2009

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Boughton, Elizabeth, "Understanding Plant Community Composition In Agricultural Welands: Context Dependent Effects And Plant Interactions" (2009). *Electronic Theses and Dissertations, 2004-2019*. 3819.
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UNDERSTANDING PLANT COMMUNITY COMPOSITION IN AGRICULTURAL WETLANDS: CONTEXT DEPENDENT EFFECTS AND PLANT INTERACTIONS

by

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A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Summer Term
2009

Major Professor: Pedro F. Quintana-Ascencio

ABSTRACT

Community composition results from an integrated combination of random processes, regional habitat spatial structure, local environmental conditions, and species interactions. For example, the outcome of plant interactions can change depending on local environmental conditions such as nutrient availability, land management, or herbivory intensity. In particular, plant interactions may vary between facilitation and competition depending on ecological context, with facilitation expected to be prevalent under stressful conditions. I present the results of four studies that address different aspects of the community assemblage and dynamics emphasizing the synergistic effect of different processes. In the first, I investigated the importance of habitat isolation in determining species richness of wetlands with contrasting land use. The second describes an experiment to test the hypothesis that plant interactions with an unpalatable plant (*Juncus effusus*) would range from competition in ungrazed areas to facilitation in grazed areas and predicted that facilitative effects of *Juncus* would differ among functional groups of beneficiary species and be strongest when grazing was intense. In the third, I examine the community composition impacts of *Juncus* and predicted that *Juncus* would preserve functional diversity in grazed wetlands but that the effects of *Juncus* would vary along a grazing gradient. The fourth study investigated the relative importance of competition and nutrients in determining wetland invasion in two different land use types. Broadly, I demonstrate that the importance of different processes (habitat isolation, nutrient availability, competition/facilitation) to community composition is dependent on ecological conditions. This integrated view of community dynamics is interesting from a purely ecological perspective but also can be applied to understanding ecological problems such as exotic invasions and restoration of disturbed habitats.

For my husband, Raoul, who with unwavering optimism encouraged me to accept new challenges,
For my children, Bryce and Russell, who taught me balance and determination, and
For my parents, Karl and Cindy, who always provided unconditional love and support.

ACKNOWLEDGMENTS

This work would not have been possible without the guidance and knowledge of my mentor and friend, Pedro F. Quintana-Ascencio. I owe much of my growth and confidence as a scientist to Pedro because from the very start he treated me as a colleague and valued my work. Pedro spent long hours in the field with me throughout my research and I never felt alone throughout the whole process. One of the most unique things about Pedro that I will always be grateful for was that he respected my dual role as mother and scientist. He never once doubted my ability to "do it all" and for that I feel deep gratitude.

I also gained from the support and broad perspectives of my committee members. Special thanks to Patrick Bohlen who provided me with valuable logistical support in every aspect of my research. Thanks to Dave Jenkins for his insights on community assembly and for careful editing. Thanks also to John Weishampel for always being knowledgeable about program procedures and for the multivariate analysis class where I was introduced to vector analysis.

The execution of my large *Juncus* experiment would not have been possible without the help of numerous people. Raoul Boughton and Adam Peterson put in the most grueling hours and I am indebted for their help. Others include: K. and C. Ewing, A. Tweel, R. West, S. Saha, D. Jenkins, J. Fauth, C. Brescacin, G. Morgan, A. Feswick, M. Rickey, A. Wieler, R. Morrison, K. Windes, E. Morales-Quintana, J. Hermanson, R. Pickert, M. Rensel, M. Cooper, I. Pfingsten, A. Fraulo, G. Bobeche, O. Page, K. Horn, and B. Stephens.

I also would like to thank the whole family at Archbold Biological Station, including, Eric Menges, Hilary Swain, Carl Weekley, and Roberta Pickert, who always provided insightful comments and encouragement through the years.

Special thanks to my husband, Raoul, who taught me so much about grant writing, scientific writing in general, and was always available as a sounding board to bounce ideas. He gave up his valuable time when I was setting up my field work and has always been a giving partner in both family and work responsibilities.

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CHAPTER 1 INTRODUCTION

Understanding the factors that drive community composition is of broad ecological interest. Ecologists seek to understand why certain species may be present or absent within a community and why composition may change over time and in response to environmental alterations (Lortie et al. 2004). Understanding community assembly has important implications for solving major ecological problems such as invasion by exotic species, restoration of disturbed communities, and the effect of climate change on natural communities (Lortie et al. 2004).

The quest to understand community composition has a long history. In the past, a debate developed over the importance of deterministic processes versus stochastic processes in determining the composition of communities (Grossman et al. 1982). Ecological determinism is the view that community composition is shaped by environmental conditions and species tolerances or niche requirements. Determinism leads to communities that are predictable given knowledge of environmental conditions and species traits. Ecological stochasticity refers to the view that community composition is a product of the order of arrival of propagules which is mainly a random process (Jenkins and Buikema 1998) as well as random extinctions (Hubbell 2001). Increasing evidence shows that a combination of both deterministic and stochastic processes shape community composition and a new view of community assembly is surfacing among ecologists (Chase 2007). The question has changed from which type of process is more important to a framework which considers that the relative importance of different processes is mediated by ecological conditions (Lortie et al. 2004). For example, disturbance may influence the relative importance of community assembly processes (Chase 2007). In harsh environments where disturbance is frequent, species' tolerance limits may drive community composition (i.e.

deterministic processes may dominate) (Chase and Liebold 2003). In less disturbed habitats, dispersal-driven assembly may become more important as species tolerance limits become less vital for survival (i.e. stochastic processes may dominate) (Chave 2004).

In the plant ecology literature, a long standing debate initiated between Frederic Clements and Henry Gleason is similar to the debate on whether deterministic or stochastic processes shape community composition. Clements held the view that individuals within a community are strongly linked by both interactions and niche requirements, and viewed plant communities as following predictable trajectories through time (Clements 1916). Gleason on the other hand emphasized that plant communities are not tightly defined because they change from year to year and many different plant communities share the same species. Gleason went so far as to ask if plant communities are “merely a coincidence” (Gleason 1926). As in the community assembly literature, ecologists are beginning to believe that neither Gleason or Clements views were correct but that community composition can be understood by a melding of random processes, local environmental conditions, and species interactions. This new view of plant communities has been called the integrated community (Figure 1-1; Lortie et al. 2004).

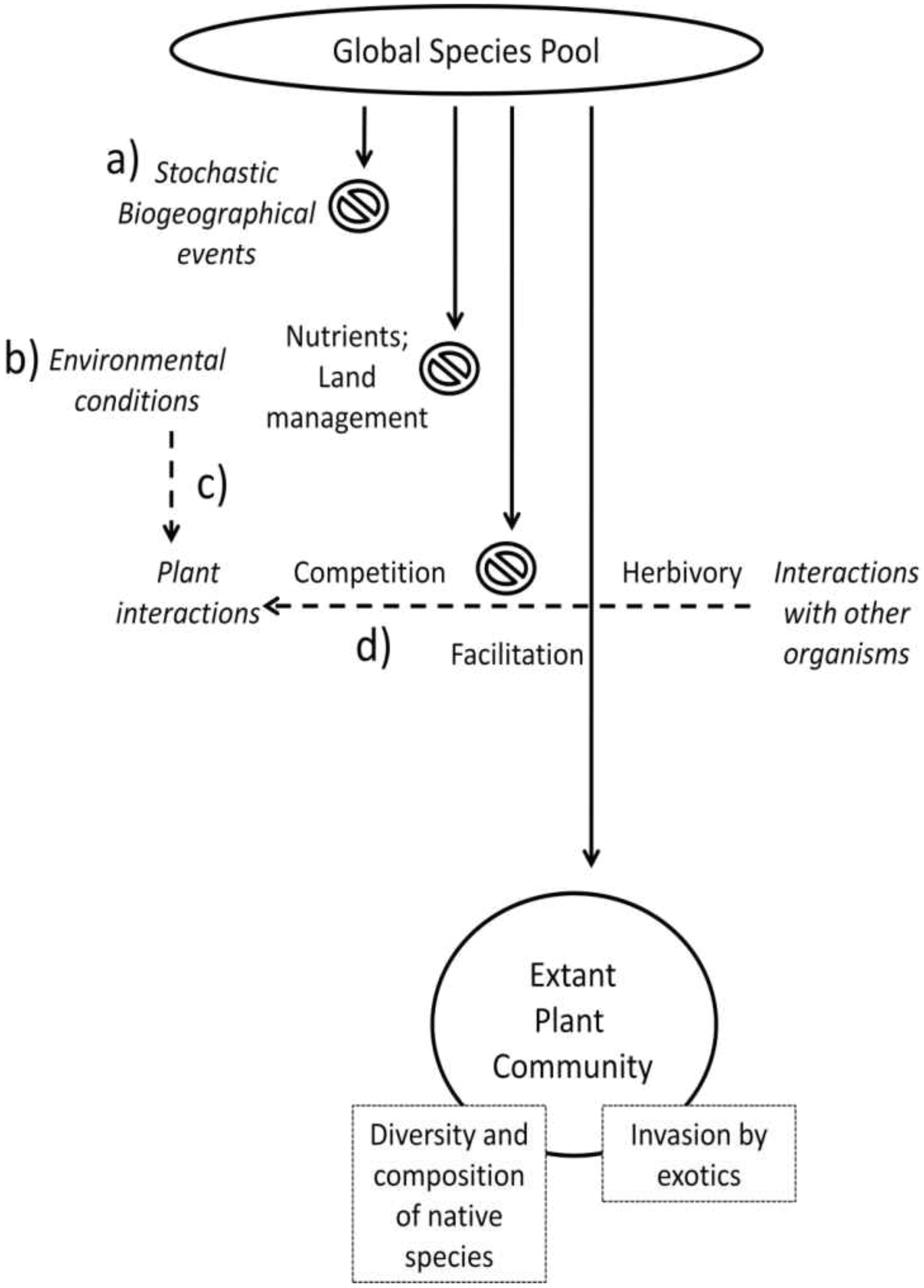

Figure 1-1 The integrated community view (adapted from Lortie et al. 2004). Composition of plant communities is determined by both random and deterministic processes. See text for further details.

In the integrated community view, which combines stochastic and deterministic processes, three basic filters exist that plant species must pass to be present in the extant community: a) random and deterministic biogeographical events (i.e. dispersal, distance to new environment); b) local environmental conditions (i.e. nutrients, management); and c and d) species interactions (i.e.plant interactions and interactions with other organisms (herbivores)). Interestingly, it appears that these filters can also interact or behave synergistically (dotted lines in Figure 1-1). The outcome of plant interactions can change depending on local environmental conditions such as nutrient availability, land management (**c** in Figure 1-1), or herbivory intensity (**d** in Figure 1-1). In particular, plant interactions may vary between facilitation (positive interactions) and competition (negative interactions) depending on ecological context, with facilitation expected to be prevalent under ecologically stressful conditions (Bertness and Callaway 1994). Additionally, in humanized environments, filters may be removed or added to the community assembly process.

My dissertation addresses all three of the basic filters to plant community composition and focuses primarily on the effect of environmental conditions on the outcome of plant interactions (dotted lines **c** and **d** in Figure 1-1). Chapter One (*submitted to Ecography*) addresses the effect of distance between habitats (**a** in Figure 1-1) and soil nutrients (**b** in Figure 1-1) on both native and exotic species richness across two land management types (**b** in Figure 1-1). Chapters Two (*submitted to Ecology*) and Three (*in prep for Plant Ecology*) address the interaction of environmental conditions, plant interactions, and herbivory (**b**, **c**, and **d** in Figure 1-1). Chapter 4 (*in prep for Journal of Vegetation Science*) addresses the effect of environmental conditions and plant interactions on wetland invasibility (**b** and **c** in Figure 1-1). In the broad ecological sense, the primary objective of my dissertation was to investigate the effects of environmental conditions and herbivory on the outcomes of plant interactions. Many questions remain unanswered about how plant interactions are affected by ecological context, including determining the shape (linear or quadratic) of the relationship between ecological stress and facilitative intensity and how species with different strategies respond to plant interactions along stress gradients. In addition, my research addresses applied ecological problems such as exotic invasions and restoration of disturbed habitats; therefore I have focused on both native and exotic plant responses. Understanding native plant composition is important for sustainable management and restoration of ecosystems impacted by human activities. Understanding exotic plant composition has both ecological and economic implications. For example, weed growth in rangelands results in decreased forage and costs ~ $6 billion annually in the USA (Mack et al. 2000) and invasions by exotics are one of the top three causes of biodiversity loss (Mack et al. 2000).

Below, I review the relevant literature and pressing unanswered questions about the nature of plant interactions along ecological gradients that are the main focus of my dissertation in greater detail.

Plant interactions and ecological context

Plants interact in many different ways ranging from negative interactions or competition to positive interactions or facilitation. In the past, ecological researchers tended to fixate on either one or the other of these interactions in their studies (Callaway and Walker 1997). However, it has been suggested that the focus on plant-plant interactions should be flexible, and recognize that interactions between the same set of plants change along spatial and temporal gradients, the outcomes of which are dependent on ecological context (Callaway and Walker 1997, Bertness 1998).

In many studies of plant-plant interactions, there is a tendency to *a priori* identify the interaction as either competitive or facilitative (Callaway and Walker 1997). Competition studies have dominated the literature of plant interactions since the 1960's (Brooker et al. 2008). However, in the late 1980's early 1990's, many researchers suggested that facilitation is ubiquitous in plant communities and not just as a process in primary succession (Connell and Slatyer 1977, Hunter and Aarssen 1988, Bertness and Callaway 1994, Bruno et al. 2003, Brooker et al. 2008). Today, plant ecologists are still working to have facilitation incorporated into ecological theory (Bruno et al. 2003, Michalet et al. 2006). However, a switch from focusing on competition to focusing on facilitation is not the goal of these ecologists (Walker and Callaway 1997, Lortie et al. 2004). Given that multiple studies have been conducted on plant-plant interactions along environmental gradients and have found switches in competition and facilitation between the same species, it seems that understanding how and why plant-plant interactions vary through space and time may be more relevant to our understanding of plant interactions.

Early work focused on how plant interactions change along productivity gradients; a controversial topic that resulted in the Grime-Tilman debate (Goldberg and Novoplansky 1997, Goldberg et al. 1999). The focus of this debate was how intensity of competition changes along productivity gradients in which Grime hypothesized that competition would be unimportant in unproductive environment whereas Tilman postulated that competition would be important in both unproductive and productive areas but that the resource that plants compete for would change in importance (Grime 1973, 1974, Tilman 1988). Further complexity was introduced when Bertness and Callaway 1994 hypothesized that facilitation would be frequent in unproductive areas. Since the Bertness and Callaway model, much empirical work on plant-plant interactions along gradients has been conducted in salt marshes, arid ecosystems, and alpine tundras and these studies have been crucial in demonstrating that not only are positive interactions important in structuring communities but also that plant-plant interactions are variable and the range of outcomes depends on the context of the environment where they occur.

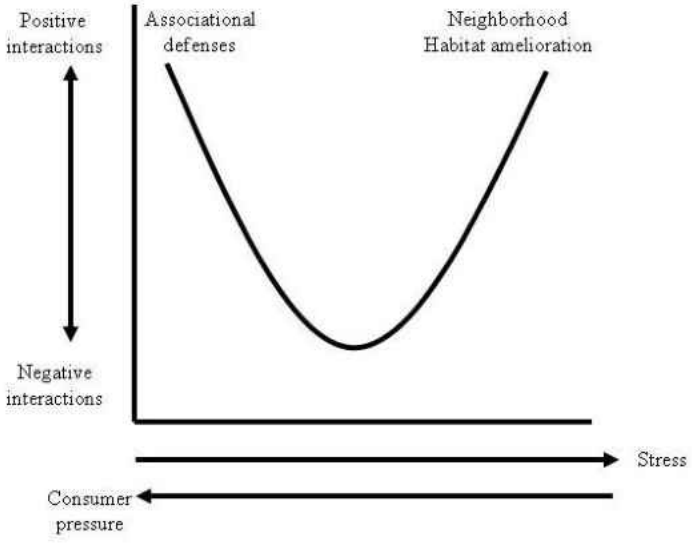

Figure 1-2 The Bertness and Callaway (1994) conceptual model predicting when competition and facilitation will be important in structuring plant communities.

This review will cover three sections corresponding to relevant topics in my research. The first section covers when facilitation and competition are most likely to be important processes structuring plant communities. Secondly, species specific responses to plant interactions will be addressed. Third, the relationship of plant interactions to ecological stress will be covered.

When are facilitation and competition most prevalent?

Bertness and Callaway 1994 proposed that the frequency of positive interactions between plants will increase as physical stress increases (Figure 1-2). This simple conceptual model also predicts that in benign environments with low stress and low consumer pressure, the frequency of negative interactions or competition will be high. Additionally encompassed in the model is the hypothesis that as consumer pressure increases associational resistance (protection from herbivory) will increase in frequency. Of the predictions in this model, most studied are the predictions that correspond to increases in facilitative interactions along abiotic stress gradients. The generality of this model has been questioned although many studies support its predictions (Pennings et al. 2003, Maestre et al. 2005, Michalet et al. 2006). In addition to the problem of actually defining "stress", it has been suggested that differences in types of gradients studied may contribute to contrasting results (Brooker et al. 2008).

Spatial Gradients

Two types of spatial gradients have been studied, non-resource and resource, although the two are often difficult to separate. Non-resource gradients can be characterized by differences in temperature (extreme cold), wind exposure, salinity, herbivory, or disturbance although many stress gradients have multiple stressors acting at once (le Roux and McGeoch 2008). For example in alpine areas, elevation is often used as a surrogate for stress because there are multiple stressors associated with increasing elevation such as cold temperatures and strong wind. Resource gradients include a gradient in which nutrients, water, light, or mycorrhizae are limiting productivity in an ecosystem (Callaway 2007). Vacillations between facilitation and competition will occur when benefits of a facilitator species increasing resources outweighs competitive effects. In many studies, it is often difficult to discern between resource and non-resource gradients. For example, in deserts, often the limiting resource is water, and in these systems shrubs often benefit annuals, cacti, or grasses (Holzapfel et al. 2006). However the benefits of shrubs increasing water availability cannot be easily separated from beneficial shade effects in intense heat. Callaway (2007) suggests that due to complexity of stress gradients, ecologists should measure productivity as a surrogate for stress. If environments are highly stressful, productivity will be limited because producers cannot easily transfer energy to biomass (Grime 1973). Grime (1973) argued that defining stress by productivity may enable comparisons across ecosystems. For the purpose of this review, the focus will be on non-resource gradients because they are most widely studied and because they are more applicable to my dissertation.

Non-Resource Gradients

In the context of non-resource gradients, species that act as benefactors are able to tolerate rough conditions and buffer other species from them (Callaway 2007). For example, in arctic and sub-Antartic areas, where extremely cold temperatures and strong winds prevail, there have been many studies that show a switch from a dominance of competitive interactions at lower elevation environments to a dominance of facilitative interactions at high elevation environments. le Roux and McGeoch (2008) found that spatial associations between species increased with elevation (a surrogate for stress). Spatial associations between species have been found to be associated with positive interactions (Tirado and Pugnaire 2005).

In one of the first studies of plant interactions along an environmental stress gradient, (Wilson and Keddy 1986) found that the importance of diffuse competition decreased as environmental stress increased. They studied competition from the surrounding community along a gradient of wave action which encompassed both disturbance (waves limiting biomass) and resource stress (soils with less nutrients and organic matter). They found that at the harshest end of the gradient, the surrounding community benefitted transplants while as the environment became more benign, competition suppressed transplants. They showed that productivity (standing crop) was positively correlated with diffuse competition, in line with the predictions of both Grime's predictions and the Bertness and Callaway model.

Salinity is another non-resource gradient where variations in plant interactions have been found. Bertness and Yeh (1994) found that when they removed neighbors around focal species, salinity levels increased due to high evaporation levels in direct sun, having a negative impact on the growth and survival of a focal plant. In plots with neighbors, salinity levels were lower due to shading. However, when water was added to all plots, the positive effects of neighbors on the focal plant disappeared due to the dilution of the saline environment.

Variation in herbivory is another type of non-resource gradient but differs from the above non-resource gradients in that it is a biotic factor. Plant interactions can be highly affected in ecosystems with intense herbivory or grazing (Hay 1986, Bertness and Callaway 1994, Callaway et al. 2000). Unpalatable species can provide refuges for more palatable species by shared defense or by associational resistance in which palatable species are either protected by spines or toxins or hidden from predators (McAuliffe 1984, Brown and Ewel 1987, Callaway 2007). Unpalatable plants (benefactors) have been found to provide protective benefits to an array of species (beneficiaries) in many grazed ecosystems ranging from marine areas, deserts, marshes, meadows, to shrublands (McNaughton 1978, Hay 1986, Rousset and Lepart 2000, Rebollo et al. 2002, Rousset and Lepart 2003, Callaway et al. 2005, Smit et al. 2007) with a diversity of consumers (insects (Hamback et al. 2000), crabs (Alberti et al. 2008), fish (Hay 1986), sheep (Callaway et al. 2005, Graff et al. 2007), cattle (Rebollo et al. 2002), beaver (Parker et al. 2007), and deer (Brooker et al. 2006)). However, there have not been many studies that investigate interactions between palatable and unpalatable species along true grazing gradients; most often only two extremes are studied (but see Brooker et al. 2006). In three studies conducted along grazing gradients, two (Graff et al. 2007, Smit et al. 2007) found that facilitative interactions peaked at moderate grazing intensities and declined at higher grazing pressure whereas the other study found that grazing refuges increased in importance as grazing pressure increased (Rebollo et al. 2005). Differences in grazing intensities among studies could have caused apparent discrepancies or could be due to sampling only a portion of the gradient.

Since most research on plant interactions along stress gradients have been conducted along abiotic gradients versus biotic gradients it is unknown whether plant interactions will vary similarly. More work should be conducted along biotic or grazing gradients of stress in both productive and unproductive environments (Michalet et al. 2006).

Species-specific complexity: Traits of beneficiaries matter

In addition to stress gradients affecting outcomes of plant-plant interactions, it has been found that a species' particular strategy (*sensu* Grime 1977) such as competitive ability or stress tolerance plays a role. In 1977, Grime outlined three major strategies in plants: competitive, stress-tolerant, and ruderal (C-S-R). The intensity of disturbance and stress in a plant community determines what group a plant community will be dominated by. For example, in communities with low stress and low disturbance, competitive species dominate, while in communities with high disturbance and low stress, ruderal species dominate. Most plants have intermediate strategies between these three main strategies. It has been hypothesized that these three plant strategies may help predict if certain species will exhibit facilitative responses (Michalet et al. 2006). Ruderal species (*sensu* Grime 1977) may not be able to obtain facilitative effects from benefactor species because they are too sensitive to competition (Michalet et al. 2006). Michalet et al. (2006) also predict that the species most likely to experience strong facilitative effects are competitive species because they are most able to tolerate competitive effects from benefactor species and may be most vulnerable to ecological stressors. Stress-tolerant species are less likely to exhibit strong facilitative effects because they are not highly vulnerable to ecological stressors and may not require amelioration of conditions that benefactors provide.

Thus, species traits must be accounted for when examining plant-plant interactions along gradients (Liancourt et al. 2005, Baraza et al. 2006, Osem et al. 2007, Crain 2008, Eskelinen 2008, Pihlgren and Lennartsson 2008). For example, Crain (2008) found that while *Solidago* seedlings benefited from associational protection from herbivory, seedlings of *Iva*, a stress-tolerant species adapted to saline marsh habitat did not, presumably because competition overrode any effects of associational defenses from the community when *Iva* was planted outside of its typical saline habitat. Likewise, in frequently studied interactions between shrub and annual species in arid systems, potential beneficial effects of shrubs are determined in part by the characteristics of the annuals. Osem et al. 2007 found that only a few annuals benefitted from shrubs because most annuals could not reproduce in shade.

Although many studies have tested species responses to plant interactions along environmental stress gradients, and have found variation in responses based on species' strategies *sensu* Grime (1977), it is not known if species responses will vary in the same way along biotic gradients of stress such as grazing gradients (Michalet et al. 2006). Since protective effects of unpalatable plants inherently encompass competitive interactions, species with low competitive ability (such as ruderals) may not be able to take advantage of associational resistance. Tall generalist species with a wide range of tolerance to environmental conditions may be less susceptible to negative neighbor effects and therefore likely to gain benefits from associational resistance (Eskelinen 2008). Similarly, Pihlgren and Lennartsson (2008) found that tall species (grasses) were more likely than short statured species to obtain protection from grazing from shrubs in semi-native pastures due to the superior ability of grasses to compete for light. Additionally, for facilitation to occur, the focal species must be intolerant to grazing. For example, Callaway et al. 2005 found that palatable species were protected from grazing within unpalatable *Cirsium* sp. and *Veratrum* sp. refuges while less palatable species were not. Palatability is often associated with a species' ability to tolerate grazing with unpalatable species being grazing tolerant. This result emphasizes that competition occurs between the unpalatable plant and potential beneficiaries and for a positive effect of the refuge to occur, grazing must be a negative influence on the beneficiary. From the results of previous studies along grazing gradients, it can be predicted that competitive grazing-intolerant species will most likely benefit from facilitation by associational resistance.

Studies that do not support the SGH: Facilitation is highest at intermediate stress levels

Overall, most empirical studies support the predictions of the model proposed by Bertness and Callaway (1994) and in general show that facilitation is more prevalent in ecologically stressful habitats. However, there has been some controversy over the shape of the relationship between stress and facilitative intensity. Central to the Bertness and Callaway model is that the shape of the relationship between stress and facilitation is linear (Callaway 2007). However, increasingly, studies report that the shape of the relationship between stress and facilitation is quadratic (Michalet et al. 2006, Brooker et al. 2006, Smit et al. 2007, Graff et al. 2007) with facilitative intensity peaking at intermediate levels of stress and then decreasing as stress becomes so severe that benefactor plants no longer ameliorate conditions (Michalet et al. 2006).

The original model of Bertness and Callaway (1994) does not encompass extremely stressful conditions but Michalet et al. (2006) considered how competition/facilitation could shape the hump backed diversity model proposed by Grime (1973) which takes into account extremely stressful conditions. Grime's hypothesis was that competitive exclusion would dominate at high productivity environments causing reduction in diversity while at low productivity environments, stress would limit diversity; thus diversity was predicted to be highest at intermediate productivity sites. Michalet et al. (2006) suggested that facilitation may cause the increase in diversity at intermediate levels of productivity by maintaining competitive species into stressful conditions, but at highly stressful low productivity areas, facilitation declines and environmental conditions determine which species can persist – mainly only the stress-tolerant species.

The prediction which poses that as consumer pressure increases, associational defenses (protection from herbivory) will increase in frequency is less studied (Bertness and Callaway 1994, Michalet et al. 2006). The few experimental studies investigating biotic gradients of stress suggest that protection from herbivory may also wane in importance as herbivory becomes more and more intense (Brooker et al. 2006, Graff et al. 2007, Smit et al. 2007). This loss of associational resistance is due to decreased feeding selectivity in herbivores. Site productivity may also alter the relationship between facilitative interactions and consumer pressure. In lower productivity sites, lower food availability often causes herbivores to be less selective and palatable species are less easily hidden by unpalatable plants (Milchunas and Noy-Meir 2002) (Rebollo et al. 2005, Smit et al. 2007). Therefore, facilitation via associational resistance is thought to be more important in productive sites because increased food availability allows herbivores to feed selectively around unpalatable plants and associational resistance occurs (Milchunas and Noy-Meir 2002, Smit et al. 2006).

Do small scale plant interactions affect community composition?

An extremely important issue to address is that most studies of plant interactions occur between only one or two species and are pairwise experiments in which the performance of a focal plant is assessed both with and without a benefactor species. Usually, these studies do not address the population or community level impacts of such interactions (Brooker et al. 2006, 2008). For changes in competition/facilitation to be important processes in community composition, studies must scale up to determine if small scale interactions result in larger effects. Additionally, if competition/facilitation processes are to be incorporated into restoration and/or management plans large effects must be demonstrated. For example, Gomez-Aparicio et al.

(2004) conducted one of the largest cross-community experiments including 18,000 transplants of 11 possible beneficiary species and 16 different nurse shrubs and found that in general shrubs enhance restoration efforts in Mediterranean forests. On the other hand, Brooker et al. (2006) investigated the protective effects of heather shrubs on Scot's pine seedlings and found that although heather protected seedlings from deer herbivory in the first year after transplanting, this facilitative effect did not translate into a biomass effect because in the second year when pines grew taller than heather, facilitative effects disappeared. Thus, facilitation as a potential restoration tool can vary among habitats and cannot be recommended as a general management tool unless substantial population or community level effects can be demonstrated.

Understanding plant community composition in agricultural wetlands: Context dependent effects and plant interactions

In conclusion, this review identifies several areas in need of research to clarify the relationship between plant interactions and ecological stress gradients. The main questions I address in my dissertation are the following:

1) Most work has been conducted on plant interactions along abiotic stress gradients. Consumer pressure gradients are less frequently studied and it is not known whether patterns observed along abiotic stress gradients will hold on consumer pressure gradients.
2) Do strategies of beneficiaries affect the outcome of plant interactions on consumer pressure gradients? Are facilitative responses most likely for competitive species as models predict?
3) Is the shape of the relationship between facilitation and consumer pressure linear as predicted by Bertness and Callaway (1994) or quadratic as some studies suggest?

4) Does habitat productivity alter the relationship between facilitation and consumer pressure?
5) Do observable plant interactions at the pairwise species scale translate into community composition effects?

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CHAPTER 2 LAND USE AND ISOLATION INTERACT TO AFFECT WETLAND PLANT ASSEMBLAGES

Abstract

Different management regimes imposed on similar habitat types provide opportunities to investigate mechanisms driving community assembly and changes in species composition. We examined the effect of pasture management on vegetation composition in wetlands with varying spatial isolation on a Florida cattle ranch. We hypothesized that increased pasture management intensity would dampen the expected negative effect of wetland isolation on native species richness due to a change from dispersal-driven community assembly to niche-driven assembly by accentuated environmental tolerance. We used native plant richness, exotic plant richness and mean coefficient of conservatism (CC) to assess wetland plant assemblage composition. Sixty wetlands were sampled, stratified by three levels of isolation across two pasture management intensities: semi-native (less intensely managed, mostly native grasses, never fertilized) and agronomically improved (intensely managed, planted with exotic grasses, and fertilized). Improved pasture wetlands had lower native richness and CC scores, and greater total soil phosphorus and exotic species coverage compared to semi-native pasture wetlands. Increased wetland isolation was significantly associated with decreases in native species richness in semi-native pasture wetlands but not in improved pasture wetlands. Additionally, the species-area relationship was stronger in improved wetlands than semi-native wetlands. We suggest that our results indicate that (a) native species transition from dispersal-based community assembly in semi-native pastures to a species-sorting process in the environmentally-stringent "improved" pastures, and (b) recently-introduced exotic species already adapted for ranch conditions are primarily undergoing dispersal-based community assembly. That land-use may alter the relative

importance of assembly processes and that different processes drive native and exotic richness has implications for both ecosystem management and restoration planning.

Introduction

The conversion of land to agricultural or other human use has reduced wetland area and number (Dahl and Johnson 1991) and disrupted natural processes governing species composition and structure (de Blois et al. 2002). Wetlands subject to human activities often have increased nutrients (Bedford et al. 1999) and are smaller and more spatially isolated than wetlands in undisturbed landscapes (Lachance and Lavoie 2004). Because wetlands contribute valued ecosystem services (USEPA 2001), understanding factors that degrade wetlands is essential to maintain and maximize benefits to humans and wildlife. This is especially crucial on agricultural lands where numerous wetlands could potentially lessen negative impacts of agricultural activities such as nutrient export (Tweel and Bohlen 2008).

