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GRASS-SHRUB SPATIAL ASSOCIATIONS OVER PRECIPITATION AND
GRAZING GRADIENTS IN THE GREAT BASIN, USA

by

Maike F. Holthuijzen

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

ECOLOGY

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2015

ABSTRACT

Grass-shrub Spatial Associations Over Precipitation and Grazing Gradients in the Great
Basin, USA

by

Maike Freija Holthuijzen

Utah State University, 2015

Major Professor: Kari Veblen
Department: Wildland Resources

Plant spatial patterns have been studied to gain insight into plant interactions such as competition and facilitation (positive plant interactions). The stress gradient hypothesis predicts that as environmental stress increases facilitation dominates, while competition dominates in less stressful conditions.

Beneficial plants (nurses) can create favorable abiotic conditions for subnopy plants. Additionally, palatable herbaceous species growing under nurse shrub canopies benefit from physical protection.

I investigated spatial associations between Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) and three native grasses (*Poa secunda*, *Elymus elymoides*, and *Pseudoroegneria spicata*) across a rainfall gradient in the Great Basin, USA. I also explored the effect of grazing on grass-shrub spatial associations. I hypothesized that positive shrub-grass spatial associations would become more frequent at lower rainfall levels; I further hypothesized that 1) at intermediate levels of stress, positive grass-shrub

spatial associations would dominate and 2) at extreme levels of stress, positive grass-shrub spatial associations and interactions would no longer dominate. At high moisture stress, the addition of grazing stress may limit the nurse's ability to provide to benefits to subcanopy plants.

Cover of *P. secunda* was greater in shrub canopy microsites than interspaces at low to moderate levels of rainfall. Cover and density of *E. elymoides* were greater in sagebrush canopies over most rainfall levels. *Elymus elymoides* and *P. spicata* were taller and narrower in basal width and less likely to be grazed in canopy versus interspace microsites.

I next investigated the effects of grazing intensity over a rainfall gradient and found a significant interaction of rainfall and microsite on *P. secunda* cover. *Poa secunda* formed positive interactions with *A. tridentata* at lower rainfall levels, regardless of grazing intensity. Its cover was significantly greater in interspaces at high rainfall compared to low rainfall sites. *Elymus elymoides* density was greater in canopy vs. interspace microsites, regardless of rainfall level or grazing intensity.

Plant spatial associations can indicate which nurse microsites are favorable to plant growth and may improve seeding or planting success during ecological restoration. My results suggest that exploiting sagebrush canopy microsites for restoration of native perennial grasses would improve plant establishment, growth or survival particularly in drier areas.

PUBLIC ABSTRACT

Grass-shrub spatial associations over precipitation and grazing gradients in the Great Basin, USA

Maike Freija Holthuijzen

As environmental stress increases, positive plant interactions dominate, resulting in clumped, plant-plant spatial patterns. Positive plant-plant interactions have received more attention in recent years due to their importance to the structure and function of arid ecosystems. Certain plants (nurses), provide benefits that help neighboring plants grow or survive

I investigated spatial associations between Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) and three native grasses (*Poa secunda*, *Elymus elymoides*, and *Pseudoroegneria spicata*) across a rainfall gradient in the Great Basin, USA. We also explored the effect of grazing on grass-shrub spatial patterns. I hypothesized that positive grass-shrub spatial associations would become more frequent at lower rainfall levels; we also hypothesized that : 1) at intermediate levels of stress (i.e. low rainfall, low grazing intensity conditions or high rainfall, high grazing intensity conditions), positive grass-shrub spatial associations would become more frequent and 2) at extreme levels of stress (low rainfall, high grazing intensity conditions), positive grass-shrub spatial associations and interactions would no longer dominate. At extreme levels of moisture stress, the addition of high grazing stress may cause positive interactions to break down.

I sampled perennial grass cover, density, height, basal width, grazing status, and reproduction in sagebrush canopies and interspaces at 32 sites over a rainfall gradient. I

found that cover of *P. secunda* was higher in canopy microsites than interspaces at low to moderate levels of rainfall. Cover and density of *E. elymoides* were higher in sagebrush canopies at almost all rainfall levels. Both *E. elymoides* and *P. spicata* plants were taller, narrower, and were less likely to be grazed in canopy microsites.

To investigate the effects of grazing, I sampled 5 site pairs over a rainfall gradient. I did not find any significant effects of grazing, consistent with the results of other studies. For *P. secunda*, shrubs were more facilitative at low rainfall compared to high rainfall sites, regardless of grazing intensity. *E. elymoides* was facilitated regardless of rainfall level or grazing intensity.

Plant spatial associations can indicate which types of microsites are favorable for plant growth, which could in turn help improve seeding or planting success during ecological restoration of the Great Basin. My results suggest that using sagebrush canopy microsites for restoration of native perennial grasses would likely improve plant establishment, growth, especially in drier areas.

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Maike Freija Holthuijzen

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CHAPTER 1

INTRODUCTION

Our natural world is full of spatial patterns, from the distribution of trees in a forest [1], to the arrangement of termite mounds in an African savanna [2], to the fine-scale arrangements of plant species relative to one another [3]. What underlying biological processes may result in these patterns? Providing an answer to that question has been one of the main goals of ecological research for decades [4]. Plant spatial patterns, for example, may be the result of seed dispersal, resource heterogeneity, or disturbance; in addition, they can provide important insight into plant-plant interactions [5-7]. Aggregated plant spatial associations can indicate positive or “facilitative” relationships [7, 8], whereby one plant (a beneficiary) gains an advantage by growing in close proximity to its neighbor (nurse plant). In contrast, segregated or random patterns among plants may result from intense competition for resources such as light and soil moisture [1, 6]. Facilitative plant-plant relationships have recently received more attention and are now considered critical to the structure and function of a variety of ecosystems [9]. However, positive relationships among plants may change in response to changes in abiotic and/or biotic environmental stresses: according to the stress gradient hypothesis, facilitative plant-plant interactions will increase in intensity or frequency as abiotic and/or biotic environmental stress increases, while competitive interactions will dominate in less stressful environments [10]. Enhanced knowledge of how plant-plant associations and interactions change over environmental stress gradients can not only add to ecological theory and our understanding of plant-plant interactions [9]—it can help us

in a practical sense as well: if we can predict how plant patterns respond to stressors, we can make informed management decisions, especially in response to climate change.

The effects of abiotic stresses on plant spatial associations and interactions have been well-documented in various ecosystems and generally support the stress gradient hypothesis [11]. The majority of studies have taken place in arid or semi-arid regions, where abiotic environmental stress such as moisture limit plant growth [12]. For example, facilitative grass-tree interactions increase over a decreasing precipitation gradient in African savannas [13]; other examples of this pattern have been observed between a native shrub and various understory species in Eastern Spain [14] and the Iberian Peninsula [15], as well as between a native shrub and perennial grasses [16] and conifer seedlings in the Great Basin desert of North America [17]. In subalpine environments, positive interactions among graminoids and forbs [18] and two species of conifer [19] have been shown to increase along altitudinal gradients. Even studies in less stressful regions provide evidence for the stress gradient hypothesis: Liu, Ren (20) observed an increase in positive interactions between two native shrubs as soil nutrients decreased along an elevational gradient in the subtropics, while [21] found that cushion plants facilitated a native species at the upper limits of an altitudinal gradient in a tropical Andean forest. Evidence contradicting the stress gradient hypothesis suggests that at extreme levels of abiotic stress, any beneficial effects of neighbors are outweighed by competition for resources [22, 23].

Ungulate grazing also has been shown to affect the strength of plant-plant spatial associations, resulting in more complex species' interactions [24] and has been studied in many ecosystems. Generally, positive plant-plant interactions increase in response to

increases in ungulate grazing stress [25-27], mainly due to the physical protection of vulnerable, palatable species by less palatable or thorny neighbors [26, 28, 29]. This phenomenon, known as associational resistance [30], may be enhanced when the difference in palatability between nurse and beneficiary plants increases [29]. However, as grazing stress progressively increases, competitive plant-plant interactions may predominate again at the highest grazing levels [24, 31], which could be due to an overall breakdown in facilitative processes as grazing ungulates, for example, become less selective when foraging for food. The interaction of environmental stress gradients such as grazing and moisture are less well-studied and poorly understood [32]. Perea and Gil (33) observed that concomitant increases in moisture and ungulate grazing stresses resulted in additive facilitative effects between shrubs and tree seedlings in a Mediterranean ecosystem [but see 27]. However, Veblen (26) reported grazing-mediated facilitation between two grasses in an African savanna, suggesting that the presence of a biotic stress may result in positive plant-plant interactions. On the other hand, Verwijmeren, Rietkerk (32) and Noumi, Chaieb (34) reported plant-plant interactions became slightly less negative at low moisture stress only if grazing intensity was sufficiently high, indicating only a slight degree of facilitation due to grazing [but see 35]. The combined effects of moisture and grazing stresses can be difficult to study and have not received much attention in the literature; thus, the influence of both moisture and grazing stresses on plant-plant interactions warrant further study.

The general premise of the stress gradient hypothesis, namely that positive plant-plant interactions increase monotonically along environmental stress gradients, has been refined in recent years to account for species traits, type of stress (resource versus non-

resource) [36, 37], and ontogenic stage of the species involved [38, 39]. Facilitation is likely to occur for competitive plant species at the upper limits of their stress tolerance, while competition is likely to occur for stress-tolerant species at their ecological optimums [16, 40]. In the presence of a resource based stress (e.g., moisture), facilitation between plants will likely dominate, due to the amelioration of abiotic environmental conditions by a nurse plant [36]. However, non-resource based stresses, such as temperature, will only result in facilitative plant-plant interactions if a nurse plant can sufficiently mitigate that stress for beneficiaries; thus, this type of facilitation is less frequently observed [36]. Finally, juvenile plants, rather than adult plants, are more likely to gain benefits by growing near neighbors [39].

Shrubs are the most commonly studied nurse plants worldwide [12], as their canopies can ameliorate harsh environmental conditions for sub-canopy beneficiaries by improving soil moisture conditions [41, 42], mediating soil temperature [43, 44], providing increased soil nutrient availability [20] and organic matter [44] and decreasing solar radiation [41, 45]. In shrub canopies, lower temperatures decrease transpiration rates and vapor pressure deficits, which can positively influence the soil water potentials of sub canopy plants [46]. Nurse shrubs may further promote positive soil water potentials of neighboring plants via hydraulic lift, a process in which the shrub's deep roots redistribute soil water to surface layers [47].

Positive interactions among nurse and beneficiary plants also have important implications for the restoration of degraded ecosystems. The use of nurse shrubs in expediting ecological restoration of native species is receiving increasing attention [48, 49]. The Great Basin, an endangered and degraded semi-arid region in which ecological

restoration is extremely difficult [50], may benefit from an enhanced understanding of positive plant-plant interactions. It is also an ideal area to study plant spatial associations, as the main environmental stresses are drought and domestic cattle grazing, the latter of which was introduced in the 1850s [51]. Evidence suggests that positive perennial grass-shrub associations occur between the common Wyoming big sagebrush, *Artemisia tridentata* ssp. *wyomingensis* and perennial grasses such as *Achnatherum thurberianum*, *Poa secunda*, *Elymus elymoides*, and *Pseudoroegneria spicata* [16, 52, 53, in review]. However, no studies have investigated the efficacy of *A. tridentata* in facilitating native herbaceous species for ecological restoration over a large extent of the Great Basin.

In chapter one, I characterized grass-shrub spatial associations between *A. tridentata* and three native, perennial grasses in the Great Basin. My objective was to infer how the role of *A. tridentata* as a nurse plant shifted over a Great Basin-wide precipitation gradient. For chapter two, since livestock grazing is also a prominent biotic stress of the Great Basin, and grazing has been shown to affect grass-shrub spatial associations, I investigated the role grazing may have had in influencing the plant spatial associations results from Chapter 1. To date, the combined effects of abiotic and biotic stresses on grass-shrub spatial associations are not well understood.

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CHAPTER 2

GRASS-SHRUB SPATIAL ASSOCIATIONS OVER A PRECIPITATION GRADIENT
IN THE GREAT BASIN, USA¹**Abstract**

As environmental stress increases facilitative plant interactions often predominate, often resulting in positive plant-plant spatial associations. Plant spatial associations can indicate which types of microsites (e.g., shrub canopies of potential “nurse plants”) are favorable to plant growth and can improve seeding or planting success during ecological restoration. Despite geographic and abiotic variation among sites requiring restoration, no studies have specifically examined how spatial associations between nurse plants and restoration species change over large-ranging, regional stress gradients in the context of ecological restoration. We investigated spatial associations between Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) and three native grasses (*Poa secunda*, *Elymus elymoides*, and *Pseudoroegneria spicata*), representing short-, medium-, and deep-rooted growth forms, respectively, across a rainfall gradient in the Great Basin, USA. We hypothesized that positive shrub-grass spatial associations would become more frequent at lower rainfall levels. We sampled perennial grass cover, density, height, basal width, grazing status, and reproductive status in sagebrush canopies and interspaces at 32 sites over a rainfall gradient. We found that cover of the shallow rooted grass, *P. secunda*, was higher in canopy microsites than interspaces at low to moderate levels of rainfall. Cover and density of the medium-rooted grass, *E. elymoides* were higher in sagebrush canopies at all but the highest rainfall

¹ Co-authored by Kari Veblen

levels. Neither annual rainfall nor sagebrush microsite significantly affected *P. spicata* cover. Both *E. elymoides* and *P. spicata* plants were taller, narrower, and were less likely to be grazed in canopy microsites. Our results suggest that exploiting sagebrush canopy microsites for restoration of native perennial grasses would improve plant establishment, growth, survival, or some combination thereof, particularly in drier areas. We suggest that land managers should consider the nurse plant approach as a way to improve the ecological health and resilience of the Great Basin. Controlled experimentation will provide further insights into the life stage-specific effectiveness and practicality of a nurse plant approach in this region.

Introduction

Plant spatial patterns reflect biological processes and are studied to gain insight into plant-plant interactions [1-3]. Aggregated plant spatial associations can indicate positive or “facilitative” relationships [3, 4], whereby one plant (a beneficiary) gains an advantage by growing in close proximity to its neighbor (nurse or benefactor). In facilitative relationships, the neighbor often provides a favorable microclimate [5-8] or defense against herbivory [9, 10]. The frequency and importance of facilitative plant interactions are predicted to increase with increasing environmental stress [11] [but see 12, 13], including in arid and semi-arid ecosystems [4, 14].

Better understanding of nurse-beneficiary plant spatial associations may improve establishment of native plants in ecological restoration settings [15-17], particularly when nurse plants can ameliorate extreme abiotic stress imposed by drought and extreme temperatures [15, 16, 18]. Nurse plant canopies create microsites with favorable abiotic conditions characterized by improved soil moisture [6, 18], moderated soil temperature

[8], increased soil nutrient availability [19] and decreased solar radiation [18, 20]. In some cases, restoring facilitative processes may be a requirement for native species growth [21]. A growing number of studies have specifically considered use of nurse plants to improve establishment of native species in ecological restorations. Examples include use of tussock grasses to improve native shrub establishment [18] and shrubs to improve herbaceous seedling survival [22] and tree seedling establishment [15]. These studies provide evidence that using existing vegetation (e.g. shrubs) as nurse plants can be a viable tool for maximizing establishment and survival of restoration plantings [16].



Figure 2-1. Sampling sites in UT, ID, and NV, USA. Sites were located in five major land resource areas across the Great Basin (inset).

Because plant-plant interactions often shift from positive at high stress to negative at low stress [11, 23, 24], the efficacy of nurse plants as restoration tools likely also varies over regional gradients (that range from high to low stress). One region that would benefit from a better understanding of the utility of nurse plants is the Great Basin, USA (Fig. 2-1), one of the most endangered ecosystems of western North America [25]. Invasive annual species such as *Bromus tectorum* (cheatgrass) threaten this region [26], and native plant restoration is a major priority [27]. *Artemisia tridentata* ssp. *wyomingensis* (Wyoming big sagebrush) the dominant shrub throughout much of this region at low to mid elevations, creates favorable sub-canopy microsites by reducing evapotranspiration [5], mediating soil temperatures [7], increasing soil water via hydraulic lift [28], and concentrating soil nutrients [29].

It is not clear, however, if or to what extent *A. tridentata* would be beneficial as a nurse plant for native plants throughout the Great Basin region (~32 million hectares) [27] that receives a wide range of annual rainfall (~200-450 mm) [30]. Field studies of grass-shrub spatial associations in the Great Basin [7, 31] have been limited to the northwestern portion of this ecosystem (Oregon, USA), and only one [31] explicitly examined stress gradients. Nurse shrubs may improve restoration of herbaceous plants in drier areas of the Great Basin where environmental stress is high, but may be less beneficial in more moderate (i.e., wetter) areas. Alternatively, herbaceous plants may perform better in interspace microsites where there is less woody plant competition [17]. Moreover, species-specific traits such as growth form or life history, may influence how different species respond to potential nurse shrubs [11, 32].

