montana (Table 3). During the first sampling period, (week 5), ammonification in Romanian soils treated with (±)-catechin was 35% of that of *C. maculosa* soils ($F_{treatmnet}$=5.579, df=2,5, $P=0.023$). The grass soils exhibited an ammonification rate that was intermediate between the two treatments, however this could not be tested statistically because of a lack of replication. This treatment effect was not present four or eight weeks later (weeks 9 and 12, respectively; Table 3).

Differences in pH were unlikely to be the cause of the treatment effects. There was no effect of treatment on pH ($F_{treatment}$=1.234, df=2,23, $P=0.310$). Furthermore, across all treatments, soils from Montana were more acidic than those from Romania (5.66 ± 0.21, 7.16 ± 0.12 respectively; $F_{country}$=43.570, df=1,23, $P<0.0005$), the opposite of what would be expected if pH was driving biogeographic differences.

## DISCUSSION

My results suggest that *Centaurea maculosa* has the capacity to alter soil nitrogen cycling. Reductions in $NO_3^-$ in the invaded range of North America appear to be driven at least partially by biochemical effects on nitrifying bacteria by the root exudate, (±)-catechin. These results join a growing body of evidence that suggests that invasive species may have significant effects on soil ecosystem processes (Vitousek 1986, Kourtev et al. 1998, Vitousek and Walker 1989, Kourtev et al. 1999, Ehrenfeld et al.

2001, Ehrenfeld and Scott 2001, Kourtev et al. 2002, Ehrenfeld 2003, Hawkes et al. 2005, Heneghan et al. 2004).

### *Reduction of inorganic nitrogen in the invaded range*

Plants can affect soil $NH_4^+$ and $NO_3^-$ through plant uptake, microbial immobilization and inhibition, and/or stimulation of denitrification (Figure 1). Here, I focused on belowground processes mediated by soil microbes, particularly decomposition, immobilization, and inhibition. Denitificiation may be altered by invasion by *C. maculosa* (J.L. Pollock and W. Holben, *personal communication*), but I did not explicitly address this microbial function.

The results of the litter decomposition experiment (Table 2) suggest that differences in the rates of nutrient release from the litter of *C. maculosa* and native species do not cause the observed reduction in inorganic nitrogen in invaded communities. Over the two years of the decomposition experiment, the C:N ratio of all litter treatments remained above the level at which immobilization occurs (30:1; Stevenson and Cole 1999) and there was no difference in the C:N ratio of native litter in uninvaded communities and *C. maculosa* litter in invaded communities. In contrast, during the last two sampling periods, the N:P ratio of native litter in both invaded and uninvaded communities exceeded the Redfield ratio (16:1; the ratio above which P becomes limiting for biological processes relative to N (Redfield 1958 though see discussion by Hedin 2004). The N:P ratio of *C. maculosa* litter remained below the Redfield ratio throughout the experiment, likely due to the high concentration of P in *C. maculosa* tissue relative to native tissue (Thorpe et al. 2006). Therefore, because N:P ratios have been found to be negatively correlated with decomposition (Swift et al. 1979, Heal et al. 1997), decomposition and the subsequent release of nitrogen would be expected to be *higher* in invaded communities than in uninvaded communities. It is unlikely that differences in the nutrient concentration of *C. maculosa* litter, relative to native litter, explains the reduced levels of inorganic nitrogen observed in this study. However, I did not measure the levels of polyphenols present in the plant tissues, which may also inhibit decomposition (Spalding 1980, Northup et al. 1995, Schimel et al. 1998, Hättenschwiler and Vitousek, 2000).

For all soils from Montana, there was a trend towards reduced $NH_4^+$ in *C. maculosa* communities (Fig. 2). Neither (±)-catechin nor *C. maculosa* affected the activity of ammonifying bacteria in the isotope dilution experiment (Table 4). Because *C. maculosa* has high levels of tissue N (A. Thorpe, *unpublished data*) and grows rapidly, uptake is a likely cause of decreasing $NH_4^+$, but a significant three-way interaction between site, community, and species when $NH_4^+$ measured *in situ* suggests that microsite differences may be important in determining $NH_4^+$ levels. This agrees with Hook et al. (2004), who measured potential net N mineralization in nine paired sites, and found higher rates in the native sites of two pairs, but no signification differences between the other seven paired sites. In general, soil $NH_4^+$ tends to vary more among sites and years than $NO_3^-$ (Hart et al. 1993, Hooper & Vitousek 1998, Jackson et al. 1988, Herman et al. 2003). Thus, more replicated sampling over a range of times and sites may be required to accurately determine the effects of *C. maculosa* on $NH_4^+$.

In contrast, the reduction of $NO_3^-$ in communities invaded by *C. maculosa* appears to be at least partially caused by altered activity of nitrifying bacteria. Soils from *C.*

*maculosa* rhizospheres had lower resin extractable $NO_3^-$ (Fig. 2) and gross nitrification (measured using isotope pool dilution; Fig. 6) than native soils. The only significant deviation from this pattern was in the greenhouse experiment (Fig. 4). In pots with *L. sericeus* and *F. idahoensis*, the roots of the relatively small *C. maculosa* may not have been sufficient to significantly alter nitificiation in the native soils used in this experiment. Surprisingly, when *P. spicata* was grown with *C. maculosa*, the $NO_3^-$ level was approximately five times that found in soil from just *P. spicata*, a species that tends to be more resistant to (±)-catechin and *C. maculosa* than other native species (Callaway et al. 2004a, Thelen et al. 2005). When under stress, some plants increase production of root exudates (Hamilton and Frank 2001, Weir et al., *in press*), which can alter microbial growth and inorganic nitrogen pools (Hamilton and Frank 2001). It is possible that when competing with *C. maculosa, P. spicata* increases its production of root exudates, as does another native species, *Gaillardia grandifolia* (Weir et al. *in press*), which may increase microbial growth and result in increased inorganic nitrogen.

When (±)-catechin was applied to soils from Montana, I observed a decrease in the maximum potential rate of nitrification in the field (Table 3; Fig. 5) and gross nitrification in laboratory incubated soils (Table 4; Fig. 6), suggesting potential inhibition of nitrifying bacteria (Rice 1964, Lodhi and Killingbeck 1980, Thibault et al. 1982). Since this compound can be exuded from *C. maculosa* in large quantities (Perry et al. 2005b, but see Blair et al. 2005), this biochemical is a likely driver of chages in $NO_3^-$ in soils occupied by *C. maculosa.* The effect of (±)-catechin was generally greater than the reduction caused by *C. maculosa* itself. These differences are probably due to the manner in which catechin was applied. The levels of catechin I applied in all experiments are thought to be within the range observed in field soils invaded by *C. maculosa* (Bais et al. 2002, 2003, Thelen et al. 2005, J. Vivanco and L. Perry, *personal communication*), but *C. maculosa* probably exudes (±)-catechin continuously over a period of time (J. Vivanco and L. Perry, *unpublished data*), where-as I applied catechin in one or two doses. Application of (±)-catechin in doses may also explain the lack of inhibition in weeks 9 and 12 of the isotope dilution experiment. Although under investigation (L. Perry, *personal communication*) it is currently unknown how long (±)-catechin remains active in the soil. However, metal ions contribute to a very rapid disappearance of (±)-catechin in solution (J. Pollock and W. Holben, *unpublished data*), and break-down of (±)-catechin as it combines with metal ions may reduce its inhibitory effect, allowing populations of nitrifying bacteria to recover. Furthermore, some bacteria degrade (±)-catechin (Arunachalum et al., 2003; W. Holben *personal communication*), which may also reduce its inhibitory effect over time.

### *Biogeographical differences in the effects of* C. maculosa *on nitrogen cycling*

A criticism of many studies of invasive plant species has been that the studies have not been conducted in both the invaded and native ranges of the species (Hierro et al. 2005). As the majority of hypotheses attempting to explain the success of invasive species invoke a difference between the two ranges [escape from natural enemies (Darwin 1859, Elton 1958), evolution of increased competitive ability (Blossey and Notzold 1995), and the novel weapons hypothesis (Rabotnov 1982, Callaway and Aschehoug 2000, Mallik and Pellissier 2000, Baldwin 2003, Callaway and Ridenour

2004, Callaway and Hierro *in press*)], biogeographic comparisons are essential (Hierro et al. 2005).

In both the invaded (Montana) and native (Romania) ranges, there was a trend towards reduced soil ammonium in communities invaded by *C. maculosa*. As discussed in the previous section, the variability and lack of clear mechanism suggests that local and seasonal variability play a major role in the observed reduction of $NH_4^+$ in invaded soils in Montana. In Romania, in addition to reduced $NH_4^+$ in communities with a high proportion of *C. maculosa*, $NH_4^+$ was reduced in the rhizospheres of *C. maculosa* but not the rhizospheres of other species in a community where *C. maculosa* was evenly mixed with other species (Valea David), suggesting that reduced $NH_4^+$ is caused by the presence of *C. maculosa*, rather than colonization of low $NH_4^+$ soils. Exudation of (±)-catechin may alter the actvitiy of ammonifying bacteria in Romanian soils as (±)-catechin initially reduced gross ammonification rates in the lab. However, application of (±)-catechin did not result in a reduction of $NH_4^+$ *in situ* in Romania.

Even though the maximum rate of nitrification was reduced when (±)-catechin was injected *in situ* in Romania, most of my results indicated that nitrifying bacteria in Romania are more resistant to (±)-catechin than those in Montana. In the field experiments, although twice the amount of (±)-catechin was applied to rhizospheres in Romania, the maximum rate of nitrification was reduced by only 25%, compared to a 41% reduction in Montana. Furthermore, there was no difference in nitrification rates found in rhizosphere soils of *C. maculosa* and other Romanian species. This difference is particularly noteworthy as *C. maculosa* appears to exude less (±)-catechin in European sites versus those in Montana (Bais et al. 2003). My strongest evidence for biogeographical differences in the effect of *C. maculosa* was for soils collected from a large number of sites and incubated in the laboratory. In these experiments there was no effect of *C. maculosa* or (±)-catechin on nitrification in Romanian soils, but a strong negative effect of *C. maculosa* on soil nitrate availability and (±)-catechin on nitrification in soils from Montana. Although there can be an effect of (±)-catechin at relatively large concentrations in Romania, populations of nitrifying bacteria there appear to be more resistant to this chemical. These results are consistent with the Novel Weapons Hypothesis (Rabotnov 1982, Callaway and Aschehoug 2000, Mallik and Pellissier 2000, Baldwin 2003, Callaway and Ridenour 2004, Callaway and Hierro *in press*), which suggests that the success of an exotic species may be at least partially because species native to the invaded range (here, Montana) lack a co-evolutionary based response to biochemical traits of the invasive species. In contrast, due to a co-evolutionary history, species from the native range of the invasive (here, Romania) would exhibit some level of resistance to biochemicals produced by the plant. These results are the first to suggest that this hypothesis may apply to interactions between an invasive plant and microbially mediated soil processes.

The lack of difference in nitrification rates in the soils collected from the rhizospheres of *C. maculosa* and other native species in Romania (measured by both aerobic soil slurry and isotope pool dilution) but not Montana (measured by isotope pool dilution) suggest the existence of microbial species in Romanian soils that can utilize and degrade (±)-catechin. It is possible that over time, microbial populations in Montana will also become adapted to (±)-catechin (see Thorpe and Callaway, 2006), a possibility that is currently being explored. Importantly, changes in soil chemistry and nutrient cycles may lead to further and unpredictable changes in the soil ecosystem, and such changes may be more difficult to restore than native plant communities.