Vegetation in many wetland types can shift in response to nutrient inputs from land-use in the resident watershed (Ehrenfeld and Schneider 1991, Cohen et al. 2004), including decreases in native species and increases in non-natives and/or weedy species. Eutrophication in wetlands may increase plant biomass (Wisheu et al. 1990) and decrease plant species diversity at high production levels (Wilson and Keddy 1988, Mountford et al. 1993). Eutrophication of wetlands is a large and continuing problem in Florida where wetlands that were historically low in phosphorus (P) occupy a significant proportion of the landscape (Qualls and Richardson 1995, Gathumbi et al. 2005).

Effects of wetland spatial configuration on wetland plant assemblages are less well-known but should interact with land-use effects. The theory of island biogeography (MacArthur and Wilson 1967), is potentially valuable for testing hypotheses about community assembly in hydrologically-isolated wetlands embedded in terrestrial landscapes. The theory predicts that smaller and/or more isolated islands will have fewer species. The species-area relationship has been well-studied in wetlands (Møller and Rørdam 1985, Lopez et al. 2001, Matthews et al. 2005, Houlahan et al. 2006) but effects of isolation on wetland plant species richness remain unclear. Some studies reported negative isolation effects on species richness (or floristic quality) consistent with island biogeography theory (Lopez et al. 2001, Lopez and Fennessy 2002, Mulhouse and Galatowitsch 2003, Matthews et al. 2005) but others reported no relationship between isolation and wetland plant species richness (Møller and Rørdam 1985, Brose 2001, Wright et al. 2003).

Contrasting results on the effect of isolation on species richness may be due to different techniques for measuring wetland isolation across studies. Commonly used measures of isolation include distance to the nearest wetland or nearest three wetlands; these measures may lack biological relevance (Møller and Rørdam 1985). Isolation measures may be improved by including wetland density and sizes because many species may require stepping stones for dispersal (Møller and Rørdam 1985). Additionally, the effect of wetland isolation on species richness may depend on land-use type, because environmental conditions can influence community assembly (Chase 2007). In harsh environments where disturbance is frequent, species' tolerance limits may drive community composition (Chase and Liebold 2003). In less disturbed habitats, dispersal-driven assembly may become more important as species tolerance limits becomes less vital for survival (Chave 2004). Therefore, wetland isolation may become less significant as land-use management intensifies and local conditions override effects of dispersal limitation.

In this paper we focus on wetland vegetation responses to management of surrounding pastures and wetland isolation on a cattle ranch in Florida. Aquatic plants are central to wetland functions such as nutrient cycling and habitat structure (Bouchard et al. 2007) and are influenced by management regime and spatial configuration (Lopez et al. 2002). We measured native and exotic species richness and mean coefficient of conservatism scores (CC) because we expected that more disturbed wetlands would have higher weedy species richness. Wetlands in Florida ranches present an excellent model for examining the effects of P-based eutrophication on wetland plant assemblages because they are embedded in both highly impacted (improved pastures; high P) and less impacted pastures (semi-native pastures; low P) (Gathumbi et al. 2005). They are also an ideal system for examining landscape-level processes because numerous small wetlands are dispersed throughout areas with different land-use intensity. The main purposes of this study were to determine:

1) how land-use intensity (improved vs semi-native pasture types) influences wetland plant species richness and floristic quality (coefficient of conservatism scores);

2) how wetland size and isolation affect species richness of native and exotic wetland plants, and

3) if the importance of wetland isolation differed between the two land-use intensities.

Specifically, we expect that wetland isolation would predict species richness better in semi-native pasture wetlands than in improved pasture wetlands. Such a result would be consistent with the hypothesis that greater disturbance intensity in improved pastures elevates the importance of niche-based community assembly relative to dispersal-based community assembly in semi-native pastures (Chase and Liebold 2003, Chase 2007).

Methods

Site Description

This study was conducted at the MacArthur Agro-ecology Research Center, a division of Archbold Expeditions, in south central Florida (27°09' N, 81°11' W). The Center is located at Buck Island Ranch, a 4,252-ha commercial cattle ranch with over 600 isolated, seasonal wetlands embedded throughout the property and evenly distributed among intensely managed improved pastures and less intensely managed semi-native pastures (Figure 2-1). Improved pastures are composed primarily of the introduced forage grass, Bahia grass (*Paspalum notatum* Flueggé), are fertilized annually with N, were historically fertilized with P (1960's -1986), and are grazed intensely. Semi-native pastures are composed of a mixture of Bahia grass as well as native grasses (i.e. *Andropogon* spp. L., *Axonopus* spp. P. Beauv., and *Panicum* spp. Schult.), have never been fertilized and are moderately grazed. During 2005-2008, the average annual stocking rate was 0.52 cows/ha in improved pastures and 0.28 cows/ha in semi-native pastures. Cattle use wetlands for forage, drinking water, and cooling and can spend considerable time in wetlands on hot days. Because improved pastures are grazed more heavily than semi-native pastures, it is possible that cows may aid some wetland plants in dispersing among wetlands;

however, because we do not have detailed records of cow movements among wetlands, this hypothesis is difficult to test.

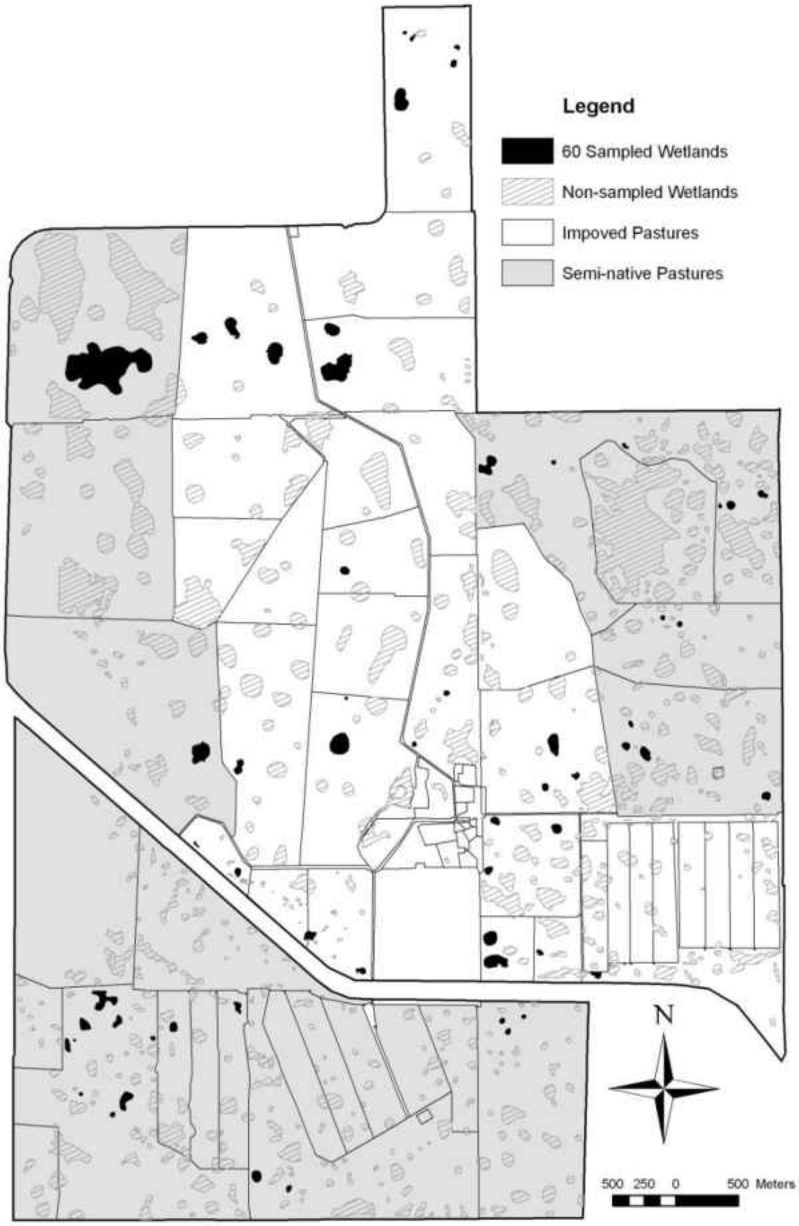

Figure 2-1 Map of the study area on Buck Island Ranch, the location of MAERC.

In addition to grazing intensity differences between pasture types, fertilizer regimes differ. Improved pastures are generally fertilized annually with N (~50 kg*ha^{-1}) and were regularly fertilized with NPK fertilizer (~20 kg P*ha^{-1}) for over two decades until 1987 (Capece et al. 2007). In 1987, P fertilizer was discontinued (Kidder et al. 2002) though N fertilizer continues to be applied. Because the historical and present differences between pasture types were unable to be quantified, we used pasture type as a proxy to incorporate differences in grazing, fertilizer, and soil disturbance effects.

In May-August 2005 we surveyed plant communities in thirty wetlands in each of intensively managed improved pastures and semi-native pastures. In addition to wetland isolation and size, we also evaluated soil phosphorus and soil pH to determine how these factors relate to wetland vegetation characteristics.

Wetland Selection

We randomly selected wetlands, first by pasture type (30 in each type) then by isolation index values (10 in each of three isolation categories per pasture type). Isolation index was calculated using the equation (Hanski and Thomas 1994):

$$S_i = -\sum_{j}^{n} exp(dij) * Aj \qquad \text{eqn (1)}$$

where d_{ij} is distance (m) from focal patch i to j through n, where n=628 (total number of wetlands at MAERC), A_j is the area (ha) of the wetland, and α is a constant for strength of distance and area affects; we used α =1 as a conservative estimate (Quintana-Ascencio and Menges, 1996). Three categories of isolation (high, medium, low) were determined using the distribution of isolation scores calculated within a 5000 m^2 radius. Highly isolated wetlands had higher index scores, low isolation had lower scores, and wetlands with scores in the middle of the distribution were classified as having medium isolation. Because we multiplied by -1 and ln transformed the isolation index, we expected a positive correlation of species richness with isolation index if isolation negatively affects plant species richness.

We were interested in the effect of different isolation measures on results, so we also evaluated isolation using other isolation measures: distance to the nearest wetland and the average distance to the nearest three wetlands. We evaluated scale-dependence of the isolation index (equation 1) and identified all wetlands within circular buffers around each of the sixty wetlands. Buffer radii (m) were: 100, 200, 300, 400, 500, 1000, 1500, 2000, 2500, and 3000. We then calculated isolation (equation 1) for each of the central wetlands at each buffer scale. For this analysis, we used National Wetland Inventory maps to include buffer wetlands on neighboring properties. We calculated distances among wetlands based on centroid-to-centroid (c-c), centroid-to-edge (c-e), and edge-to-edge (e-e) for each of the buffer radii. Centroid-to-centroid distances are easier to calculate in GIS than other measures, but we questioned the biological relevance of this measure since it may underestimate the density of wetlands within a particular buffer, considering that wetlands often have irregular shapes and distance between wetland centroids are farther than distances measured between wetland edges. Analyses were conducted with Arc View GIS 9.0. Log transformations were performed on both nearest-neighbor distances and average distance to the nearest three wetlands for analyses. Additionally, c-c isolation indices calculated within radii of 100-600 m and c-e and e-e isolation index values were log transformed to meet normality requirements. After preliminary analyses, we found that edge-to-edge distance in a radius of 400 m was the best fit for a model relating isolation to species richness, explaining 44% (r^2=0.44, p<0.001) of the variation in species richness in semi-native pasture wetlands. Thus we used this isolation index in all subsequent analyses (Figure 2-2). In comparison, distance to the nearest wetland (improved wetlands: r^2=0.158, p = 0.030; semi-native wetlands: r^2=0.141, p = 0.041) and the average distance to the nearest three wetlands (improved wetlands: r^2=0.099, p = 0.090; semi-native wetlands: r^2=0.074, p = 0.146) explained little or no variation in species richness.

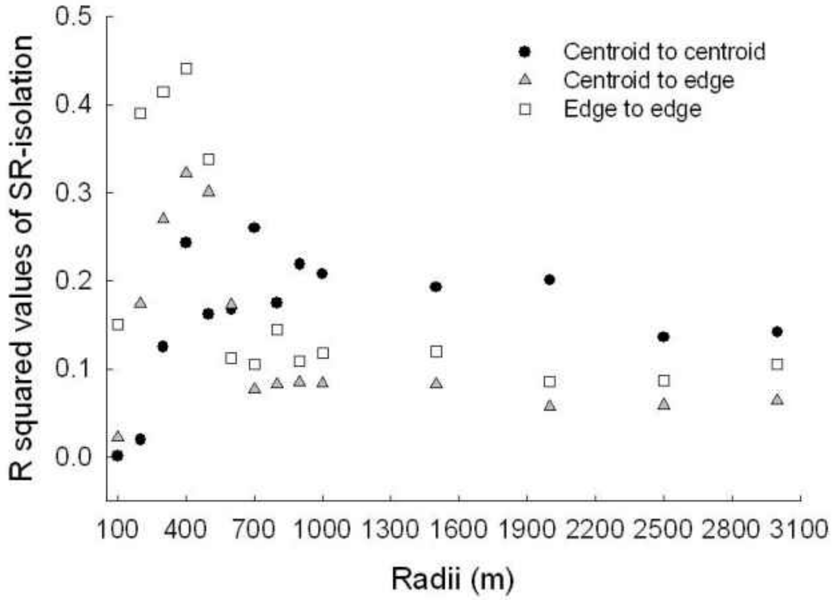

Figure 2-2 R-square values of the regressions between species richness (SR) and the isolation calculated within different sizes of buffers (m) around each sampled wetland. Semi-native wetlands results only.

Vegetation sampling

Within each wetland, vegetation was sampled along two transects, beginning at the center and traversing to the edge of the wetland using randomly selected compass directions. A 1-m^2 quadrat was placed randomly every 5 m along the transect to sample vegetation as percent cover.

Transects were used to ensure that all vegetation zones were sampled. For the 60 sampled wetlands 1,005 1 m^2 plots were surveyed. Species nomenclature followed Wunderlin (1998).

We calculated species-area curves and asymptotic estimates of species richness to assess sampling adequacy using PC-ORD v4. Our sampling effort fit within 95% confidence intervals for bootstrap asymptotic estimates of species richness for 58 of 60 wetlands. Also, results did not differ between bootstrap or observed species richness; we present results from analyses using observed species richness.

We also calculated mean coefficient of conservatism (CC) because native species richness alone may not accurately indicate the "quality" of the wetland plant assemblage if exotic and weedy species are present. The CC score indicates the specificity of a plant species to a particular habitat or tolerance to disturbance intensity (Lopez and Fennessy 2002, Cohen et al. 2004, Matthews et al. 2005). These coefficients range from 0 to 10, where species with a CC of ten exhibit very limited tolerance to disturbance and a high degree of specificity to ecological conditions, and a CC of zero indicates exotic or invasive native taxa (Cohen et al. 2004). We used the average CC score of a wetland plant assemblage as an index of the level of disturbance in the wetland. We did not use the standard floristic quality assessment index (FQAI; Andreas and Lichvar 1995) because it includes species richness and because average CC score is a better predictor of wetland condition (Lopez and Fennessy 2002, Cohen et al. 2004, Matthews et al. 2005). We calculated the average CC of each wetland as:

$$\overline{C}\overline{C}_j = \sum_{i=1}^{n} CC_{ij} / N_j \qquad \text{eqn (2)}$$

where the mean CC_j is the average coefficient of conservatism score for the wetland, and CC_{ij} is the coefficient of conservatism scores of species i through j and N_j is the number of species in the wetland.

Soil sampling and analysis

Three soil samples were taken along one transect in each wetland: at the center of the wetland, transect midpoint, and at the edge. Soil samples were collected with a soil probe to a depth of ~15 cm. Upon return from the field, soil samples were oven dried and then passed through a 2 mm sieve and stored for analysis.

Soil organic matter was measured as loss-on-ignition using 0.5 kg of soil ashed at 450°C for 16 hours. Ash was analyzed for total P (Allen et al. 1974) by extracting with aqua regia (Murphy and Riley 1962). Phosphorus was analyzed for both soil and detritus samples and expressed as soil total P and detrital total P (μg/g). Soil pH was measured in a slurry (5 g soil, 25 mL distilled water) using an Orion pH meter (model 230A). The slurry was shaken and then allowed to incubate for 30 minutes before measuring pH. A microplate spectrophotometer (μQuant Microplate Spectrophotometer, BioTek Instruments, Winooski, Vermont) was used to analyze samples for total P using a modified malachite green method (D'Angelo et al. 2001).

Data Analysis

Species data were converted to a presence-absence matrix consisting of 60 wetlands by 128 species. We used Non-metric Multidimensional Scaling (NMS; PC-ORD v. 4) to ordinate wetland vegetation and compare vegetation composition among pasture-types. The effect of pasture, total P, soil pH, wetland size, isolation, and mean CC/wetland were compared to the ordination with a joint plot and correlations with axis scores. NMS is suitable for heterogeneous data sets with many shared zeros among sampling units (wetlands in our case) and does not assume linear relationships among variables (McCune and Grace 2002). Sørenson distance, a city block distance measure expressed as a proportion of the maximum distance, was selected because it fits heterogeneous vegetation patterns better than other distance measures (e.g. Euclidean distance; McCune and Grace 2002). Coefficient of variation among wetlands and species were 35.9% and 128.4% respectively; no transformations of the matrix were necessary. We selected a three dimensional solution based on the lowest stress. A randomization test of the final stress based on 500 runs showed significant structure in the data (p=0.02). This solution explained 83% of the variation in the data set (r^2= 0.33, 0.17, and 0.33 for Axis 1, 2, and 3 respectively). Multi-response permutation procedures (MRPP) was used to test if wetland plant composition was different between the two pasture-types. Before testing hypotheses, we conducted correlation analyses to determine collinearity of the variables (Table 2-1). Wetland area was log transformed to reach normality for analyses.

Table 2-1. Correlation matrix of the independent variables used in ANCOVA.

	Size	Isolation	pH	Total P
Size	1.0	--	--	--
Isolation	0.39	1.0	--	--
pH	0.19	0.28	1.0	--
Total P	-0.34	-0.44	-0.44	1.0

To better evaluate the quality of the wetland in relationship with community composition, we used both native and exotic species richness as dependent variables in analysis of covariance

(ANCOVAs) to assess the effect of pasture-type and the covariates, total P, soil pH, wetland size and isolation, on vegetation. We used a general linear model assuming the Poisson distribution (appropriate for count data such as species richness). We tested 29 possible models, iteratively fitting models starting with the full factorial model and systematically assessing models with all four-way, three-way, and two-way interactions and then single main effects of each of the five covariates (pasture, pH, total P, wetland isolation, and wetland size). There were 120 different possible model combinations with these five covariates, however, we narrowed our model selection to test only 29 of them because we were specifically interested in the pasture:isolation interaction (Burnham and Anderson 2002) To determine the best model (out of the 29 tested) we used Akaike Information Criterion (AIC). AIC allowed us to determine the most parsimonious model with the most retained information. We calculated model AIC weights which indicate the likelihood of the model, given the data. Model weights range between 0 and 1 and a weight closer to 1 indicates more explanatory power.

All univariate statistical analyses were carried out in R software (v 2.6.1; R Development Core Team 2005).

Table 2-2. Characteristics of wetlands by surrounding upland pasture-type (Mean ± standard deviation). * indicate that the differences in the values are significant, One-way ANOVA, $p \leq 0.05$.

	Improved Pasture Wetlands (N=30)	Semi-Native Pasture Wetlands (N=30)
Area (ha)	1.41 ± 3.15	0.93 ± 1.93
Species Richness*	20.67 ± 1.22	24.37 ± 1.65
Exotic cover (%)*	7.62 ± 5.26	0.69 ± 0.98
Mean CC*	2.46 ± 0.51	3.12 ± 0.52
Soil Total P (µg/g)*	264.14 ± 122.83	195.75 ± 77.00
Soil pH	4.52 ± 0.57	4.51 ± 0.36

Results

Plant assemblages and some soil properties differed between wetlands in improved pastures and in semi-native pastures. Intensely managed improved pasture wetlands had lower species richness (One-way ANOVA, $F_{(1,58)}$=4.87, P=0.031; Table 2-2), CC scores (One-way ANOVA, $F_{(1,58)}$= 24. 484, P< 0.0001; Table 2-2), and higher soil total P and percent cover of exotic species than in less intensively managed semi-native pasture wetlands (Table 2-2). Semi-native pasture wetlands had lower soil P levels, higher CC scores and greater native species richness and were dominated by grasses (*Panicum* spp.), sedges (*Rhynchospora* spp. Michx. (Vahl.)), and emergent vegetation such as *Pontederia cordata* L. and *Sagittaria lancifolia* L.

The NMS ordination indicated two distinct plant communities defined by pasture type (Figure 2-3). MRPP revealed these groups were statistically different (P< 0.0001). Axis 1 of the ordination was associated with a gradient in total phosphorus that increased towards the area of improved pasture wetlands. Axis 2 was associated with pH, though pH was not clearly related to pasture type. Wetland area was weakly associated with axis 3 and isolation was weakly associated with both axis 1 and 2 (Table 2-3). Axis 1 was significantly positively associated to wetland mean coefficient of conservatism scores (Table 2-3); with mean CC scores increasing towards the semi-native pasture wetland cluster (Figure 2-4). Axis 3 was significantly positively related to native species richness (Table 2-3).

Table 2-3. Pearson correlations (r) between variables and ordination axes. * is significant at the 0.05 level and ** is significant at the 0.01 level.

	Axis 1	Axis 2	Axis 3
ln(Size)	0.09	0.26*	0.34**
ln(Isolation)	0.37**	0.38**	0.27*
pH	0.10	0.56**	-0.26*
Total P	-0.52**	-0.33*	0.02
Native Species Richness	0.32*	0.27	0.53**
Mean CC score/wetland	0.78**	-0.04	0.30*

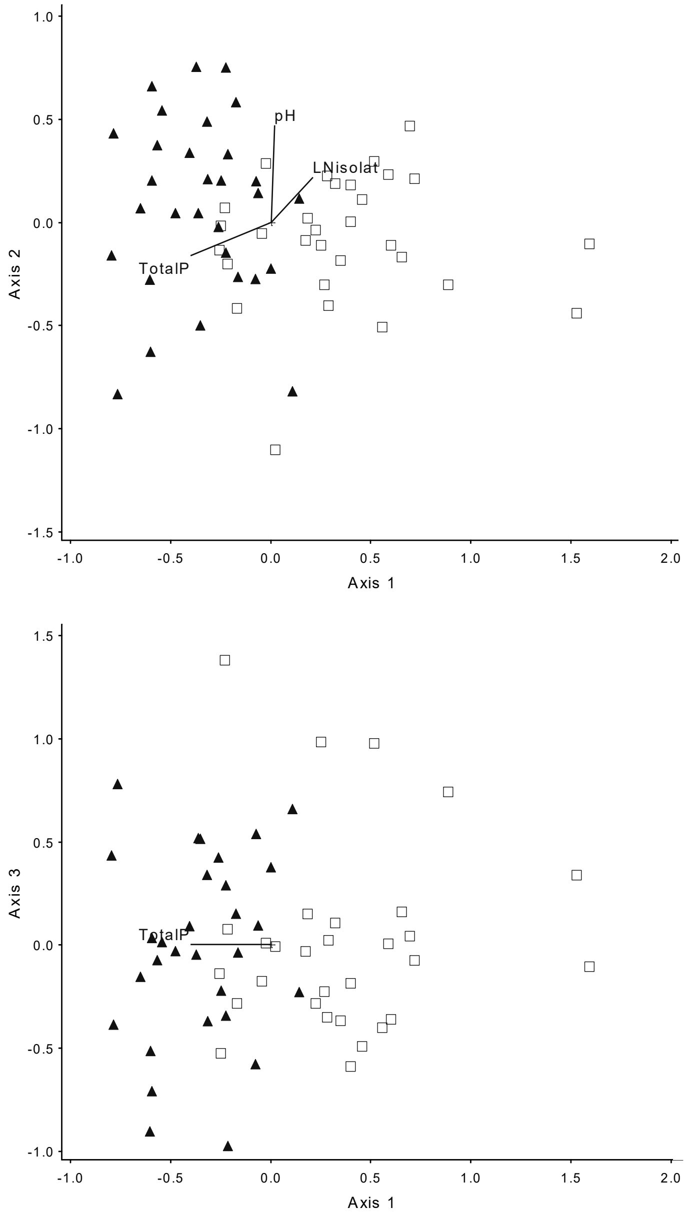

Figure 2-3 NMS ordination of wetlands in plant species space with joint plot of wetland size, isolation, pH, and total P. Radiating lines indicate the strength and direction of correlations between individual variables and the strongest gradients in species composition. Each symbol represents one wetland. Black triangles represent improved pasture wetlands and white squares represent semi-native pasture wetlands.

The best model of native species richness included the main effects of wetland size, isolation, total P, and pasture as well as various two and three-way interactions (Tables 2-4, 2-5). The first five models all included wetland size and isolation, and the second and third models with high information contained total P. In the best model, the coefficient for the main effect of wetland size was significant, with diversity increasing with area (Table 2-5). Pasture-type was also significant, with higher richness in the semi-native pasture wetlands (Table 2-5).

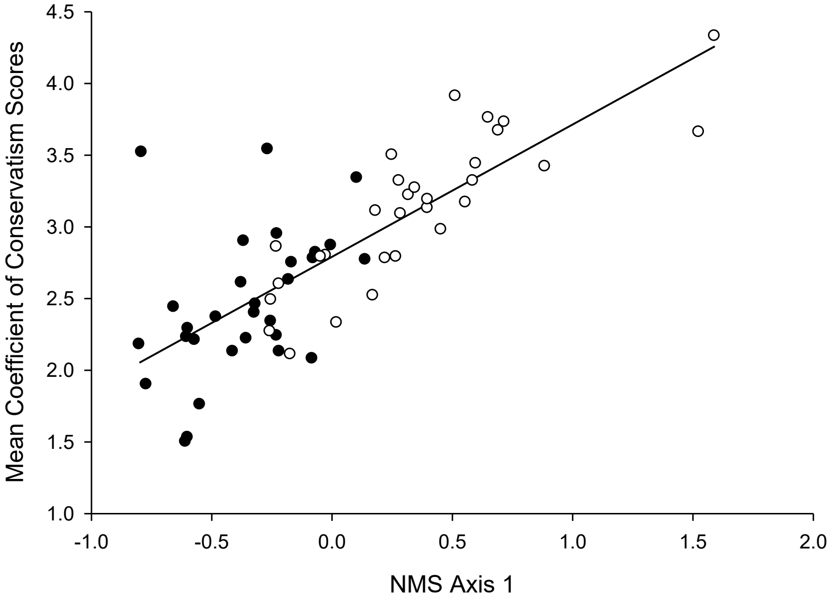

Figure 2-4 NMS Axis 1 is significantly related to mean coefficient of conservatism scores. Each symbol represents one wetland. Black circles represent improved pasture wetlands and white circles represent semi-native pasture wetlands. $F=89.80$, $R^2=0.61$, $p<0.001$.

We found a significant interaction of wetland size and pasture-type caused by a strong species-area relationship in improved pasture wetlands, but not in semi-native pasture wetlands (Figure 2-5). There was a highly significant interaction between pasture-type and isolation with a strong relationship of wetland isolation to native species richness in semi-native pasture wetlands but not in improved pasture wetlands (Figure 2-6a), supporting our hypothesis that land-use intensity alters the relationship between isolation and species richness. The significant three-way interactions (Table 2-5) indicated that the relationship between total P, size, and isolation contributed to variation in native richness. Smaller and more isolated wetlands had greater total P levels (Figure 2-7a,b) and total P was significantly greater in improved pasture wetlands than in semi-native wetlands (Table 2-2).

The best model predicting exotic species richness included only the main effect of wetland isolation, where exotic richness decreased with greater wetland isolation in both pasture types ($z= 2.28$, $p=0.02$; Figure 2-6b).

Table 2-4. ANOVA table of significance of coefficients in the best model predicting native species richness.

	Coeff.	s.e	DF	z	p
ln(Size) (S)	0.40	0.26	1	2.59	**0.009**
ln(Isolation) (I)	-0.19	0.13	1	-1.41	0.16
Total P (T)	-0.002	0.0009	1	-1.66	0.09
Pasture (P)	-1.75	0.57	1	-3.08	**0.002**
S:I	-0.11	0.08	1	-1.43	0.15
S:P	-0.48	0.19	1	-2.55	**0.01**
I:P	0.70	0.20	1	3.46	**0.0005**
S:T	-0.0008	0.0004	1	-1.73	0.08
I:T	0.0008	0.0005	1	1.69	0.09
T:P	0.007	0.003	1	2.50	**0.01**
S:I:T	0.0003	0.0002	1	1.22	0.22
S:T:P	0.002	0.0007	1	2.07	**0.04**
I:T:P	-0.003	0.0009	1	-2.66	**0.008**

Discussion

Pasture intensification (nutrient enrichment and/or intense grazing) affected wetland vegetation by promoting exotic and/or invasive species and decreasing native species richness. Many species native to Florida developed in low soil P environments and are replaced by weedier, more generalist species when P levels increase (Qualls and Richardson 1995, Gathumbi et al. 2005, Tweel and Bohlen 2008). In particular, *Juncus effusus* L. var. *solutus* (Fernald and

Wiegand) becomes dominant in improved pasture wetlands, in turn affecting vegetation structure and distribution of organic matter and soil nutrients (Gathumbi et. al. 2005, Tweel and Bohlen 2008). *Juncus effusus* is unpalatable to cattle and increases with grazing pressure possibly due to release from competition by selective grazing (Tweel and Bohlen 2008).

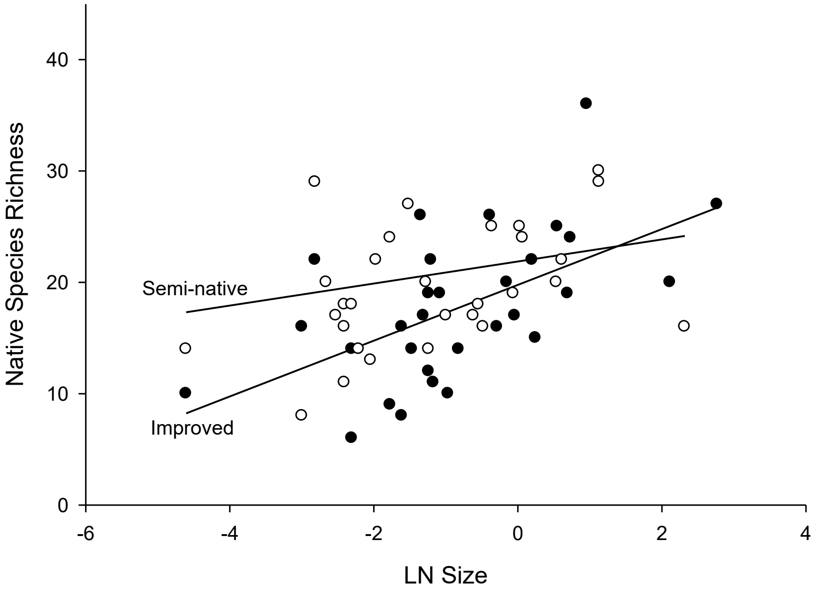

Figure 2-5 Native species richness is significantly positively related to wetland size in improved pasture wetlands (black circles, F=11.62, R^2=0.29, p=0.002), but not in semi-native pasture wetlands (white circles, F=3.95, R^2=0.12, p=0.06).

