

University of Nevada, Reno

Can three native Mojave Desert perennials adapt to increasing atmospheric nitrogen deposition and annual grass invasion in Joshua Tree National Park?

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
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By

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ABSTRACT

Anthropogenic emissions have increased atmospheric nitrogen deposition nearly four-fold in the industrial era. Although increased N availability can be beneficial for individual plants, it can increase community susceptibility to invasion and have detrimental effects on native plant communities. We grew three native Mojave Desert perennials, *Ambrosia salsola*, *Hilaria rigida*, and *Stipa speciosa*, collected across an N deposition/invasion gradient in Joshua Tree National Park, in greenhouse and field common gardens to determine if populations possessed the heritable variation required to adapt to increasing N deposition and annual grass invasion, and asked if there was any evidence for adaptation to these factors. We collected seed from individual plants (families) at six sites representing three levels of N deposition and invasion within the park, and grew plants in a greenhouse for 90 days with and without competition from the invasive *Bromus madritensis* ssp. *rubens* at varying N levels. We analyzed differences in leaf number, total biomass, allocation to above and below ground biomass, and allocation to fine and coarse roots using a nested ANOVA. As expected, *B. madritensis* competition decreased biomass of all native plants, and N addition increased biomass, except for *H. rigida*. Families of all species differed in many measured traits, and there were site differences in some traits for *A. salsola* and *H. rigida*. Populations from different N deposition and invasion levels showed some differences in growth strategies, especially in *A. salsola*, for which plants from high N/invaded sites saw a smaller relative decrease in biomass and leaf number when grown with *B. madritensis*, consistent with adaptation to annual grass competition. We also conducted a reciprocal transplant experiment using seed collected from the same six sites. Plants were transplanted reciprocally after one year in the greenhouse. Transplant survival, vigor, and size were affected by transplant site and N status, whereas origin site and N status did not significantly impact these traits. Although there

was no evidence of evolutionary response to N deposition level for any species, families exhibited differences in responses to N, suggesting that there may be a small amount of heritable variation that could allow for future adaptation to conditions that are likely to be prevalent in the future. Use of populations or individuals that show increased performance under likely future conditions could allow for more successful restoration projects.

Keywords: Global change, rapid adaptation, nitrogen deposition, invasion, Joshua Tree National Park, *Ambrosia salsola*, *Hilaria rigida*, *Stipa speciosa*, *Bromus madritensis* ssp. *rubens*

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INTRODUCTION

Human expansion and development bring myriad disturbances to natural ecosystems, including invasions of non-native organisms and changes to geochemical cycles, such as the nitrogen cycle, which have substantial impacts on native plant communities (Vitousek et al. 1997b). On a global scale, anthropogenic fixation has doubled the input of reactive forms of nitrogen (Galloway et al. 2004), and nitrogen emissions into the atmosphere from fossil fuel combustion and agricultural activity have risen to nearly three times the rate of natural emissions, increasing total nitrogen emissions into the atmosphere from 34 Tg N in preindustrial times to 124 Tg N today (Fowler et al. 2005). These emissions result in greater atmospheric deposition of nitrogen in nitrate and ammonia forms, essentially fertilizing land by as much as ten times the natural nitrogen input in areas such as the eastern United States, and, for example, by up to 100 times in heavily affected areas of western Europe (Vitousek et al. 1997a).

Human activity also transports biological invaders and allows their proliferation via disturbance, both physical and geochemical, often at great ecological and economic cost (Vitousek et al. 1996). In low nutrient sites, greater nutrient availability can lead to greater invasibility in plant communities, as exotic plants that previously could not tolerate harsh local conditions can establish themselves under conditions of surplus resources (Burke and Grime 1996, Davis et al. 2000, Milbau and Nijs 2004). Weedy life-history strategies that often characterize non-native invaders, for example an annual life cycle, quick and early opportunistic growth, and high investment in reproduction, are only feasible in an environment with adequate resources to sustain these strategies (Grime 1977). Invasive plants often exhibit greater ability to utilize available soil nitrogen, taking up more nitrogen and converting it into biomass, whereas

many native plants use more conservative stress-tolerating strategies better adapted to low nitrogen environments (Grime 1977, Tilman 1987, Blackshaw et al. 2009).

Inland southern California is currently experiencing the effects of concurrent increases in nitrogen deposition and invasion. Due to pollution from the greater Los Angeles metropolitan area, the Mojave Desert, and Joshua Tree National Park (JTNP), in particular have seen atmospheric nitrogen deposition rise dramatically, creating a deposition gradient, with western areas of the park receiving up to five-fold the deposition of eastern areas (Tonnesen et al. 2007). Such increases are likely to have more substantial impacts in areas with low nitrogen levels, like the Mojave, where plants are adapted to low resource conditions. Species adapted to nutrient-poor soils can show a small response or no response at all in growth and nutrient uptake when presented with more available nutrients, whereas species from areas with more fertile soils would more likely convert additional nutrients to biomass (Chapin 1980, Chapin et al. 1986). For example, Williams and Bell (1981) found that some Mojave Desert winter annuals exhibited no increase in biomass or tissue nitrogen content with nitrogen addition, whereas other annual species consistently increased in both these characteristics. Other studies on Mojave species, both native and invasive, have shown conditional increases in biomass, as plants are only able to utilize additional nitrogen under specific types of conditions. These include variable responses to nitrogen addition when grown in different soil types, and, most importantly, variable responses due to water availability; some species only responded to nitrogen fertilization when available water was also increased (Romney et al. 1978, Williams and Bell 1981, Gutierrez and Whitford 1987).

Nitrogen addition alone can have a positive or neutral effect at the individual level on low-nutrient adapted native plants, but it has been proposed that invasive plants can benefit from increased resources more than native species (Blumenthal 2005). Fertilization often results in

increased biomass of non-native plants and decreased biomass and species richness of native plants (Tilman 1987, Huenneke et al. 1990, Tyler et al. 2007), presumably due to increased competitive ability of non-native species. Additionally, native plants can suffer from disproportionate increases in herbivory under elevated nitrogen conditions, as insects preferentially increase herbivory on natives rather than invaders (Throop and Lerdau 2004, Blumenthal 2005) (Fig. 1).

Invasive annual grasses, most notably various annual species of the genus *Bromus*, first established in the arid West around the turn of the 19th century and have since become dominant over much of the western rangeland, especially disturbed areas (Mack 1981, Hunter 1991). Brooks (1999) found that in the Mojave Desert, disturbance increased both richness and dominance of annual invasive plants, but to a much lesser degree than did high nutrient levels. Experimental addition of nitrogen in Mojave ecosystems has been shown to increase biomass and density of *Bromus madritensis* ssp. *rubens* and *Schismus* spp., common invasive annual grasses, and *Erodium cicutarium*, a common annual invasive forb, and decreased native plant biomass (Brooks 2003).

Local adaptation in plants is common (Leimu and Fischer 2008), but in an era of rapid global change, the question of whether local populations can adapt to new, anthropogenic disturbances like climate change, increased nitrogen deposition, and non-native plant invasion becomes increasingly important. Reduced populations of natives still exist and compete with non-natives in invaded areas (Gurevitch and Padilla 2004). Several studies in the arid West have shown that remnant populations of native plants have undergone rapid natural selection for traits that make them better competitors in invaded environments (Mealor and Hild 2007, Leger 2008, Goergen et al. 2011, Oduor 2013). The ability of populations to adapt to various aspects of global change presupposes that heritable variation in traits relevant to the selective pressure exists in the

population (Linhart and Grant 1996, Strauss et al. 2006), although such variation may not be present in all populations or for all possible selective pressures. Further, the combination of simultaneous changes can be even more difficult for native plants to tolerate, and multiple types of disturbances may be more difficult for plants to adapt to, leading to greater risk of population diminishment or extinction than in areas with fewer types of disturbances (Davis 2003).

The anthropogenic gradient in atmospheric nitrogen deposition and annual grass invasion in Joshua Tree National Park allows us to test questions pertaining to the potential of native plants to adapt to these novel potential selective forces using greenhouse and field experiments. We collected seed from three different native plant species across the disturbance gradient and grew them in a greenhouse with and without competition and with different nitrogen treatments to determine:

- 1) If populations contained the heritable variation necessary for adaptation to occur;
- 2) If there is evidence that adaptation to N deposition or annual grass competition has already occurred in these populations; and
- 3) Whether populations differ in their ability to adapt to each of these possible selective pressures.

We focused on changes in growth, allocation to roots, and allocation to different size classes of roots, as these traits are important for nutrient acquisition and would likely be affected by the changes in nutrient availability associated with nitrogen deposition and annual grass invasion.

We also performed a reciprocal transplant experiment in Joshua Tree NP, using seedlings grown from the same seed collections to determine:

- 4) If there is any evidence for local adaptation to nitrogen deposition level in the field.

In addition to our manipulation experiments, we observed plant community composition and quantified herbivory of these species in the field. Additionally, we measured size distributions of plants at seed collection sites across the disturbance gradient to examine differences in populations *in situ*, asking if size cohort structures were consistent with recruitment of new plants. The answers to these questions are highly relevant to any future restoration of sites in the Mojave that are likely to see high levels of invasion and nitrogen deposition in the future, and for which restoration stock that is pre-adapted to these conditions will result in greater efficiency and success.

METHODS

Study Species

Three Mojave Desert perennial species, burrobrush (*Ambrosia salsola* (Torrey & A. Gray ex A. Gray)), big galleta grass (*Hilaria rigida* (Thurb.) Benth. ex Scribn.), and desert needlegrass (*Stipa speciosa* (Trin. & Rupr.) Barkworth), were selected for this study. Each of these species is common across the nitrogen deposition/invasion gradient in Joshua Tree National Park and is routinely used by the park in restoration projects. *Ambrosia salsola* is an early successional monoecious shrub in the Asteraceae that reproduces primarily from seed, with a lifespan of several decades (Tesky 1993). *Hilaria rigida* is a shallow-rooted C4 bunchgrass that reproduces primarily rhizomatously (Robberecht 1988); Nobel (1980) measured in *H. rigida* what was at the time the highest photosynthetic rate recorded. *Stipa speciosa* is a C3 bunchgrass that reproduces both vegetatively as well as by seed under advantageous conditions (Pavek 1993).

Site Selection and Seed Collection

Joshua Tree National Park is located east of Los Angeles in southern California and is subject to deposition of pollutant products of that metropolitan area. Allen et al. (2009)

documented a nitrogen deposition gradient spanning JTNP, with deposition decreasing from west to east across the park. Generally corresponding with this depositional gradient, the density of invasive species has been observed to decrease from west to east (J. Hoines, pers. com). In a seed bank study, Schneider and Allen (2012) found that the density of invasive seeds increased with increasing N deposition. Seeds of both the native perennials and the invasive annual grass red brome (*Bromus madritensis* ssp. *rubens* (L.) Husnot (hereafter *B. madritensis*)), the dominant invasive annual grass in the western Mojave Desert (Brooks and Berry 2006), were collected in June of 2012 and 2013 from six sites representing three levels of deposition (Fig. 2). The two Covington Road sites (CR1 and CR2) represent the high end of deposition in JTNP at ~12.4 kgN/ha/yr, and the two Pine City sites (PC1 and PC2) represent the lower end, experiencing ~6.2 kgN/ha/yr (Allen et al. 2009). The Lost Horse sites (LH1 and LH2) represent an intermediate deposition value. Deposition at Lost Horse was not directly measured by Allen et al. (2009), but they did report soil extractable nitrogen, which correlated with measured deposition, was measured and found to be intermediate between the high and low deposition sites. The National Atmospheric Deposition Program (NADP) modeled deposition at a coarse 4km grid for which the deposition value at the Lost Horse sites was ~7 kgN/ha/yr. Seed collection sites ranged in elevation from 1213 to 1441 m and in annual precipitation from 181 to 196 mm (PRISM 30 year average). Temperature was similar across all sites (PRISM 30 year average).

A. salsola, *H. rigida*, and *S. speciosa* seeds were collected from a haphazard sampling of 15-20 individual plants at each site that produced enough seed for our experimental purposes. These individual collections are hereafter called “families.” *B. madritensis* seeds were collected from sites where it was present and collections were bulked together. Both 2012 and 2013 were very dry years with low seed production, and we were unable to collect enough *H. rigida* seed at PC2 to use in our study.

Community Composition, Soil Characterization, and Size Distribution

Plant community composition at the seed collection sites was sampled in May of 2014 (see appendix 1). Additionally, for each species and collection site, we sampled the size distribution of the population as a proxy for determining the age structure at each site. At each site, we laid a 50 m tape in a northerly direction starting from a random point. A 50 m transect heading west was laid out every five meters along this tape, starting at zero. Six of these east-west transects were randomly selected to sample, and at every five meters along the tape, we measured the height and two perpendicular axes of width for the target species centered closest to the tape mark and within a 5 X 5 m square centered at the tape mark. This method was continued until at least 50 individuals of each of the three species were measured. Additional transects were sampled if six did not achieve this sample size. *H. rigida* did not have sufficient representation at PC2 to achieve an adequate sample. An elliptical basal area was calculated for each plant using both width measurements. It should be noted that older *H. rigida* can also become small, as the process of senescence starts in the center of the bunchgrass and can split individual bunchgrasses. However, no clear evidence of small plants occurring from senescence was seen. These plants were also examined for evidence of herbivory (see appendix 2). To characterize soil properties at each site, nine field soil samples of the top 20 cm were collected from the 0, 25, and 50 m marks of the transects described in appendix 1 and bulked from each seed collection site and kept as cool as possible in a cooler. These six soil samples were analyzed by A & L Labs, Memphis, TN, for soil chemistry and texture variables (Table 1).

Greenhouse Methods

Greenhouse experiments were conducted in the spring to summer of 2013 (*A. salsola*) and 2014 (*H. rigida* and *S. speciosa*). Plants were grown from seed in small pots (3.8 cm X 10 cm, 164 mL, Ray Leach SC10 cone-tainers, Steuwe and Sons., Inc.) in a 1:1 mixture of field-

collected Nevada topsoil and decomposed granite sifted through a 5 mm screen. This soil mix was also analyzed by A & L Labs, and provided a rough approximation of typical JTNP soils (Table 1), which range from sandy to sandy loamy with low levels of available nitrogen; for logistical reasons, it was not possible to use field soil from JTNP.

Seeds were grown in a factorial design either with or without competition and at either two (*H. rigida* and *S. speciosa*) or three (*A. salsola*) nitrogen treatments, depending on the availability of seed for each species. Treatment pots received nitrogen via an ammonium sulfate (Lilly Miller 21-0-0) solution in a fixed volume of water every three weeks equal to the prorated deposition rate of 15 kgN/ha/yr (high N) or 10 kgN/ha/yr (medium N), similar to high and medium levels of deposition at JTNP. No nitrogen was added to low nitrogen pots. *Bromus madritensis* seeds were planted in each competition treatment pot at the same time as the native perennials. The competition treatment of 4 seeds per pot approximated a *B. madritensis* seed density of 3,500 seeds/m². For each site and species, seeds from fifteen families were planted. *Ambrosia salsola* produced adequate seed for four seeds to be planted into each treatment combination (3 N X 2 competition). *Hilaria rigida* and *S. speciosa* did not produce enough seed to include the medium N treatment. For these two species, most families had adequate representation for four planted seeds per treatment combination (2 N X 2 competition), and those that did not were assigned treatments such that each treatment was represented as many times as possible. Plants were watered every other day, adequately wetting the soil but minimizing leaching from the bottom of the pot. We used PRS probes (Western Ag. Inc.), which approximate both ammonium and nitrate available to plant, to estimate the effect of our treatments on plant nitrogen uptake. In the second year of the study, 2014, probes were placed in 12 pots of each of the four N by competition treatments in the place of the focal plant. Probes were bulked into three sets of four probes per treatment combination and analyzed for nitrate, ammonium, and total N uptake.

Not all seeds planted germinated and survived until harvest. For the subset of seeds that did, growth measurements were taken (leaf number and shoot length), and each plant was harvested three months after its emergence. Roots of both native perennials and *B. madritensis* were carefully rinsed from the soil. We scanned the roots of the native perennials and analyzed the images with WinRhizo 2007 (Regent Instruments Inc.) to determine the total root length and length of fine roots (diameter < 0.3 mm), intermediate roots (0.3 mm < d < 0.6 mm), and coarse roots (d > 0.6 mm). Native perennial and *B. madritensis* aboveground and belowground biomass were then dried and weighed. In each year, 20 *B. madritensis* pots (4 seeds) for each N treatment level were grown without a native focal plant, and aboveground and belowground biomass were dried and weighed.

Field Reciprocal Transplant Methods

A. salsola, *H. rigida*, and *S. speciosa* plants were grown in pots in the UNR greenhouse from seed bulk collected from the six collection sites in 2012. No *S. speciosa* was collected from the medium N deposition sites (LH1 and 2) due to lack of availability. In January 2014, these one-year old plants were reciprocally transplanted into planting sites at JTNP. These sites were near, but not identical to the original collection sites, with locations selected for their acceptability for soil disturbance, as determined by park biological and archeology staff. Planting proceeded according to standard JTNP restoration planting procedures. All plants were caged, provided with a slow water release gel, individually tagged, marked using GPS, and watered every three months. 708 plants of all three species were transplanted in total, with distribution at each of the six planting sites as follows: for *A. salsola*, 10-11 plants from CR1, 7 from CR2, 9 from LH1, 8 from LH2, 9-10 from PC1, and 7 from PC2; for *H. rigida*, 9 plants from CR1, 9 from CR2, 9-10 from LH1, 9 from LH2, 9 from PC1, and 9 from PC2; for *S. speciosa*, which was only

transplanted into the low and medium N deposition sites, 9 plants from CR1, 8-9 from CR2, 6-7 from PC1, and 7-8 from PC2.

We collected survival data as well as several measures of vegetative growth of plants one year after transplanting in order to examine the role that origin and transplant site and nitrogen deposition status might have on growth of native transplants. Before planting, we measured height for all species, branch number and stem diameter for *A. salsola*, and elliptical basal area for *H. rigida* and *S. speciosa* to use as a covariate in size analyses. In January 2015, transplant survival was recorded and all growth variables were remeasured. Additionally, qualitative measures of greenness (a whole plant average of how green each plant was compared to the maximum level seen for each species) and bushiness (a whole plant average of branch/leaf densities compared to the maximum level seen for each species) were scored using a 1-5 scale upon remeasurement in the field. Twelve pairs of PRS probes (Western Ag. Inc.) were placed in each transplant site at planting, six in interspaces between plantings and six in the watered area around plantings, in order to estimate the N available to plants. These probes were removed and analyzed in May 2014, and results reported are the total uptake of N (in $\mu\text{g}/10\text{cm}$ of probe surface area) over the entire deployment period.

Data Analysis

Differences in size distribution (in cm^2) of target species measurements at each collection site were analyzed with N site and site (nested within N site) as factors. For graphical purposes, sizes were log transformed in order to display the large variation in size within populations.

We analyzed data using JMP 10.0.2 (SAS Institute Inc.). Greenhouse experiment response variables were shoot length, leaf number, total biomass, root weight ratio (RWR, root weight/total biomass), specific root length (SRL, root length/root weight), and proportion of total

root length allocated to fine, intermediate, and coarse roots. These response variables fall into three categories: those indicative of gross size and status of the plant (emergence time, leaf number, shoot length, and total biomass), those indicative of allocation of resources to above and belowground biomass (RWR), and those indicative of allocation of resources to fine versus coarse roots (SRL and proportions of fine, intermediate, and coarse roots). These traits are all important in resource acquisition (Chapin 1980) and would likely be traits under selection if selection is occurring due to competition and nitrogen addition, as has been seen in previous research (Reynolds and Antonio 1996, Rowe and Leger 2011, Phillips and Leger 2015). For the greenhouse experiment, analysis of variance was used for each species to determine the effects of the N deposition level of the seed collection site (N site), competition, and N treatment, and all possible interactions of these three, as well as collection site and family included as random factors, nested within N site and within site and N site respectively. The restricted maximum likelihood (REML) method for ANOVA was used except in a few cases when it failed to converge, in which case the expected mean squares (EMS) method was used. To quantify variation in family responses to treatments, the same model plus all family interactions with competition and N treatment was analyzed for the subset of families which were represented in each N by competition treatment (eliminating some families was necessary because not all seeds germinated, yielding 56 *A. salsola* families, 61 *H. rigida* families, and 28 *S. speciosa* families). Significance of the random family factor was determined using a likelihood ratio test comparing the full models to a model without the random factor. Effects were deemed significant at an α of 0.05, and Tukey's HSD tests were performed *post hoc* on significant effects. Differences in PRS probe N uptake between greenhouse competition and N treatments were also tested using ANOVA, with N treatment, competition treatment, and the interaction between the two included in the model. *Bromus madritensis* pots grown alone were analyzed using ANOVA with N treatment and year as factors. All tests were conducted on data transformed to improve normality

and homogeneity of variances, and untransformed means and standard errors are reported in text and figures.