Understanding how a Great Basin-wide rainfall gradient affects spatial associations between *A. tridentata* and perennial grasses could help restoration practitioners determine where, within the Great Basin, *A. tridentata* nurse plants have the highest potential to improve growth and establishment of native grasses. We focused on three perennial grass species of differing growth forms that are of interest for use in restoration of Great Basin plant communities. In accordance with the stress gradient hypothesis [33], we hypothesized that the frequency of positive, grass-shrub spatial associations would increase as moisture stress increased (Fig. 2-2).

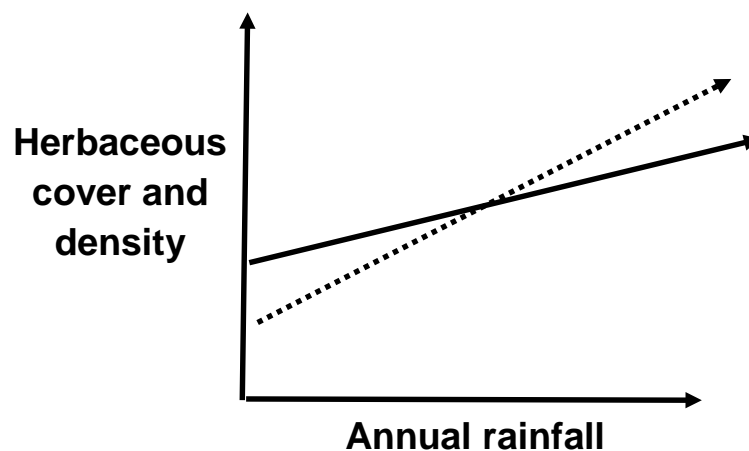


Figure 2-2. Hypothesis: Absolute cover and density of herbaceous species is greater in canopy microsites (solid line) versus interspace microsites (dashed line) at low rainfall levels. As rainfall increases, the relationship reverses, and absolute cover and density of herbaceous species is greater in interspace than canopy microsites.

Methods

Study area and site selection

The study area encompassed *A. tridentata* ssp. *wyomingensis*-dominated areas across the Great Basin, USA (Fig. 2-1). We selected sites within five major land resource areas (MLRAs), geographically associated land units [34] (Fig. 2-1). Sites sampled in 2012 (n=20), 2013 (n=9), and 2014 (n=3) ranged in mean annual rainfall from 220 mm to 378 mm (Appendix 1, Table S1-1). All sites were located on public land managed by the Bureau of Land Management (BLM).

We used a database of 826 candidate sites [35] to identify sites characterized by <40% *Bromus tectorum* cover (where native plant restoration efforts are most likely to be successful [Rodhouse *et al.* 2014]), presence of native perennial grasses, and no 50 year history of burning or land treatments. All sites had a history of spring and fall grazing in the five years prior to sampling. We used the Natural Resources Conservation Service Ecological Site Description (ESD) system [34] to select sites described as having loamy soils, a shrub overstory of *Artemisia tridentata* spp. *wyomingensis* (Wyoming big sagebrush), a dominant perennial grass understory of *Pseudoroegneria spicata* (bluebunch wheatgrass) or *Achnatherum thurberianum* (Thurber's needlegrass), and a sub-dominant herbaceous understory of *Elymus elymoides* (squirreltail) and *Poa secunda* (Sandberg's bluegrass). We retained 195 sites that met these criteria.

We determined rainfall for each of the 195 candidate sites with a Parameter-Elevation Regressions on Independent Slopes Model (PRISM) one of the most widely used and accurate weather models available [30] for the study area. The ~80-year average total spring (March-June) rainfall for weather stations located closest to our field sites

(Boise, ID; Twin Falls, ID; Winnemucca, NV; Tuscarora, NV; and Tooele, UT) is 102.33mm. Averages were 96.15 mm (2012) and 74.2 mm (2013) [Utah Climate 37].

We prioritized sites with the greatest perennial grass cover and least cover of *Bromus tectorum* (cheatgrass), an invasive, widespread plant of great economic and ecological concern in the Great Basin [26]. We then visited sites that represented a rainfall gradient as well as geographic variability across and within MLRAs. Final selection of our 32 field sites was based on field visits confirming minimal *B. tectorum* invasion and dominance by native perennial grasses (which were *P. secunda* and *E. elymoides*, the grasses described as being sub-dominant for sites in their ESD reference state), evidence of minimal to moderate recent livestock grazing, and accessibility (150-600 m from access roads). Evidence of livestock grazing was based on field inspections and grazing records maintained by the BLM.

Focal species

We selected three cool-season, native perennial grass species, *Poa secunda*, *Elymus elymoides*, and *Pseudoroegneria spicata* to represent general growth forms of bunchgrasses seen in the Great Basin, namely, shallow-rooted tufted [38], medium-rooted [39], and deep-rooted, respectively [40]. *Poa secunda* and *E. elymoides* were common across the entire range of rainfall whereas *P. spicata* was less common (Table S1). All three species are common and desirable components of plant communities and restoration native seed mixes in the Great Basin [41, 42]. *Artemisia tridentata ssp. wyomingensis* (Wyoming big sagebrush) is a perennial, evergreen shrub that can reach ages of over 100 years [43]. The root system includes a taproot that can reach depths of 1.5-2.4 meters and a shallow network of lateral roots reaching 0.9-1.5 meters from the shrub base [43].

Sampling

We investigated how spatial association between Wyoming big sagebrush (*A. tridentata ssp. wyomingensis*) and three common native perennial grasses (*P. secunda*, *E. elymoides*, and *P. spicata*) responded to an annual rainfall gradient. In 2012 - 2014 field sampling occurred between May 15th and July 30th to capture peak herbaceous biomass. We sampled 32 sites (*P. secunda* = 29 sites, *E. elymoides* = 27 sites, and *P. spicata* = 14 sites) (Appendix 1, Table S1-1). At each of the 32 sites we sampled 25-33 sagebrush individuals. Our sampling criteria for sagebrush shrubs included: height > 40cm (at the tallest portion of live canopy), width > 40cm (at the longest axis of canopy and its perpendicular length), > 50% live canopy (visually estimated), and presence of a single clearly discernable base stem; approximately 80% of shrubs fit within this criteria. To select focal shrubs we walked along three 50-m lines that radiated, at equal spacing, from a single randomly selected point. Every five meters we selected the closest shrub, alternating between left and right sides of the line. If a shrub did not fit our sampling criteria we chose the next closest shrub until we found an appropriate shrub.

At each shrub (780 across all sites), we sampled transects extending from the shrub base in each cardinal direction. We used canopy-intercept to record cover of *E. elymoides* and *P. spicata* along the four transects extending from each shrub base. On each transect the “canopy” zone extended from shrub base to canopy dripline. When this distance was zero (in the case of asymmetric shrubs), no canopy sampling occurred. The “interspace” zone extended from the canopy dripline to either a) the mid-point between canopy dripline and the nearest neighboring shrub canopy or b) 200 cm if the nearest neighboring shrub was more than 300 cm away. In cases of overlapping canopies only

canopy measurements were taken for that transect. Due to the patchy growth form of *P. secunda* at our study sites, its cover was visually estimated to the nearest 1% in 20 cm x 20 cm quadrats placed in the canopy (midpoint of canopy zone), edge (at canopy dripline, extending into interspace), and interspace (at both midpoint and end of the interspace) microsites (Appendix 1, Fig. S1-1).

We also assessed *E. elymoides* and *P. spicata* densities within a 40cm belt transect along each of the four transects. A third microsite (edge), extended from 10 cm inside to 10 cm outside the canopy dripline (canopy and interspace zones were adjusted accordingly). When no canopy existed (e.g. an asymmetrical shrub), the interspace on that transect extended from shrub base to 200 cm or the midpoint to the nearest neighboring shrub canopy (whichever came first). For each *E. elymoides* and *P. spicata* plant recorded in our density counts, we also measured its basal width, height and evidence of grazing (yes/no). We also recorded flowering (yes/no) of randomly selected *E. elymoides* and *P. spicata* plants at 10 sites between May 11 and July 1, 2014 (283 canopy / 249 interspace plants for *E. elymoides*, and 79 canopy / 98 interspace for *P. spicata*) (Appendix 1, Table S1-1). Due to difficulty in distinguishing *P. secunda* individuals, we did not record densities, sizes, height and grazing for *P. secunda* individuals.

We characterized general vegetation conditions at each of the 32 field sites along the three 50-m focal shrub selection line transects described above. Following the methods of Herrick, Van Zee (44), we assessed annual grass, perennial grass, annual forb and perennial forb cover, as well as bare ground, litter, duff, soil crust, and rock/gravel cover with line-point intercept; basal and canopy gap width of perennial vegetation;

density of livestock dung, perennial grasses, shrub density in 2m wide belt transects; and shrub volume of the first 15 shrubs >10cm tall per line transect.

Data Analysis

We used sites as replicates in our analyses (n=29 for *P. secunda*; n=27 for *E. elymoides*; n=10 for *P. spicata* cover; n=14 for *P. spicata* density), pooling shrub-level means. Sample sizes differ among species and response variables because cover and density were too low at some sites to be included in our analyses. We used individual mixed-effects ANCOVA models with interaction terms to model the spatial association of perennial grass covers and densities with *A. tridentata* over a continuous rainfall gradient. All analyses were conducted with SAS 9.3 [45] using the PROC GLIMMIX procedure with the Kenward-Roger method for calculating degrees of freedom. The categorical predictor variable for all analyses was shrub microsite (canopy or interspace), and the continuous covariate was total annual site rainfall. We included the interaction term microsite*rainfall, and the random effects were site and site*rainfall. Our response variables for nine separate main analyses were site levels means for cover of *P. secunda*, *E. elymoides*, and *P. spicata*; density of *E. elymoides* and *P. spicata*; height of *E. elymoides* and *P. spicata*; and basal width of *E. elymoides* and *P. spicata*. The analysis for *E. elymoides* and *P. spicata* density also included “edge”, a third level of shrub microsite. Since preliminary analyses indicated there was no year effect on percent cover and densities of perennial grasses, we combined field data from 2012, 2013, and 2014.

We used pairwise comparisons to test if regression slope coefficients of the rainfall*microsite interaction term differed among sagebrush microsites (canopy, edge and interspace). We inferred facilitation from a significantly positive shrub-grass spatial

association (e.g. canopy > interspace cover) and competition from a significantly negative shrub-grass spatial association (e.g. canopy < interspace cover). To avoid obtaining extrapolative values in type III tests of fixed effects, we centered rainfall values around a mean of 0. To achieve residual homogeneity of variance and assumptions of normality we log-transformed *E. elymoides* cover data, and square-root transformed *E. elymoides* density data. *Pseudoroegneria spicata* cover and density data were analyzed with beta and lognormal distributions, respectively.

We used a mixed logistic regression model to determine if the proportion of grazed plants differed among canopy, edge, and interspace sagebrush microsites for *P. spicata* and *E. elymoides*. For each species our response variable was the number of grasses grazed over the total number of grasses in each microsite (canopy, edge, or interspace). The model was run using PROC GLIMMIX [45]. We used a logistic regression model to determine if the proportion of reproducing *E. elymoides* and *P. spicata* grasses differed among canopy, edge, and interspace microsites. The two separate analyses were conducted using PROC LOGISTIC; we applied the Firth adjustment for small sample sizes [46]. For each species the response variable was the number of grasses flowering over the total number of grasses in each microsite (canopy, edge, or interspace). For all four analyses, counts were pooled across sampling sites, which were the replicating factors and random variables in the model (n=26 and n=13 for *E. elymoides* and *P. spicata*, respectively).

We examined variables associated with general site conditions to explore broadscale patterns among rainfall and other site-level covariates. We examined bivariate (Pearson) correlations between biologically meaningful covariates and cover and

density of focal species in canopy and interspace microsites using PROC CORR [45].

We did this as an exploratory analysis to determine if rainfall was correlated with other site covariates. We did not perform additional model selection procedures due to the limited number of sites (i.e., replicates) in the study.

Results

Exploratory analyses: site characteristics and correlations

Bromus tectorum cover was negatively correlated with sagebrush density ($r = -0.38$, $p = 0.04$) and positively correlated with perennial basal gap size ($r = 0.45$, $p = 0.01$). However, *Bromus tectorum* cover was not significantly correlated with cover of *E. elymoides*, *P. spicata*, or *P. secunda* in either sagebrush canopy ($r = -0.02$, $p = 0.92$; $r = -0.35$, $p = 0.32$; $r = -0.06$, $p = 0.75$, respectively) or interspace microsites ($r = -0.19$, $p = 0.33$; $r = 0.15$, $p = 0.66$; $r = -0.09$, $p = 0.64$, respectively). Dung density (our proxy of recent grazing intensity) was not significantly correlated with *E. elymoides*, *P. spicata*, *P. secunda* in either sagebrush canopy ($r = -0.04$, $p = 0.83$; $r = -0.23$, $p = 0.61$; $r = 0.0007$, $p = 0.99$, respectively) or interspace. Several site variables were correlated with rainfall (canopy gap size $r = -0.47$, $p = 0.01$; basal gap size $r = -0.39$, $p = 0.04$; bare ground $r = -0.57$, $p = 0.0011$; *Bromus tectorum* cover $r = -0.37$, $p = 0.04$; annual forb cover $r = -0.37$, $p = 0.05$; perennial forb cover $r = 0.36$, $p = 0.06$); and overall perennial grass cover $r = 0.44$, $p = 0.02$).

Cover

We found a significant effect of a microsite*rainfall interaction on mean *P. secunda* cover ($F_{1, 24.1} = 12.78$, $p = 0.002$; Fig. 2-3a), suggestive of a facilitative effect of *A.*

tridentata canopies on *P. secunda* at low to moderate rainfall levels. In shrub interspaces, mean percent cover of *P. secunda* increased significantly from 4% at the lowest to 13% at the highest annual rainfall ($t_{27}=2.7$, $p=0.01$; Fig. 2-3a). However, in sagebrush canopy microsites, there was no evidence to conclude that percent cover of *P. secunda* changed over increasing rainfall values ($t_{27}=0.0$, $p=1.0$; Fig. 2-3a). Percent cover of *P. secunda* in shrub canopies was significantly greater than that of interspaces at low (25th percentile, 249mm) to medium (50th percentile, 278 mm) rainfall levels ($t_{24.4}=5.1$, $p < 0.0001$, and $t_{24.4}=3.8$, $p=0.0008$, respectively). Differences were no longer significant at the 75th percentile of annual rainfall (310 mm) ($t_{24.41}=0.51$, $p=0.61$).

There was a strong effect of shrub microsite on *E. elymoides* cover which was significantly greater in canopy versus interspace microsites over all rainfall levels ($F_{1, 25.52}=63.07$, $p<0.0001$). However, the effect was strongest at lower annual rainfall as evidenced by a marginally significant effect of the interaction between shrub microsite and rainfall on mean *E. elymoides* cover ($F_{1, 25.52} = 3.98$ $p=0.06$; Fig. 2-3b). In contrast to *P. secunda*, cover of *E. elymoides* decreased significantly in shrub canopies from about 5% at low rainfall levels to less than 1% at the highest rainfall levels ($t_{23.46}= -2.13$, $p=0.04$). *Elymus elymoides* cover did not change significantly over rainfall values in interspace microsites ($t_{23.46}= -0.68$, $p=0.45$). In contrast to *P. secunda* and *E. elymoides*, there was no significant effect of either microsite or rainfall on *P. spicata* cover ($F_{1, 19} =7.18$, $p = 0.68$; $F_{1, 19}=7.218$, $p = 0.88$; Fig. 1-3c).