Semi-native pastures were composed of species that are indicative of the wet savannah/calcareous “Indian Prairie” vegetation type that historically covered the region in which this study took place (Bridges and Orzell 2005). Although ranchlands in Florida are impacted by humans, semi-native pastures are less impacted and provide a refuge for many native plant species (e.g. *Muhlenbergia sericea* Michx. and the endangered *Hypericum edisonianum* (Small) P. Adams and N. Robson), increasing the biodiversity of the Florida landscape (Bridges and Orzell 2005). Unfortunately, native and semi-native ranchland habitats are increasingly being converted to improved pasture or in some cases sold for development.

Table 2-5. Top five models from AIC model comparisons. Log(£)=maximized log-likelihood, K=# of parameters, AIC=Akaike Information Criterion value, Δ_i=difference between the lowest AIC value and AIC_i, w_i=model weight given the data.(:) denotes an interaction term. S: ln(size); I: ln(isolation); T: total Phosphorus; P: Pasture; pH: pH

Model	Log(£)	K	AIC	Δ_i	w_i
Native Species Richness					
S+I+T+P+S:I+S:P+I:P+S:T+I:T+T:P+S:I:T+S:T:P+I:T:P	-179.33	14	**386.7**	0	**0.62**
S+I+T+P+S:I+S:P+S:T+I:T+T:P+I:P+S:I:T+S:I:P+I:T:P+S:I:T:P	-178.77	16	389.5	2.87	0.15
S+I+T+pH+S:I+S: pH +S:T+I:T+T: pH +I: pH +S:I:T+S:I: pH +I:T: pH +S:I:T: pH	-179.13	16	390.3	3.59	0.10
S + I + P +S:I+S:P+I:P+S:I:P	-187.20	8	390.4	3.74	0.09
S + I + T +S:I+S:T+I:T+S:I:T	-188.32	8	392.6	5.97	0.03
Exotic Species Richness					
I	-107.86	2	**219.7**	0	**0.38**
I * P	-107.19	4	222.4	2.66	0.10
P	-109.20	2	222.4	2.68	0.09
I * T	-107.23	4	222.5	2.73	0.09
I * S	-107.50	4	223.0	3.28	0.07

This trend reduces valuable ecosystem services of ranch wetlands, including high biodiversity value, nutrient cycling, recreational and hunting activities, important winter forage for livestock, and habitat for rare wildlife (Swain et al. 2007).

Our finding that native species richness increased with wetland size is in agreement with other observations of a species-area relationship in wetland plants (e.g. Møller and Rørdam 1985, Matthews et al. 2005). However, the species-isolation relationship in wetland plants has been less clear, possibly because different measures of isolation have been used among studies. Møller and Rørdam (1985) recommended that isolation measures should integrate size and number of neighboring patches, topography, and other factors. We found that isolation effects were most apparent when more sophisticated measures of isolation were used and when distances were calculated between wetland edges rather than between wetland centroids (Figure 2-1). Distances between wetland centroids are farther than distances between wetland edges and do not realistically portray the amount of terrestrial habitat wetland plant propagules must traverse, especially because many wetland plants occupy wetland edges rather than deeper centers. Other commonly-used isolation measures (i.e., distance to the nearest wetland or average distance between the nearest three wetlands) were relatively poor measures of isolation and may not apply well to species that rely on habitat patches as stepping stones or whose dispersal vectors (e.g. waterfowl) are sensitive to habitat patch aggregation (Møller and Rørdam 1985, Brown and Dinsmore 1986).

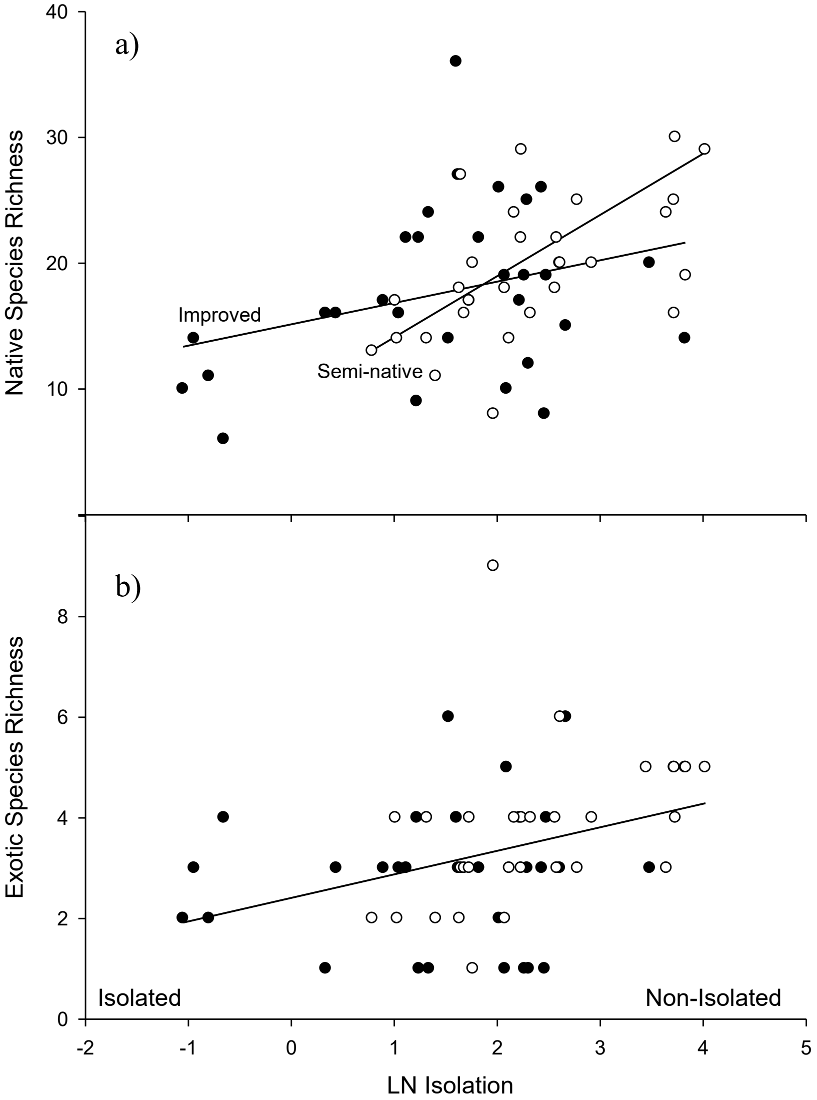

Figure 2-6 Native species richness is significantly related to isolation in semi-native pasture wetlands (white circles, F=22.05, R^2=0.44, p<0.001), but weakly in improved pasture wetlands (black circles, F=4.78, R^2=0.15, p=0.04). b) Exotic species richness is related to isolation in both pasture types (F=8.22, R^2=0.12, p=0.006.)

Our analyses emphasized the importance of spatial scale on the relationship between species richness and isolation (Figure 2-2). We found that isolation had the greatest impact on species richness within radii of 400-700 m in semi-native pastures, roughly comparable to results in Swedish grasslands (Lindborg and Eriksson 2004, Cousins 2006, Cousins et al. 2007). Semi-native pastures are similar to native prairie habitat (Orzell and Bridges 2006), and thus the results of our study may be generalized to other seasonal wetlands embedded in prairies if species have similar dispersal abilities as those of our study.

Native species richness was affected by wetland isolation in semi-native pasture wetlands but weakly related to wetland isolation in improved wetlands. Additionally, the species-area relationship was stronger in improved wetlands than semi-native wetlands. Together, these results suggest that semi-native wetland vegetation reflected persistent dispersal-based assembly processes (Fukami et al. 2005), whereas community assembly was filtered in improved wetland vegetation by extreme environmental conditions (fertilization, grazing, and soil disturbance) to tolerant species. This result suggests that the strength of isolation effects and residual variance in a species-area curve may indicate the relative strengths of dispersal- and niche-based processes in metacommunity assembly (Chase and Liebold 2003).

While pasture intensification (nutrient enrichment and/or intense grazing) predicted how native species assemblages responded to wetland isolation, this was not true for exotic species richness. Exotic species richness decreased with increasing wetland isolation suggesting some ongoing dispersal limitation (i.e., ongoing colonization) for exotics in both pasture-types. Since exotic species are more likely to be tolerant to disturbance, pasture-intensification does not prevent them from becoming established in improved pastures wetlands as it does for some native species.

A caveat to this study is that historical data are not available for these wetlands: we cannot evaluate differences between these wetlands in these pasture types prior to conversion to pastures. Though some differences may be likely due to differences in community assembly mechanisms from slight (1-3 m elevation) topographical differences, wetlands in such close proximity (Figure 2-1) with similar hydroperiods (~6 months) were probably once governed by comparable processes with similar diversity (Kushlan 1990).

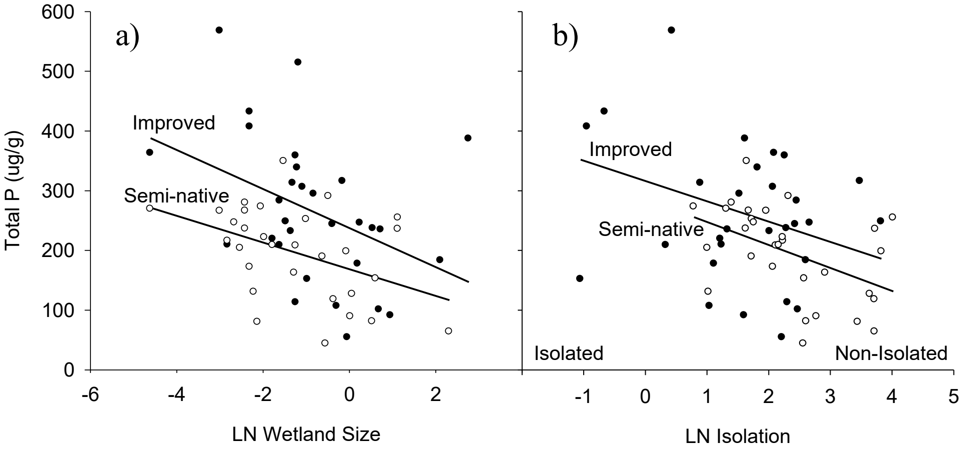

Figure 2-7 Total P declines with increasing wetland size in both pasture-types (improved wetlands=black circles, semi-native wetlands=white circles, F=7.45, R^2=0.11, p=0.008). b) Total phosphorus declines as wetland isolation declines (F=14.67, R^2=0.20, p<0.001.)

In summary, more intensive land-use in pastures and increased soil phosphorus was associated with declines in native plant species richness and coefficient of conservatism scores. Isolation affected native plant composition in wetlands embedded within semi-native pastures, but isolation was less important to native species richness than the effects of harsh conditions in wetlands embedded within intensively-managed pastures. Exotic species richness was only affected by wetland isolation, suggesting exotic colonization of wetlands continues. We suggest that our results indicate that (a) native species transition from dispersal-based community assembly in semi-native pastures to a species-sorting process in the environmentally-stringent "improved" pastures, and (b) recently-introduced exotic species already sorted for ranch conditions are primarily undergoing dispersal-based community assembly. That land-use may alter the relative importance of assembly processes and that different processes drive native and exotic richness has implications for both ecosystem management and restoration planning.

Acknowledgements

We thank Jenna Smith and Adam Peterson for help with lab and field work. Marina Morales-Hernandez contributed valuable GIS and GPS ground-truthing of wetlands. E. H. Boughton was supported in part as a graduate research intern by Archbold Biological Station and in part by the United States Environmental Protection Agency (EPA) under the Science to Achieve Results (STAR) Graduate Fellowship Program.

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CHAPTER 3 DIFFERENTIAL FACILITATIVE AND COMPETITIVE EFFECTS OF A DOMINANT MACROPHYTE ON NATIVE AND NON-NATIVE GRASSES AND FORBS IN GRAZED SUBTROPICAL WETLANDS

Abstract

Plant-plant interactions fluctuate between competition and facilitation depending upon ecological conditions and species traits. Along gradients of high consumer pressure or stress, facilitative interactions are expected to increase in frequency via associational defenses or amelioration. However, because species involved in the interaction vary in their tolerance to negative conditions provided by the benefactor, competitive species may be more likely to take advantage of facilitation than for example, ruderal species. Additionally, at high levels of stress, benefactor species may become less effective at ameliorating conditions, although this has been less studied along gradients of consumer pressure. We used grazed wetlands to investigate interactions between a dominant unpalatable plant, *Juncus effusus* L., and four potential beneficiary species: two species each of grasses and forbs, including one native and one non-native species of each. We hypothesized that plant interactions with *Juncus* would range from competition in ungrazed areas to facilitation in grazed areas. When grazing was intense, we predicted that facilitative effects of *Juncus* would differ among functional groups of beneficiary species. To examine these predictions, we transplanted potential beneficiary species into 1 m^2 plots either with or without *Juncus* and with or without grazing in wetlands experiencing a range of grazing intensities. In grazed plots, facilitation occurred with increased survival for three of four species and increased biomass for the two grass species when *Juncus* was present. The native forb did not obtain grazing refuge from *Juncus* and benefited by experimental clipping of the surrounding community, suggesting that it is a poor competitor. When grazing was removed, *Juncus* had negative effects on survival and biomass for all species except the non-native forb. Facilitative effects of *Juncus* varied in magnitude among species, depending on species' competitive ability. As the first study of plant-plant interactions along a biotic gradient in a productive subtropical system, we found that nutrient availability may alter facilitative responses across grazing gradients, possibly through effects on productivity. In lower nutrient wetlands, we found that facilitation decreased as grazing intensity increased, while in higher nutrient wetlands, facilitation remained constant across the grazing gradient.

Keywords: associational resistance; grazing refuge; herbivory; Juncus effusus*; indirect facilitation; plant-plant interactions; rangeland*

Introduction

Plant interactions result in a range of outcomes that vary between competition and facilitation. Competition and facilitation may occur simultaneously and the balance may be tipped one way or another depending on environmental stress or consumer pressure (Bertness and Callaway 1994, Callaway and Walker 1997, Holmgren et al. 1997, Brooker and Callaghan 1998, Smit et al. 2007, Crain 2008). Thus, it is important to recognize that interactions between the same set of plants may change along spatial and temporal gradients, the outcomes of which are dependent on ecological context (Grime 1977, Callaway and Walker 1997, Bertness 1998). Understanding how and why plant-plant interactions vary through space and time may be relevant to addressing pressing ecological problems, such as predicting plant species responses to climate change or developing effective restoration of ecological communities.

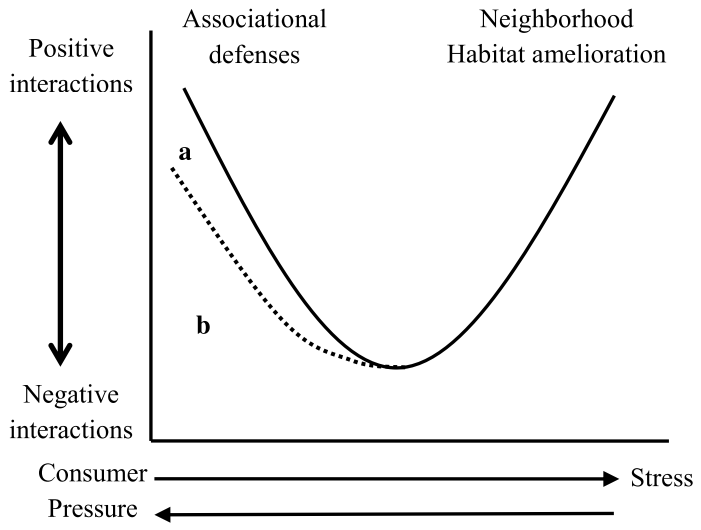

Figure 3-1 The conceptual model proposed by Bertness and Callaway 1994. The dotted lines depict the range of species responses to associational defenses as a function of their life history strategy. (a) grazing-intolerant competitive species (b) ruderal species. See Discussion for more details.

The stress gradient hypothesis (SGH), which arose from the conceptual model proposed by Bertness and Callaway (1994), hypothesizes that the frequency of positive interactions between plants will increase as physical stress increases (Figure 3-1). This hypothesis has been studied extensively and although generally supported, it has been found that when extreme levels of stress are present, the frequency of positive interactions may gradually decrease as benefactor species no longer ameliorate conditions (Michalet et al. 2006). However, the hypothesis also poses that as consumer pressure increases, associational defenses (protection from herbivory) will increase in frequency. The few experimental studies investigating biotic gradients of stress suggest that protection from herbivory may also wane in importance as herbivores become increasingly less selective and the effectiveness of the benefactor declines (Graff et al. 2007, Smit et al. 2007).

In addition to stress gradients affecting outcomes of plant-plant interactions, it has been found that a species' particular strategy (*sensu* Grime 1977) such as competitive ability or stress tolerance plays a role. Thus, species traits must be accounted for when examining plant-plant interactions along gradients (Liancourt et al. 2005, Baraza et al. 2006, Osem et al. 2007, Crain 2008, Eskelinen 2008, Pihlgren and Lennartsson 2008). For example, Crain (2008) found that while *Solidago* seedlings benefited from associational protection from herbivory, seedlings of *Iva*, a stress-tolerant species adapted to saline marsh habitat did not, presumably because competition overrode any affects of associational defenses from the community when *Iva* was planted outside of its typical saline habitat. Likewise, in frequently studied interactions between shrub and annual species in arid systems, potential beneficial effects of shrubs are determined in part by the characteristics of the annuals. Osem et al. 2007 found that only a few annuals benefitted from shrubs because most annuals could not reproduce in shade. Although many studies have tested species responses to plant interactions along environmental stress gradients, and have found variation in responses based on species' strategies *sensu* Grime (1977), it is not known if species responses will vary in the same way along biotic gradients of stress (Michalet et al. 2006).

Given that the strategy of beneficiary species can determine whether they can benefit from facilitation, it is possible to make some predictions about which types of functional groups might persist in areas exposed to abiotic or biotic stress. For example, Pihlgren and Lennartsson (2008) found that tall species (grasses) were more likely than short statured species to be protected from grazing by shrubs in semi-native pastures due to the superior ability of grasses to compete for light. Ruderal species (*sensu* Grime 1977) may not be able to obtain facilitative effects from benefactor species because they are sensitive to competition (Michalet et al. 2006). Additionally, non-native species are often good competitors due to traits that allow them to become established in novel communities. Therefore, it is reasonable to predict that some non-native species would be likely to experience facilitative effects from even some of the most competitive benefactor species. In disturbed systems, such as grazed lands, grazing refuge provided by benefactor species may improve restoration efforts, especially vegetation regeneration (Pihlgren and Lennartsson 2008, Uytvanck et al. 2008). However, if highly competitive species such as non-natives are likely to take advantage of facilitation from benefactor species, then this restoration technique could be counterproductive in some cases (Badano et al. 2007, Bulleri et al. 2008).

For grazed systems, it has been hypothesized that facilitation is likely to be most important to community structure when grazing intensity is high but not extreme (Smit et al. 2007). In this scenario, unpalatable species (benefactors) provide protection from herbivory to palatable species (beneficiaries) under high grazing intensity, but become less effective refuges when grazing becomes too intense. Changes in the importance of facilitation have not been extensively examined along grazing gradients (but see Rebollo et al. 2005, Smit et al. 2007, Graff et al. 2007), although unpalatable plants have been found to protect an array of species in grazed ecosystems ranging from marine areas, deserts, marshes, meadows, to shrublands (McNaughton 1978, Hay 1986, Rousset and Lepart 2000, Rebollo et al. 2002, Callaway et al. 2005, Smit et al. 2007) with a diversity of grazers (insects; Hamback et al. 2000, crabs; Alberti et al. 2008, fish; Hay 1986, sheep; Callaway et al. 2005, cattle; Rebollo et al. 2002, beaver; Parker et al. 2007, and deer; Brooker et al. 2006).

In three studies conducted along grazing gradients, two (Graff et al. 2007, Smit et al. 2007) found that facilitative interactions peaked at moderate grazing intensities and declined at higher grazing pressure whereas the other study found that grazing refuges increased in importance as grazing pressure increased (Rebollo et al. 2005). Apparent discrepancies among studies could be due to differences in grazing intensities among studies, differences in sampling breadth along grazing gradients, or variation in methods of estimating grazing intensity.

In this study, we considered the effect of an unpalatable plant on vital rate variation (survival and growth) of plants of different functional groups and origins (native and non-native forbs and grasses) in wetlands embedded in pastures of differing grazing intensity and in ungrazed exclosures. The wetlands were dominated by the unpalatable, tussock-forming species, *Juncus effusus,* with many species co-existing within the *Juncus* tussock, suggesting facilitation. While previous studies used grazing intensity classes, we take a different approach and use realistic levels of grazing on a working cattle ranch. We selected numerous wetlands across an entire ranch to sample a range of grazing intensities across two pasture-types that have been found to differ in nutrient content: semi-native (SNP) and improved (IP) (Steinman et al. 2003). We tested if interactions with *Juncus* varied along the grazing intensity gradient and hypothesized that interactions would range from competition in non-grazed exclosures to facilitation in intensely grazed wetlands. We predicted that facilitative effects of *Juncus* would differ depending on the functional group of the beneficiary species. To account for potential environmental factors that could influence the outcome of our experiment we measured soil nutrients (nitrogen and phosphorous) and organic matter and assessed their effects as covariates.

Methods

Study Site

This study took place at the MacArthur Agro-Ecology Research Center (MAERC), a division of Archbold Expeditions, located in south-central Florida (27°09' N, 81°11' W). MAERC is located at Buck Island Ranch (BIR), a 4252-ha commercial cattle ranch with approximately 630 isolated, mostly small seasonal wetlands embedded throughout the property. Approximately half of the land area of BIR is occupied by intensely managed improved pastures (IPs) and the other half is occupied by less intensely managed semi-native pasture (SNPs). IPs are composed primarily of Bahia grass (*Paspalum notatum* Flueggé) an introduced forage grass, are usually fertilized annually with N (~52 kg ha^{-1}), and were historically fertilized also with P (1960's-1986). SNPs are composed of a mixture of *P. notatum* and native grasses (i.e. *Andropogon* spp. L., *Axonopus* spp. P.Beauv., and *Panicum* spp. Schult.) and have never been fertilized. The climate is subtropical with a mean annual temperature of 22°C, and summer maximums of 33°C. Mean annual precipitation is 1300 mm, of which 69% falls during the wet season (June-October). Cattle ranching is a major land use in Central Florida (40-50% by area), especially in the watershed north of Lake Okeechobee which is a main water source for the Everglades.

Wetlands embedded in pastures are generally small (< 1 ha) and serve as refuges for wetland plants in the drained landscape. In IPs, wetland edges are dominated by the native, *Juncus effusus* L.var. *solutus* Fernald and Wiegand, an unpalatable tussock plant which cattle generally avoid, and centers are dominated by emergent vegetation (e.g. *Pontederia cordata* L.). We observed several plant species growing within *Juncus* tussocks including both native species (*Panicum hemitomon* Schult., *Centella asiatica* (L.) Urb., *Diodia virginiana* L., *Ipomoea* *sagittata* Poir.) and non-native species (*Alternanthera philoxeroides* (Mart.) Griseb. and *Panicum repens* L.). In SNPs, *Juncus* is less abundant, but exists in more disturbed wetlands (i.e. along canals or roads). The presence of *Juncus* depends to some degree on selective cattle grazing; in 5-year grazing exclosures within IPs, *Juncus* declined while *P.hemitomon* and other wetland grasses became dominant (Tweel and Bohlen 2008). In a survey of 40 wetlands, 20 in each pasture-type of similar size (0.32 ha-1.25 ha), we found SNP wetlands have higher diversity, with more than 50 species in some wetlands. These diverse wetlands are dominated by an array of sedges (*Rhynchospora* spp. (Oakes) Fernald and grasses (*Aristida* spp. L. and *Panicum* spp. Schult.), shrubs (*Hypericum* spp. Lam.), and emergent macrophytes (*Sagittaria lancifolia* L. and *P. cordata*). In a separate study, peak standing biomass in grazing exclosures within wetlands was ~ 720±378 g/m^2 in IP wetlands and ~400±185 g/m^2 in SNP wetlands (Bohlen & Quintana-Ascencio, *unpublished data*). In grazed areas peak standing biomass was ~345±172 g/m^2 and ~280±76 g/m^2 in IP and SNP wetlands, respectively. It is clear that wetlands across the ranch vary in many different ways including nutrient content, diversity, and productivity. Because of these differences, we selected eight wetlands randomly in each pasture-type and measured nutrients to attempt to account for these differences.

Grazing intensity varies across the ranch, although IPs usually experience higher grazing pressure than SNPs. For the years of this study (~January 2006-December 2007), average stocking rate was 1.08 cows/ha in IPs and 0.59 cows/ha in SNPs. As IPs and SNPs are subdivided into several smaller pastures by fences, there is a wide range of grazing intensities within the two pasture-types. Within the IPs in our study, grazing pressure ranged from 0.57-1.7 cows/ha and within SNPs, grazing pressure ranged from 0.15-1.12 cows/ha.

Experimental Design

Our experiment was a factorial design with three factors: pasture (improved vs. semi-native), grazing (grazed or ungrazed), and *Juncus* (*Juncus* or no *Juncus*). We randomly selected 16 wetlands stratified by pasture (eight in each). To ensure an evenly distributed sample of the ranch property, we divided the property into eight pie sections and chose one IP wetland and one SNP wetland randomly within each pie slice. Wetlands were chosen for use in the study only if they contained a large population of *Juncus*. At each wetland site, two random directions were chosen from eight possible directions (N, NE, E, SE, S, SW, W, and NW). These directions were used to determine the positioning of two experimental 4 m x 2 m plots within a wetland. We divided each experimental plot into a 2 m x 2 m grazed subplot and a 2 m x 2 m non-grazed exclosure subplot, each of which was further subdivided into four 1 m^2 quadrats for the *Juncus* treatments. Grazing exclosures were constructed from four 2.5-m long sections of galvanized cattle panels attached to steel t-posts with heavy duty wire. These exclosures excluded all large herbivores (i.e. cattle, hogs, and deer) from experimental plots. A total of 32 exclosures were built for the study (2 pasture-types x 8 wetlands x 2 exclosures per wetland). Within each experimental plot, quadrats were assigned randomly as either a *Juncus* or non-*Juncus* treatment. However, since some areas within the plot did not have *Juncus* present, some plots were non-*Juncus* by default (~1/3 of plots). All *Juncus* plants were removed from the non-*Juncus* treatment quadrats using a machete and large clippers. Non-*Juncus* treatments were kept clear of *Juncus* by clipping during subsequent visits until no resprouts were found.

Native and non-native grasses and forbs were collected during February-March 2006 for use in the transplant experiment. We selected four common species: *Panicum hemitomon* (native grass), *Panicum repens* (non-native grass), *Diodia virginiana* (native forb), and

Alternanthera philoxeroides (non-native forb). Eight hundred plants each of the four species were collected from two to three different wetlands on the ranch property. Both non-native species used in this experiment are abundant on Buck Island Ranch. Prior to transplanting we collected each species by digging up plants and placing one ramet into a 12 inch pot along with soil from the collection area. Each individual ramet of each species was given an aluminum tag number with a wire flag. All species were kept in pots and watered daily for three months until July 2006, when transplanting took place.

Within each 1 m^2 quadrat, two individuals each of the four species were planted (8 plants per quadrat x 16 quadrats/wetland =128 transplants/wetland). Because of the extreme disturbance by cattle at some sites, especially in IPs, there was a possibility that all transplants within the grazed areas would be eradicated by cattle; therefore, we tried to minimize the loss of replicates by planting eight of each species in each treatment (grazing with *Juncus*, grazing without *Juncus*, ungrazed with *Juncus*, and ungrazed without *Juncus*) per wetland and averaged their response. Plants were transplanted into 10-cm diameter holes made by pounding a PVC pipe into the ground with a sledge hammer, creating a circular pattern of holes approximately 20 cm apart in each meter square subplot. In *Juncus* treatments, holes were cored so that they were butted up as close as possible to the *Juncus* individual in the subplot. This technique of coring minimized disturbance to the quadrat. A plant was then taken from a pot and excess soil was shaken off until the roots could fit into the cored hole. Plants were planted so that forbs and grasses were alternating to minimize competition between transplants. A colored telephone wire was attached to each plant and maps were made of each of the 256 quadrats to enable transplant location during subsequent visits. The height of all transplants was measured prior to planting and we paired one relatively large plant (≥ median) and one relatively small plant (< median) of each species within the 1 m^2 quadrats to attempt to minimize the confounding effect of initial height. Transplants were not chosen for inclusion in the study if their size was outside the 95% C.I. of the potted population of that species. Individuals of the same species within the same treatment from the same wetland were averaged for analyses to remove pseudoreplication. A total of 2048 transplants were planted (2 pastures x 8 wetlands x 2 subplots x 4 treatments x 2 sub-treatments x 4 species x 2 sub-transplants).

Each of the transplants was measured in November 2006, April 2007, and November 2007 and transplants were harvested in December 2007 (after two growing seasons). We measured survival, height, and number of stems at each census period. Harvested vegetation was divided into shoots and roots and then dried at 70°C for 48 hours. The samples were weighed for biomass determination. Here we present results based on the final evaluation. Belowground biomass was not analyzed due to the difficulty of obtaining all of the roots.

We also conducted a clipping experiment within ungrazed exclosure plots. The clipping treatment was conducted to simulate the effects of biomass removal of the surrounding community on the transplants. Three main consequences of grazing include: direct biomass removal from a target plant, trampling, and removal of surrounding vegetation. We chose to simulate the third grazing effect to determine if this allowed plants to survive in the presence of a strong competitor (*Juncus*). In non-*Juncus* plots we removed aboveground biomass in the plot to 10 cm above the soil, while leaving transplants intact. In *Juncus* plots, aboveground biomass was removed while leaving both transplants and *Juncus* unclipped. This allowed us to compare the interactions between *Juncus* and transplants with and without clipping. Clipping was conducted within the second growing season of the experiment and half of the plots within each exclosure were clipped three times (May, July, and Oct 2007). Clipped biomass was weighed after being oven dried at 70° C for 48 hours.

To quantify differences between pasture-types and effects of treatments on edaphic factors, soil samples were collected in July 2007 (after transplants had been in the experimental treatments for a year), within each of the 256 subplots to analyze organic matter, available phosphorus (P) and available N (NH_4^+ and NO_3^-). One 15-cm core was collected in the middle of each 1-m^2 quadrat. Samples of the same treatment within the same wetland were aggregated for a total of four soil samples/wetland (grazing w/ *Juncus*, grazing w/o *Juncus*, ungrazed w/ *Juncus*, and ungrazed w/o *Juncus*). Fresh soil samples were sieved (2-mm) and refrigerated until analysis. Soil subsamples were dried, weighed, and organic matter levels were determined by loss-on-ignition (450 °C for 16 h). Mehlich-1 extractable P was determined by the dilute double acid method developed by (Mehlich 1953) and modified by (Sims 2000). Ammonium (NH_4^+) was extracted using salicylate (Sims et al. 1995) and nitrate (NO_3^-) was determined using vanadium chloride method (Doane and Horwath 2003). All samples were analyzed in a microplate spectrophotometer (µQuant Microplate Spectrophotometer, BioTek Instruments, Winooski, VT).

To estimate the intensity of cattle grazing in each wetland, we calculated cows/ha for each pasture that contained a study wetland. This was calculated by multiplying the number in the herd by the number of days spent in a study wetland pasture for each event that there were cows in that particular pasture. These numbers were then summed for each study wetland pasture and divided by the area of the pasture in hectares to calculate livestock unit*days/ha.

This number was then divided by 365 days to obtain cows/ha. Cows/ha was calculated for the years of 2006 and 2007 and averaged.