Because plants change their allocation to different structures as a function of size and developmental stage of the plant in a process known as ontogenetic drift, as well as due to environmental factors like nutrient availability, it is important to distinguish between these two processes (Wright and McConnaughay 2002, Weiner 2004). For example, small or young plants often allocate more of their biomass to roots than larger plants by virtue of their size or age alone, thus comparisons of root allocation in treatments that result in different sized individuals among treatments (e.g. generally smaller plants grown with competition) can yield results that appear to be active changes in allocation, but are actually due to differences in plant size and ontogenetic drift. To determine if differences in root allocation between competition and nitrogen treatments were an active response to the treatments or a passive product of ontogenetic drift, we analyzed each significant root allocation effect using analysis of covariance with each allocation trait as a response and competition or nitrogen treatment, biomass, and the interaction of the two as factors. Species with significant interaction terms were considered to have actively changed their root allocation in response to the treatment. We then regressed the root trait against total biomass to determine the difference in slopes between treatments. The same method, using site, biomass, treatment, and the treatment by biomass interaction for each N site, was used to determine if different populations had differences in allocation traits.

For the reciprocal transplant experiment, categorical data (survival, greenness, and bushiness) were analyzed using nominal logistic regression. Continuous data (height, basal area, stem diameter, and branch number) were analyzed using ANCOVA using the value at planting was included as a covariate. Analysis on all variables except survival was conducted on the subset of plants alive at measurement. Factors for each model were the N deposition level of both

the seed origin site (N origin) and the transplant site (N transplant), origin site and transplant site nested within N origin and N transplant respectively. Continuous data were transformed to improve normality, and least square means are reported in text and figures. Field nitrogen probe data were analyzed using ANOVA with N site, site as a random factor nested within N site, and watering status as factors.

RESULTS

Size Distribution and Community Composition within Native Plant Populations

Population size distributions skewed slightly toward larger plants to varying degrees for all species and sites, with differing size responses to N deposition level among species (Fig. 3). At all sites there was a long left tail to the distributions, meaning that smaller plants were present at all sites. *Ambrosia salsola* plants differed in size with N deposition/invasion (significant N site effect, $F=20.2$, $df= 2,295$, $p<.0001$), averaging 6001 ± 516 , 7599 ± 521 , and 4121 ± 426 cm² at high, medium, and low N depositional sites, respectively, with all N sites significantly different from each other. *Ambrosia salsola* plants from medium deposition sites were on average larger, with fewer small plants relative to other populations, possibly indicating less recruitment at these sites. Sites were similar within N site except at low deposition, where PC2 had a much higher representation of the smallest plants than did PC1.

For the two grass species, size increased with increasing N deposition. *H. rigida* averaged 2820 ± 354 , 303 ± 37 , and 139 ± 20 cm², and *S. speciosa* averaged 245.5 ± 26.6 , 147.4 ± 16.9 , and 68.7 ± 8.8 cm² at high, medium, and low N depositional sites, respectively (significant N site effects: *H. rigida*, $F=77.8$, $df= 2,263$, $p<.0001$; *S. speciosa* $F=25.1$, $df= 2,295$, $p<.0001$). Sites were similar within N site except for *H. rigida* at medium N deposition, where plants from LH1 were significantly larger than those from LH2.

Plant communities varied according to both N site and site (see Appendix 1 for detailed analysis). Sites with higher deposition showed greater diversity overall and greater native diversity. We did not see direct evidence of an invasion gradient concurrent with the N deposition gradient during this very dry study period, but litter was higher in the high N deposition sites.

Greenhouse Experiment

Emergence and Treatment Effects

Ambrosia salsola seeds emerged and survived to harvest at three months at a rate of 52.6%, *H. rigida* at 70.5%, and *S. speciosa* at 30.3%.

Nitrogen uptake by PRS probes, which served as a proxy for N available to the three focal species, indicated that available N was mostly in nitrate form, and availability followed expected patterns, greatly decreasing with *B. madritensis* competition ($F=139.6$, $df= 1,12$, $p<.0001$) and increasing with high N treatments, a trend that approached significance ($F=4.48$, $df= 1,12$, $p=.056$) (Table 2).

The nitrogen treatments had a significant effect on *B. madritensis* biomass. *Bromus madritensis* grown without a native focal plant increased in size with increasing nitrogen ($F=5.57$, $df= 2,99$, $p=.014$) across the three N treatments in the *A. salsola* experiment in 2013, although the increase in biomass was smaller and non-significant in the second year (Fig. 4).

Main Effects of N Site and Site

Both the seed collection sites and N site differed in some of the growth traits measured, with differences among species (Table 3). For *A. salsola*, sites differed in shoot length, RWR, and coarse root proportion (Fig. 5a-c), but these differences were not related to the level of N

deposition. Sites also differed in RWR for *H. rigida* (Fig. 5d), but with no N site effects.

Biomass and RWR for *S. speciosa* differed between N sites: plants from medium deposition sites had both higher biomass and higher allocation to roots than plants from high or low deposition sites (Fig. 6).

Main Effects of Competition and N treatment

Competition with *B. madritensis* affected nearly all growth traits measured for all three species (Table 3). Competition decreased measures of plant size for all species, with *A. salsola* decreasing the most in shoot length and *S. speciosa* exhibiting the greatest declines in leaf number and total biomass (Fig. 7). Competition also affected allocation of biomass to roots and shoots for all species. *Ambrosia salsola* actively increased root allocation in response to competition (Fig. 8; competition X biomass interaction, $F=40.88$, $df= 1,1132$, $p<.0001$), whereas both grasses allocated less biomass to roots when grown in competition with *B. madritensis* (Fig. 8), actively according to the ontogenetic drift model for *H. rigida* and passively for *S. speciosa* (competition X biomass $F=13.35$, $df= 1,721$, $p=.0003$ and $F=1.69$, $df= 1,358$, $p=.19$ respectively). *Ambrosia salsola* plants grown in competition also had a lower specific root length, an active response according to the ontogenetic drift model, indicating that roots were on average thicker (Fig. 9; competition X biomass $F=67.89$, $df= 1,1130$, $p<.0001$). Conversely, *S. speciosa* actively produced more fine roots in competition (competition X biomass $F=9.10$, $df= 1,358$, $p=.0027$), as fine root proportion increased from $.71\pm.004$ to $.81\pm.005$ while intermediate root proportion decreased from 0.22 ± 0.003 to 0.17 ± 0.005 (competition X biomass $F=12.37$, $df= 1,358$, $p=.0005$). *H. rigida* actively increased allocation to intermediate roots from 0.22 ± 0.003 (competition X biomass $F=5.75$, $df= 1,721$, $p=.0167$), while passively decreasing allocation to fine roots from 0.66 ± 0.004 to 0.60 ± 0.007 (competition X biomass $F=.002$, $df= 1,721$, $p=.968$) and actively

decreasing coarse root proportion from 0.11 ± 0.002 to 0.05 ± 0.002 (competition X biomass $F=43.84$, $df= 1,721$, $p<.0001$).

Nitrogen addition affected some, but not all, measured traits for *A. salsola* and *S. speciosa*, but had no significant effect on any trait for *H. rigida* (Table 3). Nitrogen addition increased *A. salsola* shoot length under the high N treatment and increased both biomass and shoot length of *S. speciosa* (Fig. 10). *Ambrosia salsola* produced a slightly but significantly higher proportion of fine roots at low N (0.51 ± 0.008) than at either medium (0.49 ± 0.007) or high N (0.49 ± 0.008) however this change was a passive response (competition X biomass $F=.50$, $df= 2,1128$, $p=.60$).

N Site by Competition Interactions

There were interactive effects of competition and collection site deposition level for at least one trait in all species, with the most interactions observed for *A. salsola* (Table 3). This interaction was significant for *A. salsola* shoot length, leaf number, and total biomass. Because differences among N sites were small, post-hoc Tukey's tests failed to differentiate significant effects of competition, but for all three traits, plants from high N deposition sites experienced less of a negative effect of competition, as competition lowered shoot length by 71.4 %, as opposed to 76.9% and 78.1% for medium and low N sites (Fig. 11). Similarly, total biomass was reduced by competition by 73.8% for high N plants, 78.0% for medium, and 78.5% for plants from low N deposition. Competition decreased leaf number by 56.1% at high and 56.8% at medium N sites, as opposed to 60.3% at low N sites. Allocation to fine roots decreased in response to competition for *A. salsola* plants from all levels of deposition, but the decrease was smallest in plants from high N deposition sites: 16.9%, compared to 25.9% for medium deposition and 27.4% for low (Fig. 12). All responses were active according to the ontogenetic drift model except fine root proportion from high N sites (analysis not shown).

The interactive effects between N site and competition were less pronounced in *H. rigida* and *S. speciosa*, with specific root length the only trait affected. Specific root length was constant across N site and competition treatments for *H. rigida* with the exception of plants from medium N deposition sites, which grew significantly coarser roots without competition (Fig. 13). Specific root length was actively higher under competition in *S. speciosa* from high and low N sites, with plants from medium deposition sites experiencing no significant relative change (Fig. 13).

N Site by N Treatment Interactions

Populations from different levels of nitrogen deposition did not differ in response to N treatment. No trait for any of the three species showed a significant interaction between N site and N treatment (all $p > 0.05$, a complete table of means from the greenhouse experiment can be found in appendix 3).

Family Level Variation and Family Interactions

Individual families showed a great deal of variation in almost all measured traits. *Ambrosia salsola* showed family-level variation for all the traits measured (Table 3). Families of both *H. rigida* and *S. speciosa* differed in shoot length, RWR, and SRL. In addition, *H. rigida* families differed in proportion of fine, intermediate, and coarse roots, and *S. speciosa* families differed in total biomass and number of leaves (Table 3).

Plants showed family level variation in response to competition (family x competition interactions) for fewer traits: leaf number for *H. rigida* and intermediate root proportion for *H. rigida* and *A. salsola* (Table 4). Competition always decreased *H. rigida* leaf number, but relative magnitude of these losses ranged from 32 to 79% (Fig. 14). Both species generally produced more intermediate roots with competition, but family responses were positive, negative, and

neutral (data not shown). There was no variation in family response to competition for *S. speciosa*.

Similarly, differences in family level trait responses to N treatment were few, but there was much variation among families for the few traits that exhibited differences. *Ambrosia salsola* families' RWR exhibited both positive (26 families) and negative (30 families) responses to increased N, with a range of -15 to 12% (Fig. 15). Similarly, there was much variation in total biomass response of *S. speciosa* to N addition, with 17 families showing positive responses and 11 showing negative ones, ranging from -41 to 207% (Fig. 16).

Reciprocal Transplant Results

Total nitrogen and nitrate uptake by soil probes trended upward with increasing nitrogen deposition, but these trends were not significant (Total nitrogen, $F=1.32$, $df= 2,3.178$, $p=.38$; nitrate $F=1.52$, $df= 2,3.186$, $p=.34$) (Table 5). Individual sites did not significantly differ within N site, and watered areas and dry interspaces also did not differ in total nitrogen uptake (all $p>0.05$).

Herbivory on native plants at the seed collection sites was more related to site conditions than N deposition (see Appendix 2 for detailed analysis). *Ambrosia salsola* did not show evidence of herbivory at any site, whereas both bunchgrass species suffered more herbivory at the two medium deposition sites, which may have provided greater rodent habitat (D. Hibbard, pers. obs.).

Of the 708 plants transplanted, 92.1% of *A. salsola*, 60.9% of *H. rigida*, and 64.3% of *S. speciosa* survived in the first year. For both *A. salsola* and *H. rigida* no factor in our model significantly affected first-year transplant survival. Only transplant site significantly affected *S. speciosa* survival ($\chi^2=11.84$, $df= 2$, $p=.0027$), with plants transplanted into CF1 and PC2 suffering

high mortality, 51.5% and 46.9% respectively, whereas 28.1% and 15.6% of the plants transplanted into CF2 and PC1 died in the first year.

Deposition level of the transplant site significantly affected at least one plant trait for all three species. Greenness, bushiness, height, basal area, and branch number in *A. salsola* all increased with increasing transplant site nitrogen deposition (Fig. 17). For *H. rigida*, bushiness increased with N deposition ($2.5 \pm .14$ low, $2.55 \pm .17$ medium, and $2.88 \pm .19$ high) as did *S. speciosa* greenness ($1.30 \pm .09$ low and $2.31 \pm .25$ high) (Table 6). There was a significant interaction between origin and transplant N deposition for *A. salsola* basal area and branch number (Table 6), but patterns were not consistent with local adaptation (data not shown).

Origin nitrogen deposition level affected only one trait, bushiness, for only one species, *A. salsola* (Table 6). Plant bushiness decreased with increasing nitrogen deposition of the seed collection site, from $2.75 \pm .13$ at low to $2.49 \pm .10$ and $2.36 \pm .09$ at medium and low deposition.

Plants from all collection sites performed similarly except for basal area of *H. rigida*. In contrast, transplant site affected many vegetative traits: *A. salsola* greenness, bushiness, height, and basal area as well as *H. rigida* height and basal area (Fig. 18).

DISCUSSION

Anthropogenic global change is a reality to which species must respond, be it through adaptation to new environments, migration or other changes in distribution patterns, physiological acclimation, or extinction (Holt 1990, Vitousek et al. 1997b, Kozlowski and Pallardy 2002). Arid systems and other systems that are historically nutrient poor have been shown to be particularly susceptible to change in resource availability such as increases in atmospheric nitrogen deposition (Burke and Grime 1996, Davis et al. 2000, Milbau and Nijs 2004). Such changes can affect plant

community composition and productivity both directly and indirectly, by facilitating the process of invasion (Bobbink et al. 2010) and changing patterns of herbivory (Throop and Lerdau 2004). Native plants may need to adapt to changing conditions in order to persist. In a recent review, Oduor (2013) documented several instances of rapid evolution in response to novel selective pressures in plants, a process by which native plants could improve their situations in a changing ecosystem.

We used a combination of field and greenhouse experiments to determine if these processes could be at work across a gradient of increased nitrogen deposition and invasion in Joshua Tree National Park (JTNP). For three native, long-lived species commonly used in restoration in JTNP (*Ambrosia salsola*, *Hilaria rigida*, and *Stipa speciosa*), we asked whether populations: 1) possessed the necessary heritable variation in traits related to resource acquisition for adaptation to occur, 2) currently showed evidence of adaptation to increased N deposition and invasion, 3) showed greater ability to adapt to either increased deposition or invasion. Although all species showed a large degree of heritable variation in the traits we measured in the greenhouse, *A. salsola* was the only species that showed a pattern of response to disturbance consistent with local adaptation. Specifically, there were small but significant increases in the ability of *A. salsola* to tolerate *B. madritensis* competition in populations collected from high N deposition sites. Preliminary results from reciprocal transplanting showed no evidence of local adaptation with regards to first year survival, though there were differences in performance among sites, with most species growing more vigorously at high deposition sites. Further adaptation may be possible in these populations, given the high degree of heritable variation in the root traits that we examined, which are likely to be important in a landscape of changing nutrient availability.

Competition with *B. madritensis* affected all three species as expected, as all measures of size decreased greatly, and all species displayed some changes in growth strategy in response to competition. The increase in allocation of biomass to roots in both bunchgrasses is a typical plastic response to resource reduction (Hodge 2004, 2009), however, the reduction in RWR and coarser roots of *A. salsola* in competition is less typical. The response of *A. salsola* might be indicative of a strategy of spatial segregation of roots from those of a competitor, which is common among shrubs which typically root deeper than grasses (Casper and Jackson 1997). Such a strategy would have had little chance of success in small experimental pots, but might be an effective response in a field setting. Field studies comparing the success of families with different levels of phenotypic plasticity could determine if the observed root responses of these three species are adaptive in the field.

The effects of nitrogen on desert plants can be mixed, with some plants being more capable of using the excess nitrogen than others (Romney et al. 1978, Williams and Bell 1981, Gutierrez and Whitford 1987). For *A. salsola* and *S. speciosa*, nitrogen addition in the greenhouse had overall positive influences on plant size, increasing biomass without changing RWR or allocation to fine and coarse roots. In contrast, no trait of *H. rigida* responded to N addition, a conservative strategy that would theoretically typify plants from very nutrient-poor environments. Both our PRS probe data and the increased growth of *B. madritensis* in high nitrogen treatments show that the nitrogen was available to support a strategy of greater N uptake. *H. rigida* is a C4 species and therefore more nitrogen-use efficient than C3 plants and perhaps better adapted to low nitrogen environments (Brown 1978). Species adapted to low nutrient levels often show low nutrient uptake rates as well (Chapin 1980). Thus, *H. rigida* may not possess the plasticity to increase uptake in the presence of luxury nitrogen, whereas *A. salsola* and *S. speciosa* appear to have some capacity to increase uptake.

Rapid evolution to strong selective pressures is common in plants (Buswell et al. 2011), and adaptation of native species to invasion is expected if a given population possesses the heritable genetic variation to respond to the new selective pressure (Strauss et al. 2006). Previous studies have shown that plants from invaded areas can have greater ability to tolerate or compete with invaders and that natural selection is likely driving these differences (Callaway et al. 2005, Meador and Hild 2006, Leger 2008, Goergen et al. 2011). However, not all populations have the necessary genetic variation in traits under selection to adapt (Blows and Hoffmann 2005). We observed some evidence for population-level differences in response to *B. madritensis* invasion for all three species, especially *A. salsola*. Competition consistently negatively affected shoot length, leaf number, and biomass of *A. salsola*, but the effect lessened for plants from higher N deposition levels and likely greater exposure to invasive competitors. Plants from high N deposition sites were not the largest when grown without competitors but were the most tolerant of competition, similar to the pattern seen by Leger (2008) in the Great Basin bunchgrass *Elymus elymoides* in response to competition with *B. tectorum*. Relative to plants from low deposition sites, *A. salsola* plants from high deposition sites also allocated more resources to fine rather than intermediate diameter roots under competition, an expected root competition response (Schenk 2006). As above, field experiments with families of known plasticity could help determine if such plasticity is adaptive.

In the greenhouse, *H. rigida* and *S. speciosa* did not exhibit evidence of adaptation to *B. madritensis* competition. Both RLR and SRL in *S. speciosa* from medium N depositional levels showed no response to competition, whereas plants from high and low N sites responded to competition equally and as expected by producing finer, nutrient foraging roots. Plants from medium N sites seem to lack the plasticity that plants from the other sites possess in responding to *B. madritensis* competition. *H. rigida* exhibited the opposite pattern, as only plants from medium N deposition possessed the ability to respond to competition by making finer roots.

These populations do not necessarily lack the ability to adapt to competition: within populations we observed heritable variation in some of the traits, such as leaf number in *H. rigida*, but at this point there is no evidence that natural selection has led to differentiated responses to competition among sites of differing N deposition.