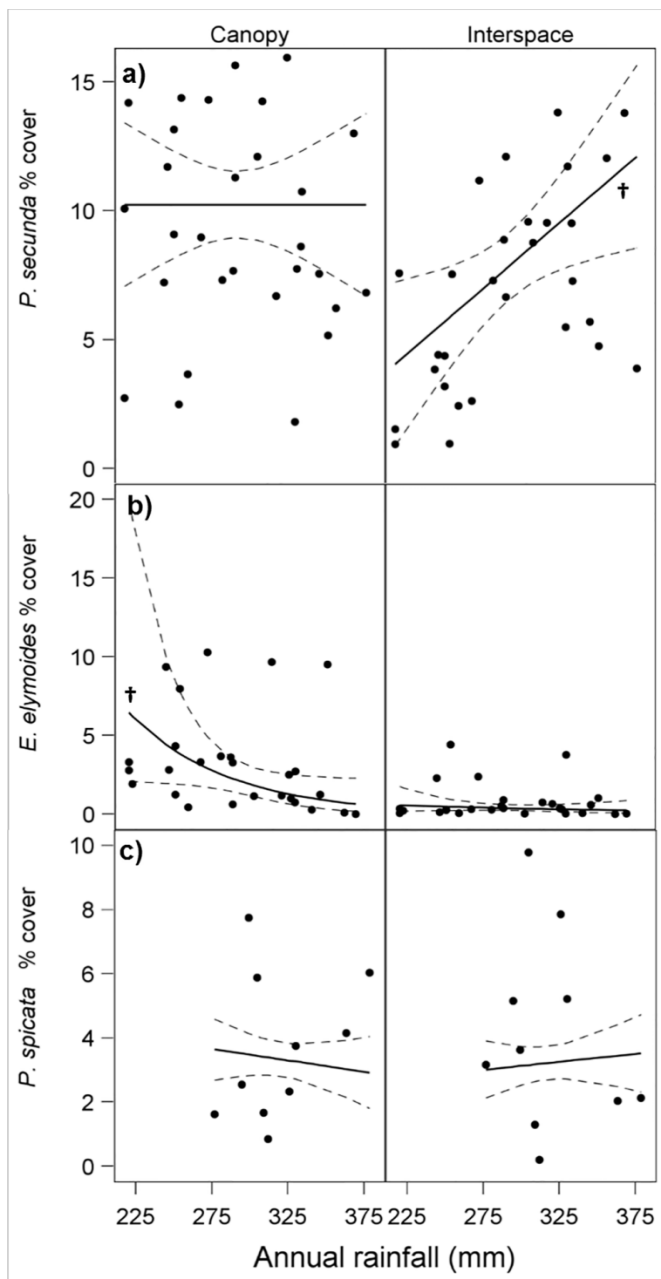


Figure 2-3. Mean percent cover and predicted regression lines (with 95% confidence bands) for a) *P. secunda* (n= 29 sites), b) *E. elymoides* (n= 27 sites) and c) *P. spicata* (n= 10 sites). Regression lines with slope significantly different from zero are denoted with (†). Sites are distributed across a rainfall gradient, and at each site cover was assessed for two microsites (shrub canopy and interspace). *E. elymoides* and *P. spicata* data are back-transformed.

Density

Elymus elymoides density results were consistent with a pattern of a stronger *A. tridentata* facilitative effect at lower rainfall levels. We found a significant interaction between shrub microsite and rainfall for *E. elymoides* mean densities ($F_{2, 38.26}=4.10$, $p = 0.02$; Fig. 2-4a). The slopes in both canopy and edge microsites were significantly more negative than that of the interspace microsite (canopy vs. interspace: $t_{49.5} = -3.14$, $p=0.003$; edge vs. interspace: $t_{49.5} = 2.02$, $p = 0.05$; Fig. 2-4a). Differences in *E. elymoides* densities were significant for all pairwise microsite comparisons up to the 90th percentile of the rainfall gradient (Table 2-1). *Pseudoroegneria spicata* density responded significantly only to rainfall, increasing with increasing rainfall ($F_{1, 12}=4.58$, $p = 0.05$; Fig. 2-5a).

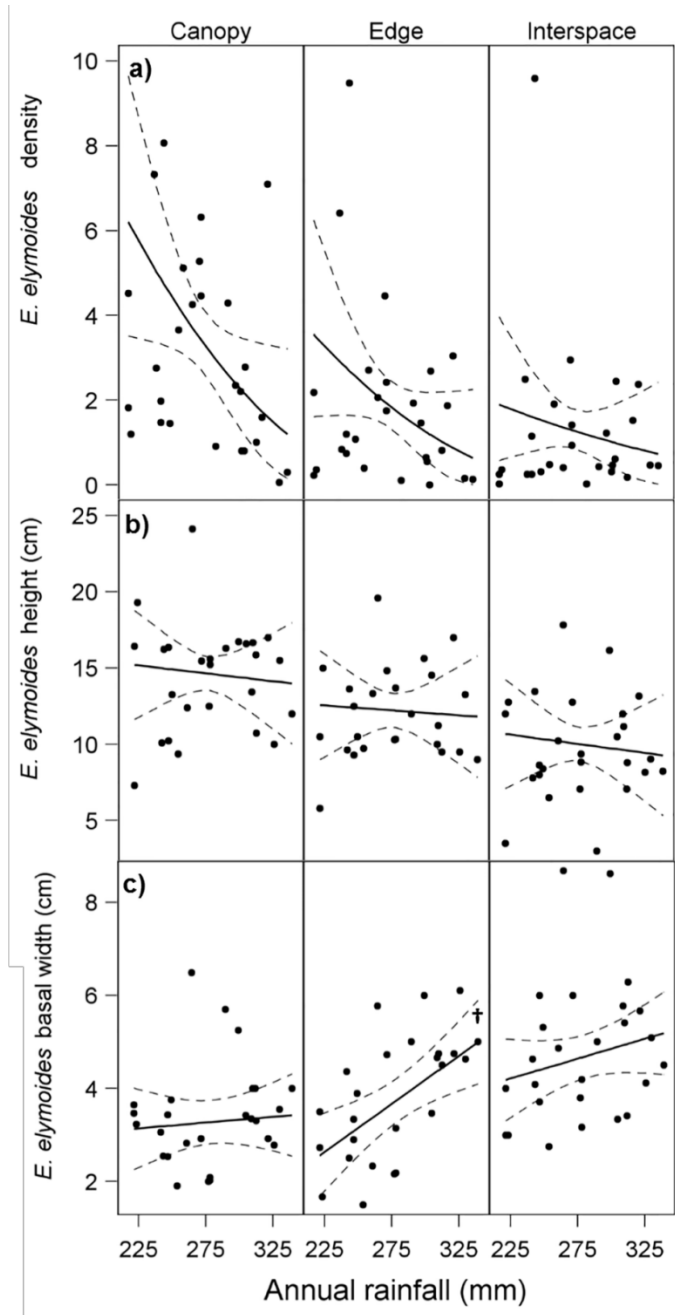


Figure 2-4. Site means and predicted regression lines (with 95% confidence bands) for *E. elymoides* a) density (number of plants / m²), b) height (cm), and c) basal width (cm) across rainfall and shrub microsites. Regression lines with slope significantly different from zero are denoted with (†). Sites are distributed across a rainfall gradient, and at each site cover was assessed for three microsites (shrub canopy, edge, and interspace).

Plant size

Height of *E. elymoides* (n=26 sites) was greater in canopy microsites across all rainfall levels ($F_{2,47}=40.64$, $p<0.0001$, Fig. 2-4b); mean heights were as follows: canopy $\bar{x}=14.6\text{cm}$ ($\sigma_{\bar{x}}=0.55$), edge $\bar{x}=12.2\text{cm}$ ($\sigma_{\bar{x}}=0.55$), interspace $\bar{x}=10\text{cm}$ ($\sigma_{\bar{x}}=0.93$). Mean heights differed significantly for all pairwise microsite comparisons (canopy vs. interspace: $t_{47}=9.01$, $p<0.0001$; canopy vs. edge: $t_{47}=4.64$, $p<0.0001$; edge vs. interspace: $t_{47}=4.24$, $p=0.0001$; Fig. 2-4b). Mean *E. elymoides* basal width (n=26 sites) followed the opposite pattern; basal width was overall greatest in interspace microsites, followed by edge and canopy ($F_{2,47}=7.45$, $p=0.002$; canopy $\bar{x}=3.27\text{cm}$ ($\sigma_{\bar{x}}=0.23$), edge $\bar{x}=3.72\text{cm}$ ($\sigma_{\bar{x}}=0.23$), interspace $\bar{x}=4.66\text{cm}$ ($\sigma_{\bar{x}}=0.23$); Fig. 2-4c). In addition, rainfall had a strong increasing effect on mean basal width for all microsites ($F_{2,47}=24.84$, $p<0.0001$), although the increase in basal width was significant only for edge microsites ($t_{41,97}=3.13$, $p=0.0032$; Fig. 2-4c) and was steeper than canopy slopes ($t_{47,21}=2.72$, $p=0.01$). Mean basal widths generally differed significantly for canopy-interspace and edge-interspace microsite comparisons up to the 90th percentile of rainfall (Table 2-1).

Neither sagebrush microsite nor rainfall significantly affected *P. spicata* height (n=13 sites; microsite $F_{2,18}=1.61$, $p=0.23$; rainfall $F_{1,11}=0.84$, $p=0.38$; Fig. 2-5b), although average height showed a trend of being highest in canopy microsites, followed by edge and interspace microsites: canopy $\bar{x}=28.8\text{cm}$ ($\sigma_{\bar{x}}=2.4$), edge $\bar{x}=27.6\text{cm}$ ($\sigma_{\bar{x}}=2.5$), interspace $\bar{x}=22.8\text{cm}$ ($\sigma_{\bar{x}}=2.3$). *Pseudoroegneria spicata* basal width (n=13 sites) was significantly affected only by microsite ($F_{2,18}=7.3$, $p=0.0048$) and was greatest in interspace microsites, followed by edge and canopy (canopy $\bar{x}=9.13\text{cm}$, $\sigma_{\bar{x}}=1.28$; edge $\bar{x}=11.2\text{cm}$, $\sigma_{\bar{x}}=1.3$; interspace $\bar{x}=13.4\text{cm}$, $\sigma_{\bar{x}}=1.24$; Fig. 2-5c).

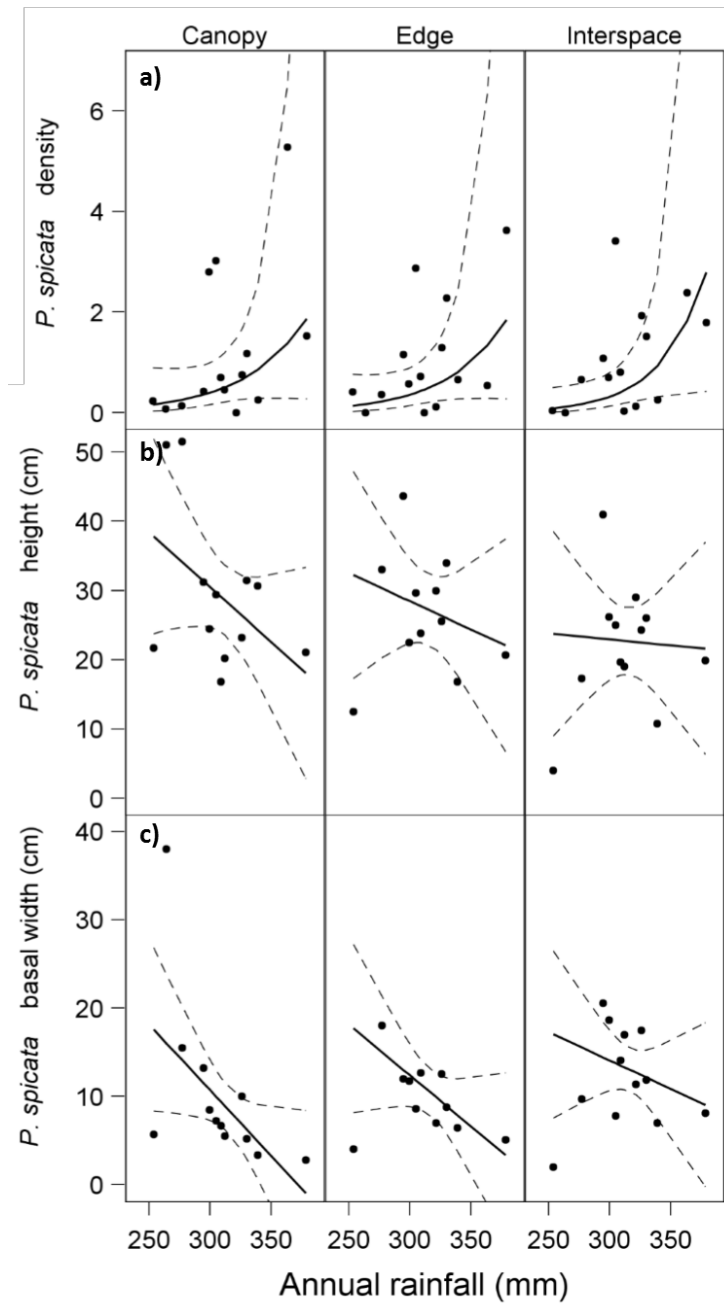


Figure 2-5. Site means and predicted regression lines (with 95% confidence bands) for *P. spicata* a) density (number of plants / m²), b) height (cm), and c) basal width (cm) across rainfall and shrub microsites. All regression lines were not significantly different from 0. Sites are distributed across a rainfall gradient, and at each site cover was assessed for three microsites (shrub canopy, edge, and interspace).

Grazing response and reproduction

The proportion of grazed grasses differed significantly among microsites for both *E. elymoides* and *P. spicata* ($F_{2,49} = 75.9$, $p < 0.0001$; $F_{2,14} = 75.9$, $p < 0.0001$, respectively) The proportion of grazed *E. elymoides* and *P. spicata* grasses was significantly greater in interspace vs. canopy microsites ($t_{49} = -11.98$, $p < 0.0001$; $t_{14} = -3.09$, $p = 0.02$, respectively), as well as interspace vs. edge microsites ($t_{49} = -6.27$, $p < 0.0001$; $t_{14} = -2.68$, $p = 0.04$, respectively).

The proportion of reproducing grasses did not differ significantly among microsites for either *E. elymoides* or *P. spicata* (Wald Chi-sq₂=0.38, $p=0.84$ and Wald Chi sq₂=0.79, $p=0.67$).

Discussion

We found that spatial patterns of two native perennial grasses, *P. secunda* and *E. elymoides*, were strongly and positively associated with *A. tridentata* canopy microsites at the lowest annual rainfall levels. These patterns are suggestive of net facilitative plant relationships in more stressful conditions (low annual rainfall) shifting to more neutral or competitive relationships in mesic conditions (high annual rainfall). These results, from across a regional rainfall gradient, provide support for the generality of the stress gradient hypothesis [33] and the prediction that stronger facilitation occurs in more stressful environmental conditions [11, 33, 47]. We suggest that shrub canopy microsites could be advantageous microsites for restoration plantings of *P. secunda* and *E. elymoides* in moisture-limited areas of the Great Basin. Lack of evidence for positive spatial associations between *A. tridentata* and the third grass species, *P. spicata*, however, also indicates the importance of considering species-specific relationships [48]. Our results

are generally consistent with, but much broader in geographical scope, than previous work in northwestern portions of the Great Basin that has shown positive spatial associations between *A. tridentata* and native perennial grasses under stressful, moisture-limited conditions [7, 31].

Shrub-grass patterns

We identified distinctly different positive patterns for the different grass species. At high rainfall, the net positive effect of *A. tridentata* on cover of the first grass, *P. secunda*, shifted to neutral, likely due in part to increased competition with *A. tridentata* and other herbaceous vegetation for soil moisture. Although subcanopy cover of the second grass, *E. elymoides*, decreased with increasing rainfall, *A. tridentata* still had a net facilitative effect on *E. elymoides* cover over almost the entire range of annual rainfall. Cover of the third grass species, *P. spicata*, was unaffected by proximity to *A. tridentata* nurse shrubs due to either a lack of nurse plant effect or lower sample size than the other two grass species. Our results are consistent with Davies, Bates (7), who found increased *E. elymoides* cover under *A. tridentata* canopies at drier sites but no differences in subcanopy *P. spicata* cover between dry and mesic sites. Our results for all three grass species contrast with those reported by Reisner (31) who found evidence for net competitive shrub-grass relationships for *P. secunda* and *E. elymoides* in wetter conditions and positive relationships at drier sites for *P. spicata*. Our study encompassed a greater geographical extent and more site variability than Reisner (31), which may explain our contrasting results.

Patterns of higher *Elymus elymoides* cover in canopy microsites were driven by higher plant densities rather than bigger plant sizes. Both *E. elymoides* and *P. spicata*

canopy microsite plants were taller and had smaller basal widths than those growing in edge or interspace microsites. The narrow, tall stature of shrub canopy plants may be a growth response to the increased ratio of far to near-red light found in canopy conditions [49], or it could be due to increased inter- or intraspecific competition among adult plants for other resources [50, 51]. Alternatively, interspace plants may have been shorter due to grazing (see *Grazing* section below). Although reduced plant size could reduce fitness, we found that flowering was not reduced in canopy microsites. Together our size and reproduction results suggest that targeting restoration plantings in canopy microsites might yield smaller plants but not at the expense of establishing a self-sustaining (i.e., reproductive) population.