Data Analysis

Statistical analyses were carried out using R software (R Development Core Team, 2007). We used logistic regression to analyze survival (Crawley 2007). We included pasture, *Juncus*, and grazing as main effects, the interactions, and nutrients and initial transplant height as covariates. Since our data did not have enough degrees of freedom to support the use of all four covariates in the models at the same time, we tested models entering one covariate at a time and chose the model with the lowest Akaike Information Criterion (AIC).

We used linear models to analyze growth for each species (LM; Crawley 2007). We included aboveground biomass ln(*x*+1) as dependent variable in an ANCOVA, with pasture, *Juncus*, and grazing as fixed effects, the interactions of the fixed effects, and initial height, phosphorus, NH_4^+, and NO_3^- as covariates. We used AIC to select the best model. If our hypothesis that the nature of interactions with *Juncus* depends on the presence of grazing was supported, we would expect a significant interaction between *Juncus* and grazing treatments (J x G). Plots that had the clipping treatment in the exclosure were included in the above analyses. This is a conservative analysis because clipping was expected to reduce competition within the exclosure. We used separate analyses to examine survival and aboveground biomass in the clipping experiment conducted within non-grazed exclosures.

To investigate the net outcome and intensity of species interactions across pasture-types and grazing treatments, we calculated relative interaction intensity (RII; Armas et al. 2004), where RII= (B_w-B_o/B_w+B_o). B_w is the biomass of the plant with *Juncus* and B_o is the biomass of the plant without *Juncus*. We calculated RII for both ungrazed and grazed plots and unclipped and clipped plots. This index is centered on zero, with positive values indicating facilitation and negative values indicating competition. When calculating RII, we compared the *Juncus* effect on biomass with and without grazing. We used ANOVA to determine how grazing and pasture-type affected RII. To examine the effect of clipping on the plant interactions, we compared the *Juncus* effect on transplant biomass with and without clipping. We expected that clipping away the surrounding community from transplants with *Juncus* would reduce competition and result in a neutral RII compared to non-clipped plots. We used ANOVA to assess the affect of clipping and pasture-type on RII.

To test if species had different facilitative responses to *Juncus* in grazed areas of each pasture-type, we calculated the difference between survival with *Juncus* and survival without *Juncus* (Smit et al. 2007) for each of the sixteen wetlands. We also tested if RII (relative facilitation on biomass) differed among species and pasture-types. For these ANOVAs all species were analyzed together with species and pasture-type as fixed factors. No transformations of the data were necessary. Tukey's HSD post-hoc tests were used to determine differences among species.

To determine how grazing intensity affected RII, we used ANCOVA with pasture-type as an independent variable and cows/ha as a covariate. Species were analyzed together excluding *Diodia* because *Diodia* did not show a facilitative response. RII was expected to increase (greater facilitation) as grazing intensity increased (Bertness and Callaway 1994).

We used linear models (LM; Crawley 2007) to compare available P, NH_4^+, and NO_3^- among pasture-types and after one year of grazing and *Juncus* treatments to quantify environmental characteristics and to determine if *Juncus* created more favorable environmental conditions for transplants; an alternative hypothesis to protection from herbivory as the mechanism of facilitation. Phosphorus and ammonium were divided by grams of organic matter because these two variables were linearly related and transformed as natural logarithm. Nitrate was also transformed as natural logarithm prior to analyses.

Results

Table 3-1. ANOVA table of *Diodia* survival results. NF= native forb; values in bold are considered significant. Dev.= Deviance, Resid. Dev.= Residual deviance, Resid. Df = Residual Df.

	*Diodia (*NF)			
	Df	Dev.	Resid. Df	*P*
Juncus (J)	1	13.13	62	**<0.001**
Grazing (G)	1	**9.95**	**61**	**0.002**
Pasture (P)	1	0.40	60	0.53
J x G	1	0.47	59	0.50
J x P	1	0.21	58	0.64
G x P	1	1.00	57	0.32
J x G x P	1	1.67	56	0.20
Resid. Dev.	63	72.78	56	

Survival

We found strong effects of *Juncus* and grazing on survival of transplants in both pasture-types. *Juncus* had a positive effect on survival for three of the four transplant species (*Alternanthera*, *P. repens*, and *P. hemitomon)* when grazing was present but negative effects when grazing was removed (Tables 3-1-3-3; Fig. 3-2). This interaction of *Juncus* and grazing was significant in both pasture-types. Survival of *Diodia* was inhibited by *Juncus* and there was no significant interaction between *Juncus* and grazing (Table 3-1; Fig.3-2). Pasture-type significantly affected *Alternanthera* survival, with higher survival occurring in IP wetlands (mean±st.dev: 45%±24) compared to SNP wetlands (31%±24; Table 3-2; Fig. 3-2). *P.hemitomon* had higher survival in SNP wetlands (54%±35) compared to IP wetlands (46%±35; Table 3-3). The effect of grazing was negative on survival for all species (Tables 1-3; Fig. 3-2). Different covariates were important to survival among species but inclusion of covariates did not remove any experimental treatment effects (Tables 3-1-3-3).

Table 3-2. ANOVA table of *Alternanthera* survival results. This was the best model identified by AIC and included NO_3. NNF= non-native forb; values in bold are considered significant. Dev.= Deviance, Resid. Dev.= Residual deviance, Resid. Df = Residual Df.

	Alternanthera (NNF)			
	Df	Dev.	Resid. Df	*P*
NO3 (N)	**1**	**7.77**	**62**	**0.01**
Juncus (J)	1	1.03	61	0.31
Grazing (G)	**1**	**17.89**	**60**	**<0.001**
Pasture (P)	**1**	**12.79**	**59**	**<0.001**
N x J	1	0.71	58	0.40
N x G	1	3.12	57	0.08
J x G	**1**	**15.23**	**56**	**<0.001**
N x P	1	0.00	55	0.96
J x P	1	0.01	54	0.94
G x P	1	0.00	53	1.00
N x J x G	1	2.64	52	0.11
N x J x P	1	0.24	51	0.62
N x G x P	1	0.67	50	0.41
J x G x P	1	0.66	49	0.42
N x J x G x P	**1**	**7.07**	**48**	**0.01**
Resid. Dev.	63	83.59	48	

Biomass

Juncus had a positive effect on biomass in grazed areas and a negative effect in ungrazed exclosures for the two grass species but not for the forb species (Tables 3-4,3-5; Fig. 3-3). The lack of a significant interaction between *Juncus* and grazing for *Alternanthera* was due to a negligible effect of *Juncus* in the ungrazed treatments, although *Alternanthera* shows a pattern similar to the response of the two grasses (Fig.3-3). Biomass of *Diodia* was significantly lower with *Juncus* compared to without *Juncus*. Biomass of 3 of the 4 species did not respond to any covariates so covariates were dropped from these analyses (Table 3-5).

Table 3-3. ANOVA table of *P.hemitomon* and *P.repens* survival results. These were the best models identified by AIC and included initial height. NG = native grass, NNG=non-native grass; values in bold are considered significant. Dev.= Deviance, Resid. Dev.= Residual deviance, Resid. Df = Residual Df.

			P. hemitomon (NG)		*P.repens* (NNG)	
	Df	Resid. Df	Dev.	*P*	Dev.	*P*
Initial Ht (I)	1	62	0.00	0.95	8.83	0.003
Juncus (J)	1	61	0.07	0.79	3.58	0.06
Grazing (G)	1	60	**104.7**	**<0.001**	**13.60**	**<0.001**
Pasture (P)	1	59	**5.59**	**0.02**	0.00	0.95
I x J	1	58	2.79	0.10	2.67	0.10
I x G	1	57	**9.85**	**0.002**	0.02	0.90
J x G	1	56	**46.12**	**<0.001**	**27.47**	**<0.001**
I x P	1	55	**8.41**	**0.004**	**5.59**	**0.02**
J x P	1	54	0.19	0.66	0.71	0.40
G x P	1	53	1.03	0.31	**7.10**	**0.01**
I x J x G	1	52	0.01	0.93	0.76	0.38
I x J x P	1	51	1.50	0.22	1.96	0.16
I x G x P	1	50	0.83	0.36	1.22	0.27
J x G x P	1	49	**3.92**	**0.05**	0.45	0.50
I x J x G x P	1	48	0.22	0.64	0.01	0.94
Resid. Dev.	63	48	119.3		170.8	

The effect of grazing strongly reduced aboveground biomass for all four species (Fig.3-3). Within exclosures, all species had lower survival and biomass with *Juncus* except *Alternanthera*. For *Alternanthera*, a significant effect of pasture was found with higher survival and biomass in the IP wetlands (survival:[*D*=8.42, df=1,64, *P*=0.004]; 55.5%±26 surviving in IPs vs. 38%±29 in SNPs; biomass:[*F*=8.52 df=1,64, *P*=0.01]; 1.11g±0.85 in IP vs. 0.35g±0.95 in SNP).

Clipping Experiment

In the clipping experiment that occurred within exclosures, clipping the biomass around transplants had no direct effects on survival and final biomass for any of the transplants, although there were several significant interactions. A significant *Juncus* x clipping interaction was found for *Diodia* survival (*D*=4.20, df=1,64, *P*=0.04) where clipping positively affected survival in non-*Juncus* plots but not in *Juncus* plots (mean percentage of surviving plants±st.dev.: *Juncus* w/clipping: 7.8%±12; *Juncus* w/o clipping: 15.63%±20; non-*Juncus* w/clipping: 34.4%±18; non-*Juncus* w/o clipping: 21.9%±22). A *Juncus* x clipping interaction was also found for *Diodia* biomass, (*F*=6.63, df=1,64, *P*=0.01) caused by a positive effect of clipping on biomass in non-*Juncus* plots (1.14 g ±1.33) but no effect of clipping on biomass with *Juncus* (0.14 g ±0.49).

Clipping interacted with pasture-type and soil P on *P.repens* survival (*D*=4.31, df=1,64, *P*=0.04). This was caused by a negative relationship between survival and soil P in both clipped and non-clipped treatments in IP wetlands, while in SNP wetlands, there was a negative relationship between survival and soil P in non-clipped plots but no relationship between survival and soil P in clipped plots.

A significant *Juncus* x Clipping x Pasture (*D*=5.69, df=1,64, *P*=0.02) interaction was found for *P.hemitomon* survival caused by a significant positive effect of clipping in non-*Juncus* plots within SNP wetlands but not in IP wetlands (mean percentage of surviving plants±st.dev.: non-*Juncus* w/clipping SNP: 100%±0.0; non-*Juncus* w/o clipping SNP: 75%±18.9; non-*Juncus* w/clipping IP: 84.4%±22.3; non-*Juncus* w/o clipping IP: 84.4%±35.2). The clipping results emphasize that the four species differ in their competitive abilities and that competitive intensity may be higher in IP wetlands (in ungrazed plots) perhaps due to higher nutrients and productivity.

Table 3-4. ANOVA table of *Diodia* biomass results. This model had the lowest AIC value compared to all other considered models with other covariates. NF=native forb. Values in bold are considered significant.

		Diodia (NF)		
Source of variation	Df	MS	*F*	*P*
Initial Height (I)	1	**0.88**	**10.64**	**0.002**
Juncus (J)	1	**0.81**	**9.71**	**0.003**
Grazing (G)	1	**0.65**	**7.75**	**0.007**
Pasture (P)	1	0.05	0.59	0.46
I x J	1	0.14	1.67	0.20
I x G	1	0.07	0.87	0.36
J x G	1	0.28	3.35	0.07
I x P	1	**0.40**	**4.79**	**0.03**
J x P	1	0.02	0.28	0.60
G x P	1	0.00	0.00	0.98
I x J x G	1	0.16	1.89	0.18
I x J x P	1	0.26	3.13	0.08
I x G x P	1	0.00	0.06	0.80
J x G x P	1	0.08	0.91	0.35
I x J x G x P	1	**0.53**	**6.38**	**0.02**
Residuals	48	0.08		

Relative Interaction Intensity

In ungrazed treatments, RII was below zero (suggesting competition) while in grazed plots RII was higher than zero (suggesting facilitation) for three of the species (*P. repens*: *P. hemitomon*; *Alternanthera*; Fig. 3-4). For *Diodia*, interactions with *Juncus* were competitive regardless of consumer context (Fig. 3-4). We also compared RII in clipped vs. non-clipped plots within exclosures to determine if clipping away the surrounding community (excluding *Juncus* and the transplants) allowed transplants to better tolerate being next to *Juncus*. Clipping had no effect on RII with *Juncus* for any of the species (*P. repens*: with clipping = -0.46±0.56, without clipping = -0.44±0.47; *P. hemitomon*: with clipping = -0.39±0.48, without clipping = -

0.37±0.52; *Alternanthera*: with clipping = -0.30±0.63, without clipping = 0.01±0.68; *Diodia*: with clipping = -0.72±0.59, without clipping = -0.27±0.87).

When all species were combined into one analysis to determine if their relative facilitative responses in grazed conditions differed for survival and biomass, we found that species exhibited different responses to *Juncus* (Survival: species [*F*=8.55, df=1,64, *P*<0.001]; Biomass: species [*F*=7.58, df=1,64, *P*<0.001]). Relative facilitation on survival was lowest for *Diodia* which significantly differed from the response of both *P. hemitomon* and *Alternanthera*, but not *P. repens*. *P.repens* survival response did not differ from either *P.hemitomon* or *Alternanthera*. RII (relative facilitation on biomass) of species with *Juncus* was similar among all species except for *Diodia*.

Table 3-5. ANOVA table of *Alternanthera*, *P.repens, and P.hemitomon* biomass results. These models without any covariates had the lowest AIC value compared to other considered models with covariates. NNF=non-native forb; NNG=non-native grass; NG=native grass. Values in bold are considered significant.

		Alternanthera (NNF)			*P.repens* (NNG)			*P. hemitomon* (NG)		
	Df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Juncus (J)	1	0.00	0.03	0.87	0.48	2.40	0.13	0.43	0.76	0.39
Grazing (G)	1	**2.70**	**30.34**	**<0.001**	**3.76**	**18.86**	**<0.001**	37.24	65.43	**<0.001**
Pasture (P)	1	**0.36**	**4.03**	**0.05**	0.05	0.24	0.63	0.77	1.35	0.25
J x G	1	0.16	1.76	0.19	**1.80**	**9.00**	**0.004**	7.15	12.56	**<0.001**
J x P	1	0.00	0.00	0.98	0.00	0.01	0.92	0.06	0.11	0.74
G x P	1	**0.36**	**4.01**	**0.05**	0.05	0.25	0.62	0.06	0.10	0.75
J x G x P	1	0.00	0.00	0.96	0.02	0.09	0.78	0.21	0.36	0.55
Residuals	56	0.09			0.20			0.57		

The magnitude of facilitation differed across the grazing intensity gradient but depended on pasture-type. In SNP wetlands, RII decreased with increasing grazing intensity while in IP wetlands, there was no relationship (pasture x cows/ha: *F*=7.80, df=1,47, *P*=0.008; Fig. 3-5) . There was a main effect of pasture in which RII was higher in SNP wetlands (*F*=6.34, df=1,47, *P*=0.02) The same trends were found when analyzing relative facilitation on survival across the grazing intensity gradient. Relative facilitation decreased with increasing grazing intensity in SNP wetlands while there was no trend in IMP wetlands (pasture x cows/ha: *F*=5.07, df=1,47, *P*=0.03) and overall there was higher RII in SNP wetlands (*F*=3.99, df=1,47, *P*=0.05).

Nutrient Differences among Treatments

Differences in nutrients were found among treatments. Wetlands in IPs had higher available phosphorus (*F*=4.0, df=1,64, *P*=0.05) but similar available ammonium and nitrate concentrations compared to SNP wetlands. A significant effect of *Juncus* was found on available nitrate (*F*=6.23, df=1,64, *P*=0.02), with *Juncus* plots having more nitrate than non-*Juncus* plots. However, despite these differences in nutrients between treatments, they did not contribute significantly to the analysis of the experimental *Juncus* and Grazing treatments, although soil P may have played a role in generating the observed pasture-type differences.

Discussion

Our study supports the general idea that facilitation occurs when consumer pressure is high while competition dominates when consumer pressure is low (Bertness and Callaway

1994). However, we found that not all species obtained protection from grazing from *Juncus* presumably because *Juncus* created conditions that were outside the tolerance of some of the species. This result is supportive of the many studies that have shown competition and facilitation occur in unison and that the net outcome is determined by the stronger interaction (Callaway and Walker 1997, Holmgren et al. 1997). In productive systems, such as subtropical wetlands, competition is expected to be the dominant interaction occurring between plants (Tilman 1988, Bertness and Callaway 1994). However, in productive systems with large herbivores, indirect facilitation may increase in importance (Milchunas and Noy-Meir 2002, Callaway et al. 2005).

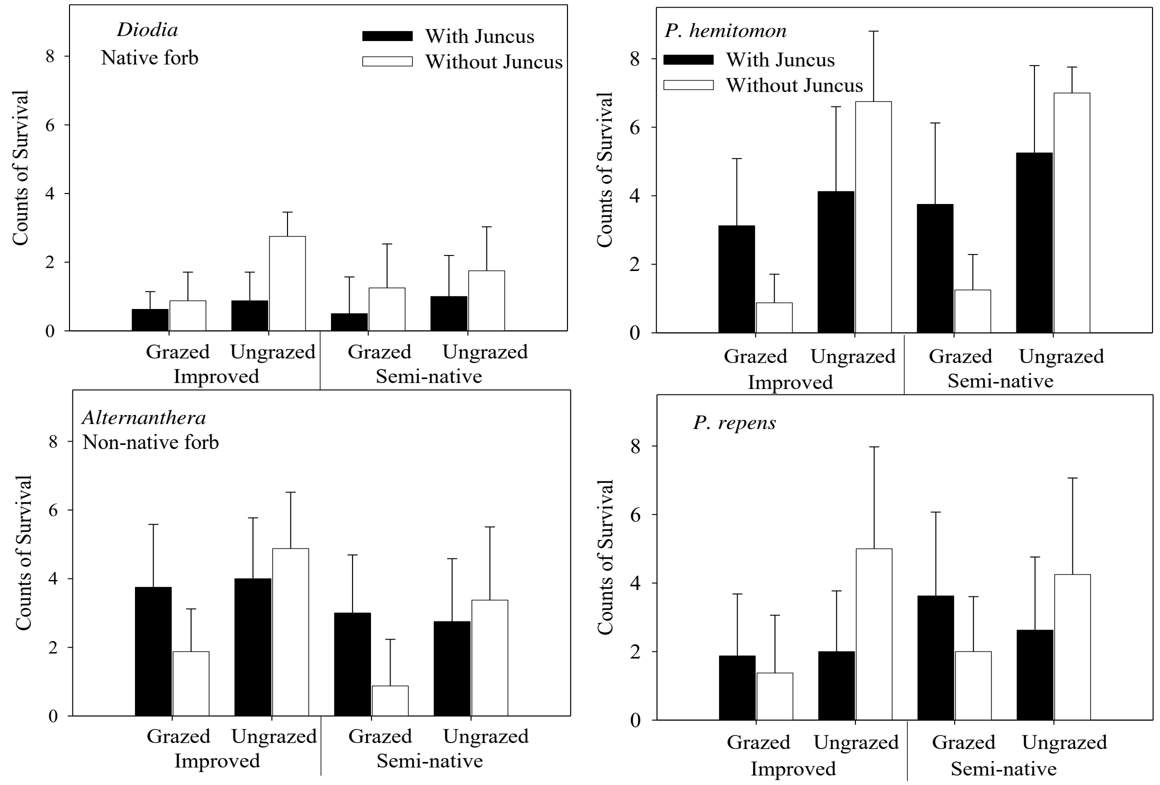

Figure 3-2 *Alternanthera, P.hemitomon*, and *P.repens* had higher survival with *Juncus* in grazed plots indicating facilitation occurred. Positive effects on survival disappeared in ungrazed treatments. *Diodia* had lower survival in all *Juncus* treatments regardless of grazing treatment suggesting strong competitive effects of *Juncus*. Data are mean ± standard deviation.

The species-specific nature of plant-plant interactions has been recognized, and for studies investigating plant-plant interactions along stress gradients it has been recommended to account for species traits (Brooker et al. 2008). Developing an understanding that benefactor species can interact with different species in variable ways could increase our ability to predict plant community composition changes as environmental conditions change. Also, because some investigators have recommended that facilitation be incorporated into restoration plans (Padilla and Pugnaire 2004, Halpern 2007) knowledge of suites of traits that make it more or less likely for a species to be facilitated is needed, especially since highly competitive non-native species may take advantage of facilitation.

In our system, the intensity of interactions with *Juncus* varied between species. *P. hemitomon*, a native grass, and *Alternanthera*, a non-native forb, had the highest facilitative responses to *Juncus* whereas *P. repens*, non-native grass, had moderate facilitative response and *Diodia*, a native forb, had none. One of the hypotheses proposed to explain variability in species' responses to associational defenses focuses on palatability (Baraza et al. 2006). Palatability may in part explain the differences between responses of the two grasses. *P. hemitomon* is known to be a valuable forage grass and its biomass was more strongly depressed by grazing than *P. repens* biomass suggesting that *P. hemitomon* may be more palatable. However, all four of our species have relatively high forage value (Bohlen, *unpublished data*), although forage quality measurements do not necessarily indicate grazing preferences.

Additionally, all species decreased in both survival and biomass in grazed areas indicating that grazing occurred for all species and was a negative influence (Figure 3-3).

An explanation for the differential responses of the forb species to *Juncus* may be related to their competitive abilities and physiological tolerances for shade. Liancourt et al. (2005) found that a facilitative outcome is likely for a species that has both a low tolerance to a particular stress and a strong competitive ability. Both *Alternanthera* and *Diodia* appear to have low tolerance to grazing, however *Diodia* appears to be a poor competitor and *Alternanthera* appears to be a good competitor or highly tolerant to conditions provided by *Juncus*, which may explain their differential facilitative responses. *Juncus effusus* is known to depress species diversity in beaver wetlands due to its ability to produce dense shade (Ervin and Wetzel 2002). *Alternanthera* appears to be shade tolerant as evidenced by the neutral RII in non-grazed treatments and by the lack of difference in *Alternanthera* biomass in both *Juncus* and non-*Juncus* plots in the ungrazed treatments (Figure 3-4). *Diodia* was strongly inhibited by *Juncus* and furthermore was benefitted by clipping. Although *Diodia* had low survival throughout the experiment, we believe that this low survival is indicative of the life history strategy of this species. *Diodia* produces ample seeds, unlike any of the other focal species which mainly reproduce vegetatively (E. Boughton, *personal observation*). It is likely that *Diodia* is a ruderal species (*sensu* Grime 1977) that maintains a large seed bank to take advantage of disturbed open areas where it can be free of competition for light.

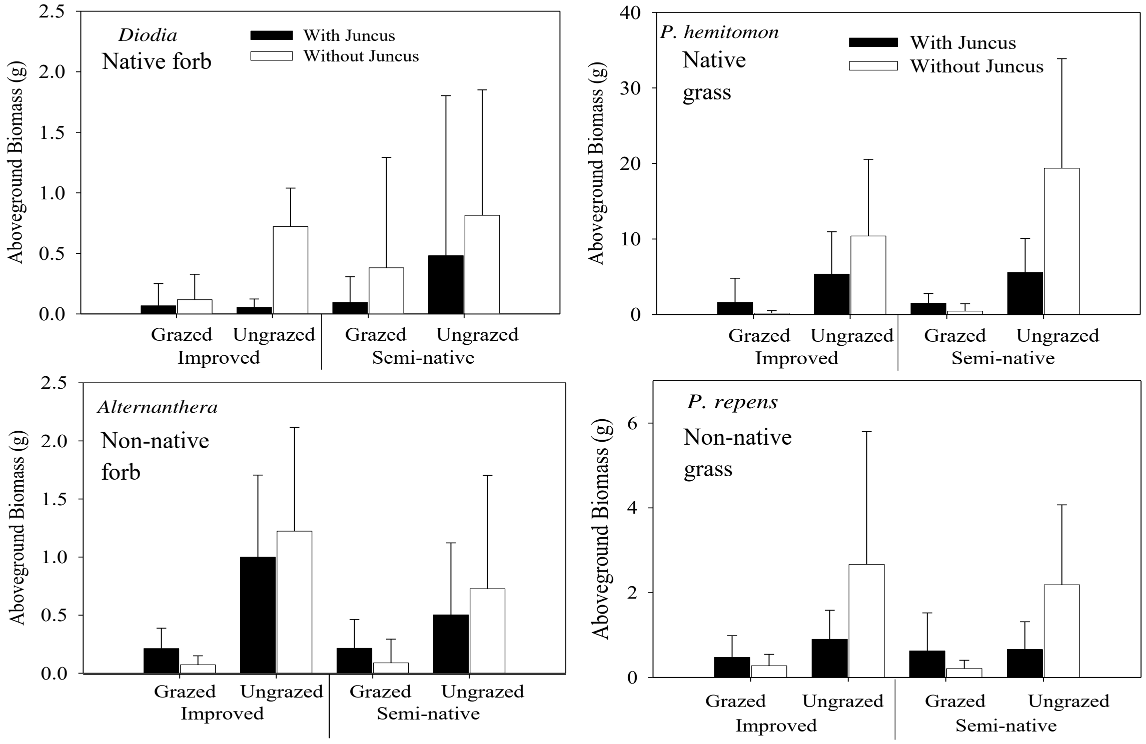

Figure 3-3 Grazing by cattle significantly depressed biomass for all species. In both pasture-types, *Alternanthera*, *P.hemitomon*, and *P.repens* had higher biomass with *Juncus* in grazed plots indicating facilitation occurred. Positive effects on biomass disappeared in ungrazed treatments. Biomass of *Alternanthera* in *Juncus* and non-*Juncus* plots was no different in ungrazed treatments. *Diodia* had lower biomass in all *Juncus* treatments regardless of grazing treatment suggesting strong competitive effects of *Juncus*. Data are mean ± standard deviation.

Our study indicates that broad functional groups based on morphology are not indicative of how a species will respond to plant interactions. Life history characteristics coupled with adaptive strategies (Grime 1977) may be more important for predicting which species will benefit from facilitation. Tewksbury and Lloyd (2001) found that ephemeral species were less likely than perennial species to be facilitated by shrubs in the Sonoran desert. Since ephemeral species are adapted to avoid drought stress and invest in short life spans with heavy flowering these species are less likely to benefit from facilitation because they only grow when water is available. Similarly, our study suggests that a range of responses to plant interactions along biotic stress gradients is possible depending on the life history strategy of beneficiary species, with ruderal species showing little or no facilitative response, and species with higher competitive ability showing a range of responses (Figure 3-1). Alternatively, the four species could have been affected by a drought that occurred in the second growing season of the study and it is possible that *Diodia* was the most drought sensitive species. However, after the first growing season, which was wet, analysis of preliminary data showed that even during this wet period, *Diodia* did not show any signs of benefitting from the presence of *Juncus* in grazed areas (data not shown).

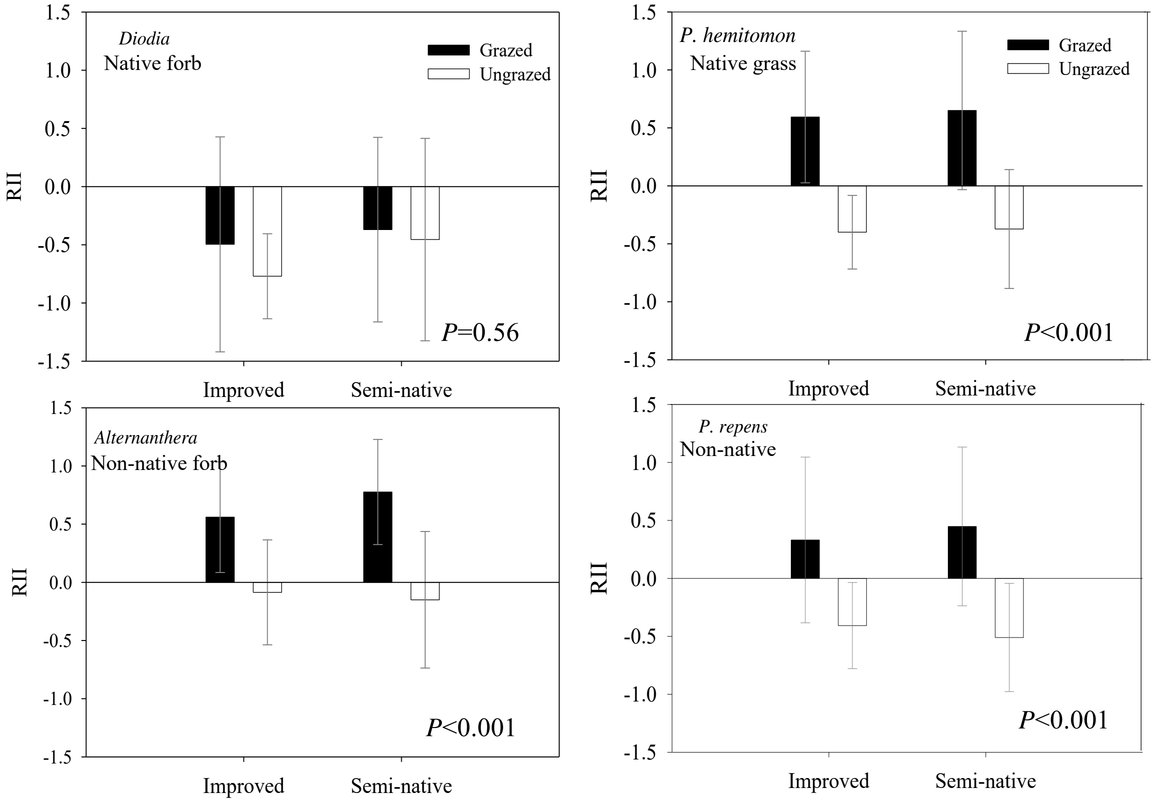

Figure 3-4 All species except *Diodia* showed positive RII values in grazed areas in both pasture-types, indicating facilitation occurred with *Juncus*. Negative RII values in ungrazed areas suggest competition with *Juncus*. *Diodia* had negative RII values with *Juncus* regardless of grazing treatment. *P* values indicate the grazing effect. Data are mean ± standard deviation.

Soil nutrients (P and N) differed between the pasture-types and treatments but including nutrients as covariates in the analyses of survival and biomass did not remove any experimental treatment effects indicating that nutrients did not contribute significantly. For example, even though *Juncus* plots had higher nitrate than non-*Juncus* plots, the only direct effects of *Juncus* were negative. Possible explanations for the higher nitrate in *Juncus* compared to non-*Juncus* plots include oxygen release into the soil or turnover in fine roots (Engelaar et al. 1995, Wiessner et al. 2002, Fornara et al. 2008). A direct positive effect of *Juncus* on either survival or biomass of the transplants would be an alternative hypothesis to protection from grazing in explaining facilitative effects, but we did not find any evidence to support this hypothesis.

