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Indirect interactions and plant community structure

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INDIRECT INTERACTIONS AND PLANT COMMUNITY STRUCTURE

By

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Competition in diverse communities promotes coexistence

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Abstract

Increased species diversity has strong positive effects on both the productivity and functioning of ecosystems. Therefore, understanding the ecological processes that drive coexistence in communities is of fundamental importance. A characteristic held in common for many of these processes, such as niche partitioning, consumer effects, and disturbance, is that they counteract, in theory, the fundamental effects of the competitive exclusion principle. Much ecological theory is based on the assumption that competition, if not neutralized by other factors, will drive weaker competitors out of the system and decrease diversity. Other theory poses that competition can be non-transitive (non-hierarchical) and that networks of competing species may actually promote coexistence, but empirical evidence for this theory is minimal. Here, I used a novel replacement species experiment in the field to demonstrate that competition among species assembled into complex groups is fundamentally different from competition among the same species in classic pairwise experiments. In groups, competition was much weaker than in pairwise arrangements and facilitative interactions emerged so that competition was considerably dampened, which should promote coexistence, but without the occurrence of non-transitive loops. These results suggest that ecological differentiation is not necessary for highly attenuated competition among plant species and their coexistence, and that current theory based on competitive hierarchies and exclusion, or on non-transitive competition in complex networks, is incomplete. Instead, coexistence may be enhanced in species rich assemblages by emergent processes derived from complex suites of direct and indirect interactions among groups of competitors that neutralize the competitive exclusion principle.

Keywords: competition, coexistence, *Centaurea stoebe*, hierarchy, indirect interactions, non-transitive.

High species diversity increases the productivity and functioning of ecosystems (Hooper 2005), therefore understanding the processes that promote coexistence in communities is crucial. Most theory for coexistence finds common ground in explaining how the effects of interspecific competition, or the "competitive exclusion principle" (Hardin 1960), might be avoided (Chesson 2000). This theory can be most simply organized into three categories: 1) avoidance of competition through spatial or temporal niche partitioning (Hutchinson 1959, MacArthur and Levins 1964, 1967, Warner and Chesson 1985), 2) weakening of strong competitors thru non-equilibrium forces such as disturbance and herbivory (Connell 1978, McNaughton 1979, Pickett 1980), and 3) disrupting competitive exclusion through non-transitive competitive interactions (Buss and Jackson 1979). This theoretical focus on mechanisms that reduce the effects of interspecific competition derives from the perspective that unfettered competition must lead to lower diversity. In other words "ecological differentiation is the necessary condition for coexistence" (Hardin 1960), a perspective based to a large degree on classic two-species models and experiments (Volterra 1926, Gause 1934). However, competitors rarely occur in pairs in nature, and theoretical models suggest that non-transitive direct interactions or "competitive loops" among *groups* of competitors may promote coexistence among competitors – a striking contrast to the competitive exclusion principle (Laird and Schamp 2006). Non-transitive interactions require that at least one competitively subordinate species in a group outcompete at least one species higher on the competitive hierarchy and provides an explanation for how direct competition might be attenuated. Experiments have demonstrated strong indirect interactions among competitors (Miller 1994, Levine 1999, Callaway and Pennings 2000); however, there is no empirical evidence for such loops in communities of competing plants.

Here I show that strong interspecific competition is highly attenuated, and even reversed to facilitative interactions, when species compete simultaneously in groups. In some cases, the "modified interactions" that occur in groups of competitors may help to explain why some diverse communities in nature appear to be far more inherently stable than predicted by two species models and experiments. In a three year field experiment, five native North American prairie grasses and forbs and one highly invasive European forb, *Centaurea stoebe*¸ were grown alone, in all pairwise combinations, and in a novel replacement series with Latin square matrix plot combinations that allowed for all possible combinations of contacts among species. By iteratively omitting each species from multispecies plots I was able to compare the net competitive effects and response of species in two-species and multi-species community settings.

I calculated direct effects using Relative Interaction Intensity indices (RII; Armas et al. 2004) to make comparisons of competitive performance. RII is a measure of the strength of interaction between species centered on zero with negative interactions (competition) indicated by values between 0 and -1, and positive interactions (facilitation) indicated by values between 0 and +1. RII allows for simple comparisons of interaction strength across taxa and treatments.

In pairwise competition 8 of 16 interactions were significantly negative, or competitive, interactions (Figure 1), consistent with the idea that competition has the potential to eliminate some species. In addition, three interactions tended towards competition, but were not significant ($P<0.10$). However, when the same native species competed in complex communities with other natives, only 4 of the 16 interactions were significant and all were competitive. Lastly, when natives competed with other natives in complex communities that had been invaded by *C. stoebe*, only 2 of the 16 interactions among natives were significant; and one of the significant interactions was competitive and one was facilitative. Two other interactions

in the invaded community tended towards significance ($P<0.10$), one negative and one positive. These apparently strong differences in the number of interactions between treatments, however, are subjective due to limitations of the existing testing strategy which prevents statistical comparisons.

The summed competitive effects of native species on each other (the total additive net direct effects) were much weaker in multi-species communities. In pairwise competition the mean of the summed competitive effects (RII) for each native species on each other was -1.03±0.30 (Figure 2). In multi-species communities composed only of natives the mean of the summed RIIs decreased by a factor of three, to -0.31±0.09 ($P = 0.008$). When native communities contained *C. stoebe*, the mean of the summed RIIs was significantly different than pairwise competition ($P = 0.05$) but not different from that in the uninvaded communities ($P = 0.47$). However, the means of summed RIIs in invaded communities were not significantly different from zero ($P = 0.971$).

When natives competed with each other in multispecies communities invaded by *C. stoebe*, the individual effects of each single species on another either diminished in strength or in one case, changed direction. In the presence of *C. stoebe*, *Gaillardia aristata*, a perennial forb, significantly facilitated *Pseudoroegneria spicata*, a dominant bunchgrass. *Gaillardia aristata* also tended to facilitate *Festuca idahoensis* in the invaded community, but this effect was not significant.

The competitive effects of the invasive *C. stoebe* on native species were far stronger than those measured among natives, ranging from RIIs of -0.20±0.09 to -0.80± 0.08 in pairwise interactions (Figure 3), but from -0.08±0.11 to -0.34±0.10 in multispecies communities. The competitive effects of *C. stoebe* were significantly dampened for three of the four native species

when in communities than in pairwise arrangements, but the RII for the dominant native competitor, *Pseudoroegneria spicata*, was not significantly different in multi-species communities. The average net direct effect of *C. stoebe* on native species was an RII of -0.50±0.16 in pairwise arrangements versus -0.26±0.06 in multispecies arrangement, but these did not differ significantly ($P = 0.22$), suggesting that stronger invaders (Ortega and Pearson 2005) do not compete by the same rules as natives.

As the diversity of competitors increases in native communities, the strong competitive effects of species on each other that are predicted by two-species models and measured in simple experiments waned substantially and shifted toward weak facilitative interactions (Figure 1). Such shifts suggest that the fundamental role of interspecific competition in communities may be profoundly different than presumed in the ecological theory of the competitive exclusion principle – I found little evidence that competition among groups of native species, whether invaded or not, would lead to competitive exclusion. However, the consistently strong competitive effects of the invader were consistent with the competitive exclusion principle. Thus competition among species may actually promote diversity in some species-rich communities, and therefore the loss of some species may have cascading or accelerating effects on the diversity of communities. The idea that competition may *promote* coexistence through the emergence of indirect positive effects as a result of complex multi-species interactions is not predicted by classic competition theory.

The strong decrease in competitive intensities that occurred in multi-species communities are almost certainly due to indirect effects. Indirect effects among competitors have been demonstrated in a number of field experiments (Miller 1994, Levine 1999, Callaway and Pennings 2000, Callaway and Howard 2007, Weigelt et al. 2007, Saccone et al. 2010, Michalet

et al. 2011) but have proved difficult to separate from direct effects or separate fully from the effects of disturbance. In a groundbreaking study of indirect interactions among plant competitors Miller (1994) used removal experiments to model important indirect positive effects among five exotic old-field plant species. Our empirical results strongly support his findings but are derived from experimental communities in which the effect of a single native perennial species on another has been experimentally quantified both in pairwise interactions and in complex communities without the disturbance of removals – i.e. without creating potential non-equilibrium conditions. To our knowledge all field studies of indirect interactions have utilized removal experiments. In addition to introducing non-equilibrium conditions, removal experiments do not account for interactions that occur prior to removal, which may leave confounding legacies on the strength and direction of competition.

Centaurea stoebe appears to be an exceptionally strong competitor in intermountain prairies, based on its ability to replace natives and form near monocultures in nature (Ridenour and Callaway 2001) and its very strong effects in experiments (Maron and Marler 2008). I included this strong competitor in pairwise interactions against all natives and in full native communities and found that the invader had very strong net effects in all experimental contexts. Surprisingly, in the presence of the invader the total net effect of native species on each other when competing in communities collapsed to summed values not significantly different from zero. This provides a novel perspective on the way that invaders alter native landscapes through their unusually strong competitive effects (Maron and Marler 2008). Not only do they suppress natives themselves, they may alter the way that natives interact with each other. In this same context, our results add to the potential mechanisms by which diversity might resist exotic invasion. Strong invasive competitors may eventually exclude natives, as indicated by my

experiments, but also alter interaction intensities among native species in ways so that they compete far more weakly with each other and even facilitate each other. If increasing native diversity consistently decreases net interaction strengths among natives in invaded systems and the mean competitive effects of invaders, this may provide a novel form of biotic resistance that is inherent to diverse communities. To be clear, I did not test the effects of diversity, *per se*, in our experiment, yet our results infer a new mechanism by which diversity may contribute to biotic resistance to invasion.

An explicit understanding of how species on the same trophic level interact in groups is crucial if we are to understand the mechanisms that drive the emergent properties of groups – e.g. the relationship between species diversity and ecosystem function. It is clear that niche complementarity (Hector et al. 1999, Tilman et al. 2001) and non-equilibrium processes (Connell 1978, White 1979, Miller 1982) have powerful effects on coexistence and diversity; however our results empirically demonstrate that classic theory based on two species models and experiments substantially overestimate the competitive exclusion principle in multi-species communities, but that this overestimation is not dependent on non-transitive interactions (Stone and Roberts 1991, Laird and Schamp 2006). Instead, indirect effects within trophic levels in species rich communities are maintained in part by the emergent properties of species interacting simultaneously in groups.

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Figure Legends

Figure 1. Net direct effects between species grown in either pairwise, native multi-species groups, or invaded multi-species groups. Solid arrows are negative interactions and dotted lines are positive interactions. Black arrows represent significant interactions ($P < 0.05$) and light gray arrows represent non-significant interactions. Arrow thickness represents interaction strength. All native pairwise interactions are redrawn (light gray are non significant and dark gray are significant) for reference in the *C. stoebe* pairwise panel.

Figure 2. Total summed effects (RII values) of all native species on each other in either pairwise, native multi-species community plots, or invaded multi-species community plots. Asterisks represent significant interactions and letters represent significant difference between means of treatments ($P < 0.05$).

Figure 3. Direct effects (RII) of the invader, *Centaurea stoebe*, on native species in either pairwise or invaded multi-species communities. Asterisks represent significant interactions ($P < 0.05$).

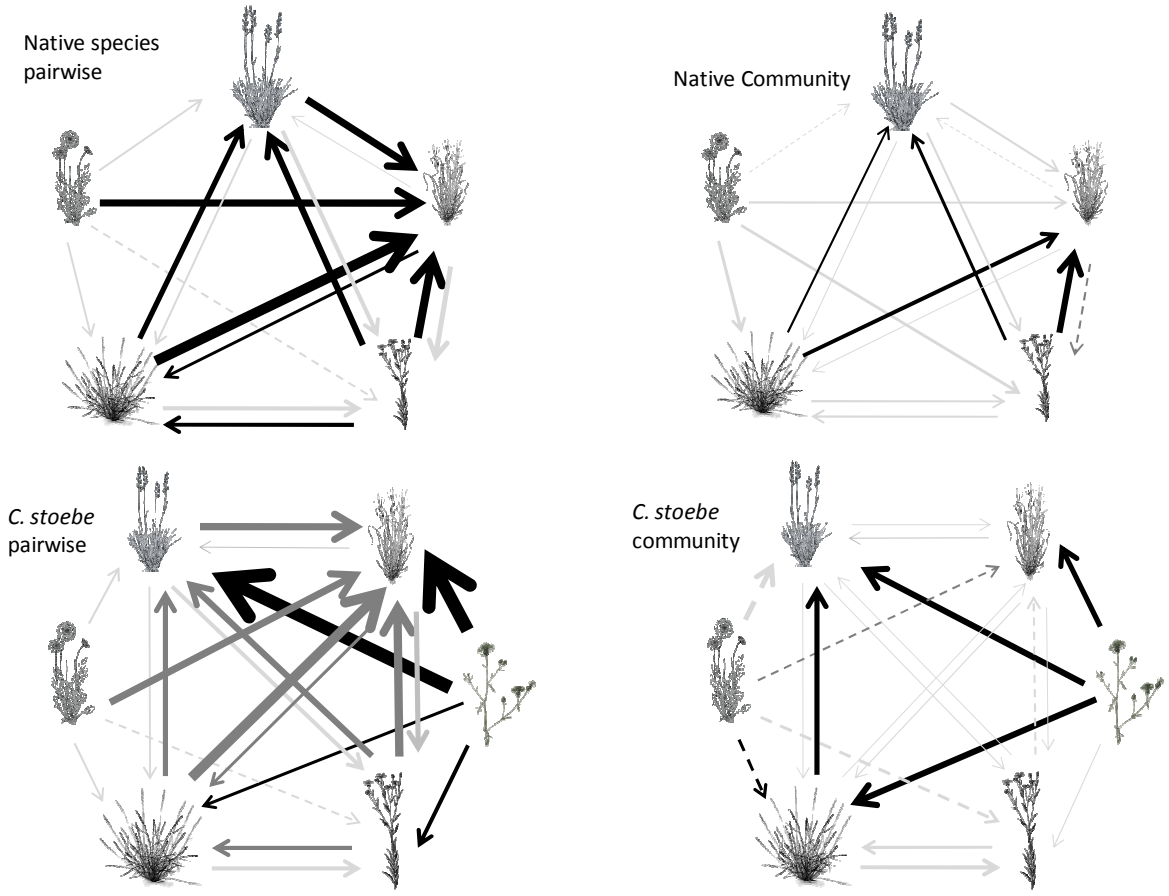
Native species
pairwise
Native Community
C. stoebe
pairwise
C. stoebe
community

Figure 1

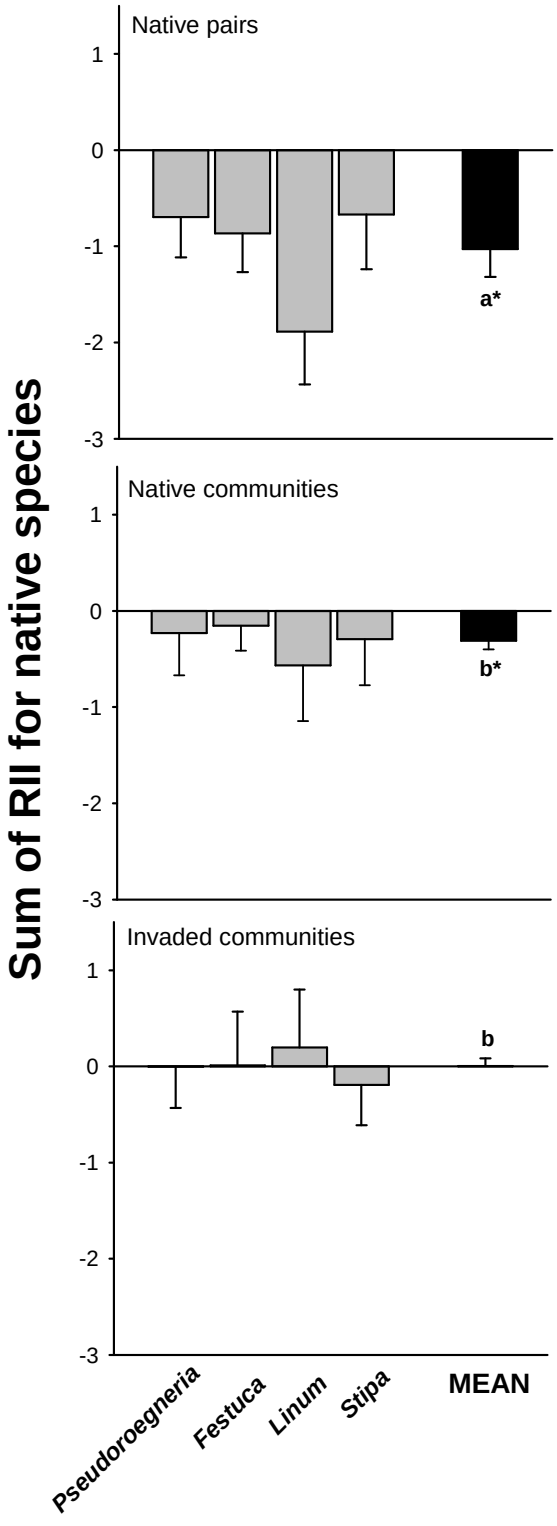
Native pairs
Native communities
Invaded communities
Sum of RII for native species
1
0
-1
-2
-3
a*
b*
b
Pseudoroegneria
Festuca
Linum
Stipa
MEAN

Figure 2

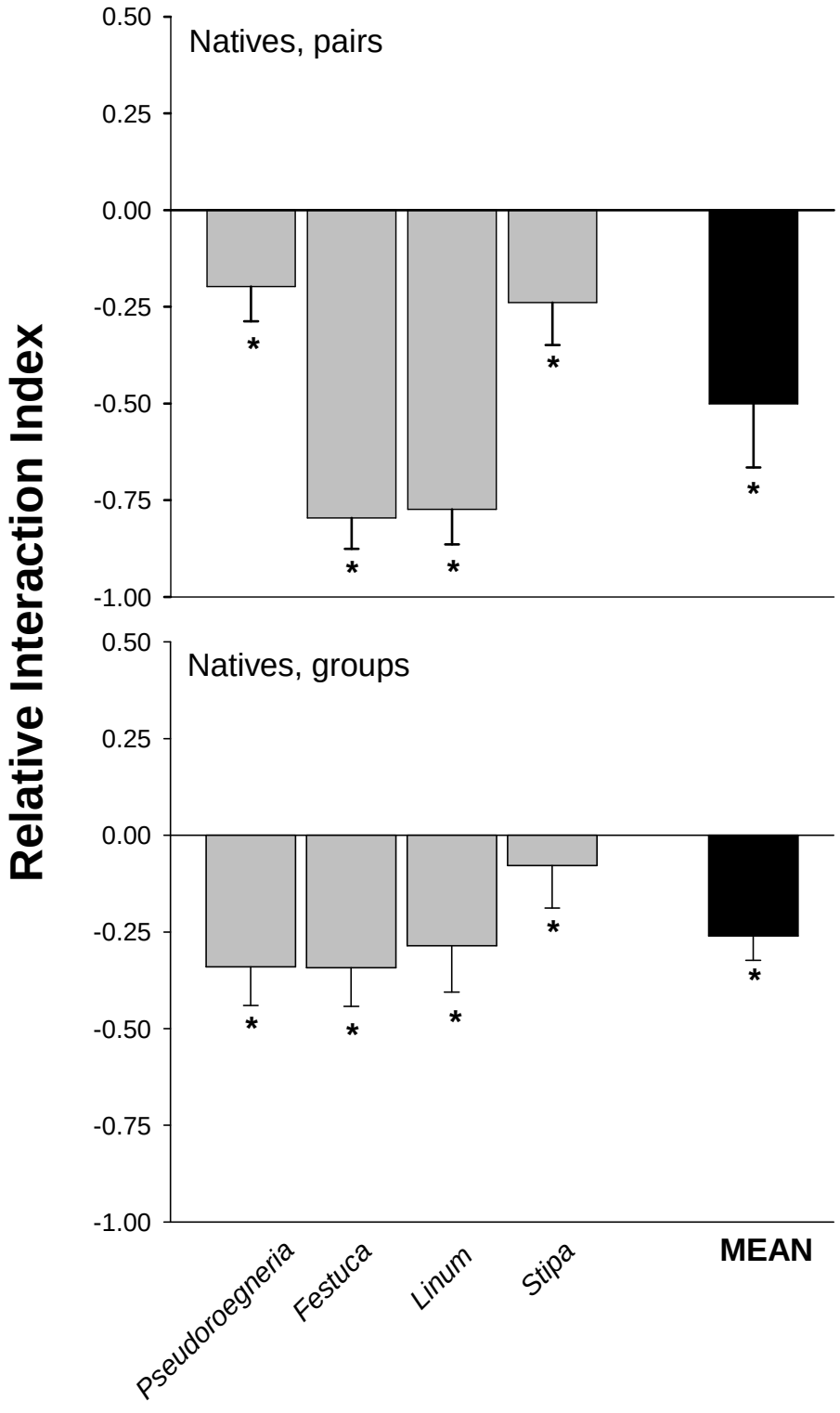
Relative Interaction Index
Natives, pairs
Natives, groups
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
*
Pseudoroegneria
Festuca
Linum
Stipa
MEAN

Figure 3

Supplementary Material

Methods

In the summer of 2007 I created 322 experimental plots varying in species composition and complexity. I chose three perennial bunchgrasses (*Pseudoroegneria spicata, Festuca idahoensis, Stipa comata*), two forbs (*Linum lewisii and Gailardia aristata*) and one highly invasive species (*Centaurea stoebe*) that are common in intermountain prairie in the northern Rocky Mountains. Communities were built in 0.25 m^2 plots in an experimental site at the Fort Missoula Biological Field Station, Missoula, Montana (46°50'16.92"N, 114° 3'24.58"W; 960 m). Plots were established in an area with homogeneous soil and solar exposure, and were subjected to the same water regimes. The site was fenced and thus free from large herbivores and disturbance. Prior to planting, the site was sprayed with the herbicide glyphosate to remove all vegetation and tilled to ensure highly uniform conditions.