Species differences

We have shown shrub-grass spatial associations vary across grass species. *Poa secunda* appears to benefit from growing in *A. tridentata* canopies at lower rainfall levels, perhaps because it is short-rooted and benefits from hydraulic lift from *A. tridentata*. However, its shallower root system may be sufficient when site moisture is higher, thereby explaining its neutral association with *A. tridentata* at higher rainfall. Additionally, the benefits of sub-canopy microsites might be limited except under the driest conditions because *P. secunda* enters dormancy sooner than most Great Basin perennial grasses, [52] enabling it to largely avoid drought during the summer months [38, 52]. In contrast, *E. elymoides* generally has a deeper root system than *P. secunda* but continues to grow later into the summer months. As moisture stress increases over the summer, *E. elymoides* may increasingly benefit from the improved microsite conditions (e.g., lower temperatures, reduced solar radiation, or increased organic matter) in shrub

canopies, even at higher rainfall levels. The deep-rooted *P. spicata*, on the other hand, can survive under a very wide range of moisture conditions [53], which may allow it to persist in both canopy and interspace microsites. Understanding these types of species-specific patterns is key toward reconciling opposing views on the importance and nature of the stress-gradient hypothesis [48].

Another explanation for differences in plant spatial patterns among grass species may be contrasting reproductive strategies and seed dispersal patterns. *Elymus elymoides*, which only reproduces via seed, may be more likely to establish near or under *A. tridentata* canopies because shrubs can act as seed traps, simultaneously limiting the dispersal of seeds dropped within shrub canopies and trapping windblown seeds at their periphery [54]. The large seedheads of *E. elymoides* were observed in great numbers under sagebrush canopies (MFH *pers. obs*). In contrast, because *P. spicata* produces less seed and propagates mainly by tillering [40], distribution of this species may depend more on location of parent plants than seed dispersal (and trapping by *A. tridentata*). Small *P. secunda* seeds may be trapped by microtopography of the soil surface [55] of either shrub canopies or interspaces.

Grazing

Herbivory also can mediate plant facilitation and even intensify abiotically-driven facilitation [9, 56]. *A. tridentata* canopy microsites may provide physical protection against large herbivore grazing [9], particularly for palatable grasses [57]. In our study all sites had been spring grazed at least once in the past five years, and *E. elymoides* and *P. spicata* plants, both of which are palatable to livestock [39, 42], were grazed more in interspace than canopy microsites. However, we did not find our livestock grazing

intensity covariate (dung density) to be correlated with *E. elymoides* or *P. spicata* cover. Further work should explore whether canopy-interspace patterns for these two grasses differ between low and high grazing intensity sites.

Applications to ecological restoration and future research needs

We found that naturally occurring *P. secunda* and *E. elymoides* plants were associated with *A. tridentata* canopy microsites, particularly in drier areas. These patterns suggest that exploiting canopy microsites for restoration seedings or plantings in Great Basin sagebrush communities could improve plant establishment, growth, survival, or some combination thereof [19, 58]. Although *E. elymoides* distributions may be driven at least partially by trapping of seeds and increased propagule pressure in shrub understories, our results clearly show that plants will persist to maturity in understory microsites. Future studies examining responses of plants at multiple life stages would help clarify the mechanisms behind the patterns we observed.

Our results are most applicable to areas of the Great Basin that contain shrub overstories but lack a robust perennial understory and are at risk of invasion by undesirable annuals. Typically, however, restoration efforts in the Great Basin target areas where sagebrush canopies are no longer intact such as burned areas. Our results highlight the potential utility of shrubs to serve as nurse plants in restoration settings and provide important ecosystem functions.

Based on the results of our study we suggest that land managers should consider the utility of a nurse plant approach as way to improve the ecological health and resilience of the Great Basin. To translate our results into practice will require controlled experimentation that explicitly investigates, across species and rainfall levels, canopy

microsite effects on multiple plant life stages, from germination to establishment, survival, and long-term population viability (e.g., reproductive potential and dispersal/establishment beyond canopy microsites). Nonetheless, our results highlight that, when planning and prioritizing restoration activities across the Great Basin, average annual rainfall level can be used as a tool to make coarse predictions about where positive plant associations can be expected to occur.

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Table 2-1. Pairwise microsite (canopy, edge, interspace) comparisons for *E. elymoides* mean density and basal width (cm) at 25th, 50th, 75th, and 90th percentile values of rainfall (mean diff = mean difference estimate) across 27 sites in the Great Basin, USA.

		Density			Basal width		
percentile		canopy vs. edge	canopy vs. interspace	edge vs. interspace	canopy vs. edge	canopy vs. interspace	edge vs. interspace
	25th	mean diff (± 1 SE)	0.54 \pm 0.13	0.94 \pm 0.13	0.39 \pm 0.13	0.11 \pm 0.50	-1.20 \pm 0.50
<i>t</i>		4.34	7.48	3.14	0.22	-2.42	-2.65
<i>P</i>		0.0001	<0.0001	0.003	0.8252	0.0193	0.0111
50th	mean diff (± 1 SE)	0.46 \pm 0.09	0.71 \pm 0.09	0.25 \pm 0.09	-0.44 \pm 0.37	-1.39 \pm 0.37	-0.95 \pm 0.37
	<i>t</i>	5.03	7.75	2.72	-1.2	-3.7	-2.5
	<i>P</i>	<.0001	<.0001	0.0098	0.0475	0.0027	0.2858
75th	mean diff (± 1 SE)	0.38 \pm 0.12	0.47 \pm 0.12	0.10 \pm 0.12	0.05 \pm 0.37	0.003 \pm 0.37	0.29 \pm 0.37
	<i>t</i>	3.13	3.92	0.8	-1.2	-3.75	-2.51
	<i>P</i>	0.0034	0.0003	0.4302	0.2349	0.0005	0.0154
90th	mean diff (± 1 SE)	0.33 \pm 0.15	0.35 \pm 0.15	0.01 \pm 0.15	-1.32 \pm 0.63	-1.68 \pm 0.63	-0.36 \pm 0.63
	<i>t</i>	2.16	2.26	0.1	-2.08	-2.69	-0.56
	<i>P</i>	0.037	0.0298	0.9246	0.0428	0.0099	0.576

CHAPTER 3

GRAZING WEAKENS RAINFALL-DRIVEN SPATIAL ASSOCIATIONS BETWEEN
SAGEBRUSH AND PERENNIAL GRASSES²**Abstract**

As environmental stresses such as limited moisture and grazing pressure increase, the frequency and/or importance of positive (i.e., facilitative) interactions between benefactor plants (nurses) and beneficiaries are predicted to increase. At extreme levels of biotic or abiotic stress these facilitative interactions may break down. While many studies have sought to explain the effect of individual stresses on plant-plant associations, few studies have addressed the effects of co-occurring stresses. We therefore investigated spatial associations between Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) and two native grasses (*Poa secunda*, and *Elymus elymoides*) across different combinations of grazing (biotic stress) and annual rainfall levels (abiotic stress) in the Great Basin, USA. We hypothesized that 1) competitive interactions and negative shrub-grass spatial associations would occur under the least stressful conditions (low grazing intensity- high rainfall); 2) positive shrub-grass spatial associations would occur most frequently at intermediate levels of stress (high grazing intensity-high rainfall and low grazing intensity -low rainfall); and 3) at extreme levels of stress (high grazing-low rainfall), positive grass-shrub relationships would no longer dominate. We sampled five site pairs (high vs. low grazing intensity) that occurred over low to high rainfall levels. At each of these 10 locations we assessed the strength of interactions between *A. tridentata* and *P. secunda* and *E. elymoides* (via the relative

² Co-authored by Kari Veblen.

interaction index [RII]), as well as responses of *P. secunda* cover and *E. elymoides* density to sagebrush microsite (canopy vs. interspace), grazing intensity, and a continuous rainfall gradient. We found that *P. secunda* and *E. elymoides* were positively associated with *A. tridentata* canopy microsites at low annual rainfall levels, but the two species had slightly different responses to grazing. For *P. secunda*, grazing stress weakened this effect, indicating, as we predicted, a potential breakdown in facilitation in highly stressful conditions. Facilitation dominated in one moderately stressful scenario (low grazing-low rainfall) supporting our hypothesis, but did not in the second moderately stressful scenario (high grazing-high rainfall). Our results provide guidance for Great Basin managers who are balancing the health of sagebrush systems with livestock grazing needs.

Introduction

Positive plant-plant interactions have been documented across ecosystems worldwide [1], including many rangeland systems [2]. According to the stress gradient hypothesis [3], the frequency and importance of positive (i.e., facilitative) plant-plant interactions are predicted to increase with increasing abiotic or biotic environmental stress. Positive plant-plant interactions become more frequent or intense with increasing moisture stress, a common and pervasive type of abiotic stress [4-6] [but see 7]. At high levels of moisture stress, a “nurse” plant can benefit a neighboring beneficiary plant by ameliorating harsh environmental conditions, for example by improving soil moisture conditions [8, 9], mediating soil temperatures and reducing evapotranspiration [10, 11], enhancing soil nutrient availability [12] and organic matter [11], or decreasing solar radiation [8, 13]. Some research, on the other hand, suggests that at very high levels of

abiotic stress (e.g., extreme drought), facilitative effects of neighbors on beneficiaries are outweighed by competition for severely limited resources [14].

Biotic stress, such as ungulate grazing, also has been shown to affect the strength of nurse-beneficiary relationships, resulting in more complex species interactions [15]. Generally, positive plant-plant interactions increase in response to increases in grazing stress [16-18], mainly due to the physical protection of vulnerable, palatable species by less palatable or thorny neighbors [17, 19, 20]. This phenomenon, known as associational resistance [21], may be enhanced when the difference in palatability between nurse and beneficiary plants increases [20]. However, as biotic stress progressively increases, competitive plant-plant interactions may predominate again at the highest stress levels [15, 22]. For example, under heavy grazing pressure, ungulate grazers are less selective and browse less-preferred (thorny or unpalatable) nurse plant species, which may, in turn, damage nurse plants and reduce their effectiveness at protecting palatable species growing under their canopies [23]. At higher grazing pressure, grazers may also search more intensely for food resources [24]. Thus, an increase in biotic stress may cause facilitative plant-plant interactions to follow a hump-shaped pattern, wherein facilitative plant-plant interactions peak at intermediate stress levels, and negative interactions dominate at extreme levels of biotic stress [15, 25].

How nurse-beneficiary relationships respond to the interaction of environmental stresses such as herbivory and moisture stress is less studied and poorly understood [26]. There have been a number of studies suggesting that co-occurrence of herbivory and moisture stress increases positive plant interactions. Perea and Gil (27) observed that concomitant increases in moisture and ungulate herbivory stresses increased facilitative

effects between shrubs and tree seedlings in a Mediterranean ecosystem [but see 18], and Veblen (17) reported that grazing-mediated facilitation between two grasses only occurred during the most abiotically stressful (dry) times. Similarly, Verwijmeren, Rietkerk (26) and Noumi, Chaieb (28) reported only a slight degree of grazing-mediated facilitation when abiotic conditions were less stressful (i.e., under higher moisture) [but see 29].

It is also possible that under dual (biotic and abiotic) stresses, facilitation is more likely to break down. For example, in abiotically stressful environments where forage availability is limited, grazing ungulates may search more intensely for palatable plants that may be growing under nurse plant canopies [24]. Unraveling the relative effects of moisture and herbivory stresses on plant facilitation is difficult because moisture and grazing stresses often covary in water-limited systems. Better understanding of the separate and combined influence of biotic vs. abiotic stresses on nurse plant relationships will provide important insights into what drives plant distributions, particularly in arid and semi-arid ecosystems where moisture and herbivory stresses are common [30].

An ideal place to address questions about the relative roles of biotic and abiotic stresses in nurse plant relationships is the Great Basin, USA. In areas of the Great Basin dominated by sagebrush (*Artemisia tridentata*) annual rainfall can be as low as 177mm (7 inches) [31], and the vast expanse of land is heavily utilized for livestock grazing [32]. It previously has been shown that native perennial grasses form positive spatial patterns with *A. tridentata* under more abiotically stressful environmental conditions [5, 33, 34, in review]. *Artemisia tridentata* can facilitate neighboring species via amelioration of harsh conditions in its sub-canopy by reducing evapotranspiration [35], mediating soil

temperatures [33], increasing soil water via hydraulic lift [36], and concentrating soil nutrients [37]. Because *A. tridentata* has relatively low palatability to domestic cattle [38] and its canopy provides a physical barrier to grazing cattle, *A. tridentata* may also facilitate perennial grasses by providing them with a grazing refuge. However, only one study has explicitly considered both moisture and grazing stress gradients [5], and it was confined to the Three Rivers Resource Area of the BLM Burns District located in the Southwestern portion of Oregon. Thus it is not clear how moisture vs. grazing stresses affect facilitation between *A. tridentata* and perennial grasses over a broader geographical area.

Our objective was to determine how shrub-grass interactions and spatial associations varied under two grazing intensities (high and low) over a rainfall gradient in the Great Basin. We hypothesized that facilitative interactions and positive spatial associations would be strongest in moderately stressful conditions (i.e., high grazing intensity- high rainfall and low grazing intensity-low rainfall conditions). We expected to observe net competitive interactions and negative spatial associations in two scenarios: a) the least stressful conditions (low grazing intensity-high rainfall conditions) because resources are less limiting and b) under the highest stress (high grazing intensity-low rainfall conditions) due to the breakdown of facilitation at extreme stress [25]. The disintegration of facilitative interactions may result from increased nurse plant – beneficiary competition at low moisture levels and/or a decrease in the protective benefit of shrub canopies at low moisture levels when vegetation production is limited and ungulates may forage more intensely under shrub canopies [15].

Methods:**Focal species:**

Artemisia tridentata ssp. *wyomingensis* (Wyoming big sagebrush) is a long-lived perennial, evergreen shrub that can live over 100 years [39]. The root system includes a long taproot and a shallow network of lateral roots [39]. It is relatively unpalatable to domestic cattle (Howard 1999) and has a rigid, closed canopy which is difficult for cattle to penetrate. *Poa secunda* (Sandberg bluegrass) and *Elymus elymoides* (bottlebrush squirreltail) are common, native Great Basin perennial grasses that are palatable to domestic livestock [40, 41]. *Poa secunda* is tolerant of some drought due to its shallow, fibrous root system [42]. It is moderately grazing tolerant and is one of the first grasses to begin seasonal growth [42]. *Elymus elymoides* is a short-lived, medium-rooted, perennial bunchgrass. It is not as tolerant of drought as *P. secunda* [43] and is slightly more vulnerable to grazing, especially in the spring [44].

Study area and site selection:

The study area encompassed Wyoming big sagebrush-dominated areas across two Great Basin, USA states: Idaho and Nevada (Fig. 3-1). We selected five paired high grazing/low grazing sites; 4 were located in the Central Owyhee High Plateau, and one was located in the Humboldt Area Major Land Resource Area [45]. Sites ranged in rainfall from 220mm to 357mm, a typical range of annual rainfall values for Wyoming big sagebrush ecological sites in the region. All sites were located on public land managed by the Bureau of Land Management (BLM).

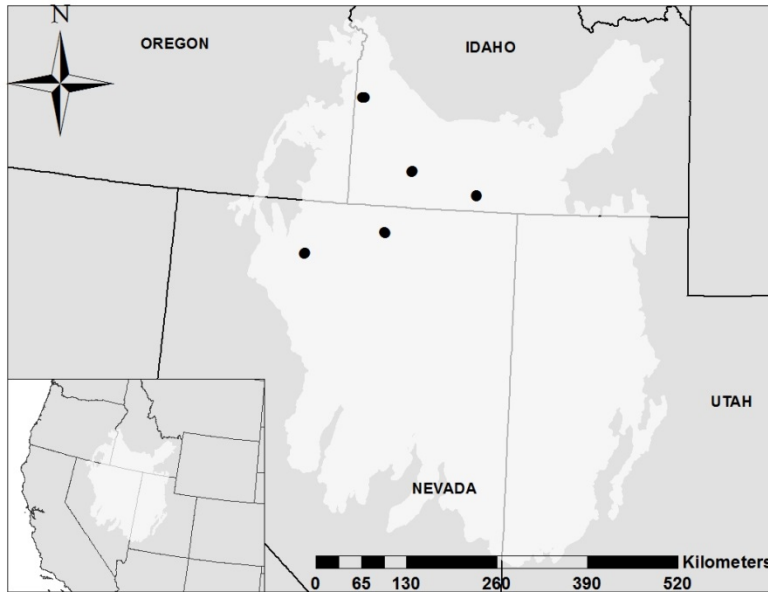


Figure 3-1. Sampling sites in the northwest Great Basin (inset). Each dot represents a paired high grazing and low grazing site.

Our initial 29 candidate sites were those sampled in a previous study investigating grass-shrub spatial associations over a rainfall gradient [34, in review]. The 29 sites were characterized by < 40% cheatgrass (*Bromus tectorum*) cover, presence of native deep- or short-rooted perennial grasses, and no recent history of burning or land treatments. All sites were described as having a shrub overstory of *Artemisia tridentata* spp. *womingensis*, a dominant herbaceous understory of bluebunch wheatgrass (*Pseudoroegneria spicata*), or Indian ricegrass (*Achnatherum thurberianum*), a sub-dominant herbaceous understory of *Elymus elymoides* and *Poa secunda*, and loamy soil, according to Ecological Site Descriptions (NRCS 2006). Although sites were described as being dominated by *P. spicata* or *A. thurberianum* in their reference state we found that the major herbaceous species present at our sites were *P. secunda* and *E. elymoides*.