General support has been found for the stress gradient hypothesis, which predicts increased frequency in facilitative interactions as productivity decreases (Callaway et al. 2002 and references therein). Few studies have been conducted along biotic gradients of consumer pressure and results are inconclusive as to how plant-plant interactions will behave at higher levels of grazing (Rebollo et al. 2005, Baraza et al. 2006, Graff et al. 2007, Smit et al. 2007). We found that facilitative responses across the grazing gradient differed depending on pasture-type. In low nutrient SNP wetlands, facilitation decreased as grazing intensity increased while there was no difference in facilitative effects across the grazing gradient in IMP wetlands (Figure 3-5). Additionally, higher levels of facilitation were found in SNP wetlands than in IP wetlands. One possible explanation is that because the grazing gradient differed slightly among pastures (0.15-1.1 cows/ha in SNPs compared to 0.5-1.7 cows/ha in IPs) we were not able to observe the peak in facilitation that might have occurred in IP wetlands at lower grazing intensities (<0.5 cows/ha). However, this does not seem likely as RII remained high in IPs even between 1.2 and 1.7 cows/ha (Figure 3-5). Alternatively, plant interaction intensities may differ between the two pasture-types due to nutrient and productivity levels which are higher in IP wetlands. Higher nutrient levels and productivity are usually associated with increased competitive intensity possibly resulting in decreased facilitative intensity in IP wetlands. Another possible explanation is that facilitation quickly declined as grazing intensity increased in SNP wetlands because in these lower nutrient wetlands, cows may have become less selective at a lower grazing intensity level than in IPs due to decreased food availability therefore resulting in a decline in the protective effects of *Juncus* (Smit et al. 2007). In IP wetlands, facilitation may have remained constant across the gradient even at high grazing intensities due to increased food availability in wetlands with higher productivity; therefore cattle remained selective even at high grazing intensities and *Juncus* maintained its protective benefits (Smit et al. 2007).

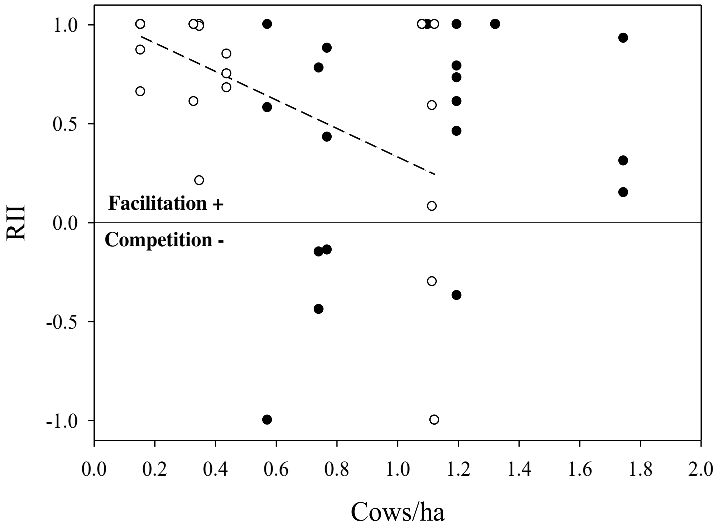

Figure 3-5 Relative Interaction Intensity (RII) with increasing grazing pressure. For three of the studied plant species, RII decreased with grazing intensity in SNP wetlands (white symbols, F=7.2, R^2=0.25, p=0.02), but not in IP wetlands (black symbols, F=1.9, R^2=0.09, p=0.18).

In conclusion, our results suggest that species with a ruderal strategy do not benefit from facilitation along biotic stress gradients while competitive species do. This is similar to how species respond along physical stress gradients (Michalet et al. 2006). As the first study of plant-plant interactions along a biotic gradient in a productive subtropical system, we found that nutrient availability may alter facilitative responses across grazing gradients possibly through effects on productivity. In lower nutrient SNP sites, facilitation via protection from herbivory may decline as grazing intensity increases while in higher nutrient IP sites, although facilitative intensity may be slightly reduced, the importance of facilitation may remain constant across the consumer pressure gradient.

Acknowledgements

We thank the many people whom helped in the set-up of this experiment. We are grateful to G. Lollis, BIR staff, and Archbold Biological Station for valuable logistical support. E.H.B. was supported by an EPA STAR Fellowship. Society of Wetlands Scientists Student Research Grants and The Florida Native Plant Society also provided valuable funding. Additional support was through USDA CSREES National Research Initiatives - Managed Ecosystems Program, Grant 2006-35101-17204).

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CHAPTER 4 REFUGE EFFECTS OF *JUNCUS EFFUSUS* ON WETLAND PLANT COMMUNITIES ACROSS A GRAZING DISTURBANCE GRADIENT

Abstract

Unpalatable plant species often act as biotic refuges by protecting neighboring plants from herbivores. This positive interaction can increase functional diversity in grazed ecosystems by protecting species sensitive to grazing that may otherwise be eradicated. Studies of plant interactions along stress gradients such as grazing intensity have shown contrasting results as to whether positive interactions will increase linearly with stress. Additionally, while many studies investigate pair-wise interactions between benefactors and beneficiaries, few show that these interactions result in community composition effects. We studied the effect of an unpalatable plant, *Juncus effusus*, on wetland plant communities across a cattle grazing gradient. We tested several predictions: 1) *Juncus effusus* presence would have significant effects on plant assemblage composition 2) the effects of *Juncus* on plant assemblage composition would vary along the grazing gradient; and 3) *Juncus* would increase plant functional diversity in grazed wetlands. We found that *Juncus* preserved functional diversity in grazed wetland communities by protecting species that decrease with grazing pressure. The effect of *Juncus* was highest at intermediate levels of grazing pressure and decreased as grazing became intense. In multivariate analyses, grazing was the strongest driver of species composition but we found significant effects of *Juncus* on both vegetation change and species composition in grazed plots. These results indicate that *Juncus* has significant effects on the composition of plant assemblages in grazed wetlands although these positive effects wane when grazing becomes intense. Understanding the

effects of plant interactions at the community level is essential to applying plant interactions to restoration or management.

Keywords facilitation, herbivory, plant-plant interactions, stress gradients

Introduction

Unpalatable plant species often benefit neighboring plants by protecting them from herbivores (Atsatt and O'Dowd 1976; Milchunas and Noy-Meir 2002). For this indirect interaction to occur, an herbivore species must be present; however, if the herbivore were absent, interactions between unpalatable and palatable species would be mostly competitive (Bertness and Callaway 1994; Callaway 2007). Even though protection from herbivory is an indirect interaction, it is one of the most important ways one plant can benefit another (Callaway et al. 2005). Unpalatable species which protect others have often been called biotic refuges or benefactors (Callaway et al. 2000; Rebollo et al. 2002; Oesterheld and Oyarzabal 2004). As a biotic refuge, unpalatable plants may have important implications for diversity, conservation, and management in grazed ecosystems (Callaway et al. 2005) and may provide a safe site for species that would otherwise be eradicated by grazing. Because unpalatable plants may maintain populations of certain species in a grazed context, unpalatable plants can promote stability in habitats with high consumer pressure (Callaway et al. 2000; Rebollo et al. 2002; Callaway et al. 2005; Rebollo et al. 2005).

Often, the plants that are protected by unpalatable plants are palatable species that grazers prefer (Callaway et al. 2005; Baraza et al. 2006) and are sensitive to grazing pressure. Species have been divided into functional groups based on the way they respond to grazing (Olff and Ritchie 1998; McIntyre et al. 2003). Increasers are species that gain relative dominance with grazing pressure and decreasers are species that reduce relative dominance with grazing pressure (McIntyre et al. 2003). These two functional groups correspond to the adaptive strategies proposed by Grime (1977), with increasers being “stress-tolerant” species (in this case tolerant to grazing) and decreasers being either “competitive” or “ruderal”. In grazed ecosystems, biotic refuges are usually stress-tolerant and are increasers themselves, while the species they protect are usually competitive decreasers; ruderal species likely do not benefit from facilitation due to sensitivity to competition (Michalet et al. 2006, Boughton et al. Chapter 2). In intensely grazed ecosystems, decreasers may be lost and replaced by increasers (McIntyre et al. 2003). With the loss of the decreaser functional group, functional diversity of the ecosystem is reduced. However if unpalatable plants are present and can protect decreasers from herbivory, they can preserve functional diversity in grazed ecosystems (Figure 4-1).

Plant interactions, indirect or direct, are often a complex balance of positive and negative interactions influenced by ecological context (Callaway and Walker 1997). Therefore, beneficial refuge effects of unpalatable species may not occur for many reasons. Potential beneficiary plants may vary in their response to biotic refuges due to differences in palatability. For example, Callaway et al. (2005) found that palatable species were protected from grazing within unpalatable *Cirsium* sp. and *Veratrum* sp. refuges while less palatable species were not. This result emphasizes that competition occurs between the unpalatable plant and potential beneficiaries and for a positive effect of the refuge to occur, grazing must be a negative influence on the beneficiary. Therefore, species that are tolerant to grazing (increasers) do better outside the refuge than inside the refuge within a grazed context (Figure 4-1).

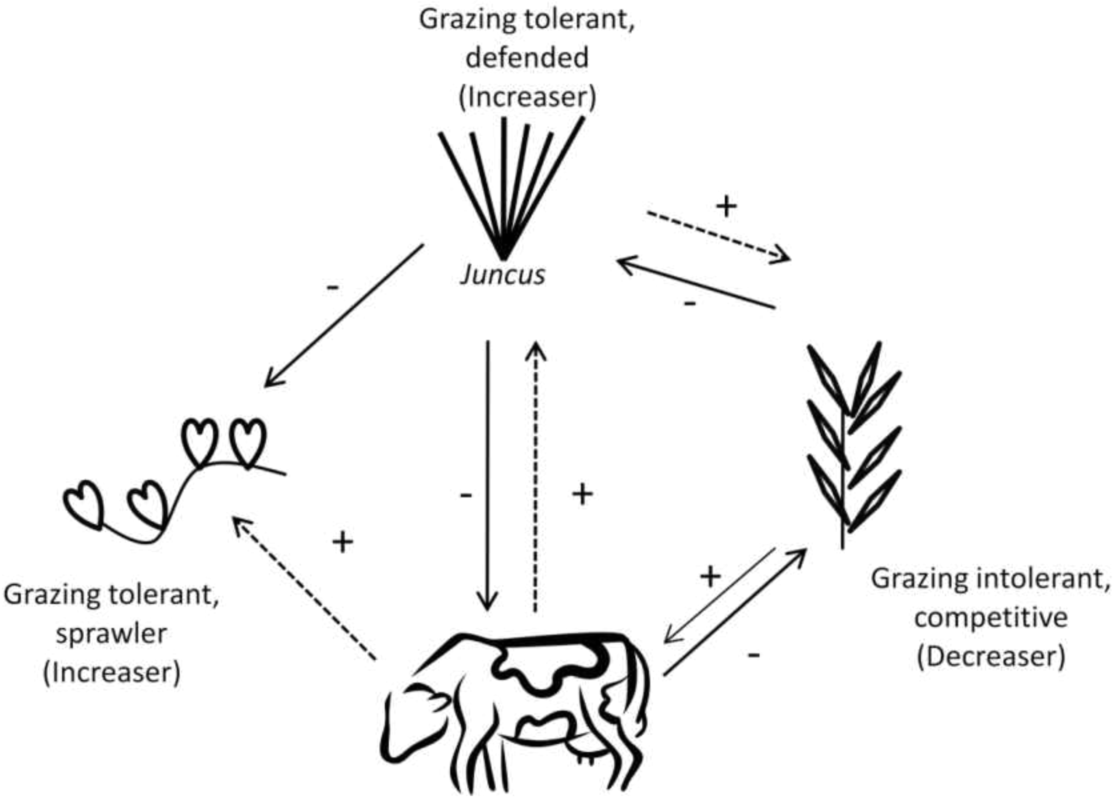

Figure 4-1 Interaction web between a herbivore, *Juncus*, and increaser and decreaser species. Herbivores indirectly benefit both increaser, grazing tolerant defended species (such as *Juncus*) and short-statured sprawling species by reducing their competitors (decreasers). *Juncus* indirectly positively affects grazing intolerant competitive species by protection from herbivory which results in a direct negative effect of *Juncus* on cattle.

Grazing intensity may also play a role in determining if positive interactions occur between unpalatable and palatable species. The effect of grazing must be a highly negative force on the palatable species so that protection from the unpalatable plant outweighs competitive effects of being near the unpalatable. Rebollo et al. (2005) found that interactions between the biotic refuge, *Opuntia* sp. and the palatable species, *Bouteloua gracilis* were negative in lightly grazed areas while they became positive in intensely grazed areas. Conversely, in intensely grazed situations, other studies have found that unpalatable plants lose their ability to function as a refuge and have observed highest facilitative intensity at intermediate levels of grazing (Brooker et al. 2006; Graff et al. 2007; Smit et al. 2007). Of the few studies that have assessed plant interactions along stress gradients, most have only been able to compare two (high vs. low) points along the gradient (Brooker et al. 2006; Brooker et al. 2008). More studies are needed that sample entire stress gradients as it appears that quadratic rather than linear relationships may describe the relationship between stress and the outcomes of plant interactions (Brooker et al. 2006; Michalet et al. 2006).

In south-central Florida cattle ranching is the dominant land use. There are many isolated seasonal wetlands embedded within cattle ranches and many of these wetlands become invaded by the native tussock-forming rush, *Juncus effusus* L.var. *solutus* Fernald and Wiegand (Boughton et al. Chapter 1). *Juncus* is unpalatable to cattle due to its tough spiky culms and many species grow within its tussock. The central goals of this study were to determine if *Juncus* preserves functional diversity in wetlands embedded in subtropical pastures, and to determine how *Juncus* influences the composition of grazed wetland communities. In a previous study, we found that *Juncus* provided refuge for three of four species in a transplant experiment (Boughton et al. Chapter 2), providing evidence that *Juncus* has the potential to preserve some plant species in grazed wetland communities. However, it has been demonstrated that plant interactions may not always translate into population or community composition effects (Brooker et al. 2006). This is an important issue to consider if the goal is to increase understanding of how plant communities respond to altered ecological conditions as well as application of plant species interactions to restoration or management.

In this study, we tested the following hypotheses : 1) If *Juncus* influences wetland plant community composition in grazed wetlands then we expected that species richness and abundance of different functional groups (specifically increasers and decreasers) would differ between non-*Juncus* and *Juncus* plots, and 2) that positive effects of *Juncus* would change across the grazing gradient.

Methods

Study site

This experiment was conducted at the Mac-Arthur Agro-Ecology Research Center (MAERC), a division of Archbold Biological Station, located in south-central Florida (27°09’ N, 81°11’ W). MAERC is located within Buck Island Ranch, a 4170 ha commercial cattle ranch which is a combination of improved (IMP) and semi-native pastures (SNP) with approximately 630 isolated, seasonal wetlands embedded throughout the property. Approximately half of the land area of Buck Island Ranch is occupied by intensely managed IMP and the other half is occupied by less intensely managed SNP. Improved pastures are composed primarily of Bahia grass (*Paspalum notatum* Flueggé, an introduced forage grass), are fertilized annually with N and were historically fertilized with P (1960’s-1986). Semi-native pastures are composed of a mixture of *P.notatum* as well as native grasses (i.e. *Andropogon* spp. L., *Axonopus* spp.P.Beauv., and *Panicum* spp. Schult.) and have never been fertilized. The climate is subtropical with a mean annual temperature of 22°C, and summer maximums of 33°C. Mean annual precipitation is 1300 mm, of which 69% falls during the wet season (June-October). Cattle are the main herbivore in this system, but feral pigs also are present and often create large soil disturbances in and around wetlands. In a separate study, peak standing biomass in grazing exclosures within wetlands estimated productivity as ~ 720±378 g/m^2 in IMP wetlands and ~400±185 g/m^2 in SNP wetlands (Bohlen and Quintana-Ascencio, *unpublished data*). In grazed areas peak standing biomass was ~345±172 g/m^2 in IMP wetlands and ~280±76 g/m^2 in SNP. More detailed information about the study site can be found in (Boughton et al. Chapter 2).

We selected 16 wetlands across Buck Island Ranch to sample a range of grazing intensities from low to high across two pasture-types: SNP and IMP. Grazing intensity varies across the ranch, although IMPs usually experience higher grazing pressure than SNPs. For the duration of this study which covered two growing seasons, (~July 2006-December 2007), average stocking rate was 1.08 cows/ha in IMPs and 0.59 cows/ha in SNPs. However, as the IMP and SNP are subdivided into several smaller pastures by fences, there is a wide range of grazing intensities within these two pasture-types. Within the IMPs in our study, grazing pressure ranged from 0.57-1.7 cows/ha and within SNPs, grazing pressure ranged from 0.15-1.12 cows/ha. To estimate the intensity of cattle grazing in each wetland, we calculated cows/ha for each pasture that contained a study wetland. This was calculated by multiplying the number of days spent in a study wetland pasture by the number in the herd for each event that there were cows in that particular pasture. These numbers were then summed and divided by the area of the pasture in hectares to calculate livestock unit*days/ha. This number was then divided by 365 days to obtain cows/ha. Cows/ha was calculated for the years of 2006 and 2007 and averaged. However, as Rebollo et al. (2005) pointed out, local differences in grazing intensity can occur and even in a lightly stocked pasture, grazing pressure can be intense in some areas. Therefore, we attempted to estimate a local grazing intensity for each study wetland; we measured six heights (one from the grazed subplot) outside the exclosure and averaged them. Lower heights indicate higher grazing intensity. This local measure of grazing intensity was significantly related to cows/ha (R^2=0.40, F = 9.3, p=0.009).

Experimental Design

Our experiment was a factorial design with three factors: pasture (improved vs. semi-native), grazing (grazed or ungrazed), *Juncus* (*Juncus* or no *Juncus*). This study took place within a larger experiment to assess pairwise interactions between *Juncus* and two native and two non-native species (Boughton et al. Chapter 2).

To ensure an evenly distributed sample of the ranch property, we divided the property into eight pie sections and chose one IMP wetland and one SNP wetland randomly within each pie slice for a total of 16 wetlands. Wetlands were chosen for use in the study only if they contained a large population of *Juncus*. At each wetland site, two random directions were chosen from eight possible directions (N, NE, E, SE, S, SW, W, and NW). These locations were used to determine the positioning of two experimental subplots within a wetland. At each experimental subplot we set up a grazed 2 m x 2 m plot containing four 1 m^2 quadrats and a non-grazed 2 m x 2 m exclosure containing four 1 m^2 quadrats. Grazing exclosures were constructed with four t-posts, four 2.5 m cow panels (Tractor Supply) and thick wire. Grazing exclosures successfully excluded all large herbivores (i.e cattle, hogs, and deer) from experimental plots. We constructed 32 exclosures (2/wetland) for the study. Within each experimental subplot, quadrats were then randomly assigned either as a *Juncus* treatment or non-*Juncus* treatment. All *Juncus* plants were removed from the non-*Juncus* treatment quadrats using a machete and large clippers. *Juncus* was kept out of the non-*Juncus* treatment quadrats by clipping during subsequent visits until no resprouts were found. Community composition of each of the quadrats was assessed by categorizing visual estimates of percent cover into seven cover classes (1: 0-1%, 2: 2-5%, 3: 6-25%, 4: 26-50%, 5: 51-75%, 6: 76-95%, 7: 96-100%; Daubenmire 1959). Composition estimates were obtained in September 2006 after all fences and *Juncus* treatments had been established and again in October 2007 at the end of the experiment. Percent cover midpoints of each species from the same treatment/wetland were averaged to remove pseudoreplication (Abrams and Hulbert 1987).

Data Analysis

Indicator species analysis in PC-ORD v. 5 was used to identify species that were significantly associated with either ungrazed or grazed plots to determine which species were increasers (species that increase with grazing) or decreasers (species that decrease with grazing). The percent cover of increasers and of decreasers was summed for each treatment/wetland to obtain abundance of that functional group.

We used ANOVAs to determine if *Juncus*, grazing, and pasture treatments affected species richness and decreaser abundance. Analyses were conducted in SPSS 16.0. Decreaser abundance was natural log transformed prior to analysis. We could not obtain normality in increaser abundance so non-parametric Kruskal Wallis tests were used. We did three Kruskal Wallis tests, one for each main effect (pasture, *Juncus*, and grazing). Since this results in three tests on the same data set, we used a Bonferroni correction to determine the correct p-value to denote significance (0.05/3) which resulted in α=0.02 (Sokal and Rohlf 1995).

To assess the effect of grazing intensity on decreaser cover within *Juncus* clumps, we calculated relative facilitation as (Smit et al. 2007):

$$(\ln(decreaser_{Juncus} - decreaser_{no\_juncus} + 1)$$

We analyzed the relationship between relative facilitation and the two measures of grazing intensity (cows/ha and vegetation height) with ANCOVA, including pasture-type as a fixed factor. If pasture had no effect, it was dropped from the model.

We analyzed the effect of grazing, *Juncus,* and pasture-type on community composition, using non-metric multidimensional scaling (NMS) ordination in PC-ORD v.5 with Sørenson distance, a random starting configuration and 50 runs of both real data and random data. A total of 84 species were included in the ordination. The percent cover of *Juncus* was removed to prevent circularity when comparing treatment effects. Before conducting the ordination we assessed descriptive statistics in PC-ORD of each plot (rows) and found the coefficient of variation was 34.78% indicating no transformations were necessary. Ordination scores were compared between treatments using overlap of 25% and 75% quartiles calculated and graphed in SigmaPlot v. 10 and the medians of different treatments. Community changes were compared between treatments by analyzing the differences in length and direction of successional vectors (McCune and Grace 2002). For each sample unit, a vector extends from the position in species space at the beginning of the experiment (vector tail) to the position in species space at the end of the experiment (vector head). The vectors show movements of the sample units in species space. We analyzed vector length and vector direction separately (McCune 1992).

Table 4-1. Indicator species analysis for decreasers and increasers.

	Decreaser	Increaser	*p*
Centella asiatica	x		0.005
Cynodon dactylon		x	0.06
Cyperus haspans	x		0.01
Luziola flutans		x	0.03
Ludwigia repens		x	0.09
Panicum hemitomon	x		0.001
Saciolepis striata	x		0.004
Bare ground		x	0.001

Vector length translates into magnitude of change in species composition from the start of the experiment to the end of the experiment (Harcombe et al. 2002), while vector direction represents how similar species composition is between treatments at the end of the experiment. We tested the hypothesis that species composition among the factorial *Juncus* and grazing treatments would differ either between treatments or in magnitude of change. We expected that pasture-type would affect length and direction of vectors of the *Juncus* and grazing treatments due to differences in nutrient and grazing intensity characteristics. Additionally, we expected direction of vectors to differ between pasture types because wetlands in these two pastures have been shown to have different species composition (Boughton et al. Chapter 1). To separate and test magnitude (vector length) from vector direction, we translated the vectors to a common origin (0, 0 in 2-dimensional space) by subtracting the score of the tail from the scores of both the head and tail (McCune 1992; McCune and Grace 2002). We chose to calculate both vector length and vector direction using city block distances rather than Euclidean distances due to less weight given to outliers in city block space (McCune 1992; Harcombe et al. 2002). Because the ordination had a two dimensional solution, the city block vector length was calculated as the sum of the absolute values of the x and y coordinates after the vectors had been centered on a common origin (McCune 1992). To assess differences in vector direction between treatments, we first centered all vectors so that tails were on 0,0 and then standardized them to unit length by dividing each coordinate (x_i and y_i) by sum of their absolute values (McCune 1992). This allows the coordinates of each vector head to sum to one in city block space (McCune 1992). These standardized vectors were then analyzed in a MANOVA in SPSS 16.0 to test whether the heads of the vectors occupy the same region in 2-dimensional space with the x and y coordinates of the vector heads as dependent variables and grazing, *Juncus*, and pasture-type as fixed factors. Vector length was analyzed with an ANOVA, with length as the dependent factor and grazing, *Juncus*, and pasture-type as fixed factors. Dependent factors were checked for normality and no transformations were necessary.

Results

Table 4-2. ANOVA results for species richness among treatments.

	df	MS	*F*	*p*
Juncus (J)	1	49.0	2.90	0.09
Grazing (G)	1	1.0	0.06	0.81
Pasture (P)	1	27.56	1.63	0.21
J x G	1	0.56	0.03	0.86
J x P	1	0.25	0.02	0.90
G x P	1	0.25	0.02	0.90
J x G x P	1	0.56	0.03	0.86
Error	56	16.89		

Indicator species analysis identified four significant decreasers and two significant and two marginally significant increasers (Table 4-1). None of the experimental treatments or their interactions were significant in explaining variation in species richness (Table 4-2). As expected, decreasers were significantly less abundant in grazed plots (mean±st.error: 9.2%±1.8) vs. ungrazed plots (37.6%±3.7) and in IMP wetlands (19.9%±3.6) compared to SNP wetlands (26.9%±3.9) (Table 4-3). Decreaser abundance was significantly higher in plots with *Juncus* in grazed areas, but the opposite was true in ungrazed areas (Table 4-3, Figure 4-2). There was a significant pasture-by-grazing interaction in which decreaser species were equally abundant in the ungrazed plots in both pasture types (IMP: 35.2%±4.0; SNP: 39.9%±4.0), but decreasers were more abundant in SNP grazed plots (13.8%±4.0) compared to IMP grazed plots (4.7%±4.0) (Table 4-3).

Table 4-3. ANOVA results for decreaser abundance among treatments.

	df	MS	*F*	*p*
Juncus (J)	1	0.62	1.21	0.28
Grazing (G)	**1**	**40.34**	**78.99**	**<0.001**
Pasture (P)	**1**	**4.97**	**9.73**	**0.003**
J x G	**1**	**4.53**	**8.86**	**0.004**
J x P	1	0.01	0.01	0.92
G x P	**1**	**2.76**	**5.41**	**0.02**
J x G x P	1	0.31	0.60	0.44
Error	56	0.51		

This is likely due to lower grazing intensity in SNP wetlands compared to IMP wetlands. The coverage of *Juncus* was the same inside the exclosure (75.5%±3.4) and outside the exclosure (78.9%±3.4) at the beginning of the experiment (df=1,32, F=0.49, p=0.49) but was lower inside the exclosures (50.3%±4.6) at the end of the experiment (df=1,32, F=4.48, p=0.04) suggesting that *Juncus* was declining and was beginning to be outcompeted by other species when grazing was removed.

Increasers were significantly higher in grazed areas (mean rank in grazed: 40.88; mean rank in ungrazed: 24.12; χ^2=13.37, p=0.003) and higher in IMP wetlands compared to SNP (mean rank in IP: 37.7; mean rank in SNP: 27.3; χ^2=5.2, p=0.02). The main effect of *Juncus* was not significant (mean rank w/*Juncus*: 28.6; mean rank w/o *Juncus*: 36.4; χ^2=2.89, p=0.09).

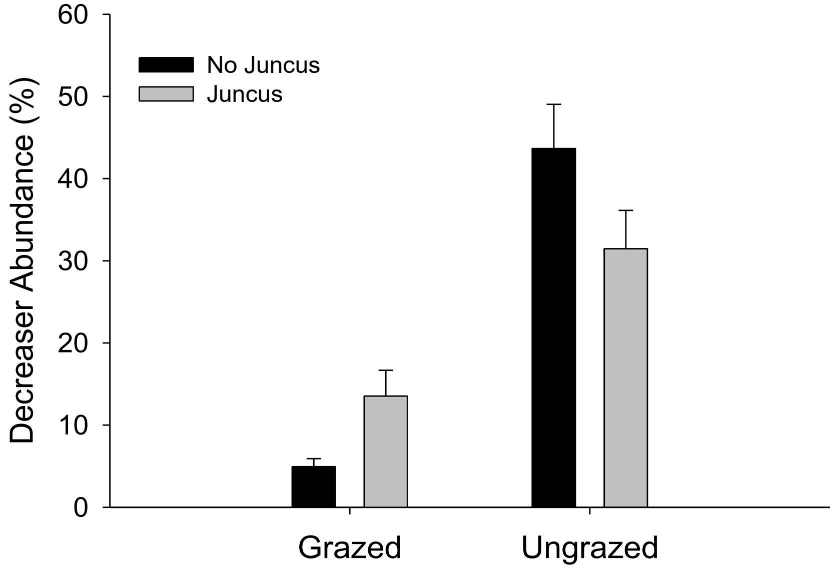

Figure 4-2 In grazed plots, decreaser abundance was significantly higher with Juncus than without, while the opposite was true in ungrazed plots. This suggests a switch from facilitative to competitive effects of *Juncus* depending on consumer context.

Local grazing intensity affected relative facilitation on decreaser abundance, but cows/ha had no effect (df =1,15, F=0.97, P=0.34). Relative facilitation (R^2=0.34, df=1,15, F=6.98, P=0.021) decreased as local grazing became more intense. Pasture-type had no effect on how grazing intensity affected relative facilitation and was dropped from analyses. Curve estimation showed that the quadratic relationship was a better model than a linear relationship for relative facilitation vs. grazing intensity. The quadratic relationship showed that relative facilitation peaked at moderate grazing intensities (R^2=0.49, df=1,15, F=5.71, P=0.018) (Figure 4-3).

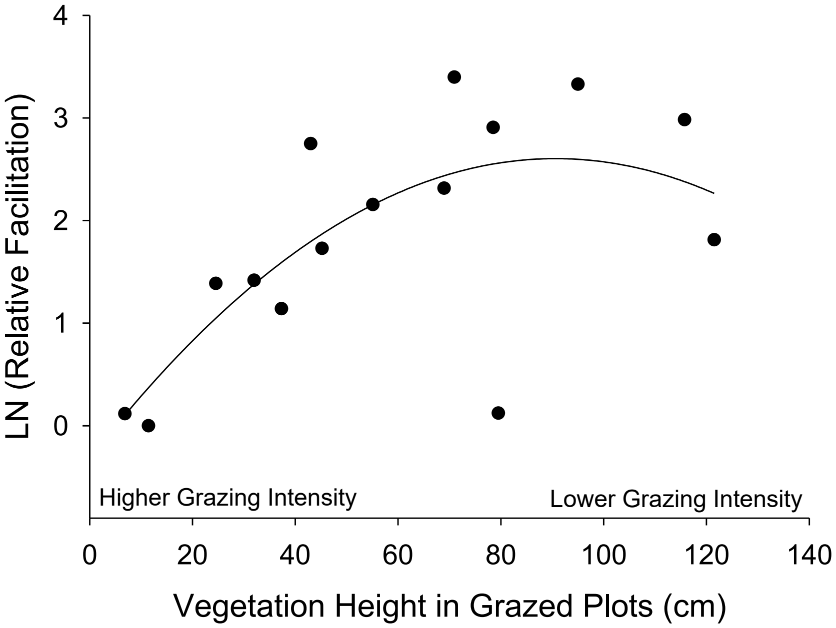

Figure 4-3 The highest levels of relative facilitation occurred at intermediate grazing levels. Facilitation declines at higher grazing intensity. Fit of the quadratic curve: R^2=0.49, F=5.71, P=0.018.

NMS ordination showed that species composition varied among pasture and grazing treatments (Figure 4-4). At the beginning of the experiment, there were no major differences between treatments except for a slight pasture effect (Figure 4-4a). In year two, grazing treatments differed strongly in IMP wetlands and less so in SNP wetlands (Figure 4- 4b). A two dimensional solution was selected and both axes were significant ($p=0.019$). The percent of variance explained by the entire ordination was 0.54 (Axis 1 $r^2=0.23$, Axis 2 $r^2=0.31$). Final stress was 25.98 with a final instability of 0.00002. Analysis of the length of successional vectors (rate of vegetation change) showed that the main effect of pasture (df = 1,64, F=4.92, p=0.03) was significant, but the main effects of *Juncus* (df = 1,64, F=1.13, p=0.29) and grazing (df = 1,64, F=0.46, p=0.50) and all interactions were not. Vector length indicates how much species composition changed over the course of the experiment and this result suggests that the amount of vegetation change depends on pasture-type, with longer vectors in SNP wetlands indicating greater species turnover compared to short vector lengths in IMP wetlands, indicating more stable species composition (Figure 4-5a).