To quantify and compare direct and indirect interactions, all species were grown alone, and in pairwise and iterative omission Latin square matrix plot designs that allowed for all possible combinations of contact among species (Figure 1). Plants were first grown in 100 mL "conetainers" (2.5cm x 16.5 cm; Stuewe and Sons, Corvallis, OR) from purchased seed in a greenhouse. We outplanted seedlings in July 2007 and provided supplemental water during the first growing season to improve transplant success. Following planting and for three growing seasons, I weeded all plots by hand to minimize the effects of garden weeds on the interactions among target species. After three growing seasons, I harvested, dried in drying ovens for 72 hours at 60° C, and weighed all above ground biomass for each individual.

Drought either killed or prevented the resprouting of large numbers of one native forb, *Gaillardia aristata*, and the invasive forb, *Centaurea stoebe*, during the last two months of the

experiment. Thus I was able to quantify 16 of the 20 possible direct effects among native species, and the effects, but not the response, of *C. stoebe* in our experimental treatments. I calculated direct effects using the Relative Interaction Intensity index (RII; Armas et al. 2004). RII is calculated by dividing the difference between the biomass of the treatment and control by the sum of the biomass of the treatment and control ($(B_T-B_C)/(B_T+B_C)$). Standard error was calculated using the formula provided by Armas et al. (2004, Appendix A). RII is a measure of the strength of interaction between species centered on zero with negative interactions (competition) indicated by values between 0 and -1, and positive interactions (facilitation) indicated by values between 0 and +1. RII allows for simple comparisons of the impact of species on each other across taxa and treatments.

For all species in pairwise plots I calculated RIIs (Table 1) using the mean biomass of plants grown alone as controls and the mean biomass of plants in pairwise competition as the treatment. For all species grown in multi-species experimental plots (native and invaded treatments), RIIs for the net direct effect of a single species on another in the context of communities were calculated by using the mean biomass of individuals grown in four species plots as controls and the mean biomass of individuals grown in complete five species assemblages as the treatment. In order to avoid pseudoreplication I took the mean size of the surviving individuals of each species in each plot and used it to calculate overall treatment means and standard errors. I excluded all mortalities from our analysis. Statistical tests were performed on RIIs using t-tests (Excel 2007) to determine if interactions were significantly different from zero. All statistical comparisons of RIIs among different species or between treatments utilized either a one-way ANOVA with Tukey post hoc comparisons or two sample means t-tests (Sigmaplot 11.2, Systat Software).

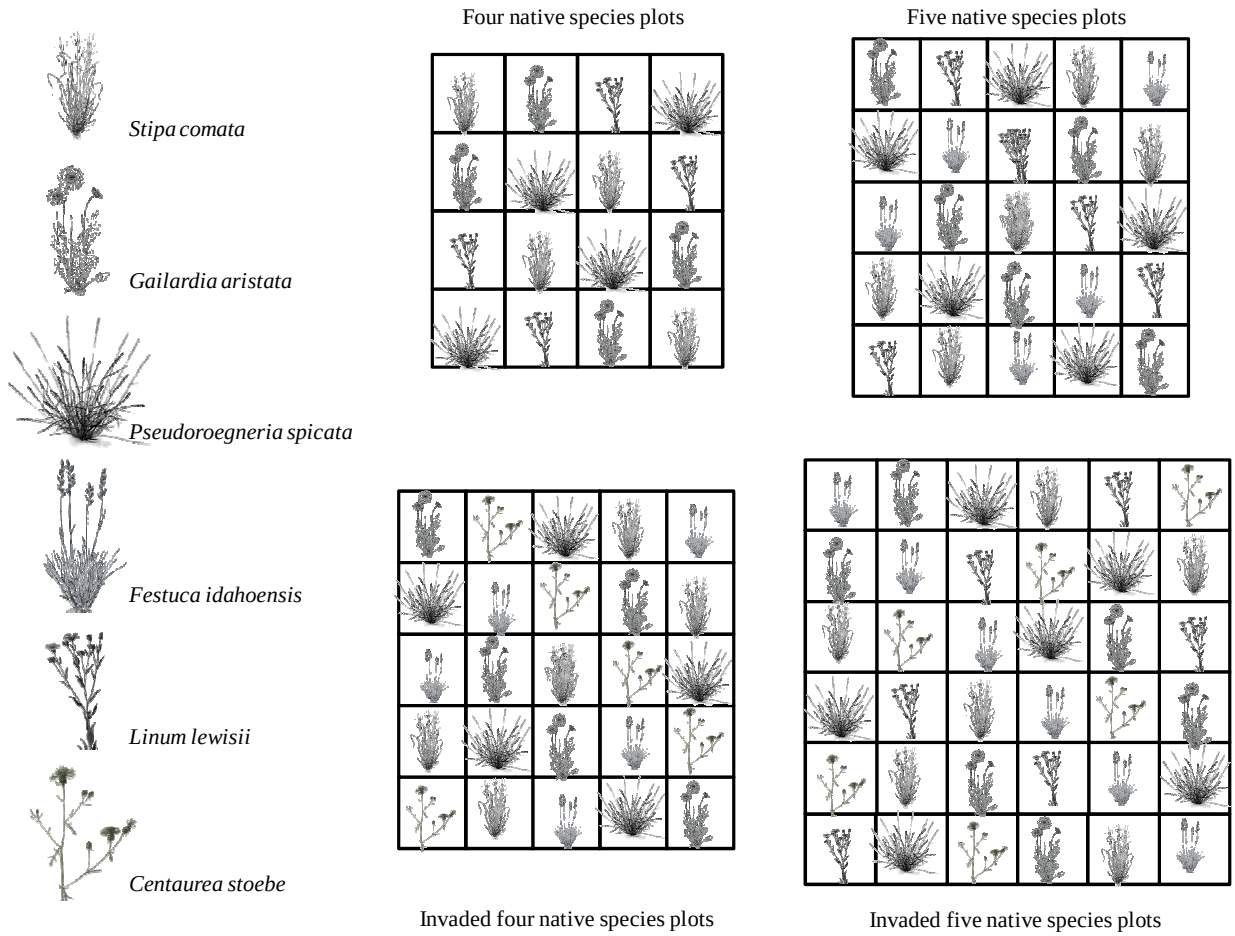

Figure 1. Experimental design for iterative omission, Latin square plot design. All species were planted 10 cm apart horizontally and 14 cm on diagonal. All plot designs were replicated (n=10 for species grown alone and in pairs, n=8 for species grown in native four species plots, n=10 for native five species, n=10 for invaded four native species plots, and n=12 for invaded five species plots) with all possible combinations of species. All species occupied all possible locations within each plot via replication to avoid bias in location within the plot.

Table 1. Net direct effects (RII) of each species interaction in pairwise, native multi-species community and invaded multi-species community plots. Bold values represent significant interactions ($P<0.05$) and asterisks indicate marginally significant interactions ($P<0.10$).

Pairwise Interactions

	Pseudoroegneria spicata	***Festuca idahoensis***	***Stipa comata***	***Linum lewisii***
P. spicata		**-0.30 ± 0.09**	**-0.58 ± 0.11**	-0.23 ± 0.13*
F. idahoensis	-0.13 ± 0.11		**-0.43 ± 0.19**	-0.24 ± 0.13*
S. comata	**-0.20 ± 0.08**	-0.07 ± 0.10		-0.30 ± 0.15*
L. lewisii	**-0.26 ± 0.10**	**-0.38 ± 0.11**	**-0.45 ± 0.12**	
Gailardia aristata	-0.11 ± 0.13	-0.12 ± 0.10	**-0.43 ± 0.13**	+0.10 ± 0.16
Centaurea stoebe	**-0.20 ± 0.09**	**-0.80 ± 0.08**	**-0.77 ± 0.09**	**-0.24 ± 0.11**

Native Community Interactions

	Pseudoroegneria spicata	***Festuca idahoensis***	***Stipa comata***	***Linum lewisii***
P. spicata		**-0.14 ± 0.07**	**-0.22 ± 0.09**	-0.10 ± 0.11
F. idahoensis	+0.02 ± 0.13		-0.10 ± 0.15	-0.11 ± 0.10
S. comata	-0.01 ± 0.12	+0.08 ± 0.06		+0.12 ± 0.11
L. lewisii	-0.10 ± 0.10	**-0.18 ± 0.08**	**-0.36 ± 0.14**	
Gailardia aristata	-0.15 ± 0.09	+0.08 ± 0.05	+0.11 ± 0.20	-0.20 ± 0.16

Invaded Community Interactions

	Pseudoroegneria spicata	***Festuca idahoensis***	***Stipa comata***	***Linum lewisii***
P. spicata		**-0.29 ± 0.12**	-0.02 ± 0.15	-0.25 ± 0.12*
F. idahoensis	0.00 ± 0.11		+0.02 ± 0.12	-0.02 ± 0.11
S. comata	-0.04 ± 0.13	+0.08 ±0.16		-0.08 ± 0.10
L. lewisii	-0.16 ± 0.10	-0.05 ± 0.14	+0.11 ± 0.19	
Gailardia aristata	**+0.20 ± 0.09**	+0.27 ± 0.14*	+0.10 ± 0.14	+0.15 ± 0.09
Centaurea stoebe	-0.34 ± 0.10	-0.34 ± 0.10	-0.29 ± 0.12	-0.08 ± 0.11

No evidence for community assembly through classic competition models: the importance of indirect “interaction modifications”.

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Abstract

The role of competition in assembling communities has been conceptualized in two fundamentally different ways. The first is hierarchical, or transitive, and in this view competition functions as a strong force against coexistence. The second conceptual perspective is non-hierarchical, or non-transitive, and in this view competition does not act as a strong force against coexistence and can even increase the potential for coexistence among species. I used a novel replacement series field experiment with prairie plant species from the northern Rocky Mountains and path analysis models in order to compare the outcomes of pairwise and multispecies competition and to search for non-transitivity in multispecies communities. Competition had profoundly different outcomes in pairwise experiments than in multispecies communities indicating that pairwise experiments and thus hierarchical models function poorly to predict the role of competition in the assembly of complex, diverse plant communities. In addition, I found no evidence for explicitly non-transitive assembly rules; there were no reversals in competitive rankings resulting in competitive loops. Thus I propose a third perspective on the role of competition in the organization of communities that has not been previously formalized in the literature in which "interaction modifications" function to strongly buffer, attenuate, or even reverse competitive outcomes, *without* non-transitive interactions, in ways that sustain coexistence and biological diversity in plant communities.

Introduction

The processes that determine species coexistence, and thus determine the distributions of species in communities, have interested ecologists for decades. These processes include those that occur when communities are at or near equilibrium such as species-specific adaptation to unique niches (Hutchinson 1959, Silvertown 2004), regulation by regional "species pools" (Zobel 1997), and temporal niche fluctuation such as storage effects (Chesson 2000). Also, non-equilibrium processes such as disturbance and herbivory can allow species with different life histories to coexist by creating situations where species that tolerate disturbance and compete well after the effect of the disturbance subsides are favored (Connell 1978, Reice 1994). Importantly, both equilibrium and non-equilibrium processes are thought to counteract the effects of competition in some way in order to promote coexistence. This is because classic competition theory suggests that competition always leads to the exclusion of less competitive species – the competitive exclusion principle (Hardin 1960). However, classic competition theory is largely derived from models and experiments that evaluate interactions between two species (Volterra 1926, Gause 1934, Tilman 1982) or sets of pairwise interactions (Keddy and Shipley 1989). But natural communities generally have many more species which interact with each other and the same time, thus it is crucial to ask questions about coexistence in diverse communities where the effects of interspecific competition at equilibrium may be much different than predicted by classic theory (Stone and Roberts 1991, Miller 1994).

Early attempts to characterize the fundamental nature of plant communities, and thus the forces that organize them, produced largely mutually exclusive concepts. For example, Clements (1916) theorized that plant communities were tightly knit and predictable associations, and that these associations may even possess coevolved relationships among plants. In contrast,

Gleason (1926) suggested that plant communities are loosely assembled by random dispersal events into areas where abiotic conditions either permit or exclude a particular species. This Gleasonian view led to a wide acceptance of the individualistic perspective on communities (Whittaker 1951, 1975, Curtis 1959, McIntosh 1967, Peet 1981, Ter Braak and Prentice 1988, Austin 1990). These divergent views on the nature of plant communities have influenced much of modern plant community theory, including theory on how species coexist (Lortie et al. 2004). For instance, the individualistic paradigm is reflected in theory that focuses on explaining species coexistence in the context of competition for resources (Tilman 1982), the null model of community assembly (Connor and Simberloff 1979), and neutral theory (Hubbell 2001). Classic competition theory, in which exclusion is the inevitable outcome of competition at equilibrium, fits well with the construct of individualistic theory. As a result, while we know much about the effects of species on each other via direct, pairwise interactions, less is understood regarding how competition functions within communities.

Recently, ecologists have suggested that rather than communities being organized along the extremes advocated by Gleason or Clements, a "middle ground" view is more in line with empirical evidence (Lortie et al. 2004, Brooker et al. 2008). This is in part due to research over the last 30 years that suggests indirect interactions may be important in determining the structure of plant communities. For example, Miller (1994) manipulated the presence-absence of five old-field annual plant species in a field study and consistently found indirect facilitative effects among competitors. Levine (1999) experimentally demonstrated that *Carex nudata*, a riparian sedge, had strong direct competitive effects on most species that occurred within its tussocks. At the same time, *C. nudata* had a strong positive indirect effect on the liverwort *Conocephalum conicum* by reducing the abundance of *Mimulus guttatus* which strongly suppresses

Conocephalum. Theory and experimentation with indirect effects as potentially important drivers of coexistence have provided an intriguing alternative to the dominant paradigm for the way that competition may influence the assembly and maintenance of biodiversity in plant communities. This has also stimulated a renewed interest to understand indirect interactions among competitors of the same trophic level (Stone and Roberts 1991, Callaway and Pennings 2000, Pages and Michalet 2003, Callaway and Howard 2007, White et al. 2006, Weigelt et al. 2007, Koorem and Moora 2010, Saccone et al. 2010). If in multispecies groups species interact in ways that attenuate the intensity of competition through the creation of indirect facilitative interactions, then competitive exclusion may be less of a fixed outcome in communities in nature (Huisman and Weissing 2001).

One broad approach that has been used to link competitive interactions into a predictive framework for community structure has been the study of "assembly rules". However, to my knowledge there have been no efforts to link conceptual progress made through research on indirect interactions and coexistence with the study of assembly rules. Such a link has a great deal of potential for exploring the two general categories of assembly rule theories for how plant communities assemble under equilibrium conditions. The first assumes that plant communities are competitively transitive in nature (Goldsmith 1978, Mitchley and Grubb 1986; Keddy and Shipley 1989). In other words, all species in a given pool, or community, can be ranked in a simple linear hierarchy of competitive ability. The strict "pecking order" that results provides a predictive tool for community organization. The crucial conceptual outcome of a transitive, or hierarchical, perspective on assembly rules is that communities will consistently move towards dominance by the best competitor in the hierarchy through competitive exclusion. In transitive or hierarchical communities the weaker competitors will be excluded given enough time and the

absence of non-equilibrium processes. In this paradigm only non-equilibrium filters or abiotic heterogeneity can prevent the formation of monocultures or dominance by a small number of the most competitive species in the pool.

A major criticism of hierarchal assembly theory is that transitive properties have rarely been demonstrated in the field in natural communities (although see Miller and Werner 1987; but see Silvertown and Dale 1991). In addition, the majority of evidence supporting hierarchical assembly rules is derived from highly artificial protocols (Silvertown and Dale 1991). The most common experiments investigating hierarchical assembly rules are performed with "phytometers". Generally conducted in greenhouses, each member of a community is grown in competition in a pot with a species that is not found in the community of interest, and generally thought to be a moderate to weak competitor. The competitive effect of the phytometer species on each individual in the community is quantified and used to rank all species along a continuum. By this approach a hierarchy is established and community assembly is predicted.

There are major problems with this approach. First, the resource-rich and low-stress life in a greenhouse is likely to overemphasize the importance of competition relative to what might be observed in natural systems where physical stress has the potential to reduce competitive intensities (Grime 2001, Besaw et al. *in press*). In addition, this approach assumes that plants compete only in ways that can be encapsulated in general traits such as the mean size or allocation ratios of a species, species-specific light requirements, or species-specific nutrient uptake rates (Gaudet and Keddy 1988), all of which are likely influenced by greenhouse conditions. This overemphasis of simple competitive traits is further exaggerated by forcing plants to grow in the same space in pots, which eliminates most potential for spatial niche partitioning. Importantly, spatial niche partitioning is considered to be a fundamental

determinant of coexistence (Parrish and Bazzaz 1976) even within the paradigm of hierarchical assembly rules. The phytometer approach also ignores facilitative interactions (Bertness and Callaway 1994, Callaway 2007), indirect interactions (Miller 1994, Levine 1999), and evolved relationships that may attenuate the potential effects of competition and promote coexistence (Callaway and Aschehoug 2000, Thorpe et al. *in press*). It is hard to see how anything *but* a hierarchy could be determined from phytometer-based measurements, and not surprisingly, phytometer-based approaches consistently support theory for hierarchical community organization (Keddy et al. 2000; Fraser and Keddy 2005; Storkey *et al.* 2010).

While most attempts to detect and quantify community assembly rules have used the phytometer approach described above, other methods account for natural differences in competitive *effect* and competitive *response* between species within a community. Competitive effect, or the ability of a species to suppress its neighbor, differs from competitive response, which is the ability of a plant to resist its neighbor's negative impacts (Goldberg and Werner 1983, Goldberg and Landa 1991). Pairwise studies have been used to quantify the competitive effects and responses of species that comprise communities in fully iterative designs. This approach allows all plants to directly compete with all other species in pots and although pairwise comparisons have some of the same experimental constraints as phytometer experiments using pairwise comparisons among all or most community members more accurately incorporates ecologically relevant direct effects among species. Most importantly, pairwise experiments are not experimentally restricted to a hierarchical outcome.

Objections to the theory of hierarchal assembly rules generally derive from evidence that the nature of interactions among plants interacting in natural communities, or simultaneously in groups, are far more complex than those measured in simplified or highly controlled conditions

(Herben and Krahulec 1990, Perkins et al. 2007, Engel and Weltzin 2008). The incorporation of complex interactions among *groups* of plant species may lead to very different conclusions about how competition acts within plant communities. When many species interact simultaneously in space and time, non-transitive, or non-hierarchical, community assembly rules can emerge (Jackson and Buss 1975, May and Leonard 1975, Petraitis 1979). For example, theoretical models and experiments have shown that if community members interact in non-transitive ways, coexistence may be maintained among large pools of species even in the absence of abiotic heterogeneity or non-equilibrium processes (Buss 1980, Laird and Schamp 2006). Hierarchical organization is best described mathematically as A>B>C, whereas non-hierarchical organization occurs when loops form in the hierarchy such as A>B>C>A. In other words, species C indirectly benefits species B by having a direct negative impact on species A. Given the right starting point, a simple loop within a suite of competing species can result in a perpetually shifting state in which all three species coexist indefinitely. This coexistence is based entirely on the balance of direct competitive interactions, but leads to the formation of complex networks of species interactions. Mathematical evaluations of non-transitive loops among competitors have demonstrated that communities of multiple species can coexist (Karlson and Jackson 1981, Laird and Schamp 2006, 2008).

Theory for non-hierarchical community assembly is well developed; however, experimental demonstration of the effects of competitive networks on community diversity and the coexistence of species has proved highly intractable. Because of this, most evidence for non-hierarchical assembly has relied on mathematical modeling or indirect evidence derived from removal experiments. For example, Miller (1994) used a field based removal design to model direct and indirect effects among five old-field annual plant species and consistently found

important *indirect* positive effects among species that also had strong *direct* competitive effects on each other. This approach can address how an existing set of plant species responds to the absence of a single community member, but the remaining species may still be interacting in either transitive or non-transitive ways that are not measured, and disturbance from the removal of the species can introduce non-equilibrium effects. Regardless, removal experiments that show strong increases in abundance for some species and strong decreases for other species provide critical evidence for a non-hierarchical perspective on the effects of competition on coexistence in natural and complex communities.

Hierarchical and non-hierarchical paradigms for how competition affects the way species assemble into communities are fundamentally different. Hierarchical assembly rules function under the assumption that competition inexorably acts to exclude poor competitors from communities, whereas non-hierarchical assembly rules suggest that competition can actually *contribute* to coexistence among species in communities.

