

Differential facilitative and competitive effects of a dominant macrophyte in grazed subtropical wetlands

Elizabeth H. Boughton^{1*}, Pedro F. Quintana-Ascencio², Patrick J. Bohlen² and David Nickerson³

¹MacArthur Agro-Ecology Research Center, 300 Buck Island Ranch Road, Lake Placid, FL 33852, USA; ²Department of Biology, University of Central Florida, Orlando, FL 32816-2368, USA; and ³Department of Statistics and Actuarial Science, University of Central Florida, Orlando, FL 32816, USA

Summary

1. Plant–plant interactions fluctuate between competition and facilitation depending upon ecological conditions and species traits. Facilitative interactions are expected to increase in frequency via associational defences with increasing consumer pressure. The ability of species to cope with competition and/or ecological stressors may alter the outcome of plant–plant interactions.
2. We conducted a transplant experiment to determine if native and non-native grasses and forbs respond similarly to interactions with *Juncus effusus* L., an unpalatable benefactor species, along a grazing intensity gradient in two contrasting pasture types: intensively managed and semi-natural. We expected competitive taller, erect species (grasses) and non-natives to obtain stronger facilitative effects than shorter, sprawling species (forbs) and natives.
3. We transplanted two species each of grasses and forbs, including one native and one non-native species of each, into 1-m² grazed plots or enclosure plots either with or without *Juncus* in wetlands experiencing a range of grazing intensities across the two pasture types.
4. Interactions with *Juncus* varied from facilitation in grazed plots to competition in enclosures for the two grasses and the non-native forb. *Juncus* did not facilitate the native forb in grazed conditions. Grazing negatively influenced all species. For the grasses, facilitation intensity increased as grazing intensity increased, while facilitation intensity for forbs was unrelated to grazing intensity.
5. *Synthesis.* Facilitation intensity of a dominant macrophyte increased with consumer pressure for tall grasses in productive subtropical wetlands. These findings suggest that facilitation can allow native and non-native species to pass through human-induced ecological filters, such as cattle grazing, with implications for understanding forces governing community composition in disturbed environments.

Key-words: associational resistance, grazing refuge, herbivory, indirect facilitation, *Juncus effusus*, plant–herbivore interactions, 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 & Callaway 1994; Callaway & Walker 1997; Holmgren, Scheffer & Huston 1997; Smit *et al.* 2007; Crain 2008). In addition, the strength and direction of plant interactions is in part determined by traits of the interacting species such as the ability to tolerate particular stressors or disturbance (Liancourt, Callaway & Michalet 2005; Maestre

et al. 2009). A model proposed by Bertness & Callaway (1994) provides a conceptual foundation for generating predictions about the outcome of plant interactions under varying productivity and consumer pressure.

A main component of the Bertness & Callaway model is the stress gradient hypothesis (SGH), which hypothesizes that the frequency of positive interactions between plants will increase as physical stress increases. While many studies show results in support of this prediction, other studies have found that when extreme levels of stress are present, the frequency of positive interactions may gradually decrease, as benefactor species no longer ameliorate conditions for other species (Michalet *et al.* 2006). The other prediction of the model, that associational defences (protection from herbivory) increase in frequency as

*Correspondence author. E-mail: eboughton@archbold-station.org

consumer pressure increases, is less studied. However, unpalatable plants have been found to protect an array of species in grazed ecosystems ranging from marine areas, deserts, marshes and meadows to shrublands (Hay 1986; Rebollo *et al.* 2002; Callaway *et al.* 2005) with a diversity of grazers (invertebrates, Hambach, Agren & Ericson 2000; Alberti *et al.* 2008, and vertebrates, Hay 1986; Callaway *et al.* 2005; Rebollo *et al.* 2002). Experimental studies investigating biotic stress gradients suggest that protection from herbivory may wane as consumer pressure increases and herbivores become increasingly less selective. When herbivores are less selective, the effectiveness of the benefactor declines. This scenario would result in a hump-shaped relationship between stress and facilitation (Graff, Aguiar & Chaneton 2007; Smit *et al.* 2007; Levenbach 2009). In other words, unpalatable species (benefactors) provide protection from herbivory to palatable species (beneficiaries) as grazing pressure increases up to a point, but become less effective at providing protection at very high grazing intensity.

Studies incorporating several species have also found that co-occurring species may not show the same response to a common neighbour under the same environmental conditions, suggesting that species differ in their response to stress or in their competitive ability. Therefore, it is important to account for species traits when examining plant–plant interactions along gradients (Liancourt, Callaway & Michalet 2005; Baraza, Zamora & Hodar 2006; Osem, Perevolotsky & Kigel 2007; Crain 2008; Pihlgren & Lennartsson 2008). For example, Pihlgren & Lennartsson (2008) found that tall species (grasses) were more likely than short species to obtain protection from grazing from shrubs in semi-native pastures because they were better able to compete for light. In disturbed systems, such as managed grazing lands, refuge provided by benefactor species may improve restoration efforts, especially vegetation regeneration (Pihlgren & Lennartsson 2008). However, if undesirable non-native species take advantage of facilitation from benefactor species, then using facilitative relationships as a basis for restoration may be counterproductive in some cases (Badano *et al.* 2007; Bulleri, Bruno & Benedetti-Cecchi 2008).

In this study, we considered the effect of an unpalatable plant on variation in vital rates (survival and growth) of plants of different functional groups (grasses and forbs) and origins (native and non-native) in wetlands embedded in pastures of differing grazing intensity, including ungrazed exclosures. The wetlands were dominated by the native, unpalatable, tussock-forming perennial rush species, *Juncus effusus* L., with many species co-existing within the *Juncus* tussock, suggesting a potential for facilitation. While previous studies used grazing intensity classes, we took a different approach and used actual levels of grazing on a cattle ranch. We selected numerous wetlands across an entire ranch to sample a range of grazing intensities across two pasture types: semi-natural (SNP) and intensively managed (IP) (Steinman *et al.* 2003; Boughton, Quintana-Ascencio & Bohlen 2011). We tested whether interactions with *Juncus* varied along the grazing intensity gradient and hypothesized that interactions would range from competi-

tion in non-grazed exclosures to facilitation in intensely grazed areas. We predicted that facilitative effects of *Juncus* would differ depending on the functional group of the beneficiary species with taller species (grasses) obtaining stronger facilitative effects than the shorter species (forbs). To account for potential environmental factors that could influence the outcome of our experiment, we measured soil nutrients (nitrogen and phosphorus) and organic matter and assessed their effect as covariates.