Our reciprocal transplant experiment was conducted across a range of sites that were selected for their gradient of N-deposition and invasion. Soil nitrogen can be highly variable at small scales in arid systems (Schlesinger et al. 1996), and the trend of soil N availability along the east-west gradient, while not significant, likely reflected the general differences in available nitrogen across the deposition gradient previously observed in other studies (Allen et al. 2009, Schneider and Allen 2012). We did not observe a gradient in annual plant invasion within these sites, but our field transplant experiments were conducted in a very dry year when *B. madritensis* cover was low, and litter, often associated with annual grasses, increased with increasing N deposition, suggesting that this invasion gradient may have existed in past years (Appendix 1). There were no differences in field survival due to either origin or transplant nitrogen deposition level or any interaction between the two. Rather, individual sites differed, and then only for *S. speciosa*, indicating that some site characteristic other than nitrogen deposition caused higher mortality at one high deposition and one low deposition site. Because the plants were already a year old when they were transplanted, any seedling establishment barriers, common in arid environments (e.g. James et al. 2011), were most likely already overcome at the time of transplantation, and thus may differ from survival patterns experienced by plants in natural populations. As conditions were improved by being watered under JTNP restoration practices, we might not have expected to see differential survival, although mortality was not negligible for any species.

We were interested in whether there was more variation in the ability of plants to respond to competition from invaders or to N deposition. While family level variation in the traits we measured was widespread, there was less variation in response to invasive annual grass competition, and families had even lower variability in their responses to nitrogen addition. Thus, there is perhaps a greater potential to adapt to competition within these populations, though our limited sampling, necessitated by drought conditions, may be obscuring further variation within populations. Competition and atmospheric nitrogen deposition in isolation affect native plants through resource availability in opposite directions, which may be challenging (Etterson and Shaw 2001). In the Mojave, invasive annual grass competition is a contest for scarce soil resources, resulting in depletion of available water and nutrients for native plants (Brooks 2000, DeFalco et al. 2003). Increased nitrogen deposition in isolation increases available resources and would favor plants that can adapt to utilize luxury nitrogen whereas nutrient depletion due to annual grass competition would likely evolutionarily favor plants that adopt strategies that favor greater acquisition or greater resource use efficiency.

However, increased nitrogen deposition may also indirectly reduce resource availability due to increased invasibility (Davis et al. 2000, Brooks 1999). Supporting this, those traits that did show adaptation in *A. salsola* exhibited root trait response patterns consistent with a decrease in nitrogen availability (Chapin 1980), suggesting that although overall nitrogen availability increases with greater deposition, less nitrogen was actually available to native perennial plants at higher levels of deposition and invasion. Nitrogen probe data bear this out, as total available nitrogen was lower in high nitrogen and competition pots than in low nitrogen pots without *B. madritensis* competition. Native plants may have greater plasticity in response to the effective nutrient reduction resultant from annual grass competition than to nutrient addition from deposition, as suggested by the greater number of family traits that responded to competition than did to nitrogen addition. DeFalco et al. (2003) found that *B. madritensis* takes up nitrogen and

water at similar rates independent of resource availability and population density and at higher rates than native grasses, which could explain the greater effect of competition. The annual invasive grass *Schismus arabicus* performs better in the Mojave under constant, low level inputs of nitrogen than it does under more intermittent resource inputs (James et al. 2006), meaning the constant nitrogen deposition regime of the present and the foreseeable future is likely more advantageous for annual invaders. However, if extended dry periods become more prevalent, decreasing invasive annual grass cover, then increased nitrogen could have neutral or positive effects on individual plants and native species, if not necessarily on the native community as a whole.

For evolution to occur, recruitment is needed as well as natural selection on heritable variation. Because these are long-lived plants, we undertook field surveys to determine if there was evidence of reproduction within these populations. The size distributions we observed indicate that recruitment is likely ongoing at all sites for all three species, with the possible exception of *A. salsola* at the two medium N deposition sites. Except for these two sites, the smallest, youngest plants comprised the greatest percentage of the population, likely indicating that invasion and increased nitrogen deposition have not created an environment where recruitment has ceased and only large plants remain. Because size is a good indicator of reproductive output for many perennial bunchgrasses (Lefkovitch 1965, Kirkpatrick 1984, Moloney 1988), and the only plants for which we could collect adequate quantities of usable seed were large, there may be a lag in our ability to assess the current level of adaptation, as our sampling was biased towards larger individuals of earlier cohorts.

Interestingly and not unexpectedly, *A. salsola*, the species with the greatest reliance on seed for reproduction, exhibited the greatest adaptive potential and the greatest evidence of possible local adaptation, followed by *S. speciosa*, which is less reliant on seed, and *H. rigida*,

even less reliant on seed. A project similar to this one focusing on native annual species or shorter lived perennials might have different results due to the even shorter generation time, as increased opportunity for recombination and more rapid response to natural selection could occur for those species.

Our results document effects on seedlings and not mature plants, and thus results may change as plants mature. However, seedling establishment in the arid West is a major barrier for restoration and establishment of native species (James et al. 2011), therefore the effects of competition on seedlings are likely to have a large impact on plant fitness. Rice and Knapp (2008) found that local adaptation in two northern California bunchgrasses was most pronounced on measures of reproduction in older plants. We are continuing to monitor our reciprocal transplant experiment in order to assess the longer-term fitness impacts of *B. madritensis* competition and nitrogen deposition in JTNP. These plants, which did not endure the rigors of seedling establishment in the Mojave Desert, did not show differential survival due to either origin or transplant site nitrogen level, but it is yet to be seen whether population differences in measures of reproductive output exist. It is also unclear whether the adaptive responses seen here represent a general response to increased competition or a specific response to *B. madritensis* competition. Species-specific competition experiments would be needed to determine whether there is heritable variation in response to competition in general or to specific competitors, native or invasive.

These results suggest that the Joshua Tree National Park's current procedures for seed collection with regards to restoration are appropriate for these three species and their current level of adaptation to nitrogen and invasion. The National Park Service's management principles already advise that restoration be done using stock as genetically similar as possible (United States 2006). Continuing to do so will not disrupt ongoing evolutionary processes and, given the

general lack of local adaptation to nitrogen deposition and annual grass invasion, is not likely to put certain naïve populations at a disadvantage relative to more experienced populations under probable future conditions. If native populations are found to be suffering from these pressures, further screening can be done for populations inside the park or outside, likely to the west, that might be more successful, which can aid in restoration efforts.

The shadow of global change looms over all current and future restoration projects, and the question of what geographic and genetic properties restoration stock should possess is a matter of debate (McKay et al. 2005, Broadhurst et al. 2008, Jones 2013, Breed et al. 2013). Evolution is increasingly viewed as meaningful on ecological timescales (Thompson 1998, Rice and Emery 2003, Hairston et al. 2005, Hoffmann and Sgrò 2011). Although less common than studies of adaptation in invaders, recent work has shown that native plant populations can show rapid evolutionary change in response to invasion (Callaway et al. 2005, Lau 2006, Meador and Hild 2007, Leger 2008, Ferrero-Serrano et al. 2011, Bergum et al. 2012, Sebade et al. 2012). Given that this rapid adaptive ability seems common, it is prudent to build this process into restoration plans to maximize the adaptive potential of native populations while minimizing that of invaders (Leger and Espeland 2010). Eradication of invaders is unlikely, but this type of restoration strategy increases the chance that a native population will put itself into an acceptable balance with invasive species. Consideration should also be given to using stock for native seedlings and plantings derived from populations that have gone through the gauntlet of invasion and are likely to be better competitors with probable invaders.

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FIGURE CAPTIONS

Figure 1: Diagram showing both direct and indirect effects of increased nitrogen availability on native plants. Direct effects of increased nitrogen are usually positive for native plants, whereas the indirect effects of increased invasion and herbivory are almost always negative. Whether the net effect on native plants is positive or negative depends on whether the positive direct effect outweighs the negative indirect effects of invasion and herbivory.

Figure 2: Location of the study site in western JTNP in the Mojave Desert of southern California. Black squares represent native plant collection sites. Pine City 1 (PC1) and Pine City 2 (PC2) are low N deposition sites, Lost Horse 1 (LH1) and Lost Horse 2 (LH2) are medium deposition, and Covington Road 1 (CR1) and Covington Road 2 (CR2) are high deposition sites. Western sites experience more atmospheric nitrogen deposition than eastern sites. Inset shows National Atmospheric Deposition Program modeling of total nitrogen deposition in kg/ha in southern California averaged from 2011-2013. Warm colors represent higher levels of deposition, and cooler colors represent lower deposition. The JTNP boundary is marked is outlined in black, and the study area within the park is marked by the white box.

Figure 3: Log transformed size distribution (elliptical area in cm^2) of populations of *Ambrosia salsola*, *Hilaria rigida*, and *Stipa speciosa* at each seed collection site, arranged by N deposition level. Untransformed means and standard deviations for each population are reported. $n \geq 50$ at for each species at each site, except for *H. rigida*, which was not present at PC2.

Figure 4: Mean and SE of *B. madritensis* biomass grown alone across low, medium, and high nitrogen treatments in 2013, when three N treatments were used, and in 2014, when two N treatments are shown. Bars with the same letters are similar according to Tukey's HSD test.

Figure 5: Mean and SE of a) shoot length, b) RWR, and c) coarse root proportion of *A. salsola* and d) RWR of *H. rigida* from different seed collection sites. No *H. rigida* was collected at PC2, thus there are five sites represented. Different lettered bars indicate significant differences according to Tukey's HSD test.

Figure 6: Mean and SE of a) total biomass and b) RWR of *S. speciosa* from different N deposition levels. Different lettered bars are different according to Tukey's HSD test.

Figure 7: Means and standard errors for three measures of plant size, shoot length, leaf number, and biomass, for *A. salsola* (left), *H. rigida* (middle) and *S. speciosa* (right) grown with and without *B. madritensis* competition. All differences significant at $p < .0001$.

Figure 8: Mean and SE of root weight ratio for a) *A. salsola*, b) *H. rigida*, and c) *S. speciosa* grown with and without *B. madritensis* competition ($p = .006$, $p = .0004$, and $p < .0001$ respectively).

Figure 9: Mean and SE of specific root length for *A. salsola* grown with and without *B. madritensis* competition ($p < .0001$).

Figure 10: Mean and SE of a) shoot length of *A. salsola* and b) shoot length and c) biomass of *S. speciosa* under different N treatments. For *A. salsola*, different lettered bars are different according to Tukey's HSD test. For *S. speciosa*, $p=.0131$ and $.0067$ respectively.

Figure 11: Mean and SE of a) shoot length, b) leaf number, and c) total biomass of *A. salsola*. Bar color represents the competition treatment for plants from different N sites. The N site x competition interaction was significant ($p=.0028$, $p=.0119$, and $p=.0086$ respectively), but Tukey's HSD test did not differentiate between N-sites.

Figure 12: The effect of competition and N site on allocation to fine, intermediate, and coarse root length of *A. salsola*, represented by different shades of grey. Mean proportion of root in each size diameter class is given as a percentage on the bar. The percent decline in total root length for plants grown in competition is also given.

Figure 13: Mean and SE of SRL for a) *S. speciosa* and b) *H. rigida*. Dark bars represent plants grown without competition and light bars represent plants grown with *B. madritensis* competition for each N site. Different lettered bars are different according to Tukey's HSD test.

Figure 14: Mean family responses of *H. rigida* leaf number to competition. Each line represents the mean response for a single family of seeds. The inset displays the range of percent change for each family.

Figure 15: Mean family responses of *A. salsola* RWR to N treatment. Each line represents the mean response for a single family of seeds. The inset displays the range of percent change for each family. For the sake of clarity, only low and high N treatments are shown.

Figure 16: Mean family responses of *S. speciosa* total biomass to N treatment. Each line represents the mean response for a single family of seeds. The inset displays the range of percent change for each family.

Figure 17: Differences in *A. salsola* across the gradient of nitrogen deposition at the transplant site in a) mean greenness and bushiness scores, b) height, c) basal area, and d) branch number (LS means \pm SE shown for b, c, and d). Columns marked with the same letter indicate similar values according to Tukey's HSD.

Figure 18: Differences across transplant site in *A. salsola* a) mean greenness and bushiness scores, b) height, c) basal area, and in *H. rigida* d) height and e) basal area (LS means \pm SE shown for b, c, d, and e). Columns marked with the same letter indicate similar values according to Tukey's HSD.

TABLES

Table 1: Soil texture and chemistry properties for seed collection sites and greenhouse (GH) soil. The average and SE of all field soil samples are also shown.

N site		Low N		Med N		High N		Field	
Site		PC1	PC2	LH1	LH2	CR1	CR2	Avg.	GH
Texture	% Sand	72.2	84.2	84.2	90.2	92.2	80.2	83.9± 2.9	92.2
	% Silt	18.5	8.5	8.5	4.5	0.5	8.5	8.2± 2.4	2.5
	% Clay	9.3	7.3	7.3	5.3	7.3	11.3	8.0± 0.8	5.3
	Classification	Loam y Sand	Loam y Sand	Loam y Sand	Sand	Sand	Loam y Sand		Sand
Chemistry	pH	7.5	7.1	7.1	7.2	7.5	7.3	7.3± 0.1	6.8
	P ³⁻	73	49	62	40	57	61	57.0± 4.7	34
	K ⁺	301	180	198	178	184	201	207± 19.2	49
	Ca ²⁺	2368	1102	1126	745	1370	1553	1377± 227	1671
	Mg ²⁺	320	196	158	133	126	133	177.7± 30.4	133
	Organic Matter	1.6	0.7	0.9	0.6	1.1	1.1	1.0± 0.14	1.1
	NO ₃ ⁻	6	5	6	3	9	8	6.2± 0.9	3
	NH ₄ ⁺	2	0	0	2	0	2	1.0± 0.44	0
CEC	12.5	6.3	6.2	4.4	6.8	7.7	7.3± 1.1	8	

Table 2: Total nitrogen, nitrate, and ammonium uptake of PRS probes under each of the four competition and nitrogen treatment combinations in the greenhouse.

	Total N		Nitrate		Ammonium	
	Low N	High N	Low N	High N	Low N	High N
No Comp.	246.3±16.7	329.1±14.1	244.1±16.9	326.3±14.0	2.26±0.23	2.78±0.42
Comp.	16.3±2.9	28.6±4.3	13.6±2.7	26.0±4.1	2.73±0.21	2.62±0.21

Table 3: ANOVA results for differences in plant traits among N site, sites, in response to competition and N addition treatment, the interactions of these two treatments and N site, and among families. Site was nested under N site and family under site and N site. Reported values are $F_{(\text{numerator df, denominator df})}$, and * signifies a p-value <0.05 , ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$. Models run as EMS are denoted with ⁺. Δ log likelihood between models with and without the factor is reported for random factors (site and family). Transformations used are indicated as follows: $\log_{10} = ^a$, box Cox = ^b, and sq. rt. = ^c.

Site	Competition	N Treatment	N Site X Comp.	N Site X N Treat	Comp. X N Treat	N Site X Comp. X N Treat	Family
10.2**	1433.7 _(1,1062) ****	6.4 _(2,1062) **	5.9 _(2,1061) **	0.3 _(4,1061)	1.7 _(2,1063)	0.9 _(4,1062)	18.9****
0.1	2965.5 _(1,1065) ****	2.0 _(2,1065)	4.4 _(2,1064) *	0.5 _(4,1065)	0.4 _(2,1067)	1.5 _(4,1066)	15.4****
4.2	2251.2 _(1,1066) ****	8.8 _(2,1065) ****	4.8 _(2,1066) **	0.7 _(4,1065)	7.2 ₍₁₀₆₉₎ ****	0.2 _(4,1067)	14.5****
10.3**	7.6 _(1,1074) **	0.4 _(2,1073)	1.5 _(2,1074)	1.4 _(4,1073)	2.6 _(2,1078)	1.1 _(4,1077)	5.0*
3.8	105.9 _(1,1068) ****	1.0 _(2,1068)	0.4 _(2,1068)	0.2 _(4,1068)	0.1 _(2,1071)	2 _(4,1070)	15.1****
0.0	293 _(1,1075) ****	3.4 _(2,1076) *	5.3 _(2,1074) **	0.4 _(4,1075)	0.1 _(2,1078)	1.4 _(4,1077)	10.1**
0.3	280.6 _(1,1081) ****	2.3 _(2,1081)	10.7 _(2,1081) ****	0.6 _(4,1081)	0.3 _(2,1084)	1.2 _(4,1083)	7.8*
16.6****	1.1 _(1,1076)	0.5 _(2,1075)	5.1 _(2,1075) **	0.5 _(4,1075)	0.7 _(2,1079)	0.8 _(4,1078)	6.8*
1.0	886.7 _(1,6667) ****	0.9 _(1,6619)	0.1 _(2,6669)	1.3 _(2,6622)	0.1 _(1,6694)	0 _(2,6691)	31.0****
0.1	1283.2 _(1,6757) ****	0.2 _(1,6689)	0.3 _(2,6758)	0.5 _(2,6692)	1.4 _(1,6802)	0.4 _(2,6794)	4.0
3.9	1148.6 _(1,6768) ****	0.0 _(1,6682)	0.6 _(2,6768)	0.5 _(2,6684)	0.5 _(1,6815)	0.3 _(2,6805)	0.9
8.7**	12.6 _(1,6604) **	3.5 _(1,6543)	0.3 _(2,6605)	0.3 _(2,6545)	2.3 _(1,6631)	0.2 _(2,6627)	20.9****
1.4	3.5 _(1,6612)	0.7 _(1,6567)	6.4 _(2,6614) **	0.1 _(2,6569)	0 _(1,6656)	1.2 _(2,6653)	44.0****
0.5	39.5 _(1,6704) ****	0.9 _(1,6652)	1.2 _(2,6704)	0.7 _(2,6655)	0.5 _(1,6739)	3.4 _(2,6734) *	16.1****
1.1	317.1 _(1,6765) ****	0.5 _(1,6698)	0.8 _(2,6765)	0.7 _(2,670)	0.4 _(1,6806)	3.5 _(2,6799) *	4.9*
0.1	376.4 _(1,6707) ****	1.0 _(1,665)	1.9 _(2,6708)	1.4 _(2,6652)	0.2 _(1,674)	1.1 _(2,6756)	14.8****
0.3	159.3 _(1,3407) ****	6.2 _(1,3336) *	1.6 _(2,3353)	1.3 _(2,329)	1.1 _(1,337)	1 _(2,332)	12.6****
0.0	2071.5 _(1,3387) ****	1.1 _(1,3303)	1.0 _(2,3319)	0.3 _(2,3241)	0.3 _(1,3348)	0.1 _(2,329)	18.9****
0.0	3079.5 _(1,3425) ****	7.4 _(1,3357) **	3.0 _(2,3385)	0.4 _(2,3315)	0.2 _(1,3392)	0.2 _(2,3353)	6.7*
0.1 _(3,1765)	39.7 _(1,288) ****	0.9 _(1,288)	0.2 _(2,288)	0.9 _(2,288)	0.1 _(1,288)	1.2 _(2,288)	1.0 _(9,288)
0.2	70 _(1,3425) ****	0.0 _(1,3367)	5.9 _(2,3394) **	0 _(2,3337)	0.8 _(1,3394)	0.9 _(2,336)	5.5*
0.9	141.2 _(1,3434) ****	0.6 _(1,3416)	1.2 _(2,3436)	0.8 _(2,3412)	0 _(1,342)	0.2 _(2,3411)	1.0
1.8	50.6 _(1,3441) ****	0.1 _(1,3416)	2.1 _(2,3444)	0.6 _(2,3414)	0 _(1,3427)	0.2 _(2,3419)	1.2
0.1	565.1 _(1,3372) ****	3.7 _(1,3361)	0.4 _(2,3361)	2 _(2,3339)	0.2 _(1,335)	0 _(2,333)	0.4

<i>A. salsola</i>	n	N Site
Shoot Length ^a	1133	0.1 (2,2.979)
Leaf Number ^b	1131	1.2 (2,2.795)
Biomass ^c	1136	1.6 (2,3.001)
RWR ^b	1136	0.2 (2,2.996)
SRL ^a	1135	0.3 (2,2.999)
Fine Root Proportion	1135	6.2 (2,2.975)
Intermediate Root Proportion	1135	9.6 (2,3.03) *
Coarse Root Proportion ^b	1135	0.7 (2,2.985)
<i>H. rigida</i>		
Shoot Length ^c	726	1.3 (2,1.885)
Leaf Number ^c	726	1.9 (2,1.725)
Biomass	725	0.4 (2,1.961)
RWR ^b	725	1.3 (2,2.024)
SRL ^a	726	0.5 (2,1.952)
Fine Root Proportion ^b	726	4.4 (2,1.055)
Intermediate Root Proportion ^c	726	0.7 (2,1.956)
Coarse Root Proportion ^b	726	5.0 (2,1.892)
<i>S. speciosa</i>		
Shoot Length ^c	362	2.7 (2,9.623)
Leaf Number ^b	362	2.7 (2,4.422)
Biomass ^b	362	6.5 (2,5.642) *
RWR ^b	362	7.5 (2,14.8) ***
SRL ^c	362	0.3 (2,4.23)
Fine Root Proportion ^b	362	2.3 (2,2.758)
Intermediate Root Proportion ^c	362	2 (2,3.155)
Coarse Root Proportion ^b	362	5.8 (2,1.411)

Table 4: ANOVA results for family level responses of different plant traits to competition and N treatment for the subset of families with representation in all treatments. Δ log likelihood between models with and without the factor is reported for these random factors. No family by competition by N treatment interactions were significant and are not shown. N was 862-865 for *A. salsola* from 56 families, 685-686 for *H. rigida* from 61 families, and 260 for *S. speciosa* from 28 families. * signifies a p-value <0.05, ** signifies p<0.01, *** signifies p<0.001, and **** signifies p<0.0001. Models run as EMS are denoted by an ⁺ and are reported as F_(numerator df, denominator df). Transformations used are indicated as follows: log=^a, box Cox=^b, and sq. rt.=^c.