From the pool of 29 sites, we selected five sites that fell across a rainfall gradient, where we were able to determine locations of natural (streams, ponds) or artificial water sources (troughs, artificial lakes) known to have been utilized to some degree by livestock cattle for at least 5 years. At each site we defined two plot types: low and high grazing intensity. High intensity areas were located 200-400m from a water source, and low intensity plots were 800+ m from a water source. Because grazing intensity attenuates with increasing distance from a water source, distance from water sources has been used to quantify long-term grazing intensity [46]. We verified recent high grazing intensity with observations of hoofprints, fresh dung, and bite marks on grasses, and presence of nearby cattle. Low grazing plots showed no to little signs of recent livestock activity.

Sampling

We investigated how spatial association between Wyoming big sagebrush (*A. tridentata ssp. wyomingensis*) and two common native perennial grasses (*P. secunda* and *E. elymoides*) responded to grazing intensity. In 2013 field sampling occurred between May 15th and June 30th to capture peak herbaceous biomass. At each of the 5 paired sites (10 total), we sampled 25 sagebrush individuals. We randomly selected focal shrubs by walking along three 50-m lines that radiated, at equal spacing, from a single randomly selected point. Every five meters we chose the nearest shrub, alternating between left and right sides of the transect. Our sampling criteria for sagebrush shrubs was: height > 40cm (at the tallest part of live canopy), width > 40cm (at the longest axis of canopy and its perpendicular length), > 50% live canopy (visually estimated), and presence of a single,

distinguishable base stem. If a shrub did not fit our sampling criteria we selected the next closest shrub until we found an appropriate shrub.

At each shrub (250 across all sites), we sampled transects extending from the shrub base in each of the four cardinal directions. At each transect the “canopy” zone extended from shrub base to canopy dripline. When this distance was zero (in the case of asymmetric shrubs), we did not sample the canopy zone. The “interspace” zone extended from the canopy dripline to either a) the mid-point between the canopy dripline and the nearest neighboring shrub canopy or b) 200 cm if the nearest neighboring shrub canopy was more than 300 cm away. In cases of overlapping canopies only canopy measurements were taken for that transect.

We used canopy-intercept to record cover of *E. elymoides* along the four transects extending from each shrub base. We assessed *E. elymoides* density within a 40cm belt transect along each of the four transects. In cases where no canopy measurements were taken (e.g. an asymmetrical shrub), the interspace on that transect extended from shrub base to 200 cm or the midpoint to the nearest neighboring shrub canopy (whichever came first). Due to the patchy growth form of *P. secunda* at our study sites, its cover was ocularly estimated to the nearest 1% in 20 cm x 20 cm quadrats placed in the canopy (midpoint of canopy zone), edge (at canopy dripline, extending into interspace), and interspace (at both midpoint and end of the interspace) microsites (Appendix 2, Fig. S2-1).

Finally, to characterize the overall site, we measured the following attributes along all three transects: cattle dung density, cover of perennial grasses, density of

perennial grasses, basal gap size, annual grass cover, perennial forb cover, annual forb cover, soil crust cover, and bare ground cover using the methods of [47].

Analysis

We used percent cover data for all *P. secunda* analyses and density data for all *E. elymoides* analyses. We were not able to use percent cover data for *E. elymoides* due to the large number of zeros (primarily in high grazed sites) in that dataset. Previous work shows that *E. elymoides* density and cover respond to rainfall in similar ways [34, in review]. We used shrubs as replicates for *P. secunda* cover analyses (n= 267). Due to the large number of zero density values for *E. elymoides*, shrub-level means were pooled thereby treating sites as replicates (n=10).

To characterize grass-shrub interactions, we used the Relative Interaction Index (RII), a widely used metric in ecology that characterizes the strength of plant-plant interactions [48]. We calculated the RII for each grass species according to the following formula:

$$\text{(eq. 1)} \quad \text{RII} = (\text{Canopy}_C - \text{Interspace}_C) / (\text{Canopy}_C + \text{Interspace}_C)$$

where (Canopy_C) is percent cover in the canopy microsite of the target *A. tridentata* shrub and Interspace_C is percent cover in the interspace adjacent to the shrub. RII values range from -1 (intense competition) to +1 (intense facilitation). For each grass species we used a general linear mixed model to analyze the response of each species' RII to two levels of grazing intensity and a continuous rainfall gradient (220mm to 340mm). The categorical fixed effects factor for this model was grazing intensity (low, high), and annual site rainfall was the continuous fixed effects factor. The model also included the interaction

of grazing intensity with rainfall, and total variance was partitioned into variance among site pairs.

For the general linear mixed model testing of *P. secunda* cover response, grazing intensity (low/high) and microsite (canopy/interspace) were treated as categorical fixed effects factors. Rainfall was treated as a continuous fixed effects factor, and the model included rainfall* grazing intensity and rainfall*microsite interactions. Total variance was partitioned into variance among site pairs: variance among sites nested within pairs and associated with different levels of grazing intensity, variance among locations nested within sites and associated with different microsites, and residual variance. For analysis of *E. elymoides*, fixed effects were grazing intensity (low/high), microsite (canopy/interspace), and rainfall (continuous). The model included the interaction of grazing intensity and microsite. Total variance was partitioned into variance among site pairs: variance among sites nested within pairs and associated with different levels of grazing intensity.

Percent cover values for *P. secunda* were arcsine-square root transformed, and density values for *E. elymoides* were square-root transformed prior to analysis to better meet assumptions of normality and homogeneity of variance. For all analyses, pairwise comparisons were used to test whether coefficients of the regression slopes of rainfall*microsite and rainfall* grazing intensity interactions differed among sagebrush microsites and grazing intensity levels. To avoid obtaining extrapolative values in type III tests of fixed effects, rainfall values were centered around a mean of 0; all type III tests of fixed effects were reported for standardized rainfall data. Data calculations were made

using the GLIMMIX procedure using the Kenward-Roger method for calculating degrees of freedom in SAS v. 9.3 for Windows [49].

To identify differences between high and low grazing sites with respect to site characteristics, we calculated site means for density of cattle dung patties, density of perennial grasses, perennial grass cover, bare ground cover, perennial forb cover, annual forb cover, soil crust cover, and perennial plant canopy gap size (cm). We also conducted t-tests to determine if these variables differed between low and high grazing intensity sites. Basal gap size data were log-transformed prior to conducting t-tests to achieve residual normality and homogeneity of variances. These analyses were conducted in SAS using PROC GLIMMIX.

Results

Site characteristics

High grazing intensity sites were characterized by significantly greater dung density ($p=0.0062$) and lower perennial grass density ($p=0.03$) than low grazing intensity sites (Table 3-1). Perennial grasses showed a non-significant trend toward lower cover, and basal gap sizes were non-significantly greater in high grazing intensity sites (Table 1). All other measurements did not differ statistically between high and low grazing intensity sites (Table 3-1)

Table 3-1. Site attribute means (\pm 1 SD) and t-test results for high vs. low grazing intensity sites

Variable	High grazing intensity	Low grazing intensity	t value (DF=10)	Standard error	p
Density dung patties (patties / m ²)	0.14 (0.05)	0.05 (0.07)	3.45	0.027	0.0062
Density perennial grasses (plants / m ²)	0.35 (0.22)	0.88 (0.62)	-2.56	0.21	0.03
Perennial grass % cover	31.9 (19.0)	45.3 (14.5)	-1.75	0.077	0.1110
Bare ground % cover	16.4 (9.9)	15.2 (11.7)	0.05	0.25	0.81
Perennial forb % cover	3.8 (8.0)	4.6 (5.8)	0.03	-0.23	0.82
Annual forb % cover	8.9 (9.7)	3.4 (4.1)	1.63	0.03	0.13
Annual grass % cover	9.0 (13.5)	8.7 (8.7)	0.05	0.05	0.96
Soil crust % cover	1.2 (2.0)	3.5 (5.9)	0.02	-1.18	0.26
Perennial plant basal gap size (cm)	275 (355)	110 (71.29)	0.35	1.18	0.26

RII overall results

Poa secunda showed an overall pattern of positive (i.e., facilitative) RII values at the lowest rainfall levels and a decrease to negative (competitive) values at the highest annual rainfall levels (Fig. 3-2). In contrast, *E. elymoides* interactions with *A. tridentata* were positive, regardless of annual rainfall, with the exception of one site (Fig. 3-2; RII >0 for nine out of ten sites). For *P. secunda*, the shift from positive RII values at low rainfall to negative RII values at high rainfall was driven by greater absolute *P. secunda* cover in *A. tridentata* canopy versus interspace microsites (i.e., positive spatial

associations between *P. secunda* and *A. tridentata*) at low rainfall levels (10th percentile: canopy cover = 8.3%; interspace cover = 2.6%; Appendix 2, Fig. S2-2) and also by higher cover in interspace versus canopy microsites at high rainfall (90th percentile: canopy cover = 8.4%; interspace cover = 12%; Appendix 2, Fig. S2-2; rainfall*microsite: $F_{1, 3.014} = 15.23$, $p = 0.005$; Appendix 2, Fig. S2-2). The dominance of positive RII values for *E. elymoides* was due to the consistently greater absolute densities of *E. elymoides* in canopy versus interspace microsites (canopy density = 2.07 plants/m²; interspace density = 0.67 plants/m²; effect of sagebrush microsite ($F_{2,16} = 13.43$, $p = 0.002$; Fig. 3-4).

For *P. secunda*, the pattern of facilitative interactions at low rainfall becoming increasingly competitive with increasingly wet conditions differed according to grazing intensity, while for *E. elymoides*, interactions with *A. tridentata* were positive, regardless of both grazing intensity and annual rainfall, with the exception of one site (Fig. 3-2; RII >0 for nine out of ten sites). For *P. secunda*, the pattern was strongest under low grazing (RII rainfall*grazing interaction $F_{1, 262.9} = 5.38$, $p = 0.02$; percent cover rainfall*grazing interaction $F_{1, 3.014} = 12.92$, $p = 0.0362$; Fig. 3-2.). Under low grazing intensity, *P. secunda* RII decreased significantly from facilitative (+0.7) at low rainfall to competitive (-0.25) at high rainfall (slope = -0.17, $t_{3.35} = -3.77$, $p = 0.02$; Fig. 3-2). Although the trend of increasingly competitive plant interactions with increasing rainfall was also present at high grazing intensity (+0.3 at low rainfall to -0.1 at high rainfall), the magnitude of the difference was smaller and had only a marginally significant (slope = -0.11, $t_{3.297} = -2.52$, $p = 0.08$; Fig 3-2). For *E. elymoides*, only the effect of rainfall was significant ($F_{1, 3.014} = 13.43$, $p = 0.03$); grazing was not significant ($F_{1, 3} = 0.82$, $p = 0.43$), nor was the interaction of grazing*rainfall ($F_{1, 3.024} = 2.17$, $p = 0.24$).

The contrasting RII patterns for high and low grazing intensities, specifically for *P. secunda*, were driven by two main factors. First, under low rainfall, the magnitude of the difference between canopy and interspace cover was smaller under high grazing intensity (difference of ~3.7%; Fig. 3-3b) than low grazing intensity (difference of ~7.6%, Fig. 3-3a). Second, the relationship (direction of slope) between absolute cover of *P. secunda* and rainfall differed according to grazing intensity and shrub microsite. At low grazing intensity, cover in canopy microsites decreased, while in interspace microsites, cover increased over rainfall (canopy microsites: slope = -.02, $t_{8.736} = -2.03$, $p=0.07$; interspace microsites: slope = 0.02, $t_{8.736} = 1.4$, $p=0.20$; Appendix 2, Fig. S2-2). In contrast, under high grazing intensity, absolute cover of *P. secunda* increased significantly in response to rainfall in both canopy and interspace microsites (canopy microsites: slope = 0.03, $t_{9.082} = 2.19$, $p=0.06$; interspace microsites: slope = 0.07, $t_{8.736} = 5.81$, $p=0.0002$; Appendix 2, Fig. S2-2). As a result, at low annual rainfall (10th percentile), grazing reduced absolute percent cover of *P. secunda* in both microsites (Fig. 3-3 a & b), while at high rainfall (90th percentile), there was instead a small increase in canopy and interspace covers due to grazing (Fig. 3-3 a & b).

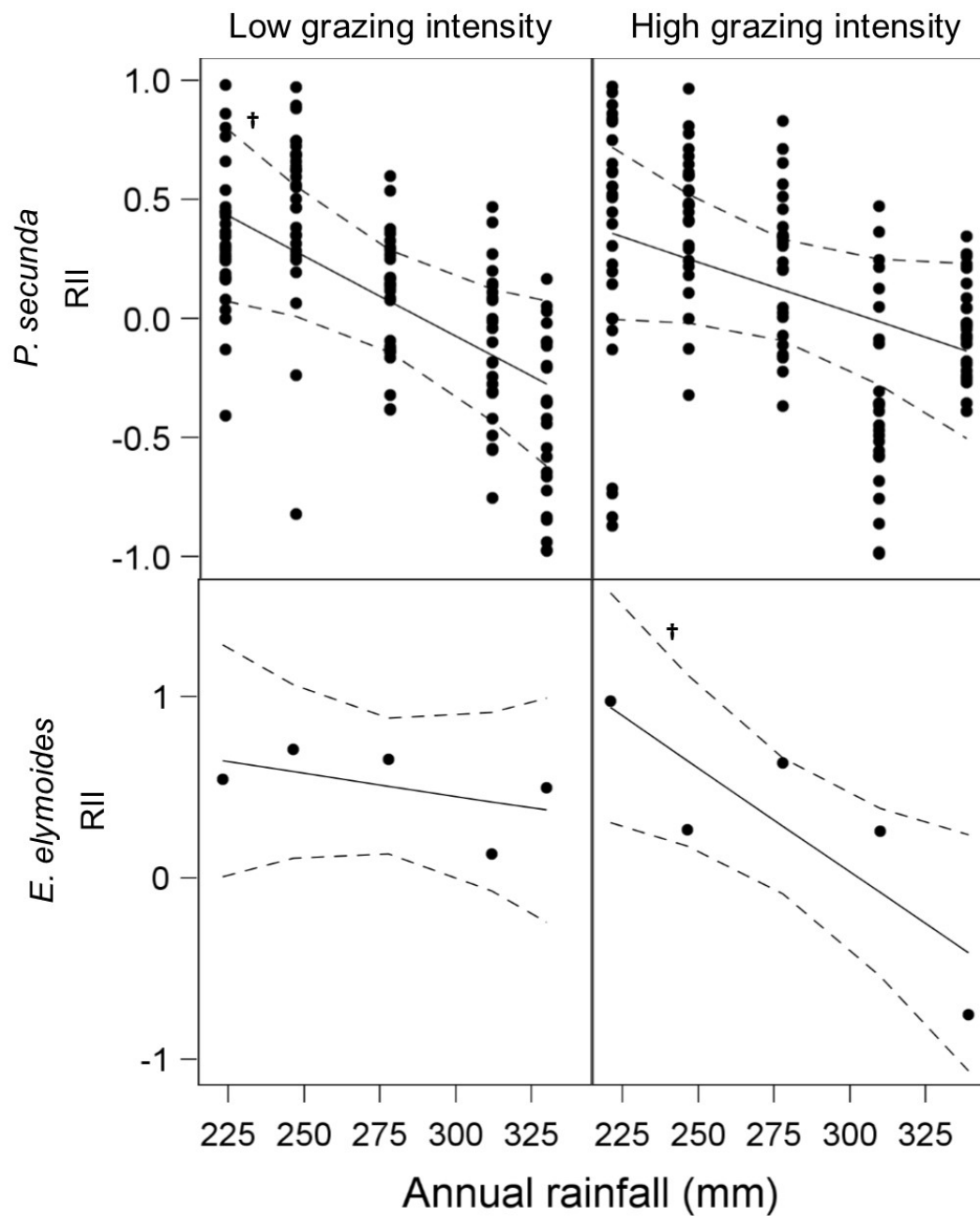


Figure 3-2. Shrub-level means and predicted regression lines (with 95% confidence bands) for RII of *P. secunda* and *E. elymoides* across rainfall. Regression lines significant from zero are denoted with (†).