Materials and 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, USA (27°09' N, 81°11' W). MAERC is located at Buck Island Ranch (BIR), a 4252-ha commercial cattle ranch. The climate is subtropical with a mean annual temperature of 22 °C and a summer maximum of 33 °C. Mean annual precipitation is 1300 mm, of which 69% falls during the wet season (June–October).

The ranch has *c.* 630 isolated, mostly small seasonal wetlands embedded throughout the property. Approximately half of the land area of BIR is occupied by IPs and the other half is occupied by less intensively managed SNPs. IPs composed primarily of Bahia grass (*Paspalum notatum* Flueggé), an introduced forage grass, are usually fertilized annually with N (*c.* 2 kg ha⁻¹), and were historically fertilized annually also with P (1960s–1986, 40 kg P₂O₅ ha⁻¹). 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. Grazing intensity across the ranch varies but is usually higher in IPs than SNPs. For the period of this study (January 2006–December 2007), average stocking rate was 1.08 cows ha⁻¹ in IPs (range: 0.57–1.7 cows ha⁻¹) and 0.59 cows ha⁻¹ in SNPs (range: 0.15–1.12 cows ha⁻¹).

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 *J. effusus* L. var. *solutus* Fernald & Wiegand (hereafter *Juncus*), a native tussock-forming species that cattle generally avoid, and wetland centres are dominated by emergent vegetation (e.g. *Pontederia cordata* L.). Cattle rarely graze *Juncus* and preferentially graze between *Juncus* tussocks (Humphrey & Patterson 2000). In SNPs, *Juncus* is much less abundant; it exists mainly in more disturbed wetlands (i.e. along canals or roads). The presence of *Juncus* depends to some degree on selective cattle grazing of surrounding species; in 5-year grazing exclosures within IPs, *Juncus* declined significantly while *Panicum hemitomon* Schult. and other wetland grasses became dominant (Tweel & Bohlen 2008). In a separate study, peak standing biomass in fenced wetlands was estimated at *c.* 720 ± 378 g m⁻² in IP wetlands and *c.* 400 ± 185 g m⁻² in SNP wetlands, and in unfenced grazed wetlands it was *c.* 345 ± 172 g m⁻² and *c.* 280 ± 76 g m⁻² in IP and SNP wetlands respectively (Bohlen & Quintana-Ascencio, unpublished data).

EXPERIMENTAL DESIGN

Our experiment was a split-split plot design with three factors: pasture type (IMP versus SNP), grazing (grazed or exclosure) and *Juncus* (*Juncus* or no *Juncus*). We divided the property into eight blocks, and within each block we randomly chose one IP wetland and one SNP

wetland from wetlands containing significant populations of *Juncus*. At each wetland, we chose two random directions from eight possible directions radiating from the centre of the wetland (N, NE, E, SE, S, SW, W and NW), and positioned two 4 × 2-m plots in the selected areas. Each 4 × 2-m plot consisted of a 2 × 2-m grazed subplot and a 2 × 2-m non-grazed exclosure subplot, each of which was further subdivided into four 1-m² 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. There were a total of 32 exclosures (2 pasture-types × 8 wetlands × 2 exclosures per wetland). Within each experimental grazing subplot, each 1-m² quadrat was assigned randomly as either a *Juncus* or non-*Juncus* treatment. Average diameter of *Juncus* was 0.85 m. The non-*Juncus* treatment involved removing all *Juncus* plants from selected quadrats using a machete and large clippers, and clipping *Juncus* repeatedly during two subsequent visits until no resprouts were found. *Juncus* was completely removed from non-*Juncus* plots within 2 weeks of clipping.

We collected 800 plants each of four common species for use in the transplant experiment: *P. hemitomon* (native grass), *Panicum repens* L. (non-native grass), *Diodia virginiana* L. (native forb) and *Alternanthera philoxeroides* (C.F.P. Martius) Griseb. (non-native forb). The grasses are tall: *P. hemitomon* grows 0.5–1.8 m tall and *P. repens* grows to 1 m (Hitchcock 1951; Leithead, Yarlett & Shiflet 1971). The forbs are short, sprawling species, with *A. philoxeroides* growing 1–2 m horizontally (eFloras, 2008) and *D. virginiana* 0.15–0.45 m (United States Geological Survey 2003). Collections took place during February and March 2006 in three different wetlands on the ranch property. Prior to transplanting we collected each species by digging up plants and placing one ramet into a 30.5-cm pot along with soil from the collection area. Each individual ramet was given an aluminium tag number with a wire flag. All collected plants were kept in pots and watered daily for 3 months until July 2006, when they were transplanted into the experimental plots.

We transplanted two individuals each of the four species into each 1-m² quadrat in the experimental plots (8 plants per quadrat × 16 quadrats per wetland = 128 transplants per wetland). Plants were transplanted into 10-cm diameter holes (20 cm depth) arrayed c. 20 cm apart in a circular pattern in each 1-m² quadrat subplot. In *Juncus* treatments, holes were cored so that they were as close as possible to the individual *Juncus* plant in the subplot. Plants were planted so that forbs and grasses were alternating to minimize competition between transplants. A coloured wire was attached to each plant and maps were made of each of the 256 quadrats to identify transplant locations. One each of a tall (≥median) and a short (<median) plant were transplanted into each 1-m² quadrat to attempt to minimize the confounding effect of initial height. Transplants were not chosen if their size was outside the 95% CI of each potted population. A total of 2048 transplants were planted (2 pasture types × 8 wetlands × 2 locations within wetland × 2 grazing treatments (grazed/ungrazed) × 2 *Juncus* treatments (*Juncus*/no *Juncus*) × 2 sub-replicates (two *Juncus* and two non-*Juncus* treatments in each of the grazed and exclosure plots) × 4 species × 2 transplants of each species in each 1-m² plot.

Each plant was measured in November 2006 and November 2007 and harvested in December 2007. Data for individuals of the same species within the same treatment from the same wetland were averaged for analyses to remove pseudo-replication. We measured survival, height and number of stems at each census period. Harvested shoots were dried at 70 °C for 48 h and weighed for biomass determination.