<i>A. salsola</i>	n	Family X Competition	Family X N Treatment	Family X Comp. X N Treat.
Shoot Length ^a	862	1.3	2.6	0.0
Leaf Number ^{+b}	861	1.7 _(50,105.4) **	1.2 _(100,100)	1.0 _(100,540)
Biomass ^c	864	0.2	1.3	2.0
RWR ^b	864	0.8	5.7*	4.4
SRL ^a	864	1.4	2	2.6
Fine Root Proportion ⁺	864	1.3 _(50,103.8)	1.1 _(100,100)	1.0 _(100,543)
Intermediate Root Proportion	864	7.4*	0.9	0.3
Coarse Root Proportion ^b	864	0.0	0.0	1.0
<i>H. rigida</i>				
Shoot Length ^c	686	3.1	0.2	0.2
Leaf Number ^c	686	4.7*	1.8	1.8
Biomass	685	0.5	0.0	0.0
RWR	685	3.5	0.0	0.0
SRL ^a	686	0.4	0.1	0.0
Fine Root Proportion	686	2.3	0.2	0.2
Intermediate Root Proportion ^c	686	5.1*	2.5	2.5
Coarse Root Proportion ^a	686	0.0	0.0	0.0
<i>S. speciosa</i>				
Shoot Length ^c	260	0.8	2.7	0.6
Leaf Number ^c	260	0.1	0.1	0.1
Biomass ^c	260	1.6	6.9*	4.0
RWR	260	0.1	0.4	2.0
SRL ^c	260	0.7	0.3	1.6
Fine Root Proportion	260	0.6	1.2	1.5
Intermediate Root Proportion ^c	260	0.6	0.8	1.1
Coarse Root Proportion ^c	260	3.3	2.7	2.3

Table 5: Total nitrogen, nitrate, and ammonium uptake of PRS probes for each N site at transplant sites in JTNP.

N Site	Total N	Nitrate	Ammonium
Low	89.7±17.2	83.0±17.7	6.8±1.0
Medium	99.1±20.4	91.3±19.8	7.8±1.2
High	133.0±24.9	127.6±25.0	5.3±0.6

Table 6: Logistic regression results for categorical variables and ANCOVA results for continuous variables for transplant responses due to N of origin site, N of transplant site, the interaction between the two, origin site (nested within N of origin site), and transplant site (nested within N of transplant site). Values at transplant for continuous variables were used as a covariate, but results are not shown. Reported values are $\chi^2_{(df)}$ for categorical variables and $F_{(\text{numerator df, denominator df})}$ for continuous variables. * signifies a p-value <0.05, ** signifies p<0.01, *** signifies p<0.001, and **** signifies p<0.0001.

Categorical Variables						
<i>A. salsola</i>	n	N Origin	N Transplant	N Orig. X N Trans.	Orig. Site	Trans. Site
Survival	305	0.13 ₍₂₎	2.10 ₍₂₎	0.13 ₍₄₎	7.69 ₍₃₎	6.65 ₍₃₎
Greenness	282	1.35 ₍₈₎	28.26 ₍₈₎ ***	14.02 ₍₁₆₎	11.05 ₍₁₂₎	38.58 ₍₁₂₎ ****
Bushiness	281	21.21 ₍₈₎ ***	32.80 ₍₈₎ ****	16.95 ₍₁₆₎	5.14 ₍₁₂₎	23.38 ₍₁₂₎ *
<i>H. rigida</i>						
Survival	274	3.39 ₍₂₎	3.65 ₍₂₎	3.78 ₍₄₎	4.15 ₍₂₎	4.65 ₍₃₎
Greenness	167	0.0000095 ₍₂₎	0.0000056 ₍₂₎	3.04 ₍₄₎	0.01 ₍₂₎	4.20 ₍₃₎
Bushiness	167	7.19 ₍₈₎	17.64 ₍₈₎ *	20.62 ₍₁₆₎	5.32 ₍₈₎	16.97 ₍₁₂₎
<i>S. speciosa</i>						
Survival	129	0.08 ₍₁₎	1.09 ₍₁₎	2.25 ₍₁₎	0.97 ₍₂₎	11.84 ₍₂₎ *
Greenness	83	0.75 ₍₄₎	20.78 ₍₄₎ ***	0.26 ₍₄₎	11.78 ₍₈₎	11.69 ₍₈₎
Bushiness	83	7.67 ₍₄₎	2.90 ₍₄₎	3.42 ₍₄₎	3.75 ₍₈₎	11.59 ₍₈₎
Continuous Variables						
<i>A. salsola</i>	n	N Origin	N Transplant	N Orig. X N Trans.	Orig. Site	Trans. Site
Height	280	0.71 _(2,264)	6.55 _(2,264) **	0.62 _(4,264)	0.52 _(3,264)	6.01 _(3,264) ***
Basal Area	282	0.91 _(2,266)	11.35 _(2,266) ****	3.00 _(4,266) *	0.69 _(3,266)	2.80 _(3,266) *
Branch No.	279	0.08 _(2,263)	16.85 _(2,263) ****	3.77 _(4,263) **	1.17 _(3,263)	1.96 _(3,263)
<i>H. rigida</i>						
Height	167	0.76 _(2,152)	0.94 _(2,152)	1.92 _(4,152)	0.56 _(2,152)	3.85 _(3,152) *
Basal Area	167	2.12 _(2,152)	0.17 _(2,152)	0.79 _(4,152)	3.60 _(2,152) *	3.37 _(3,152) *
<i>S. speciosa</i>						
Height	81	0.0002 _(1,72)	0.41 _(1,72)	0.16 _(1,72)	0.64 _(2,72)	0.13 _(2,72)
Basal Area	82	1.65 _(1,73)	0.0057 _(1,73)	0.51 _(1,73)	0.97 _(2,73)	0.26 _(2,73)