Thus we have three general ways in which to conceptualize the role of competition in assembling communities. The first is hierarchical, or transitive, and in this view competition functions as a strong force against coexistence and the stability of diversity. The fundamental problem with this perspective is that how species interact in communities is assumed to be the same as how they act in pairwise interactions. The second conceptual perspective is non-hierarchical, or non-transitive, and in this view competition may reduce coexistence, but with the appropriate competitive loops among species, competition can function to sustain or promote coexistence. The fundamental problem with this perspective is that no empirical data from complex plant communities support the existence of competitive loops. The third perspective has not been clearly articulated or formalized in the literature prior to my research and stems

from a small but growing body of literature showing that competition in multispecies communities is "modified" in ways that strongly buffer, attenuate, or even reverse competitive outcomes, but *without* non-transitive interactions. Despite the importance of resolving these three conceptual perspectives on the effects of competition on coexistence and diversity in complex communities, there have been no experiments designed to isolate the ways that competition functions in multispecies experiments. This is in part due to methodological problems, but also because detection of very subtle changes in how plants interact can easily be confounded by covariation in the environment, consumer effects, and disturbance. Here, I studied the role of competition in assembling or disassembling communities by experimentally comparing the outcome of a classic pairwise competition experiments to that of a novel replacement series experimental design with Latin square matrix plot combinations that allowed for all possible combinations of contact among species. By iteratively omitting each species from multispecies plots I measured the net competitive effects and response of species in two-species and multi-species community settings without the potentially confounding effects of pre-removal interactions, microsite preference, disturbance caused by removals, and age-based priority effects. By comparing the results of pairwise to multispecies interactions I test the assumption that pairwise effects are equivalent to effects in multispecies communities- i.e. do hierarchical assembly rules predict interaction outcomes in both pairwise and multispecies communities? I then tested whether or not non-transitive interactions occurred in the multispecies communities – i.e. are reversals in competitive outcomes necessary to explain differences between pairwise and multispecies interactions or do emergent "interaction modifications" occur without non-transitive competition?

Methods

In the summer of 2007 I built 322 experimental plots varying in species composition and complexity. I chose three perennial bunchgrasses (*Pseudoroegneria spicata, Festuca idahoensis, Stipa comata*), two forbs (*Linum lewisii and Gailardia aristata*) and one highly invasive species (*Centaurea stoebe*) that are common in intermountain prairie in the northern Rocky Mountains. Communities were built in 0.25 m^2 plots in an experimental common garden at the Fort Missoula Biological Field Station, Missoula, Montana (46°50'16.92"N, 114° 3'24.58"W; 960 m elevation). Plots were established in an area with homogeneous soil and solar exposure, and were subjected to the same water regimes. The garden was fenced and thus free from large herbivores and disturbance. Prior to planting, the site was sprayed with the herbicide glyphosate to remove all vegetation and tilled to ensure highly uniform conditions.

To quantify and compare direct and indirect interactions, all species were grown alone, and in pairwise and iterative omission Latin square matrix plot designs that allowed for all possible combinations of contact among species (Figure 1). Plants were first grown in 100 mL "conetainers" (2.5cm x 16.5 cm; Stuewe and Sons, Corvallis, OR) from purchased seed in a greenhouse. I outplanted seedlings in July 2007 and provided supplemental water during the first growing season to improve transplant success. Following planting and for three growing seasons, I weeded all plots by hand, but without disturbing the soil, to minimize the effects of garden weeds on the interactions among target species. After three growing seasons, I harvested, dried in drying ovens for 72 hours at 60° C, and weighed all aboveground biomass for each individual.

I built pairwise and multispecies communities with only native species in order to evaluate hierarchical and non-hierarchical interactions, but also included the invader *C. stoebe*

(Figure 1) in all treatments to test whether or not a very powerful competitor might demonstrate strong hierarchical effects or alter interactions among natives in ways that produce non-transitive competition or more easily measured interaction modifications.

Unusual heat and drought either killed or prevented the resprouting of large numbers of one native forb, *G. aristata*, and the invasive forb, *C. stoebe*, during the last two months of the experiment. Thus I was able to quantify 16 of the 20 possible direct effects among native species, and the effects, but not the response, of *C. stoebe* in my experimental treatments. I calculated direct effects using the Relative Interaction Intensity index (RII; Armas et al. 2004). RII is calculated by dividing the difference between the biomass of the treatment and control by the sum of the biomass of the treatment and control ($(B_T-B_C)/(B_T+B_C)$). RII and the standard error was calculated after Armas et al. (2004, Appendix A). RII is a measure of the strength of interaction between species centered on zero with negative interactions (competition) indicated by values between 0 and -1, and positive interactions (facilitation) indicated by values between 0 and +1. RII allows for simple comparisons of interaction strength across taxa and treatments.

For all species in pairwise plots I calculated RIIs using the mean biomass of plants grown alone as controls and the mean biomass of plants in pairwise competition as the treatment. For all species grown in multi-species experimental plots (native and invaded treatments), RIIs for the net direct effect of a single species on another in the context of communities were calculated by using the mean biomass of individuals grown in four species plots as controls and the mean biomass of individuals grown in complete five species assemblages as the treatment. In order to avoid pseudoreplication I took the mean size of the surviving individuals of each species in each plot and used it to calculate overall treatment means and standard errors. I excluded all mortality from my analysis. Statistical tests were performed on RIIs using t-tests (Excel 2007) to

determine if interactions were significantly different from zero. All statistical comparisons of RIIs among different species or between treatments utilized either a one-way ANOVA with Tukey post hoc comparisons or two sample means t-tests (Sigmaplot 11.2, Systat Software).

I used path analysis models to quantitatively explore two interaction scenarios for indirect interactions in my experimental system. I built path analysis models using RII effects from pairwise interaction plots as path coefficients to predict the effects of a single species on another in multi-species assemblages. In these models, I included all 20 native species interactions. For all other analyses, I omitted the effects of native species on *Gailardia aristata* because of the low sample sizes at harvest. Three of the four pairwise effects on *G. aristata* appear to be facilitative and one competitive. These effects on biomass are unreliable because of the very low sample size, but mortality data strongly supports the patterns of facilitative responses that were calculated from biomass results. When grown alone nine of the ten (90%) *G. aristata* individuals either died or failed to re-sprout by the time of harvest. In contrast, in pairwise interactions with other native species only 68% of *G. aristata* individuals either died or failed to resprout at harvest. To statistically compare predicted interaction strengths to experimentally measured strengths, I performed a parametric bootstrap using experimentally derived pairwise RII and SD data to bound randomly drawn distributions of 1000 iterations. These data were used to estimate 95% confidence intervals around path analysis model outputs (Excel 2007).

Results

Pairwise interaction intensities

In pairwise interactions, native species exerted significant negative (competitive) effects on each other in 8 of 16 measured interactions ($P < 0.05$; Table 1). An additional 3 of 16

competitive interactions showed trends towards competition, but were not significant ($P < 0.10$). Significant RII values ranged from -0.20 ± 0.08 to -0.58 ± 0.11 with a mean of -0.38 ± 0.04.

Multispecies native communities

RII indices derived among natives in multispecies community plots were substantially lower than those derived from pairwise interactions. In multispecies interactions only 4 of 16 interactions had significant net direct effects, and all were competitive. Furthermore, these significant RII values ranged from -0.14 ± 0.07 to -0.36 ± 0.14, with a mean of -0.23 ± 0.04, 36% lower than the mean significant RII in pairwise competition ($P = 0.053$). For all species, significant interactions in multispecies native community plots had lower RII values than the RIIs in pairwise interactions, but these differences were not significant. However, 4 of the 8 significant interactions from pairwise interactions were reduced to non-significance in native multispecies plots. In addition, the mean RII for all species in all pairwise interactions was -0.26 ± 0.04, whereas the mean RII value for all species in multispecies communities was -0.08 ± 0.03; $P = 0.003$).

Invaded multispecies native communities

The invader *C. stoebe* had strong significant competitive effects on all native species in pairwise interactions. Interaction strengths varied from -0.20 ± 0.09 ($P = 0.051$) against *P. spicata* to -0.80 ± 0.08 ($P < 0.001$) against *F. idahoensis.* *Centaurea stoebe* maintained strong competitive effects on three of the four native species when competing in communities. In multispecies communities RII values for the significant effects on natives ranged from -0.29 ± 0.12 to -0.34 ± 0.10. The reduction in intensity from pairwise interactions to community plots

was significant for *F. idahoensis* (difference in competitive effect =+0.46; $P = 0.002$) and *S. comata,* (difference in competitive effect =+0.48; $P = 0.006$). In contrast, the interaction between *C. stoebe* and *L. lewisii* shifted from significant in pairwise plots (RII= -0.24 ± 0.11; $P = 0.047$) to non-significant in community plots (RII = -0.08 ± 0.11; $P = 0.480$).

The mean RII for interactions among natives in multispecies communities that contained the invader *C. stoebe* were significantly less than mean pairwise interactions (0.0008 ± 0.04; $P < 0.001$) but were not significantly different from mean native community interactions ($P = 0.33$). There were two significant interactions among native species in plots containing *C. stoebe*; one facilitative (+0.20 ± 0.09 between *Gaillardia aristata* and *Pseudoroegneria spicata*) and one competitive (-0.29 ± 0.12 between *P. spicata* and *Festuca idahoensis*). There were also two interactions that were marginally significant ($P < 0.10$); one facilitative and one competitive. RII values among natives ranged from +0.27 ± 0.14 to -0.29 ± 0.12.

Indirect interaction scenarios

For two interaction scenarios, I examined sets of direct interaction strengths in greater detail in order to look for congruence between the modeled net effects from pairwise interactions and the measured net effects from experimental communities. In other words, are pairwise effects additive, do non-transitive loops occur, or do emergent interaction modifications occur? This allowed me to quantify the potential for indirect effects among competitors and potentially explain the general differences between pairwise models for competitive outcomes and multispecies models. The interaction scenarios I modeled were the effects of *Pseudoroegneria spicata* on *F. idahoensis* and the effects of *L. lewisii* on *F. idahoensis. Pseudoroegneria spicata*, a dominant bunchgrass significantly suppressed *F. idahoensis* in pairwise interactions (RII = -

0.30 ± 0.09; $P < 0.005$). *Linum lewisii*, a perennial forb, also had strong competitive effects on *F. idahoensis* (RII = -0.38 ± 0.11; $P = 0.003$).

Pseudoroegneria spicata had moderate negative net direct effects on *F. idahoensis* in native community plots (RII = -0.14; $P = 0.053$). These effects were smaller than the effects of *P. spicata* measured in pairwise plots, but the difference between the two treatments was not significant ($P = 0.193$). *Linum lewisii* exerted strong competitive effects on *F. idahoensis* in native community plots (RII = -0.18 ± 0.08; $P = 0.043$). The difference between effects measured in pairwise interactions and effects measured in native community plots was large (-0.20), but this difference was not significant ($P = 0.143$).

In path analysis models for these two scenarios, I found that for one species, *P. spicata*, pairwise based models of indirect interactions involving the other species (RII = -0.12) accurately predicted the measured effects between *P. spicata* and *F. idahoensis* at the community level (RII =-0.14). In contrast, predictions from pairwise interactions for the net effects of *L. lewisii* on *F. idahoensis* significantly overestimated (RII = -0.34) the net effects measured in communities (RII = -0.18).

The estimated 95% confidence intervals for the modeled interaction between *P. spicata* and *F. idahoensis* were +0.14 to -0.38 and the estimated 95% confidence intervals for the modeled interaction between *L. lewisii* and *F. idahoensis* were -0.05 to -0.61. Thus, the modeled results were not significantly different from measured results for both cases.

Discussion

When species experience more complex competitive environments due to an increased diversity of competitors and thus greater numbers of simultaneous interactions, the strength of the interactions between species declined. In addition, I found no evidence of competitive loops

among species, suggesting that processes other than non-transitive competition caused declines in interaction strengths in multispecies communities. Further, my results indicate that the measured effects of competition in multispecies communities cannot be easily predicted from pairwise interactions. This suggests that competition among groups of species in communities has emergent, or non-additive, properties that are quite different than the sum of the pairwise interactions. These differences may be due to the presence of strong, but not easily quantified or modeled, indirect effects that reduce the net direct effects of one species on another. I found little evidence for clear hierarchical rules, in one case additive pairwise indirect effects explained the discrepancy between pairwise and multispecies competitive interactions, and in one case the strength of multispecies interactions appear to be explained by interaction modifications. In other words, interaction modifications occur and appear to have important effects on the way competition functions in multispecies communities.

The degree to which we can predict community interactions and species performance when in complex, multi-species assemblages from simple experiments remains a largely unanswered question in ecology. My results are not consistent with theory that establishes competition as a solely exclusionary process at all levels of organization (Hardin 1960). In fact, my results suggest that pairwise experiments that are analyzed as purely additive direct effects (Goldberg and Werner 1983, Keddy and Shipley 1989, Shipley and Keddy 1994, Keddy et al. 1994, 1998, 2000, Fraser and Keddy 2005, Storkey et al. 2010) are poor predictors of assembly rules, for how species become organized in communities.

In contrast to theory developed from pairwise experiments in the context of hierarchical assembly rules, there has been a developing body of theory over the last 30 years highlighting the importance of indirect facilitative interactions (Stone and Roberts 1991, Miller 1994, Levine

1999, Callaway and Pennings 2000, Pages and Michalet 2003, Callaway and Howard 2006, White et al. 2006, Weigelt et al. 2007, Koorem and Moora 2010, Saccone et al. 2010), which suggests that pairwise interactions may overestimate the competitive effects of species that are interacting in complex communities. More recently, explicit tests of the predictive power of pairwise interactions have further suggested that non-additive, or higher order, interactions greatly change the way that species compete in complex, multispecies groups (Dormann and Roxburgh 2005, Dormann 2007, Perkins et al. 2007, Weigelt et al. 2007, Engel and Weltzin 2008). For example, Dormann and Roxburgh (2005) found that Lotka-Volterra (LV) type models built from pairwise outcomes did not accurately predict biomass and coexistence for three species mixtures in five out of the six combinations grown. Similarly, LV models did not predict biomass and coexistence in an experimental seven species mixture. However, when a 'non-additive', or higher order, competition coefficient was added to the model, predictions more closely matched experimental outcomes. This suggests that indirect interactions can be important for determining the outcomes of competition within communities, but whether or not non-transitive interactions played any role in these indirect effects was not tested.

Weigelt et al. (2007) measured the effect of one, two and three species neighborhoods on a target species to test the assumption that competitive effects in multi-species communities are additive. Yield density models suggested that competitive intensity in most multi-species assemblages could be accurately predicted by pairwise interaction outcomes. However, certain combinations of species showed significant deviations from the predictions of the model generated from pairwise interactions. But when non-additive parameters were added to the model, the predictive power of the model was significantly increased, indicating that indirect

interactions among specific combinations of species can result in non-additive effects that are difficult to predict from more simplistic, pairwise derived models.

I found that in direct pairwise interactions, all native species exerted significant competitive effects on at least one other species. However, when assembled into native multispecies communities, only the two most dominant competitors, *Pseudoroegneria spicata* and *Linum lewisii,* were able to significantly suppress other native species (Table 1). Concomitantly, I found the mean interaction strength between native species significantly decreased from -0.26 ± 0.04 in pairwise interactions to -0.08 ± 0.03 ($P = 0.003$) in multispecies community interactions. I also found the mean interaction strength among significant interactions to decrease significantly from -0.38 ± 0.04 in pairwise interactions to -0.23 ± 0.04 ($P = 0.053$) in multispecies community interactions.

I also found that *C. stoebe* exerted very strong competitive effects on all native species in pairwise interactions, potentially establishing the only clear single species competitive hierarchy in my experiment. But the effects of *C. stoebe* were greatly reduced for three of the four natives in invaded multispecies interactions. In addition, the mean interaction strength among all natives in invaded multispecies communities was significantly lower than the mean interaction strength of natives in pairwise interactions suggesting that even extraordinarily strong competitors cannot maintain pairwise level competitive effects when in multispecies communities.

Considered together, my results and similar results from the recent literature, provide strong support for the argument that indirect facilitative interactions buffer against competitive exclusion. As an example, the competitive effect of *P. spicata* on *F. idahoensis* in pairwise arrangements is much larger than the competitive effect in multispecies communities, suggesting that indirect effects are altering interaction strengths. However, it is not possible to evaluate

whether these indirect effects are additive or non-additive without building models of interactions using path analysis methods. To do this, I used the direct effects (RII) from pairwise experiments of *P. spicata* on all other native species and all other species on *F. idahoensis* to estimate the additive direct and indirect effects of *P. spicata on F. idahoensis* in multi-species communities. I found approximate congruence between the estimated (-0.12) and measured effects (-0.14), suggesting that the competitive effects of a dominant, highly competitive bunchgrass appear to be the result of additive direct and indirect effects – in other words, by adding the key competitive interactions among all community members interacting in pairwise fashion I am able to predict the competitive effect of *P. spicata* on *F. idahoensis* in *multispecies communities*. I also estimated the competitive effect of *L. lewisii* on *F. idahoensis* in a multi-species community context in an attempt to tease out the cause of differences in interaction strength between pairwise and multispecies community treatments. Unlike the scenario for *P. spicata* and *F. idahoensis*, the path analysis estimated effect (-0.33) and the measured effects (-0.18) of *L. lewisii* on *F. idahoensis* are not congruent. This suggests that there are powerful *non-additive* indirect interactions between species that greatly reduced the competitive effect of *L. lewisii* when growing in complex communities. These non-additive effects cannot be predicted from pairwise interactions and most importantly this suggests that interaction modifications rather than non-transitivity determine competitive outcomes at the community level.

For both path analysis scenarios the calculated 95% confidence intervals from parametric bootstrap techniques yielded only non-significant differences for all effects. In fact, the 95% confidence intervals were so large that it raises questions about whether or not predictions can be made from path analyses using such experimental data. I conducted my experiment in as near equilibrium conditions as possible, and yet my data lacked the precision necessary to use path

analysis models for estimates of community level effects. Other studies have suggested that in complex, diverse communities the outcome of competition between species simply cannot be predicted because of the inherently complicated nature of multi-species competition (Huisman and Weissing 2001). Because of this, I argue that if we are to understand how competition shapes community structure, we must seek to use sophisticated experiments in natural conditions that actually measure interaction strengths between species within communities and not rely on models for predictions about community level interactions between species.

Pairwise and highly controlled experiments can teach us much about the mechanisms by which plants interact (Caldwell et al. 1985, Callaway and Aschehoug 2000), how conditional factors affect interactions (Tilman 1982, Besaw et al. *in press*), how particular traits might contribute to competitive outcomes (Harris 1967), and how other organisms may affect competitive outcomes (Aschehoug et al. *in press)*, but a growing body of literature indicates that these outcomes cannot be used to accurately predict community assembly. Far more importantly, these outcomes cannot be used to estimate the strength or importance (Brooker et al. 2005) of competition in given community scenarios. My results suggest that the most useful information on the importance of competition on the distribution and abundance of species, or the assembly of species into communities comes from field experiments conducted within the natural matrix of communities in which the mixture of neighbors is manipulated (Grace and Wetzel 1981, Gurevitch 1986, D'Antonio and Mahall 1991, Pennings and Callaway 1992, Callaway et al. 1996).

My results also provide strong support for the inclusion of indirect interactions and non-additive processes into theory that seeks to make predictions about how communities assemble through competitive interactions at a single trophic level. For example, hierarchical assembly

theory is grounded in the basic premise that competition continually acts to exclude species from complex systems. But I found that complex suites of multi-species interactions greatly reduce competitive intensities among species and therefore also reduce the likelihood of competitive exclusion within communities. In other words, multi-species competition may reduce the strength of plant-plant interactions to such low levels that coexistence becomes possible despite the presence of competition.

Importantly, my results did not support the current non-transitive alternative to hierarchical assembly rule theory, which requires that competitive loops form in order for competition to allow or drive coexistence among different species. These non-hierarchical processes have been demonstrated in some systems (Buss 1980, Sinervo and Lively 1996) but have not, to my knowledge, been empirically demonstrated in plant communities. I found no evidence of competitive loops in my novel experimental system; however, I did find evidence of powerful indirect interactions and non-additive effects without the formation of competitive loops indicating that emergent "interaction modifications" were a key process. Such interaction modifications have not received formal attention in the ecological literature.

Despite clear differences between hierarchical and non-hierarchical assembly rule theory, common ground can be found in the power of direct interactions. Hierarchical assembly rule theory assumes that direct interactions are always more powerful than any indirect effects and therefore, direct competitive effects lead to competitive exclusion. Non-hierarchical assembly rule theory requires a requisite set of direct interactions - indirect interactions are a necessary by-product of competitive reversals - but such explicit reversals, at least so far, appear to exist more in theory than in nature for plant communities. A central proposition of the work here is that I propose that plants may assemble in communities based on "modified interactions". That is to

say indirect interactions, additive and non-additive, are potentially more important in the context of complex communities than direct interactions. Instead of assembly rules based solely on direct interactions, the suite of interactions experienced by individuals in space and time within communities may be highly emergent in ways that are specific to the particular combination of neighbors and their indirect effects on the system.