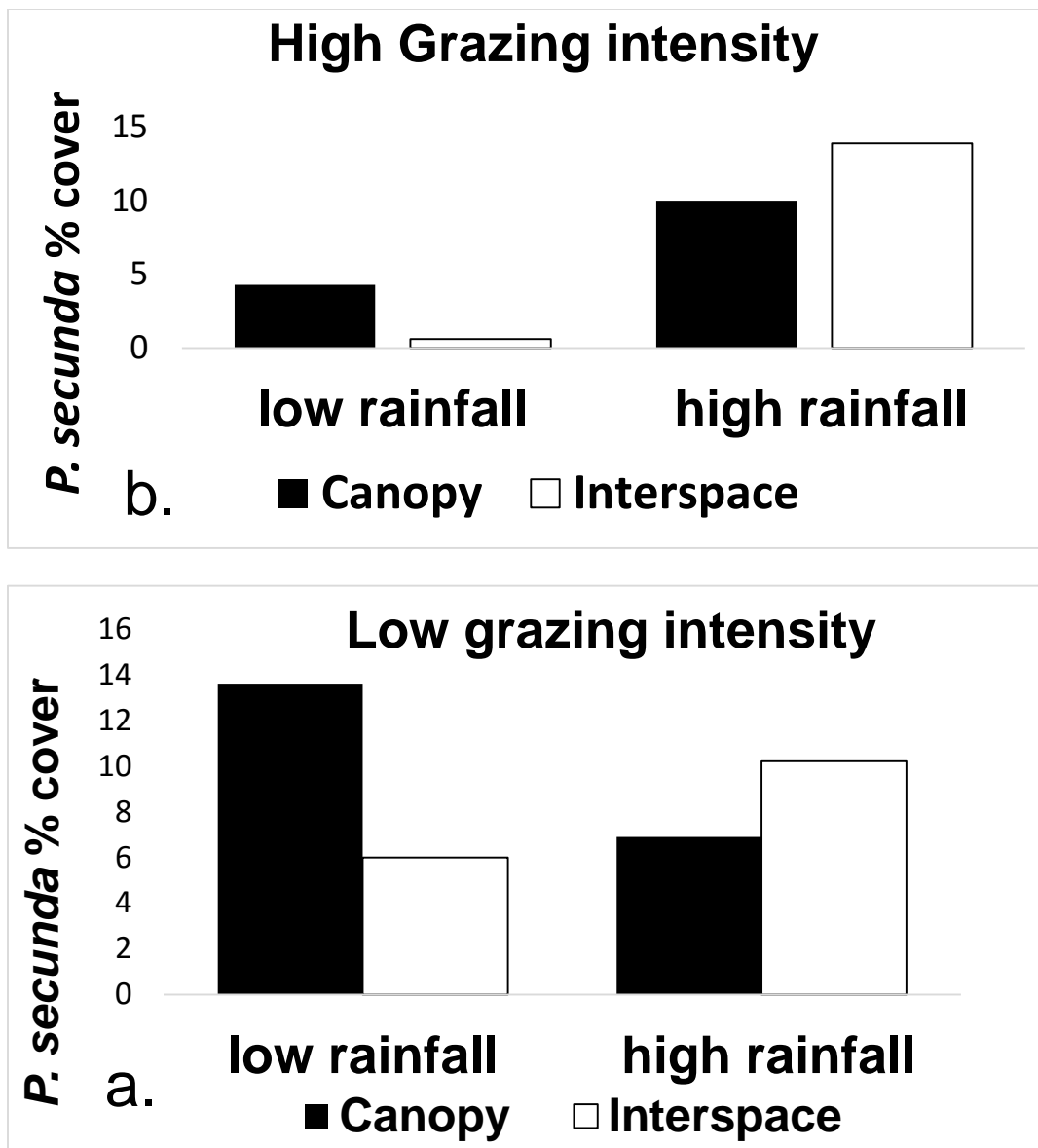


Figure 3-3. Percent cover of *P. secunda* in canopy and interspace microsites at low and high annual rainfall (10th and 90th percentiles) over low (a) and high (b) grazing intensities.

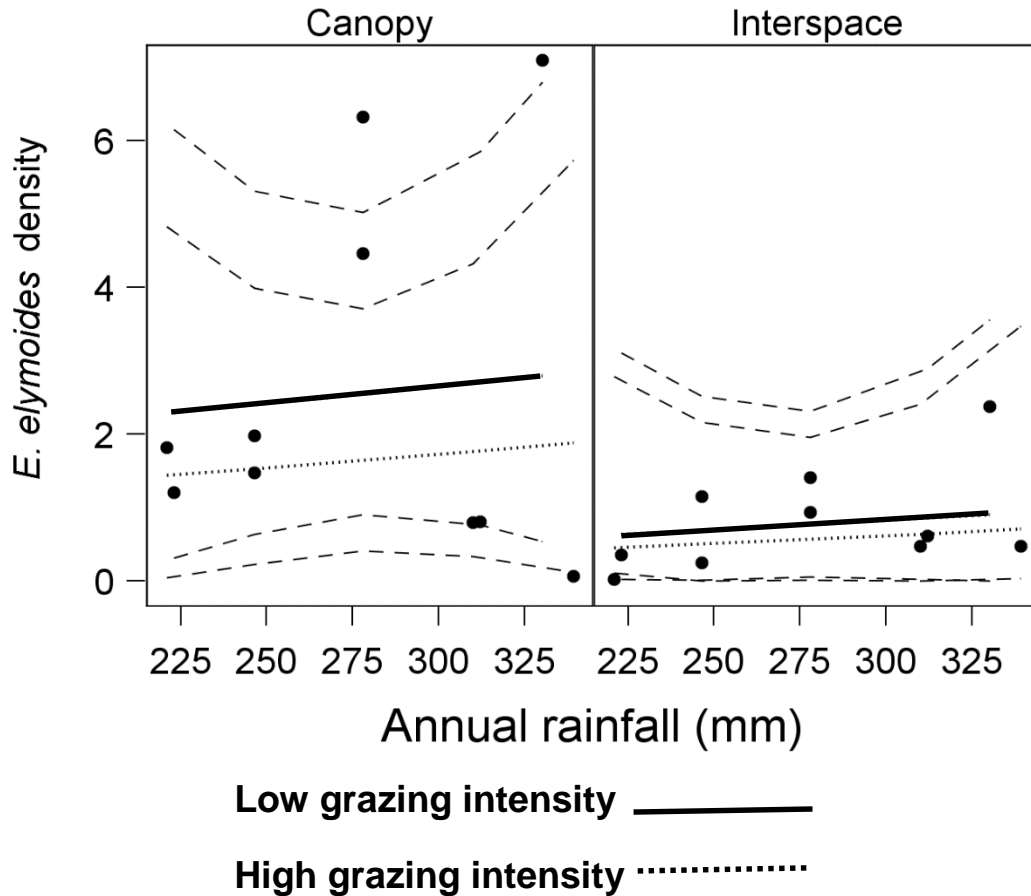


Figure 3-4. Back-transformed means and predicted regression lines (with 95% confidence bands) for absolute densities of *E. elymoides* at ten sites across a rainfall gradient, in canopy and interspace microsites, and at high and low grazing intensity. No slopes were significantly different from zero.

Overall, our results show that *P. secunda* and *E. elymoides* formed positive associations with *A. tridentata* canopy microsites at low rainfall levels (stressful conditions).

Discussion

Relatively little is known about how biotic and abiotic stresses interact to influence plant-plant relationships, and in particular whether facilitative relationships will break down under the most stressful conditions. We found that the native perennial grasses *P. secunda* and *E. elymoides* both were positively associated with *A. tridentata* canopy microsites in stressful conditions at the lowest annual rainfall levels. For *P. secunda*, addition of biotic (grazing) stress weakened this effect, indicating a potential breakdown in facilitation in highly stressful conditions (e.g., low moisture conditions and high grazing stress), as we had predicted. Our results did not entirely support our prediction that facilitation would dominate in the two scenarios we considered to be moderately stressful; for both grass species, facilitation dominated in one scenario, low grazing-low rainfall, but did not in the second, high grazing-high rainfall.

Poa secunda

Interactions between *A. tridentata* and *P. secunda* generally shifted from facilitative to competitive as annual rainfall increased, which is consistent with the results of other Great Basin studies [5, 33, 34, in review]. However, we found that grazing weakened this pattern; facilitative interactions between *P. secunda* and *A. tridentata* were not as strong when moisture was limiting and grazing intensity was high. Specifically, under dry conditions, the magnitude of the benefit of growing in canopy microsites (relative to interspaces) was less under high than low grazing.

We suggested that canopy microsites offered less protection to *P. secunda* from grazing animals in dry conditions due to covariance of moisture and grazing stresses; in semi-arid systems, grazing often is more intense under low rainfall [17, 50]. We found

that at low annual rainfall, high grazing intensity resulted in a considerable reduction of *P. secunda* cover (relative to low grazing intensity) in canopy microsites. This reduction in cover could be attributed to an increase in the search intensity of grazing animals when low moisture limits overall vegetation production and forage resources [24]. In this scenario, *A. tridentata* may be less effective in shielding subcanopy *P. secunda* plants from grazing. This reduction in associational resistance has been documented at extreme levels of consumer pressure [23]. Verwijmeren, Rietkerk (26) found that as grazing pressure increased on (drier) south facing slopes in southeastern Spain, plant-plant facilitation was reduced. In contrast, herbivory has been shown to intensify abiotically-driven facilitation elsewhere [17, 20]. In the Great Basin, Reisner (5) found that a gradient of increasing moisture and grazing stress resulted in strong facilitative interactions between *P. secunda* and *A. tridentata*. The rainfall gradient used by Reisner (5) was similar to ours, but our contrasting results could be due to different tests of grazing stress. Reisner (5) used a grazing gradient based on sampling at various distances from a water source, while we considered only two distances and levels of grazing intensity.

We also found that grazing reduced the magnitude of the net competitive interactions we observed at high annual rainfall for *P. secunda*. At high rainfall, RII was slightly less negative (i.e., more weakly competitive) under high than low grazing intensity, suggesting that grazing stress either reduced competition between *P. secunda* and *A. tridentata* or increased any facilitative interactions between the two. These results are consistent with Verwijmeren, Rietkerk (26), who also found that, in wetter conditions,

net competitive interactions between two plant species were reduced as grazing pressure and the effect of associational resistance increased.

Although we observed net facilitative effects of *A. tridentata* on *P. secunda* under some intermediate stress conditions (low grazing-low rainfall), we observed net competitive effects under other intermediate stress levels (high grazing intensity, high rainfall conditions), not facilitative effects as we had predicted. These latter results were surprising because grazing often drives net facilitation when unpalatable or thorny neighbors protect vulnerable understory plants [e.g., 16]. However, there is some evidence that grazing-mediated facilitation only occurs in drier conditions [17]. At high rainfall levels where moisture should be more readily available in both canopy and interspace microsites, grazing tolerant species such as *P. secunda* may benefit more by growing in interspaces to avoid competition with nurse shrubs, i.e., the benefit of lower competition in interspaces may outweigh any protection from grazing offered by shrub canopies.

Elymus elymoides

Interactions between the second perennial grass species, *E. elymoides*, and *A. tridentata* were indicative of net facilitation at most sites, regardless of rainfall or grazing intensity. Although we were not able to use percent cover data for *E. elymoides* in our analyses, a previous study [34, in review] showed that *E. elymoides* cover and density responded similarly to *A. tridentata* canopy and interspace microsites. The facilitative effects we observed were mainly due to greater densities of *E. elymoides* in sagebrush canopy than interspace microsites. These results are consistent with Reisner (5), Davies,

Bates (33), and Holthuijzen and Veblen (34, in review) who also found evidence that *E. elymoides* is facilitated by *A. tridentata* in most areas of the Great Basin.

We found one exception to this pattern of facilitation, at high grazing intensity under high rainfall, where *E. elymoides* showed a net competitive response. This site was grazed more heavily than all other sites (*pers. obs*), as evidenced by low grass cover in both canopy and interspace microsites (and despite the high rainfall and expectation of higher productivity and cover at the site); sufficiently heavy grazing could cause animals to preferentially select more nutritious under-canopy plants, which was also found by Smit et al. (23). Because our regressions were each based on only five points, a greater sampling effort would help determine whether the shift from facilitative to competitive is genuine or an artefact of small sample size.

E. elymoides, which is more susceptible to both grazing and drought than *P. secunda*, may be facilitated by *A. tridentata* in two ways. First, it may benefit to a greater extent from improved soil moisture conditions in sagebrush canopies as compared to *P. secunda* because it is less drought tolerant. Second, it may benefit more from physical protection from grazing. In a previous study, Holthuijzen and Veblen (34) found that a smaller proportion of *E. elymoides* plants were grazed in canopy versus interspace microsites. It is important to note that, although both species are considered relatively tolerant of grazing by domestic livestock, *P. secunda* is more grazing tolerant than *E. elymoides* [42, 44].

We found that naturally occurring *P. secunda* and *E. elymoides* plants were associated with *A. tridentata* canopy microsites, particularly in drier areas. Additionally, we found that the combination of two stressors, moisture limitation and grazing, cause grass-shrub interactions to become more complex. In particular, grazing weakened facilitation when rainfall was limiting, but also seemed to weaken competition when rainfall was high. We also found that facilitation did not always dominate under moderate stress. Rather, in the moderate conditions of high grazing under high rainfall, the benefits of increased rainfall appeared to outweigh any negative effects of grazing on *P. secunda*. An alternative explanation to this result is that high site-site variability (e.g., in historic stocking rates) prevented us from detecting a strong grazing effect.

Our results may assist land managers in predicting how Great Basin plant communities will shift over time in response to the separate and combined effects of grazing and climate change. Facilitation between plants may increase the fundamental niche of species whose ranges are limited due to harsh environmental conditions (limited moisture, high temperatures, etc.) [51]. Our results suggest that future decreases in rainfall would result in an increase in the frequency (or importance) of facilitative grass-shrub interactions, causing perennial grasses to preferentially grow under sagebrush canopies. This outcome could leave interspaces even more vulnerable to invasion by undesirable annual grasses such as *Bromus tectorum*. Competitive interactions may dominate in higher rainfall conditions, resulting in increased perennial grass cover in sagebrush interspaces, which, in turn, could reduce site invasion potential. Depending on how plant distributions change, mitigation of climate change impacts may have to occur via modification of grazing practices.

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CHAPTER 4

CONCLUSION

My results suggest that the native, Great Basin grasses (*P. secunda* and *E. elymoides*) are associated with *A. tridentata* canopy microsites, particularly in drier areas. Additionally, I have shown that the combination of two stresses, moisture limitation and grazing, cause grass-shrub interactions to become more complex. Grazing by domestic livestock cattle weakens facilitation when rainfall is limiting, but also seems to weaken competition when rainfall is high.

I found that canopy microsites could be used during ecological restoration of the Great Basin to help improve the growth, establishment, germination, or recruitment of seedlings and or seeds. The findings presented here may help managers predict how Great Basin plant communities will shift over time in response to the separate and combined effects of grazing and climate change. Future studies examining responses of plants at multiple life stages would help clarify the mechanisms behind the patterns we observed.

My results apply to areas of the Great Basin that still contain shrub overstories but lack a robust perennial understory and are at risk of invasion by undesirable annuals. Typically, however, restoration efforts in the Great Basin target areas where sagebrush canopies are no longer intact such as burned areas. In my study, I have demonstrated the potential utility of shrubs to serve as nurse plants in restoration settings and provide important ecosystem functions.

The results of my thesis I suggest that land managers should consider the utility of a nurse plant approach as way to improve the ecological health and resilience of the Great

Basin. To translate these results into practice will require controlled experimentation that explicitly investigates, across species and rainfall levels, canopy microsite effects on multiple plant life stages, from germination to establishment, survival, and long-term population viability

In conclusion my results may help managers predict how Great Basin plant communities will shift over time in response to the separate and combined effects of grazing and climate change. Facilitation between plants may increase the fundamental niche of species whose ranges are limited due to harsh environmental conditions (limited moisture, high temperatures, etc.). Future decreases in rainfall may result in an increase in the frequency (or importance) of facilitative grass-shrub interactions, causing perennial grasses to preferentially grow under sagebrush canopies. This could leave interspaces vulnerable to invasion by undesirable species such as *Bromus tectorum*. However, if rainfall increases, competitive interactions may dominate, resulting in increased perennial grass cover in sagebrush interspaces, which could reduce site invasion potential. Future studies on multiple stress gradients in this region would benefit from controlled tests of grazing intensity across a greater number and diversity of sites.

APPENDICES

APPENDIX A

Table S1-1. 2012, 2013, and 2014 sampling sites and attributes.