Figure 1

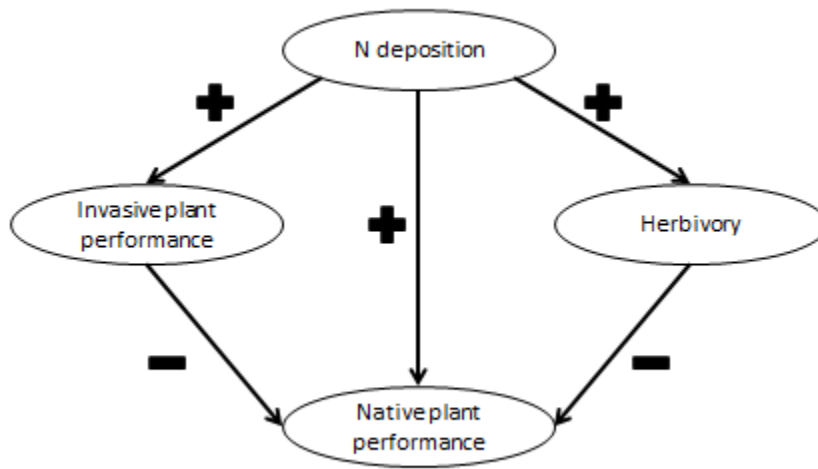


Figure 2

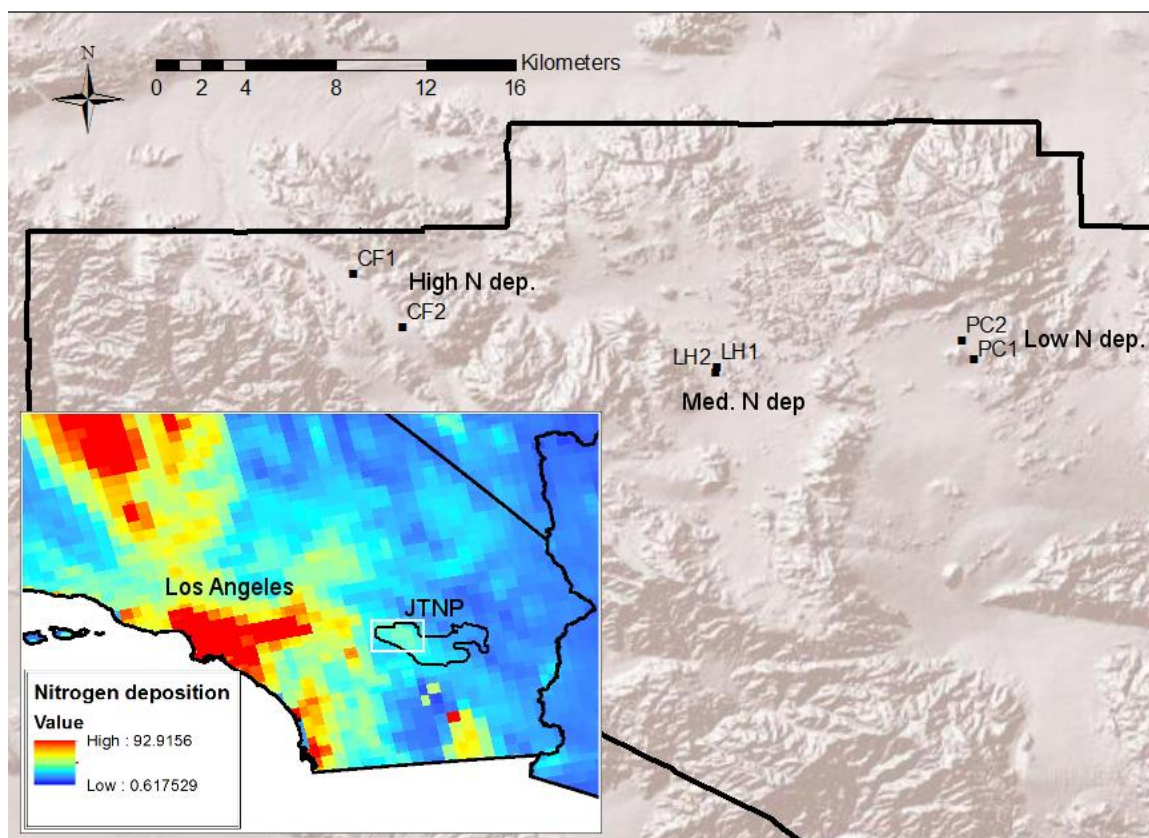


Figure 3

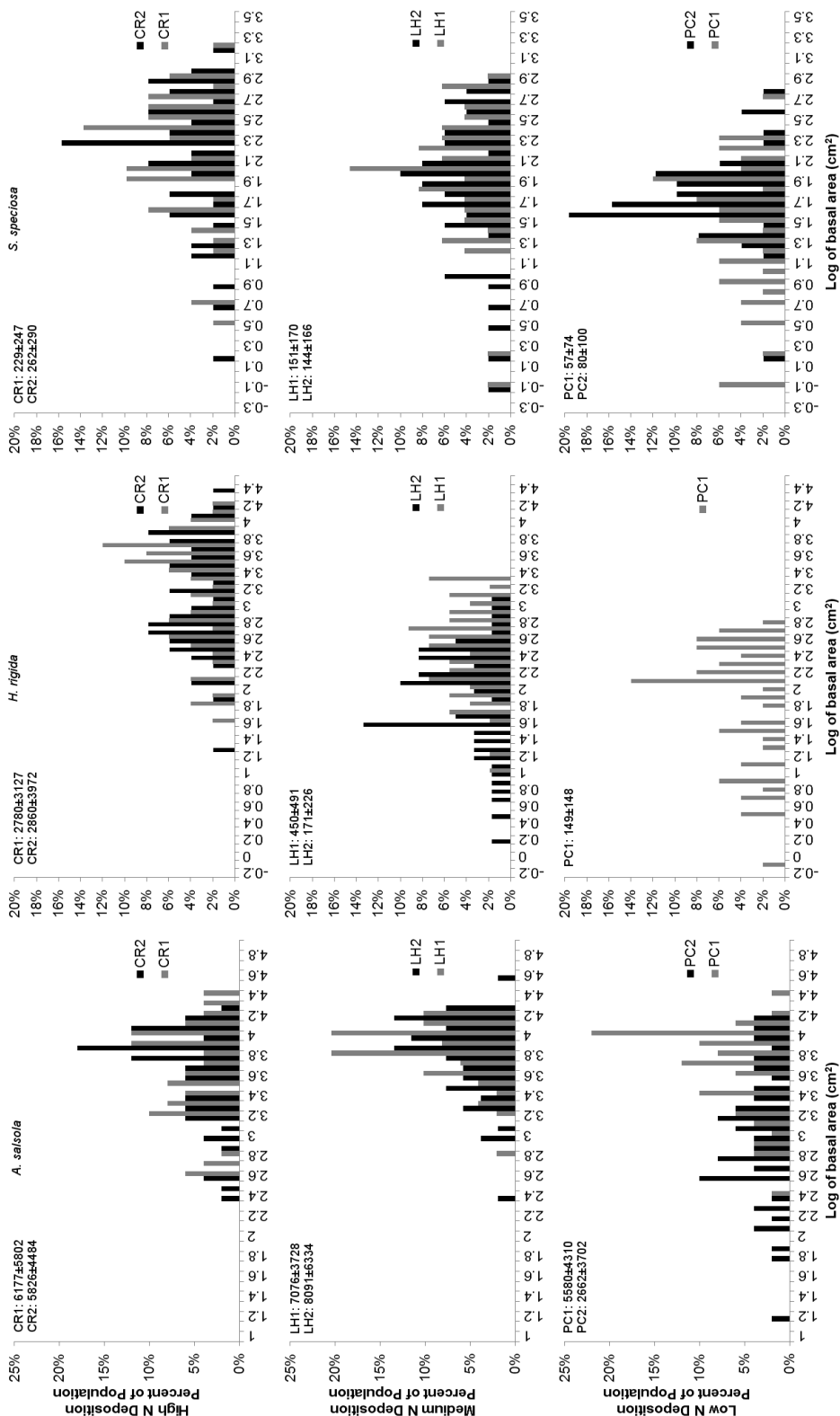


Figure 4

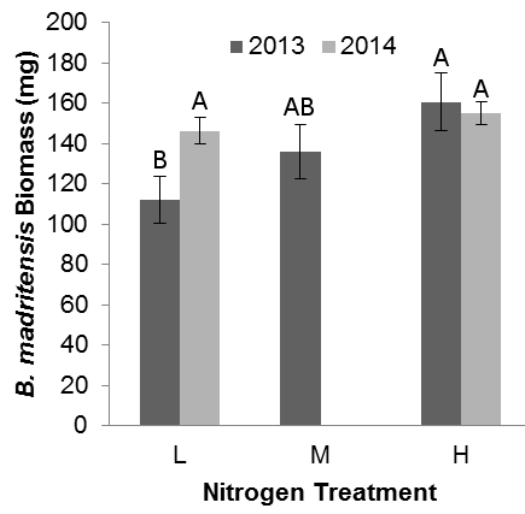


Figure 5

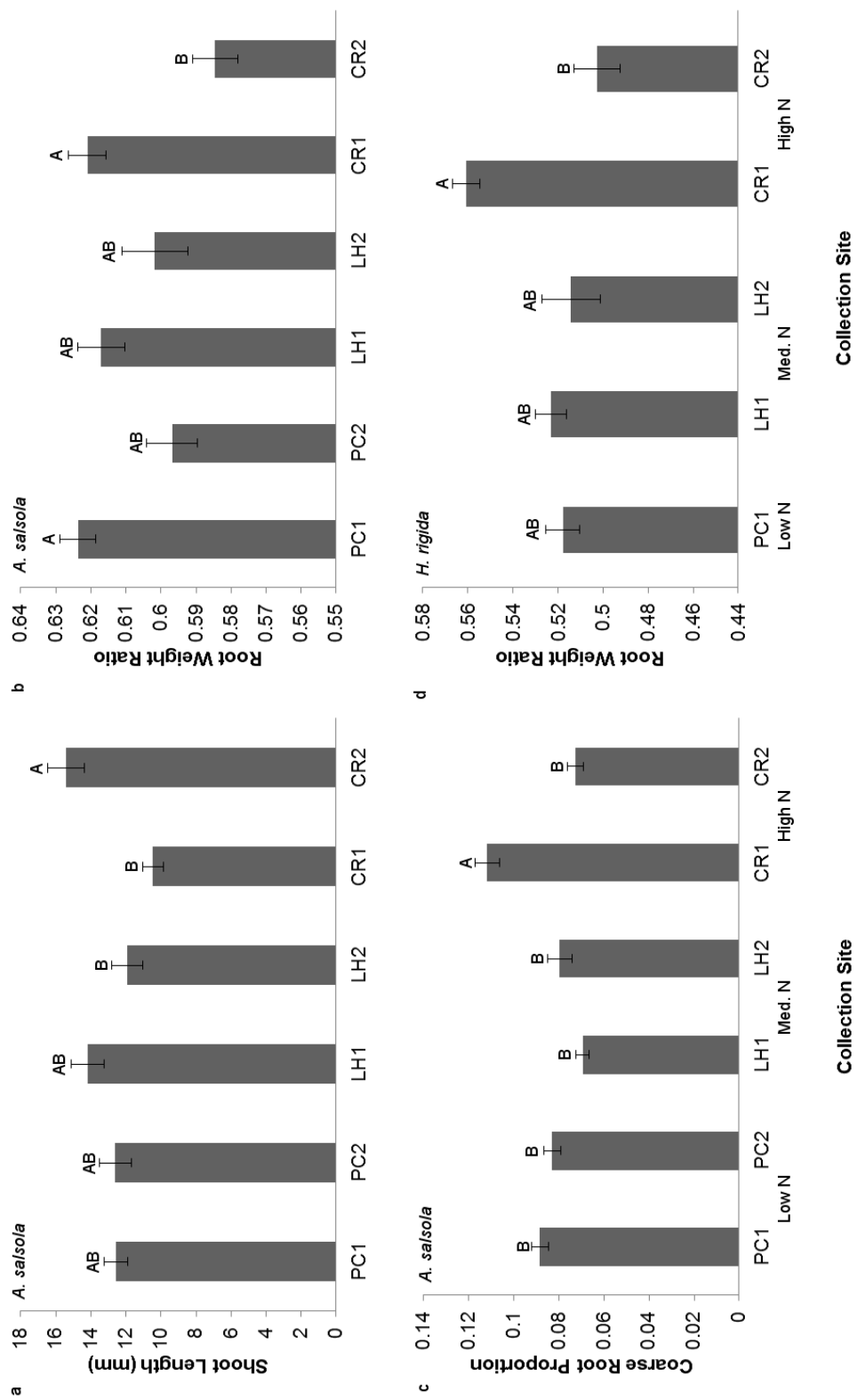


Figure 6

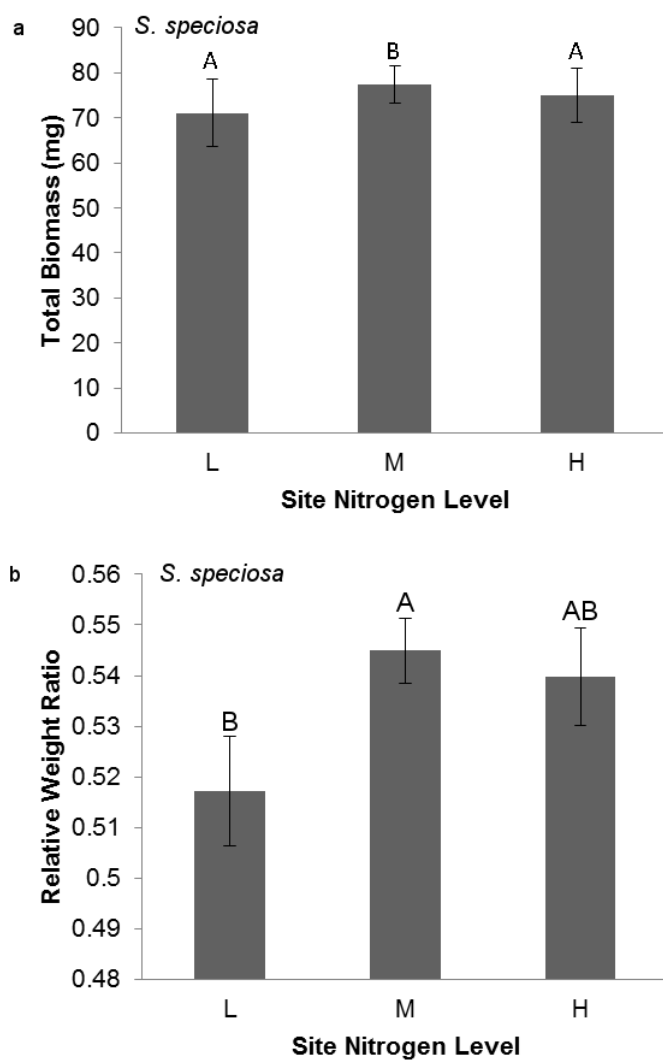


Figure 7

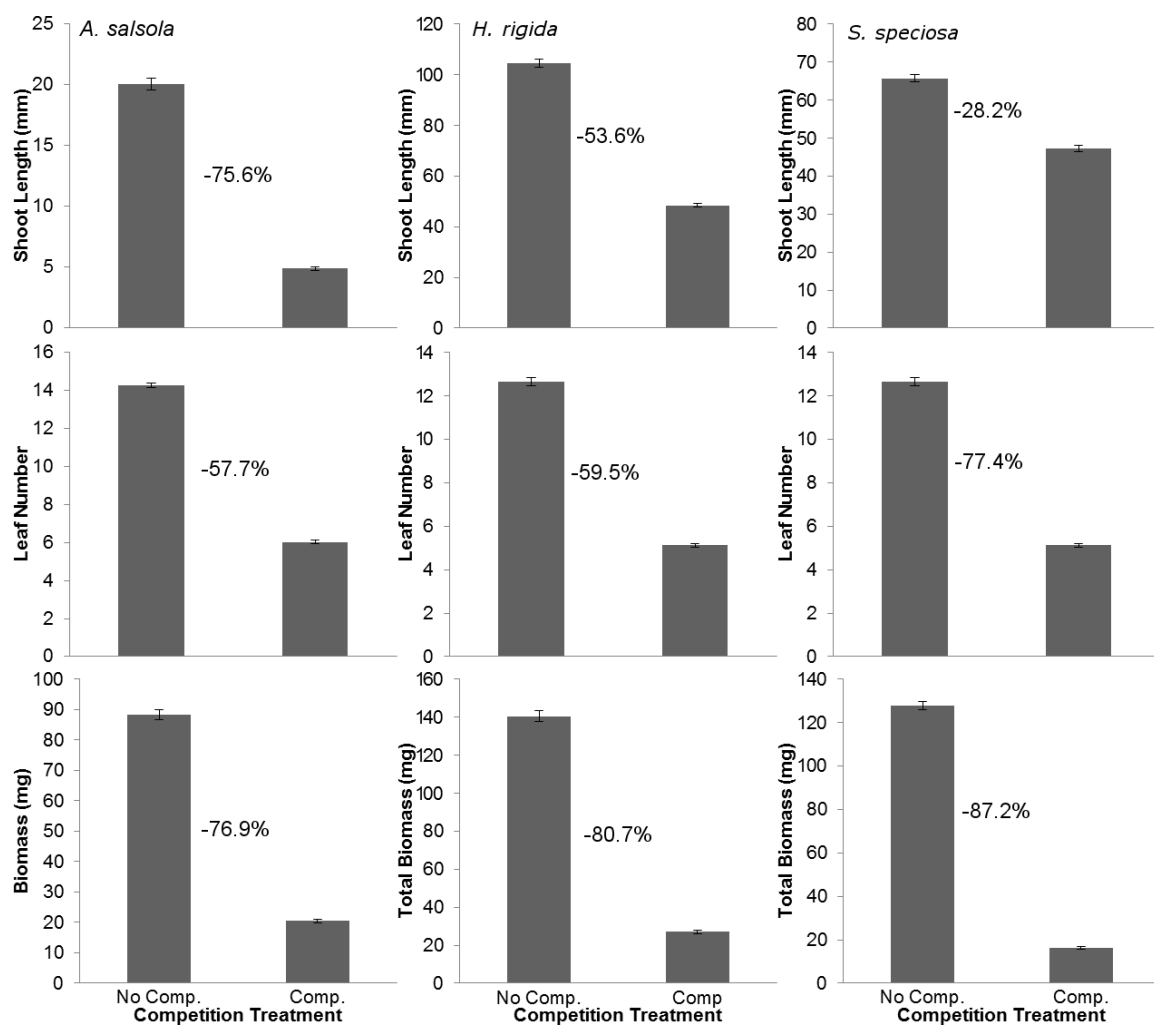


Figure 8

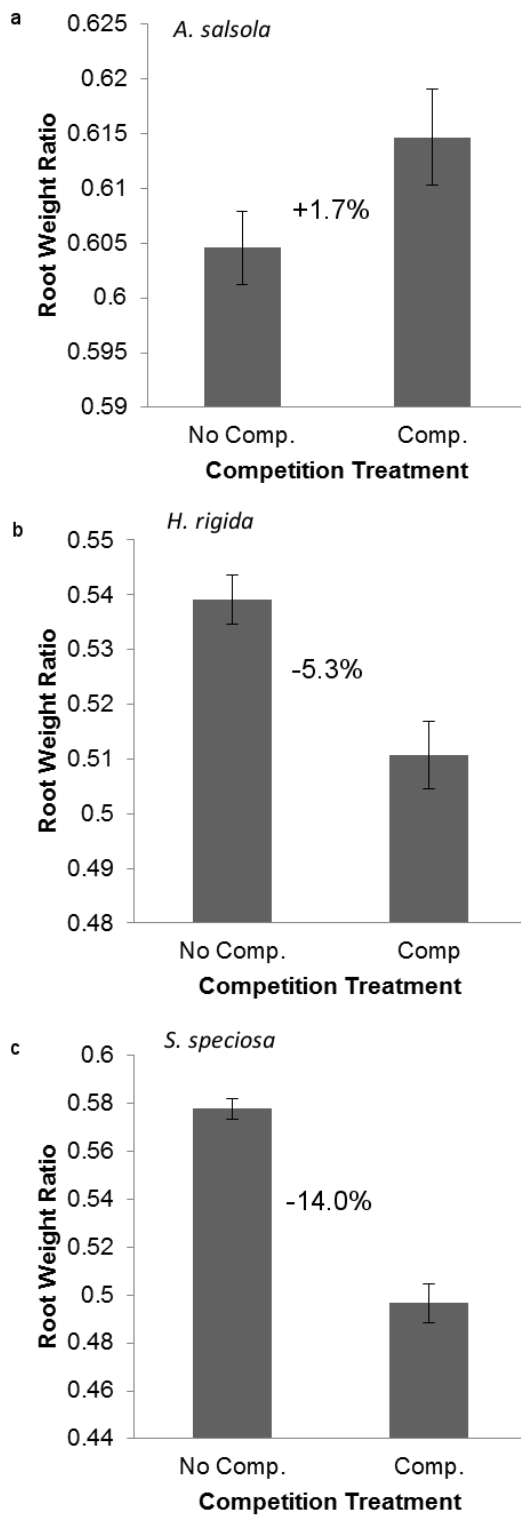


Figure 9

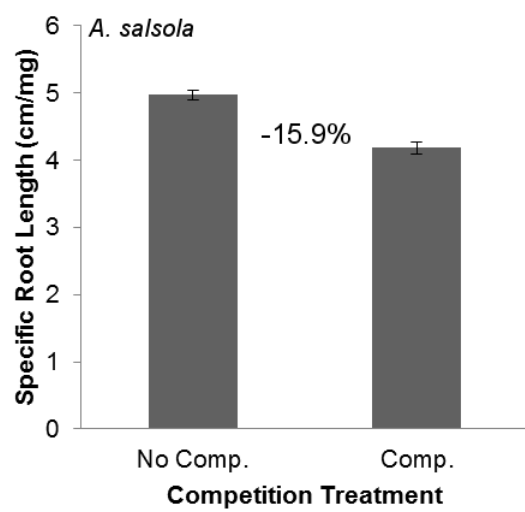


Figure 10

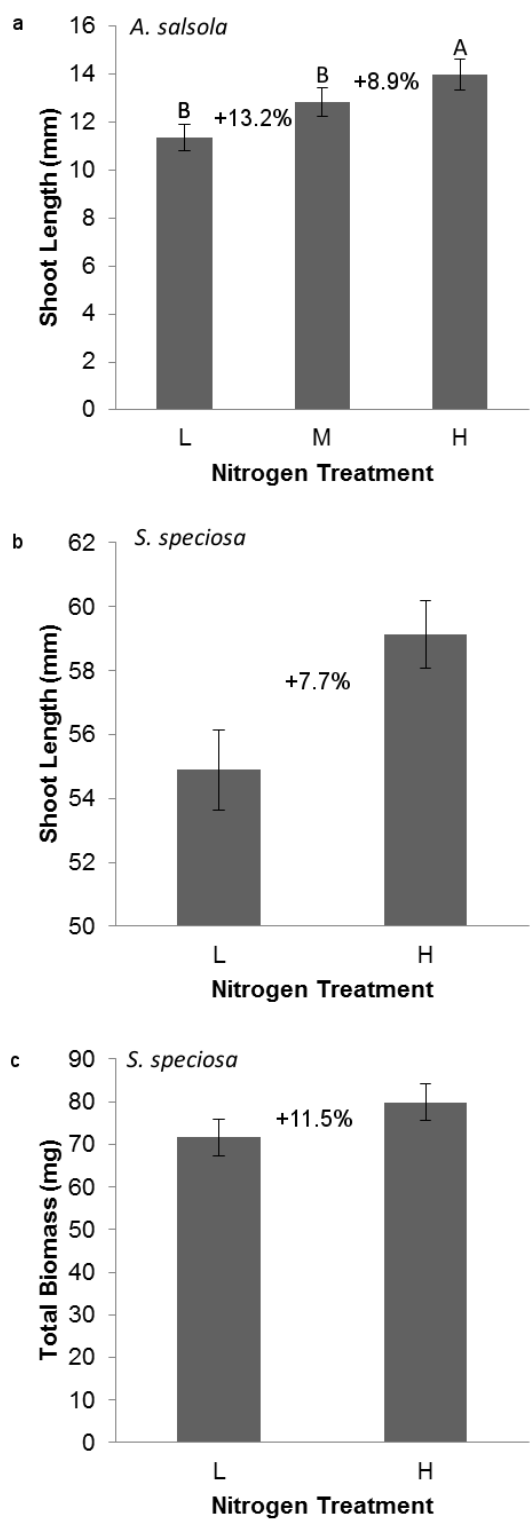


Figure 11

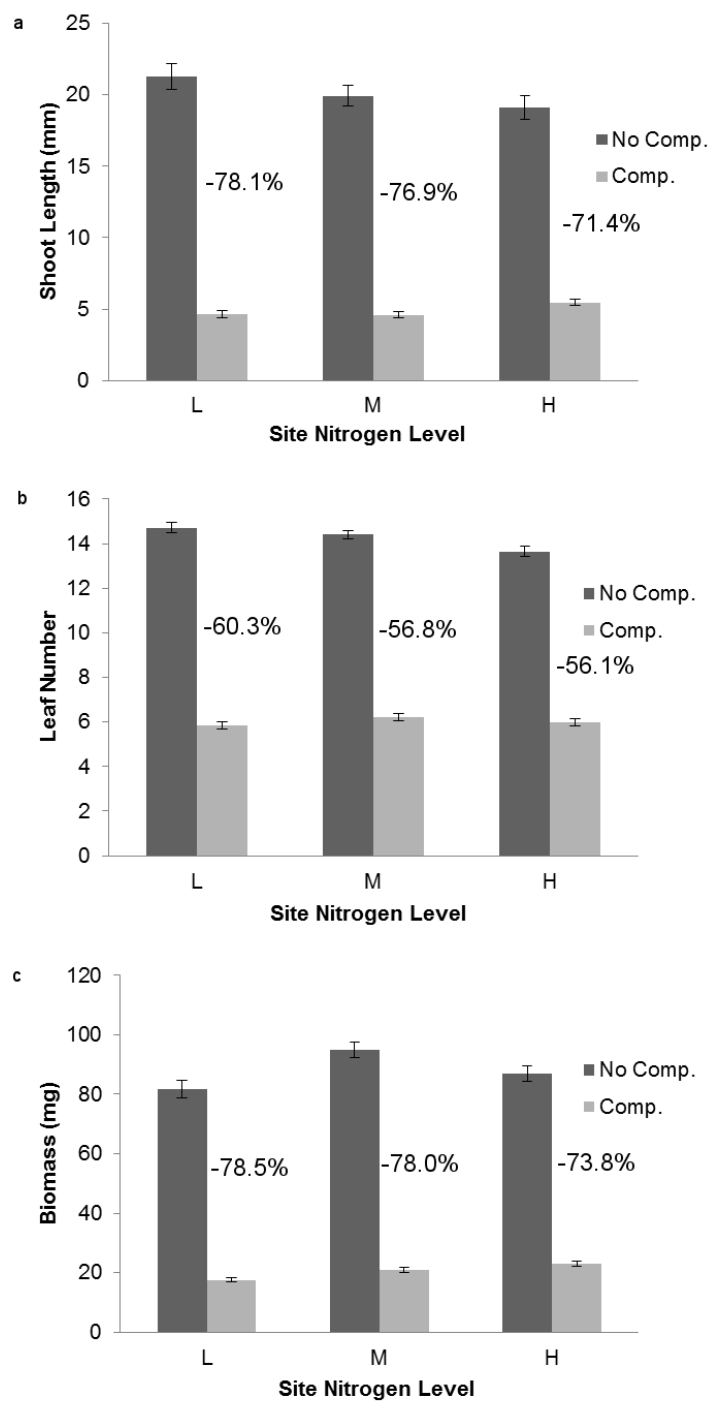


Figure 12

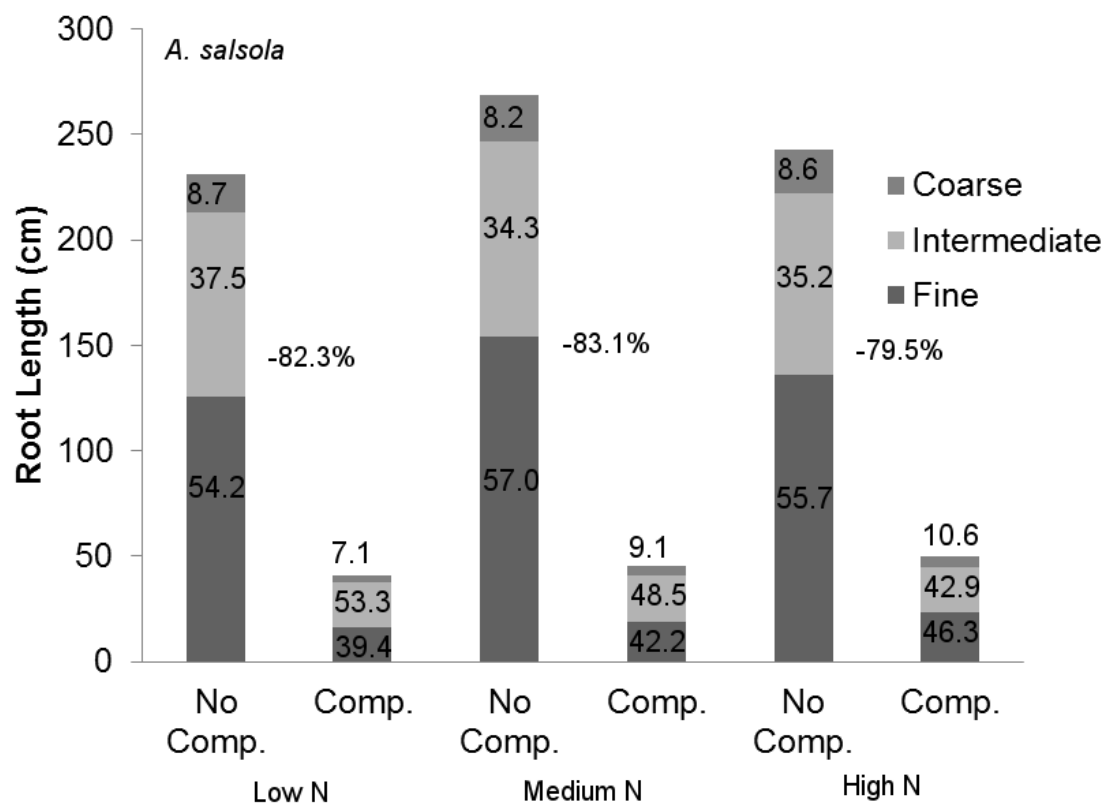


Figure 13

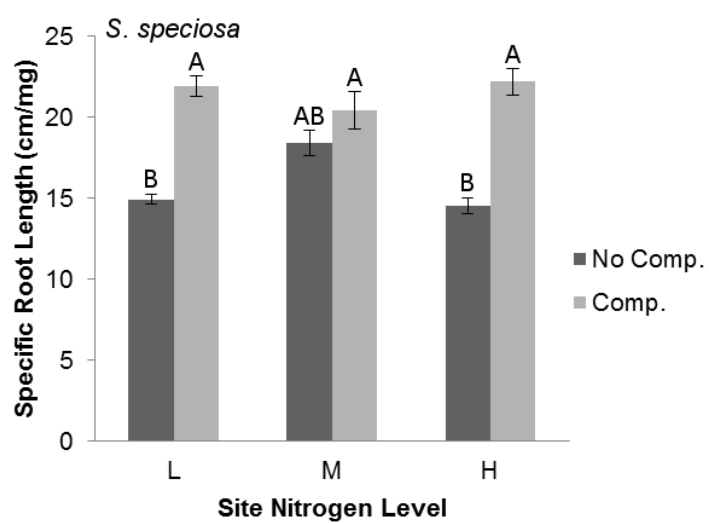
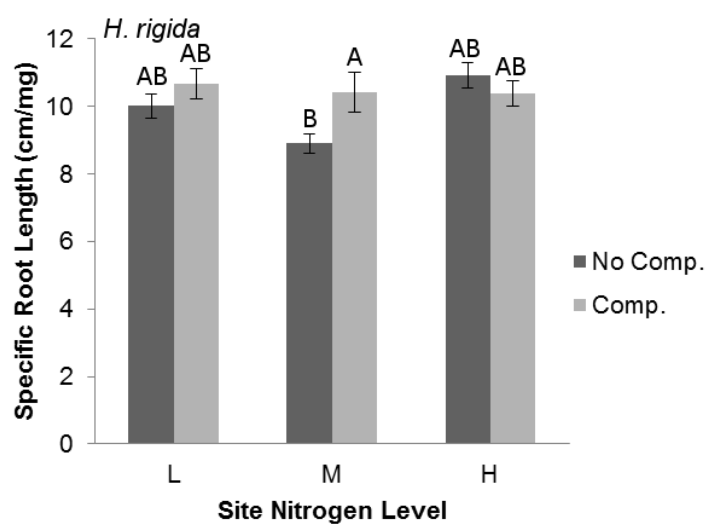