We measured soil nutrients, organic matter and moisture to evaluate effects of treatments on nutrient availability. Soil samples were collected in July 2007, within each of the 256 subplots to analyse available P and available N (NH₄⁺ and NO₃⁻). These nutrients had been regularly added as fertilizer to IMP and were therefore expected to help characterize differences between the two pasture types. One 15-cm core was collected in the middle of each 1-m² quadrat. Samples of the same treatment within the same wetland were aggregated for a total of four soil samples per wetland (2 grazing treatments × 2 *Juncus* treatments). Fresh soil samples were sieved (2-mm mesh) and refrigerated until analysis. Soil moisture was determined by determining the weight lost after drying fresh soil at 105 °C for 24 h. Soil subsamples were dried, weighed and organic matter levels were determined by loss-on-ignition (450 °C for 16 h). Mehlich-I extractable P was determined by the dilute double acid method developed by Mehlich (1953) and modified by Sims (2000). Ammonium (NH₄⁺) was extracted using salicylate (Sims, Ellsworth & Mulvaney 1995) and nitrate (NO₃⁻) was determined using the vanadium chloride method (Doane & Horwath 2003). All samples were analysed in a microplate spectrophotometer (µQuant Microplate Spectrophotometer; BioTek Instruments, Winooski, VT, USA).

To estimate the intensity of cattle grazing in each wetland, we calculated cows ha⁻¹ for each pasture that contained a study wetland by multiplying the number of individuals in the herd by the number of days spent in each pasture for time during which 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 units × days ha⁻¹. This number was then divided by 365 days to obtain cows ha⁻¹ a⁻¹. Cows ha⁻¹ was calculated for the years 2006 and 2007 and averaged. Although these values provide a relative measure of grazing intensity within a pasture, local differences in grazing intensity can occur; even in a lightly stocked pasture, grazing pressure can be intense in some areas (Rebollo, Milchunas & Noy-Meir 2005). Therefore, we evaluated an alternative estimate of local grazing intensity for each study wetland, in which we measured six plant heights within each wetland and averaged them. As expected, we observed a negative association between vegetation height and our calculation of cows ha⁻¹ ($R^2 = 0.40$, $F = 9.3$, $P = 0.009$). Vegetation height was unrelated to measured abiotic variables, except for a positive correlation with soil organic matter ($r = 0.376$, $P = 0.034$).

DATA ANALYSIS

To analyse survival, we used a mixed model with pasture, grazing and *Juncus* plus their interactions as fixed effects and blocks plus their interactions as random effects to analyse binomial counts with the logit function. We followed a restricted pseudo-likelihood described in Wolfinger & O'Connell (1993). We tested the effect of pasture, *Juncus* and grazing and their interactions on final above-ground biomass, number of stems and height separately for each of the four species. The analysis was conditional on at least one plant surviving in a plot for a particular combination of block, pasture, exclosure and *Juncus*. If a particular plot had no surviving plants for a given species, then that observation was considered missing. Given that at least one plot was missing for every species and that there was more than one source of random variation, the data were analysed using the MIXED procedure (SAS Institute Inc 2010). This procedure allowed for the analysis of mixed models for unbalanced data. The models were fit using the restricted maximum likelihood option in MIXED. The F statistics were calculated using quadratic forms. By default, the denominator degrees of freedom of the F statistics were calculated using the

containment method (SAS Institute Inc 2010). In some instances, the data were missing to such an extent that there were no denominator degrees of freedom. Consequently, no *P*-value could be calculated. This method of computing degrees of freedom and model fitting match the ANOVA results for a balanced split-split plot design. In one instance regarding the response biomass (*P. hemitomon*) the log-transformation was used to stabilize the variance. There was such a substantial number of missing data for *D. virginiana* that no analysis was done for biomass, stem number and height for that species. If our hypothesis that the nature of interactions with *Juncus* depends on the presence of grazing was supported, we expected a significant interaction between *Juncus* and grazing treatments (*J* × *G*). Analyses were carried out using the R statistical environment (R Development Core Team 2007) and SAS. Initial height and nutrient availability did not significantly alter the effect of the experimental treatments so they were not included in the final analyses.

To compare the magnitude of *Juncus* effects on the four target species across the grazing intensity gradient, we calculated relative interaction intensity (RII; Armas, Ordiales & Pugnaire 2004), where $RII = (B_w - B_o)/(B_w + B_o)$ [correction added after online publication 27 June 2011: $RII = (B_w - B_o/B_w + B_o)$ corrected to $RII = (B_w - B_o)/(B_w + B_o)$]. B_w is the biomass of the plant with *Juncus* and B_o is the biomass of the plant without *Juncus*. This index is centred on zero, with positive values indicating facilitation and negative values indicating competition. We used a relative measure of interaction intensity because our goal was to quantify the observed consequences of interactions with *Juncus* and eliminate confounding effects due to local environmental conditions (Goldberg *et al.* 1999). When calculating RII, we compared the *Juncus* effect on biomass with and without grazing. An ANCOVA was used to determine how pasture type and grazing intensity (vegetation height) affected RII while accounting for the variation contributed by blocks for each species separately. Organic matter was included as a covariate in this analysis to statistically account for its effects on vegetation height. RII was expected to increase (greater facilitation) as grazing intensity increased (Bertness & Callaway 1994).

We used linear models to compare organic matter, available P, NH_4^+ and NO_3^- among pasture types after one year of grazing and *Juncus* treatments to quantify environmental characteristics and to determine if *Juncus* created more favourable environmental conditions for transplants. Organic matter and soil moisture were highly correlated (Pearson correlation: 0.917, $P = 0.01$); therefore, soil moisture was removed from the analysis. If *Juncus* enhances organic matter and/or nutrients and plants are found to perform better in *Juncus* plots in both grazed and ungrazed conditions, this would suggest that *Juncus* directly benefits plants rather than indirectly via protection from herbivory. All nutrient data were transformed as natural logarithm prior to analyses, and P and N data were divided by organic matter to account for differences in soil organic matter among wetlands.

Results

RESPONSES OF GROWTH AND SURVIVAL TO MANAGEMENT INTENSITY

Semi-natural wetlands provided a more favourable growing environment for the native grass, *P. hemitomon*, which was significantly taller within SNP wetlands (mean ± SD = 87.69 ± 24.9 cm) than in IP wetlands (mean ± SD = 61.72 ± 29.67 cm; Table 1). This native grass also grew taller in

enclosures within SNP wetlands compared with enclosures within IP wetlands (significant $P \times E$ on height; Table 1). No other significant pasture effects were observed for the other three study species.

PLANT GROWTH AND SURVIVAL RESPONSE TO GRAZING

Exclusion of cattle had positive effects on survival of *P. hemitomon*, *D. virginiana* and *A. philoxeroides* but there was no difference in survival between grazed and enclosure plots for *P. repens* (Table 1, Figs 1a,b and 2a,b).