### *Conclusion*

My results provide strong evidence that *C. maculosa* alters soil nitrogen availability and that this effect is at least partially driven by altered activity of nitrifying bacteria by the novel biochemical, (±)-catechin. To my knowledge, this is the first study demonstrating that such effects of an invasive species in its invaded range can be very different that those in the native range. This type of biogeographical approach is critical if we are to truly understand invasions (Hierro et al. 2005). Finally, my research joins a growing body of work demonstrating that invasive species can alter microbial communities in ways that can have significant impacts on basic ecosystem functions (Vitousek 1986, Kourtev et al. 1998, Vitousek and Walker 1989, Kourtev et al. 1999, Ehrenfeld et al. 2001, Ehrenfeld and Scott 2001, Kourtev et al. 2002, Ehrenfeld 2003, Hawkes et al. 2005, Heneghan et al. 2004). As several authors have previously noted (e.g. Vitousek 1986, Ehrenfeld 2003, Hawkes et al. 2005) these impacts may be the legacy of invasive species even after they have been removed.

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Table 1. Location and Characteristics of soil at sites used in Montana and Romania. Within each sites, native communities were free from *C. maculosa* because they had either previously been invaded but *C. maculosa* had been removed at least two years prior to the study through the use of herbicide (sprayed) or the site had not yet been invaded.

| Dependent variable | Location | Soil classification | Maintenance of native site |
|---|---|---|---|
| Montana | | | |
| Bandy Ranch | 47°04’ N, 113°15’ W | Loamy-skeletal Haploxerolls | Sprayed (soil nitrogen survey)<br>Never-invaded (greenhouse experiment) |
| National Bison Range | 47°19’ N, 114°13’ W | Loamy-skeletal, mixed, frigid, typic Haploxerolls | Never-invaded |
| Calf Creek | 46°17’ N, 114°81’ W | Loamy-skeletal, mixed, frigid, typic Haploxerolls | Sprayed |
| Moccasin Ridge | 46°45’ N, 113°45’ W | loamy, skeletal, mixed andic Cryochrepts and udic Ustochrepts | Never-invaded |
| Mt. Jumbo | 46°53’ N, 113°56’ W | Loamy-skeletal, mixed, frigid, typic Haploxerolls | Sprayed |
| Mt. Sentinel | 46°53’ N, 113°57’ W | Loamy-skeletal, mixed, frigid, typic Haploxerolls | Sprayed |
| Thompson Falls | 47°38’ N, 115°27’ W | *Information not available* | Never-invaded |
| Romania | | | |
| Breazu | 47°13’ N, 27°31’ E | Alfic udic Argiustolls, Chernozems | *Not applicable* |
| Craşna | 47°10’ N, 22°54’ E | Enoquic Udifluvents | *Not applicable* |
| Şorogari | 47°13’ N, 27°34’ E | Entic Hapludolls, Chernozems | *Not applicable* |
| Ştenga | 47°14’ N, 25°32’ E | Typic Calciustolls, Chernozems | *Not applicable* |
| Suceava | 47°28’ N, 26°16’ E | Typic Glossaqualfs | *Not applicable* |
| Timişesti | 47°14’ N, 26°31’ E | Hapludalfs | *Not applicable* |
| Valea David | 47°11’ N, 27°19’ E | Entic Hapludolls, Chernozems | *Not applicable* |

Table 2. Percent N, C, and P, C:N, and C:P of native litter placed in a native community, *C. maculosa* litter placed in an invaded community, and native litter placed in an invaded community. Values are means ± 1 SE. Letters indicated differences ($P < 0.05$) between treatments within each sampling period.

| | Native community | Invaded community | |
|---|---|---|---|
| | Native litter | *C. maculosa* litter | Native litter |
| % Nitrogen | | | |
| Fall 2002 | 0.80 ± 0.05 | 0.79 ± 0.05 | 0.80 ± 0.05 |
| Spring 2003 | 0.89 ± 0.06 | 0.89 ± 0.06 | 0.86 ± 0.06 |
| Fall 2003 | 0.90 ± 0.05 | 0.84 ± 0.04 | 0.94 ± 0.07 |
| Spring 2004 | 1.06 ± 0.07$^{a}$ | 1.11 ± 0.07$^{ab}$ | 1.27 ± 0.08$^{b}$ |
| Fall 2004 | 0.72 ± 0.08$^{a}$ | 0.73 ± 0.03$^{a}$ | 1.10 ± 0.09$^{b}$ |
| % Carbon | | | |
| Fall 2002 | 43.73 ± 0.20$^{a}$ | 45.31 ± 0.09$^{b}$ | 43.73 ± 0.20$^{a}$ |
| Spring 2003 | 42.67 ± 0.29$^{a}$ | 45.47 ± 0.24$^{b}$ | 42.86 ± 0.28$^{a}$ |
| Fall 2003 | 40.28 ± 0.55$^{a}$ | 43.23 ± 0.51$^{b}$ | 40.48 ± 0.43$^{a}$ |
| Spring 2004 | 38.27 ± 1.02$^{a}$ | 43.68 ± 0.46$^{b}$ | 40.77 ± 0.58$^{a}$ |
| Fall 2004 | 38.26 ± 1.07$^{a}$ | 43.67 ± 0.40$^{b}$ | 39.41 ± 0.29$^{a}$ |
| % Phosphorus | | | |
| Fall 2002 | 0.075 ± 0.005$^{a}$ | 0.13 ± 0.00$^{b}$ | 0.075 ± 0.005$^{a}$ |
| Spring 2003 | 0.10 ± 0.01 | 0.12 ± 0.01 | 0.11 ± 0.01 |
| Fall 2003 | 0.083 ± 0.004 | 0.077 ± 0.005 | 0.085 ± 0.007 |
| Spring 2004 | 0.063 ± 0.006 | 0.082 ± 0.006 | 0.077 ± 0.007 |
| Fall 2004 | 0.048 ± 0.005$^{a}$ | 0.068 ± 0.003$^{b}$ | 0.080 ± 0.004$^{b}$ |
| C:N Ratio | | | |
| Fall 2002 | 55.36 ± 3.87 | 61.74 ± 3.71 | 57.96 ± 3.64 |
| Spring 2003 | 52.40 ± 4.07 | 55.36 ± 3.87 | 55.56 ± 4.81 |
| Fall 2003 | 47.31 ± 3.20 | 53.95 ± 3.16 | 47.13 ± 4.02 |
| Spring 2004 | 37.98 ± 2.43 | 41.74 ± 2.82 | 33.69 ± 1.94 |
| Fall 2004 | 57.91 ± 5.58$^{a}$ | 41.73 ± 2.82$^{a}$ | 40.28 ± 3.63$^{b}$ |
| N:P Ratio | | | |
| Fall 2002 | 11.14 ± 0.56$^{a}$ | 6.20 ± 0.29$^{b}$ | 11.14 ± 0.58$^{a}$ |
| Spring 2003 | 9.47 ± 0.50$^{a}$ | 7.56 ± 0.32$^{b}$ | 8.58 ± 0.63$^{ab}$ |
| Fall 2003 | 10.86 ± 0.31 | 15.23 ± 4.94 | 11.36 ± 0.40 |
| Spring 2004 | 17.78 ± 0.96$^{a}$ | 13.91 ± 0.42$^{b}$ | 17.58 ± 0.98$^{a}$ |
| Fall 2004 | 15.41 ± 1.17 $^{a}$ | 10.81 ± 0.28$^{b}$ | 16.92 ± 0.81$^{a}$ |

Tests were performed using the Type three sums of squares from SPSS version 13 (SPSS Inc., Chicago, IL, USA). Montana $NH_4^+$, Montana $NO_3^-$, and Romania $NH_4^+$ were log transformed. The data for Montana was collected in 2002; the data for Romania was collected in 2003.

Table 3. Summary of two-way ANOVA of effects of species (Montana: *Festuca idahoensis*, *Lupinus sericeus*, or *Pseudorogneria spicata*; Romania: *F. valesiaca* or *Achillea setacea*) and treatment ((±)-catechin vs. control) on soil $NH_4^+$, $NO_3^-$, and nitrification rate in Montana and Romania. P<0.05 are in bold.

| Dependent variable | Factor | Df | MS | F | *P* |
|---|---|---|---|---|---|
| Montana | | | | | |
| $NH_4^+$ | Species | 2 | 0.484 | 1.027 | 0.368 |
| | Treatment | 1 | 0.211 | 1.389 | 0.246 |
| | Species x Treatment | 2 | 0.129 | 0.418 | 0.661 |
| | Error | 38 | 0.155 | | |
| $NO_3^-$ | Species | 2 | 0.111 | 1.231 | 0.303 |
| | Treatment | 1 | 0.205 | 2.271 | 0.140 |
| | Species x Treatment | 2 | 0.114 | 1.269 | 0.293 |
| | Error | 38 | 0.090 | | |
| Nitrification rate | Species | 2 | 0.002 | 0.122 | 0.885 |
| | Treatment | 1 | 0.065 | 4.558 | **0.039** |
| | Species x Treatment | 2 | 0.00251 | 0.001 | 0.998 |
| | Error | 39 | 0.014 | | |
| Romania | | | | | |
| $NH_4^+$ | Species | 2 | 0.539 | 6.038 | **0.005** |
| | Treatment | 1 | 0.030 | 0.337 | 0.565 |
| | Species x Treatment | 2 | 0.218 | 2.440 | 0.126 |
| | Error | 39 | 0.892 | | |
| $NO_3^-$ | Species | 2 | 0.105 | 1.082 | 0.349 |
| | Treatment | 1 | 0.010 | 0.104 | 0.748 |
| | Species x Treatment | 2 | 0.112 | 1.152 | 0.290 |
| | Error | 38 | 0.098 | | |
| Nitrification rate | Species | 2 | 0.014 | 0.554 | 0.579 |
| | Treatment | 1 | 0.132 | 5.052 | **0.030** |
| | Species x Treatment | 1 | 0.018 | 0.701 | 0.407 |
| | Error | 40 | 0.026 | | |

Tests were performed using the Type three sums of squares from SPSS version 13 (SPSS Inc., Chicago, IL, USA). Montana $NH_4^+$, Montana $NO_3^-$, and Romania $NH_4^+$ were log transformed. The data for Montana was collected in 2002; the data for Romania was collected in 2003.