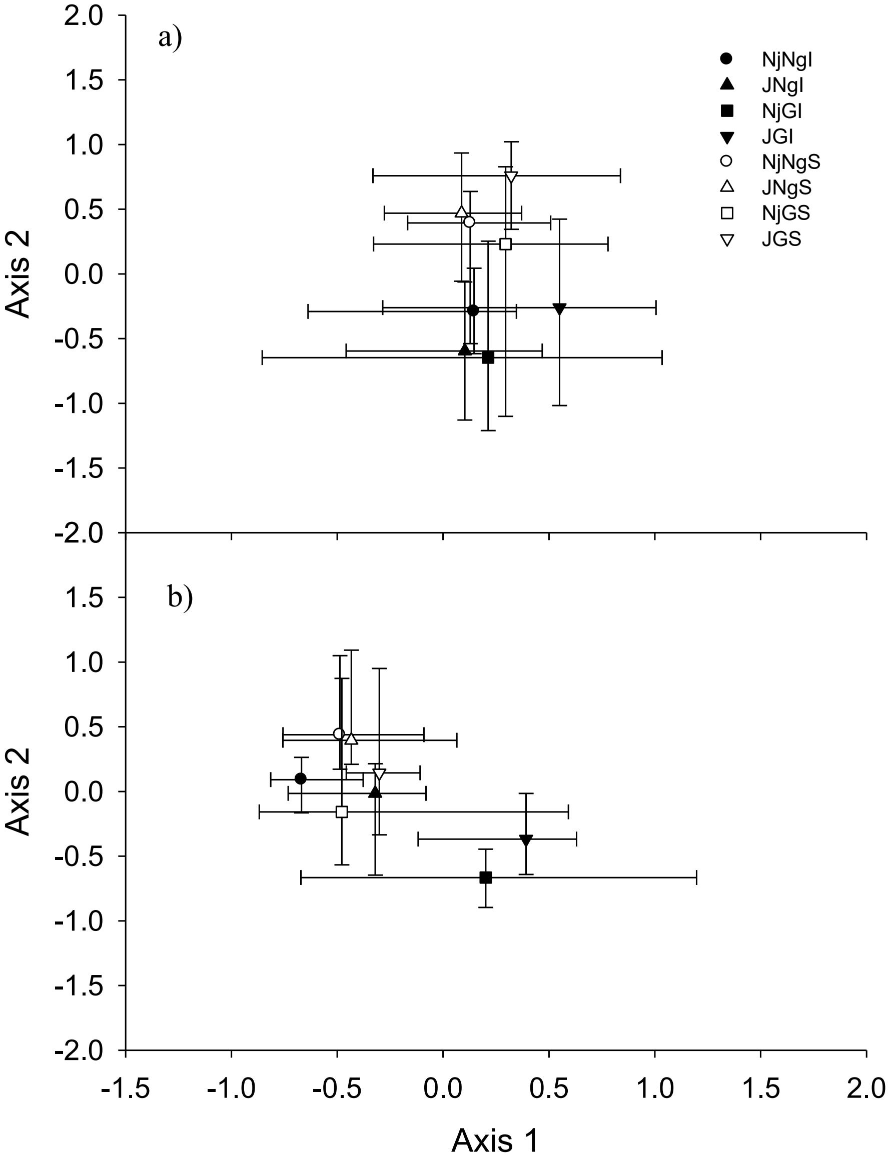

Figure 4-4 NMS ordination for the effects of pasture and grazing and Juncus treatments (white= SNP, black =IMP). Symbols represent the medians of 8 plots and bars represent 25 % and 75% quantiles. (a) Community composition at the beginning of the experiment. (b) Community composition at the end of the experiment. NjNg =non-*Juncus*, non-grazing; JNg=*Juncus*, non-grazing; NjG=non-*Juncus*,grazing; JG =*Juncus*,Grazing. I or S after the abbreviations indicates improved or semi-native wetlands, respectively.

When analyzing vector direction, which represents similarity in species composition between treatments at the end of the experiment, the MANOVA showed the main effect of grazing (Pillai's Trace, df =1,64, F=4.95, p=0.01) was significant while effects of *Juncus* (Pillai's Trace, df=1,64, F=0.37, p=0.69) and pasture (Pillai's Trace, df=1,64, F=0.47, p=0.63) and all interactions were non-significant. This result indicates that grazed and ungrazed plots differed significantly in species composition at the end of the experiment (Figure 4-6).

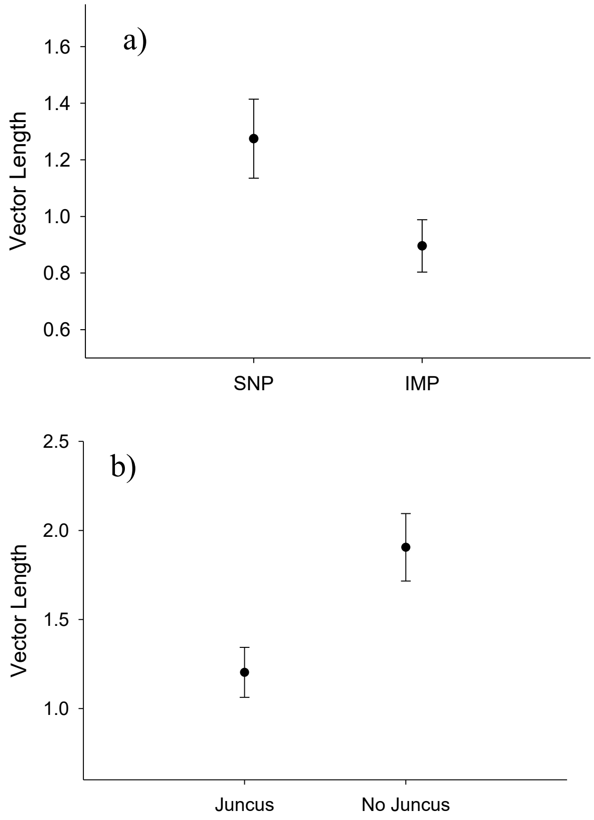

Figure 4-5 **a)** SNP wetlands show greater vegetation change over the course of the experiment than IMP wetlands **b)** No *Juncus* plots show greater vegetation change than *Juncus* plots. Symbols represent means and bars represent st.error.

Because the grazing effect was so strong and may have obscured any effects of *Juncus* on species composition, we ran a second ordination of only the grazed plots to determine if *Juncus* and non-*Juncus* plots differed in vector length and direction. A three dimensional solution was selected and all axes were significant ($p=0.019$). The percent of variance explained by the entire ordination was 0.64 (Axis 1 $r^2=0.17$, Axis 2 $r^2=0.21$, Axis 3 $r^2=0.26$). Final stress was 17.70 with a final instability of 0.00007. In this analysis, we found that *Juncus* significantly affected vector length (df=1,32, $F=10.51$, $p=0.003$), with shorter vectors in *Juncus* plots compared to non-*Juncus* plots (Figure 4-5b). The MANOVA of vector direction showed the main effect of *Juncus* (Pillai's Trace, df =1,32, $F=4.17$, $p=0.02$) was significant while the effect of pasture (Pillai's Trace, df=1,32, $F=1.99$, $p=0.14$) and their interaction was not (Pillai's Trace, df=1,32, $F=0.58$, $p=0.63$). These results suggest that *Juncus* plots remained more stable in species composition compared to non-*Juncus* plots over the course of the experiment and also that in grazed areas, *Juncus* plots differed significantly in species composition compared to non-*Juncus* plots.

Discussion

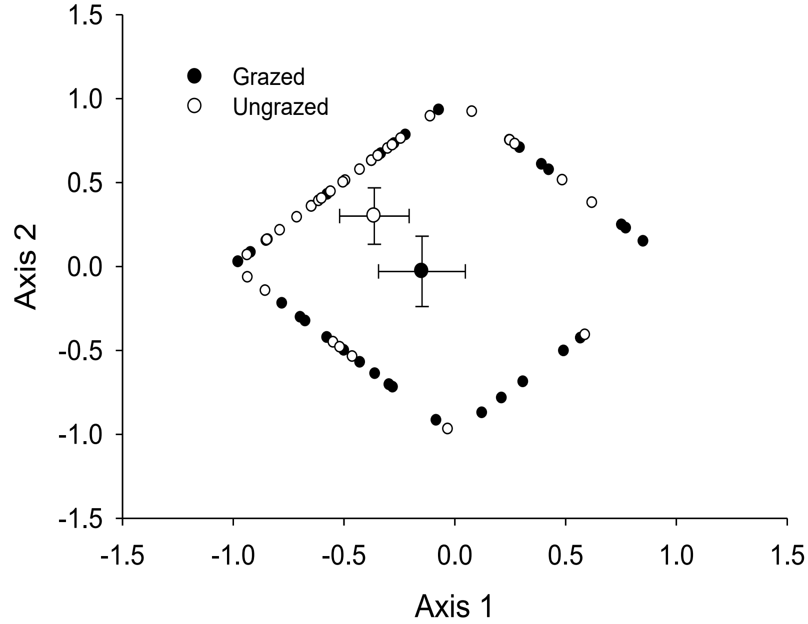

Figure 4-6 Grazed and ungrazed plots differed significantly in species composition at the end of the experiment. Smaller symbols represent each treatment point, while larger symbols represent medians of ordination scores and bars represent 25-75% quartiles. The diamond-shaped scatter results from the standardization of vectors for length in city-block space.

Refuge effects of Juncus effusus

Juncus effusus, an unpalatable tussock forming plant, protects some species from grazing, specifically species that are sensitive to grazing or decreasers. Although species richness was not higher with *Juncus* tussocks, decreaser abundance was significantly higher within *Juncus* tussocks compared to plots without *Juncus* in a grazed context supporting our hypothesis that *Juncus* preserves functional diversity in grazed wetlands. Similarly, Oesterheld and Oyarzabal (2004) found that an unpalatable grass provided refuge for a palatable grass preventing the palatable species from becoming locally extinct. Our results support evidence that unpalatable plants preserve functional diversity in grazed ecosystems and eliminating these species (often the goal of rangeland managers) could result in the eradication of palatable species with consequences for loss of both diversity and ecosystem services (McNaughton 1978; Callaway et al. 2000; Oesterheld and Oyarzabal 2004; Callaway et al. 2005).

We identified four species that were significant decreasers in these wetlands (Table 4-1). Since our study was limited to wetlands dominated by *Juncus* and only occurred in wetland edges, it is likely that more grazing decreasers would be identified in larger wetland studies that examine whole wetlands and wetlands not dominated by *Juncus*. Two species that were identified as decreasers, *Panicum hemitomon* and *Sacciolepis striata* are native wetland grasses beneficial for both wildlife and cattle forage. Decreasers increased in cover within exclosures while *Juncus* cover decreased, suggesting that *Juncus* may be outcompeted by the species it benefits when grazing is removed.

Facilitation across a Consumer Pressure Gradient

We observed a peak in facilitative effects of *Juncus* at moderate levels of grazing and decreasing facilitation by *Juncus* as grazing intensity increased (Figure 4-3). This finding is in agreement with other studies that have found that facilitative effects are greatest at intermediate levels of consumer pressure (Brooker et al. 2006; Graff et al. 2007; Smit et al. 2007). Facilitation by *Juncus* may be reduced when grazing becomes more intense because cattle become less selective as food availability decreases. Thus, in high consumer pressure situations, our data suggest that *Juncus* loses the ability to provide refuge for palatable species.

In this study we compared two measures of grazing intensity. Cows/ha was calculated at the pasture-level and was not highly correlated with relative facilitation while vegetation height within the grazed areas of wetlands was associated with relative facilitation. “Stress” is difficult to quantify, especially in terms that are applicable to the focal organism, and it has been suggested that stress be measured in terms of productivity (Callaway 2007). In grazed systems, high levels of grazing intensity result in lower biomass present, presumably equating to high levels of stress induced by grazing. Since grazing intensity can vary spatially and temporally, in terms of quantifying plant responses to grazing stress, local measures of grazing intensity such as vegetation height seem more appropriate than global measures such as cows/ha in large pastures.

Experimental Effects of Juncus, Grazing, and Pasture on Species Composition

The NMS ordination confirmed that species composition of all treatments was very similar at the beginning of the experiment, while treatments diverged in species composition at the end of the experiment (Figure 4-4). Analysis of successional vectors in the ordination that included all treatments did not show a large effect of *Juncus*. Rate of change or length of vectors was affected by pasture-type, with longer vectors within SNP wetlands compared to IMP wetlands. This result suggests that species turnover is rapid in SNP wetlands but stable in IMPs. This result is supportive of our previous findings that IMP wetland plant communities may be niche-assembled while SNP wetlands may be dispersal assembled (Boughton et al. Chapter 1). Since IMP wetlands are stressful environments due to intense grazing and eutrophication, only species that are tolerant to these conditions can survive. This results in a plant community that is composed of only the tolerant native and exotic species, possibly resulting in a more or less unchanging plant community over time.

The analysis of the position of vector heads when standardized for length, which represents similarity in species composition at the end of the experiment, showed that grazing was the only factor that significantly distinguished species composition among treatments at the end of the experiment. This was probably due to a large increase in the relative abundance of decreaser species and a decrease in *Juncus* cover within exclosures. We expected that there would be a large difference between plots with *Juncus* and without *Juncus* in grazed areas, and even though the ordination shows some separation of these treatments in IMP wetlands (Figure 4-4b), the effect was not strong enough to be significant in the MANOVA. This may be due to the fact that even though *Juncus* provides protection to grazing-sensitive species in grazed plots, their cover is not maintained to levels within the exclosure (Figure 4-2) and overall the cover of many species is reduced in grazed plots even when *Juncus* is present. In the ordination of only grazed plots, we found that *Juncus* significantly affected rate of vegetation change and species composition. Lengths of successional vectors were lower with *Juncus* compared to without *Juncus* suggesting that *Juncus* may have influenced species composition to remain stable. Plots without *Juncus* are not protected from soil and grazing disturbance and this resulted in more species composition change over the course of the study. Additionally, because *Juncus* has negative effects on some species due to shading (Ervin and Wetzel 2002), it may be that only a subsample of species are able to coexist with *Juncus,* thus resulting in less change in composition over the course of the experiment and shorter vector lengths. The second ordination also showed that species composition was different between *Juncus* and non-*Juncus* plots, which is in agreement with our result that decreaser species were more abundant with *Juncus* than without in grazed plots (Figure 4-2).

Implications for Management and Conservation of Wetland Plant Communities on Florida Ranches

Changes in the outcomes of plant interactions along ecological stress gradients is interesting from a purely ecological point of view (Bruno et al. 2003), but also has implications for management in ecosystems impacted by human activities (Callaway et al. 2000, Rebollo et al. 2005). Throughout the world, many wetlands are subject to grazing by domestic animals (Brinson and Malvarez 2002; Nicol et al. 2007; Moran et al. 2008). By understanding how unpalatable plants interact with palatable neighbors along grazing gradients, appropriate management decisions can be made if the goal is to maintain plant communities that provide forage, wildlife habitat, and ecosystem functions. For example, we found that the ability of *Juncus* to function as a refuge for valuable grasses such as *P. hemitomon* and *S. striata* declines when grazing pressure exceeds ~1.0 cows/ha (~when vegetation height is between 40-60 cm). Higher stocking densities, in this system, could result in a loss of palatable species and a reduction in valuable wetland ecosystem services.

Acknowledgements

We thank R. Boughton, A. Peterson, and MAERC interns for help in the set-up of this experiment. We are grateful to G. Lollis, BIR staff, and Archbold Biological Station for valuable logistical support. R. Boughton and J. Navarra provided valuable comments on a previous draft. E.H.B. was supported by an EPA STAR Fellowship. Society of Wetlands Scientists Student Research Grants and The Florida Native Plant Society also provided valuable funding. Additional support was through USDA CSREES National Research Initiatives - Managed Ecosystems Program, Grant 2006-35101-17204).

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CHAPTER 5 RELATIVE IMPORTANCE OF PLANT COMMUNITY COMPOSITION AND NUTRIENTS IN DETERMINING WETLAND INVASIBILITY IN SUBTROPICAL RANGELANDS

Abstract

Question: What is the relative importance of resident native plant richness, resident functional group composition, soil N, soil P, and pasture-type in determining wetland invasion? Do non-native richness, frequency, and biomass respond to the same abiotic and biotic variables?

Location: MacArthur Agro-Ecology Research Center, south-central Florida, USA

Methods: We measured native and non-native species richness and frequency in 15 1 m^2 plots / 40 wetlands across two different pasture-types, agronomically improved (IMP) and semi-natural (SNP). Biomass of both non-native and native plants was collected in five 0.25 m^2 plots/wetland, sorted to species, dried and weighed. Soil cores were collected from five points/wetland to analyze soil total N and P. Preliminary analyses showed that native C3 perennial grass (C3g) abundance was the only functional group related to non-native attributes. We used residual maximum likelihood (REML) to model non-native richness, frequency, and biomass as a function of native attributes, C3g abundance, N, P, and pasture-type. Effect sizes were used to determine the strength of the covariates in relation to one another. Non-metric multidimensional scaling was used to determine if there were different suites of non-natives among pasture-types.

Results: In SNP wetlands non-native richness was negatively correlated to native richness and positively correlated to soil N while non-native frequency was positively associated with soil total P. In contrast, abiotic variables were unimportant in explaining non-native richness or abundance in IMP wetlands. However, non-native richness, frequency and biomass were all negatively correlated with resident C3g abundance in both pasture-types. The two pasture-types had different suites of non-native species due to the most abundant non-native forb occurring almost exclusively in IMP wetlands.

Conclusions: Management intensity can alter factors controlling habitat invasibility. In wetlands embedded in less intensive pastures, biotic and abiotic factors were equally important in predicting non-native richness and abundance, while in intensely managed wetlands, abiotic factors did not affect non-native abundance. However, C3g abundance played a role in inhibiting non-natives in both pasture-types. This suggests that human induced resource enrichment may remove abiotic filters that would otherwise have played a role in preventing invasion. Experiments are now needed to determine the mechanisms by which C3 grasses can potentially decrease invasion.

Keywords: functional groups, invasion, competition, resource availability, land-use

Nomenclature: Wunderlin 1998

Abbreviations: IMP=improved pastures; SNP=semi-native pastures

Introduction

The susceptibility of an ecosystem to invasion by non-native (exotic) species is a function of both biotic and abiotic factors, but the relative importance of these variables in influencing invasions is unknown (Huebner & Tobin 2006, Lonsdale 1999, Maron & Marler 2007, Stachowicz et al. 2002). Biotic resistance, a mechanism driven by competition where species-rich communities are less susceptible to invasion due to more complete resource use and less niche opportunities, is generally accepted to be functioning at small spatial scales (Elton 1958, Levine & Rees 2002, Shea & Chesson 2002) and is thought to be responsible for generating a negative relationship between non-native and native richness in experimental studies (Knops et al. 1999, Levine 2001, Symstad 2000). Despite a large amount of work conducted on this topic, the “diversity-invasibility” hypothesis is still controversial (Lonsdale 1999, Gilbert & Lechowicz 2005). Some have proposed that instead of richness, biotic resistance may be better characterized by measuring functional diversity or by examining the functional group of the dominant species (Symstad 2000, Emery 2007, Ortega & Pearson 2005, Perelman et al. 2007, Prieur-Richard et al. 2002). Adding further controversy, the native-exotic relationship becomes positive as the scale of study increases (Stohlgren et al. 1999, Levine 2000). This pattern has been explained by competitive exclusions at small neighbor-hood scales being overridden by factors that determine both native and non-native diversity at large scales, such as propagule pressure (Levine 2000, Shea & Chesson 2002).

Besides scale effects, other factors may change the slope of the native-exotic relationship. Davies et al. (2007) found that site productivity was correlated with the slope of the native-exotic relationship and after further investigation found that at small scales in high productivity sites the native-exotic relationship was negative while it was positive in low productivity sites. The relationship between native and non-native richness may also vary due to anthropogenic activities although few studies have examined this phenomenon (but see Belote et al. 2008). In human disturbed landscapes, ecological processes are disrupted and novel filters to species composition may be introduced such as enriched nutrients and increased herbivory or predation (de Blois et al. 2002). These changes could result in different factors driving non-native richness versus native richness. In extreme cases, when anthropogenic disturbances are intense, competition from native species is likely to be relaxed and communities become more similar (homogenized) as exotics and native ruderals increase (Olden 2006, Omacini et al. 1995). In these situations, the relationship between natives and non-natives may become decoupled.

Human disturbed communities, such as old fields and agricultural wetlands are often the focus of ecological restorations (Bakker & Wilson 2004, Orr et al. 2007). Recently, much interest has been shown in creating invasion-resistant restorations (Funk et al. 2008). This idea is based on the premise that focusing on native species resource-use traits has potential to increase community resistance to invasion. Therefore, determining which functional groups are important to resisting invaders is an important area of research (Perelman et al. 2007, Funk et al. 2008). Additionally, determining the relative importance of functional group composition versus abiotic factors in determining non-native abundance is essential for setting priorities for invasive species management in protected natural areas and restorations.

In this study we examined non-native species richness, frequency, and biomass in relation to a variety of abiotic and biotic variables in isolated, seasonal wetlands embedded in two pasture-types on a Florida cattle ranch. One pasture-type is intensely managed with nutrient additions, heavy grazing, and complete upland conversion to non-native forage grasses (IMP: improved pastures) and the other pasture type is semi-native (SNP) with no fertilizer additions, moderate grazing, and an upland composed of a matrix of both native and non-native grasses. We aimed to determine the relative association of attributes of native plant assemblages (richness, frequency, and biomass), C3 perennial grass composition and soil nutrients, both phosphorus and nitrogen, to attributes of non-native assemblages. Because the ecological range of conditions in our system is narrow (i.e. the spatial scale of the study is not large, ~4,170 ha, and we examine only one habitat type –freshwater wetlands–, we expected a negative relationship between non-native and native species richness, with non-natives decreasing as native richness increases in less intensely disturbed wetlands and no relationship of natives-exotic species richness in intensely disturbed, nutrient enriched wetlands due to homogenization (Shea & Chesson 2002, Perelman et al. 2007). Second, we examined the composition of non-native plant assemblages to determine if there are different suites of non-natives between pasture-type. By examining both community level and species level patterns in non-native abundance we aimed to determine the relative importance of abiotic and biotic forces affecting the susceptibility of wetlands to non-native invasion.

Methods

Study area

This study was conducted at the MacArthur Agro-Ecology Research Center, a division of Archbold Biological Station, located in south-central Florida (27°09' N, 81°11' W). The Center is located within Buck Island Ranch, a 4170 ha commercial cattle ranch which is a 50:50 combination of improved and semi-native pastures with approximately 600 isolated, seasonal wetlands embedded throughout the property (Figure 5-1). Agronomically "improved" pastures (IMP) are composed primarily of the introduced forage grass, *Paspalum notatum* Flueggé, are fertilized annually with N, and were historically fertilized with P, and have higher stocking rates. Semi-native pastures (SNP) are composed of a mixture of *P. notatum* as well as native grasses (i.e. *Andropogon* spp. L., *Axonopus* spp. P. Beauv., and *Panicum* spp. Torr.) and have never been fertilized. IMP wetlands have been shown to harbor decreased native species richness compared to SNP wetlands (Boughton et al. unpublished). For 2005-2008, the average stocking rate was 0.512 cows/ha in improved and 0.28 cows/ha in semi-native. Improved pastures are fertilized annually with N (~50 kg*ha^{-1}) and were fertilized historically with P fertilizer up until 1987 (~20 kg*ha^{-1}).

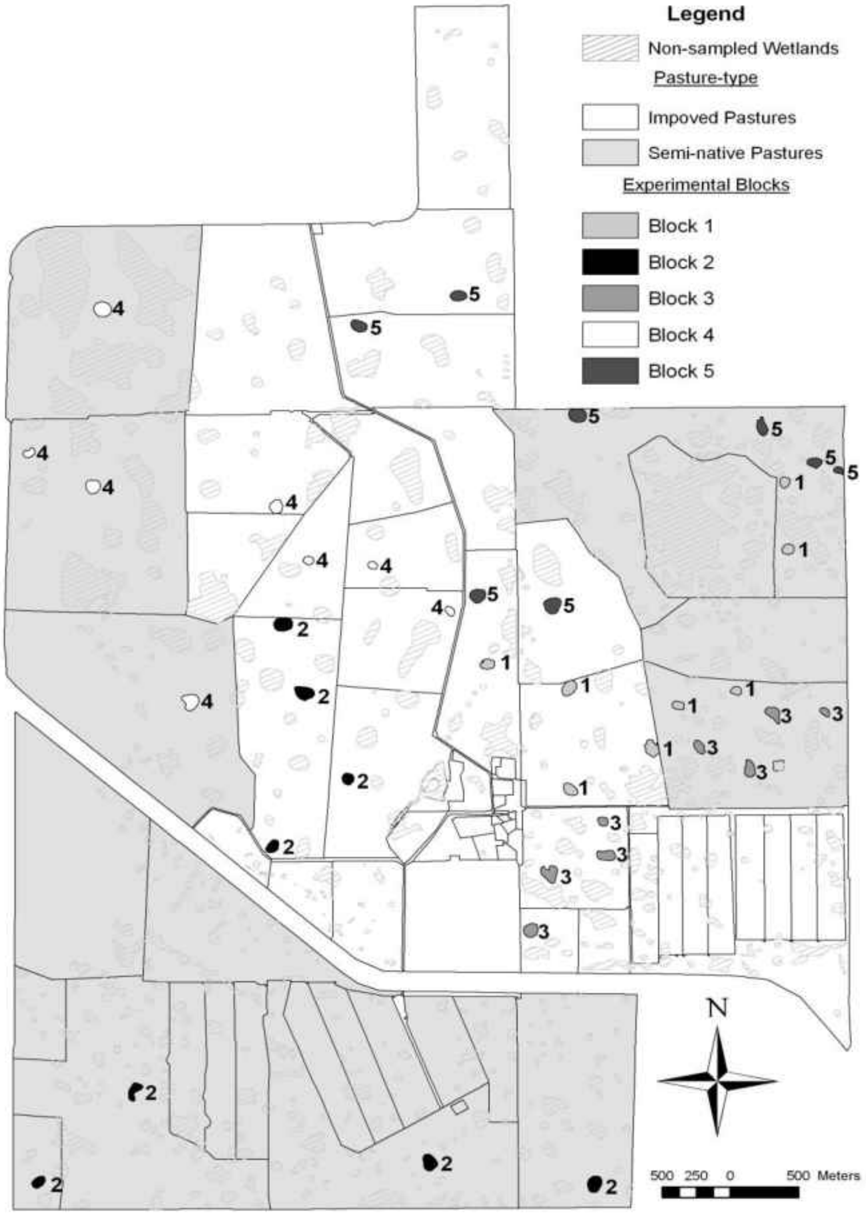

Figure 5-1 Map of the study site, MacArthur Agro-Ecology Research Center (MAERC). The forty sampled wetlands are solid with number labels denoting the five experimental blocks.

Wetland selection and plant sampling

Forty wetlands were randomly selected after stratification by blocking the ranch property into five regions. Twenty wetlands were selected within each pasture-type. Blocks were needed to account for differences among wetlands due to location, wetness and size because there was a slight gradient from higher elevation in the northwest to lower elevation in the southeast. Each block contained eight wetlands (four improved and four semi-native; Figure 5-1). Vegetation sampling was conducted at the end of the growing season in Oct-Nov 2006, at a period of peak biomass. Within each wetland we selected 15 random points (in ArcView 9.0) to sample species richness in 1 m^2 circular quadrats. To ensure equal sampling over the entire pond these fifteen points were stratified by five locations within each pond: center, northeast, northwest, southeast, and southwest. Five of the 15 points (one in each of the five regions of the pond) were randomly selected for biomass collection. The five biomass points were marked with a T-post in order to re-visit the plot during subsequent sampling events. Biomass was collected by species within a 0.25 m^2 circular quadrat. Plants were cut at approximately 10 cm from ground level and a smaller quadrat (0.0625 m^2) was used to sample growth below 10 cm. Individual species were bagged and oven dried (70°C for 48 hrs) and then weighed to obtain dry weight. Voucher specimens were collected for most species and deposited in the UCF and MAERC herbariums.

Soil collection and nutrient analysis

At each of the five vegetation biomass sampling posts, two soil samples were collected 1 m from the post in two randomly selected compass directions and aggregated into one sample for a total of five soil samples per pond. Soil was collected with a hammer core to a depth of 15 cm. Samples were oven dried at 105ºC for 24 hours and pushed through a 2mm sieve. Soil organic matter was measured as ash-free dry mass using 0.5 kg of soil ashed at 450°C for 16 hours. Ash was analyzed for total P (Allen et al. 1974) using the method of Murphy & Riley (1962) on a Technicon Autoanalyzer II (USEPA 1983). Total N was analyzed at the Analytical Chemistry Lab at the University of Georgia. The Micro-Dumas combustion technique was used and samples were analyzed on a Carlo Erba NA 1500 CHN Analyzer.

Statistical analysis

We conducted univariate statistical analyses using SAS software and multivariate analyses using PCord v. 5. We calculated native and non-native species richness, frequency, and biomass for each wetland. Frequency was defined as the number of occurrences of non-native or native species within the 15 sampled plots. We also divided species into functional groups to determine if non-native species richness was related to a particular native functional group. All species were recorded as either annual or perennial, by growth form, and by photosynthetic pathway (C3 or C4). Photosynthetic pathway information was obtained from relevant literature (Bowes 1993, Downton 1975, Looney et al. 1993, Waller et al. 1979). Growth forms included: submergent/emergent, forb (dicotyledonous herbs, including legumes), grass (all species in *Poaceae*, as well as sedges, rushes, and other monocots), shrubs, ferns, and an unknown category for plants that were unable to be identified (a maximum of 2/wetland, a total of 7 unknowns out of 154 species). The number of annual and perennial of each growth form was calculated and then further separated by resource use (C3 or C4) for a total of 18 different possible functional groups (submergent (Sub), C3 native annual forbs (CSNFa), C3 native perennial forbs (CSNFp), C3 native annual grasses (CSNGa), C3 native perennial grasses (CSNGp), C4 native annual grasses (WSNGa), C4 native perennial grasses (WSNGp), ferns (F), native shrubs (SN), exotic shrubs (SE), unknown (UK), C3 exotic annual forbs (CSEFa), C3 exotic perennial forbs (CSEFp), C3 exotic annual grasses (CSEGa), C3 exotic perennial grasses (CSEGp), C4 exotic annual grasses (WSEGa), C4 exotic perennial grasses (WSEGp), and forage grasses (FG). Of the above groups there were no species in CSEGa or WSEGa. To obtain abundance values for each of these groups, we totaled the number of occurrences for each group (number of times encountered in each of the 15 species composition plots) and then divided it by the total number of occurrences of all groups in the whole wetland. The two pasture-types differed slightly in the relative contribution of these functional types in the wetlands (Figure 5-2). Preliminary analyses showed that C3 perennial grass (hereafter, C3g) composition was the only functional group that was related to non-native richness and was the only group used in subsequent analyses.