Exclusion of cattle had positive effects on height and biomass for the two grasses and the non-native forb (Table 1, Figs 1c,g and 2c,d,g,h). Stem number of *P. hemitomon* was also lower in grazed plots compared with enclosure plots (Fig. 2f).

PLANT GROWTH AND SURVIVAL RESPONSES TO JUNCUS

There were few significant main effects of *Juncus* on the transplants. *Panicum hemitomon* was shorter without *Juncus* (72.23 ± 6.11 cm) than with *Juncus* (77.37 ± 5.26 cm, $P = 0.03$, Table 1). The survival of *D. virginiana* was greater without *Juncus* (Fig. 1b). Overall there was no net effect of *Juncus* on survival of *P. hemitomon*, *P. repens* or *A. philoxeroides* (Table 1), mostly explained by the opposite effects of this species with and without grazing (see below).

INTERACTIONS BETWEEN GRAZING AND JUNCUS

The negative effect of cattle grazing on survival was greater in non-*Juncus* plots compared with *Juncus* plots for all species except *D. virginiana* (Table 1). In enclosures, the presence of *Juncus* had a negative effect on survival for all species. Survival of *P. repens*, *P. hemitomon* and *A. philoxeroides* was significantly higher with *Juncus* than without in grazed plots, suggesting facilitation (Figs 1a and 2a,b). The effect of *Juncus* on survival was non-significant in grazed plots for *D. virginiana* (Fig. 1b). In enclosures, all species had lower survival in *Juncus* plots (Figs 1a,b and 2a,b).

Cattle grazing had a greater negative effect on above-ground biomass and stems, in plots without *Juncus*, compared to plots with *Juncus* for both the native (*P. hemitomon*) and non-native (*P. repens*) grasses (significant enclosure × *Juncus*, Table 1; Fig. 2c–f). This interaction was also significant for height of the native grass (Fig. 2h). These results suggest facilitation occurred because the presence of *Juncus* dampened the negative effect of grazing. When cattle were excluded, the two grasses had more stems, grew taller and had greater biomass in plots without *Juncus* than with *Juncus*, indicating competition.

NUTRIENT AVAILABILITY AMONG TREATMENTS

Differences in nutrients were found among treatments. *Juncus* plots had significantly more available nitrate than non-*Juncus*

Table 1. ANOVA results of biomass, stem number, height and survival of the transplants in response to the experimental treatments. ANOVAs were not conducted on *Diodia virginiana* because there were not enough surviving plants. Nu: numerator, De: denominator. Bold values are statistically significant

Survival		<i>Panicum hemitomon</i>				<i>Panicum repens</i>				<i>Alternanthera philoxeroides</i>		<i>Diodia virginiana</i>	
Source	d.f.	<i>F</i>	<i>P</i>			<i>F</i>	<i>P</i>			<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Pasture (P)	1	1.07	0.34			0.48	0.51			3.33	0.11	0.1	0.76
Exclosure (E)	1	28.4	0.001			2.45	0.16			13.15	0.01	7.42	0.03
Juncus (J)	1	0.02	0.9			3.43	0.11			4.39	0.07	6.76	0.04
P × E	1	0	0.99			1.84	0.22			0	0.96	0.34	0.58
P × J	1	0.23	0.65			3.61	0.09			0.57	0.48	0	0.95
E × J	1	50.14	0.0002			27.94	0.001			19.36	0.03	0.44	0.53
P × E × J	1	0.47	0.51			0.16	0.7			0.33	0.58	1.69	0.24

Biomass		Nu		De		Nu		De		Nu		De	
Source	d.f.	d.f.	<i>F</i>	<i>P</i>	d.f.	d.f.	<i>F</i>	<i>P</i>	d.f.	d.f.	<i>F</i>	<i>P</i>	<i>P</i>
Pasture (P)	1	7	1.61	0.25	1	5	0.22	0.66	1	6	0	0.99	
Exclosure (E)	1	4	54.73	0.002	1	5	16.29	0.01	1	5	10.36	0.02	
Juncus (J)	1	4	0.42	0.55	1	5	0.02	0.90	1	5	0.41	0.55	
P × E	1	7	0.83	0.39	1	5	0.25	0.64	1	7	0.33	0.58	
P × J	1	7	0.02	0.90	1	6	0.04	0.85	1	6	0.13	0.73	
E × J	1	4	13.42	0.02	1	5	12.75	0.02	1	5	0	0.99	
P × E × J	1	1	0.35	0.66	1	0	N/A	N/A	1	2	1	0.42	

No. of stem		Nu		De		Nu		De		Nu		De	
Source	d.f.	d.f.	<i>F</i>	<i>P</i>	d.f.	d.f.	<i>F</i>	<i>P</i>	d.f.	d.f.	<i>F</i>	<i>P</i>	<i>P</i>
Pasture (P)	1	7	1.75	0.23	1	5	0.67	0.45	1	6	0.15	0.71	
Exclosure (E)	1	7	21.88	0.01	1	5	2.74	0.16	1	6	1.22	0.31	
Juncus (J)	1	5	10.97	0.21	1	5	1.55	0.27	1	6	2.32	0.18	
P × E	1	7	3.75	0.09	1	5	0.32	0.60	1	7	0.01	0.93	
P × J	1	7	0	0.97	1	5	1.98	0.22	1	6	0.3	0.60	
E × J	1	5	12.92	0.02	1	6	6.39	0.04	1	6	1.18	0.32	
P × E × J	1	2	0	0.96	1	1	0.76	0.54	1	2	0.89	0.45	

Height		Nu		De		Nu		De		Nu		De	
Source	d.f.	d.f.	<i>F</i>	<i>P</i>	d.f.	d.f.	<i>F</i>	<i>P</i>	d.f.	d.f.	<i>F</i>	<i>P</i>	<i>P</i>
Pasture (P)	1	7	12.13	0.01	1	5	0.12	0.74	1	6	0.12	0.74	
Exclosure (E)	1	5	110.74	0.0001	1	5	48.3	0.001	1	6	12.86	0.012	
Juncus (J)	1	5	8.32	0.03	1	5	2.06	0.21	1	6	3.74	0.10	
P × E	1	7	6.47	0.04	1	5	0.54	0.50	1	7	0.49	0.51	
P × J	1	7	0.01	0.93	1	5	0.33	0.59	1	6	0.28	0.62	
E × J	1	5	7.17	0.04	1	6	0.96	0.37	1	6	0.03	0.87	
P × E × J	1	2	0.01	0.92	1	0	N/A	N/A	1	2	1.18	0.39	