Table 4. Summary of one-way ANOVA of effect of treatment ((±)-catechin, *C. maculosa* soil, or grass soil) ammonification and nitrification rates in soils from Montana and Romania, measured through $^{15}N$ pool dilution. $P<0.05$ are in bold.

| Dependent variable | Factor | df | MS | F | *P* |
|---|---|---|---|---|---|
| Montana | | | | | |
| Week 5 | | | | | |
| Ammonification | Treatment | 2 | 9.852 | 0.176 | 0.845 |
| | Error | 4 | 55.981 | | |
| Nitrification | Treatment | 2 | 1033.407 | 4.091 | **0.047** |
| | Error | 11 | 252.579 | | |
| Week 9 | | | | | |
| Ammonification | Treatment | 2 | 3.140 | 0.968 | 0.413 |
| | Error | 10 | 3.244 | | |
| Nitrification | Treatment | 2 | 18.714 | 7.17 | 0.510 |
| | Error | 11 | 26.112 | | |
| Week 12 | | | | | |
| Ammonification | Treatment | 2 | 0.181 | 0.909 | 0.429 |
| | Error | 12 | 0.199 | | |
| Nitrification | Treatment | 2 | 5.661 | 0.275 | 0.765 |
| | Error | 11 | 20.614 | | |
| Romania | | | | | |
| Week 5 | | | | | |
| Ammonification | Treatment | 2 | 5.579 | 8.819 | **0.023** |
| | Error | 5 | 0.633 | | |
| Nitrification | Treatment | 2 | 51.507 | 1.338 | 0.302 |
| | Error | 11 | 38.492 | | |
| Week 9 | | | | | |
| Ammonification | Treatment | 2 | 3.566 | 1.426 | 0.281 |
| | Error | 11 | 2.500 | | |
| Nitrification | Treatment | 2 | 156.993 | 1.964 | 0.191 |
| | Error | 10 | 79.948 | | |
| Week 12 | | | | | |
| Ammonification | Treatment | 2 | 0.158 | 0.459 | 0.643 |
| | Error | 12 | 0.344 | | |
| Nitrification | Treatment | 2 | 36.591 | 1.767 | 0.213 |
| | Error | 12 | 20.708 | | |

Tests were performed using the Type three sums of squares from SPSS version 13 (SPSS Inc., Chicago, IL, USA).

Figure 1. Diagram of potential effects of invasion by *Centaurea maculosa* on the soil nitrogen cycle. (1) Soil nitrogen levels may be altered if *C. maculosa* takes up greater or lesser amounts of ammonium or nitrate than native species. (2) Differences in litter quality between *C. maculosa* and native species may result changes in the amount of ammonium or nitrate withheld by microbes during immobilization. (3) Exudation of root chemicals, particularly (±)-catechin may reduce soil nitrogen by inhibiting soil microbes. (4) Changes in the rate of denitrification may alter the levels of soil nitrogen.

Figure 2. Differences in soil ammonium and nitrate in communities with and without *C. maculosa* in Montana and Romania. Data are means +1 SE. Within in each country, asterisks (*) show significant differences ($p<0.05$) between communities with and without *C. maculosa.* Note that y-axes are not on the same scale.

Figure 3. C:N ratio of remaining litter within each community (uninvaded or invaded) for each litter type (*C. maculosa* or native). *C. maculosa* litter was not placed in uninvaded communities in order to prevent accidental introduction. Data are means ±1 SE. Within each sampling date, different letters represent significant differences ($p<0.05$) between litter/community types as determined by Bonferonni tests for multiple comparisons.

Figure 4. Soil $NO_3^-$ capture on ionic resin capsules during a greenhouse experiment when *Festuca idahoensis*, *Lupinus sericeus*, and *Pseudorogneria spicata* were grown alone, in competition with *Centaurea maculosa* or in soil treated with (+)-catechin. Data

are means +1 SE. Within each species, different letters represent significant differences ($p<0.05$) between treatments as determined by Bonferonni tests for multiple comparisons.

Figure 5. Maximum potential nitrification rate in the field experiment conducted in Montana and Romania. In Montana, 0.0625 mg (±)-catechin $ml^{-1}$ was injected into the rhizospheres of *Pseudoroegneria spicata*, *Festuca idahoensis*, and *Lupinus sericeus* in May 2003. In Romania, 0.125 mg (±)-catechin $ml^{-1}$ was injected into the rhizospheres of *F. valesiaca, Agropyron repens, Achillea setacea* in May 2004. Data are means +1 SE. Within in each country, asterisks (*) show significant differences ($p<0.05$) between treatments.

Figure 6. Nitrification rates in soils from Montana and Romania incubated in the laboratory as measured by $^{15}N$ pool dilution measured five weeks after the initial application of (±)-catechin. Rhizosphere soils were collected in May 2004 from five sites for each country. Data are means +1 SE. Within each country, different letters represent significant differences ($p<0.05$) between litter/community types as determined by Bonferonni tests for multiple comparisons.

Fig. 1

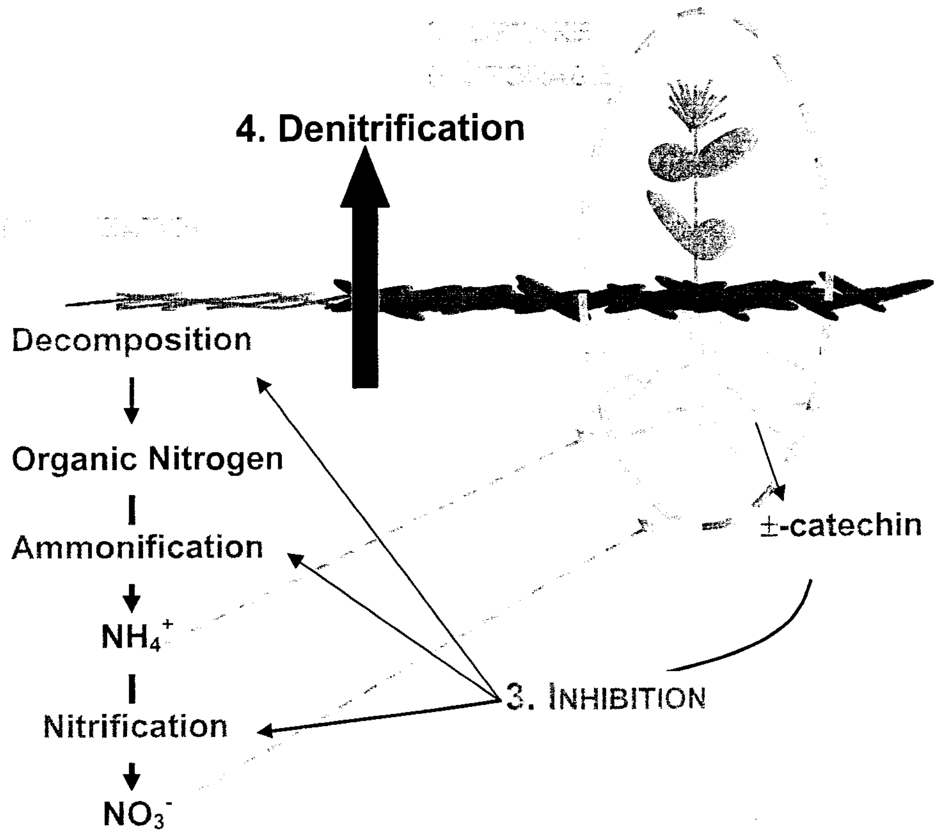
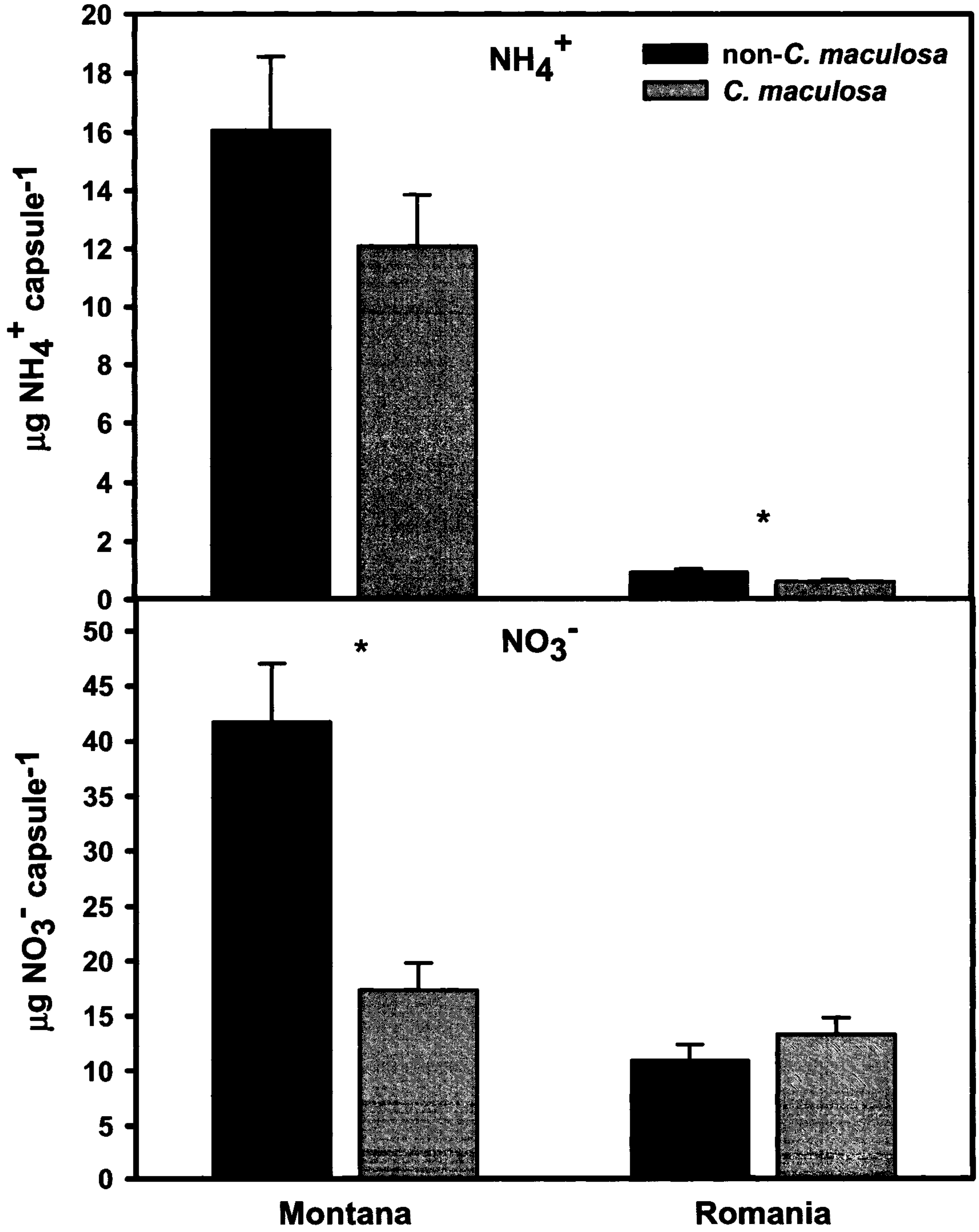

4. Denitrification
Decomposition
Organic Nitrogen
Ammonification
$NH_4^+$
Nitrification
$NO_3^-$
±-catechin
3. INHIBITION

Fig. 2



$NH_4^+$
$NO_3^-$
non-*C. maculosa*
*C. maculosa*
$\mu g\ NH_4^+\ capsule^{-1}$
$\mu g\ NO_3^-\ capsule^{-1}$
0
2
4
6
8
10
12
14
16
18
20
0
5
10
15
20
25
30
35
40
45
50
*
*
Montana
Romania