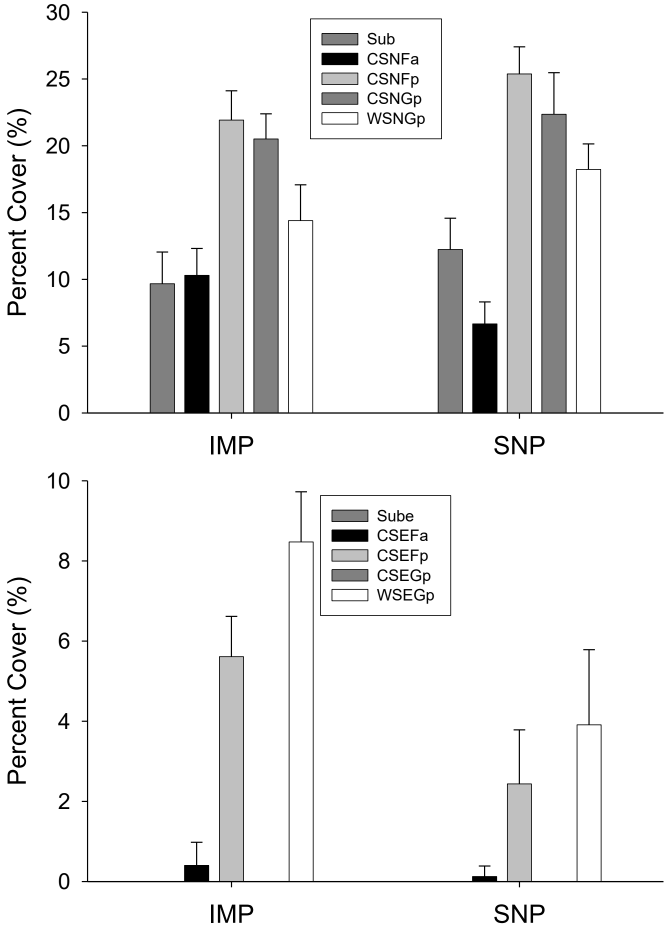

Figure 5-2 a) Percent covers of the dominant native functional groups in improved pasture (IMP) wetlands and semi-native pasture (SNP) wetlands. Letters denote significance in percent covers between pasture-types. b) Percent covers of the dominant non-native functional groups among the two pasture-types. Means ± 95% CI shown. See methods for meanings of abbreviations.

Species were considered non-native if they were not originally from Florida, following (Wunderlin 1998). We excluded forage grasses that were known to be brought in by human activities into pastures from our non-native species richness counts because these were not considered invasions; these included *Paspalum notatum* and *Hemarthria altissima* (Poir.) Stapf & C.E. Hubbard. This analysis is conservative because when the two forage grasses were included, relationships observed were even stronger. We observed ten non-native plants in this system that did not require humans to account for their presence in wetlands, including *Alternanthera philoxeroides* (Mart.) Griseb. (S. America), *Cuphea carthagenensis* (Jacq. J.F. Macbr.(S. America), *Cynodon dactylon* (L.) Pers. (S. Africa), *Eichornia crassipes* (Martius) Solms-Laubach (S. America), *Hymenachne amplexicaulis* (Rudge) Nees (S. America), *Ludwigia peruviana* (L.) H. Hara (S. America), *Panicum repens* L. (Australia), *Paspalum acuminatum* Raddi (S. America), *Paspalum urvillei* Steud. (S. America), and *Solanum viarum* Dunal (S. America). *Cynodon dactylon*, *H. amplexicaulis*, and *P. urvillei* were originally introduced as forage grasses but were included in our analyses because these grasses are known to spread through water ways, by endozoochory, or wind and do not rely on human activities for movement (Newman et al. 2003, Shiponeni & Milton 2006, Diaz et al. 2008).

In an attempt to adequately model number of exotic species as a function of pasture-type, native richness, C3g, total P and total N while incorporating the blocks, a number of models were fit (10 models for each non-native attribute (richness, frequency, and biomass). In the initial model fitting we treated the blocks as random effects. However, modeling of covariates such as native richness, C3g, total P and total N in the presence of random effects introduced imbalance. Consequently, standard least squares modeling was inefficient. To deal with this imbalance we used residual maximum likelihood (REML) estimation (see, e.g., Rencher & Schaalje 2008). Covariates were centered and scaled by their means and variances, respectively, to better assess their effects on exotic species relative to one another. Models were fit that considered each transformed covariate individually along with pasture-type (4 possible) and their interaction as well as all possible pairs of transformed covariates along with pasture-type and their interactions (6 possible). Using $\alpha = 0.05$ for each test, the highest order terms that were insignificant were eliminated from the models. We also ran models that treated blocks as fixed effects and results were identical and thus omitted from this report. When modeling non-native richness, frequency, or biomass we used the corresponding native attribute as a covariate. For example, when modeling non-native richness, native richness was a covariate and when modeling non-native frequency, native frequency was used as a covariate. Although data were transformed for analysis, we present untransformed data in all graphs.

To determine the best model (among those out of the 10 with significant covariate terms) we used Akaike Information Criterion corrected for small sample sizes ($AIC_{c;}$ Burnham & Anderson 2002). We chose not to run all possible models or combinations of the covariates. A greater number of models than the sample size of the study may increase spurious results (Burnham & Anderson 2002). We ran models that corresponded to our hypothesis that included the interaction of the covariates with pasture-type. AIC_c allowed us to determine the most parsimonious model with the best fit.

To test if different suites of exotic species were present within the two pasture-types, we used non-metric multidimensional scaling ordination (NMS; PCord v. 5). Nine wetlands had to be removed from the analysis because they did not contain any non-native species or they only contained 1 or 2 occurrences of non-native species; all of these wetlands were semi-native wetlands. The data were then relativized by the maximum (transformation in which each value for a species (column) is divided by the maximum value for that species) for each wetland because the CV for wetlands was 99.5%; a high CV that may produce unreliable results

(McCune & Grace 2002). The ordination was conducted with the Sørenson distance with 100 runs of real data and 50 runs of randomized data to test for significance.

Table 5-1. Means± SD of attributes of improved and semi-native pasture wetlands. * denotes significance at 0.05 level.

	IMP Wetlands	SNP Wetlands
Sample size	20	20
Area (ha)	0.83±0.3	0.71±0.3
Native Richness*	20.2±5.4	30.9±9.1
Non-native Richness*	4.8±0.9	2.4±1.9
Native Frequency*	73.1±19.4	102.8±28.1
Non-native Frequency*	22.1±8.9	6.2±7.0
Native Biomass (gm^{-2})	479.9±366.1	449.5±207.7
Non-native Biomass (gm^{-2})*	146.9±147.3	41.5±73.1
C3 Perennial Grass (%)	20.5±4.0	22.4±6.7
Soil Total P ($\mu g\ g^{-1}$)	162.5 ± 105.4	126.1 ± 66.7
Soil Total N ($\mu g\ g^{-1}g^{-1}$)	357.0 ± 42.9	371.9 ± 30.7

Results

There were strong differences in biotic and abiotic variables among wetlands within different pastures-types (Table 5-1). Non-native richness among wetlands was best described by the model that included the interaction of pasture and total N and the interaction of pasture and native richness (Table 5-2). The slope of the linear model of native species richness on non-native species richness in IMP wetlands was 0.18, whereas it was -1.11 for SNP wetlands. The effect estimates for soil N were 0.22 in IMP wetlands and 1.14 in SNP wetlands (Table 5-2). These results indicate that there were almost no effects for native species richness and soil nitrogen on non-native species richness in IMP wetlands while for SNP wetlands the effects for centered and scaled native richness and total N were large and similar in magnitude but in the opposite direction (Fig. 3).

Table 5-2. ANOVA table describing the best model of non-native richness.

	Estimate	S. E.	D F	t	P
Pasture (P)	2.12	0.35	1	4.76	<0.0001
Native Richness (NR)	-1.11	0.25	1	-4.45	0.0001
Total N (TN)	1.14	0.31	1	3.70	0.001
NR x P	1.29	0.50	1	2.58	0.02
TN x P	-0.92	0.37	1	-4.43	0.02

Another model describing variation in non-native richness was one that included the main effect of pasture-type and the centered and scaled covariate C3g without their interaction; since it was insignificant (p-value = 0.4250) and was excluded. This model was much less complex compared to the above model. From this fitted model the estimated effect of C3g on number of exotic species was -0.91 (Table 5-3, Figure 5-4). For the model which included the insignificant interaction between pasture-type and C3g the estimated effect of C3g on exotic species for IMP and SNP wetlands was -0.64 and -1.01, respectively (results not shown).

Table 5-3. ANOVA table of the fitted model of non-native richness with pasture-type and C3 g as factors.

	Estimate	S.E.	DF	t	P
Pasture	2.09	0.39	1	5.38	<0.0001
C3g	-0.91	0.20	1	-4.43	<0.0001

The inability of C3g to appear in the same model as native richness and total N was probably related to a strong relationship between C3g and total N (r =-0.63, P=0.003, Pearson's correlation for SNP wetlands, while r=-0.15, P=0.539 for IMP wetlands). We considered C3g as the response (although we cannot discern cause and effect from these data) and fit models similar to the ones above with pasture-type and covariates native richness and total N. The model with a total N and pasture-type interaction (p-value = 0.0084) was significant. From this fitted model the estimated effect of total N on C3g for IMP and SNP wetlands was -0.03 and -0.81, respectively. Thus, there was nearly no relationship between C3g and total N for IMP wetlands while there was a strong, negative relationship for SNP wetlands (Figure 5-5).

Table 5-4. ANOVA table of the fitted model of non-native frequency.

	Estimate	S.E.	DF	t	P
Pasture (P)	13.76	2.15	1	6.39	<0.0001
C3g (C)	-4.69	1.13	1	-4.13	0.0003
Total P (TP)	4.18	2.03	1	2.06	0.048
TP x P	-5.55	2.43	1	-2.28	0.03

Table 5-5. ANOVA table of the best model explaining non-native biomass.

	Estimate	S.E.	DF	t	P
Pasture (P)	91.06	32.51	1	2.80	0.009
C3g (C)	-42.80	18.86	1	-2.27	0.03

The best model describing non-native frequency contained C3g and an interaction between total P and pasture-type (Table 5-4). For this fitted model the estimated effect of C3g and total P on non-native frequency was -4.69 and -1.38, respectively, for IMP wetlands. For SNP wetlands these effect estimates were -4.69 and 4.18, respectively (Table 5-4). Consequently, the effect for C3g on non-native frequency remained constant across pasture-types while the effect for total P changed from small and negative in IMP wetlands to large and positive in SNP wetlands. Furthermore, the effects for C3g and total P on non-native frequency within SNP wetlands were in the opposite direction but nearly equal in magnitude (Figure 5- 6).

Non-native biomass was best explained by the model which contained main effects for pasture-type (P-value = 0.0085) and C3g (P-value = 0.0299) (Table 5-5). For this fitted model the estimated effect of C3g on exotic biomass was -42.8 indicating that the effect for C3g on non-native biomass was negative (Figure 5-7).

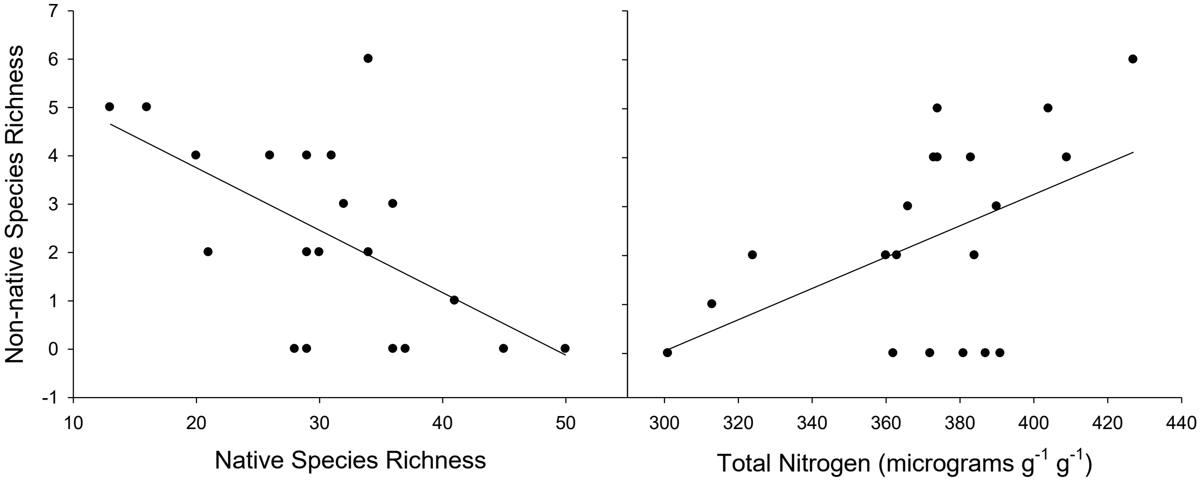

Figure 5-3 a) Non-native species richness is negatively related to native species richness, F=9.84, R^2=0.35, p=0.006). b) Non-native species richness is positively related to total nitrogen (F=5.85, R^2=0.25, p=0.03. Semi-native wetland data only.

Non-metric multidimensional scaling (NMS) ordination showed that there were slightly different suites of non-natives in each pasture-type. A 3-dimensional solution was found with final stress of 10.27. The ordination explained 93% of the variation in the matrix (Axis 1,

r^2=0.34, P=0.14; Axis 2, r^2=0.36, P=0.14; Axis 3, r^2=0.23, P=0.04). Pasture-type was a significant grouping variable (MRPP, p= 0.002) and axis 3 of the ordination represents pasture-type, although four of the semi-native wetlands grouped with the improved wetlands (Figure 5-8). These semi-native wetlands all contain a population of *Juncus effusus* L., a significant indicator species for improved pasture wetlands, and could be a sign of increased grazing pressure or soil disturbance within those wetlands. Non-native species composition was structured mainly by a perennial non-native forb, *Alternanthera philoxeroides*. *A. philoxeroides* was most abundant in IMP wetlands and was a significant indicator of IMP wetlands (p=0.004). The other abundant non-natives were *Paspalum acuminatum*, *Panicum repens*, and *Hymenachne amplexicaulis* which were distributed among both IMP and SNP wetlands.

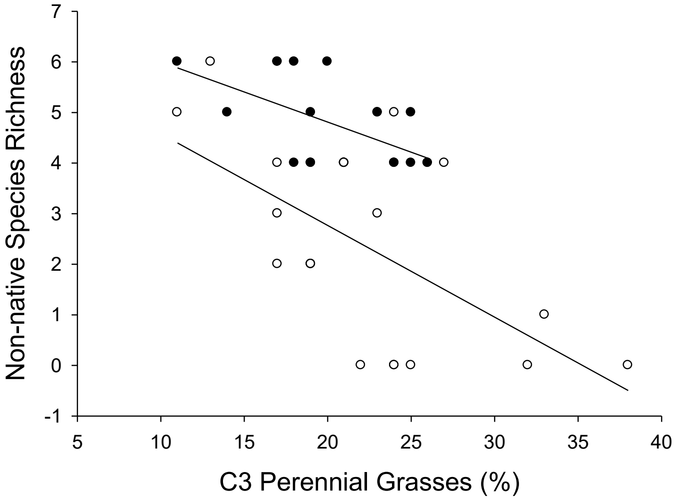

Figure 5-4 Non-native richness is negatively related to C3 perennial grass coverage in both semi-native (white, F=11.54, R^2=0.39, p=0.003) and improved pasture wetlands (black, F=8.01, R^2=0.31, p=0.01).

Discussion

The relationship between resident native plants and invading non-natives may depend on land-use alterations associated with changes in nutrient availabilities, species composition, and disturbance regimes. As we predicted, native richness and non-native richness were negatively correlated within SNP wetlands, but not related in IMP wetlands. This difference in patterns is potentially caused by increased stress for invading non-natives in SNP wetlands due to lower nutrient availability and more intense competition from resident species, while IMP wetlands have ample nutrients from ranch fertilizer regimes and lower competition from resident species due to grazing and soil disturbance. Similarly, Perelman et al. (2007) found that there was no significant association between native richness and non-native richness across mesophyte prairies in Argentinean Flooding Pampas, an area that is subjected to periodic cultivation and intensely grazed by livestock. The intense disturbances in grazed, cultivated lands coupled with increased nutrients may lessen competition from resident native species and allow communities to be invaded by exotic and native ruderal species.

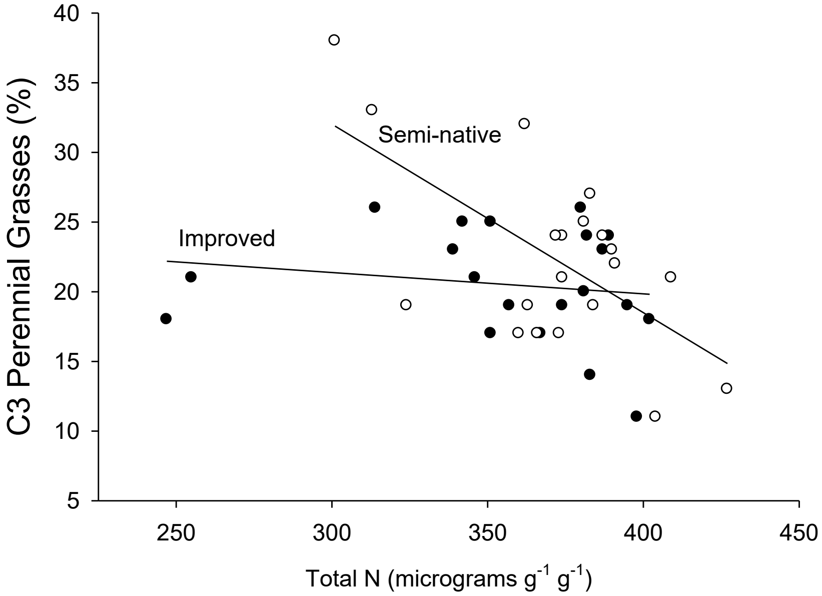

Figure 5-5 C3 perennial grass coverage is negatively related to total N in semi-native pasture wetlands (white, F=12.12, R^2=0.40, p=0.003) but not in improved pasture wetlands (black, F=0.39, R^2=0.02, p=0.54).

Different biotic and abiotic factors were important when predicting non-native richness, non-native frequency, or non-native biomass in these wetlands. In contrast to the positive affect of soil N on non-native species richness, frequency of non-natives in semi-native pasture wetlands was positively related to phosphorus. In general soils with increased nutrients are expected to directly benefit fast-growing, non-native species (Davis et al. 2000). Increased soil N is associated with factors that may decrease native plant growth, such as a loss or negative impact on associated mycorrhizal symbionts (Reynolds et al. 2003), thereby lessening competition from the resident community and causing conditions that might increase wetland invasibility. Increased N has been associated with community invasibility in many other ecosystems such as forests and grasslands (Huenneke et al. 1990, Howard et al. 2004). On the

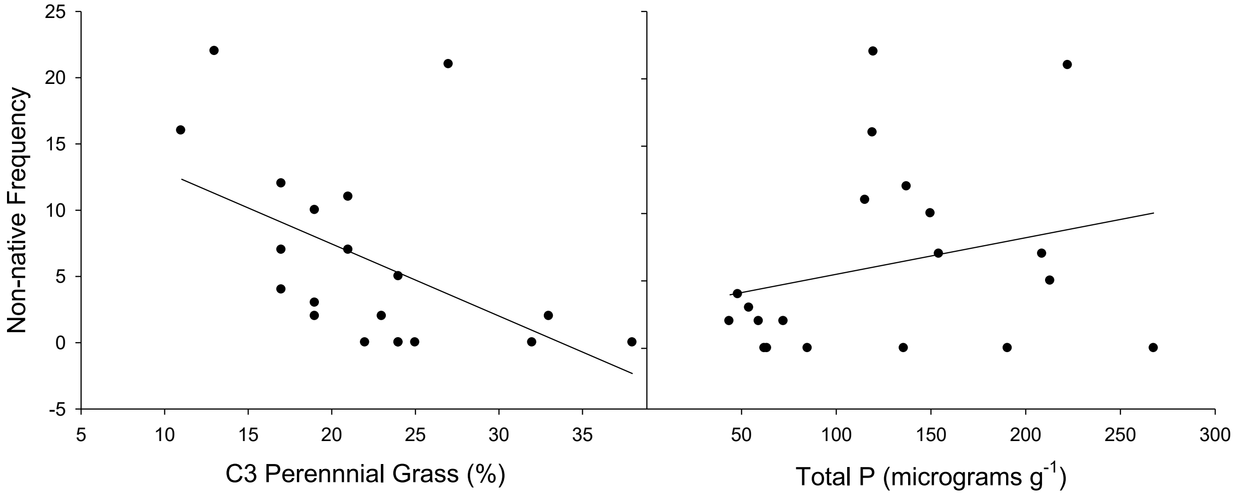

Figure 5-6 Non-native frequency is negatively related to C3 perennial grass coverage (F=7.0, R^2=0.28, p=0.02) but weakly positively related to total P in semi-native pasture wetlands (F=1.27, R^2=0.07, p=0.28).

other hand, soil P is likely associated with increased growth and vigor of the established non-natives leading to higher non-native cover. For example, phosphorus was associated with increased cover of non-natives in Californian vernal pools (Gerhardt & Collinge 2003).

The lack of relationships between non-native species richness and frequency and soil nutrients in IMP wetlands suggests that biotic homogenization may be occurring in IMP wetlands (McKinney & Lockwood 1999, Olden & Rooney 2006). For example, similar to our results, Vellend et al. (2007) found a general decoupling of species composition from environmental gradients in homogenized forests growing on former agricultural fields while there were strong species-environment relationships in ancient forests. The lack of a species-nutrient relationship could indicate that a community is composed mainly of weedy and/or exotic species that do well in enriched environments while more sensitive species may have been eradicated.

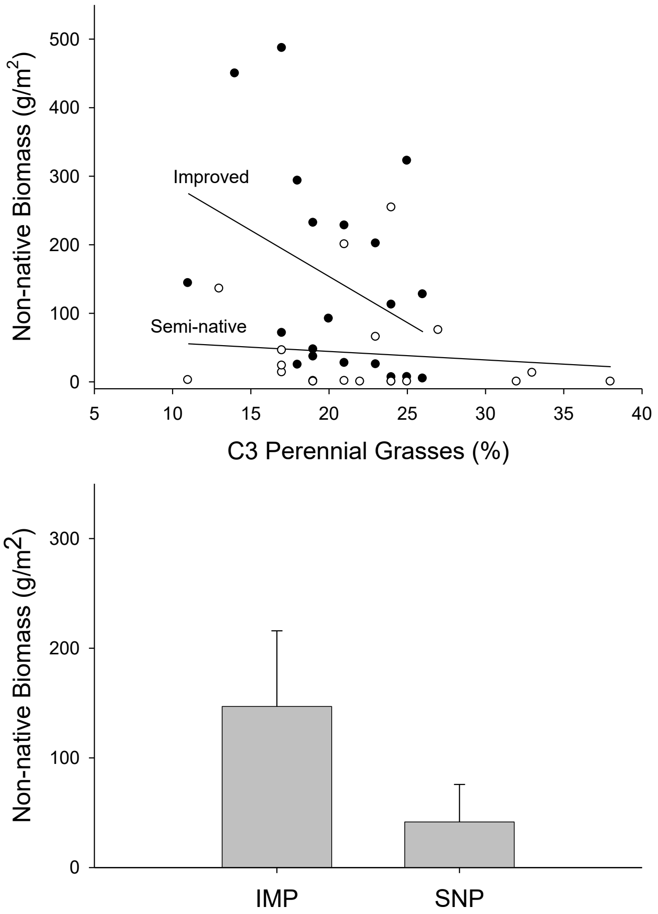

Figure 5-7 a) Non-native biomass is negatively related to C3 perennial grass coverage in both pasture-types (IMP: black and SNP: white). b) Improved pasture wetlands (IMP) contain higher non-native biomass than semi-native pasture wetlands (SNP); means ±95% CI shown.

While soil nutrients differentially influenced non-native richness and abundance, the effect of C3 grasses on non-native richness, abundance, and biomass was consistent. C3 perennial grass abundance was negatively related to non-native richness, frequency and biomass providing strong evidence that competition from native C3 grasses can reduce non-natives. The presence of C3 grass abundance in models of non-native richness, frequency, and biomass are highly suggestive that C3 grass presence may reduce wetland invasion, perhaps by competition. We observed a negative relationship between C3 perennial grass abundance and soil N (Figure 5-3) which suggests either that C3 grasses influence wetland N content by uptake or alternatively that C3 grasses are more abundant when N is low. There has recently been increased interest in determining which particular functional groups resist invasion because of the implications for management and restoration (Funk et al. 2008, Bakker & Wilson 2004). In these wetlands, C3 grasses are a candidate for further investigations as a functional group to constrain invasions, especially in the absence of N fertilization.

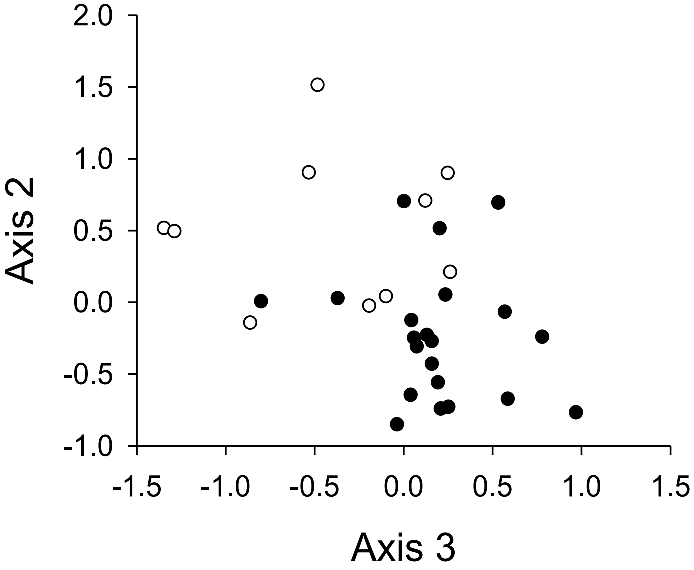

Figure 5-8 NMS Ordination of non-native species composition. Black symbols represent improved pasture wetlands and white symbols represent semi-native pasture wetlands. Each symbol represents one wetland. The closer the symbols are together, the more similar in non-native composition the wetlands are.

In the ordination analysis, we found that non-native communities were weakly structured by pasture-type (Figure 5-8). This structure is caused by the most abundant non-native perennial forb, *Alternanthera philoxeroides*, which is ubiquitous in wetlands embedded within improved pastures, but restricted to only eight wetlands out of 20 sampled within semi-native pastures. In contrast, other non-natives, such as *P. acuminatum* and *H. amplexicaulis* have colonized successfully in many of the semi-native wetlands. Additionally, almost half of the semi-native wetlands sampled did not contain any or only few non-natives. Given that most if not all of these wetlands are connected by seasonal flooding of pastures, it is unlikely that dispersal limitation explains this pattern, although we did find in a previous study that wetland isolation explains 12 % of variation in non-native richness (Boughton et al. Chapter 1). Differences between non-native species requirements may explain these results (see below).

Two previous hypotheses proposed to explain community invasion are plausible based on our results. The first hypothesis concerns empty niches in the community that would create opportunities for functionally different species to invade (Tilman 1982, Shea & Chesson 2002). We found only 10 non-native species in this system and most successful were non-native C4 grasses (Figure 5-2b). In comparison to the composition of the native community dominated by C3 species (Figure 5-2a), it seems plausible that there are empty niches for invading C4 grasses to occupy. However, the pool of available non-native species must be considered (Howard et al. 2004, Perelman et al. 2007) and it is difficult to determine if the success of C4 grasses is due to a larger available pool of non-native C4 grasses in comparison to other groups.

A second hypothesis that could explain observed patterns concerns high resource availability, which may benefit invaders by two different pathways (Davis et al. 2000). One way resource availability can increase in a community when resource uptake by residents goes down due to disturbances such as increased herbivory. Alternatively, gross resource supply could go up via eutrophication. When both increased herbivory and eutrophication occur, a community is particularly vulnerable to invasion (Davis et al. 2000). Improved pasture wetlands, had higher P content and heavy disturbance from intense grazing, and may be expected to have the highest invasibility. In SNP wetlands, where non-native richness and cover increased with higher nutrients the mechanism could be an increased gross supply of resources that the resident vegetation was unable to sequester, therefore creating conditions where competitive intensity decreased between residents and invaders because of unused resources in the wetland. It is possible that non-native species exhibit species-specific responses to increased nutrients. For example, *A. philoxeroides* may be most abundant in IMP wetlands because this species is more sensitive to competitive effects from the resident community, which is relaxed in IMP wetlands because of the combination of increased soil P and high disturbance. Other non-natives (C4 grasses mainly) may be able to colonize both SNP and IMP wetlands because they are not as sensitive to competition from the resident community and may rely less on heavy disturbance.

The main goal of our study was to assess the relative importance of biotic and abiotic drivers of non-native invasions in wetlands embedded in subtropical pastures in order to better understand and work towards preventing invasions. This objective has both ecological and economic benefits: weed growth in rangelands results in decreased forage and costs ~ $6 billion annually in the USA (Mack et al. 2000) and exotic invasions are one of the most important causes of biodiversity loss (Mack et al. 2000). In general, our study showed that in less disturbed semi-native wetlands, the biotic factors, native richness and C3 grass abundance, were equally important to abiotic drivers, soil N and P, in predicting non-native richness and abundance. This suggests that in less disturbed communities that are not directly fertilized but that may receive runoff, competition from the resident community is intense for invaders and any increases in nutrients can result in increased wetland invasibility perhaps through reduced competition. However, in disturbed wetlands with direct fertilization and increased soil disturbance, abiotic factors lose importance while some biotic resistance to invasion remains. In our case, C3 grass abundance still played a role in inhibiting non-natives in IMP wetlands, indicating that one of the first steps to reducing non-natives in these areas could be to encourage native C3 grass growth either by seeding and/or altering fertilization and grazing regimes. However, whether or not C3 grasses prevent or reduce invasion in these wetlands depends largely on the dominant mechanisms promoting invasions (Funk et al. 2008). It is unclear at this stage whether empty niches or high resource availability is causing increases in non-natives. If empty niches are causing invasion, augmenting the native community with both C3 and C4 species may reduce invasion. However, if increased resources play a major role in invasions, efforts will have to be undertaken to reduce nutrients and to prevent further nutrient increases to wetlands.

Acknowledgements

We thank the many people who helped with data collection, including Adam Peterson, Annie Wieler, Katie Windes, Andrew Tweel, Rob Morrison, Kim Medley, David Jenkins, and John Fauth. Lisa McCauley provided valuable GIS support. Funding was provided by USDA and in part by an EPA STAR Fellowship to E. Boughton.

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CHAPTER 6 GENERAL DISCUSSION

Ecologists debate whether stochastic processes or deterministic processes are more important in shaping community composition. Likewise, debates have taken place over which type of interaction is more important, competition or facilitation? However, a paradigm shift is occurring which has moved researchers from asking “which process is more important?” to asking “under which conditions are certain processes more prevalent than others?” Therefore, with context dependence in mind, I studied wetland plant communities in two different land management types and across a gradient of herbivory focusing on the idea that community assembly processes could vary between habitat types and that interactions between plant species could vary in outcome depending on ecological conditions.

Implications for understanding community assembly

My dissertation supports the view that different community assembly processes vary in importance among habitats (Figure 6-1). In my study sites, where land management intensity is an important driver of community composition, I found evidence that community assembly mechanisms differed depending on management intensity. In Chapter 1, I found that native species richness was affected by wetland isolation in semi-native pasture wetlands but weakly related to wetland isolation in improved wetlands (A in Figure 6-1). Additionally, the species-area relationship was stronger in improved wetlands than semi-native wetlands. Together, these results suggest that semi-native wetland vegetation reflected dispersal-based assembly processes, whereas community assembly was filtered in improved wetland vegetation by extreme environmental conditions (fertilization, grazing, and soil disturbance) to tolerant species (B in Figure 6-1).