plots ($F_{(1,8)} = 11.59$, $P = 0.01$). However, there was a significant interaction between *Juncus* and pasture type that suggested that *Juncus* plots had higher nitrate only in SNPs, not in IPs (pasture × *Juncus*: $F_{(1,8)} = 5.93$, $P = 0.05$). Grazing interacted with *Juncus* on available phosphorus with lower phosphorus concentrations in exclosure plots without *Juncus*, possibly due to increased uptake by plants released from both grazing pressure and competition from *Juncus* ($F_{(1,8)} = 7.08$, $P = 0.03$). A significant three-way interaction was present

between pasture type, exclosure and *Juncus* ($F_{(1,8)} = 7.17$, $P = 0.03$), which indicated that the significant decrease in phosphorus found in exclosure plots without *Juncus* only occurred in the IPs; phosphorus was similar in level between all *Juncus* and grazing treatments in SNPs. There was a significant interaction between grazing and *Juncus* on organic matter content ($F_{(1,8)} = 18.63$, $P = 0.003$). In grazed plots, soil in *Juncus* plots had more organic matter, while in exclosures soil contained more organic matter in non-*Juncus* plots.

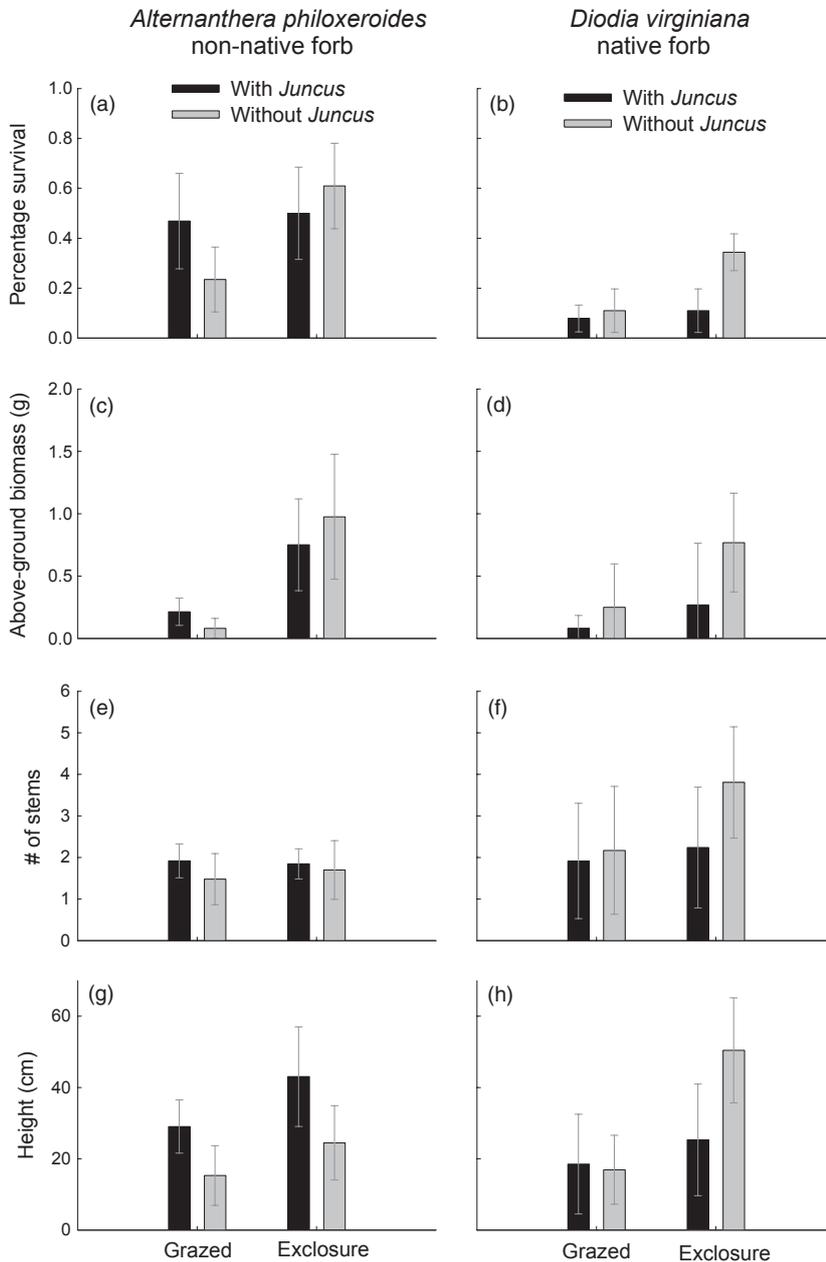


Fig. 1. Percentage survival, above-ground biomass, stem number and height of *Alternanthera philoxeroides* (non-native forb) and *Diodia virginiana* (native forb) within exclusion and *Juncus* treatments. Very little pasture effects were found; therefore data were pooled across pasture-type for *Juncus* and exclusion treatments for the figures. Error bars are 95% CI. Only survival was analysed for *Diodia*, because too few plants were alive to analyse biomass, stem number and height.

RELATIVE INTERACTION INTENSITY ALONG A GRAZING GRADIENT

Relative interaction intensity with *Juncus* became more facilitative as grazing pressure increased for the two grasses but not for the two forbs. Facilitation by *Juncus* on the native grass, *P. hemitomon*, increased as grazing pressure increased (Fig. 3; $R^2 = 0.37$, $P < 0.001$). *Panicum hemitomon* RII was significantly greater in SNPs ($F_{(1,7)} = 11.43$, $P = 0.01$). For the non-native grass, *P. repens*, RII increased with grazing pressure ($R^2 = 0.16$, $P = 0.008$), but did not differ between pasture types (Fig. 3; $F_{(1,7)} = 1.86$, $P = 0.22$). Organic matter was a significant covariate in the model analysing RII of *P. repens* ($F_{(1,7)} = 14.10$, $P = 0.02$). RII of both forbs had no relationship with grazing intensity (*A. philoxeroides*:

$R^2 = 0.04$, $P = 0.29$, *D. virginiana*: $R^2 = 0.0006$, $P = 0.90$) or pasture-type (*A. philoxeroides*: $F_{(1,7)} = 1.72$, $P = 0.23$, *D. virginiana*: $F_{(1,7)} = 1.33$, $P = 0.30$; Fig. 3).