Fig. 3

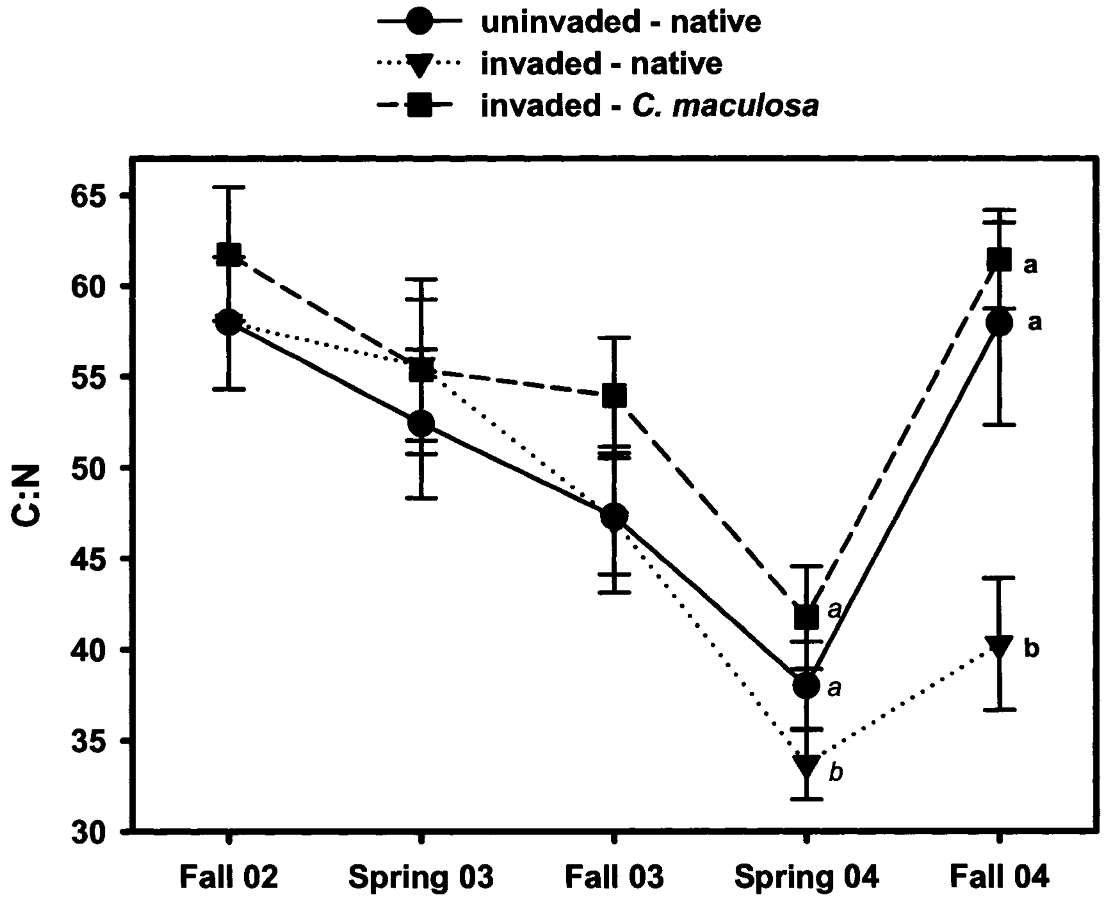
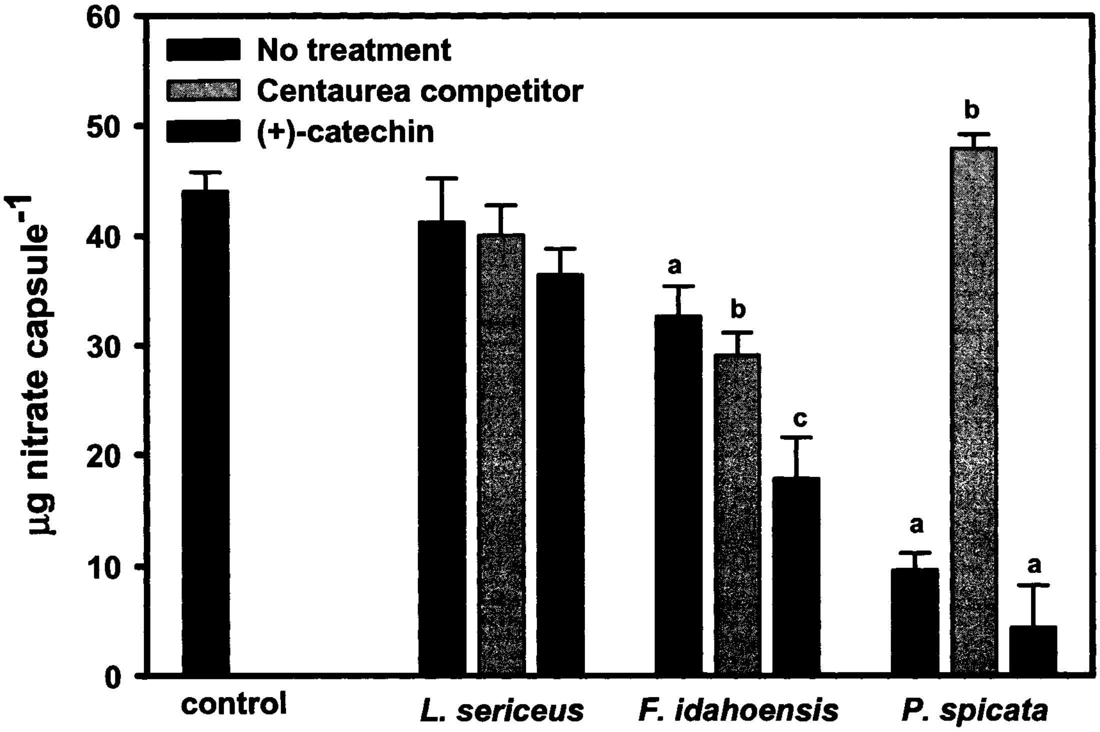

uninvaded - native
invaded - native
invaded - *C. maculosa*
C:N
65
60
55
50
45
40
35
30
Fall 02
Spring 03
Fall 03
Spring 04
Fall 04
a
a
b
a
a
b

Fig. 4



60
50
40
30
20
10
0
µg nitrate capsule$^{-1}$
No treatment
Centaurea competitor
(+)-catechin
a
b
c
a
b
a
control
*L. sericeus*
*F. idahoensis*
*P. spicata*

Fig. 5

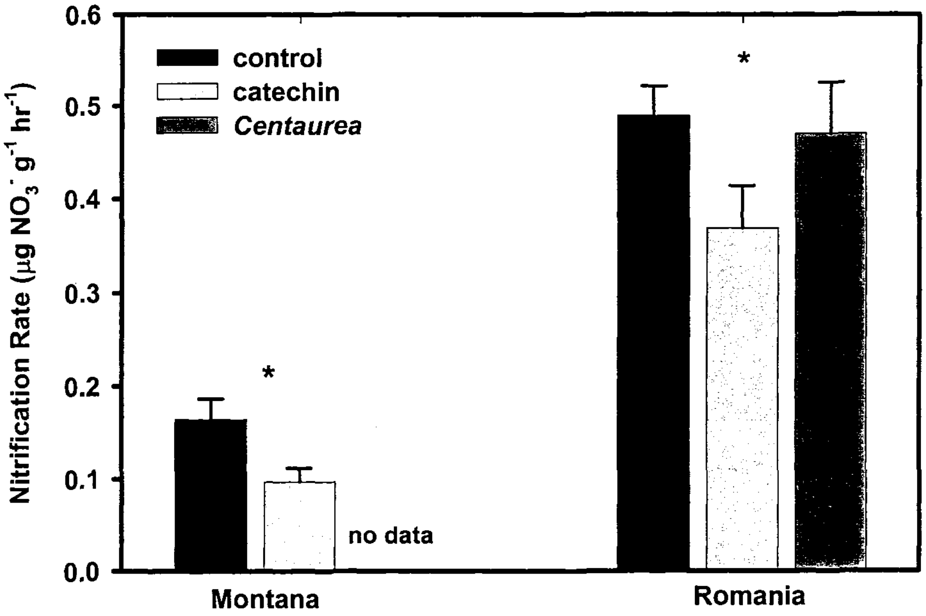
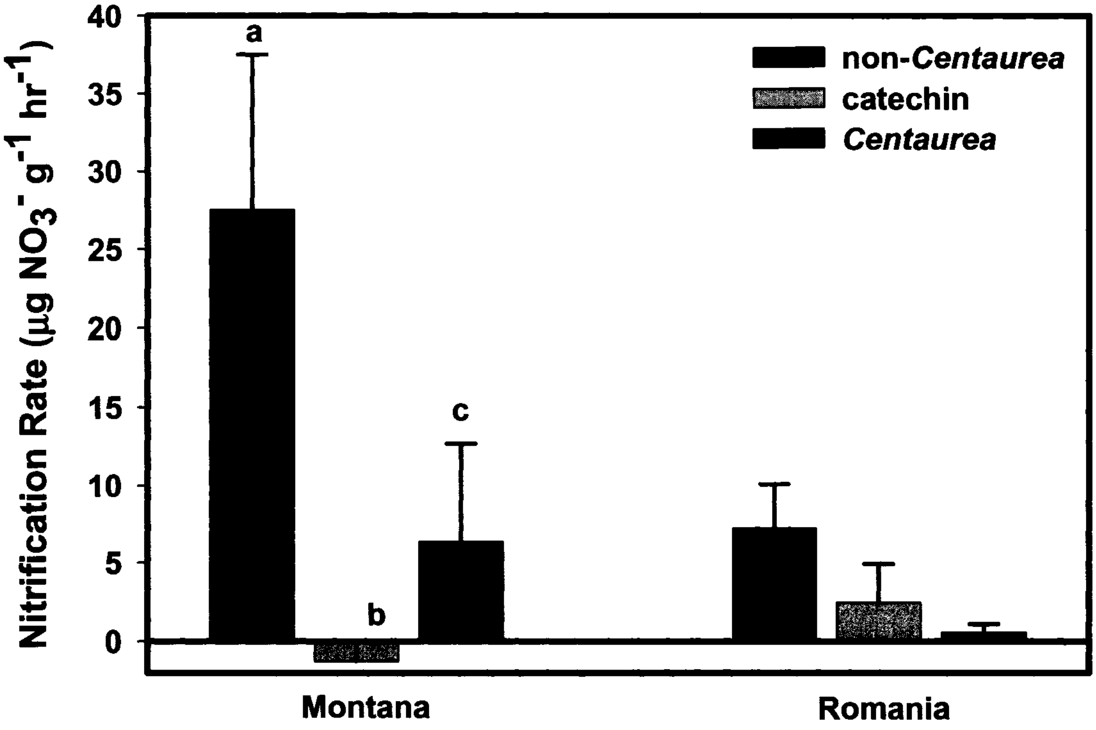

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Nitrification Rate (μg NO3- g-1 hr-1)
control
catechin
Centaurea
*
*
no data
Montana
Romania

Fig. 6



Nitrification Rate (μg NO3- g-1 hr-1)
40
35
30
25
20
15
10
5
0
a
b
c
non-Centaurea
catechin
Centaurea
Montana
Romania

# CHAPTER 3

## The invasive forb, *Centaurea maculosa*, increases phosphorus availability in Montana grasslands

### Abstract

*Centaurea maculosa* Lam. (Asteraceae, spotted knapweed) was introduced to the United States from Eurasia in the late 1800's and now covers over three million hectares in Washington, Idaho, Montana, and Wyoming. Several recent studies have suggested that the success of *C. maculosa* may be partly due to its ability to outcompete native species for phosphorus (P), through high root mass and/or association with arbuscular mycorrhizal fungi. We used a combination of field and greenhouse studies to explore the P efficiency of *C. maculosa* and its effects on soil P levels. *Centaurea maculosa* was P efficient in both a field study and greenhouse experiment. In the field, P concentration in *C. maculosa* were more than twice that of three native species (*Pseudorogneria spicata*, *Festuca idahoensis*, and *Lupinus sericeous*). In the greenhouse experiment, even at extremely low levels of soil P availability, uptake of P by *C. maculosa* was six times greater than that by the native, *Lupinus argenteus*. However, soil P levels in the field were elevated in sites invaded by *C. maculosa*, which is the opposite of what would be expected if root or mycorrhizal uptake were responsible for the higher P efficiency of *C. maculosa*. These results indicate that the success of *C. maculosa* may be due to its greater ability to acquire P than native species, but do not indicate that *C. maculosa* is actually outcompeting natives for the P that it acquires. In contrast, *C. maculosa* appears to have the ability to increase the availability of P in some soils.