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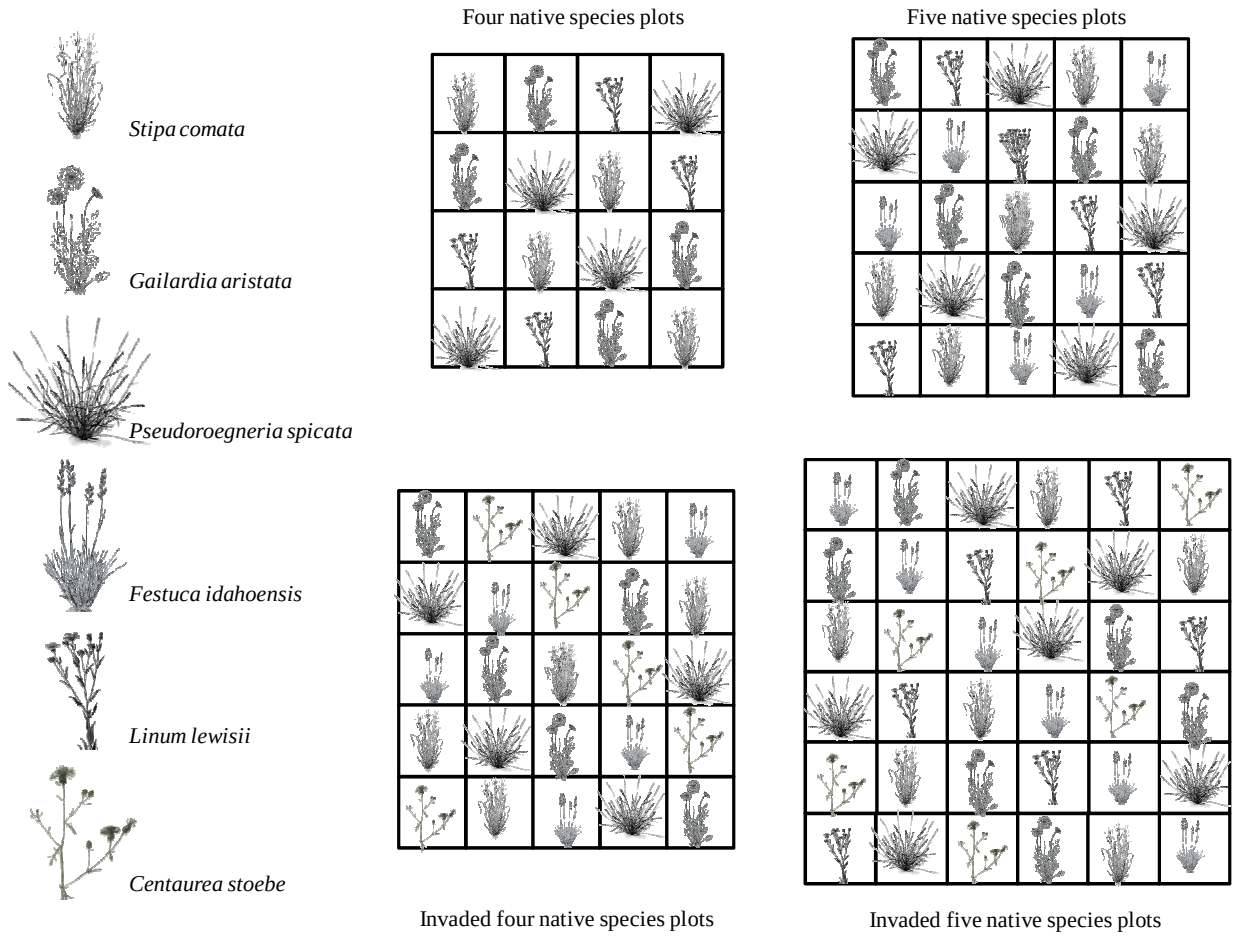

Figure 1. Experimental design for iterative omission, Latin square plot design. All species were planted 10 cm apart horizontally and 14 cm on diagonal. All plot designs were replicated (n=10 for species grown alone and in pairs, n=8 for species grown in native four species plots, n=10 for native five species, n=10 for invaded four native species plots, and n=12 for invaded five species plots) with all possible combinations of species. All species occupied all possible locations within each plot via replication to avoid bias in location within the plot.

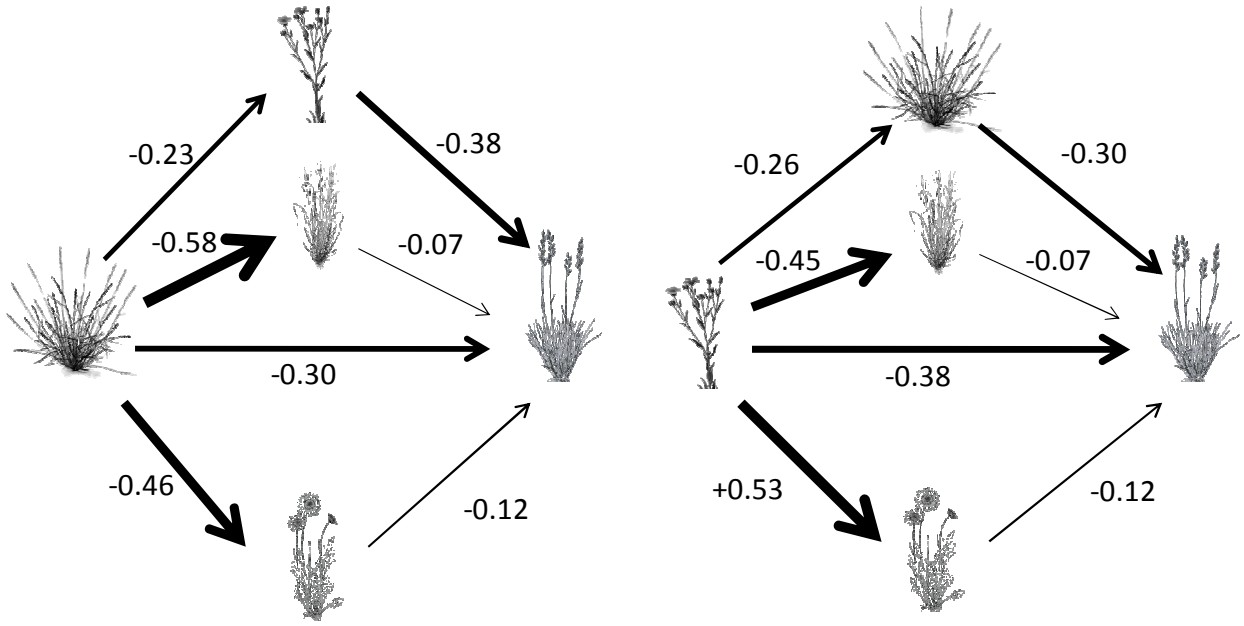

Figure 2. Path analysis models for the predicted effects of *Pseudoroegneria spicata* on *Festuca idahoensis* (left panel) and the predicted effects of *Linum lewisii* on *F. idahoensis.* All path coefficients are equal to pairwise competition derived RII values.

Pairwise Interactions

	Pseudoroegneria spicata	*Festuca idahoensis*	*Stipa comata*	*Linum lewisii*
P. spicata		**-0.30 ± 0.09**	**-0.58 ± 0.11**	-0.23 ± 0.13*
F. idahoensis	-0.13 ± 0.11		**-0.43 ± 0.19**	-0.24 ± 0.13*
S. comata	**-0.20 ± 0.08**	-0.07 ± 0.10		-0.30 ± 0.15*
L. lewisii	**-0.26 ± 0.10**	**-0.38 ± 0.11**	**-0.45 ± 0.12**	
Gailardia aristata	-0.11 ± 0.13	-0.12 ± 0.10	**-0.43 ± 0.13**	+0.10 ± 0.16
Centaurea stoebe	**-0.20 ± 0.09**	**-0.80 ± 0.08**	**-0.77 ± 0.09**	**-0.24 ± 0.11**

Native Community Interactions

	Pseudoroegneria spicata	*Festuca idahoensis*	*Stipa comata*	*Linum lewisii*
P. spicata		**-0.14 ± 0.07**	**-0.22 ± 0.09**	-0.10 ± 0.11
F. idahoensis	+0.02 ± 0.13		-0.10 ± 0.15	-0.11 ± 0.10
S. comata	-0.01 ± 0.12	+0.08 ± 0.06		+0.12 ± 0.11
L. lewisii	-0.10 ± 0.10	**-0.18 ± 0.08**	**-0.36 ± 0.14**	
Gailardia aristata	-0.15 ± 0.09	+0.08 ± 0.05	+0.11 ± 0.20	-0.20 ± 0.16

Invaded Community Interactions

	Pseudoroegneria spicata	***Festuca idahoensis***	***Stipa comata***	***Linum lewisii***
P. spicata		**-0.29 ± 0.12**	-0.02 ± 0.15	-0.25 ± 0.12*
F. idahoensis	0.00 ± 0.11		+0.02 ± 0.12	-0.02 ± 0.11
S. comata	-0.04 ± 0.13	+0.08 ±0.16		-0.08 ± 0.10
L. lewisii	-0.16 ± 0.10	-0.05 ± 0.14	+0.11 ± 0.19	
Gailardia aristata	**+0.20 ± 0.09**	+0.27 ± 0.14*	+0.10 ± 0.14	+0.15 ± 0.09
Centaurea stoebe	-0.34 ± 0.10	-0.34 ± 0.10	-0.29 ± 0.12	-0.08 ± 0.11

Table 1. Net direct effects (RII) of each species interaction in pairwise, native multi-species community and invaded multi-species community plots. The top row represents the target species and the left column the competitor. Bold values represent significant interactions ($P<0.05$) and asterisks indicate marginally significant interactions ($P<0.10$).

Plasticity and trait-mediated indirect interactions among plants

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Summary

Virtually all investigations of trait mediated indirect interactions (TMIIs) have focused on predator-prey and herbivore-plant systems. However, because of the high degree of phenotypic plasticity found in plants, plant-plant interactions offer a unique opportunity to ask questions about how changes in phenotype can influence both the strength and direction of interactions with other species. We know of no examples in which researchers have investigated TMIIs in the context of plant-plant interactions, but there are many examples of plants exhibiting plasticity to both abiotic and biotic conditions in ways that should affect the way they interact with their neighbors, and *genetically* mediated indirect interactions have been demonstrated. Furthermore, because of the complex, multi-species nature of plant communities, plants are often simultaneously subjected to combinations of direct and indirect interactions, which makes plant-plant interactions a potentially very rewarding area to experimentally test TMIIs. Here we highlight the literature in which different components of TMIIs have been demonstrated, and discuss how these examples can provide more complete tests of TMIIs. We also speculate on the potential for TMIIs to expand our understanding of how plant communities are organized.

Introduction

Ecologists have long recognized the importance of phenotypic plasticity as a mechanism by which organisms acclimate and adapt to local environments. Phenotypic plasticity is commonly defined as variation in the morphological or physiological phenotype of a given genotype in response to the abiotic and biotic environment (Bradshaw 1965). Plants are particularly plastic organisms because they must solve the fundamental problems of resource acquisition, competition, and herbivore attack without mobility (Sultan 1987; Sultan 2000). Plasticity has been demonstrated in the morphological, developmental, physiological and

biochemical traits of plant species, with many traits showing flexibility in expression both between and within individual plant species (Novoplansky 2002; de Kroon *et al.* 2005; Valladares *et al.* 2006). The plastic responses vary not only in their form, but also in their permanence. Responses may be permanent for the lifetime of an individual, fixed for long periods of time (e.g. a growing season), or dynamic at the scale of minutes (Metlen *et al.* 2009).

Research on the nature of plasticity and its potential to broaden the ecological niches of species in shifting abiotic and biotic conditions has historically focused on the morphological responses of plants. However, morphological responses tend to be slow and are largely irreversible—two parameters that are not favored by selection (Valladares *et al.* 2007). In contrast to changes in morphology, plants can also respond via biochemistry. These responses can be exceptionally rapid and highly ephemeral (Metlen *et al.* 2009); traits that lend themselves to adaptive value and are favored by natural selection, but biochemical traits have been studied very little in the explicit context of plasticity.

Plasticity and trait variation have been studied extensively in plants, but the ecological consequences of such phenotypic variation are poorly understood (Miner *et al.* 2005). Experimental settings commonly overestimate the degree to which plants can exhibit plastic responses to changes in natural conditions. This may be because of the continually changing conditions in nature which makes optimization of some plastic responses difficult, especially if plastic responses have long lag times and are largely irreversible. In addition, the diffuse nature of competition among plants within communities may limit a plant's ability to exhibit its maximum potential plasticity in response to biotic interactions. Thus there appear to be greater limits and constraints to plasticity in plants in natural systems than would be predicted from responses to controlled environments (Valladares *et al.* 2007). However, investigations that

focus on trait responses that can mediate multiple environmental stimuli in plants should have a higher potential for adaptive value. For example, secondary biochemistry responses to nutrient stress may also mediate competitive or facilitative interactions (Tharayil *et al.* 2009) and therefore can potentially provide a more stable cue for plant plasticity responses in natural systems.

It's surprising that the consequences of plasticity for interactions among plants have not been more deeply explored since high phenotypic plasticity in plants is thought to be a characteristic of ''good competitors'' (Grime 2001), and good competitors can have powerful effects on communities (Connell 1983). Species classified as good competitors generally show more rapid responses to variation in their environment, such as adjusting root:shoot ratios, leaf specific area, proportions of fine to coarse roots, and diversity of biochemistry, than species that are poor competitors (Grime 2001; Callaway *et al.* 2003). Despite the high degree of plasticity expressed by plants, and the potential for this plasticity to affect the way a species might interact with its neighbors (known as trait mediated interactions or TMI), very few studies have been conducted with plants that focus on plasticity and interactions. However, by re-examining earlier work with a focus on plasticity we can piece together direct evidence for TMIs.

For example, Callaway (1990) found that the root architecture of *Quercus douglasii* seedlings demonstrated plasticity to variation in water source. *Quercus douglasii* seedlings with experimentally restricted access to deep stores of water produced roughly twice as many fine lateral roots and more than 5x the lateral root mass as seedlings with access to a deep water source. This phenotypic plasticity demonstrated by seedlings in controlled experiments corresponded with apparent plasticity in the field, where mature trees without access to deep water possessed very dense surface lateral root systems while trees with deep water access did

not (Callaway *et al.* 1991). Plasticity in root architecture in the field appeared to create a TMI as trees with abundant shallow roots strongly suppressed understory productivity; whereas trees without abundant shallow roots had strong facilitative effects.

More recently, ecologists have extended the view of TMIs from direct interactions between plants to indirect interactions (TMIIs) among species. While we know of no studies of plant-plant interactions that test for the presence of TMIIs, conceptually all of the necessary components to produce TMIIs have been studied, making the next step ripe for empirical research. For example, in the scenario of the TMI apparently mediated by plasticity in the root architecture of *Quercus douglasii*, this plasticity also correlated with different understory community compositions. The abundance of the native *Nassella* (nee *Stipa*) *pulchra* was higher under trees with abundant shallow roots (Callaway *et al.* 1991). Simultaneously the abundance of European annuals (primarily *Avena fatua* and *Bromus diandrus*), which can competitively exclude natives like *Nassella*, was lower. This pattern suggests the occurrence of a TMII but does not demonstrate it. However, a relatively simple experiment could explore this spatial pattern in the context of TMIIs, and similar experiments could be used to study TMII in other systems. The key would be to determine whether shallow root architecture simply promoted *Nassella* directly, or altered the competitive effects of the European annuals in ways that indirectly promoted *Nassella* (see Rice & Nagy 2000). Using *Nassella* as a target species, TMIIs would be demonstrated if experimental treatments in which European annuals were removed from around *Nassella* under trees without shallow root architecture improved the growth or fitness of *Nassella*.

This link between plastic responses to environment and its affect on plant-plant interactions (direct and indirect) represents a major gap in our understanding of how plant

communities assemble. TMIs and TMIIs have the potential to create tremendous variation, or conditionality, in the outcomes of interactions among competing species, and thus have important implications for how competitors might coexist (Chesson & Rosenzweig 1991). In other words, we know that indirect interactions among groups of competitors can promote coexistence among species that would otherwise be likely to competitively exclude each other (Miller 1994; Callaway & Howard 2007), thus plasticity among species can greatly enhance the potential for indirect interactions to sustain coexistence among competing species and thus increase community diversity.

Interactions among plants

Negative direct interactions among plants appear to derive primarily from the need to acquire basic resources such as light, water and nutrients, which are often in limited supply (Goldberg 1990; Miller & Travis 1996). Because plants are sessile, resource competition between individuals can be intense, potentially making coexistence difficult when essential resources are scarce (Tilman 1982). In addition, allelopathy, the negative biochemical effects of neighbors on each other (Turlings *et al.* 1990; Williamson 1990; Mahall & Callaway 1992; Schenck *et al.* 1999), can also be a mechanism by which plant species inhibit each other.

Positive interactions among plants, or facilitation, occur when the presence of one plant enhances the growth, survival, or reproduction of a neighbor (Callaway 2007). But it is important to note that facilitation by one species on another may correspond with reciprocal negative, positive, or neutral responses. Direct positive interactions may incorporate a wider range of mechanisms than direct negative interactions (Callaway 2007). Like competition, facilitation may occur through resource effects, one species increasing nutrient, water, or light availability to another, or through chemical effects (Metlen *et al.* 2009). However, facilitation

can also be driven by non-resource processes. Most commonly, species that are physically tolerant to stresses such as cold, heat, wind, salinity, and disturbance buffer other species from these abiotic conditions.

Indirect interactions among plants can be derived from direct resource competition, allelopathy, or facilitation (Pages & Michalet 2003; Callaway & Howard 2007; Callaway 2007), but these have received far less attention than the direct impacts that plants have on one another. This may be because plants are generally embedded within a matrix of many other plants, all of which require the same basic resources of light, water and nutrients; thereby creating an environment in which direct interactions appear to be assured. However, the highly aggregate nature of plant communities also sets the stage for common and strong indirect interactions - situations in which the direct interaction between two species is caused or altered by simultaneous interactions with additional species (Miller 1994; Levine 1999; Callaway & Pennings 2000; Callaway & Howard 2007; Cuesta *et al.* 2010).

Ecological consequences of plant interactions

Competition and facilitation among plants are the basic processes through which TMIs and TMIIs can operate, and these interactions can be powerful organizing forces in structuring plant communities (Allen & Forman 1976; Grime 1977; Connell 1983; Tilman 1985; Ortega & Pearson 2005; Callaway 2007; Cavieres & Badano 2009). Because of this, evaluating species' inherent competitive abilities can likely provide some insight into how they will perform in a community context. However, assessing the relative competitive strengths of species is difficult in anything other than simple pairwise or "bioassay" experiments; and it now is becoming apparent that such experiments do not accurately predict how individuals may respond when subjected to the diffuse nature of interactions found in plant communities (Callaway & Howard

2007; Perkins *et al.* 2007; Engel & Weltzin 2008; Schmidtke *et al.* 2010; Aschehoug 2011; Chapter 1). Even more, rankings of competitive effects and responses may not be complete indicators of individual competitive abilities when plants are in real communities (Wang *et al.* 2010). Thus theory for how plant communities assemble that fails to incorporate indirect interactions is probably incomplete, and this has very important implications for studying TMIIs.

There are two general, but contrasting, theories for how plant species may assemble into communities under equilibrium conditions as a result of competition among plants. The first does not incorporate indirect interactions, and thus does not have the potential to integrate TMIIs, and poses that plant communities are competitively transitive in nature (Goldsmith 1978; Mitchley & Grubb 1986; Keddy & Shipley 1989). In other words, all species in a given pool, or community, can be ranked in a linear competitive hierarchy. The strict "pecking order" that results from hierarchical competitive abilities provides a predictive tool for community organization. A transitive, or hierarchical, perspective on assembly rules assumes that communities will consistently move towards dominance by the best competitor in the hierarchy in a homogeneous abiotic environment, and this can potentially lead to the development of monocultures. An important theoretical consequence of not allowing TMIIs among plants in such transitive or hierarchical communities is that weak competitors will be competitively excluded given enough time and the absence of non-equilibrium processes. In this paradigm only non-equilibrium forces, such as fire, herbivory (which can establish TMIIs) or abiotic heterogeneity, can prevent the dominance of a small number of species or the formation of monocultures in a local community.

In contrast to hierarchical assembly rules is the theory that plants exhibit non-transitive or non-hierarchical competitive properties as they form communities (Jackson & Buss 1975; May

& Leonard 1975; Petraitis 1979). Whereas hierarchical organization is best described mathematically as A>B>C, non-hierarchical organization occurs when loops form in the hierarchy such as A>B, B>C, but C>A. In other words, species C indirectly benefits species B by having a direct negative impact on species A, which creates the opportunity for TMIIs to be included in conceptual models. Given the right starting point, a simple loop within a suite of competing species can result in a perpetually shifting state in which all three species coexist indefinitely (Buss & Jackson 1979). This coexistence is based entirely on the balance of direct competitive interactions, but leads to the formation of complex networks of species interactions which may be mediated directly or indirectly via plant plasticity. Proponents of non-transitive competitive processes note that because plants interact with many other species simultaneously, clear pecking orders are likely rare. In addition, sporadic reversals of dominance among species can create powerful and facilitative indirect effects among competitors which can transform overall community structure.

When community members interact in complex "networks" of interactions, competitive exclusion is much less likely. In addition, coexistence may be maintained among large pools of species in the absence of abiotic heterogeneity or non-equilibrium processes. Mathematical evaluations of such interactions predict that such indirect interactions among competitors can allow large communities of species to coexist (Karlson & Jackson 1981; Laird & Schamp 2006; Laird & Schamp 2008).

Non-transitive theory requires quite specific combinations or sequences of interactions among species to produce indirect interactions, and thus TMIIs. However, groups of plant species appear to compete in ways that produce indirect interactions, but without the competitive "loops" required for non-transitive theory. In other words, some species appear to "modify"

interactions among other species without establishing the classic non-transitive combinations of competitive dominance (Callaway & Pennings 2000; Metlen 2010).