Site	PRISM rainfall mm	ESD rainfall mm	Major land resource area	State	Year sampled	Latitude	Longitude
J521DUX_02	221	203-254	Humboldt Area	NV	2012*●	41.06298 2	- 118.45537
Grasmere 5	241	178-254	Owyhee High Plateau	ID	2012, 2014*●○□	42.44437 7	- 115.84427
X378AUX_01	243	203-254	Humboldt Area	NV	2012*●	40.86218	- 118.10173
Grasmere 3	249	178-254	Owyhee High Plateau	ID	2012, 2014*●○□	42.33215	- 115.84252
z269aux01	250	203-305	Snake River Plains	ID	2012*	42.93022	- 115.31045
X022AUX_02	253	203-254	Central Nevada Basin and Range	NV	2012*●■	40.92033 9	- 114.53371
Squaw	254	254-330	Owyhee High Plateau	ID	2012*●■	43.43040 4	- 116.86181
x428DUX_02	260	203-254	Humboldt Area	NV	2012*●■	40.48552 4	- 117.43795
J489DUX_03	264	203-254	Humboldt Area	NV	2012*●■	41.26252	- 117.71296
f555aux01	271	203-305	Snake River Plains	ID	2012*●	42.36664 7	- 114.47114
Dam	277	203-305	Owyhee High Plateau	ID	2012*●	42.20645 1	- 114.76649
Palisade	292	203-254	Owyhee High Plateau	NV	2012*	40.53208	- 116.28477
Bigfoot	292	203-305	Snake River Plains	ID	2012, 2014*●○□	43.24763 6	- 116.25227
Tuscarora	305	254-305	Owyhee High Plateau	NV	2012, 2014*●○■ □	41.59531	- 116.35237
N245AUX_01	308	254-330	Owyhee High Plateau	ID	2012, 2014*●○■ □	43.446	-116.968
X039DUX_03	312	203-254	Owyhee High Plateau	NV	2012*●	41.1728	-114.867
Dugway east	321	203-305	Great Salt Lake	UT	2012*●	40.05831 2	- 112.70609

F463aux_02	326	279-330	Snake River Plains	ID	2012, 2014*●○■ □	43.27949 6	- 114.32595
R157DUX_03	357	203-305	Great Salt Lake	UT	2012*	40.2456	-112.627
WilsonHi	310	254-305	Owyhee High Plateau	NV	2013*●	41.66200 3	- 116.33469
WilsonLow	312	254-305	Owyhee High Plateau	NV	2013, 2014*●■○ □	41.65590 2	- 116.31676
AntelopeHi	221	203-254	Humboldt Area	NV	2013*●	41.30878	-117.6917
AntelopeLow	223	203-254	Humboldt Area	NV	2013*●	41.31999 4	- 117.68408
DamHigh	278	203-305	Owyhee High Plateau	ID	2013*●	42.20115	- 114.76608
DamLow	278	203-305	Owyhee High Plateau	ID	2013*●	42.19697 8	- 114.76697
Elephant	339	254-330	Owyhee High Plateau	ID	2013*	43.37321 1	- 116.84716
Buttelo	330	254-330	Owyhee High Plateau	ID	2013*●■	43.37698	- 116.88564
JackCrk	246	254-330	Owyhee High Plateau	ID	2012*●	42.46332 7	- 115.92876
JackCrkh20	246	254-330	Owyhee High Plateau	ID	2013*●	42.46546 6	- 115.91969
F174DUX_01	277	254-330	Owyhee High Plateau	ID	2014○■□	42.10286 9	-114.7864
Q613_AUX02	378	330-457	Great Salt Lake	UT	2014○■□	39.66774 9	- 112.04157
J527_AUX03	294	203-254	Humboldt Area	NV	2014○■□	41.43687 8	- 117.05473

Table S1-2. Environmental variables, measurement method, and type (continuous/categorical) used in NMS covariate matrix.

Variable	Definition, measurement method, type (continuous or categorical)
Rainfall	Annual site rainfall based on PRISM data (Daly & Bryant 2013), <i>continuous</i>
Annual rainfall category	Annual site rainfall based on PRISM data placed into categories (Low: 203-254mm/yr, Medium: 254-304 mm/yr, High: 304+ mm/yr, <i>categorical</i>)
Density of shrubs, perennial grasses, and dung	Measured in 1-m belt transects along three 50-m site characterization transects, <i>continuous</i>
Basal gap size (perennial vegetation)	Distance (cm) between bases of perennial plants; measured along three 50m site characterization transects, <i>continuous</i>
Annual canopy gap size	Distance (cm) between canopies of annual plants, measured along three 50m site characterization transects, <i>continuous</i>
Perennial canopy gap size	Distance (cm) between canopies of perennial plants, measured along three 50m site characterization transects, <i>continuous</i>
Cover of annual grasses, perennial grasses, annual forb, perennial forb, gravel, rocks, cryptobiotic soil crust, shrubs, Embedded litter	Measured along three 50m site characterization transects using line-point intercept, <i>continuous</i>
Ecological site category	Ecological site category, based on NRCS ecological site descriptions, <i>categorical</i>
Elevation	Site elevation, estimated from NRCS ecological site descriptions, <i>continuous</i>
Soil code	Site soil type, based on NRCS soil types, <i>categorical</i>

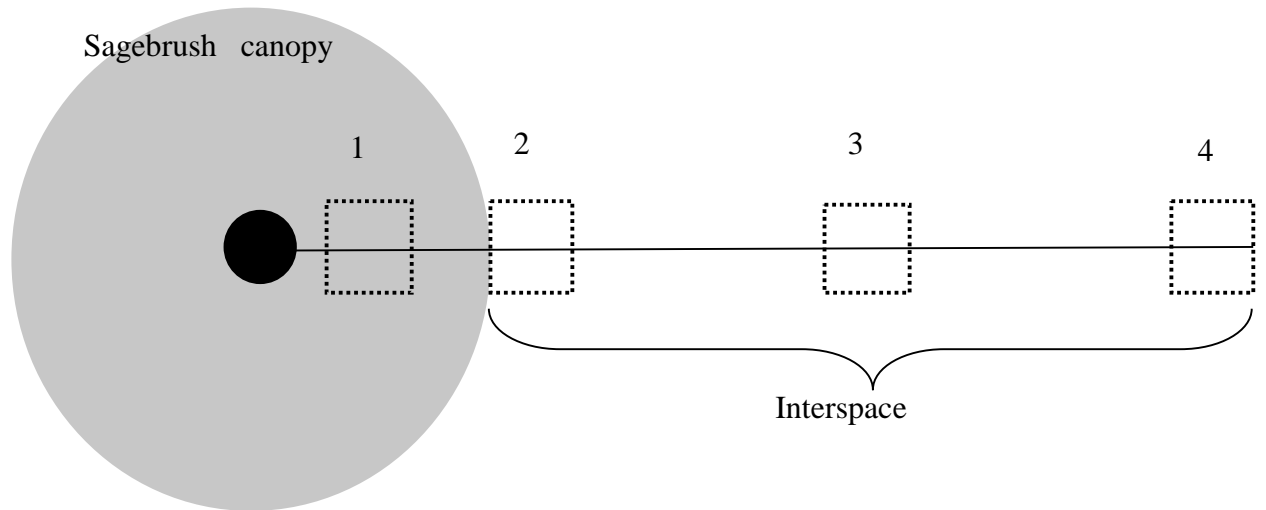


Figure S1-1. Sampling scheme for *Poa secunda* as seen from above, depicting a sagebrush canopy and transect extending from the base. Numbers correspond to placement of 20 cm x 20 cm quadrats for estimating percent cover of *P. secunda*. 1) Canopy: quadrat placed at approximate midpoint of canopy region; 2) Edge: quadrat placed directly adjacent to end of canopy. 3) Interspace: quadrat placed at approximate midpoint of transect. 4) Interspace: quadrat placed at end of transect (200cm mark).

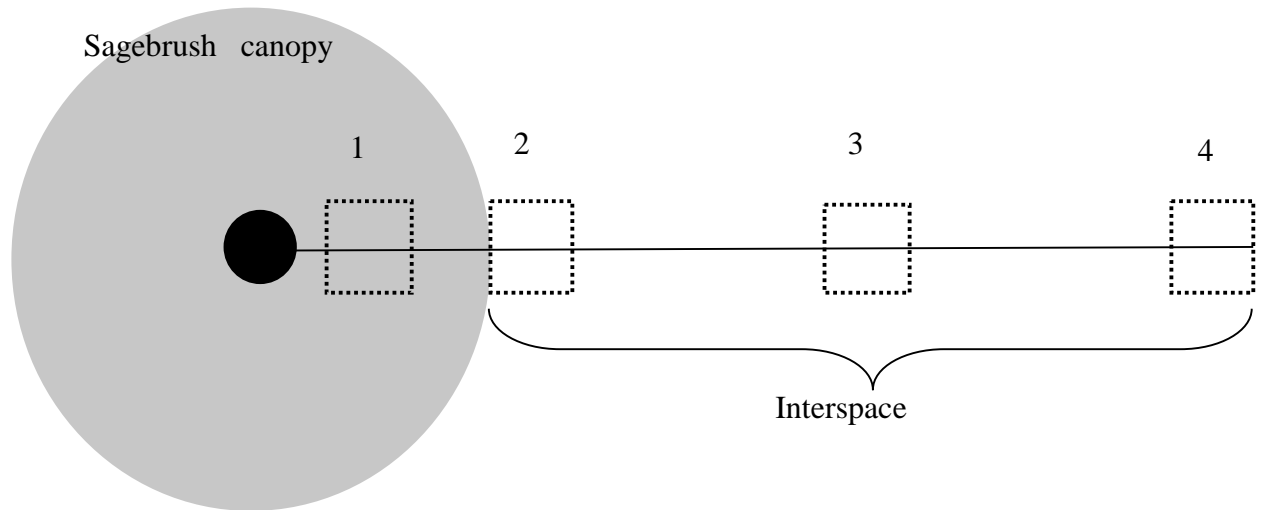


Figure S2-1. Sampling scheme for *Poa secunda* as seen from above, depicting a sagebrush canopy and transect extending from the base. Numbers correspond to placement of 20 cm x 20 cm quadrats for estimating percent cover of *P. secunda*. 1) Canopy: quadrat placed at approximate midpoint of canopy region; 2) Edge: quadrat placed directly adjacent to end of canopy. 3) Interspace: quadrat placed at approximate midpoint of transect. 4) Interspace: quadrat placed at end of transect (200cm mark).

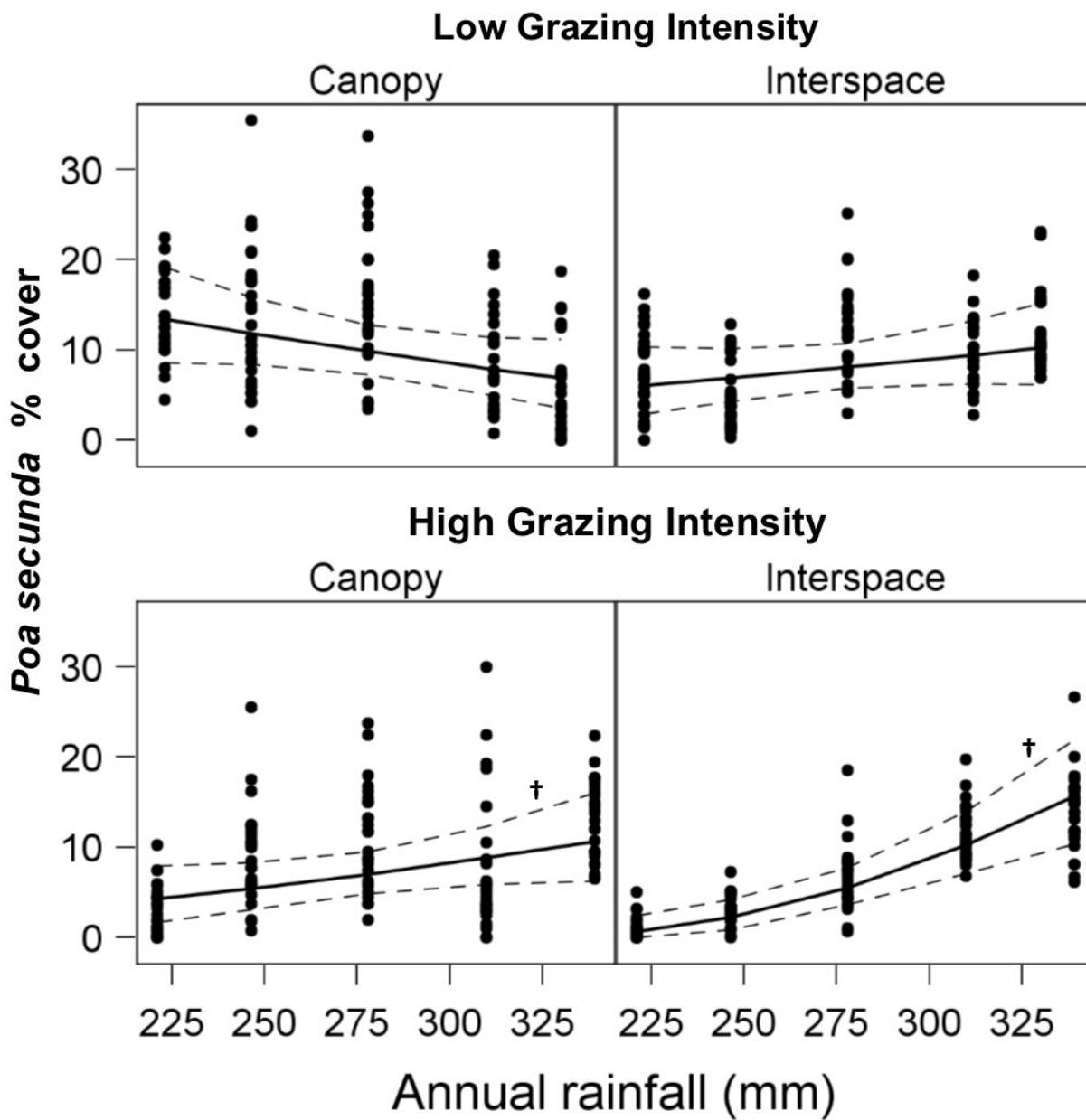


Figure S2-2. Shrub-level means and predicted regression lines (with 95% confidence bands) for % cover of *P. secunda*, across rainfall, shrub microsites, and grazing intensity (backtransformed).

APPENDIX C

MULTIVARIATE VEGETATION COMMUNITY ANALYSIS OF SAMPLING SITES

Introduction

In ecology, multivariate analyses are often used to gain insights into the interdependence of many variables in study sites [1]. These variables may include cover or density of plant or animal species or environmental correlates such as temperature, rainfall, or elevation measured at sampling sites. In the Great Basin, site characteristics are often assessed using the range monitoring methods of Herrick, Van Zee (2) and include vegetation attributes such as percent cover by species, gap sizes between perennial and annual plants, and density of vegetation by species, and other site attributes such as cover of bare ground, litter, moss, cryptobiotic crust, and gravel. We were interested in relationships among these vegetation community attributes and environmental attributes of sites sampled over an annual rainfall gradient in 2012-2013. Our objective was to determine if plant community composition was associated with site characteristics along the annual rainfall gradient.

Methods and analysis

Our study area was the Great Basin Floristic region (Fig. S3-1), which covers parts of Idaho, Utah, and Nevada. We conducted site sampling using the methods of [2] at 29 sites (n=20 in 2012 and n=9 in 2013) distributed over an annual rainfall gradient (278-340mm). We also used ranges of the rainfall gradient to assign values of either low, medium, or high annual rainfall to sites for later analysis; rainfall values were assigned as follows: 203-254mm = low; 254-304mm = medium; 304+ mm = high. Sites were

characterized by a dominant shrub overstory of *Artemisia tridentata*, an herbaceous understory of *Poa secunda*, *Elymus elymoides*, *Achnatherum thurberianum*, and *Pseudoroegneria spicata*. Common forbs included *Crepis acuminata*, *Collinsia parviflora*, and various species of *Phlox*, *Lupine*, and *Lomatium*. The invasive annual grass *Bromus tectorum* was present in small amounts, as were common invasive annual forbs: *Ranunculus testiculatis*, and *Lepidium perfoliatum*. Study sites had at least a five year history of grazing, but no other disturbances were present.



Figure S3-1. Sampling sites (n=29) in the Great Basin, USA.

Field sampling occurred between May and July in 2012 and 2013 to capture peak herbaceous biomass. Three, 50-m sampling transects extending in three, equally spaced

directions, were placed at the center of each site. Cover of individual plant species, litter, moss, gravel, rocks, and cryptobiotic crust was estimated using the line point intercept method, and density of dung, shrubs, and perennial grasses was assessed in 1m wide belt transects over each 50m transect. Annual and perennial canopy gap size, as well as perennial canopy gap size were assessed along the three transects.

The species matrix resulting from line-point intercept sampling consisted of species' cover values for 29 sites, creating a dataset of 29 rows (sites) and 30 columns (plant species). Species occurring in only one site were eliminated; data columns (species) were relativized by species' cover maxima ($x_{ij}/xMAX_j$) to maintain variation across species but standardize variation across columns (sites). Our environmental matrix consisted of 20 of the most ecologically relevant predictor variables for each of the 29 sites (18 continuous and 2 categorical variables) (Table S1-2). These variables were based on site characterization data as well other site attributes such as ecological site type, elevation, rainfall, and soil type (Table S1-2). We conducted a non-metric multidimensional scaling ordination (NMS); an NMS is a multivariate unconstrained ordination technique that assesses entire plant community datasets (e.g. site x species matrices) at variable dimensions (axes) and provides a final solution with ordination scores that reflect the lowest stress values [1]. In addition, NMS does not assume a linear relationship among variables, making it a good ordination choice for our data.