Keywords: *Centaurea maculosa*, phosphorus, invasive plant, phosphorus efficiency

**Introduction**

Invasion by exotic plant species provides an opportunity to demonstrate how the properties of individual species may affect ecosystem-level processes, such as nutrient cycling (Vitousek, 1990; Ehrenfeld et al., 2001). *Centaurea maculosa* Lam. (Asteraceae) is one of the most widespread invaders in grasslands of the United States and Canada and has greatly decreased diversity in invaded systems (Tyser and Key, 1988; Ridenour and Callaway, 2001). Recent studies have suggested that the success of *C. maculosa* may be at least partially attributed to its greater competitive ability for phosphorus (P) compared to native species (LeJeune and Seastedt, 2001, Zabinski et al., 2002). Although competition was not directly tested, soil P has been shown to be up to 88% lower in sites dominated by *C. maculosa* than in sites dominated by native grasses (Harvey and Nowierski, 1989 in Olson, 1999), suggesting higher uptake by *C. maculosa.* Higher P uptake by *C. maculosa* may be facilitated by its deep, extensive root development and/or colonization by arbuscular mycorrhizal fungi (AMF; Marler et al., 1999.; Zabinski et al., 2002).

This paper presents the results of several experiments wherein we tested soil P levels in invaded grasslands and the P efficiency of *C. maculosa* in the field and the greenhouse.

## Methods

### *Soil Phosphorus*

We studied the effect of *C. maculosa* on soil P levels using a paired comparison of plots sprayed with herbicide to eliminate *C. maculosa* and unsprayed plots located in western Montana. Soils at all three sites are moderately alkaline soils classified as Calciothidic or Typic Haploxerolls. Site 1 was sprayed with Picloram (4-amino-3,5,6-trichloropicolinic acid) in 1999. Sites 2 and 3 were sprayed with 2,4 D (2,4-dichlorophenoxy acetic acid) in 1997 and 1998, respectively. Native species composition and percent bare ground were similar between sprayed and unsprayed plots. In each plot, six samples from 0-15 cm depth were taken. Samples were dried for 48 hr at 100°C and then sieved through 2 mm mesh. Plant available P was estimated by extraction in 1.0 M $NaHCO_3$ (Kuo, 1996). Briefly, 10 g of oven dried soil were shaken in 50 ml of 1.0 M $NaHCO_3$ for 30 minutes and then filtered through Whatman no. 42 filter papers. The P concentration in the extracts was determined colorimetrically using the ascorbic acid method (Kuo, 1996). The effects of site and treatment tested using an analysis of variance (SPSS 10.0).

### *Phosphorus efficiency, field study*

To determine the relative uptake of P in field conditions, leaves of *C. maculosa*, *Pseudoroegneria spicata* (Pursh) A. Löve, *Festuca idahoensis* Elmer, and *Lupinus sericeus* Pursh were collected from three paired native and invaded sites in western Montana. The native site at the National Bison Range (Moiese) has not yet been invaded. The native sites at the University of Montana Bandy Ranch (Ovando) and Mount Sentinel

(Missoula) have been maintained by spraying with either 2,4 D or Picloram. *Centaurea maculosa* cover at the invaded sites was approximately 60% - 80%.

At each site, ten plants of each species were randomly selected. Leaves were collected in June 2002, when all species were actively growing. Leaves was dried for three days at 60°C then ground through a 1 mm-mesh using a Wiley Mill. Determination of $PO_4$-P was done by the Oregon State University Central Analytical Lab. A two-way ANOVA was used to test for the effect of species and community type (invaded vs. uninvaded) on P concentration (SPSS 10.0). Differences between species were determined by a Bonferroni test for multiple comparisons.

*Phosphorus efficiency, greenhouse study*

To investigate the effects of soil P availability on plant tissue P content, *C. maculosa* and *L. argenteus* Pursh were grown in soils with three levels of available P. This experiment was conducted in a greenhouse at the University of Montana that was kept on a 12 hr light/dark cycle. Six species/treatment combinations were replicated 14 times for a total of 84 pots. Plants were seeded into 500 g of soil in 450 ml pots. The soil was a calcareous, native mineral soil from the Missoula valley footslopes which was depleted of available P by mixing two parts mineral soil with one part sand and growing *Lolium perenne* for eight weeks prior to use.

Four weeks after *C. maculosa* began to germinate, *L. argenteus* seeds that had been scarified and aerated in water overnight were planted. Each pot was thinned to one plant per pot. Pots were watered every two to three days. Pots were fertilized with 50 ml of fertilizer solution at planting, week two, week six, and week ten. P applications were graduated for P treatment totals of 0, 20, and 100 ppm. P levels were based P efficiency studies by Johnson et al. (1994).

Plants were harvested after 16 weeks. Plant roots and shoots were separated, washed, dried at 60° C for 24 hours, and weighed for biomass. Plant P was analyzed using a combination of methods from Braum and Helmke (1995) and Kuo (1996). Fifty mg of *C. maculosa* and 20 mg of *L. argenteus* tissue were ground through a no. 4 mesh screen using a Wiley Mill. Samples were then ball milled to pass through 200 mesh, ashed at 550°C for two hours, acidified with 1 N $H_2SO_4$, brought to a 25 ml volume, and shaken for 16 hours. P analysis was performed using the ascorbic acid method (Kuo, 1996).

A two-way ANOVA was used to test for differences among all treatment means for tissue P (SPSS 10.0). Differences between treatments and contrasts were determined using a Bonferroni test for multiple comparisons.

## Results

### *Soil Phosphorus*

*Centaurea maculosa* rhizospheres contained greater soluble P at two of three sites (Site*Treatment $P = 0.028$; Fig. 1). Across all sites combined, sites that were not sprayed had twice the soluble P concentrations than sites that had been sprayed (10.09 ± 2.86 S.E. $\mu g\ P\ g^{-1}$ soil and 4.98 ± 1.27 S.E. $\mu g\ P\ g^{-1}$ soil, respectively; treatment $P = 0.015$).

*Phosphorus efficiency, field study*

While no species showed differences in tissue P concentration in invaded versus uninvaded communities (*$P = 0.058$*), there were significant differences between species (*$P < 0.0005$*). *Centaurea maculosa* tissues contained at least twice the P (3185 ± 76 S.E. μg $mg^{-1}$) than the native species tested. There was a significant difference between the nitrogen fixing *L. sericeus*_and the shallow-rooted grass *F. idahoensis* (1536 ± 72 S.E. μg $mg^{-1}$ and 1180 ± 56 S.E. μg $mg^{-1}$, respectively). The leaf P concentration of the deeply rooted grass, *P. spicata* was intermediate to the other native species (1407 S.E. ± 68 μg $mg^{-1}$).

*Phosphorus efficiency, greenhouse experiment*

Compared to *L. argenteus*, *C. maculosa* acquired greater P (*$P = 0.001$*) and biomass (*$P < 0.005$*), even in the most extremely P limited soil (Figure 2). However, *C. maculosa* concentrated less P than *L. argenteus* (*$P<0.02$*). As fertilizer levels increased, *L. argenteus* plant P content increased, but there was no change in biomass (*$P=0.2$*). This led to three times the concentration of P in the high vs. the low fertilizer treatments (*$P<0.005$*). In comparison, as fertilizer levels increased, *C. maculosa* increased both plant P content and biomass, leading to an increase in P concentrations of only 1.5 times (*$P<0.02$*).

**Discussion**

The results of our studies suggest that *C. maculosa* is more P efficient than the native species tested and it has the ability to alter soil P cycling. Soil P was elevated in two of the sites invaded (unsprayed) by *C. maculosa* (Fig. 1). The lack of detectable difference at site 2 may be due to unmeasured soil characteristics at that site. Localized soil properties may have a strong influence on the ability of plant species to impact soil P (Chen et al., 1993).

Both the field and greenhouse studies demonstrated high P efficiency for *C. maculosa*. In the field, aboveground biomass of *C. maculosa* contained approximately twice the P as the three native species studied. Although there was no difference in P concentrations in *C. maculosa* and *L. argenteus* in the greenhouse experiment at each of the fertilizer levels, there was a difference in the magnitude of response to fertilization (Fig. 2). As fertilization increased, *L. argenteus* concentrated P, whereas *C. maculosa* appeared to utilize the P for increased growth.

Previous studies have attributed the effective P uptake capacity of *C. maculosa* to association with AMF (Zabinski et al., 2002). If AMF were the mechanism for P efficiency, it would be expected that soil P levels would be lower in invaded communities due to the formation of depletion zones around colonized roots and AMF hyphae (Smith and Read, 1997), as was found by Harvey and Nowierski (1989 in Olson, 1999). However, we found higher levels of soil P in soils invaded by *C. maculosa*. This result is more consistent with increases in soluble P found in the rhizospheres of plants that exude phosphatases (Grierson and Adams, 2000) or chelating compounds (Grierson, 1992; Stevenson and Cole 1999).

*Centaurea maculosa* produces many root exudates, including the polyphenol, (±)-catechin (Bais et al. 2002, 2003). (+)-Catechin is frequently found in the extracts of the leaves of forest trees and can be important in the complexation of Fe, Al, and Ca

(Stevenson and Cole, 1999; Kidd et al, 2001). Soils in western Montana grasslands and foothills invaded by *C. maculosa* tend to be calcareous (Montagne et al., 1982), which limits P availability through the precipitation of Ca-P compounds. Thus, higher levels of P in rhizospheres of *C. maculosa* may be due to chelation of Ca by (±)-catechin (see Watt and Evans, 1999). Alternatively, the herbicide treatment itself may have caused the differences observed. However, this is unlikely due to the persistence in the difference three years post-spraying and that other than the presence of live or dead *C. maculosa*, the native species composition and percent bare ground were similar between treated and untreated sites.

It has been suggested that the success of *C. maculosa* may be due to its ability to outcompete native species for soil P (Harvey and Nowierski, 1989 in Olson, 1999; LeJeune and Seastedt, 2001), although this has not been directly tested. Our results support the hypothesis that *C. maculosa* has the ability to acquire more P than native species, potentially enhancing its competitive success. However, greater acquisition of P was not related to depletion of the resource, a requisite for competition. We suggest that there may be multiple mechanisms responsible for the P efficiency of *C. maculosa* and that these may be dependent on local soil conditions. In some invaded soils, *C. maculosa* may actually have the ability to *increase* P availability. This evidence that an invader can directly alter soil nutrient cycling has important implications for the management and restoration of invaded communities.

## Acknowledgements

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Figure 1. Soil P concentration (μg P/g soil) was higher at two C. maculosa (unsprayed) sites compared to sites sprayed to eliminated C. maculosa (Site*Treatment P = 0.028). Significant differences within sites are indicated by a *. Bars are means +1 SE.