A key commonality of the transitive and non-transitive theories of plant community assembly is the requirement of strong, species-specific, direct negative effects. In a hierarchical system, the direct effect of each species on another is linear; a single dominant species that exerts primary control of community wide species diversity via competitive interactions, and subdominant species that exert lesser degrees of control in direct proportion to their place in the hierarchy. In non-hierarchical systems, there is the requirement of at least one of the weaker competitors directly outcompeting a species of higher competitive ranking. In other words, a species that loses most of its interactions with other community members must be able to outcompete a species that wins most of its interactions in the community, an unlikely scenario without the presence of TMIs. In fact, the conditionality of competitive outcomes may be explained in large part by the plastic response of plants to competition (Cahill *et al.* 2010).

Transitive and non-transitive processes occur in communities but plants also experience "diffuse" competition in a community context. Instead of a distinct interaction with another single species, for which ranks might be determined, a plant experiences the additive effects (positive or negative) of many species interacting in space and time. Such diffuse interactions may have profound impacts on the species composition of plant communities (Davidson 1980; Vandermeer 1980; Wilson & Keddy 1986; Miller 1994; Li & Wilson 1998; Levine 1999; Callaway & Pennings 2000) and provide tremendous potential, conceptually if not logistically, to study TMIIs.

There is a substantial escalation in the complexity of assembly rules as we move from transitive to non-transitive models and as we add interaction modifications and diffuse interactions to competitive loops. And this complexity increases again once we consider that most current thinking is built on the construct, or at least the implicit assumption, that plants are fixed in their competitive abilities. However, we know that plants vary in their competitive abilities both within and between populations. For example, Grøndahl & Ehlers (2008) found that genotypic variation in the production of different terpenes by ecotypes of *Thymus pulegioides* and *T. serpyllum* altered the effects of the *Thymus* species on co-occurring plant species. The ecological effects demonstrated for *Thymus* species (also see Ehlers & Thompson 2004; Jensen & Ehlers 2010) corresponded with selective effects of *Thymus* on their neighbors. Plants that came from sites where they co-occurred naturally with a carvacrol (a terpene)-producing ecotype of *Thymus* also performed better on soil treated with carvacrol. This example of how genotypic variation can affect the competitive effects of a species derives from at least two general ways that plants interact directly, facilitation and allelopathy. If relatively subtle differences in 'fixed' competitive interactions can have such a large impact on community formation, then *phenotypic* shifts that lead to changes in interaction outcomes (TMIs and TMIIs) have the potential to be very powerful in determining how plant communities assemble.

Plasticity and direct interactions

In both transitive and non-transitive models, the intensity of direct interactions determines the degree to which plants can coexist. Therefore understanding how plasticity affects interaction intensities can greatly improve our ability to predict plant coexistence in communities. Much is known about how plant traits such as morphology, growth rates, final size, reproduction, qualitative and quantitative biochemical traits, and biomass allocation can

vary widely for a given genotype (Sultan 1987; Sultan 2000; Metlen *et al.* 2009), which creates exceptional opportunities for exploring how phenotypic plasticity within an individual species can influence interactions with other species and the subsequent effects on the structure of plant communities.

A substantial component of the way that plants affect each other (either negatively or positively) is based on plant size and growth rate. For example, Brooker *et al*. (2005) reanalyzed data from Reader *et al.* (1994) to compare the intensity of the competitive effect of neighbors on *Poa pratensis* to the importance of the competitive effect. Among grasslands that varied in productivity, both components of competition were significantly affected by total neighbor biomass. Plant size can also affect facilitative interactions. Tewksbury & Lloyd (2001) found that larger *Olneya testota* trees in the Sonoran Desert supported higher numbers of beneficiary species and larger beneficiary perennials than small canopies. Because of the importance of the size of individual plants for competitive and facilitative effects, phenotypic plasticity in size may have substantial effects on interaction outcomes.

Morphological plasticity as a response to abiotic conditions, however, is often slow and costly, which may limit the ability of plants to respond when subjected to intense competition (Novoplansky 2002). Biochemical responses, such as the release of secondary metabolites that increase nutrient availability in the rhizosphere, are less costly and more ephemeral responses that can have immediate impacts on plant performance (Metlen *et al.* 2009) and potentially effects on neighboring competitors. Li et al. (2007) found that the cluster root forming species *Vicia faba* increased phosphorus availability in the soil rhizosphere via the release of acidifying chemicals (citrate and malate). The biochemical response of *V. faba* to phosphorus deficiency is also exceptionally fast; lab tests show it reducing the pH of nutrient agar by ~2 units in 6 hours.

Such changes in soil acidity can result in 10-fold changes in phosphorus availability. In field experiments, the increase in phosphorus availability resulted in an overyielding of 26% for *V. faba*. In addition, *V. faba* directly facilitated *Zea mays* thru the shared increase in phosphorus availability leading to an overyielding of 43% by *Z. mays*. This example demonstrates the strong potential for biochemical plasticity to be a model system for understanding how TMIs and TMIIs can impact both competitive and facilitative interactions and the organization of communities.

Plasticity and indirect interactions

We know of no examples in which phenotypic plasticity in a plant trait has been shown to alter *indirect* interactions among other plant species. Conceptually, however, all of the component pieces of TMIIs can be examined from existing empirical studies. What is lacking is a comprehensive set of experiments that explicitly link plasticity and indirect interactions. Ideally, such studies would entail an experiment in which species "A" demonstrated two or more phenotypes (e.g. A_{Ph1} and A_{Ph2}), and then the indirect effects of these two phenotypes would be tested in experiments involving two or more other neighbors (Figure 1). For example, in Figure 1A, the hypothetical A_{Ph1} has weak competitive effects on species B, and species B has strong competitive effects on species C. Thus the indirect effects of A_{Ph1} on C are weak. In contrast, the hypothetical A_{Ph2} has strong competitive effects on species B, and thus strong indirect facilitative effects on species C. Figure 2 illustrates how TMIIs might occur when there is plasticity in a facilitative benefactor (species A) or a beneficiary (species B).

Next we suggest potential scenarios in nature in which these TMIIs might occur, with the goal of stimulating ideas for how such studies might be approached in the future. An intriguing scenario may exist for *Quercus agrifola*, the native perennial herb *Pholistima auritum*, and European annual grasses in California grasslands. *Pholistima* can form near monocultures

directly beneath the canopies of some *Q. agrifolia* trees, but is much less abundant in the grassland directly adjacent to the canopies where European annuals dominate (Parker & Muller 1982). However, if *Pholistima* is not present under the oaks, European grass species are intensely facilitated (as *Pholistima* itself appears to be) by *Q. agrifolia* which suggests that the low abundance of annual grass species in the understory is not due to the direct effects of the oaks. Instead, it appears that once facilitated, *Pholistima* excludes the European annual grasses through the inhibitory effects of its litter and leachates. In field experiments, fresh *Pholistima* litter reduced *Bromus* germination by 73% and *Avena* by 96%. However, when experiments were conducted with *Pholistima* litter that had been leached, at least 92% of seeds germinated in every treatment. *Quercus agrifolia* appears to have powerful negative indirect effects on grasses, and the stage is set for the next necessary step for TMIIs – if *Q. agrifolia* demonstrates plasticity in some way that affects its facilitative effect on *Pholistima*, the indirect effects of the tree on annual grasses are likely to change as well.

Quercus douglasii and *Q. agrifolia* may help us understand the *effects* of plasticity on indirect interactions (Figure 2, Model 1), but plants show a great deal of plasticity in *response* to neighbors as well (Callaway *et al.* 2003; Cahill *et al. 2010*). We know of no examples in which plastic responses have been connected to cascading indirect interactions with other species, but much like the *Quercus* example above, we can speculate about how the plastic response to competition may lead to indirect interactions.

Cahill et al. (2010) found that plants altered their root foraging strategies based on the amount and distribution of resources in the soil and the presence of competitors. When grown alone, *Abutilon theophrasti* had broadly developed root systems regardless of whether resources were uniformly or patchily distributed within the soil. However, when in competition with a

conspecific, *A. theophrasti* was highly plastic in root distribution and distance from stem depending on the distribution of resources, suggesting that plants are capable of altering the plastic response of roots to nutrients depending on the presence or absence of a competitor, a good example of a TMI. While this example highlights the plastic response of *A. theophrasti* to competition, this particular set of experiments was limited to pairwise competition, which does not test TMIIs. Nevertheless, the alteration of root system morphology based on the presence of competitors and variable resources suggests that when placed in a multi-species context, the intensity of competition that *A. theophrasti* experiences (stronger or weaker) is highly dependent on the plastic response. A test of this TMII response could easily be carried out by replicating the experiments with multiple competitors. TMIIs would be demonstrated if competition intensities differed between single and multiple competitor experiments.

Plasticity and exotic invaders

Plasticity expressed by any plant species provides an opportunity to explore TMIIs in novel ways, but exotic invaders might provide unusually good opportunities because they are thought to be unusually plastic (Richards *et al.* 2006; Hulme 2008) and unusally strong competitors (Maron & Marler 2008). High phenotypic plasticity has been suggested as a good predictor of invasiveness (Mal & Lovett-Doust 2005; Chun *et al.* 2007, but see Bossdorf *et al.* 2005; Hulme 2008). Unlike our focus here on plasticity and TMII, both Richards *et al*. (2006) and Hulme (2008) focus on the potential role of plasticity in allowing an invader to express advantageous phenotypes as they colonize a broad range of environments (Bradshaw 1965; Van Valen 1965; Whitlock 1996; Sultan *et al.* 1998; Sultan *et al.* 1998; Donohue *et al.* 2001; Richards *et al.* 2005). While likely true, exotic "invasion" is only defined in part by colonization by exotic species. Callaway & Maron (2006) and Hierro *et al.* (2005) note that exotic

"invasions" involve biogeographic shifts in the fundamental ecology of a species; generally much higher abundances and stronger apparent impacts in non-native ranges than in native ranges. Thus the essence of an "invader" as opposed to an "exotic" is not just in the processes involved in colonization, but in the processes involved in the attainment of very high densities, biomass, and impacts on other species. It is in the context of plasticity within these processes and impacts that invaders provide the best opportunities to study TMIIs.

Strong competitive interactions are likely to play an important role in establishing the dominance of some invaders (D'Antonio & Mahall 1991; Levine *et al.* 2003; Vila & Weiner 2004; Maron & Marler 2008; Munshaw & Lortie 2010). Release from specialist herbivores or pathogens may allow plants to be more competitive in non-native ranges (Keane & Crawley 2002), or successful invaders may possess competitive advantages because they come from a more competitive species pool, or happen to possess inherent traits that give them an advantage relative to their new neighbors. Invaders may have strong competitive effects in their non-native ranges through their ability to attain higher biomass, or because of novel traits that confer more subtle competitive advantages (Callaway & Pennings 2000; Callaway & Ridenour 2004). There have been quantitative biogeographic comparisons of productivity, biomass, or density in both the native and non-native ranges of invasive plant species (Woodburn & Sheppard 1996; Grigulis *et al.* 2001; Paynter *et al.* 2003; Jakobs *et al.* 2004; Beckmann *et al.* 2009), and many studies have clearly documented strong negative impacts of invaders in their non-native ranges; apparently much stronger than most if not all native species (e.g. Bruce *et al.* 1997; Ridenour & Callaway 2001; Lu & Ma 2005; Ortega & Pearson 2005; Hejda *et al.* 2009). One study has quantified the impact of an invasive species on the productivity or diversity of its neighbors in the field in both its native and non-native ranges. Inderjit *et al.* (in press) found that the canopies

of *Ageratina adenophora*, a widespread and aggressive subtropical invader, had facilitative effects on other species in its native Mexico but highly inhibitory effects in its non-native ranges in China and India.

Despite the wealth of information on the plasticity of invaders and the powerful impacts they have in their non-native ranges, we know nothing about the phenotypic plasticity of invaders within the context of TMIs or TMIIs. We can only speculate again on likely scenarios and ways in which we might experimentally explore TMIIs produced by the phenotypic plasticity expressed by exotic invasive species. For example, when the California native shrubs *Haplopappus ericoides* and *H. venetus* var. *seloides* grow in the absence of competition their root systems are concentrated near the soil surface (D'Antonio & Mahall 1991). However, when competing with the exotic *Carpobrotus edulis* the root systems of *Haplopappus* shift to a much deeper morphology as they are displaced by the mat-forming exotic (D'Antonio & Mahall 1991). This change in rooting depth by *Haplopappus* suggests that neighboring species can exert strong control over the phenotype of competitors—in this case inducing a change that may result in a tradeoff in access to nutrients and water (Ho *et al.* 2005). Further, a change in *Haplopappus* rooting depth may decrease the intensity of competition between *Haplopappus* and *Carpobrotus* but may increase the intensity of competition with other species that utilize deeper soil sections which would represent a TMII.

Carpobrotus invades different abiotic habitats (D'Antonio 1993) which is likely to elicit plastic responses by *Carpobrotus* (Weber & D'Antonio 1999). Plasticity expressed by *Carpobrotus* may change its effects on the root architecture of *Haplopappus*, creating a complex suite of plastic effects and responses between the two species. It would be intriguing to explore

the next step by experimentally subjecting *Haplopappus* to competition with other species while it is experiencing at least two different manifestations of plasticity in *Carpobrotus*.

Chemically mediated interactions among plants, such as allelopathy, can also have strong impacts on the organization of communities and represents a promising area in the search for TMIIs. *Centaurea stoebe*, an European invader in North America, exudes the compound (±)-catechin from its roots (Tharayil & Triebwasser 2010), which can inhibit the growth of neighboring competing plants (Callaway *et al.* 2005; Inderjit *et al.* 2008a; Inderjit *et al.* 2008b; Simoes *et al.* 2008; He *et al.* 2009; Pollock *et al.* 2009); but see (Blair *et al.* 2006; Duke *et al.* 2009). In addition to inhibiting neighbor performance, (±)-catechin is also a chelator, the addition of which makes phosphorus available in soils where it is bound by calcium (Thorpe *et al.* 2006; Tharayil *et al.* 2008; Tharayil *et al.* 2009) which can improve the performance of *C. stoebe* in phosphorus deficient soils. Native species vary a great deal in their susceptibility to (±)-catechin (Thorpe *et al.* 2009). Weir *et al.* (2006) found that two good competitors with *C. stoebe, Lupinus sericeus* and *Gaillardia grandiflora*, produced levels of oxalate in their root exudates that were more than an order of magnitude higher than that of three poor competitors. They also found that oxalic acid reduces the oxidative damage generated by (±)-catechin. Furthermore, exposure to (±)-catechin increased the exudation of oxalate by *G. grandifolia* by 4x and *L. sericeus* by 50x. This suggests that some native plants may respond to competition with *C. stoebe* in a plastic way, which is a demonstration of a TMI. This response creates the opportunity for a TMII involving the amelioration of (±)-catechin effects on co-occurring species. Interestingly, native grasses are highly spatially associated with *L. sericeus* in communities invaded by *C. stoebe* and field experiments show that *L. sericeus* indirectly facilitates native grasses in vegetation dominated by *C. stoebe*. This facilitation was correlated

with the presence of oxalic acid in the soil in the field. When oxalic acid was applied to the roots of native grasses it alleviated the allelopathic effects of (±)-catechin, indicating that root secreted oxalic acid may act as a chemical facilitator for plant species that do not produce the chemical. Again, this example is not an explicit test of TMIIs, but it does suggest that the chemically mediated suite of indirect interactions derives from the plastic response of some species to the presence of a novel chemical in the soil rhizosphere.

Conclusion

Although we know of no examples in which researchers have specifically investigated the effects of plasticity on indirect interactions among plants, the requisite component pieces of TMIIs in plants are well understood. Because of both the highly plastic nature of plants and a myriad of probable indirect interactions in plant communities, TMIIs among plants are clearly an important future research direction. But beyond linking existing ideas about plasticity and interactions, we also have considered how to use TMIIs to provide fundamental insight into broader ecological questions, such as how plant communities assemble, or how invasive species can act as powerful reorganizing forces in communities.

Among the more promising lines of research, the biochemical plasticity of plants (Metlen *et al.* 2009) has the potential to provide highly dynamic and inducible phenotypic shifts in plants that may also have strong allelopathic effects on some, but not all, neighbors (e.g. Thorpe *et al.* 2009), and in some, but not all abiotic contexts (Pollock *et al.* 2009). Because plant secondary biochemistry can also be specialized in purpose and unique to a family, genus, or even an individual species, the potential for plasticity, and thus TMIIs, via plant biochemistry is nearly endless. In addition, the cascading effects of induced biochemical plasticity could also be

facilitative as it can provide associational defense (Pfister & Hay 1988) and possibly alert other species to the presence of herbivores (Karban *et al*. 2006).

The absence of studies of TMIIs among plants may be due in part to the daunting matter of experimenting with highly diffuse interactions occurring among multiple species. But diffuse interactions are the product, in part, of the immobility of plants, and immobility in multi-species complexes may be why plants are so unusually plastic and provide such exceptional opportunities for studying TMIs and TMIIs. Exploring how shifts in phenotypes respond to changing abiotic and biotic conditions, and in turn affect interactions with multi-species complexes, may yield major advances towards a more mechanistic understanding of the distributions and abundances of plant species.

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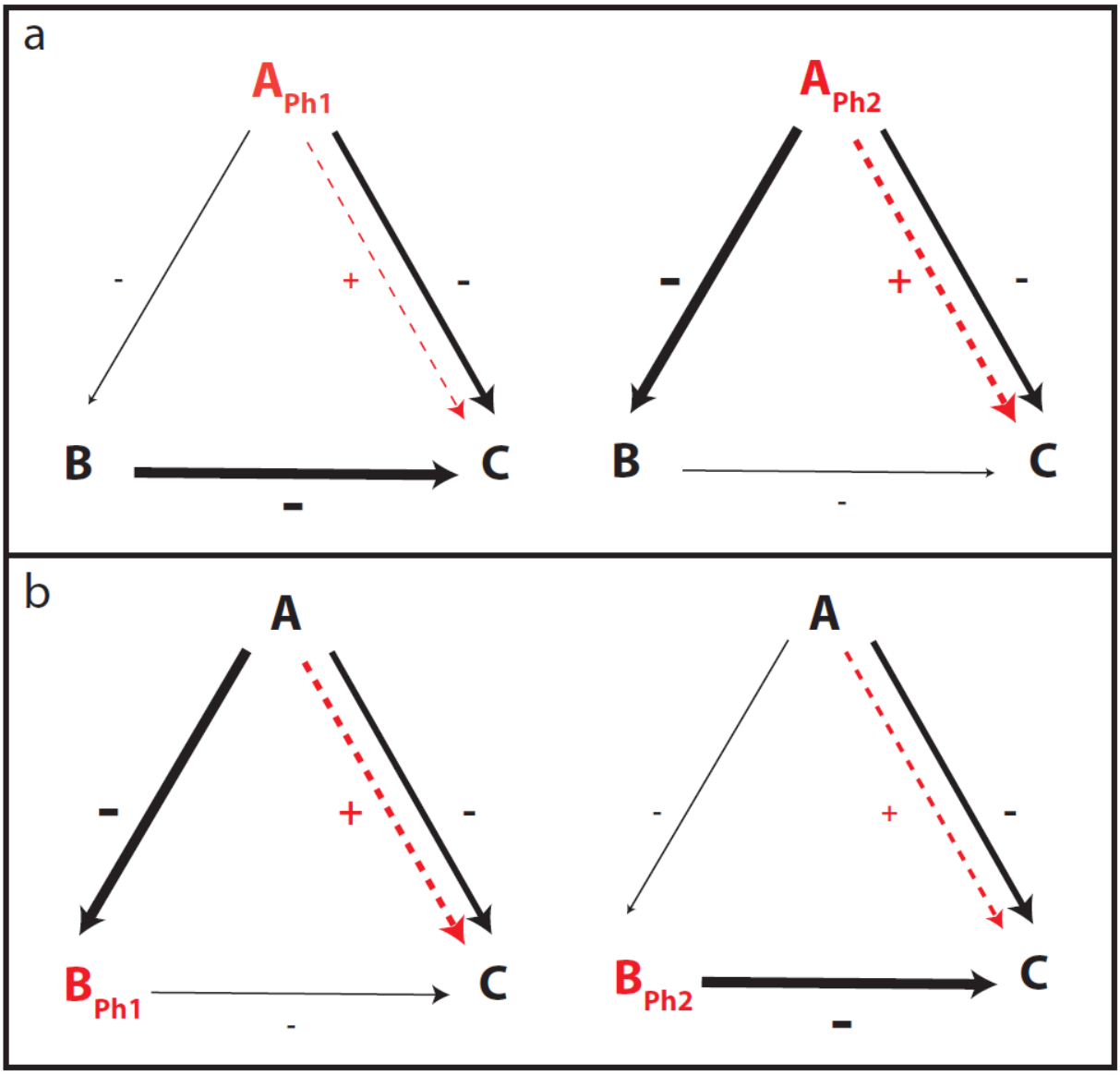

Figure 1. A conceptual model of how a phenotypic shift in species A can alter the competitive *effect* (solid line) of species A on species B, resulting in an increase in indirect (dashed line) benefit to species C. b) A model of how a phenotypic change in species B in *response* to competition by species A can result in a change in the indirect effect of species A on species C. Both models represent TMIIs as a result of competitive interactions.