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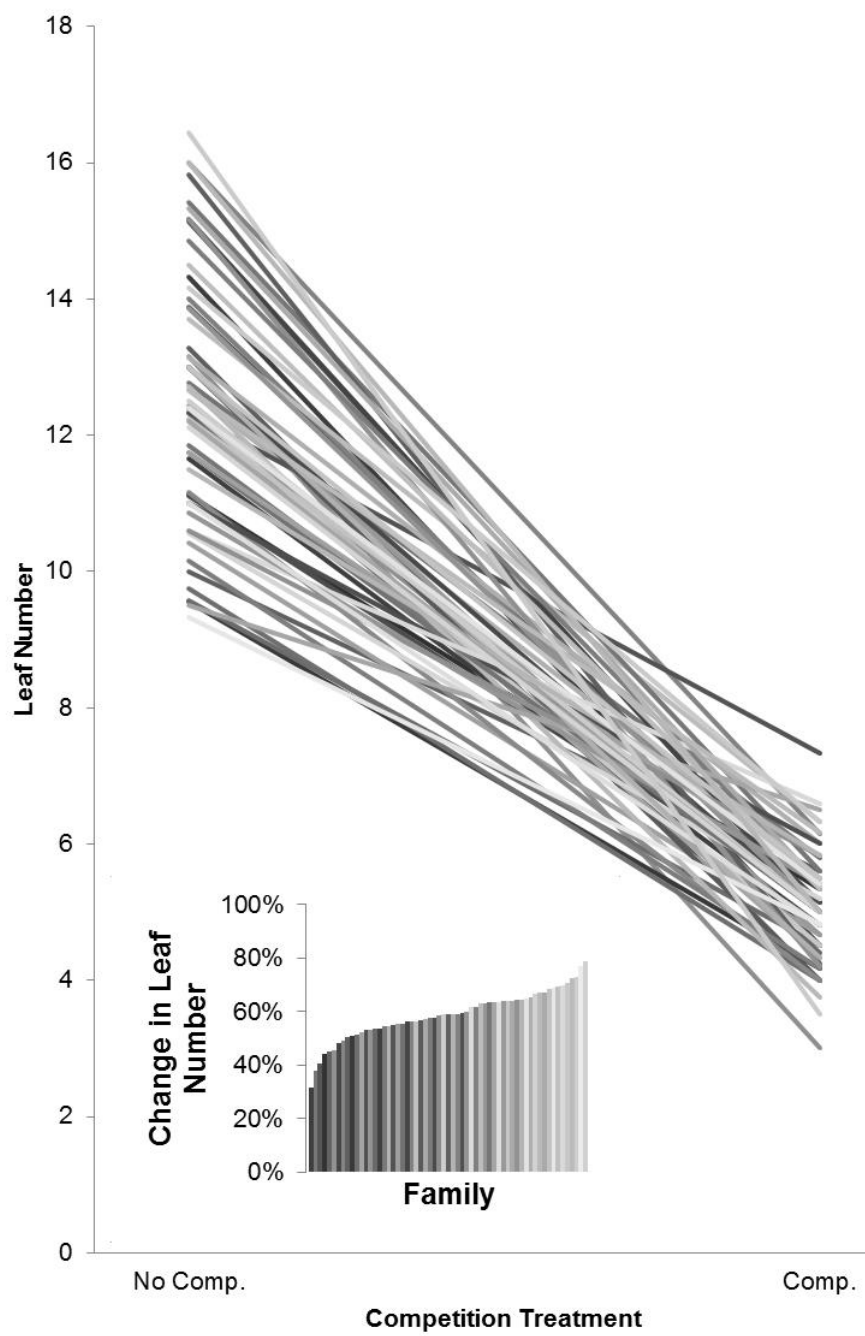


Figure 15

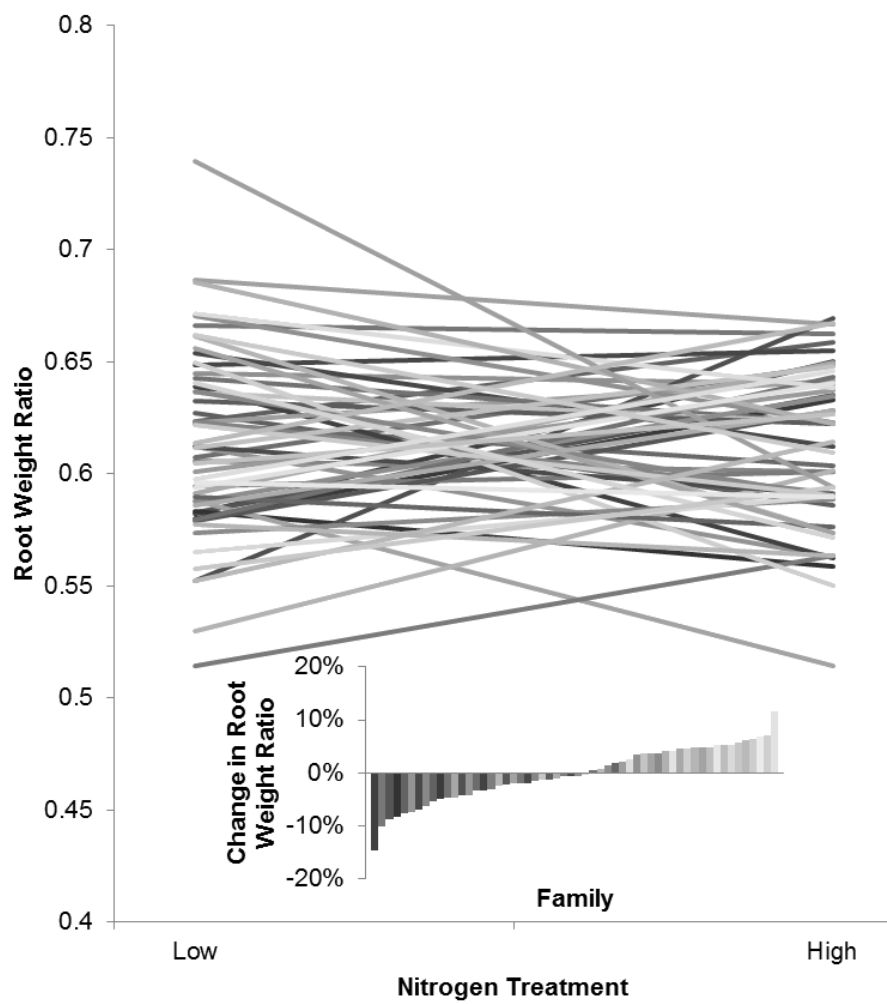


Figure 16

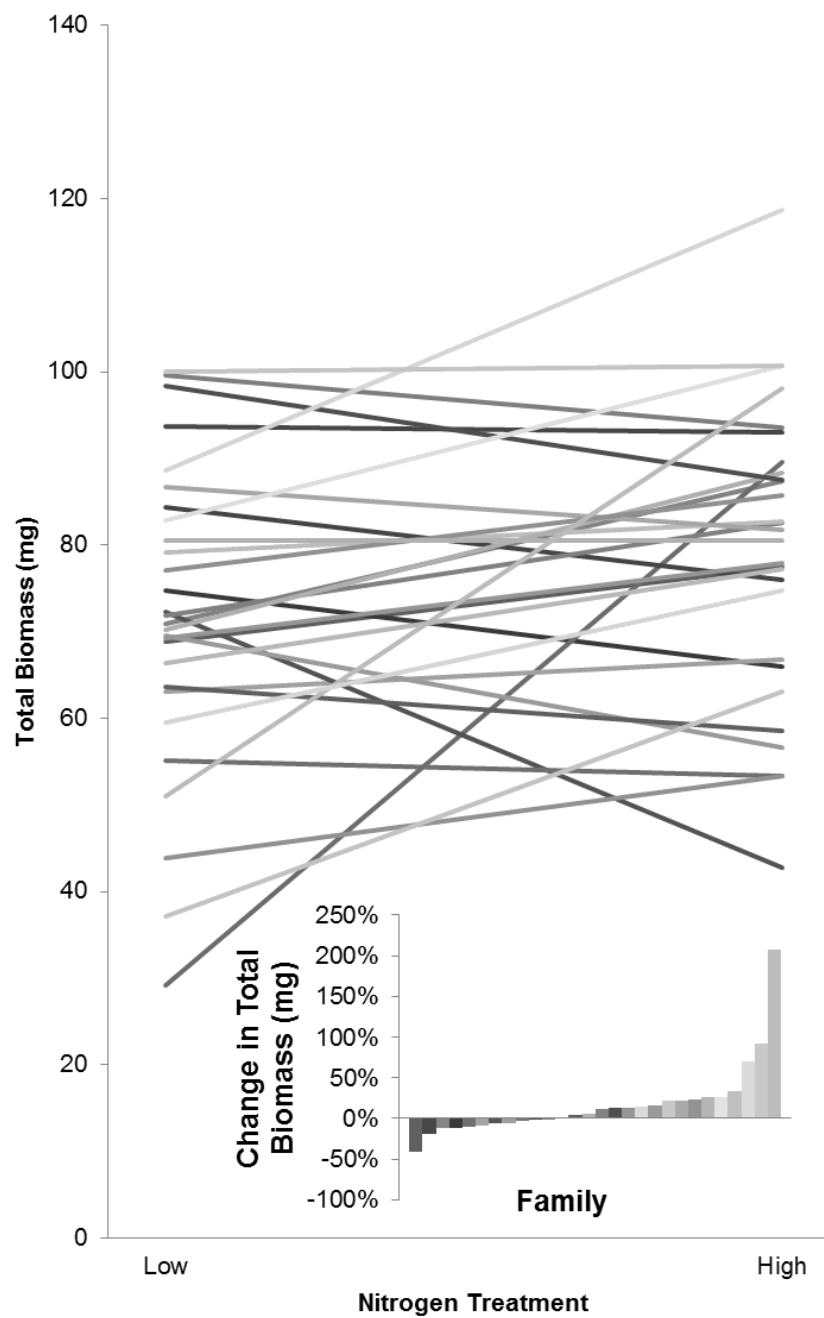


Figure 17

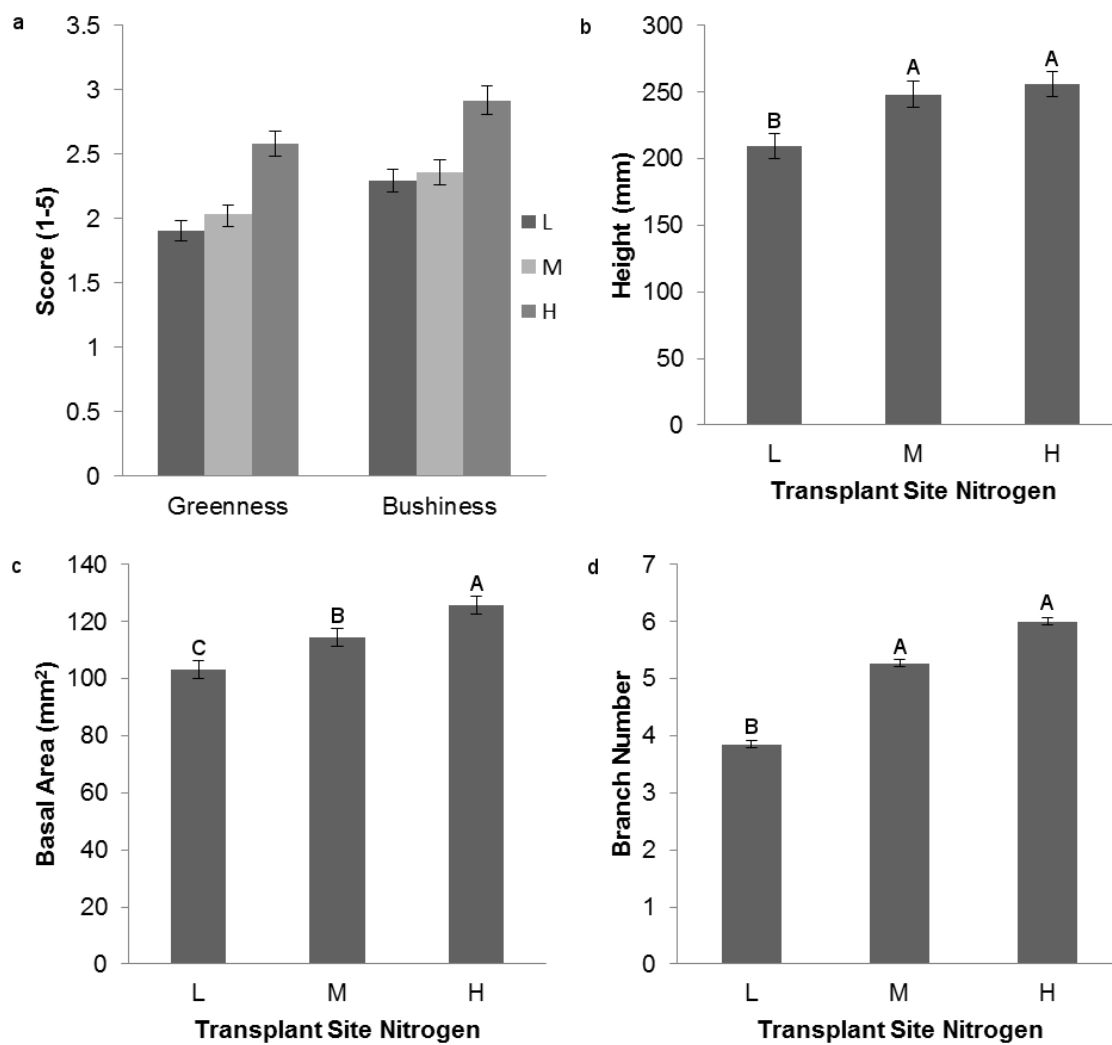
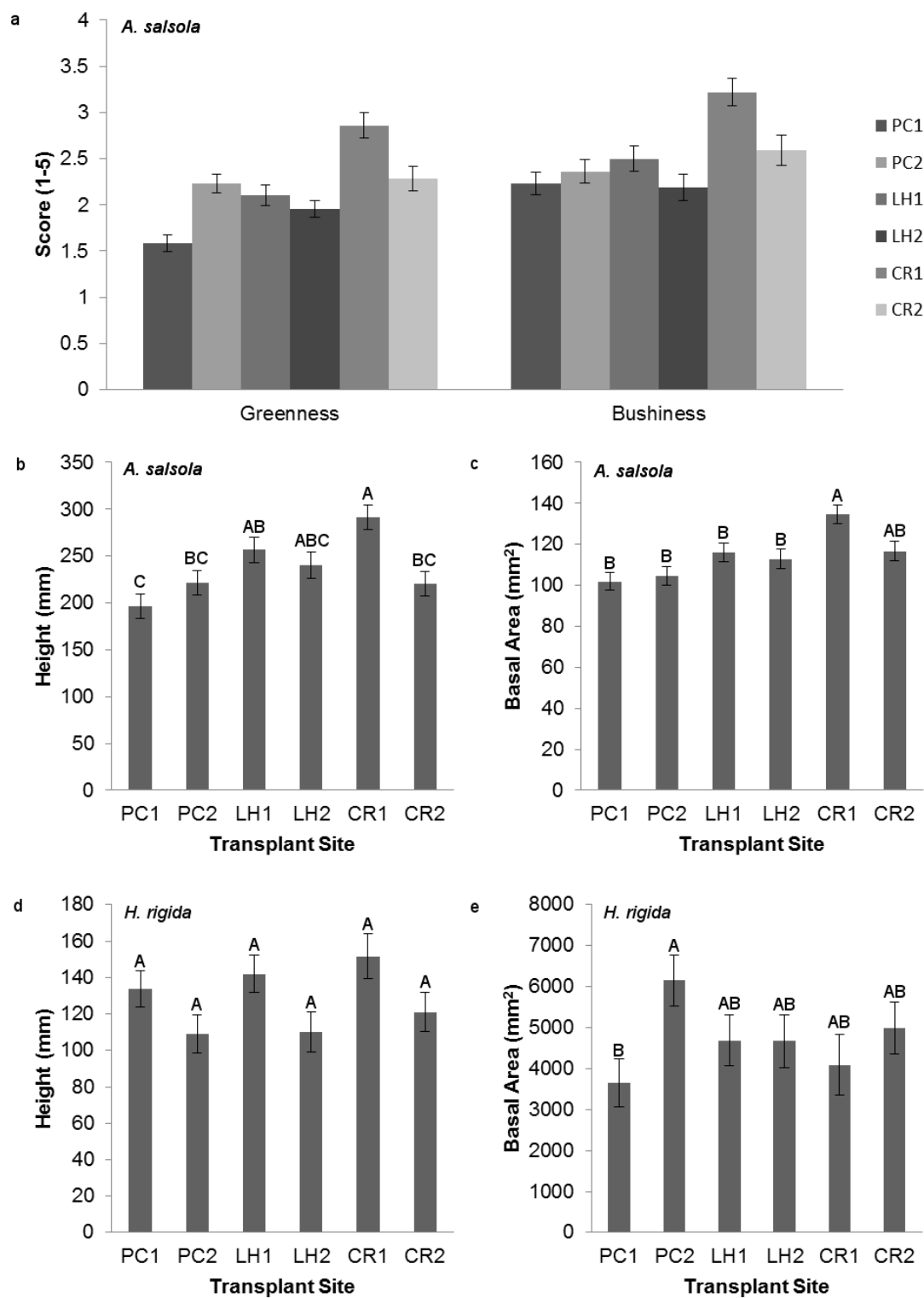


Figure 18



Appendix 1: Joshua Tree National Park plant communities across the nitrogen deposition gradient

Methods

To characterize the vegetation at each site and ask how vegetation changes across the nitrogen deposition gradient, we collected data on plant community composition at each reciprocal transplanting site in May 2014. We laid out three 50 m east/west transects at each site. All shrubs within two meters of each transect were identified to species and counted. Point-intercept data was collected each 20 cm along each transect, and vegetation encountered at each point was identified to species. To further characterize the grass and forb communities, 1 m² quadrats were centered at the 16.5 and 33.5 m marks along each transect for a total of 6 quadrats per site. We identified all species within the quadrats and estimated percent cover of each. Because we were especially interested in the density of *Bromus madritensis* across the nitrogen deposition gradient, we counted all *B. madritensis* stems in the northwest .25x.25 m corner of each quadrat.

We analyzed the three types of community data (point-intercept, shrubs, and quadrats) by functional group, first using principal components analysis to determine if sites differed in species composition and asking if N deposition level was obviously associated with any differences. Total species richness, exotic species richness, and native species richness in quadrats were analyzed using ANOVA with site nitrogen depositional status (N site), site (nested within N site), and transect (random and nested within site and N site) as factors. *B. madritensis* stem count data was analyzed using ANOVA, with N site, site (nested within N site), and transect (random and nested within site and N site) as factors.

Results

Using the point-intercept data, four clusters of similar communities emerged (Fig. 1), with the first two principal components together explaining 54.7% of the observed variation. The high N deposition site CR2 had a much greater prevalence of litter and native annual forbs than did any other site. CR1 had the second-greatest abundance of litter whereas the low N deposition site PC2 had a greater abundance of native bunchgrasses, perennial forbs, and invasive annual forbs than other sites (Fig. 1). The low N site PC1 and the medium N site LH1 had higher invasive annual grass cover and shrub cover than did the other sites, and the high N site CR1 and the medium N site LH2 were clustered near the center of the ordination space.

Total species richness and native species richness both increased with N deposition, whereas exotic species richness decreased (N Site $F=10.9$, $df= 2,12$, $p=.002$; $F=24.9$, $df= 2,12$, $p<.0001$; $F=4.8$, $df= 2,12$, $p=.029$ respectively) (Fig. 2a). The increase in native richness was driven by an increase in native annual forb richness, which increased from $1.17\pm.49$ at low deposition to $3.0\pm.44$ at medium and $5.08\pm.38$ at high N deposition. The decrease in exotic richness with increasing N was due to greater richness of invasive grasses at LH2 and PC1, the only sites that averaged more than one species. There were also significant differences between individual sites (Site, Total Richness $F=6.9$, $df= 3,12$, $p=.006$; Native $F=3.8$, $df= 3,12$, $p=.039$; Exotic $F=5.3$, $df= 3,12$, $p=.015$) (Fig. 2b). These differences were primarily driven by differences between the two medium deposition sites, LH1 and LH2. LH2 had three times the richness of native annual forbs such as *Mentzelia albicaulis*, *Gilia* sp., and *Stephanomeria exigua* while LH1 had greater invasive forb richness.