Figure 2. Tissue P content and biomass differences between L. argenteus and C. maculosa. Lupinus argenteus concentrated tissue P, while C. maculosa increased biomass with increasing added P in soil. Bars are means +1 SE.

Figure 1

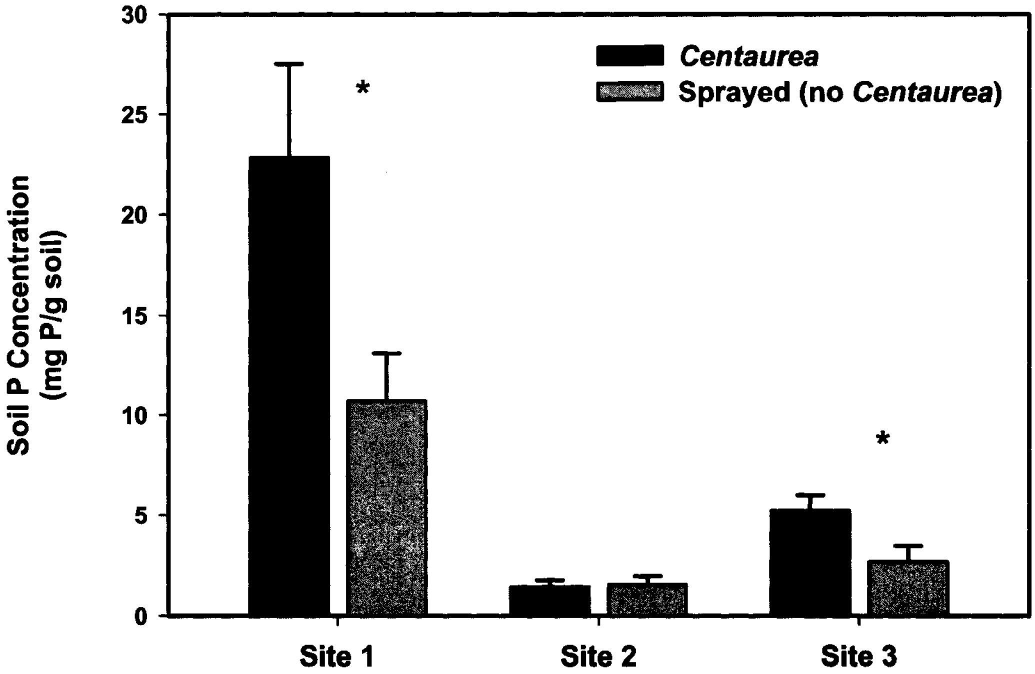
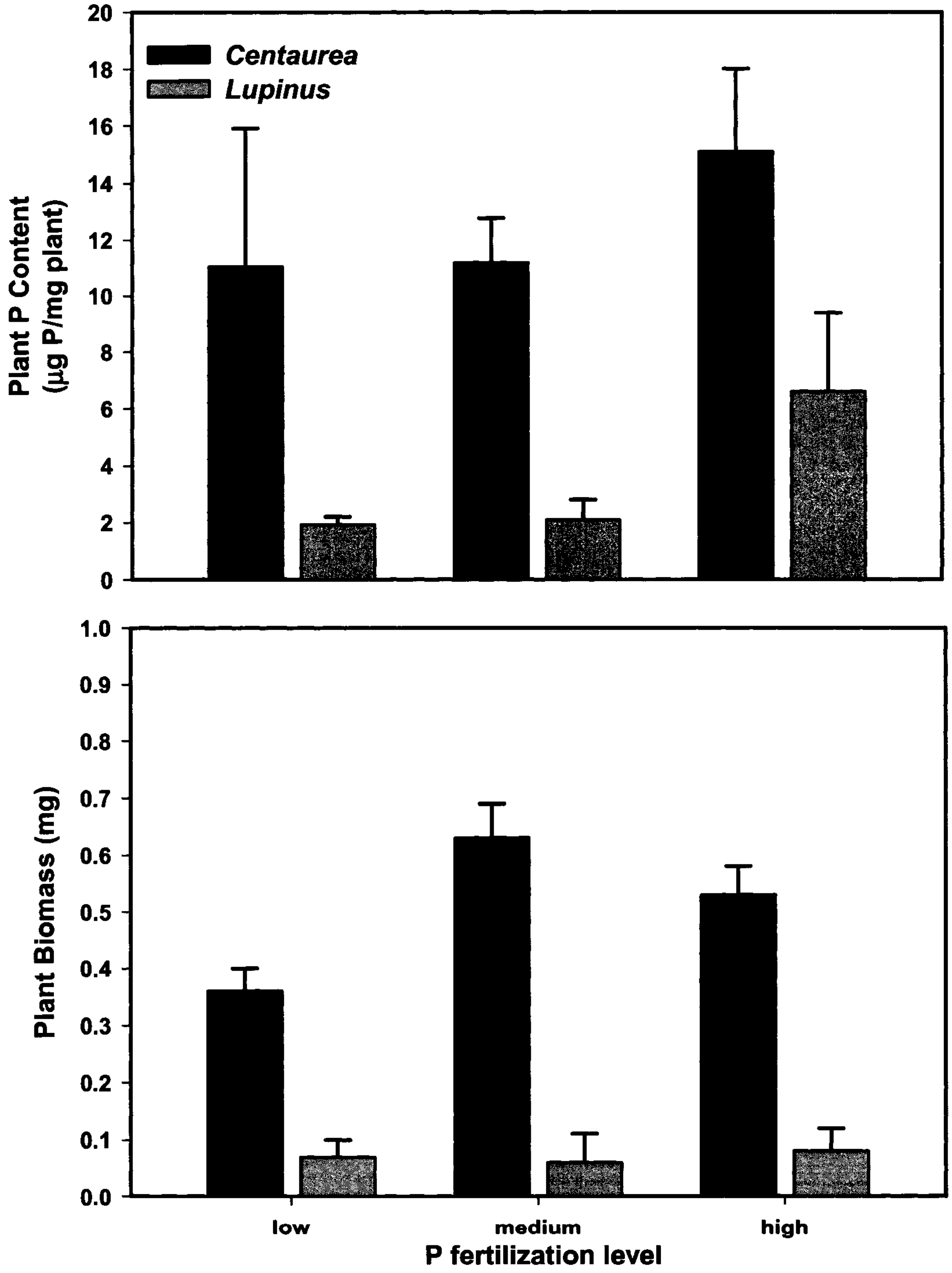

Centaurea
Sprayed (no Centaurea)
Soil P Concentration
(mg P/g soil)
30
25
20
15
10
5
0
*
*
Site 1
Site 2
Site 3

Figure 2


Centaurea
Lupinus
Plant P Content
(µg P/mg plant)
0
2
4
6
8
10
12
14
16
18
20
Plant Biomass (mg)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
low
medium
high
P fertilization level

## CHAPTER 4

# Allelopathy in the field: tolerance at home and vulnerability away

## Abstract

The novel weapons hypothesis postulates that the success of some exotic plants may be due in part to the lack of evolved tolerance of native species to new allelopathic, anti-microbial or anti-herbivore biochemicals produced by invasives. *Centaurea maculosa* produces the root exudate (±)-catechin. Controlled laboratory experiments have demonstrated that the phytotoxic effects of (±)-catechin are often stronger on North American species than on European species. Here, we test the effects of (±)-catechin in the field. We conducted experiments for two years in the native (Romania) and invaded (Montana) ranges of *Centaurea*, asking how (±)-catechin influenced related species that co-occur with *C. maculosa* in both ranges. We found substantial variability in the effects of (±)-catechin between years and among species. However, (±)-catechin caused a large reduction in the growth of most native species in Montana and had very weak effects on related species in Romania. This first *in situ* test of the novel weapons hypothesis suggests that members of natural communities may evolve tolerance to each other's biochemical effects, and that a lack of such evolutionary relationships may lead to successful invasion.

**Keywords**: *Centaurea maculosa*, (±)-catechin, allelopathy, novel weapons hypothesis

## INTRODUCTION

Invasion by exotic species threatens natural ecosystems (Wilcove *et al.* 1998) and has severe economic ramifications (Pimental *et al.* 2000). In many cases, exotic species that form near monocultures in their invaded range are much rarer in their native habitat (Lonsdale & Segura 1987; Braithwaite *et al.* 1989; Malecki *et al.* 1993; Eckert *et al.*1996; Meyer & Florence 1996; Bruce *et al.* 1997; Paynter *et al.* 1998; Memmot *et al.* 2000). Some studies comparing the interactions of invasive plants with above-ground enemies (Wolfe 2002; Mitchell & Power 2003), the soil ecosystem (Beckstead & Parker 2003; Reinhart *et al* 2003; Callaway *et al* 2004; Thorpe *unpublished data*), and other plant species (Callaway & Aschehoug 2000; Prati & Bosdorf 2004; Vivanco *et al.* 2004; Callaway *et al.* 2005a), from both the invasive plant's native and invaded ranges have found that interactions in the invaded range appear to be altered in a manner that favors the growth of the invasive plant in invaded communities. However, to our knowledge, the only *experimental* study of an invasive species *in situ* in both the native and invaded ranges is a common garden experiment with *Hypericum perforatum* by Maron et al. (2004; Hierro *et al.* 2005).

One recent hypothesis (the novel weapons hypothesis) suggests that novel biochemical constituents of invasive species may contribute to their success (Rabotnov 1982; Callaway & Aschehoug 2000; Czarnota *et al.* 2001; Bais *et al.* 2003; Vivanco et al. 2004; Cappoccino & Carpenter 2005; Carpenter & Cappoccino 2005). More specifically, the novel weapons hypothesis (Rabotnov 1982; Callaway & Aschehoug 2000; Mallik & Pellissier 2000; Baldwin 2003; Callaway & Ridenour 2004; Callaway & Hierro 2006) suggests that some invasive species owe their success to the fact that some of their biochemicals have stronger effects on native species in recipient communities that lack a coevolutionary-based tolerance to these biochemicals compared to that exhibited by co-evolved competitors in the native range. While this hypothesis has been supported in laboratory and greenhouse trials using species from the native and invaded ranges (Callaway & Aschehoug 2000; Prati & Bosdorf 2004; Vivanco *et al.* 2004; Callaway *et al.* 2005a), as for most allelopathic effects, this has yet to be demonstrated experimentally in the field.

Much of the research on the novel weapons hypothesis has been conducted on the exotic forb, *Centaurea maculosa* Lam. (Asteraceae). *Centaurea maculosa* exudes the polyphenol, (±)-catechin from its roots. In laboratory and greenhouse experiments (±)-catechin has a negative impact on the germination, growth, and survival of most grass and forb species that *C. maculosa* co-occurs with in invaded grasslands ("new neighbors"). However, (±)-catechin has little impact on similar, co-evolved species from Eurasia ("old neighbors"; Bais *et al.* 2002, 2003; Callaway *et al.* 2004; Callaway *et al.* 2005a). In a field experiment testing the effect of (±)-catechin on the growth of native grasses and forbs in Montana (new neighbors), Thelen *et al.* (2005) found that the majority of species treated with (±)-catechin exhibited mortality or significantly reduced growth compared to untreated controls. This study expands upon the experiment by Thelen *et al.* (2005) by performing a biogeographic test comparing differences in the response of native grasses and forbs in Montana (new neighbors) to the effect of (±)-catechin on the growth of and Romania (old neighbors).