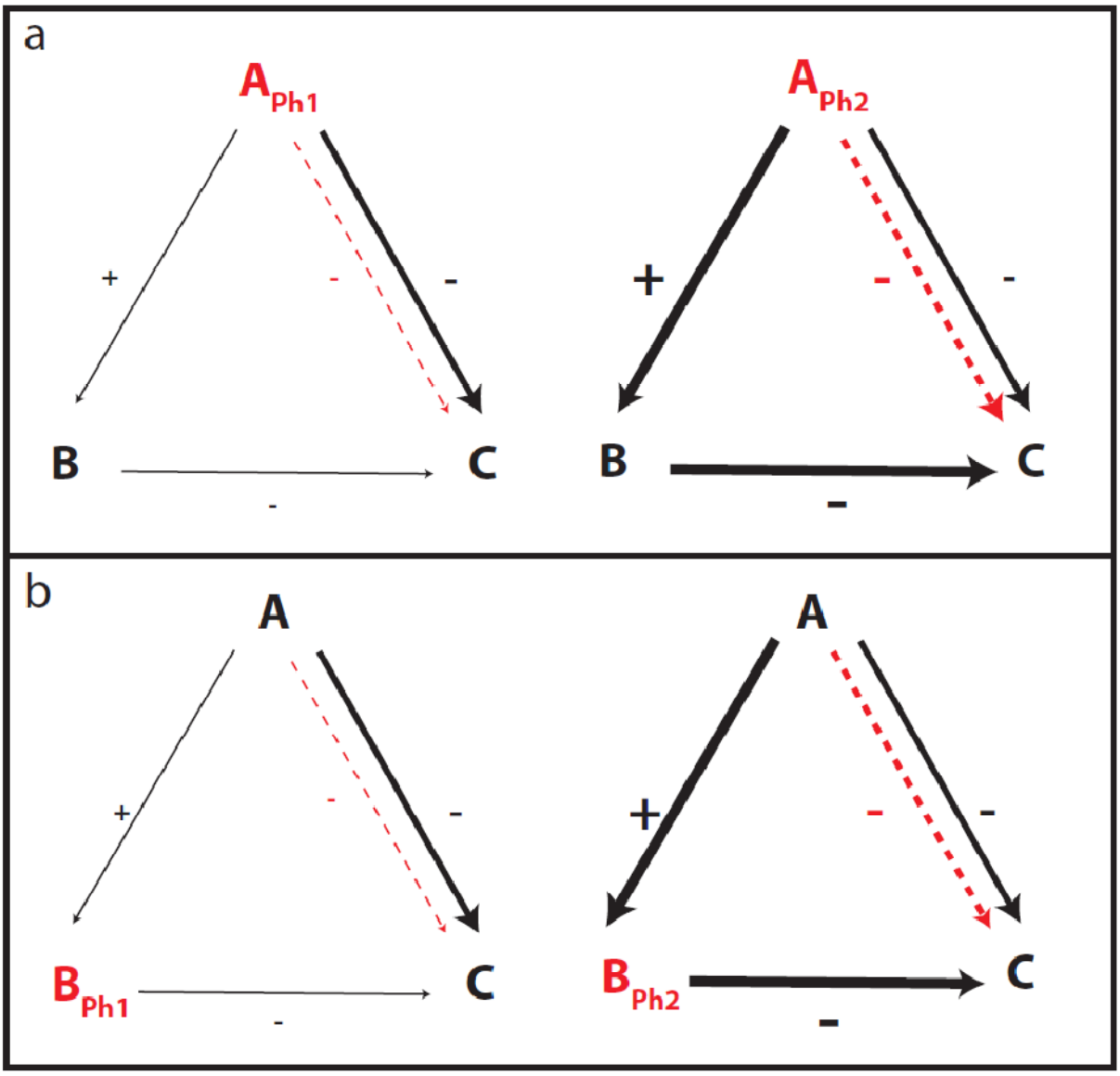

Figure 2. A conceptual model of how a phenotypic shift in species A can alter the facilitative *effect* (solid line) of species A on species B, resulting in an increase in indirect (dashed line) negative effect on species C. b) A model of how a phenotypic change in species B in *response* to facilitation by species A can result in a change in the indirect effect of species A on species C. Both models represent TMIIs as a result of facilitative interactions.

Invader alters native community composition and co-occurrence based assembly patterns: does one species change the rules of engagement?

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Abstract

Invasive plant species can have strong effects on native plants which can result in mono-dominant stands of exotics. Because of the dramatic changes in native plant community composition post-invasion much research has sought to explain how invaders can reach such extreme levels of dominance. It's possible that invaders are simply better competitors than the native species they exclude. This could occur through a release from host-specific enemies in their home ranges, specific traits that confer a competitive advantage in new ranges, or through alteration of ecosystem processes in non-native ranges. However, in addition to strong competitive effects, some studies suggest that invaders may also alter interactions among native species in ways that can facilitate community collapse. Despite the abundance of research investigating the impacts of invaders on native communities, changes in community assembly rules by invasive species remains an untested question in plant community ecology. I analyzed plot data collected from eight grassland sites in western Montana to quantitatively assess the impacts of the invader *Centaurea stoebe* on the assembly rules of native plant species. *Centaurea stoebe* had strong negative impacts on native species diversity and abundance. In addition, plots invaded by *C. stoebe* had 3-4 times lower standardized effect size (SES) C-scores than plots without *C. stoebe* in them suggesting that the level of species-specific co-occurrence between native species declines in the presence of a strong invader. Although both uninvaded and invaded plots had a high proportion of species disassociation, or "forbidden combinations," the decrease in co-occurrence suggests that *C. stoebe* may disassemble native communities by changing how the remaining native species interact with each other. This disassembly may occur through the effects of *C. stoebe* on competitive interactions among native species resulting in shifts in community composition and structure.

Introduction

Invasive species can exert strong competitive effects on natives (Levine et al. 2003, Maron & Marler 2008a), which can sometimes lead to the competitive exclusion of a large proportion of species in native communities. These powerful impacts have been explored through comparisons of invaded and uninvaded sites with similar conditions and histories (Ridenour and Callaway 2001, Levine et al. 2003, Ortega and Pearson 2005, Jäger et al. 2007, Hejda et al. 2009), comparison of canopy effects in native and non-native ranges (Inderjit et al. 2011), responses of natives as invasions proceed over time (Petsikos et al. 2007, Brewer 2008), removal of invaders (Alvarez and Cushman 2002), meta-analysis (Gaertner et al. 2009), experimental additions of invaders (Maron and Marler 2008a,b), or paired competition experiments (Callaway and Aschehoug 2000, Ridenour and Callaway 2001, Vila et al. 2004, He et al. 2009). However, it is not clear how invaders accomplish such spectacular dominance. One possible scenario is that some invasive species are simply better competitors than all of the native species they exclude. This has been proposed to occur through several mechanisms including release from host-specific enemies that occur in native ranges (Elton 1958, Keane and Crawley 2002, Callaway et al. 2004), specific traits that yield greater competitive effects (Callaway and Aschehoug 2000, Callaway and Ridenour 2004, Kim and Lee 2010, Inderjit et al. 2011), or competitive advantages through the effects of invaders on native ecosystems (Mack et al. 2001, Rout and Callaway 2009). A second and non-mutually exclusive scenario, and one that to our knowledge is untested, is that invaders do not outcompete *all* natives, but instead, through their strong competitive effects they may also disrupt assembly rule interactions among natives. In other words, invaders might change the ways that natives interact with each other in ways that contribute to their demise. In either scenario, competitive exclusion of native species may occur

because invaders are able to reach much higher biomasses and thus exert greater, but mass-symmetrical, effects (see Maron and Marler 2008a,b). Alternatively, invaders may exert stronger gram per gram effects (Besaw et al. 2011).

"Assembly rules" are the general rules that determine how species combine to form communities (Wilson et al. 1995, Wilson and Whittaker 1995, Belyea and Lancaster 1999) and provide a broad approach to link competitive interactions into a predictive framework for coexistence, competitive exclusion, and community structure. There are two general categories of assembly rule theories for how plant communities assemble under equilibrium conditions. The first assumes that plant communities are competitively transitive in nature (Goldsmith 1978, Mitchley and Grubb 1986; Keddy and Shipley 1989). In other words, all species in a given pool, or community, can be ranked in a linear hierarchy of competitive ability. The second category incorporates complex interactions among *groups* of plant species resulting in "non-transitive", or non-hierarchical, community assembly rules (Jackson and Buss 1975, May and Leonard 1975, Petraitis 1979). Experiments and models of such non-transitive processes among competitors suggest that community members interact in "networks" of interactions, not hierarchies - some positive, some negative – and raise the alternative perspective that coexistence may be maintained among large pools of species even in the absence of abiotic heterogeneity or non-equilibrium processes. Hierarchical organization is best described mathematically as A>B>C, whereas non-hierarchical organization has been proposed to occur through "competitive loops" in the hierarchy such as A>B>C>A. In other words, species C indirectly benefits species B by having a direct negative impact on species A. Given the right starting point, a simple loop within a suite of competing species can result in a perpetually shifting state in which all three species coexist indefinitely. This coexistence is based entirely on the balance of direct competitive

interactions, but leads to the formation of complex networks of species interactions. Mathematical evaluations of such interactions predict that indirect interactions among competitors can allow communities of multiple species to coexist (Karlson and Jackson 1981, Laird & Schamp 2006, 2008, Allesina and Levine 2011).

Assembly rules in natural communities have been well studied and vigorously debated (Weiher and Keddy 1999b) but we know little about how exotic invasions might affect assembly rules in native communities. Invaders can competitively suppress and exclude natives, but do they also alter the complex interactions that occur among *groups* of competing native species? Experimentally examining networks of competitive interactions in the context of invasion may provide insight into whether or not natural communities assemble in any predictable way, how they assemble, and if they disassemble when interacting with new and highly competitive species. For example, Gotelli and Arnett (2000) investigated the effects of the invasive red fire ant (*Solenopsis invicta*) on native ant communities along a 2000 km transect on the eastern coast of North America. *Solenopsis invicta* not only reduced the density of native species at local scales, it changed patterns of co-occurrence among native ants from highly segregated (low coexistence) to patterns that were fully random and suggestive of no assembly rules at all. Similarly, non-experimental analyses of spatial associations and patterns provide insight in the effects of invaders on assembly rules. Sanders et al. (2003) found strong disassembly of native ant communities by the invasive Argentine ant (*Linepithema humile*). By following *L. humile* invasions over time they found that, much like *S. invicta*, *L. humile* changed patterns within communities of native ant species from highly segregated assembly rules to patterns of random assembly.

Assembly rules have been studied extensively in plant communities (Wilson and Roxburgh 1994, Wilson et al. 1995, Wilson and Whittaker 1995, Weiher and Keddy 1999a), but to our knowledge there have been no studies, such as those on invasive ants, of the effects of invasive plants on community assembly. Exotic plant invasions are ubiquitous and have profound effects on the local abundance and diversity of native species. In addition, plant communities are often a complex matrix of direct and indirect multi-species interactions, meaning that plant communities are a rich system for investigating assembly rules (Wilson and Roxburgh 1994, Wilson et al. 1995, Wilson and Whittaker 1995). Plant communities worldwide are experiencing a large influx of new species as a result of the increased globalization of agriculture and commerce, providing the opportunity to learn much about how invasive species may disrupt the fundamental rules of how plants interact at the community level. Here I examined how invasion varied native species composition in western Montana grasslands (bluebunch wheatgrass (*Pseudoroegnaria spicatum*) type (Mueggler and Stewart 1980)) using ordination analysis and small scale assembly patterns with co-occurrence analysis.

Methods

Centaurea stoebe L. ssp. *micranthos* (Gugler) Hayek (spotted knapweed; nee C. maculosa Lam.) was introduced to North America in the early 1900s and is now an aggressive invader of western Montana grasslands. At high densities *C. stoebe* displaces native species and decreases local plant diversity (Tyser 1992, Ridenour and Callaway 2001, Ortega & Pearson 2005). I compared community composition in eight grassland sites where *C. stoebe* varied in abundance.

The percent cover of all species in 800 1 x 1 m^2 plots in western Montana grasslands was sampled in early summer (May-June) of 2000 (collected by Ortega and Pearson 2005). Eight different sites were sampled, four with virtually no invaders and four that had been moderately invaded by *C. stoebe*. All sites occurred on southwest aspects between 1300 and 1700 m elevation and were similar in slope, aspect, vegetation classification type and management history (Ortega and Pearson 2005). *Centaurea stoebe* is an aggressive invader of western Montana grasslands, but the average *C. stoebe* cover for all invaded sites was 17%, much lower cover than this species can reach at other sites (Key 1988, Tyser 1992, Ridenour and Callaway 2001,). At each site, four transects were established perpendicular to the slope approximately 50 m apart. On each transect, vegetation was sampled in 25 systematically oriented 1 m^2 quadrats placed every 10 m along each transect. Thus there were 100 plots at each site. In each plot, the percent cover of each species was estimated to the nearest 1%. For all species that occupied less than 1% of a plot, a value of 0.5% was recorded. I separated these 800 plots into two groups, those with *C. stoebe* occurring in them at any level of abundance (n=166), and those with no *C. stoebe* recorded (n=631). In this analysis the percent cover of the dominant invader *C. stoebe*, all exotic species (n=23), conifers, and all rare species (< 2 total occurrences) were excluded from all plot data to avoid bias in the ordination analysis. I compared the mean diversity of natives (total number of species) and the mean cover of natives in plots between these two groups using two sample means t-tests (Sigmaplot 11.2). I also calculated rank-abundances by summing the percent cover in all plots by species and then ordering them numerically. To plot rank-abundance curves, the abundance data were natural log transformed ($ln(x+1)$).

I conducted a non-metric multidimensional scaling (NMS) ordination analysis (PC-ORD 5.0) of the data using natural log transformed ($ln(x+1)$) percent cover data from all plots. NMS

analysis allows one to make estimates of community similarity based on the unique species combinations and relative cover data from field plots. The distance between points (plots) within the ordination space represents the degree of similarity for any two plots.

I performed a co-occurrence analysis of species in the 631 plots without *C. stoebe* and of species in the 166 plots invaded by *C. stoebe*. All percent cover data was converted into a matrix of binary values for presence (1) or absence (0) and organized by species (rows) and plot (column). For all plots, we removed the percent cover of *C. stoebe,* all exotics and two species of conifer from the analysis in order to limit the test to native species response to the presence or absence of *C. stoebe*. Using ECOSIM 7 (Gotelli and Entsminger 2009), we calculated the C-score (Stone and Roberts 1990) for both types of plots in order to quantitatively explore the level of coexistence among pairs of species; the larger the C-score, the lower degree of co-occurrence of specific species pairs. In other words, communities with large C-scores exhibit properties of species segregation, which is assumed to be driven by competition. We evaluated the statistical significance of C-scores by comparing the observed community C-score (treatment) to a null community (control) which was generated by random assemblages of the observed data. Our model assumed fixed row and column totals when calculating null communities. The random assemblages were replicated 5000 times and used to calculate a standardized effect size (SES) for each community. SES is a conversion of the C-score into units of standard deviations and allows for meaningful comparisons among matrices. SES values greater than 1.96 demonstrate patterns of segregation that are significantly different from random assemblages of species.

Results

Plots without *C. stoebe* had significantly more species per plot (mean = 6.50 ± 0.09) than plots invaded by *C. stoebe* (mean=4.65 ± 0.17; $P < 0.001$). In addition, plots without *C. stoebe* had significantly higher percent cover of natives per plot (mean = 37.38 ± 1.15) than did plots with *C. stoebe* present (mean=31.43 ± 2.21; $P = 0.018$).

Rank-abundance curves show strong differences in slope and rank position where abundance is zero (Fig. 1). The three most dominant species in uninvaded plots were *Balsamorhiza sagittata* (ln abundance = 8.57), *Pseudoroegneria spicata* (8.04), *and Festuca idahoensis* (7.74). All three species showed strong declines in plots with *C. stoebe* (*B. sagittata* = 7.91; *P. spicata* = 6.04; *F. idahoensis* = 3.76).

NMS ordination analysis showed that, as a group, the 166 plots with *C. stoebe* had substantially different species compositions than the 631 plots without *C. stoebe* (Figure 2). NMS of the samples also explained variation in the data set well with a final stress score of 22.58 for a three dimensional solution after 200 iterations. The final instability score was 0.00407. The strongest differentiation between these two groups was along NMS axis 1, and the abundant species that showed the strongest relationships to axis 1 were *Microsteris gracilis* (Kendall's Tau = 0.485, *F. idahoensis* (Tau = 0.454), *Lithophragma parviflora* (Tau = 0.414), *Lupinus* species (Tau = 0.365), *Physocarpus malvaceus* (Tau = 0.174), which were not associated with *C. stoebe*, and *B. sagittata* (Tau = -0.581), *Collomia linearis* (Tau = -0.218, *P. spicata* (Tau = -0.204), *Achillea millefolia* (Tau = -0.107) which were associated with *C. stoebe*.

Native communities appear to be structured with a high degree of segregation among species. In other words, native species demonstrate patterns of low coexistence or high numbers of "forbidden combinations" (Diamond 1975; SES C-score = 24.05). Invaded communities,

however, showed much weaker patterns of segregation (SES C-score = 7.33) indicating that the number of forbidden combinations were far fewer.

Discussion

My results provide correlative evidence from spatial patterns for the disassembly of native communities due to *C. stoebe* invasion. Many studies have shown that invasive plant species suppress and eliminate native species locally (Alvarez and Cushman 2002, Petsikos et al. 2007, Brewer 2008, Gaertner et al. 2009, Inderjit et al. 2011), and others have shown that invasive species can have disproportionally strong competitive effects (MacDougall and Turkington 2004, Vila and Weiner 2004, Maron and Marler 2008b), including *C. stoebe* (Maron and Marler 2008b, He et al. 2009, Aschehoug 2011, Dissertation Chapter 1). Importantly, most studies to date investigating the effects of invasive species on plant communities focus on the direct effects of the invader on individual native species (Callaway and Aschehoug 2000, Ridenour and Callaway 2001). Instead, these results are the first to suggest that plant invaders may fundamentally change how native species interact *with each other* and result in far less structure in the remaining native plant communities. Importantly, my results are based on spatial patterns, and thus are a correlative first step towards understanding how invaders might affect assembly rules. Experiments that control for site effects and potential indirect effects (e.g. through soil biota or herbivores) will be crucial.

To my knowledge, the only other studies of the effect of exotic species on community assembly rules and structure have been conducted on ants. Gotelli and Arnett (2000) found strong impacts on native ant community structure by the invasive red fire ant (*Solenopsis invicta*). *S. invicta* altered native ant competitive interactions by shifting patterns of low coexistence to patterns of random assembly. This significant change in how native ant species

interact is thought to be driven by the strong competitive effects of *S. invicta*. Similarly, Sanders et al. (2003) found that the invasive ant *Linepithema humile* caused native ant community structure to collapse from highly segregated to random assembly.

Understanding the rules by which plant species assemble into communities is crucial in order to make predictions about species abundances and distributions and the structure of plant communities (Wilson and Roxburgh 1994, Wilson et al. 1995, Wilson and Whittaker 1995, Weiher and Keddy 1999a). However, the search for assembly rules in ecology has not been without controversy (Diamond 1975, Simberloff and Connor 1979). Although there is a growing consensus among plant ecologists that plant communities are more than random assemblages of species and therefore exhibit properties of assembly rules (Weiher and Keddy 1995), there is disagreement regarding the nature of how assembly rules operate within communities. Currently, the debate centers on whether or not competition acts to exclude species from communities (Keddy and Shipley 1989) or whether competition can act to promote coexistence within communities (Laird and Schamp 2006). Plant invasions provide a unique opportunity for exploring the role of assembly rules and competition in structuring plant communities. The introduction of new, highly competitive species can give us insight into potentially subtle assembly rules in native communities by disrupting the interactions between native species. If assembly rules are present and detectable in native communities, changes in interactions in the presence of invaders can tell us how resilient community assembly rules are to the introduction of new species - invasive or not. My results suggest that the invader, *Centaurea stoebe,* has dramatic effects on the underlying processes of competition resulting in shifts in both community composition (Figure 2) and structure (Figure 3). Thus, my results provide further support for the presence of assembly rules - not only among native species, but also in

moderately invaded systems. This does not settle the debate about whether competition is an exclusionary force, or if competition supports coexistence in plant communities; however, these results indicate that invasive species can serve as a model system for exploring the fundamental nature of assembly rules in plant communities.

My results also suggest that assembly rules may not be fixed, but instead are dynamic. Current theory predicts that assemblages of species interact in set or fixed ways to form communities. However, plants are plastic in response to competition at the individual level (Cahill et al. 2010), thus it is also possible that plant assemblages can be plastic in the way they interact as a group in response to each other or new species. Although both uninvaded and invaded plots exhibited significant properties of segregation ($p<0.001$), I found a 70% reduction in the SES C-score, a measure of coexistence among species, from native sites to invaded sites suggesting that the rules of competition between native species are fundamentally changed in the presence of *C. stoebe.* In addition, the sampled sites represent an invasion level of *C. stoebe* (mean cover of 28% in invaded plots) that is much lower than what can occur in *C. stoebe* invasions (e.g., 60-100%; Ridenour and Callaway 2001). Despite the relatively low percent cover of *C. stoebe* in invaded plots, I still found highly significant effects of invasion on species diversity and percent cover of native species. Ortega and Pearson (2005) found that both of these measures are negatively correlated with *C. stoebe* cover, suggesting that as invasion level increases over time, native species diversity and percent cover will continue to decline. Therefore, even though our study only examines the early phases of *C. stoebe* invasion and community disassembly, it may be useful in making predictions about how assembly rules may change as invasion progresses.