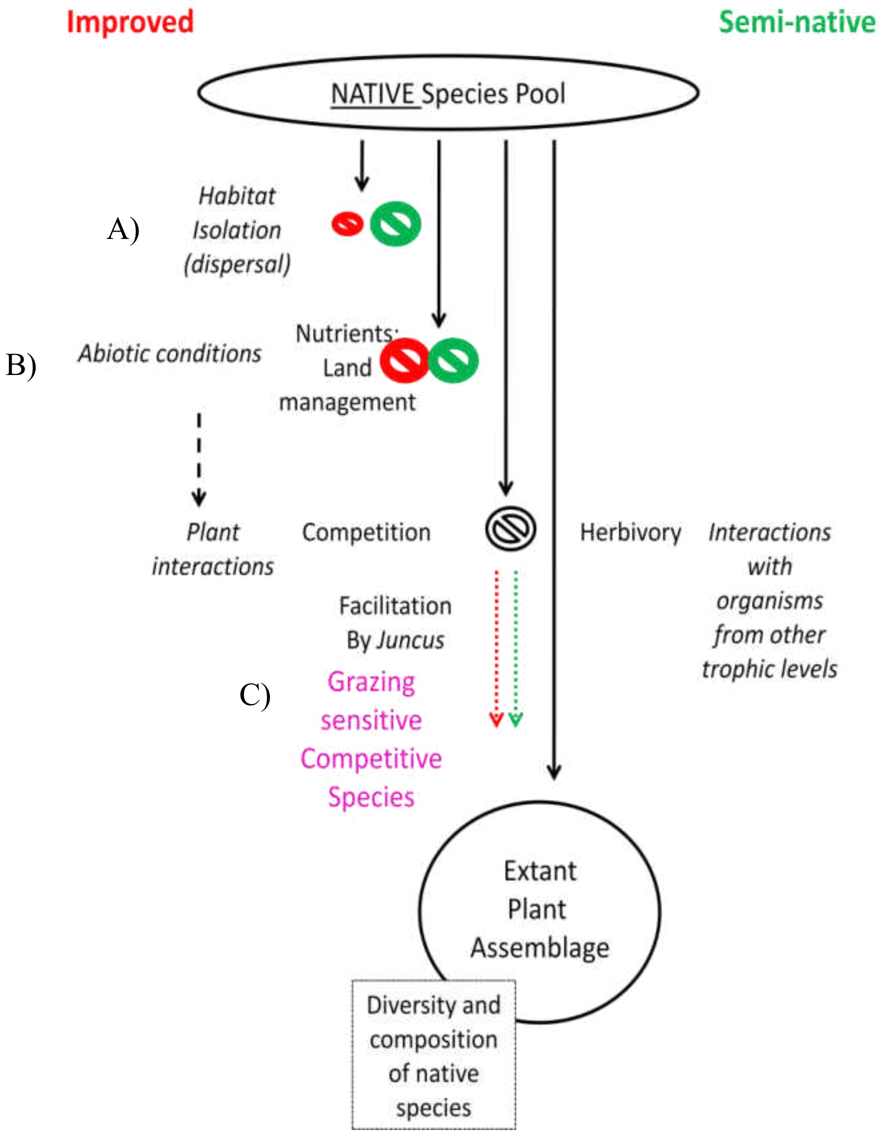

Figure 6-1 The integrated community incorporating the results of my research for the native species pool. A) Habitat isolation had a weak effect in improved pasture wetlands (red symbols) and a strong effect in semi-native wetlands (green symbols). B) Abiotic factors filtered vegetation in different ways depending on pasture-type, with high nutrients and disturbance selecting for weedy species in improved wetlands and low nutrients and less disturbance selecting for conservative native species in semi-native wetlands. C) Facilitation by *Juncus* allowed grazing sensitive, competitive species to pass through the herbivory filter.

While pasture intensification (nutrient enrichment and/or intense grazing) predicted how native species assemblages responded to wetland isolation, this was not true for exotic species richness. Exotic species richness decreased with increasing wetland isolation suggesting some ongoing dispersal limitation for exotics in both pasture-types (A in Figure 6-2). Since exotic species are more likely to be tolerant to disturbance, pasture-intensification does not prevent them from becoming established in improved pastures wetlands as it does for some native species (B in Figure 6-2).

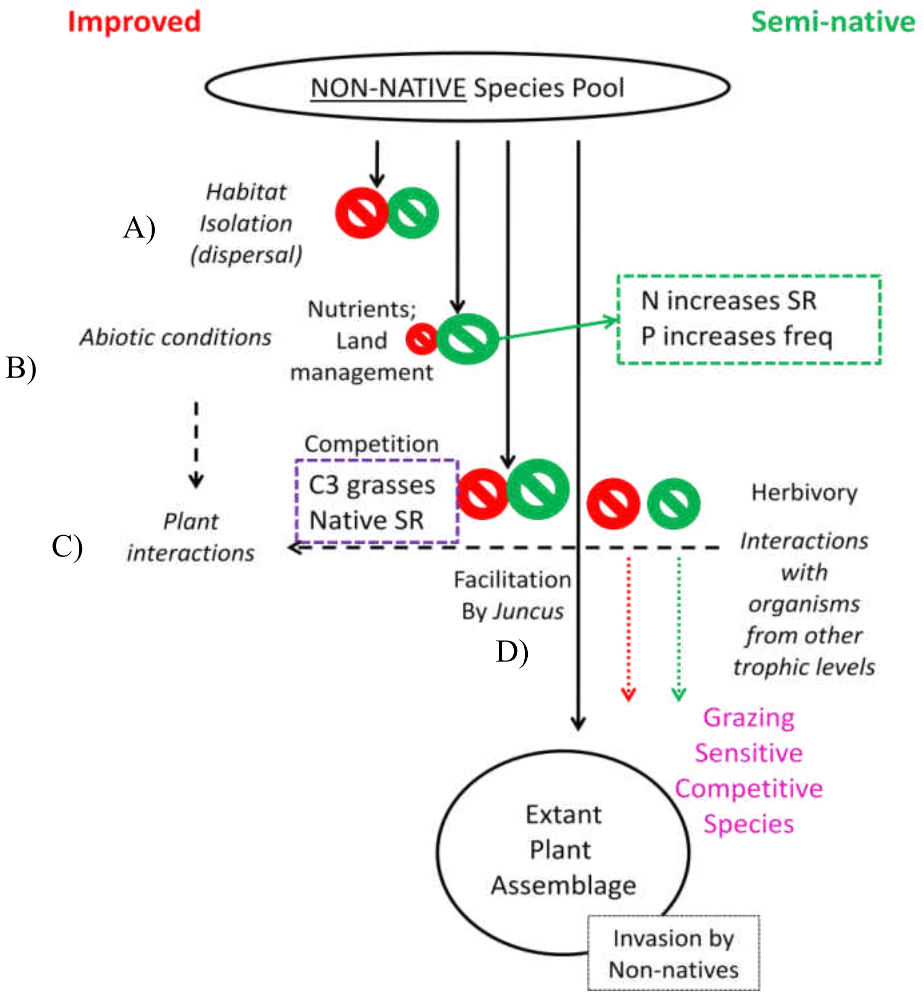

Figure 6-2 The integrated community incorporating the results of my research for the non-native species pool. See text for further details.

In Chapter 4, I found more evidence that community assembly mechanisms differed between the two pasture types in regards to a wetland's susceptibility to invasion by exotic plants. While there were strong positive effects of nutrients on exotic richness in semi-native wetlands, there was no relationship between nutrients and exotics in improved wetlands (B in Figure 6-2). These results suggest that human induced resource enrichment may have removed the abiotic filter that played a role in preventing invasion in these wetlands. However, I found that C3 grasses could possibly be creating a competitive barrier to invasion in both pasture-types (C in Figure 6-2).

Plant interactions along grazing gradients

I found that facilitation was prevalent in grazed conditions, a result in support of Bertness and Callaway's 1994 model. In general, facilitation allowed competitive species of both native and non-native origin to pass through the human induced herbivory filter (C in Figure 6-1 and D in Figure 6-2). However, I found that facilitation did not always increase with increased grazing intensity. In my pairwise interaction experiment, I found that in semi-native wetlands, facilitation intensity actually decreased with increased grazing intensity. This is possibly due to the lower productivity in these wetlands where, as grazing intensity increased and food availability decreased, herbivores became more selective and reduced the refuge effects of *Juncus*. In improved wetlands, facilitation remained high across the grazing intensity gradient and this may be because we did not sample a large enough portion of the gradient in these highly productive wetlands. Therefore, my results suggest that habitat productivity may alter the intensity of plant interactions along ecological stress gradients.

Interestingly, in the community analysis of the refuge effects of *Juncus*, I observed a quadratic relationship between facilitation and grazing intensity, where facilitation peaked at intermediate grazing intensity and there was no significant pasture effect. The difference in results that I observed among the pairwise interaction experiment and the community level analysis could be due to a dilution of species specific effects in community analysis compared to the pairwise experiment because more species were included in the multivariate analysis. As most studies are pairwise interaction experiments, I suggest that those results may not be generalized to entire communities unless many species are included. More studies of plant interactions along ecological gradients are needed that are conducted at the community level to obtain general patterns.

Of my study species in Chapter 2, I found that three of the four were grazing intolerant, competitive species which benefitted from facilitation by *Juncus* while one of the four species was ruderal which did not benefit from facilitation. This finding is in line with studies conducted on abiotic stress gradients. Both natives and exotics benefitted from facilitation as did both grasses and forbs. This suggests that neither morphology nor species origin can be used to predict if a species will exhibit a facilitative response. Useful information for predicting whether a species will be facilitated or not along a stress gradient includes its C-S-R strategy and its tolerance to particular stressors.

In future work, it would be interesting to take a community level approach and test the Michalet et al. 2006 hypothesis (adapted from Grime's diversity-productivity model) that plant diversity will peak at intermediate grazing intensities due to the overlap in incidence of different plant strategies (grazing-intolerant and grazing-tolerant), with facilitation playing an important role in preserving competitive species in the intermediate grazing zone. Although hypotheses are clear for competitive and stress-tolerant species, it is unknown what role ruderal species play in diversity in grazed, productive systems. Future work should determine where ruderal richness will peak along the grazing intensity gradient.

APPENDIX SUPPLEMENTAL TABLES AND FIGURES FOR CHAPTER THREE

Table Appendix- 1. Results of the nutrient and loss-on-ignition analyses among pasture-types and *Juncus* and grazing treatments. Data are mean ± standard deviation. IP: Improved Pasture; SNP: Semi-native Pasture; OM: organic matter.

	P $\mu g^{-1} g^{-1} g$	NH_4^+ $\mu g^{-1} g^{-1} g$	NO_3^- $\mu g^{-1} g$	OM (g)
IP wetlands	8.25 ± 3.52	3.02 ± 1.26	0.51 ± 0.28	1.22 ± 0.41
SNP wetlands	6.82 ± 3.52	5.29 ± 5.71	0.64 ± 0.68	1.56 ± 0.37
Ungrazed	7.12 ± 3.24	3.53 ± 3.05	0.55 ± 0.58	1.41 ± 0.47
Grazed	7.95 ± 3.87	4.77 ± 5.17	0.59 ± 0.47	1.36 ± 0.39
With *Juncus*	7.92 ± 3.42	3.95 ± 3.84	0.72 ± 0.65	1.33 ± 0.40
Without *Juncus*	7.15 ± 3.73	4.35 ± 4.69	0.41 ± 0.29	1.44 ± 0.45
Ungrazed w/ *Juncus*	7.80 ± 3.33	4.13 ± 4.10	0.72 ± 0.75	1.29 ± 0.43
Ungrazed w/o *Juncus*	6.44 ± 3.12	2.92 ± 1.30	0.39 ± 0.29	1. 53 ± 0.48
Grazed w/*Juncus*	8.05± 3.60	3.77 ± 3.69	0.74 ± 0.55	1.38 ± 0.39
Grazed w/o *Juncus*	7.86 ± 4.24	5.78 ± 6.28	0.44 ± 0.30	1.34 ± 0.40

Table Appendix- 2. ANOVA results for effect of treatments and pasture-type on nutrients.

		P $\mu g^{-1} g^{-1} g$			NH_4^+ $\mu g^{-1} g^{-1} g$			NO_3^- $\mu g^{-1} g$		
	Df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Juncus (J)	1	0.11	0.72	0.40	0.82	1.65	0.20	**0.45**	**6.23**	**0.02**
Grazing (G)	1	0.17	1.08	0.30	0.77	1.56	0.22	0.02	0.31	0.58
Pasture (P)	1	**0.63**	**4.00**	**0.05**	1.32	2.66	0.11	0.02	0.27	0.61
J x G	1	0.29	1.84	0.18	0.12	0.25	0.62	0.00	0.02	0.89
J x P	1	0.00	0.05	0.83	0.23	0.46	0.50	0.21	2.97	0.09
G x P	1	0.02	0.12	0.73	0.09	0.18	0.68	0.03	0.45	0.50
J x G x P	1	0.33	2.08	0.16	0.11	0.21	0.65	0.02	0.22	0.64
Residuals	56	0.16			0.50			0.07		

Table Appendix- 3. AIC values and model weights of the models tested for predicting species survivals. J= Juncus, G=Grazing, P=Pasture, DAP=Total P, I= Initial height. (x) denotes main effects and all interactions between factors.

	Alternanthera		*Diodia*		*P. hemitomon*		*P. repens*	
Model	AIC	weight	AIC	weight	AIC	weight	AIC	weight
J x G x P x DAP	261.4	0.003	183.0	0.06	267.8	0.01	314.1	0.13
J x G x P x NO3	**250.5**	**0.79**	180.6	0.19	274.4	0.00	312.9	0.23
J x G x P x I	256.5	0.04	180.7	0.18	**259.4**	**0.84**	**311.1**	**0.59**
J x G x P x NH4	259.0	0.01	191.2	0.00	263.1	0.13	317.5	0.02
J x G x P	253.8	0.15	**178.4**	**0.57**	267.3	0.02	317.4	0.03

Table Appendix- 4. AIC values and model weights of the models tested for predicting species aboveground biomass .J= Juncus, G=Grazing, P=Pasture, DAP=Total P, I= Initial height. (x) denotes main effects and all interactions between factors.

	Alternanthera		*Diodia*		*P. hemitomon*		*P. repens*	
Model	AIC	weight	AIC	weight	AIC	weight	AIC	weight
J x G x P x DAP	45.9	0.008	55.6	0.00	165.8	0.00	93.1	0.05
J x G x P x NO3	43.6	0.03	66.9	0.00	167.9	0.00	98.4	0.00
J x G x P x I	41.4	0.07	**38.4**	**0.99**	159.9	0.04	96.7	0.00
J x G x P x NH4	46.9	0.005	55.5	0.00	155.2	0.45	90.4	0.21
J x G x P	**36.46**	**0.88**	52.6	0.00	**155.0**	**0.50**	**87.9**	**0.72**

Table Appendix- 5. AIC values and model weights of the models tested for predicting species aboveground biomass within the clipping experiment.J= Juncus, C=Clipping, P=Pasture, DAP=Total P, I= Initial height. (x) denotes main effects and all interactions between factors.

	Alternanthera		*Diodia*		*P. hemitomon*		*P. repens*	
Model	AIC	weight	AIC	weight	AIC	weight	AIC	weight
J x C x P x DAP	93.94	0.01	96.29	0.00	205.05	0.00	**129.58**	**0.46**
J x C x P x NH4	96.60	0.00	76.15	0.00	**192.16**	**0.62**	131.96	0.14
J x C x P x NO3	92.93	0.02	99.30	0.00	204.09	0.00	141.29	0.00
J x C x P x I	95.74	0.00	**86.54**	**0.98**	204.23	0.00	138.94	0.00
J x C x P	**85.17**	**0.96**	96.29	0.00	193.19	0.37	129.94	0.39

Table Appendix- 6. AIC values and model weights of the models tested for predicting species survival within the clipping experiment. J= Juncus, C=Clipping, P=Pasture, DAP=Total P, I= Initial height. (x) denotes main effects and all interactions between factors.

	Alternanthera		*Diodia*		*P. hemitomon*		*P. repens*	
Model	AIC	weight	AIC	weight	AIC	weight	AIC	weight
J x C x P x DAP	212.80	0.00	151.71	0.00	182.86	0.38	**216.85**	**0.83**
J x C x P x NH4	211.97	0.00	154.14	0.00	186.48	0.06	222.87	0.04
J x C x P x NO3	205.05	0.14	140.31	0.44	190.14	0.01	226.75	0.01
J x C x P x I	214.53	0.00	151.54	0.00	185.67	0.09	221.05	0.10
J x C x P	**201.42**	**0.85**	**139.80**	**0.56**	**182.47**	**0.46**	223.80	0.03

Table Appendix- 7. ANOVA table of the RII results, comparing how interactions with *Juncus* change depending on grazing or clipping treatment. Grazing significantly alters RII with *Juncus*, resulting in positive values in grazed areas and negative values in ungrazed areas. Clipping had no effect on interactions of the transplants with *Juncus* within the exclosures. NF=native forb; NNF=non-native forb; NG=native grass; NNG=non-native grass. Values in bold are significant.*The residual values for *Diodia*, *Alternanthera*, *P.hemitomon*, and *P.repens*, respectively. Residual values differ due to different numbers of missing values among species (RII was not able to be calculated for treatments that contained all dead plants).

		Diodia (NF)			*Alternanthera* (NNF)			*P. hemitomon* (NG)			*P. repens* (NNG)		
	Df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Pasture (P)	1	0.09	0.16	0.69	0.05	0.19	0.67	0.01	0.05	0.83	0.01	0.03	0.86
Grazing (G)	1	0.20	0.36	0.56	**4.95**	**20.2**	**<0.001**	**8.13**	**28.2**	**<0.001**	**5.25**	**15.5**	**<0.001**
P x G	1	0.26	0.45	0.51	0.16	0.64	0.43	0.00	0.01	0.94	0.09	0.26	0.62
Residuals	21,28,28,25 *	0.57			0.24			0.29			0.34		
Pasture (P)	1	0.71	1.31	0.27	0.03	0.06	0.81	0.02	0.07	0.79	0.03	0.11	0.91
Clip (C)	1	1.28	2.37	0.14	0.71	1.55	0.22	0.00	0.02	0.89	0.00	0.01	0.74
P x C	1	0.03	0.06	0.81	0.16	0.34	0.56	0.04	0.15	0.70	0.04	0.12	0.73
Residuals	21,26,27,23 *	0.54			0.46			0.26			0.29		

Table Appendix- 8. Effect sizes of treatments on *P. hemitomon* survival. NG= native grass.

	P. hemitomon (NG)			
	Est.	Std. Error	z	*Pr(>|z|)*
Initial Ht (I)	0.03	0.09	0.32	0.75
Juncus (J)	1.42	6.48	0.22	0.83
Grazing (G)	0.33	4.44	0.07	0.94
Pasture (P)	14.34	5.66	2.54	0.01
I x J	-0.07	0.14	-0.47	0.64
I x G	0.00	0.10	0.04	0.97
J x G	-5.15	7.87	-0.66	0.51
I x P	-0.30	0.12	-2.46	0.01
J x P	-16.57	11.62	-1.43	0.15
G x P	6.45	9.76	0.66	0.51
I x J x G	0.19	0.17	1.07	0.28
I x J x P	0.36	0.24	1.48	0.14
I x G x P	-0.10	0.20	-0.50	0.61
J x G x P	5.21	15.97	0.33	0.74
I x J x G x P	-0.15	0.33	-0.47	0.64

Table Appendix- 9. Effect sizes of treatments on *P. repens* survival. NNG= Non-native grass.

	P. repens (NNG)			
	Est.	Std. Error	z	*Pr(>|z|)*
Initial Ht (I)	0.00	0.08	-0.03	0.98
Juncus (J)	0.12	4.00	0.03	0.98
Grazing (G)	-5.35	3.38	-1.59	0.11
Pasture (P)	3.21	6.07	0.53	0.60
I x J	-0.02	0.13	-0.13	0.90
I x G	0.17	0.10	1.59	0.11
J x G	6.73	4.95	1.36	0.17
I x P	-0.06	0.17	-0.35	0.72
J x P	-7.85	8.43	-0.93	0.35
G x P	-8.40	8.13	-1.03	0.30
I x J x G	-0.14	0.15	-0.93	0.35
I x J x P	0.20	0.24	0.85	0.40
I x G x P	0.19	0.23	0.84	0.40
J x G x P	1.51	11.04	0.14	0.89
I x J x G x P	-0.02	0.31	-0.07	0.94

Table Appendix- 10. Effect sizes of treatments on *Alternanthera* survival. NNF=non-native forb.

	Alternanthera (NNF)			
	Est.	Std. Error	z	*Pr(>|z|)*
NO3 (N)	8.42	3.54	2.38	0.02
Juncus (J)	2.04	1.71	1.19	0.23
Grazing (G)	3.75	1.66	2.26	0.02
Pasture (P)	3.07	1.67	1.84	0.07
N x J	-6.77	3.88	-1.74	0.08
N x G	-8.27	3.70	-2.23	0.03
J x G	-1.79	1.88	-0.95	0.34
N x P	-8.04	3.62	-2.22	0.03
J x P	-4.91	2.16	-2.27	0.02
G x P	-4.72	1.81	-2.60	0.01
N x J x G	7.29	4.27	1.71	0.09
N x J x P	10.13	4.66	2.17	0.03
N x G x P	9.71	3.84	2.53	0.01
J x G x P	6.28	2.39	2.63	0.01
N x J x G x P	-13.44	5.25	-2.56	0.01

Table Appendix- 11. Effect sizes of treatments on *Diodia* survival. NF=native forb.

	Diodia (NF)			
	Est.	Std. Error	z	*Pr(>|z|)*
Juncus (J)	0.37	0.61	0.60	0.55
Grazing (G)	0.37	0.61	0.60	0.55
Pasture (P)	-0.24	0.70	-0.35	0.73
J x G	1.08	0.78	1.39	0.17
J x P	0.65	0.87	0.75	0.46
G x P	0.39	0.89	0.44	0.66
J x G x P	-1.43	1.11	-1.29	0.20

Table Appendix- 12. Effect sizes of treatments on biomass. NNF=non-native forb, NNG= non-native grass, NG=native grass.

	Alternanthera (NNF)			*P.repens* (NNG)			*P. hemitomon* (NG)		
	Est.	t	*Pr(>|t|)*	Est.	t	*Pr(>|t|)*	Est.	t	*Pr(>|t|)*
Juncus (J)	-0.11	-0.78	0.44	-0.11	-0.52	0.61	-0.45	-1.2	0.24
Grazing (G)	0.46	3.61	0.003	0.24	1.07	0.29	0.91	2.41	0.02
Pasture (P)	-0.01	-0.03	0.97	0.05	0.22	0.83	0.21	0.56	0.58
J x G	0.21	0.97	0.33	0.60	1.90	0.06	1.11	2.08	0.04
J x P	0.01	0.05	0.96	-0.09	-0.29	0.77	-0.10	-0.19	0.85
G x P	-0.29	-1.38	0.17	-0.18	-0.57	0.57	-0.11	-0.19	0.84
J x G x P	-0.01	-0.05	0.96	0.14	0.31	0.77	0.46	0.60	0.55

Table Appendix- 13. Effect sizes of treatments on biomass of *Diodia*. NF=native forb.

	Diodia (NF)			
	Est.	Std. Error	z	*Pr(>|t|)*
Initial Ht (I)	0.00	0.03	0.18	0.86
Juncus (J)	0.09	0.86	0.11	0.92
Grazing (G)	0.20	0.94	0.22	0.83
Pasture (P)	-0.73	0.99	-0.74	0.46
I x J	0.00	0.04	-0.05	0.96
I x G	-0.01	0.04	-0.22	0.82
J x G	-0.33	1.24	-0.27	0.79
I x P	0.03	0.04	0.74	0.46
J x P	-1.07	1.54	-0.70	0.49
G x P	-2.00	1.37	-1.46	0.15
I x J x G	0.03	0.05	0.60	0.55
I x J x P	0.04	0.06	0.72	0.48
I x G x P	0.08	0.06	1.49	0.14
J x G x P	5.08	2.08	2.44	0.02
I x J x G x P	-0.21	0.08	-2.53	0.01

Table Appendix- 14. Means and standard deviations of survival and biomass in clipped and unclipped plots.

	Alternanthera	*Diodia*	*P. hemitomon*	*P. repens*
	Mean±St.Dev	Mean±St.Dev	Mean ±St.Dev	Mean±St.Dev
Clipped (survival)	1.88±1.07	0.84±0.81	3.06 ±1.22	1.84±1.51
Not clipped (survival)	1.84±1.25	0.75±0.84	2.72 ±1.30	1.63 ±1.34
Clipped (biomass(g))	0.95 g ±1.04	0.64 g ±1.11	10.66 g ±11.96	1.80 g ±2.59
Not clipped (biomass(g))	0.76 g ±0.80	0.39 g ±1.04	9.58 g ±10.87	1.41 g ±1.71

Table Appendix- 15. ANOVA table of survival results in the clipping experiment. NNF=non-native forb; NF= native forb; NG = native grass. Values in bold are significant. Dev.= Deviance, Resid. Dev.= Residual deviance, Resid. Df = Residual Df.

			Alternanthera (NNF)		*Diodia* (NF)		*P.hemitomon* (NG)	
	Df	Resid. Df	*Dev.*	*P*	*Dev.*	*P*	*Dev.*	*P*
Juncus (J)	1	62	1.90	0.17	**11.07**	**0.001**	**24.69**	**<0.001**
Clip (C)	1	61	0.02	0.90	0.23	0.63	2.61	0.11
Pasture (P)	1	60	**8.42**	**0.004**	1.26	0.26	2.64	0.10
J x C	1	59	1.32	0.25	**4.20**	**0.04**	1.95	0.16
J x P	1	58	0.12	0.73	1.34	0.25	0.25	0.62
C x P	1	57	0.75	0.39	3.11	0.08	2.32	0.13
J x C x P	1	57	0.01	0.91	0.64	0.42	**5.69**	**0.02**
Resid. Dev.		56	94.2		59.62		110.13	

Table Appendix- 16. ANOVA table of *P.repens* survival results in the clipping experiment. NNG = non-native grass. Values in bold are significant. DAP = Soil Phosphorus, Dev.= Deviance, Resid. Dev.= Residual deviance, Resid. Df = Residual Df.

	P. repens (NNG)			
	Df	Resid. Df	Dev.	*P*
DAP (Ph)	1	62	**20.68**	**<0.001**
Juncus (J)	1	61	**13.26**	**<0.001**
Clip (C)	1	60	0.89	0.34
Pasture (P)	1	59	0.71	0.40
Ph x J	1	58	2.98	0.09
Ph x C	1	57	0.00	0.65
J x C	1	56	0.34	0.56
Ph x P	1	55	1.59	0.21
J x P	1	54	0.01	0.94
C x P	1	53	2.14	0.14
Ph x J x C	1	52	0.02	0.89
Ph x J x P	1	51	2.74	0.10
Ph x C x P	1	50	**4.31**	**0.04**
J x C x P	1	49	1.60	0.21
Ph x J x C x P	1	48	0.32	0.57

Table Appendix- 17. ANOVA table of the biomass results in the clipping experiment for *Alternanthera*. NNF=non-native forb. Values in bold are significant.

	Alternanthera (NNF)			
	Df	MS	*F*	*P*
Juncus (J)	1	0.11	0.59	0.45
Clip (C)	1	0.11	0.55	0.46
Pasture (P)	1	**1.63**	**8.52**	**0.01**
J x C	1	0.06	0.29	0.59
J x P	1	0.00	0.01	0.91
C x P	1	0.01	0.04	0.85
J x C x P	1	0.01	0.03	0.86
Resid.s	53	0.19		

Table Appendix- 18. ANOVA table of the biomass results in the clipping experiment for *Diodia*. NF= native forb. Values in bold are significant.

	Diodia (NF)			
	Df	MS	*F*	*P*
Initial Ht (I)	1	**0.83**	**5.54**	**0.02**
Juncus (J)	1	**1.49**	**9.89**	**0.003**
Clip (C)	1	0.20	1.36	0.25
Pasture (P)	1	0.00	0.00	0.98
I x J	1	**0.59**	**3.95**	**0.05**
I x C	1	0.01	0.09	0.76
J x C	1	**0.99**	**6.63**	**0.01**
I x P	1	0.12	0.78	0.38
J x P	1	0.03	0.23	0.64
C x P	1	0.11	0.76	0.39
I x J x C	1	0.17	1.10	0.30
I x J x P	1	**1.55**	**10.24**	**0.002**
I x C x P	1	0.17	1.15	0.29
J x C x P	1	0.03	0.19	0.66
I x J x C x P	1	0.06	0.42	0.52
Residuals	48	0.15		

Table Appendix- 19. ANOVA table of the biomass results in the clipping experiment for *P.hemitomon*. NG= native grass. Values in bold are significant.

	P.hemitomon (NG)			
	Df	MS	*F*	*P*
NH4 (N)	1	**4.48**	**4.85**	**0.03**
Juncus (J)	1	**10.35**	**11.20**	**0.002**
Clip (C)	1	0.27	0.29	0.59
Pasture (P)	1	1.68	1.81	0.18
N x J	1	2.17	2.35	0.13
N x C	1	0.11	0.12	0.73
J x C	1	0.01	0.01	0.93
N x P	1	**4.50**	**4.87**	**0.03**
J x P	1	0.52	0.56	0.46
C x P	1	0.78	0.84	0.36
N x J x C	1	0.00	0.00	0.97
N x J x P	1	0.93	1.00	0.32
N x C x P	1	1.65	1.78	0.19
J x C x P	1	0.00	0.00	0.98
N x J x C x P	1	0.37	0.40	0.53
Residuals	48	0.92		

Table Appendix- 20. ANOVA table of the biomass results in the clipping experiment for *P.repens*. NNG= non-native grass. DAP = Soil Phosphorus. Values in bold are significant.

	P.repens (NNG)			
	Df	MS	*F*	*P*
DAP (Ph)	1	**2.49**	**7.16**	**0.01**
Juncus (J)	1	**2.72**	**7.83**	**0.01**
Clip (C)	1	0.08	0.24	0.63
Pasture (P)	1	0.39	1.12	0.30
Ph x J	1	0.03	0.09	0.77
Ph x C	1	0.01	0.03	0.86
J x C	1	0.07	0.20	0.66
Ph x P	1	0.54	1.54	0.22
J x P	1	0.23	0.67	0.42
C x P	1	0.04	0.11	0.75
Ph x J x C	1	0.01	0.04	0.84
Ph x J x P	1	1.23	3.54	0.07
Ph x C x P	1	1.26	3.62	0.06
J x C x P	1	0.09	0.26	0.61
Ph x J x C x P	1	0.11	0.30	0.59
Residuals	48	0.35		

Figure Appendix- 1**.** Relative facilitation of *Juncus* on survival of the beneficiary species. *P.hemitomon* and *Alternanthera* show the greatest benefit to survival from *Juncus*, while *Diodia* survival is negatively affected by *Juncus*. *P.repens* did not differ from any of the species. Data are mean ± standard deviation.

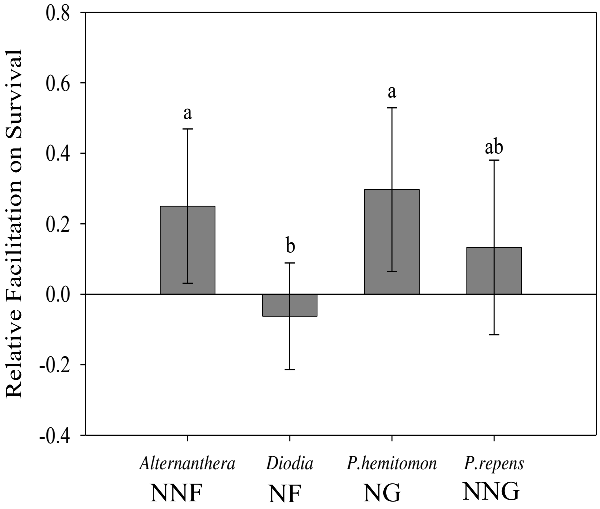

Fig.