## METHODS

In the invaded range of *C. maculosa*, the affects of (±)-catechin were tested at Moccasin Ridge (46°45' N, 113°45' W), near Clinton, Montana. The soils at this site are classified as loamy, skeletal, mixed Andic Cryochrepts and Udic Ustochrepts. The physical and biological characteristics at this site are representative of areas invaded by *C. maculosa*. However, as *C. maculosa* does not yet occur at this site, the native plants are naïve to (±)-catechin. Species used in the experiments were *Achillea millefolium* L. (Asteraceae; 2004, 2005), *Antenaria microphylla* Rydb. (Asteracae; 2004), *Arenaria nuttallii* Pax (Caryophyllaceae; 2004, 2005), *Delphinium bicolor* Nutt. (Ranunculaceae; 2005), *Eriogonum umbellatum* Torr. (Polygonaceae; 2004), *Festuca idahoensis* Elmer (Poaceae; 2004, 2005), *Geum triflorum* Pursh (Rosaceae; 2004, 2005), *Lupinus sericeus* Pursh (Fabaceae; 2005), *Poa sanbergii* Vasey (Poaceae; 2004, 2005),*Pseudoroegneria spicata* (Pursh) Á. Löve (Poaceae; 2004, 2005), *Senecio sphaerocephalus* Greene (Asteraceae; 2004, 2005), and *Zigadenus elegans* Pursh (Liliaceae; 2005).

In the native range of *C. maculosa*, the effects of (±)-catechin were tested at Valea David (47°11' N, 27°19' E), near Iaşi, Romania. The soils at this site are classified as Entic Hapludolls and Chernozems. *Centaurea maculosa* individuals in this community were rare and dispersed among other native grasses and forbs. Species used in the experiments at Valea David were *Achillea setacea* Waldst. & Kit. (Asteraceae; 2004, 2005), *Agropyron repens* (L.) Beauv. (Poaceae; 2004, 2005), *Festuca valesiaca* Schleich. ex Gaud. s.l. (Poaceae; 2005), *Medicago falcata* L. (Fabaceae; 2005), and *Salvia pratensis* L.(Lamiaceae; 2004, 2005).

Experiments took place in May 2004 and April 2005. For each species, pairs (10 pairs for each species) of nearby individuals that were of similar size were selected and each individual was randomly assigned to a treatment or control group. We attempted to select those plants that were early in their development. Using a micropipette, we applied 800 µL of 0.020 µg $\mu L^{-1}$ (±)-catechin (dissolved in methanol) to the rhizosphere of each treatment individual. 800 µL methanol was injected into the rhizosphere of each control plant. In field and greenhouse trials, methanol has not had an affect on the growth of plants compared to untreated controls (G. Thelen, *unpublished data*). Natural concentrations of (±)-catechin in North American soils, may be much higher than this, with mean concentrations reported by Perry et al. (2005) at $\approx 1000$ µg $g^{-1}$ soil. However, other studies have not found (±)-catechin in C. maculosa rhizosphere (Blair et al. 2005) and recent surveys indicate that soil concentrations are often much lower; from 10-100 µg $g^{-1}$ soil (L, Perry, J.M. Vivanco, R.M. Callaway, *unpublished data*). Based upon the rapid chemical transformation that (±)-catechin undergoes in soils (J.M. Vivanco, *unpublished data*), the active amounts of pure (±)-catechin in our experiments were probably less than the injected amounts. The allelopathically active (-)-catechin would have been present at one half of this concentration (Thelen *et al.* 2005). Measurements of leaf number and leaf length were taken for all plants prior to treatment, and three weeks after (±)-catechin application, we measured the relative change in leaf number and height (measured from the ground to the top of the longest leaf). For each year, we used a two-way ANOVA (SPSS 2004) to test for the effects of region (invaded or native) and treatment (control or (±)-catechin addition) on the percent change in the number of leaves and height for all species pooled together. For each species, individual t-tests were used to test for treatment effects on the percent change in the number of leaves and height of each individual for each country and year (Mead 1988; Hurlbert & Lombardi 2003). A two-way ANOVA was not possible to test for region and year effects within species as the same species were not present in both countries.

## RESULTS

In both 2004 and 2005, the growth of the majority of Montana plants was suppressed by treatment with (±)-catechin (Figure 1). No Romanian species was significantly affected by catechin. The relatively strong effect of (±)-catechin in North American was much more evident in 2005 than 2004 and varied substantially among North American species.

In 2004, when all species from both regions were pooled, the percent change in leaf number was reduced in plants treated with (±)-catechin (Fig. 2; $F_{treatment} = 6.074$, d.f. = 1,226, $P = 0.014$). However, in separate analyses, (±)-catechin reduced change in leaf number for three species in Montana and no species in Romania (Table 1; Fig. 1). In 2004, we found an overall trend in reduced height with the application of (±)-catechin, and when species were analyzed separately, treatment with (±)-catechin reduced the change in height of two species each in Montana and Romanian (Table 1).

In 2005, treatment with (±)-catechin significantly reduced the growth of plants in Montana, but had no effect on Romanian plants (Fig. 2; leaves: $F_{region*treatment} = 8.050$, d.f. = 1,263, $P = 0.005$; height: $F_{region*treatment} = 8.567$, d.f. = 1,262, $P = 0.004$). When species from Montana were analyzed separately, leaf growth was reduced in eight of ten species (Table 2; Fig. 2), and (±)-catechin did not affect any Romanian species (Table 2;

Fig. 2). Five of the Montana species and one Romanian species exhibited reduced height growth due to treatment with (±)-catechin (Table 2).

## DISCUSSION

Our study provides evidence that (±)-catechin, an allelochemical exuded by the roots of *Centaurea*, may act as a "novel weapon," having strong negative effects on naïve plant species from its invaded range, but weak effects on coevolved species from its native range. While this has previously been shown in laboratory experiments (Bais *et al.* 2003), this is the first experimental demonstration in the field in both the invaded and native ranges.

While trends were consistent between years, there was a large difference in the strength of the effect of (±)-catechin in Montana in 2004 and 2005 (Fig. 1). There are a few possible explanations for this variation. First, due to the timing of the experiment, the plants were more phenologically mature in 2004 than in 2005. Older plants may be more resistant to the effects of (±)-catechin. Second, differences in climate between years may have affected the activity of (±)-catechin in the soil. This may have been accentuated by the method in which (±)-catechin was applied. The levels of (±)-catechin we applied in all experiments were at the lower end the range observed in field soils invaded by *Centaurea* (Bais *et al.* 2002, 2003; Thelen *et al.* 2005; J. Vivanco & L. Perry, personal communication, but see Blair et al. 2005), but *Centaurea* probably exudes (±)-catechin continuously over time (J. Vivanco and L. Perry, *unpublished data*).

The most important caveat for the interpretation of our experiments is the potential effects of soil chemistry on the retention and activity of (±)-catechin. (±)-Catechin appears to transform rapidly into other chemical forms once injected into soils (L. Perry & J. Vivanco, unpublished data). Although not yet published, we have strong evidence that different metal concentrations in soils (lead, copper, iron, calcium) can have very large effects (positive and negative) on the concentrations of pure catechin in solution (J. Pollock, W. Holben & R.M. Callaway, unpublished data). We do not know the chemical constituency of Romanian soils at our experimental site, but differences in soils between our North American and Romanian sites could possibly alter the effects of (±)-catechin in ways that would affect our results. Determining the effects of (±)-catechin, and more importantly the derivatives of (±)-catechin, in natural soils is a difficult task, but until this task is completed our knowledge of comparative biochemical allelopathic effects will remain incomplete. Also, as this experiment was done in only once in Montana and Romania, it is difficult to generalize these results. However, the strong correlation between the biogeographic differences in field results reported here and the biogeographic differences reported in laboratory experiments with soils from multiple sites in both the the invaded and native ranges is an important step forward in understanding the potential of novel biochemical weapons to affect invasions.

In 2005, the only North American species that did not exhibit either a trend for or significant suppression in leaf and/or height growth was *Lupinus*. *Lupinus* was also resistant to (±)-catechin treatment in a similar experiment in 2003 (see Thelen *et al.* 2005). The resistance of *Lupinus* to (±)-catechin appears to be due to exudation of oxalate, which counteracts (±)-catechin (Weir *et al.* 2006). Another North American species, *Gaillardia grandiflora* has also been found to resist (±)-catechin through oxalate production (Weir *et al.* 2006). This suggests the importance of using multiple species when testing the effects of allelochemicals and may explain the variation we found among species in the field. A study using just *Lupinus* and/or *Gaillardia* would have reached very different conclusions than those we report here.

Although (±)-catechin had a significant negative effect on the change in leaf number for Romanian species in 2004, the magnitude of this difference was much smaller than in North America (Fig. 1). Over-all, Romanian species that have shared a long evolutionary history with *Centaurea* were much more resistant to (±)-catechin than North American species. Evolution can occur rapidly in reaction to anthropogenic change (Kinnison & Hendry 2001) and potentially to the introduction of novel plant species (Callaway *et al.* 2005b). It is possible that strong selection pressure from *Centaurea* will cause more North American species to develop resistance to (±)-catechin or to the general competitive or allelopathic effects of *C. maculosa.* In support of this, Callaway *et al.* (2005b) found that when grown from seed of individuals that had survived *Centaurea* invasions, some native species were more resistant to the general effects of *C. maculosa* and to (±)-catechin, adding further evidence for the importance of coevolution in the structure of plant communities. However, the "tolerant" lines of North Ameirican natives were still outcompeted by *Centaurea,* indicating that meaningful evolution of resistance in the field may take many more generations (Callaway *et al.* 2005b).

Our results join a growing body of evidence demonstrating that the success of some invasive weeds may be because of the release of novel chemicals to which naïve species are not adapted (Rabotnov 1982; Callaway & Aschehoug 2000; Czarnota *et al.*

2001; Bais *et al.* 2003; Vivanco *et al.* 2004; Cappoccino & Carpenter 2005; Carpenter & Cappoccino 2005) or that novelty in general correlates with invasive success (Strauss et al. 2006). In addition to providing information useful to understanding invasions, the indication that groups of species in the native range of *Centaurea* were adapted to a particular characteristic of this species suggests that plant communities may develop integrated relationships (Goodnight 1990; Wilson 1997; Vivanco *et al.* 2004; Lortie *et al.* 2005) through a common evolutionary history.

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Table 1. Summary of t-tests of effect of treatment with (±)-catechin on the change in leaf number and height of plants in Montana and Romania in 2004. $P < 0.10$ are italicized, $P < 0.05$ are in bold.

| Effect | Df | T | *P* |
|---|---|---|---|
| Change in leaf number | | | |
| Montana | | | |
| *Achillea* | 13 | -0.122 | 0.905 |
| *Antenaria* | 10 | 0.202 | 0.844 |
| *Arenaria* | 17 | 0.781 | 0.446 |
| *Eriogonum* | 16 | 0.738 | 0.471 |
| *Festuca* | 16 | 2.984 | **0.009** |
| *Geum* | 18 | 0.908 | **0.020** |
| *Poa* | 14 | 1.406 | 0.181 |
| *Pseudoroegneria* | 18 | 0.437 | 0.667 |
| *Senecio* | 13 | 3.156 | **0.008** |
| Romania | | | |
| *Achillea* | 17 | 0.198 | 0.845 |
| *Agropyron* | 17 | -0.162 | 0.873 |
| *Medicago* | 17 | 0.979 | 0.341 |
| *Salvia* | 18 | 0.784 | 0.443 |
| Change in height | | | |
| Montana | | | |
| *Achillea* | 13 | -0.149 | 0.884 |
| *Antenaria* | 10 | 0.627 | 0.545 |
| *Arenaria* | 17 | 0.155 | 0.879 |
| *Eriogonum* | 16 | 0.946 | 0.358 |
| *Festuca* | 16 | 3.267 | **0.005** |
| *Poa* | 14 | 1.452 | 0.168 |
| *Pseudoroegneria* | 18 | -1.567 | 0.134 |
| *Geum* | 18 | 4.434 | **0.014** |
| *Senecio* | 13 | 1.221 | 0.244 |
| Romania | | | |
| *Achillea* | 17 | -2.079 | *0.052* |
| *Agropyron* | 17 | -1.092 | 0.290 |
| *Medicago* | 17 | 0.140 | 0.890 |
| *Salvia* | 18 | 2.794 | *0.012* |

Independent samples t-tests were performed using SPSS version 13 (SPSS Inc., Chicago, IL, USA).