Discussion

In our study system plant interactions between the two grass species and *Juncus* depended on cattle grazing. Growth of both the native and non-native grasses (*P. hemitomon* and *P. repens*) indicated facilitation by *Juncus* under a context of cattle grazing, but competition with *Juncus* when cattle were excluded. *Alternanthera philoxeroides* (the non-native forb) experienced less mortality in *Juncus* plots compared with non-*Juncus* plots under grazed conditions, but *Juncus* did not facilitate the growth of this species. Only the native forb did

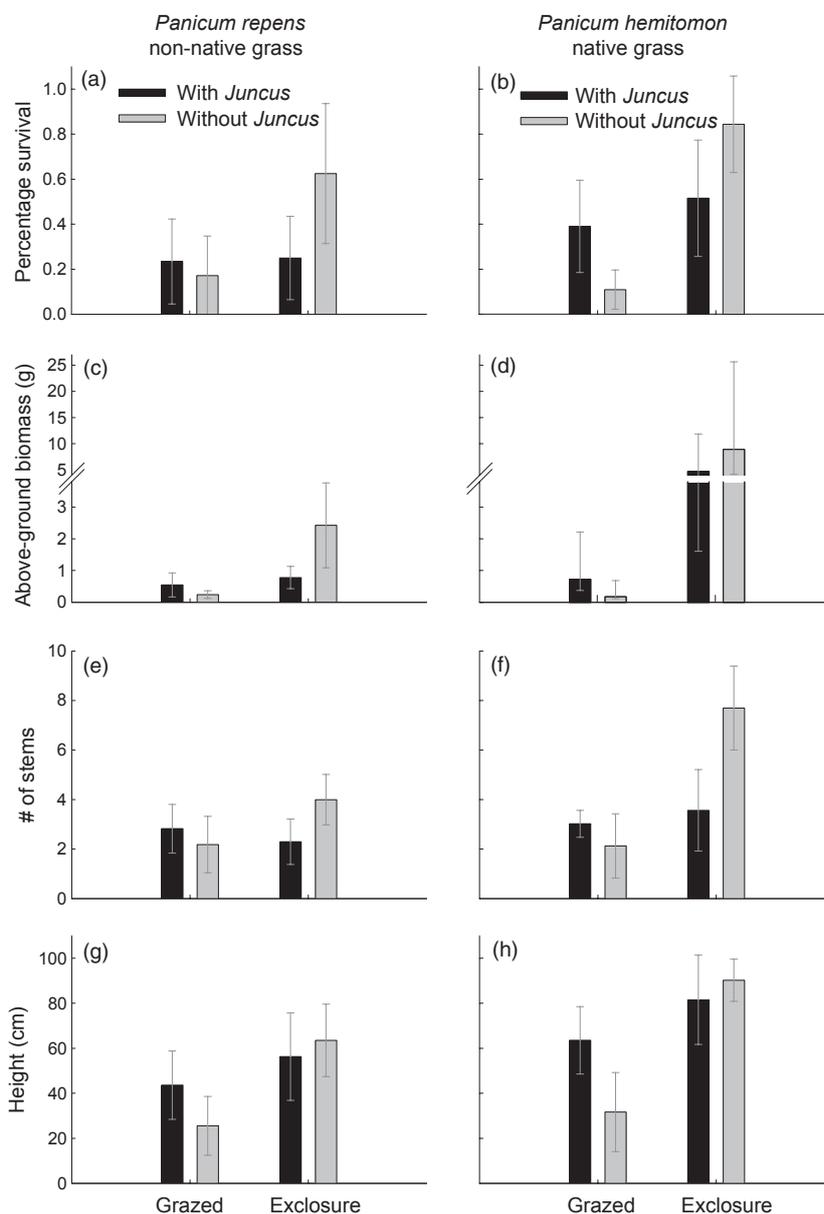


Fig. 2. Percentage survival, above-ground biomass, stem number and height of *Panicum repens* (non-native grass) and *Panicum hemitomon* (native grass) within enclosure and *Juncus* treatments. Very little pasture effects were found; therefore data were pooled across pasture type for *Juncus* and enclosure treatments for the figures. Error bars are 95% CI. Biomass of *P. hemitomon* was log-transformed, resulting in asymmetrical error bars after back-transforming.

not respond positively to *Juncus* under grazing pressure. Survival of this species was the only attribute that could be assessed due to high mortality, and *Juncus* suppressed survival of *D. virginiana* in both grazed and exclosure plots. *Juncus* facilitated survival of the other three species in grazed plots. Taken together, the growth and survival results indicate that *Juncus* can enhance survival and growth of both native and non-native species in grazed conditions. However, facilitation depended on species identity. One explanation for species-specific results is that species traits influence the outcome of interactions (Maestre *et al.* 2009).

One hypothesis to explain variability in species' responses to associational defences focuses on palatability, with more palatable species more likely to experience facilitation (Baraza, Zamora & Hodar 2006). All four of our species have relatively high forage value and good crude protein content, suggesting they are palatable (P.J. Bohlen, unpublished data; Boyd 1968).

In addition, grazing negatively influenced either survival or growth of all species. The non-native grass, *P. repens*, seemed the least susceptible to grazing; grazing did not negatively affect the survival of this non-native grass although biomass of this species was lower in grazed plots (Table 1).

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, Callaway & Michalet (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. *Alternanthera philoxeroides*, like the two grasses in our study, appears to have low tolerance to grazing as height, biomass and survival were depressed in grazed plots. However, *A. philoxeroides* appears to be a good competitor or highly tolerant to conditions provided by *Juncus* because effects of *Juncus* on this species were minimal in exclosures. *Juncus effusus* is known to