The ordination was conducted using the Bray-Sorenson distance measure, 250 runs with original data, and a random starting configuration. Significance of the final stress value was compared to random solutions using a Monte Carlo test with 50 randomizations. Correlations between NMS axes and environmental covariates (Table

S2-2) were determined with Pearson's R correlation coefficients. We assumed that environmental covariates with the largest absolute correlation coefficients had the strongest influence on NMS axes. We inspected an NMS scree plot to determine the number of dimensions in the final solution.

To test for effects due to rainfall, we conducted a multi-response permutation procedure (MRPP) [3]. An MRPP conducts a permutation test for differences between or among groups of sample units based on within group similarities [4]. The Bray-Sorenson distance measure was used. Rainfall groups were low, medium, and high rainfall. NMS and MRPP analyses were performed using PC-Ord (ver. 6.15; MjM Software Design, Gleneden Beach, OR) with significance $\alpha = 0.05$. We also conducted an indicator species analysis (ISA) to determine if distinct species were associated with rainfall levels or grazing level. An ISA determines the relationship between a species consistent occurrence and abundance in groups of sample units [5]. The resulting species indicator values are tested for statistical significance using a Monte Carlo technique.

Results

The final NMS ordination was based on a 3-dimensional solution ($p = 0.0040$), a final stress value of 13.71, a final instability of 0.00000, and 72 iterations. The total variance (R^2) explained by the solution was 0.814.

Sites were significantly grouped according to annual rainfall category based on MRPP results ($A=.033$, $p=0.006$), but this was only significant for the comparison of low rainfall versus high rainfall ($A=.048$, $p=0.0017$). Four species, *Pseudoroegneria spicata*, *Chrysothamnus viscidiflorus*, *Allium* spp, and *Phlox* spp, were significantly associated

with high rainfall sites ($p=0.0004$, $p=0.013$, $p=0.0372$, and $p=0.0196$, respectively), based on Indicator Species Analysis tests.

Based on the site by species ordination, Axis 1 was generally represented by an increase in annual forbs and two shrub species (*Tetradymia* spp and *Ephedra* spp) (Table S3-1). Axis 2 was represented by a decrease in cover of the forb *Gutteriezia sarothrae*, while Axis 3 was represented by an increase in perennial forbs (Table S3-1).

Additionally, based on correlations with environmental covariates, Axis 1 was associated with an increase in perennial plant canopy gap size (Table S3-2). Axis 2 was associated with a decrease in annual rainfall and an increase in perennial plant basal gap size; Axis 3 was associated with an increase in cover of litter & duff, gravel & rocks, perennial forbs, embedded litter (dead standing litter), and a decrease in percent cover of *Artemisia tridentata* (Fig. S3-2 through S3-4).

Table S3-1. R^2 values for species with NMS axes 1, 2, and 3. Only correlations of 0.3 or greater were considered.

Axis 1	Axis 2	Axis 3
<i>Ephedra</i> spp. (+0.497)	<i>Gutteriezia sarothrae</i> (-0.426)	<i>Allium</i> spp (+0.583)
<i>Tetradymia</i> spp. (+ 0.441)		<i>Collinsia parviflora</i> (+0.48)
Unknown annual forbs (+0.321)		<i>Crepis acuminata</i> (+0.616)
<i>Lepidium perfoliatum</i> (+0.562)		<i>Lithophragma parviflora</i> (+0.452)
		<i>Aster</i> spp (+0.52)

Table 3-2. R^2 values for environmental covariates with NMS ordination axes 1, 2, and 3. Only correlations of 0.3 or greater were considered.

Axis 1	Axis 2	Axis 3
Perennial plant canopy gap size (+0.276)	Rainfall (-0.357)	<i>Artemisia tridentata</i> percent cover (-0.273)
	Perennial plant basal gap size (+0.333)	Percent cover litter/duff (+0.27)
		Percent cover gravel/rocks (+0.281)
		Perennial forb percent cover (+0.543)
		embedded litter percent cover (+0.417)

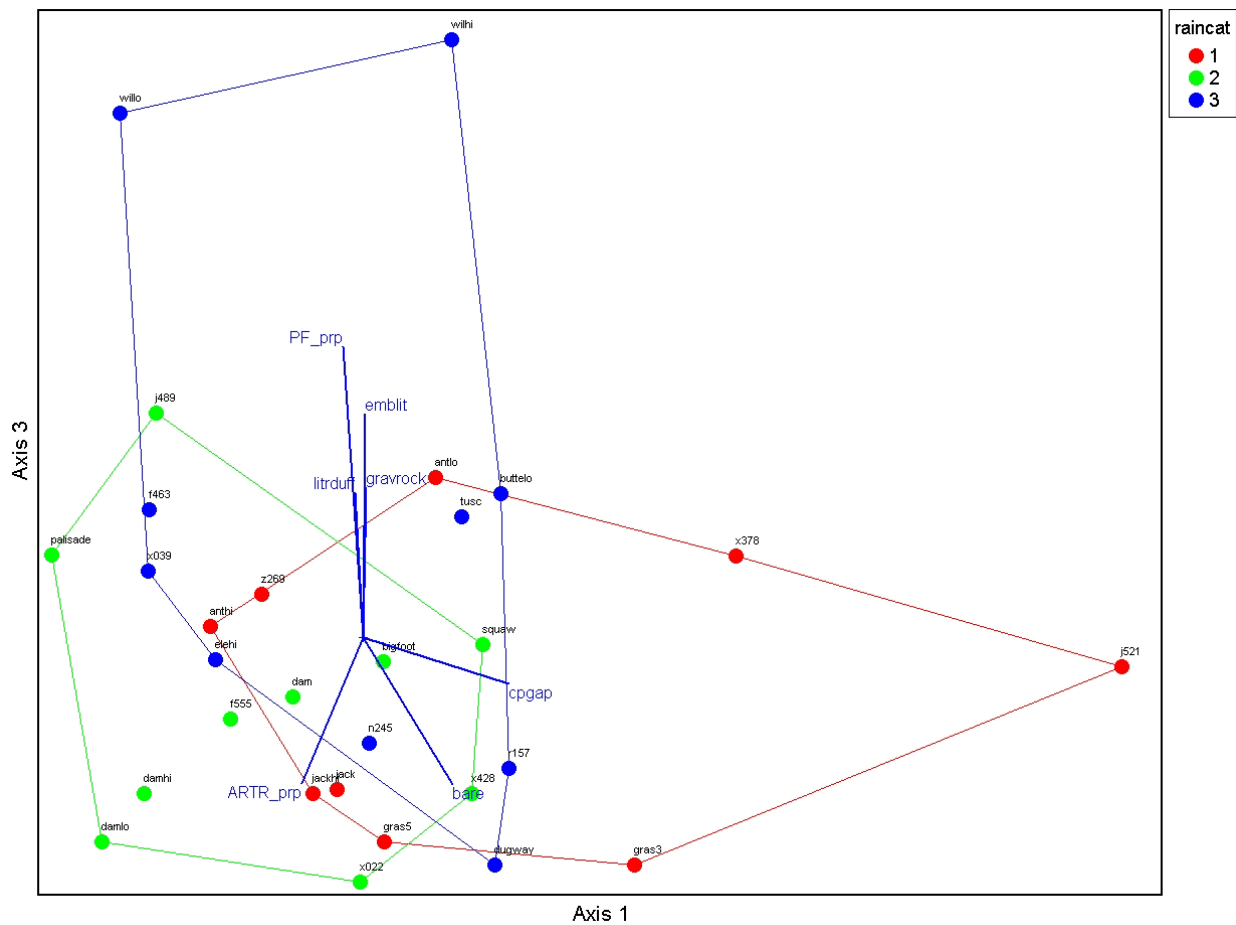


Figure S3-2. Biplot of final ordination, Axis 1 vs Axis 2 (vectors reflect environmental covariates with an r^2 value of 0.3 or greater. Vectors for species are not plotted.)

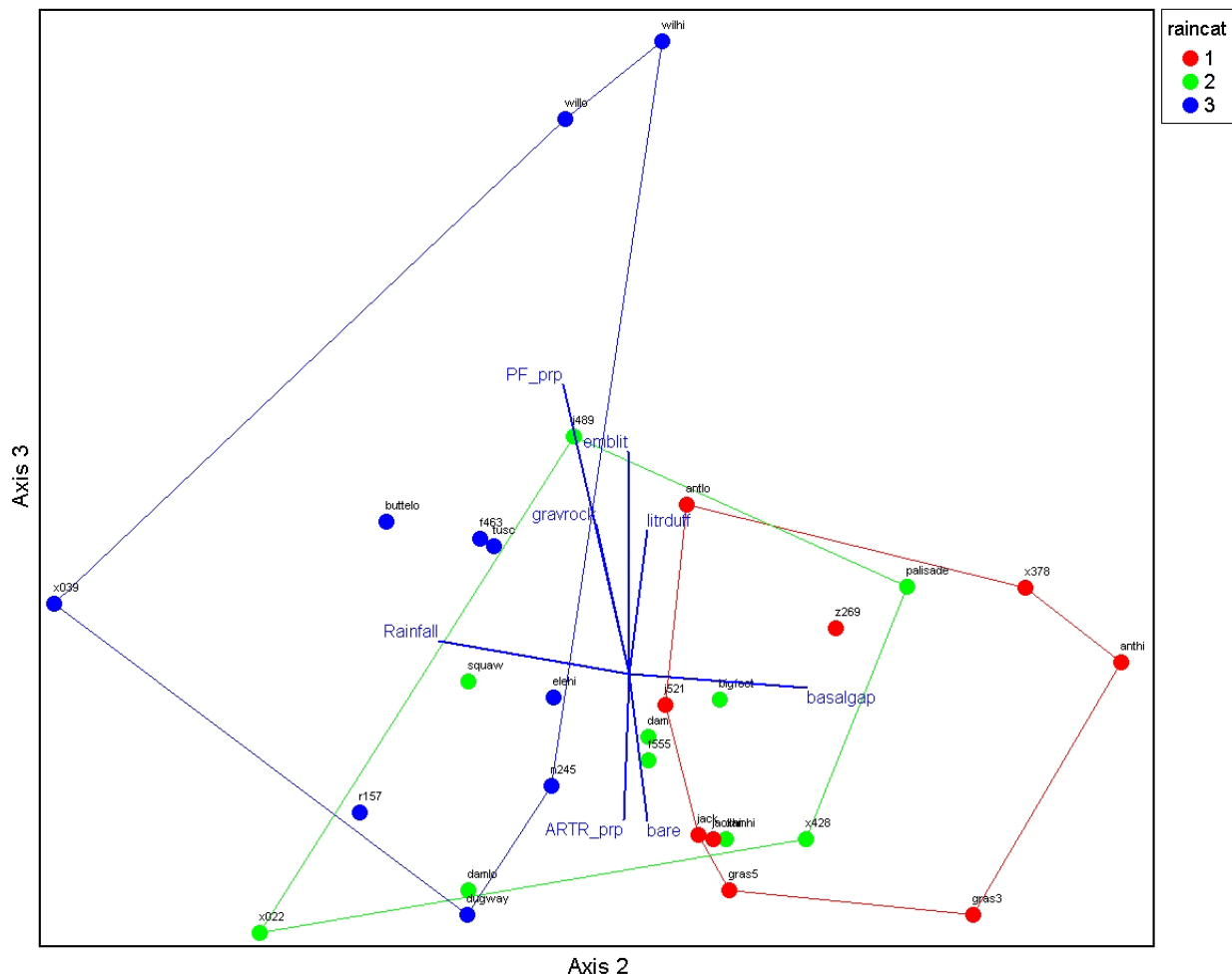


Figure S3-3. Biplot of final ordination, Axis 1 vs Axis 3 (vectors reflect environmental covariates with an r^2 value of 0.3 or greater. Vectors for species are not plotted.)

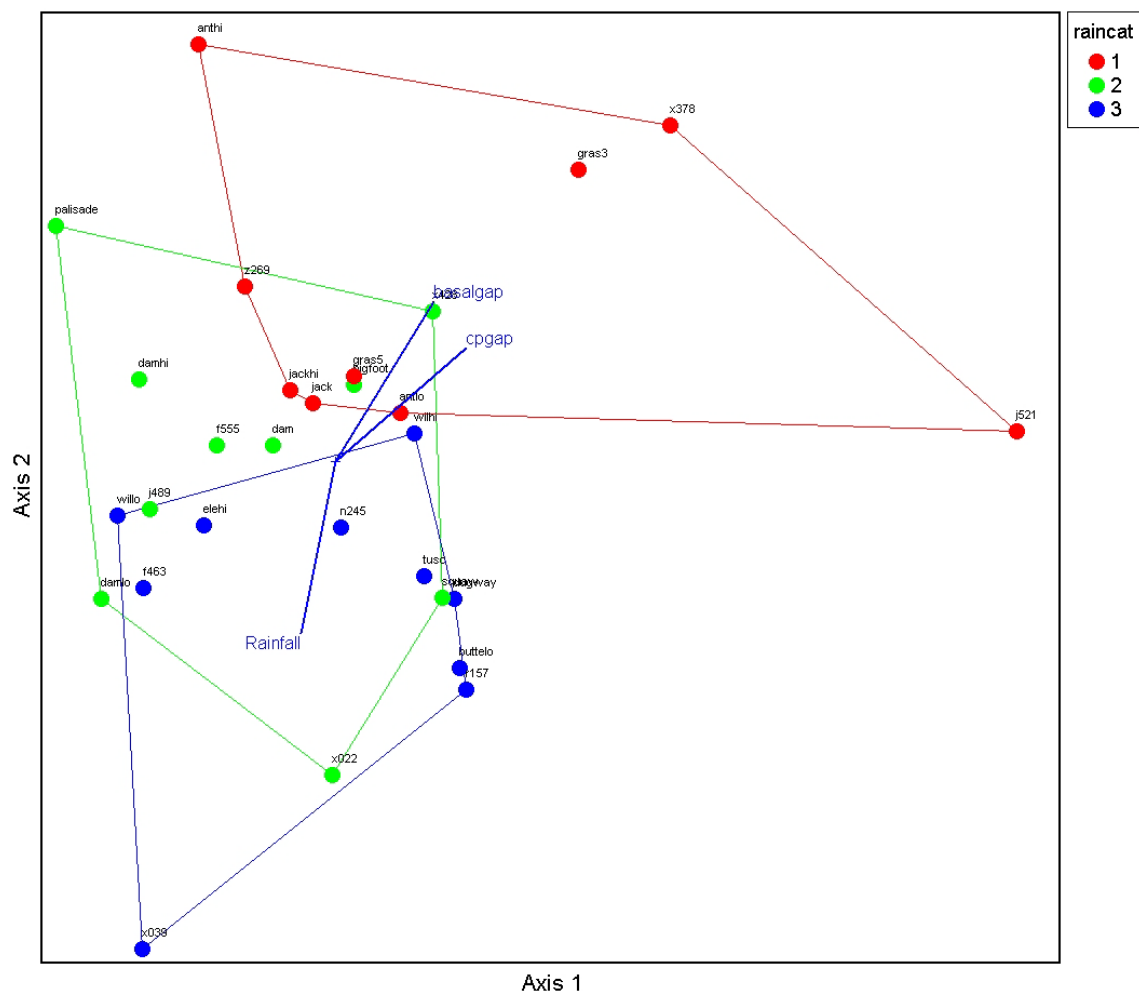


Figure S3-4. Biplot of final ordination, Axis 1 vs Axis 2 (vectors reflect environmental covariates with an r^2 value of 0.3 or greater. Vectors for species are not plotted.)

Synthesis and Conclusions

Not surprisingly, sites were generally grouped according to rainfall level. Our indicator species analysis revealed that *Pseudoroegneria spicata*, *Chrysothamnus viscidiflorus*, *Allium* spp, and *Phlox* spp were commonly associated with high rainfall sites. The increase in perennial forbs could be due to other factors than merely rainfall such as site disturbance history or site resilience to annual plant invasion. For example, high grazing pressure can reduce species richness; although dung density was not

correlated with any NMS axes, past grazing practices may still have impacted the present vegetation community. It is possible that sites with a less extensive grazing history had a more diverse perennial forb population. Additionally, ecologically resilient sites (e.g. sites more resistant to invasion by invasive annuals) are characterized by a diverse native plant community, including perennial forbs. Sites with greater cover of annual forbs had lower rainfall, and, not surprisingly, gaps among perennial plants were greater. Lower rainfall sites have less vegetation in general, which may increase risk of invasion by annual forbs and grasses.

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