Recently there has been a renewed interest in the attributes of plant communities that might confer resistance to invasion (Levine and D'Antonio 1999). Investigations have focused on how different levels of species diversity or particular combinations of species provide biotic resistance to invasions. However, the results here suggest that invaders may be successful not only because of their particularly effective competitive abilities, but also because of the cascading effects they have on changing native species interactions. Thus, biotic resistance may be conferred via not only the individual competitive abilities of native species, or their diversity *per se*, against invaders, but also by the stability of the networks of interactions occurring between natives during invasion. Given that invasions are often patchy across landscapes, we may learn much from variation in fundamental ecological processes, such as competition and assembly rules, among these patches.

My results provide circumstantial evidence for community disassembly by *C. stoebe*, especially when considered together with other studies on the effects of *C. stoebe* on North American grasslands (Tyser 1992, Kedzie-Webb et al. 2001, Ridenour and Callaway 2001, Ortega and Pearson 2005), but we cannot rule out factors other than competition as causes in our large scale correlation-based study - experimentally manipulating invasion rates and levels in intact native grasslands has ethical issues. Furthermore, we do not know the mechanisms behind the strong competitive effects of *C. stoebe* on natives or how *Centaurea* may alter the ways that natives interact with each other. *Centaurea stoebe* may gain competitive advantages through novel traits such as allelopathy (Inderjit et al. 2008, He et al. 2009), its effects on soil biota (Callaway et al. 2004), mass-based effects (Maron and Marler 2008b), or effects on resources. Regardless of the mechanism, my results are the first to suggest that invasive plants may not only

competitively suppress native species, but also strongly alter the way native species interact with each other.

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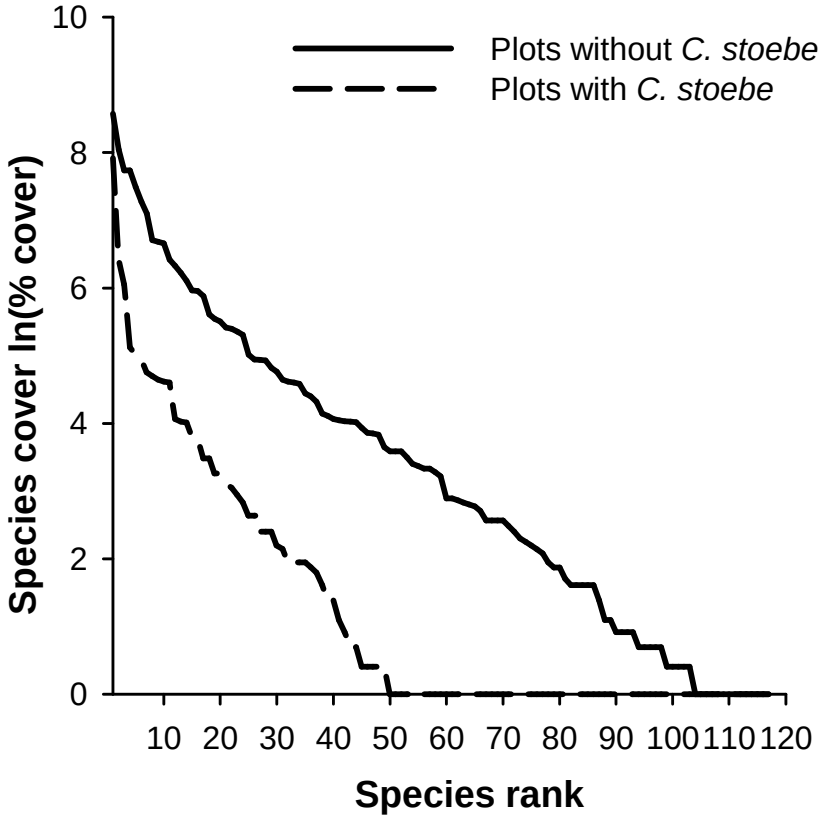

Figure 1. Species rank abundance curves for plots with and without *C. stoebe* at eight sites in intermountain grassland in western Montana.

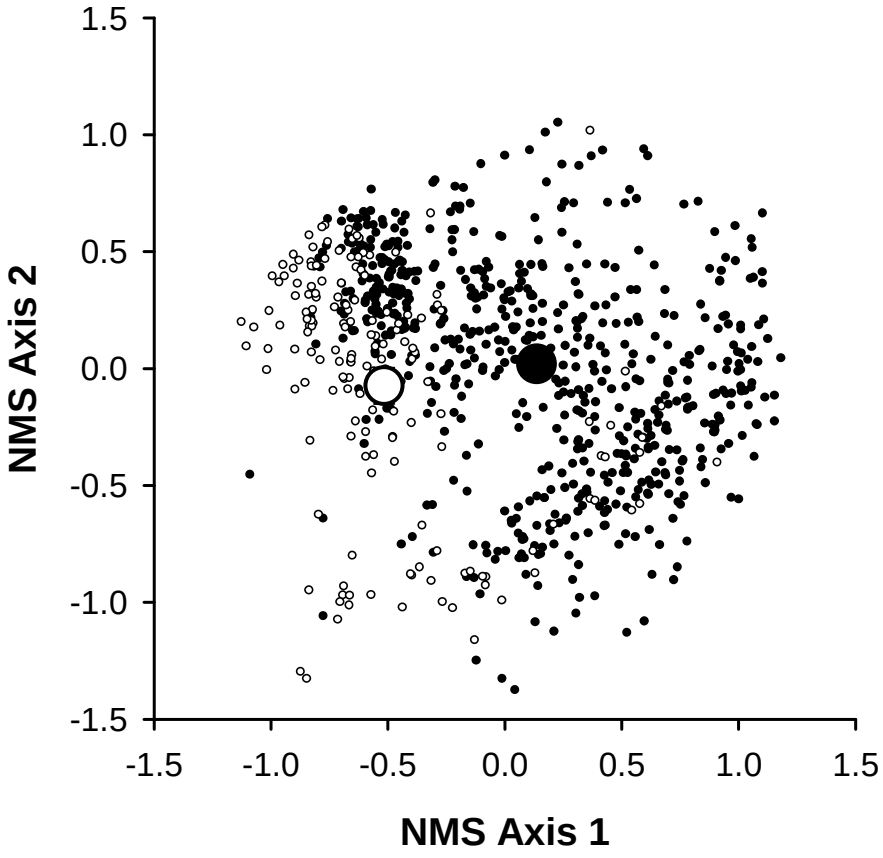

Figure 2. NMS ordination for all plots at all sites containing *C. stoebe* (n = 166, white circles) and all plots at all site not containing *C. stoebe* (n = 631, black circles). Large circles show the means for each treatment and 95% confidence limits are within the circles.

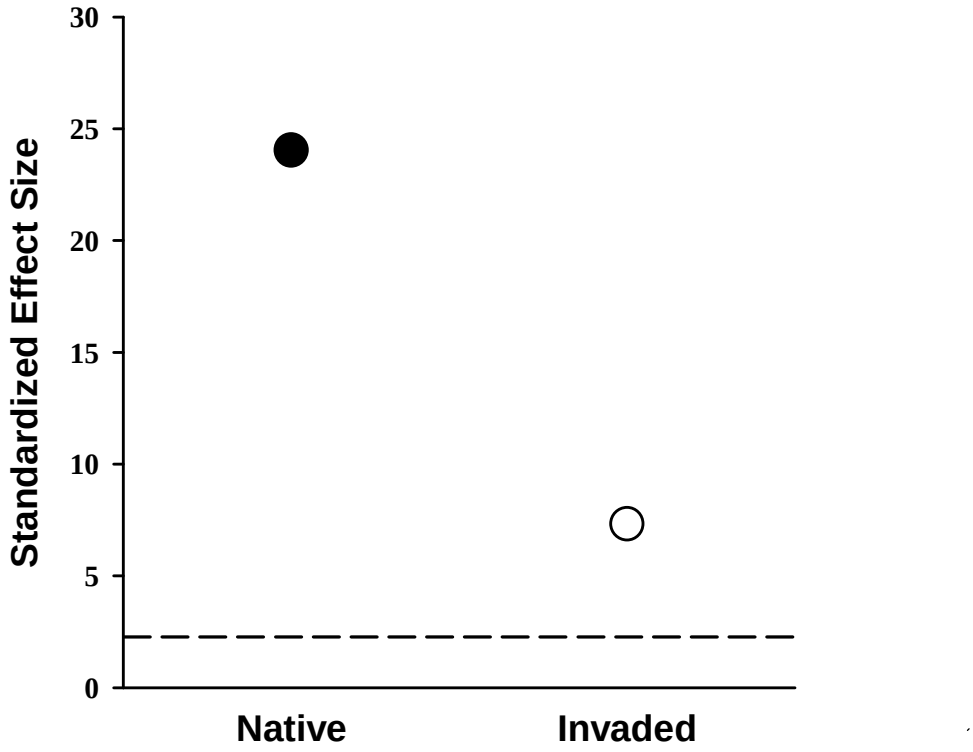

Figure 3. Effects of *C. stoebe* on native grassland community structure. Standardized effect size of the calculated C-score for plots with and without *C. stoebe*. Dashed line (SES=1.96) indicates the threshold between random assemblages (SES<1.96) and significantly segregated communities (SES>1.96).

Fungal endophytes directly increase the competitive effect of an invasive forb

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Abstract

Competitive outcomes among plants can vary in different abiotic and biotic conditions. Here we tested the effects of two phylotypes of *Alternaria* endophytes on the growth, competitive effects, and competitive responses of the exotic invasive forb *Centaurea stoebe*. *Centaurea stoebe* was a better competitor against North American grass species than native grasses from its European home range in the absence of endophytes. However, one endophyte both increased the biomass of *C. stoebe* and reduced the competitive effect of North American grasses on *C. stoebe*. The competitive effects of *C. stoebe* on grass species native to North America were enhanced by both fungal endophytes, but not for native European grasses. We do not know the mechanism by which endophytes increased *C. stoebe's* competitive ability, and particularly against biogeographically new neighbors, but one endophyte increased the competitive ability of *C. stoebe* without increasing its size, suggesting mechanisms unrelated to increased growth. We tested only a fraction of the different endophytic fungi that have been found in *C. stoebe*, only scratching the surface of understanding their indirect effects. However, our results are the first to demonstrate such effects of a fungal endophyte infecting an invasive forb, and one of the few to show that endophyte effects on competition do not have to be mediated through herbivory.

Keywords: *Alternaria, Centaurea,* biogeography, community, competition, conditionality, endophyte, fungus, invasion, mutualism

Running head: Endophytes increase competitive ability

Introduction

Competition is a strong organizing force in plant communities (Connell 1983, Grace and Tilman 1990). However, competitive outcomes are highly conditional, varying with abiotic conditions (Callaway et al. 1996), herbivore attack (Louda et al. 1990), and pathogens (Van der Putten and Peters 1997). At the scale of continents, exotic invasions also suggest strong conditionality in competition because some species become much more dominant in their non-native ranges than in their native ranges (Hierro et al. 2005). This conditionality in dominance would seem to be related, at least in part, to unusually strong competitive suppression of resident species in the newly invaded range (Maron and Marler 2008). This superior competitive ability of "invaders" in their new ranges has been primarily attributed to release from host-specific enemies (Keane and Crawley 2002), such that reduced herbivore and pathogen attack may give invaders a disproportional competitive edge in their new ranges where native species remain suppressed by their host-specific enemies. Invaders may also directly exert greater competitive effects in their non-native ranges through their ability to attain higher biomass (Maron and Marler 2008), or through novel traits that confer greater competitive ability beyond that of size (Callaway and Aschehoug 2000, Kim and Lee 2010, Inderjit et al. *in press*). However, we know much less about the conditionality of competition between invaders and natives than we do about the conditionality of competitive interactions among native species.

Mutualisms play powerful roles in some successful invasions (Richardson et al. 2000, Rout and Chrzanowski 2009, Callaway et al. *in press*) and there is evidence that the competitive ability of some invaders can be improved by associations with mutualists (Marler et al. 1999, Reinhart and Callaway 2004, 2006). Fungal endophytes are mutualists that can provide indirect benefits via herbivore defense (Clay et al. 2005, Koh and Hik 2007, but see Faeth 2002, Faeth

and Fagan 2002). Fungal endophytes can also improve plant performance by altering rhizosphere microbial communities (Rudgers and Orr 2009) and helping plants cope with water stress (Elmi and West 1995). Fungal endophytes also appear to directly increase the competitive effects of infected plants on other species (Marks et al. 1991, Rudgers and Orr 2009), but these effects are much less understood than herbivore-mediated effects. Importantly, examples of endophyte-increased competitive effects are limited so far to a relatively small group of endophytes in grasses: those belonging to the family Clavicipitaceae (e.g., *Neotyphodium*; Clay et al. 1993; Clay and Holah 1999).

There has been little investigation into the role of endophytic mutualists in the success of invasive species, with the exception of what has been learned from non-native agriculturally important grass species (Saikkonen et al. 2006). *Centaurea stoebe*, an aggressive invader of western North American grasslands, provides an opportunity to substantially broaden our understanding of how non-clavicipitaceous endophytes affect interactions among plants (Newcombe et al. 2009) and their roles in invasion because *Centaurea stoebe* harbors many fungal endophytes (Shipunov et al. 2008). Some of these endophytes have the potential to enhance the competitive and allelopathic effects of *C. stoebe*, while others may act as pathogens (Newcombe et al. 2009).

We explored the role of two fungal endophytes on the growth of *C. stoebe* and on the competitive effects and responses of the invader when interacting with North American and European grass species. Both endophytes are phylotypes of *Alternaria* (Shipunov et al. 2008). Some species in the genus *Alternaria* are pathogens of crops and trees, and are found in soils where they act as decomposers (Kwansa 1992). However, species of the polyphyletic genus *Alternaria* also act as mutualistic endophytes with some plant species. For instance, Musetti et

al. (2007) found that *Alternaria alternata* is a defense mutualist against the downy mildew *Plasmopara viticola* in grapevines (*Vitis*). Other very closely related pathogens of the order Pleosporales also appear to switch between pathogen and mutualist roles and aid plants in extreme environmental conditions (Marquez et al. 2007; McLellan et al. 2007) and can be common as endophytes (Porras-Alfaro et al. 2008).

We focused on three primary questions: 1) do fungal endophytes directly affect the growth and competitive ability of *C. stoebe*? 2) does *C. stoebe* have stronger competitive effects on and weaker competitive responses to native North American species than European species? and 3) do fungal endophytes affect competitive interactions between *C. stoebe* and North American natives more than competition with European species?

Methods

We grew *Centaurea stoebe* in three treatments: 1) endophyte free; 2) infected with *Alternaria* phylotype 'alt2f' (isolate CID120); 3) infected with *Alternaria* phylotype 'alt2b' (isolate CID73). The 'alt2f' phylotype is closely related to *Alternaria longipes* whereas the 'alt2b' phylotype is closer to *Alternaria alternata*. The CID73 isolate, or fungal individual, that we used was from seed of a *C. stoebe* plant collected along the Clearwater River, Idaho [lat.: 46.4474333; long.: -116.861917; elev.: 233 m], whereas the CID120 isolate was from Heviz, Hungary [lat.: 46.8046667; long.: 17.25566667; elev.: 454 m]. These endophytes have been found in *C. stoebe* over wider ranges, but were chosen because of differences in their relative abundances in the native and non-native ranges of *C. stoebe* (Shipunov et al. 2008). The 'alt2f' phylotype (CID73) is much less common in both the native and invaded ranges of its host than 'alt2b' (CID120), the most abundant phylotype of the native range and quite common in the invaded range as well. Endophyte infection rates of sampled populations of *C. stoebe* vary

between 0-100%; however, less than 30% of all seeds contain endophytes of any kind (Shipunov et al. 2008).

Centaurea stoebe plants were grown either alone (n=10 per endophyte treatment) or in pairwise competition (n=10 per endophyte treatment per competitor) with each of four North American and four European grass species in a greenhouse at the University of Montana, Missoula, Montana, USA. We selected grass species that are either dominant or common species in their respective native ranges. North American grasses were *Festuca idahoensis, Koeleria macranthus, Pseudoroegneria spicata,* and *Stipa comata.* European grasses were *Agropyron repens, Lolium rigidum, Melica ciliata,* and *Poa annua*. All grass seed was wild collected from native prairie surrounding Missoula, Montana and in grasslands containing *C. stoebe* near Iasi, Romania. Endophyte-free seeds of *Centaurea stoebe* were raised from a parent stock of wild collected endophyte-free seed in a greenhouse at the University of Idaho. All species were germinated in Petri dishes over a two week span prior to transplanting into 2.4 l (18 cm diameter, 22 cm depth) pots to insure germination success and reduce priority effects among competing species that germinate at different times. All pots were randomized in the greenhouse after planting to avoid the confounding effects of greenhouse microsite variability. Soil in pots comprised of a 1:1 homogenous mix of autoclave-sterilized field collected soil (Missoula, Montana) and 20/30 grit sand. All soil, sand and pots were autoclaved prior to planting to remove any confounding soil microbial effects. Fungal endophytes were cultured on potato dextrose agar and applied exogenously to *C. stoebe* roots in the seedling stage prior to planting. To ensure adequate inoculation, seedlings were placed in Petri dishes of the cultured fungal endophytes and allowed to remain in contact with fungal hyphae for 12 hours. Like other Class 2 endophytes (Rodriguez et al. 2009), the *Alternaria* phylotypes studied here can colonize roots

and leaves as well as the seeds from which they were originally isolated making our inoculation procedure an appropriate experimental manipulation that results in reliable colonization (Newcombe et al. 2009).

We tested the direct effects of endophytes on all grass species by directly applying fungal endophytes to the roots of grass seedlings using the same procedure as for *C. stoebe* seedlings. These seedlings and controls without fungal endophytes were planted alone (n=10 per species per endophyte treatment) in 500 ml cone-tainer pots. Pots were filled with a 1:1 homogenous mixture of autoclave sterilized local native soil and 20/30 grit sand.

All plants were grown for 70 days prior to harvest. Greenhouse temperatures were kept between 15 and 30° C. and natural light was supplemented by metal halide bulbs to maintain PAR above 1,200 µmols m^{-2} s^{-1}. Plants were watered two to three times per week. Entire individual plants were harvested by washing and manually disentangling roots of competing species. We subsampled the live roots of 36 individual grasses to test for horizontal transfer of endophytes from *C. stoebe* plants to grasses. Subsampled roots were surface sterilized and cultured to determine infection rates. We did live weight to dry weight conversions of all subsampled tissues using a conversion factor from the remaining root mass of subsampled plants.

Harvested plants were dried at 60°C for 72 hours and weighed. We used ANOVA (univariate GLM in PASW 18) where competitor species, region and endophyte treatment were fixed factors. We also calculated Relative Interaction Intensity indices (RII; Armas et al. 2004) using endophyte-free *C. stoebe* grown alone as the control and competitor x endophyte interactions as the treatment. RII is a measure of the strength of interaction between species centered on zero with negative interactions (competition) indicated by values between 0 and -1, and positive interactions (facilitation) indicated by values between 0 and +1. RII allows for

simple comparisons of interaction strength across taxa and treatments. Statistical analyses and the results presented in the figures for RII were calculated using t-test comparisons (Excel 2007) and one-way ANOVA with Tukeys post hoc analysis (Sigmaplot 11.2) of RII values and SE (Armas et al. 2004 Appendix A) both among and between region x endophyte treatments and to determine whether RII values significantly differ from zero.

Results

When *C. stoebe* was experimentally infected with the CID120 isolate from Hungary, plants were 46% larger (post-ANOVA Tukey test, $p=0.001$; Appendix, Table 1) than the uninfected controls and 36% larger (post-ANOVA Tukey's test, $p=0.003$) than plants infected with the CID73 isolate from the Clearwater River of Idaho.

Endophyte-free *C. stoebe* were more than twice as suppressed by European grass species as by North American grass species (Fig. 1; Appendix, Table 1); however, both European and North American species suppressed endophyte-free *C. stoebe* ($p<0.001$, $p=0.038$). When *C. stoebe* was infected by CID120, the competitive suppression by European grasses was as strong ($p<0.001$) as when *C. stoebe* was endophyte-free. In contrast, *C. stoebe* infected by CID120 was unaffected by North American grasses ($p=0.317$). When *C. stoebe* was infected by CID73, the competitive effect of the four European grass species analyzed as a group significantly suppressed *C. stoebe* ($p<0.001$) and this effect did not differ from that of either endophyte-free or CID120-infected *C. stoebe.* Unlike the effects of the CID120 endophyte, when *C. stoebe* was infected by CID73, North American plants significantly suppressed the invader ($p=0.028$).

Endophyte-free *C. stoebe* marginally suppressed European grass species analyzed as a group ($p=0.058$; Fig. 2; Appendix, Table 2). Endophyte-free *C. stoebe* tended to suppress North American grass species as a group but this effect was not statistically significant ($p=0.072$).

Centaurea stoebe infected by CID120 did not change in its effect on European grass species (p=0.074), but strongly suppressed North American grass species (p=0.005). When *C. stoebe* was infected with CID73, there was still a trend towards suppression of European grass species, but this effect was not significantly different than the effects of endophyte-free or CID120-infected *C. stoebe* (p=0.100). In contrast, North American grass species were strongly suppressed by *C. stoebe* infected with the CID73 endophyte (p=0.005).