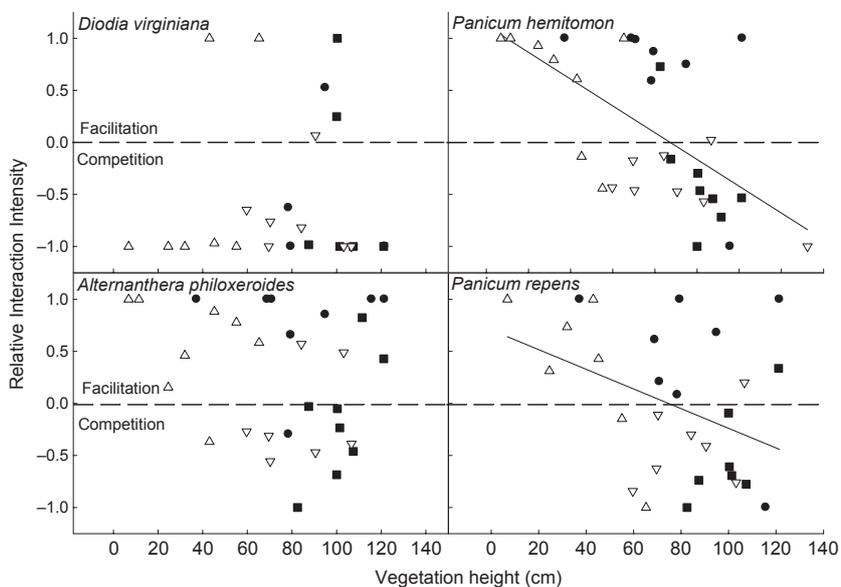


Fig. 3. Facilitation decreased in strength as grazing became less intense for the two grasses, *Panicum hemitomon* ($R^2 = 0.37$, $P < 0.001$) and *Panicum repens*. ($R^2 = 0.16$, $P = 0.008$). Grazing intensity and relative interaction intensity were unrelated for the two forb species (*Alternanthera*: $R^2 = 0.04$, $P = 0.29$, *Diodia*: $R^2 = 0.0006$, $P = 0.90$). Grazing intensity is highest at short vegetation heights. Black symbols represent semi-native pasture wetlands and open symbols represent intensively managed pasture wetlands. Circles and upward-pointing triangles are grazed plots and squares and downward-pointing triangles are exclosure plots.

depress species diversity in beaver wetlands due to its ability to produce dense shade (Ervin & Wetzel 2002). *Alternanthera philoxeroides* appears to be shade tolerant as evidenced by the lack of a significant effect of *Juncus* on biomass, height, and stem number in exclosures, whereas *D. virginiana* was inhibited by *Juncus*.

Our study indicates that broad functional groups based on morphology (i.e. grasses or forbs) are not indicative of how a species will respond to plant interactions. In our study, grasses responded similarly to plant interactions and grazing while the two forb species responded dissimilarly to *Juncus*. Both grass species examined are tall (Hitchcock 1951; Leithead, Yarlett & Shiflet 1971), and it is possible that response of short grasses may differ from that of tall grasses. Life history characteristics coupled with adaptive strategies (Grime 1977) may be more important for predicting which species will benefit from facilitation (Maestre *et al.* 2009). Tewksbury & 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 consumer gradients are possible. The net outcome of an interaction may depend on the life history strategy of beneficiary species; for example, species with better competitive ability may be more likely to obtain associational refuge from unpalatable plants, while stress-tolerant and ruderal species may show little or no facilitative response (Maestre *et al.* 2009).

Possible explanations for the higher nitrate content in *Juncus* compared with non-*Juncus* plots include oxygen release into the soil or decomposition of fine roots (Engelaar *et al.* 1995; Fornara, Tilman & Hobbie 2008). *Juncus* has abundant aerenchyma tissue and a possible mechanism for direct facilitation is soil aeration. Tweel & Bohlen (2008) investigated the effect of *Juncus* and grazing on soil redox potential, a measure of aereo-

bic conditions, and found no effect of *Juncus*. The greater organic matter under *Juncus* versus non-*Juncus* in grazed plots, and lower organic matter under *Juncus* versus non-*Juncus* in exclosures, suggests that *Juncus* may also function to protect soil structure in grazed areas. 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 because within exclosures all four species had higher survival and/or biomass in non-*Juncus* plots.

Studies conducted along gradients of consumer pressure are inconclusive as to how plant–plant interactions will behave at higher levels of grazing (Rebollo, Milchunas & Noy-Meir 2005; Baraza, Zamora & Hodar 2006; Graff, Aguiar & Chanton 2007; Smit *et al.* 2007; Levenbach 2009). The SGH predicts a positive linear relationship between facilitation and consumer pressure (Bertness & Callaway 1994). In our study, RII increased (interactions became more facilitative) as grazing intensity increased for the two grass species, supporting a positive, linear relationship. However, the relationship of RII was unrelated to grazing intensity for the forb species, suggesting that a positive relationship may not be as general as predicted and may only apply to competitive species, such as tall grasses. These results support the predictions of Maestre *et al.* (2009) that interactions would be facilitative between a stress tolerant benefactor species and competitive beneficiary species when a non-resource stress (in our case herbivory) is medium or high. When the beneficiary species is stress tolerant, interactions with a stress-tolerant benefactor were predicted to be neutral at medium stress levels because they are more likely to compete for resources (Maestre *et al.* 2009).

Conclusions

In disturbed environments, stress-tolerant species, such as *Juncus*, may be important drivers of community composition, because they allow several species to persist in environments

where they may have otherwise been eradicated. This is an important consideration for understanding both native and non-native species persistence and has implications for habitat restoration. In grazed ecosystems, maintaining populations of species functioning as benefactors may be important for conserving populations of tall grasses.

Acknowledgements

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 (F6F11250), USDA CSREES, Society of Wetlands Scientists Student Research Grant and The Florida Native Plant Society. E. Menges and two anonymous referees provided valuable comments on a previous draft. This paper is contribution number 137 from the MacArthur Agro-ecology Research Center.