Principal components analysis revealed that two sites had shrub communities that were different from the rest of the sites; together the first two principal components explained 47.2% of the observed variation. PC1, a low deposition site, was characterized by greater abundance of *Coleogyne ramossissima*, *Larrea tridentata*, *Krameria grayi*, and was the only site with the cacti

Opuntia pulchella and *O. echinocarpa* (Fig. 3). LH2 transects contained more *Yucca schidigera*, *Acacia greggii*, *Chrysothamnus* sp., *Ephedra viridis*, and *Salazaria mexicana* than did the other sites, which were characterized by greater abundance of *Lyceum cooperi*, *Mirabilis bigelovii*, and *Gutierrezia sarothrae*. There was not obvious differentiation among sites with similar N deposition levels.

Bromus madritensis density did not vary with N site ($F=1.01$, $df=2,12$, $p=.39$), but did vary significantly among sites ($F=8.13$, $df=3,12$, $p=.0032$). PC1, a low deposition site, had the highest mean density, but with a large amount of variation between quadrats (Fig. 4). *B. madritensis* density at LH2 was significantly higher than at either LH1 or CR1.

Discussion

For all three methods of plant community characterization, individual site differences other than nitrogen deposition seemed to be a greater driver of plant communities in 2014. The one exception was the clustering of high N deposition sites on the basis of high levels of litter and annual native forbs. Communities at individual sites were often closer in composition to sites with different levels of deposition than to their sister N site, despite the close geographic proximity. Sites with higher nitrogen deposition did not show greater levels of invasion by either annual grasses or forbs in 2014, contrary to expectation (e.g. Tilman 1987, Huenneke et al. 1990, Tyler et al. 2007), but rather had higher levels of annual forbs. Plant cover in general was higher with increasing N deposition, but N deposition alone did not drive the community changes we saw. These differences may vary by year, and according to PRISM data, rainfall over the period from 2010-2014 ranged between 85.6 and 95.4 mm precipitation annually, roughly half the 30 year average of 188.2 mm annually, and surveys in wetter years may have different results.

In this latest dry year in a four year dry period, we also did not find evidence of a gradient in annual grass, specifically *B. madritensis*, invasion coincident with the N deposition gradient, although JTNP officials have observed this gradient in the past (J. Hoines, pers. comm.). The higher levels of litter characteristic of annual grass invasion and associated with sites of greater N deposition suggest that such a gradient may have been present in past years, however. Schneider and Allen (2012) found that sites in Joshua Tree NP that experienced higher atmospheric nitrogen deposition also had greater presence of invasive annual seeds in the seed bank and that artificial fertilization of plots also enhanced the numbers of invasive seeds in the seed bank. Annual density in general, both native and invasive, was not high in the year we sampled, likely a product of not just the one year of drought, but due to several low precipitation years in a row (Beatley 1969). Specifically, rainfall was 92.6mm, 85.6mm, and 93.3 in the period from 2011 to 2013, the years before this survey, well below the 30 year average of 188.1mm. *B. madritensis* density was only moderately high in one quadrat, perhaps representing a favorable microsite, but otherwise was low across all sites compared to elsewhere in the Mojave (Brooks 1999). Since water availability is the greatest determining factor for success of annual plants in arid environments (Beatley 1969), our ability to detect a gradient in invasion might have been hindered by the lack of precipitation. A more extensive survey in a wet year more information on the link between N deposition and invasion across the deposition gradient.

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Tyler, A. C., J. G. Lambrinos, and E. D. Grosholz. 2007. Nitrogen inputs promote the spread of an invasive marsh grass. *Ecological Applications* 17:1886–1898.

Figure Captions

Figure 1: PCA of transect point intersect community data, labeled by site and merged into functional groups.

Figure 2: Total plant species richness in quadrats as well as exotic and native plant richness by a) N site and b) site. Columns within a richness category (total, exotic, or native) indicate similar values according to Tukey's HSD.

Figure 3: PCA of transect shrub community data, labeled by site.

Figure 4: Mean \pm SE of *B madritensis* density at each transplant site.

Figure 1

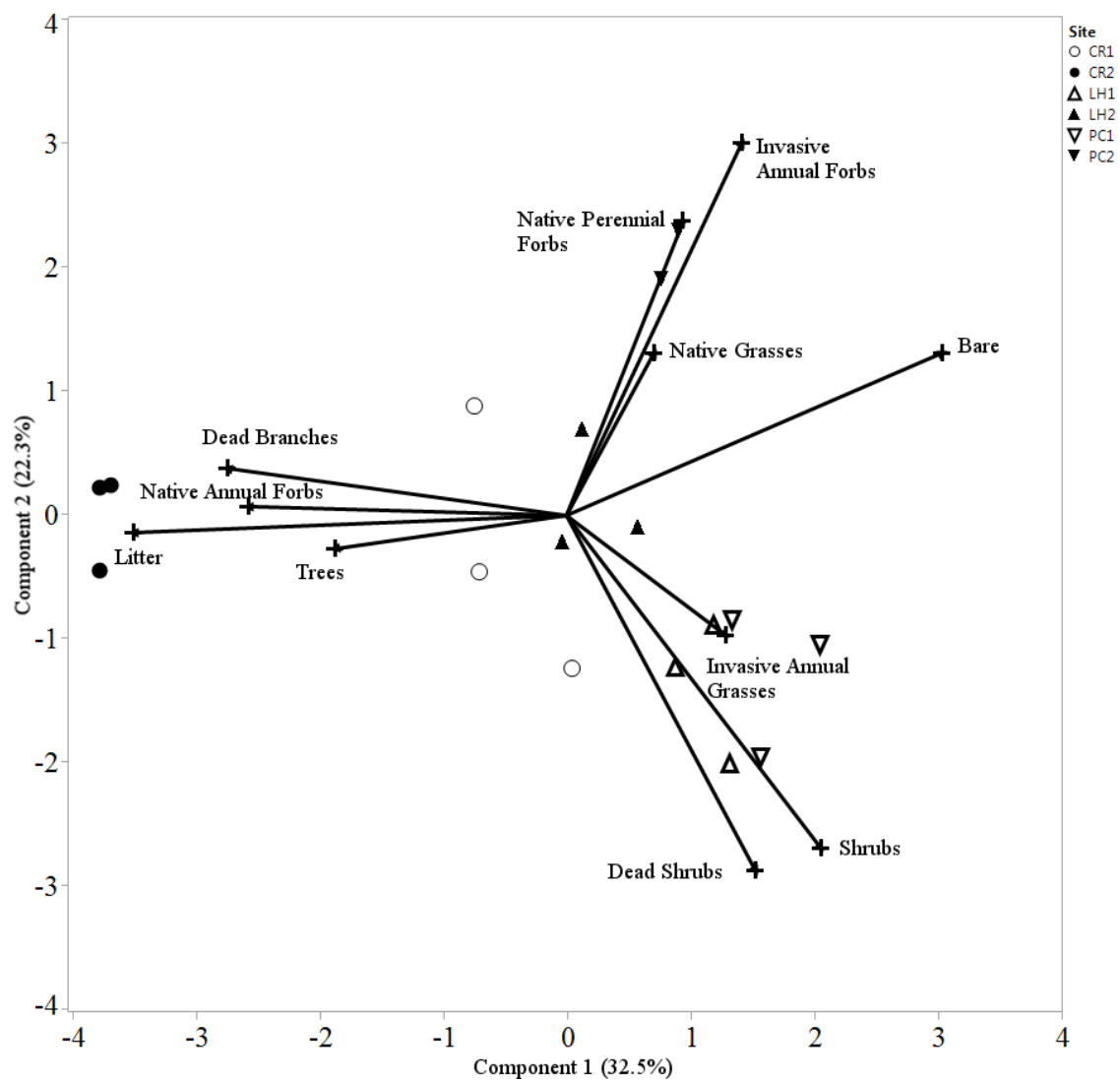


Figure 2

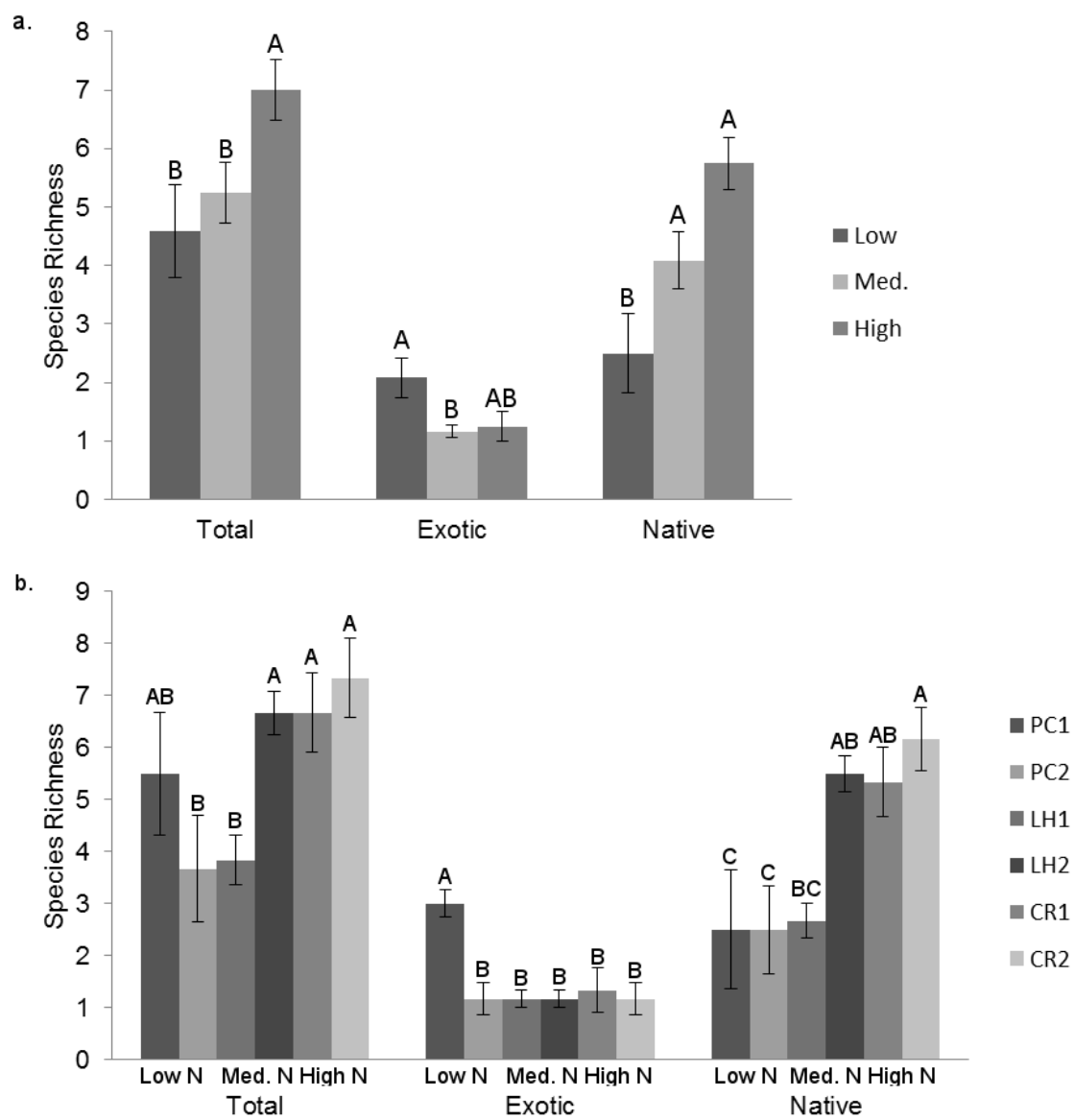


Figure 3

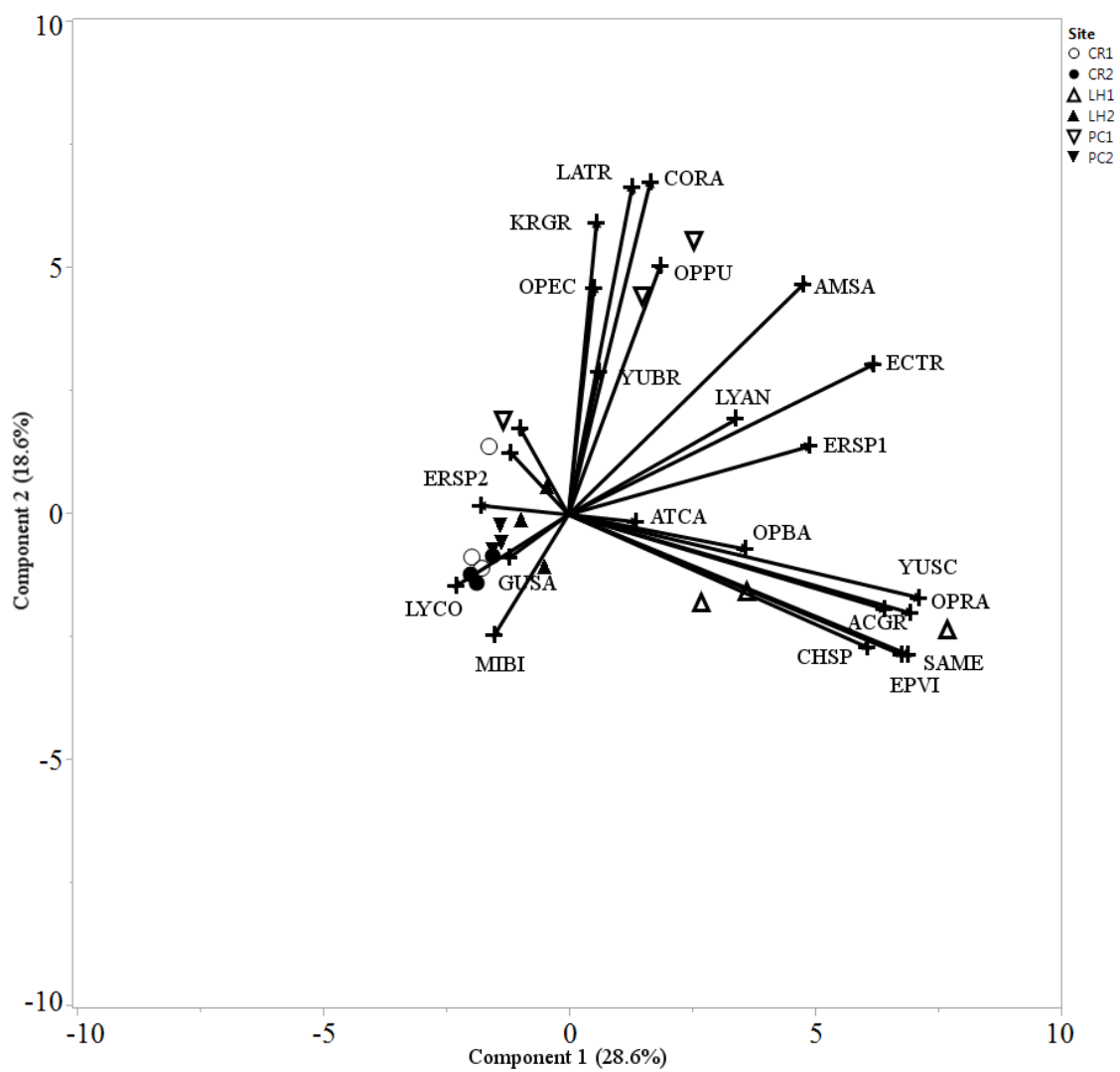
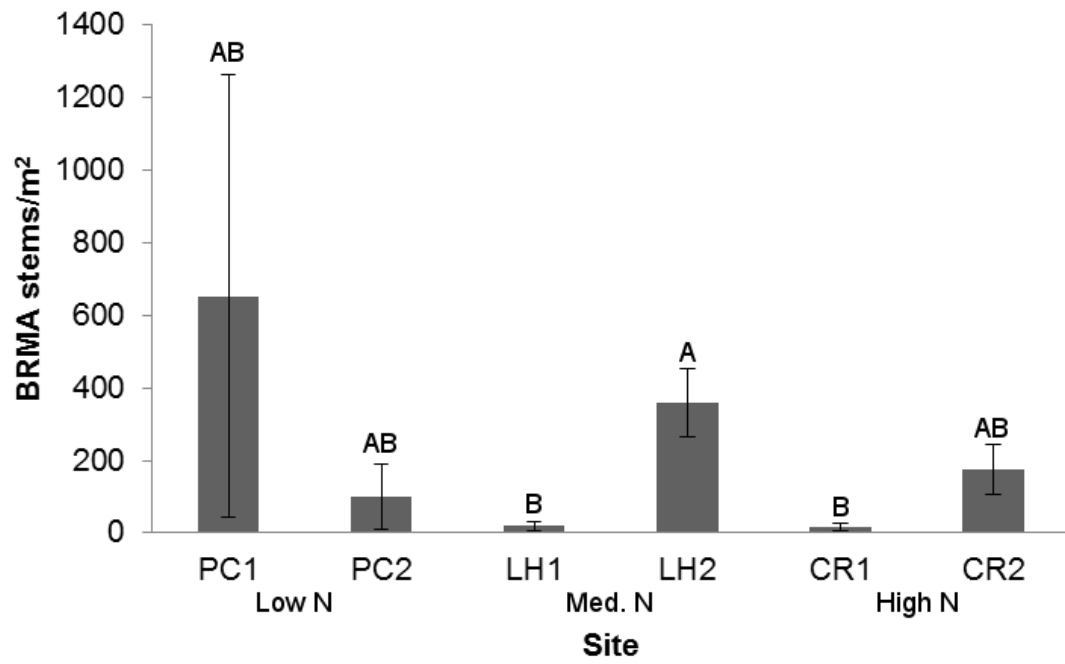


Figure 4



Appendix 2: Herbivory

Increased nitrogen deposition can indirectly increase herbivory on native plant species, as herbivores respond to changes in plant nitrogen content and overall abundance (Blumenthal 2005). This increase in herbivore pressure can act as a potential selective agent on native plant populations, which can be especially important when natives must also compete with exotic invaders, which sometimes receive less herbivory than native species (Throop and Lerdau 2004, Leger et al. 2007). We collected data on herbivory on our three focal species, *Ambrosia salsola*, *Hilaria rigida*, and *Stipa speciosa*, in order to determine if there were differences in herbivory across the nitrogen gradient and across sites, and if any differences were consistent with the hypothesis that increased nitrogen deposition can cause increased pressure from herbivory.

Methods

Plants sampled for this study were the same as those sampled for plant size distribution described in the main manuscript. Bunchgrasses herbivory primarily took the form of cut and removed stems, so the area of the herbivorized stems was measured, along with the total area of the plant, allowing us to calculate a percent of plant area herbivorized. Each plant was also given a herbivory score ranging from one, plants showing no herbivory, to five, plants very highly herbivorized. Because most plants were not herbivorized, percent herbivory data were analyzed using nonparametric Wilcoxon tests. Herbivory score was analyzed using logistic regression. Both analyses used N site and site as factors.

Results

Only the bunchgrasses displayed evidence of herbivory, as no herbivory damage was observed on any *A. salsola* plants across all sites. Both bunchgrass species showed differences between N sites in percent of each plant herbivorized (N site; *S. speciosa* $\chi^2=7.95$, $df=2$, $p=.0188$;

H. rigida $\chi^2=36.22$, $df=2$, $p<.0001$). For both grass species plants experienced more herbivory at medium deposition sites (Fig. 1). This was corroborated by herbivory scores, which showed more plants with higher scores at medium deposition sites, especially for *H. rigida* (Fig. 2). Both grass species also differed in herbivory across individual sites (site; *S. speciosa* $\chi^2=22.80$, $df=2$, $p=.0004$; *H. rigida* $\chi^2=57.15$, $df=4$, $p<.0001$). For *S. speciosa*, there were differences across high and medium N sites, but not at low deposition sites (Fig. 3). Herbivory was more constant across N sites for *H. rigida*, which suffered no damage at high N deposition, and similar levels of herbivory across the medium deposition sites and the one low deposition site with adequate representation. Herbivory scores exhibit the same patterns, with a greater frequency of plants damaged to a higher degree at CR1, LH1, and LH2 for *S. speciosa*, and at LH1 and to a lesser extent LH2 and PC1 in *H. rigida* (Fig. 4).