We found limited evidence for direct horizontal transfer of endophytes from *C. stoebe* to grass species. Of the 20 subsampled European grass species, two were infected by CID120 and two were infected by CID73. For North American grass species, we subsampled 16 individual plants and found three infected by CID120 and none infected by CID73. In our tests of the direct effect of endophytes on grass species, we found no effects on European grass species (Appendix, Fig. 1). However, North American grasses analyzed as a group were significantly inhibited by CID120. This result was highly skewed by the sensitivity of *Stipa comata* to direct infection with CID120; *S. comata* was the only species of the eight tested to show significant effects to the direct application of CID120 and CID73 (Appendix, Table 3). But when *S. comata* was grown in competition with *C. stoebe*, we found no significant differences between endophyte-free and endophyte-infected treatments (p=1.00, p=0.760; Appendix, Table 2).

Discussion

The most novel contribution of our results is that the direct competitive effects of *C. stoebe* on native species in the invaded range were strongly enhanced by fungal endophytes. Importantly, the enhanced effects of endophytic fungi only occurred against North American natives and not against European natives. The effects of endophytic fungi on competing grass

species may have been direct, as horizontal transfer from *C. stoebe* to grasses did occur. However, only 7 of 36 grasses surveyed from the competition experiment showed any evidence of fungal endophyte infection, and only *S. comata* showed negative effects of direct application of either fungal endophyte. If direct effects of the fungal endophyte via horizontal transfer are responsible for the suppression of grass species, we would have expected the highly sensitive *S. comata* to be suppressed more when in competition with endophyte infected *C. stoebe* than when in competition with endophyte free *C. stoebe*. Instead, when *S. comata* was grown in competition with endophyte-free *C.* stoebe and endophyte-infected *C. stoebe*, there was no difference in the amount of suppression of *S. comata* by *C. stoebe*. Therefore, the enhanced competitive effect of *C. stoebe* by fungal endophytes appears to be driven by changes in *C. stoebe*, or synergistic processes involving *C. stoebe* and endophytes, rather than the infection of the grass species by the endophytes themselves.

A second key finding of our study was that *C. stoebe*, with or without endophytes, was far more suppressed by European grass species than by North American grass species (Fig. 1), a general result that is consistent with other studies (Callaway and Aschehoug 2000, He et al. 2009, Thorpe et al. 2009). However, infection of *C. stoebe* by CID120 eliminated even the weak competitive effect of North American grass species on the invader that was manifest in endophyte-free and CID73-infection treatments, suggesting that CID120 improved the competitive response of *C. stoebe* as well as its competitive effect.

Infection of *C. stoebe* by CID120 also resulted in increased size when grown alone, which may explain the increased competitive effects of CID120-infected *C. stoebe* on North American grass species. However, CID73 did not increase the size of *C. stoebe* when grown alone yet CID73-infected *C. stoebe* had much stronger competitive effects on North American

grass species than endophyte-free *C. stoebe.* In addition, *C. stoebe* did not significantly differ in size when grown in competition with North American grass species regardless of endophyte treatment (Appendix, Table 1), further suggesting that the effects of fungal endophytes on competition are derived from something other than increasing the size of *C. stoebe*.

There is little information in the literature for how fungal endophytes might increase plant size or influence plant competitive ability in the absence of herbivory, but Rodriquez et al. (2009) reports a number of Class 2 endophytes that increase the root or shoot biomass of their hosts. Endophytes can increase plant defenses against herbivores through the production of alkaloids, which can indirectly increase competitive outcomes through herbivore preference for the less defended competitor (Clay et al. 1993). Fungal endophytes can also alter soil microbial communities (Rudgers and Orr 2009), and this might provide indirect competitive advantages. However, we sterilized all substrates, thus the only biota that were in the pots were the experimentally added endophytes and any organisms that colonized the pots during the experiment. To our knowledge, because we eliminated soil biota and herbivores, our results for *C. stoebe* are the first to demonstrate that endophytes can be a *direct* cause of increased competitive ability, rather than indirect. Because we worked with an invasive species and fungal mutualists for which biogeographical information is scarce, we limited our study to greenhouse experiments. However, for a better understanding the ecology of this invader-fungus mutualist field studies should be conducted in the non-native and native ranges of *C. stoebe*.

Centaurea stoebe appears to be allelopathic (He et al. 2009, Ridenour and Callaway 2001); however, the allelopathic effects of *C. stoebe* have been highly variable. The fungal endophyte community infecting *C. stoebe* throughout its native and non-native range is very diverse taxonomically, and the proportion of individual plants infected by endophytes varies

dramatically among populations (Shipunov et al. 2008). Thus variation in endophytic infection has a great deal of potential to cause variation in competitive outcomes, and perhaps explain differences among experiments and variation within experiments. Similarly, different endophytic fungi might produce different allelopathic chemicals (Rudgers and Orr 2009, Newcombe et al. 2009) or stimulate different levels of allelochemical production.

The biogeographic native ranges of the fungal isolates (Shipunov et al. 2008) is not clear, but the idea that *C. stoebe* may have picked up novel endophytic "weapons" in North America or imported an important novel weapon when it was introduced suggests important future questions. However, even endophyte-free *C. stoebe* were far more competitively superior against North American than European species, indicating that fungal endophytes enhanced an extant competitive mechanism or provided another mechanism that operated in an additive fashion.

The mechanism by which fungal endophytes increased *C. stoebe* competitive ability is unknown, but because of the strong biogeographic pattern in competitive outcomes, it would appear that the long term evolutionary histories among the interacting species is important (Callaway and Aschehoug 2000). Global *C. stoebe* populations appear to be "mosaics of uninfected and infected plants" (e.g. Faeth 2002) and vary dramatically in the genetic identity of the endophytic fungi they host. This diverse mixture of endophyte and host genotypic combinations may be maintained by different selective pressures including herbivory, abiotic factors and competition, which in turn can affect the growth, survival or reproductive costs of hosting endophytes such that net interactions can range from mutualism to parasitism (Faeth 2002). We tested only two of the more than 90 endophytes known to be found in the seeds of *C.*

stoebe, but our results suggest that endophytes can change the outcomes of competitive interactions in newly invaded ranges.

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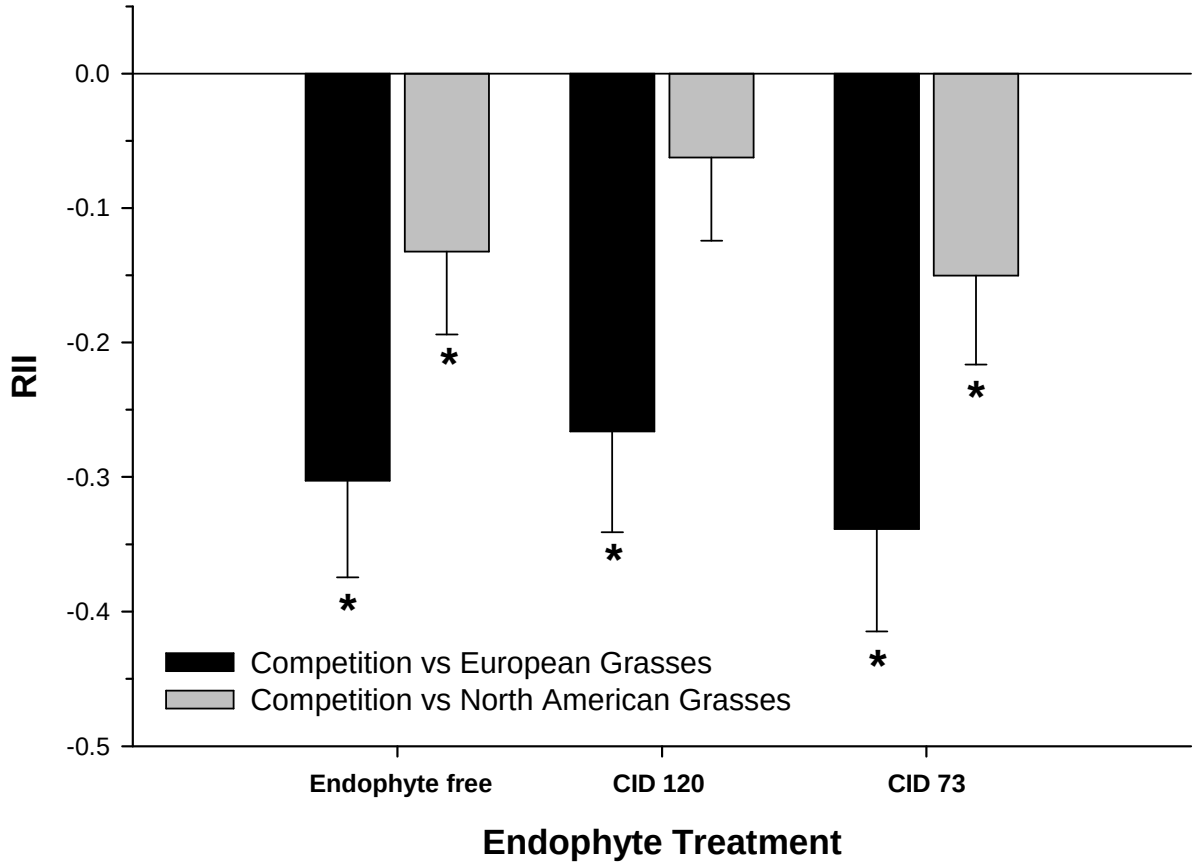
Figure Legends

Figure 1. Response of *C. stoebe* to competition with European and North American grass species when either endophyte-free, infected with the CID120 endophyte, or infected with the CID73 endophyte. Asterisks indicate a significant competitive interaction ($p<0.05$). Error bars show 1 SE.

Figure 2. Competitive effect of *Centaurea stoebe* on European and North American grass species when either endophyte-free, infected with the CID120 endophyte, or infected with the CID73 endophyte. Asterisks indicate a significant competitive interaction ($p<0.05$). Error bars show 1 SE.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RII
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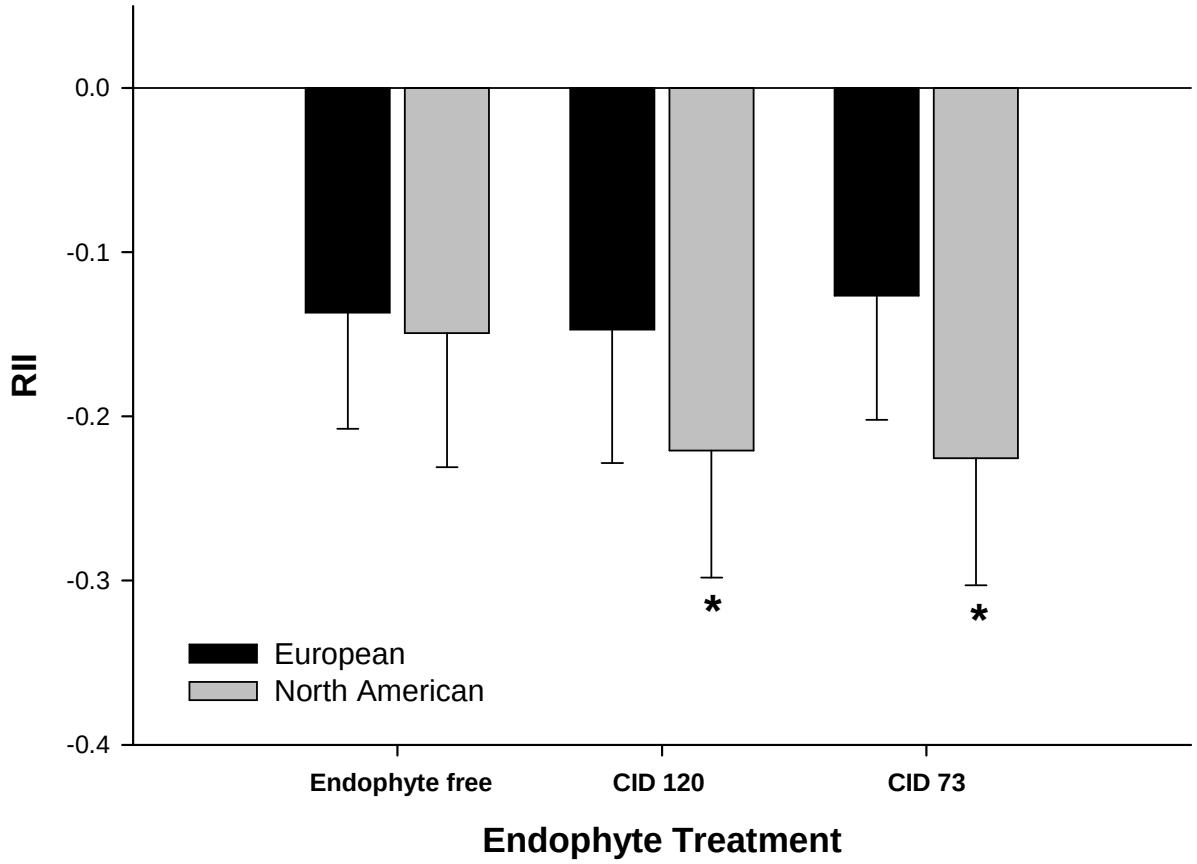
Competition vs European Grasses
Competition vs North American Grasses
Endophyte free
CID 120
CID 73
Endophyte Treatment

Figure 1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CID 73
Endophyte Treatment

Figure 2

Appendix: Table 1, Table 2, Table 3, Figure 1.

	Treatment		
	Endophyte-free	CID120	CID73
Grown alone	3.11 ± 0.19	4.57 ± 0.29	3.35 ± 0.23
versus North American Species			
Festuca idahoensis	2.78 ± 0.24	3.14 ± 0.27	2.34 ± 0.21
Koleria macranthus	2.08 ± 0.19	2.54 ± 0.26	1.60 ± 0.21
Pseudoroegneria spicata	1.72 ± 0.26	1.68 ± 0.14	1.88 ± 0.27
Stipa comata	3.18 ± 0.27	3.64 ± 0.33	3.68 ± 0.37
All species together	2.39 ± 0.15	2.75 ± 0.17	2.30 ± 0.18
versus European Species			
Agropyron repens	2.00 ± 0.34	3.03 ± 0.45	2.74 ± 0.50
Lolium rigidum	0.92 ± 0.22	0.89 ± 0.12	0.77 ± 0.11
Melica ciliata	2.82 ± 0.32	2.85 ± 0.32	2.34 ± 0.36
Poa annua	1.06 ± 0.14	0.94 ± 0.13	0.83 ± 0.09
All species together	1.67 ± 0.18	1.81 ± 0.21	1.54 ± 0.19

Table 1. Biomass (g) and SE for *Centaurea stoebe* either grown alone or in competition against grass species when either endophyte-free or infected with endophyte CID120 or CID73.

	Treatment			
	Alone	Endophyte-free	CID120	CID73
North American Species				
Festuca idahoensis	1.02 ± 0 .12	0.84 ± 0.14	0.63 ± 0.08	0.88 ± 0.18
Koeleria macranthus	3.25 ± 0.44	1.69 ± 0.23	1.60 ± 0.20	2.06 ± 0.29
Pseudoroegneria spicata	4.89 ± 0.45	4.05 ± 0.35	3.71 ± 0.31	2.82 ± 0.48
Stipa comata	1.79 ± 0.22	1.02 ± 0.22	1.01 ± 0.09	0.77 ± 0.11
All species together	2.72 ± 0.29	2.02 ± 0.26	1.74 ± 0.21	1.72 ± 0.21
European Species				
Agropyron repens	5.76 ± 0.56	4.39 ± 0.73	3.06 ± 0.49	3.98 ± 0.88
Lolium rigidum	6.80 ± 0.56	5.60 ± 0.45	5.97 ± 0.79	6.16 ± 0.50
Melica ciliata	1.86 ± 0.24	0.88 ± 0.17	0.88 ± 0.14	1.07 ± 0.17
Poa annua	7.00 ± 0.48	5.85 ± 0.25	5.68 ± 0.49	5.60 ± 0.65
All species together	5.40 ± 0.44	4.10 ± 0.42	4.01 ± 0.46	4.19 ± 0.46

Table 2. Biomass (g) and SE of all grass species grown alone (endophyte-free) or in competition with *Centaurea stoebe* that is either endophyte-free or infected with endophytes CID120 or CID73.

	Treatment		
	Endophyte-free	CID120	CID73
North American Species			
Festuca idahoensis	0.35 ± 0.08	0.31 ± 0.07	0.33 ± 0.04
Koeleria macranthus	0.66 ± 0.08	0.52 ± 0.05	0.65 ± 0.07
Pseudoroegneria spicata	0.97 ± 0.04	0.82 ± 0.08	0.88 ± 0.05
Stipa comata	1.02 ± 0.09	0.61 ± 0.09	0.60 ± 0.10
All species together	0.77 ± 0.06	0.59 ± 0.05	0.62 ± 0.46
European Species			
Agropyron repens	1.34 ± 0.17	1.01 ± 0.05	1.25 ± 0.16
Lolium rigidum	1.11 ± 0.06	1.34 ± 0.09	1.50 ± 0.06
Melica ciliata	0.72 ± 0.06	0.48 ± 0.07	0.42 ± 0.06
Poa annua	1.18 ± 0.08	1.00 ± 0.11	1.08 ± 0.09
All species together	1.06 ± 0.05	0.98 ± 0.07	1.06 ± 0.08

Table 3. Biomass (g) and SE for all grass species grown alone and either endophyte-free or directly infected with endophyte CID120 or CID73.

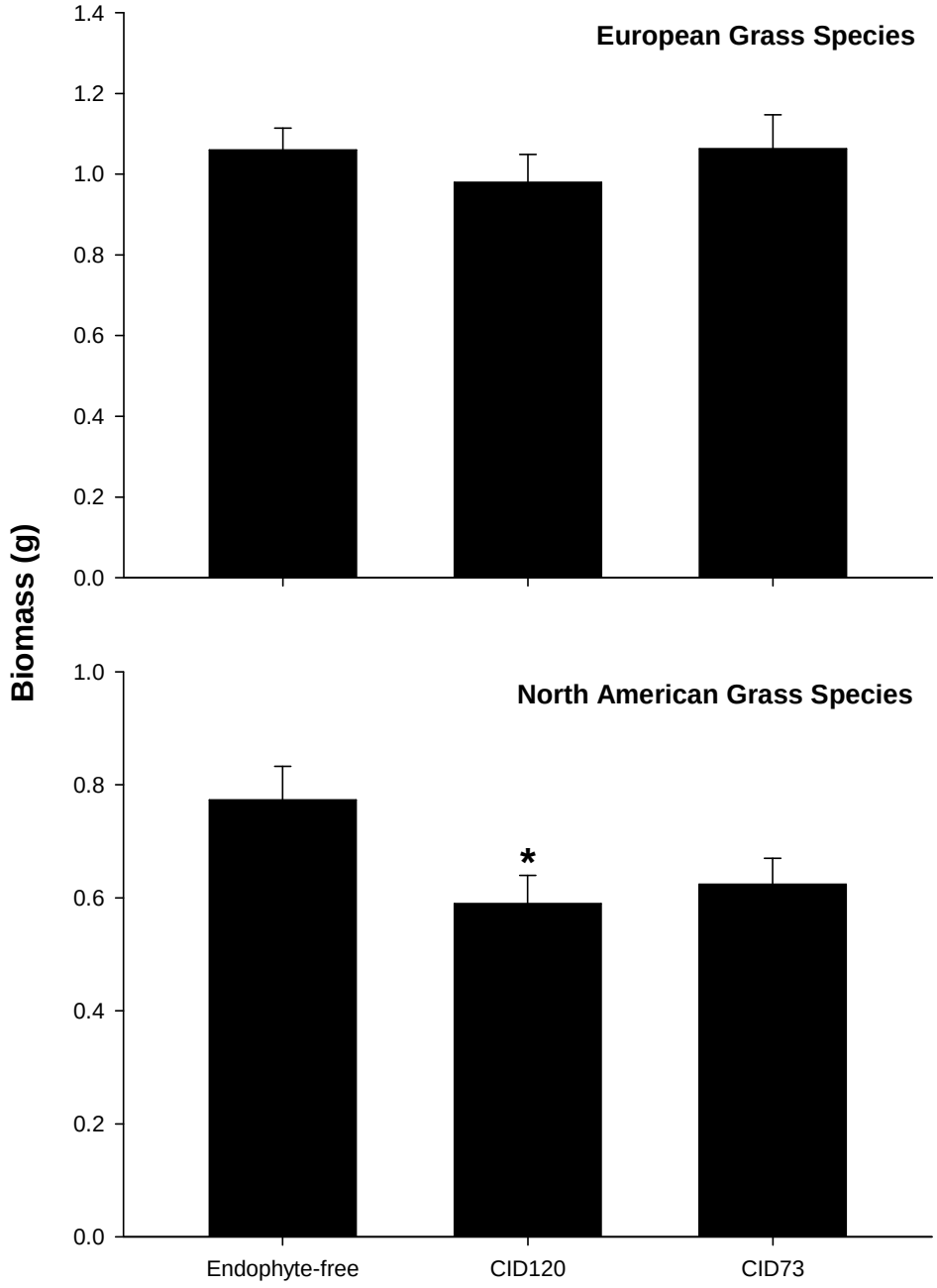

Figure 1. Direct effects of fungal endophytes on the total biomass of European and North American grass species. Bars indicate the dry mass of plants when grown alone and either endophyte-free or infected with either the CID120 endophyte or the CID73 endophyte. Asterisks indicate a significant reduction in biomass versus endophyte-free plants ($p<0.05$). Error bars show 1 SE.