References

- Alberti, J., Escapa, M., Iribarne, O., Silliman, B. & Bertness, M. (2008) Crab herbivory regulates plant facilitative and competitive processes in Argentinian marshes. *Ecology*, **89**, 155–164.
- Armas, C., Ordiales, R. & Pugnaire, F.I. (2004) Measuring plant interactions: a new comparative index. *Ecology*, **85**, 2682–2686.
- Badano, E.I., Villarreal, E., Bustamante, R.O., Marquet, P.A. & Cavieres, L.A. (2007) Ecosystem engineering facilitates invasions by exotic plants in high-Andean ecosystems. *Journal of Ecology*, **95**, 682–688.
- Baraza, E., Zamora, R. & Hódar, J.A. (2006) Conditional outcomes in plant-herbivore interactions: neighbors matter. *Oikos*, **113**, 148–156.
- Bertness, M.D. & Callaway, R. (1994) Positive interactions in communities. *Trends in Ecology & Evolution*, **9**, 191–193.
- Boughton, E.H., Quintana-Ascencio, P.F. & Bohlen, P.J. (2011) Refuge effects of *Juncus effusus* in grazed, subtropical wetland plant communities. *Plant Ecology*, **212**, 451–460.
- Boyd, C.E. (1968) Evaluation of some common aquatic weeds as possible feed-stuffs. *Hyacinth Control Journal*, **7**, 26–27.
- Bulleri, F., Bruno, J.F. & Benedetti-Cecchi, L. (2008) Beyond competition: incorporating positive interactions between species to predict ecosystem invasibility. *Plos Biology*, **6**, 1136–1140.
- Callaway, R. & Walker, L. (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology*, **78**, 1958–1965.
- Callaway, R.M., Kikodze, D., Chiboshvili, M. & Khetsuriani, L. (2005) Unpalatable plants protect neighbours from grazing and increase plant community diversity. *Ecology*, **86**, 1856–1862.
- Crain, C.M. (2008) Interactions between marsh plant species vary in direction and strength depending on environmental and consumer context. *Journal of Ecology*, **96**, 166–173.
- Doane, T.A. & Horwath, W.R. (2003) Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters*, **36**, 2713–2722.
- Engelaar, W.M.H.G., Symens, J.C., Laanbroek, H.J. & Blom, C.W.P.M. (1995) Preservation of nitrifying capacity and nitrate availability in waterlogged soils by radial oxygen loss from roots of wetland plants. *Biology and Fertility of Soils*, **20**, 243–248.
- Ervin, G.N. & Wetzel, R.G. (2002) Influence of a dominant macrophyte, (*Juncus effusus*), on wetland plant species richness, diversity, and community composition. *Oecologia*, **130**, 626–636.
- eFloras (2008) Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA. Available at <http://www.efloras.org> (accessed 28 December 2010).
- Fornara, D.A., Tilman, D. & Hobbie, S.E. (2008) Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. *Functional Ecology*, **97**, 48–56.
- Goldberg, D., Rajaniemi, T., Gurevitch, J. & Stewart-Oaten, A. (1999) Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. *Ecology*, **80**, 1118–1131.
- Graff, P., Aguiar, M.R. & Chaneton, E.J. (2007) Shifts in positive and negative plant interactions along a grazing intensity gradient. *Ecology*, **88**, 188–199.
- Grime, J.P. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, **111**, 1169–1194.
- Hambach, P.A., Agren, J. & Ericson, L. (2000) Associational resistance: insect damage to purple loosestrife reduced in thickets of sweet gale. *Ecology*, **81**, 1784–1794.
- Hay, M.E. (1986) Associational plant defenses and the maintenance of species-diversity – turning competitors into accomplices. *American Naturalist*, **128**, 617–641.
- Hitchcock, A.S. (1951) *Manual of the Grasses of the United States*. Misc. Publ. No. 200. U.S. Department of Agriculture, Agricultural Research Administration, Washington, DC, 1051 p. [2nd edition revised by Agnes Chase in two volumes. New York: Dover Publications, Inc.].
- Holmgren, M., Scheffer, M. & Huston, M.A. (1997) The interplay of facilitation and competition in plant communities. *Ecology*, **78**, 1966–1975.
- Humphrey, J.W. & Patterson, G.S. (2000) Effects of late summer cattle grazing on the diversity of riparian pasture vegetation in an upland conifer forest. *Journal of Applied Ecology*, **37**, 986–996.
- Leithhead, H.L., Yarleth, L.L. & Shifflet, T.N. (1971) *100 Native Forage Grasses in 11 Southern States*. Agric. Handb. 389. U.S. Department of Agriculture, Forest Service, Washington, DC, 216 p.
- Levenbach, S. (2009) Grazing intensity influences strength of an associational refuge on temperate reefs. *Oecologia*, **159**, 181–190.
- Liancourt, P., Callaway, R.M. & Michalet, R. (2005) Stress tolerance and competitive-response ability determine the outcome of biotic interactions. *Ecology*, **86**, 1611–1618.
- Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, **97**, 199–205.
- Mehlich, A. (1953) *Determination of P, Ca, Mg, K, Na, and NH₄*. Department of Agriculture, Raleigh, NC.
- Michalet, R., Brooker, R.W., Cavieres, L.A., Kikvidze, Z., Lortie, C.J., Pugnaire, F.I., Valiente-Banuet, A. & Callaway, R.M. (2006) Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters*, **9**, 767–773.
- Osem, Y., Perevolotsky, A. & Kigel, J. (2007) Interactive effects of grazing and shrubs on the annual plant community in semi-arid Mediterranean shrublands. *Journal of Vegetation Science*, **18**, 869–878.
- Pihlgren, A. & Lennartsson, T. (2008) Shrub effects on herbs and grasses in semi-natural grasslands: positive, negative or neutral relationships? *Grass and Forage Science*, **63**, 9–21.
- R Development Core Team (2007) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Austria, Vienna. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Rebollo, S., Milchunas, D.G. & Noy-Meir, I. (2005) Refuge effects of a cactus in grazed short-grass steppe. *Journal of Vegetation Science*, **16**, 85–92.
- Rebollo, S., Milchunas, D.G., Noy-Meir, I. & Chapman, P.L. (2002) The role of a spiny plant refuge in structuring grazed shortgrass steppe plant communities. *Oikos*, **98**, 53–64.
- SAS Institute Inc. (2010) *SAS/STAT[®] 9.22 User's Guide*. SAS Institute Inc, Cary, NC.
- Sims, J.T. (2000) Soil test phosphorus: Mehlich 1. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Water* (ed G.M. Pierzinski), pp. 15–16, North Carolina State University, Raleigh, NC.
- Sims, G.K., Ellsworth, T.R. & Mulvaney, R.L. (1995) Microscale determination of inorganic nitrogen in water and soil extracts. *Communications in Soil Science and Plant Analysis*, **26**, 303–316.
- Smit, C., Vandenbergh, C., den Ouden, J. & Mueller-Schaerer, H. (2007) Nurse plants, tree saplings and grazing pressure: changes in facilitation along a biotic environmental gradient. *Oecologia*, **152**, 265–273.
- Steinman, A., Conklin, J., Bohlen, P.J. & Uzarski, D. (2003) Influence of cattle grazing and pasture land use on macroinvertebrate communities in freshwater wetlands. *Wetlands*, **23**, 877–889.
- Tewksbury, J. & Lloyd, J. (2001) Positive interactions under nurse-plants: spatial scale, stress gradients, and benefactor size. *Oecologia*, **127**, 425–434.
- Tweel, A. & Bohlen, P.J. (2008) Influence of Soft Rush (*Juncus effusus*) on phosphorus flux in grazed seasonal wetlands. *Environmental Engineering*, **33**, 242–251.
- United States Geological Survey (2003) Virginia Buttonweed (*Diodia virginiana*). <http://www.nprc.usgs.gov/resource/plants/floramw/species/diodvirg.htm>.
- Wolfinger, R. & O'Connell, M. (1993) Generalized linear mixed models: a pseudo-likelihood approach. *Journal of Statistical Computation and Simulation*, **4**, 233–243.

Received 26 August 2010; accepted 5 May 2011
Handling Editor: Jonathan Newman