Table 2. Summary of t-tests of effect of treatment with (±)-catechin on the change in leaf number and height of plants in Montana and Romania in 2005. P < 0.10 are italicized, P < 0.05 are in bold.

| Effect | df | T | *P* |
|---|---|---|---|
| Change in leaf number | | | |
| Montana | | | |
| *Achillea* | 18 | -3.709 | **0.002** |
| *Arenaria* | 18 | -1.788 | *0.091* |
| *Delphinium* | 13 | -1.041 | 0.317 |
| *Festuca* | 18 | -1.953 | *0.067* |
| *Geum* | 15 | -3.300 | **0.005** |
| *Lupinus* | 17 | 1.301 | 0.211 |
| *Poa* | 18 | -4.255 | **<0.005** |
| *Pseudoroegneria* | 18 | -3.902 | **0.001** |
| *Senecio* | 17 | -2.512 | **0.022** |
| *Zigadenus* | 16 | 3.003 | **0.008** |
| Romania | | | |
| *Achillea* | 17 | 0.198 | 0.845 |
| *Festuca* | 18 | 0.575 | 0.572 |
| *Agropyron* | 17 | -0.162 | 0.873 |
| *Salvia* | 18 | -0.268 | 0.792 |
| Change in height | | | |
| Montana | | | |
| *Achillea* | 18 | -1.458 | 0.162 |
| *Arenaria* | 18 | -1.442 | 0.166 |
| *Delphinium* | 13 | -2.159 | **0.050** |
| *Festuca* | 18 | -2.818 | **0.011** |
| *Geum* | 15 | -0.957 | 0.354 |
| *Lupinus* | 17 | -0.403 | 0.692 |
| *Poa* | 18 | -2.138 | **0.046** |
| *Pseudoroegneria* | 18 | -3.499 | **0.003** |
| *Senecio* | 18 | -1.643 | 0.118 |
| *Zigadenus* | 16 | -4.746 | **<0.0005** |
| Romania | | | |
| *Achillea* | 18 | -2.079 | *0.052* |
| *Agropyron* | 17 | -1.092 | 0.290 |
| *Festuca* | 18 | 0.169 | 0.867 |
| *Salvia* | 18 | -0.968 | 0.346 |

Independent samples t-tests were performed using SPSS version 13 (SPSS Inc., Chicago, IL, USA).

Figure 1. Differences in the percent change in leaf number of controls and plants treated with (±)-catechin for each species in Montana and Romania in 2005. Data are means +1 SE. For each species, asterisks (*) show significant differences ($p<0.05$) between control and treated plants.

Figure 2. Differences in the percent change in height and leaf number of controls and plants treated with (±)-catechin in Montana and Romania. Experiments were conducted in the field in both 2004 and 2005. Data are means +1 SE. In 2004, there was a significant effect if treatment on the change in leaf number ($p=0.014$). Within in each country, asterisks (*) show significant differences ($p<0.05$) between control and treated plants.

Fig. 1

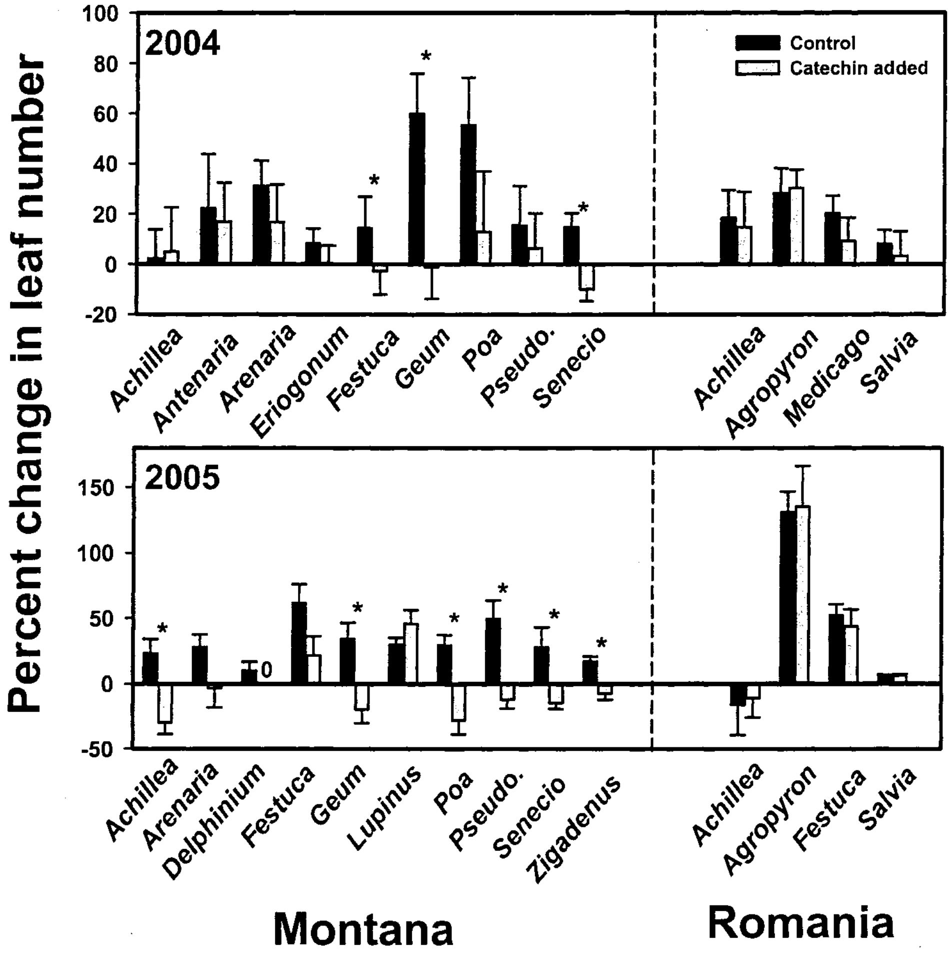
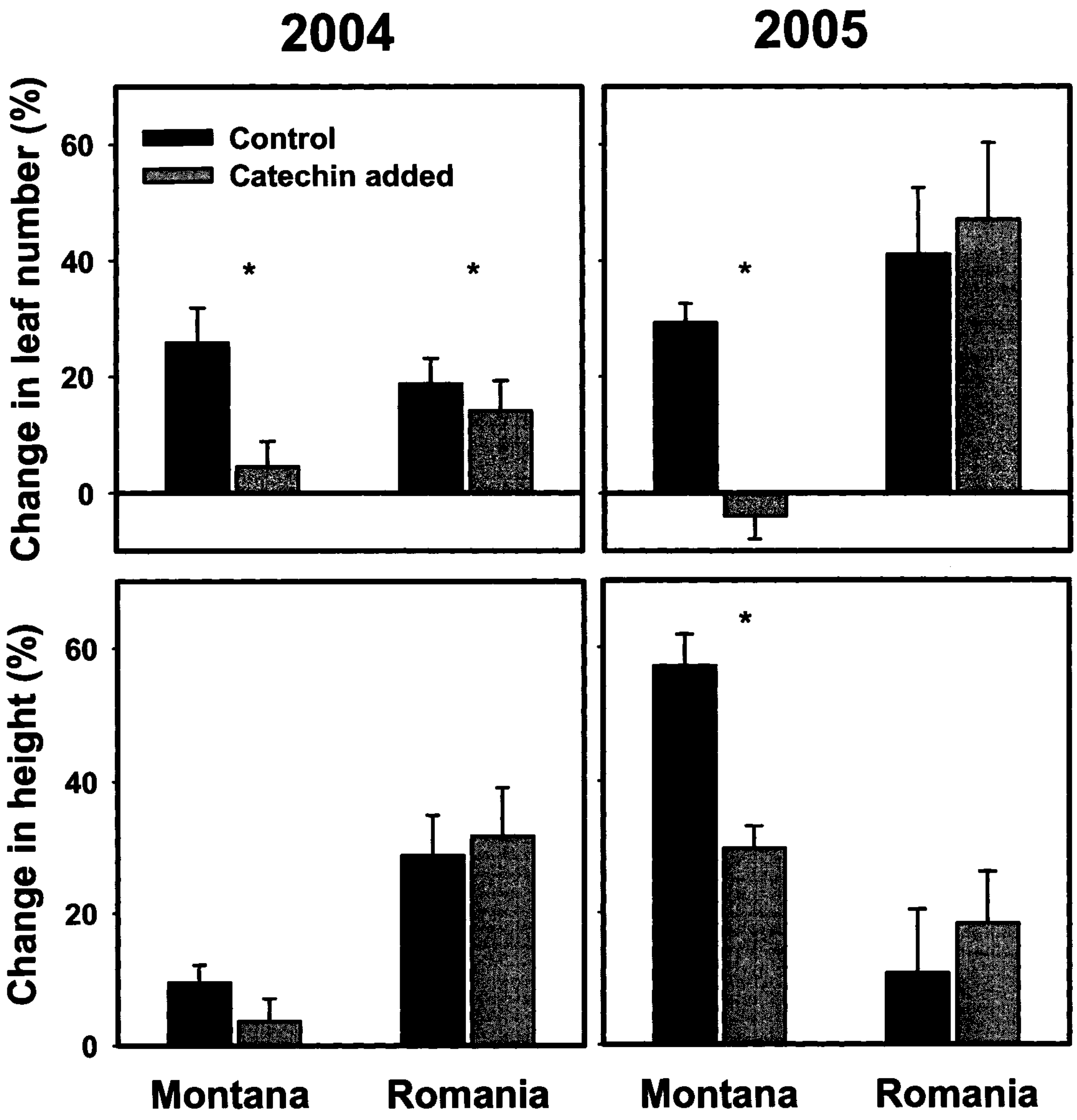

Percent change in leaf number
2004
100
80
60
40
20
0
-20
Control
Catechin added
*
*
*
Achillea
Antenaria
Arenaria
Eriogonum
Festuca
Geum
Poa
Pseudo.
Senecio
Achillea
Agropyron
Medicago
Salvia
2005
150
100
50
0
-50
*
0
*
*
*
*
*
Achillea
Arenaria
Delphinium
Festuca
Geum
Lupinus
Poa
Pseudo.
Senecio
Zigadenus
Achillea
Agropyron
Festuca
Salvia
Montana
Romania

Fig. 2


2004
2005
Change in leaf number (%)
Control
Catechin added
60
40
20
0
*
*
*
Change in height (%)
60
40
20
0
*
Montana
Romania
Montana
Romania