Discussion

The patterns of differences between sites and N sites were not consistent with the hypothesis that increased deposition would increase herbivory pressure. The signs of herbivory, which consisted of cut and removed stems, were consistent with small rodent herbivory, and little to no evidence of insect damage was found on these plants. The pattern of higher herbivory at the medium N Lost Horse sites followed by the low N Pine City sites is more likely due to differences in rodent shelter between sites than to effects of nitrogen deposition (D. Hibbard, pers. obs). Sites LH1 and LH2 are situated near large rock outcrops that could provide good habitat for granivorous rodents, and PC1 and 2 are situated in rocky areas as well, though not to the same degree as LH1 and 2. In contrast, both the high deposition sites, CR1 and CR2, are relatively open, with less obvious structure suitable for rodent habitat.

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Figure Captions

Figure 1: Mean \pm SE of the percent of each plant herbivorized for each bunchgrass species grouped by N deposition level. For *H. rigida* no herbivory was observed at high N deposition, and herbivory at low deposition is represented by one site, PC1.

Figure 2: Frequency distributions for each herbivory score class (1-5) grouped by N deposition level for A) *S. speciosa* and B) *H. rigida* with 1 meaning no herbivory and 5 indicating very high levels of herbivory.

Figure 3: Mean \pm SE of the percent of each plant herbivorized for each bunchgrass species grouped by site. For *H. rigida* no herbivory was observed at CR1 and CR2, and the population was not large enough to collect an adequate sample from PC2.

Figure 4: Frequency distributions for each herbivory score class (1-5) grouped by site for A) *S. speciosa* and B) *H. rigida* with 1 meaning no herbivory and 5 indicating very high levels of herbivory.

Figure 1

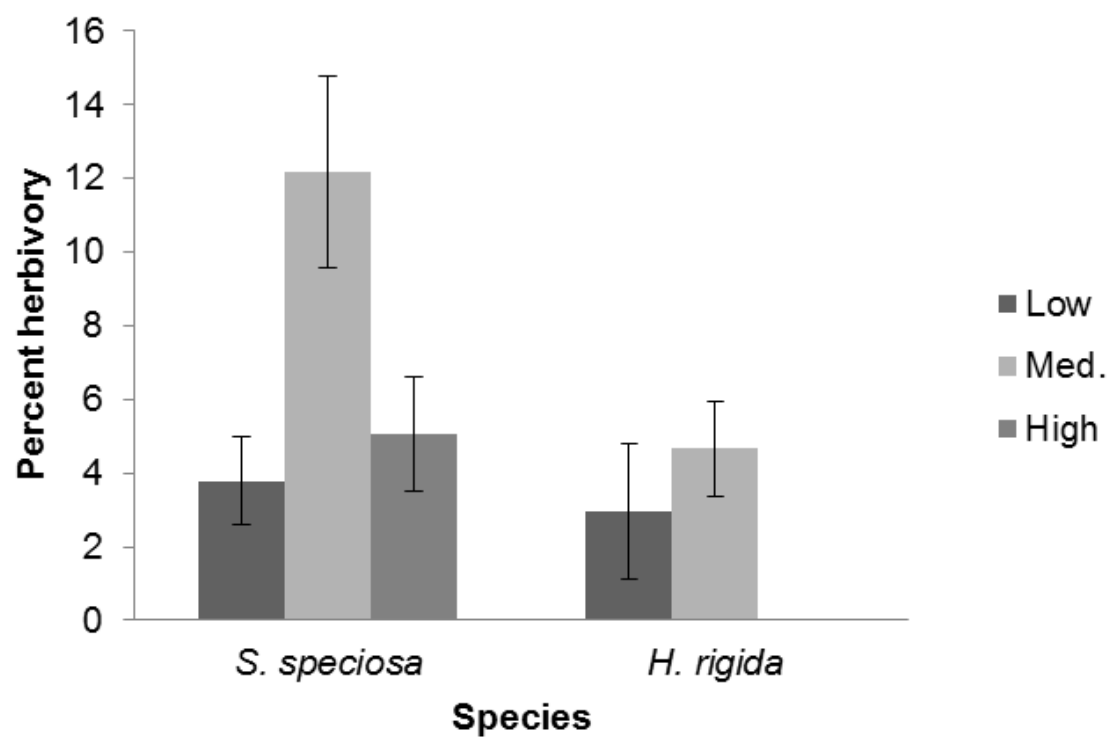


Figure 2

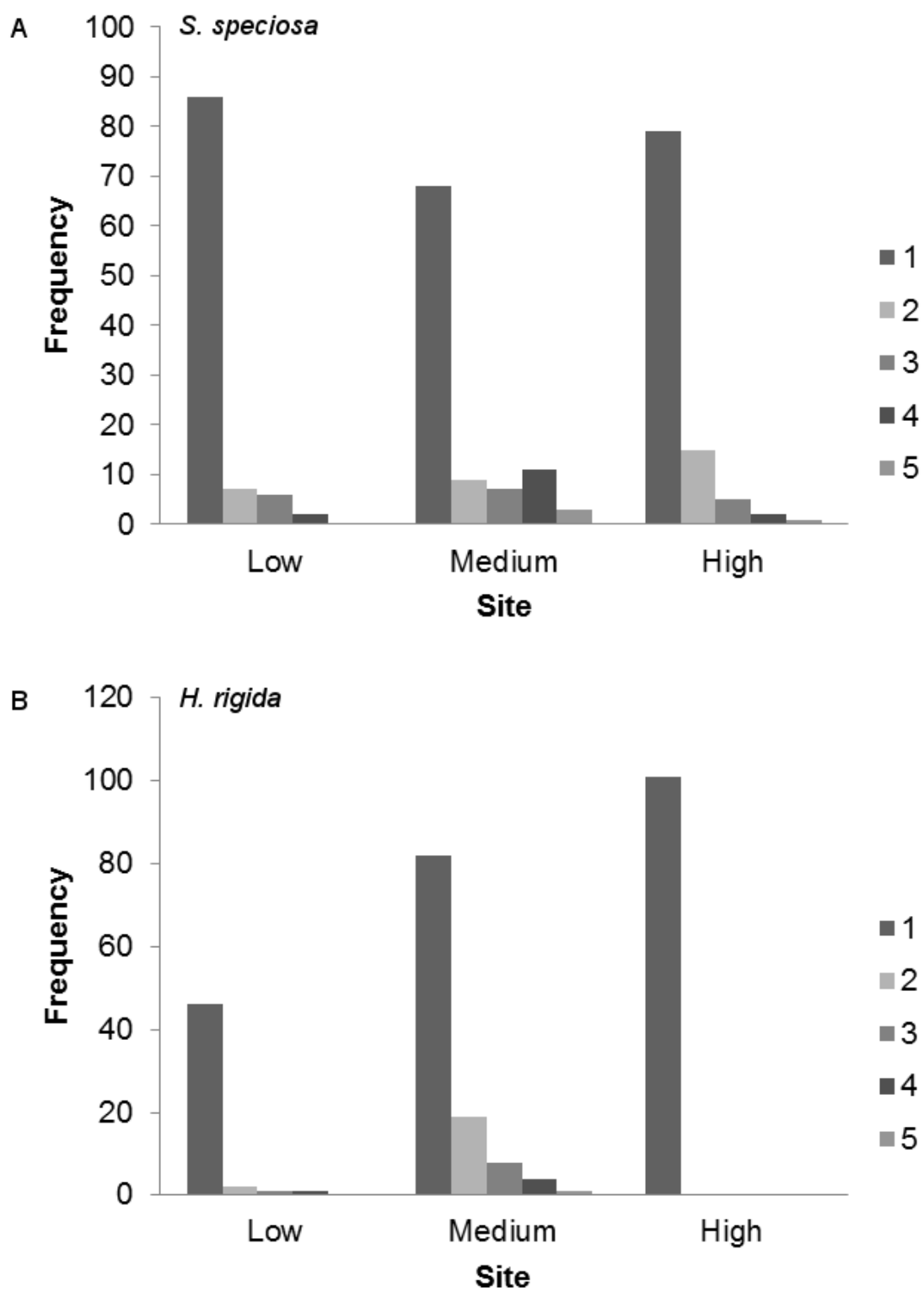


Figure 3

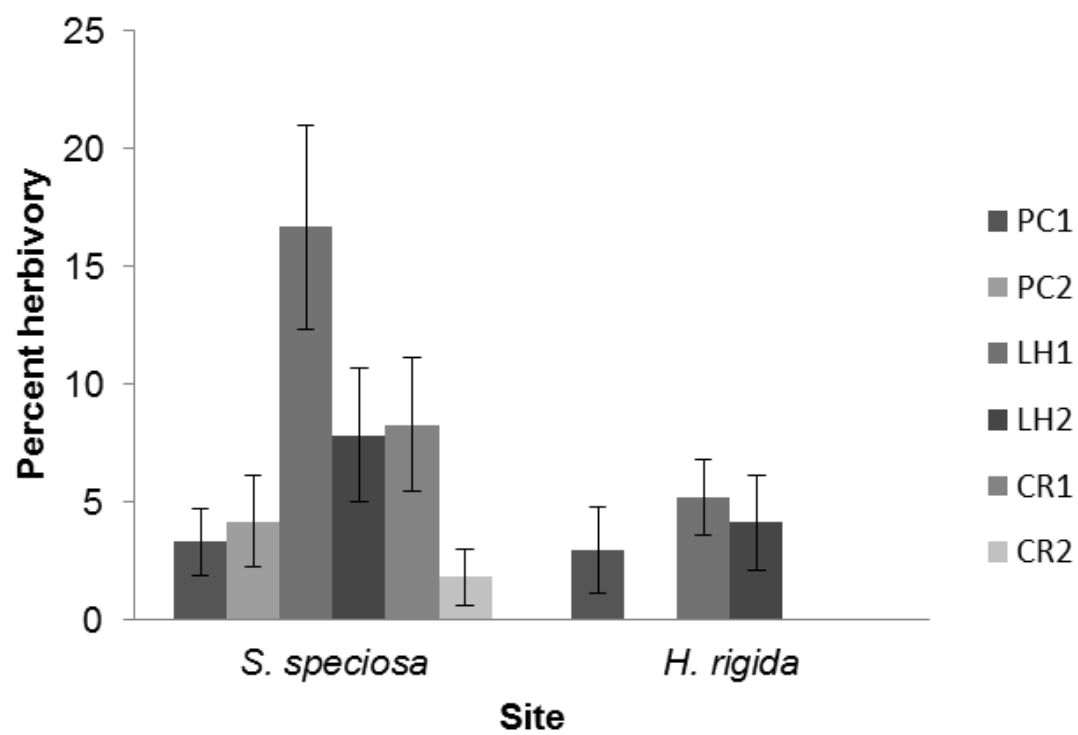
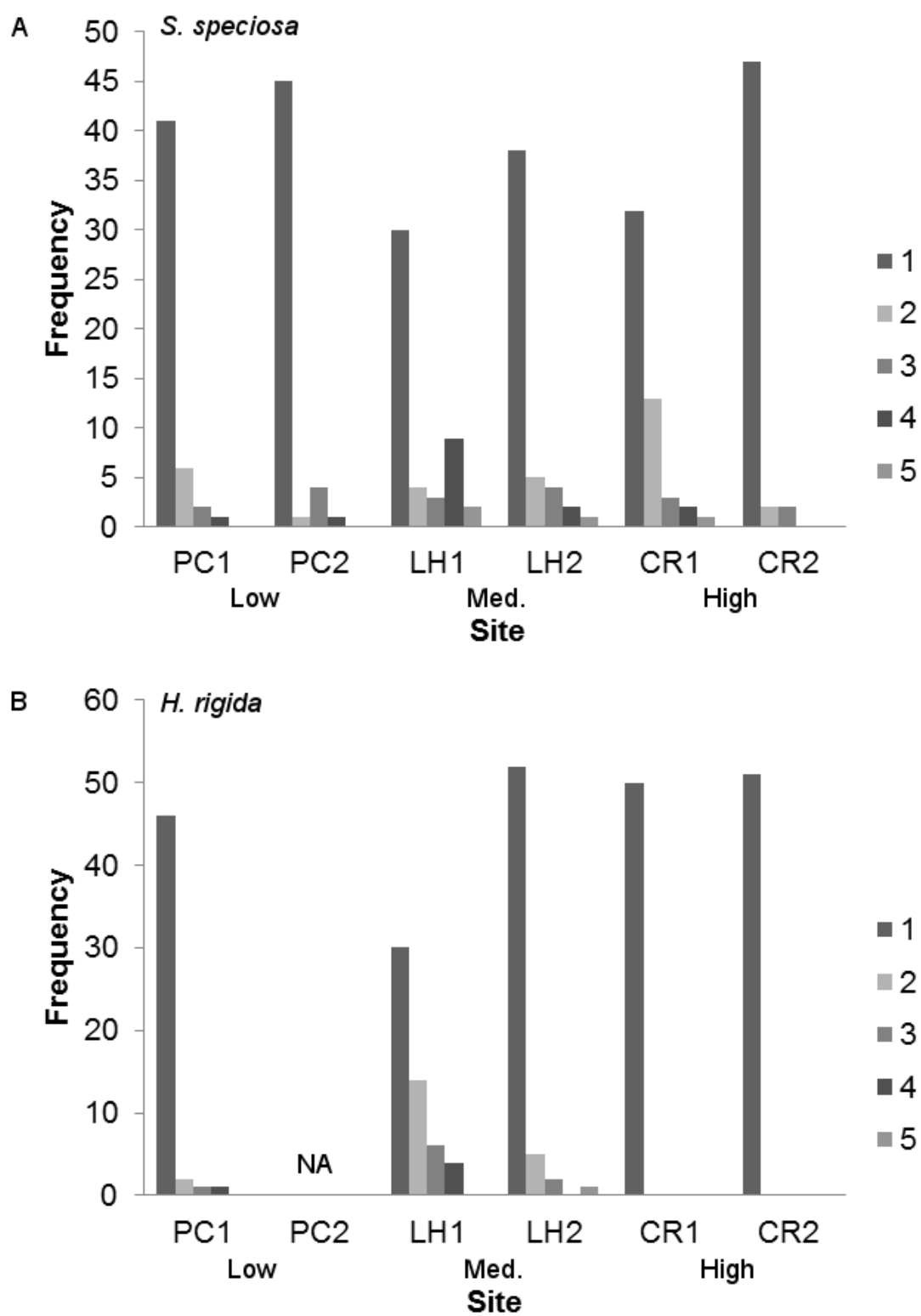


Figure 4



Appendix 3: Table of means from greenhouse experiment

Table 1: Means and standard errors for *Ambrosia salsola*, *Hilaria rigida*, and *Stipa speciosa* grouped by N site, site, competition, N treatment, N site X N treatment, N treatment X competition, and N site X competition X N treatment.

	N Site		
	L	M	H
<i>A. salsola</i>			
n	415	356	366
Shoot Length (mm)	12.6±0.5	13.2±0.7	12.6±0.6
Leaf number	10.5±0.2	10.4±0.3	10±0.2
Biomass (mg)	59±2	50±2	56±2
RWR	0.61±0.004	0.61±0.006	0.61±0.004
SRL (cm/mg)	4.51±0.09	4.78±0.11	4.5±0.1
Fine Root Proportion	0.5±0.007	0.47±0.009	0.51±0.007
Intermediate Root Proportion	0.41±0.007	0.45±0.009	0.39±0.007
Coarse Root Proportion	0.09±0.003	0.07±0.003	0.1±0.004
<i>H. rigida</i>			
n	174	240	312
Shoot Length (mm)	78.3±2.9	85.1±2.7	77.7±2.1
Leaf number	9.6±0.4	9.3±0.3	9.4±0.3
Biomass (mg)	91.1±5.3	93.6±4.9	89.8±3.9
RWR	0.52±0.007	0.52±0.006	0.54±0.006
SRL (cm/mg)	10.2±0.3	9.7±0.3	10.7±0.3
Fine Root Proportion	0.65±0.008	0.62±0.007	0.64±0.006
Intermediate Root Proportion	0.26±0.008	0.28±0.007	0.28±0.006
Coarse Root Proportion	0.08±0.004	0.1±0.004	0.08±0.003
<i>S. speciosa</i>			
n	220	45	97
Shoot Length (mm)	58.5±1	56.4±2.7	54.3±1.6
Leaf number	12.7±0.6	12.2±1.1	13.3±1
Biomass (mg)	77.4±4.1	71.1±7.4	75±6.1
RWR	0.54±0.006	0.52±0.011	0.54±0.01
SRL (cm/mg)	18.3±0.4	19.2±0.7	18.2±0.6
Fine Root Proportion	0.75±0.005	0.74±0.011	0.77±0.008
Intermediate Root Proportion	0.2±0.004	0.21±0.007	0.19±0.006
Coarse Root Proportion	0.05±0.002	0.05±0.005	0.04±0.003

	Site					
	PC1	PC2	LH1	LH2	CR1	CR2
<i>A. salsola</i>						
n	238	177	198	158	212	154
Shoot Length (mm)	12.6±0.7	12.6±0.9	14.2±0.9	11.9±0.9	10.5±0.6	15.4±1.1
Leaf number	10.5±0.3	10.5±0.4	10.5±0.4	10.2±0.4	9.9±0.3	10.1±0.4
Biomass (mg)	62±3	56±3	51±3	49±3	59±3	52±3
RWR	0.62±0.005	0.6±0.007	0.62±0.007	0.6±0.009	0.62±0.005	0.58±0.006
SRL (cm/mg)	4.32±0.11	4.75±0.13	4.97±0.17	4.54±0.13	4.31±0.11	4.77±0.18
Fine Root Proportion	0.51±0.009	0.49±0.012	0.47±0.012	0.47±0.012	0.51±0.008	0.52±0.012
Intermediate Root Proportion	0.4±0.008	0.42±0.011	0.46±0.012	0.45±0.013	0.38±0.008	0.4±0.012
Coarse Root Proportion	0.09±0.004	0.08±0.004	0.07±0.003	0.08±0.005	0.11±0.006	0.07±0.004
<i>H. rigida</i>						
n	174	-	160	80	185	127
Shoot Length (mm)	78.3±2.9	-	86.9±3.4	81.6±4.6	80.9±2.9	72.9±3.1
Leaf number	9.6±0.4	-	9.3±0.4	9.1±0.5	9.2±0.4	9.6±0.4
Biomass (mg)	91.1±5.3	-	96.3±6.1	88.3±8.2	95.7±5.4	81.1±5.6
RWR	0.52±0.007	-	0.52±0.007	0.51±0.013	0.56±0.006	0.5±0.01
SRL (cm/mg)	10.2±0.3	-	9.5±0.3	10.1±0.4	10±0.3	11.7±0.5
Fine Root Proportion	0.65±0.008	-	0.62±0.009	0.63±0.014	0.65±0.008	0.64±0.011
Intermediate Root Proportion	0.26±0.008	-	0.28±0.008	0.27±0.013	0.27±0.008	0.29±0.01
Coarse Root Proportion	0.08±0.004	-	0.1±0.005	0.09±0.006	0.08±0.004	0.07±0.004
<i>S. speciosa</i>						
n	103	117	27	18	66	31
Shoot Length (mm)	57.6±1.6	59.3±1.3	55.6±3.5	57.7±4.4	53.8±1.6	55.4±3.6
Leaf number	12.5±0.8	12.9±0.8	12.5±1.4	11.8±1.6	13.5±1.2	12.8±1.7
Biomass (mg)	78.2±6.1	76.7±5.5	71.3±9.5	70.8±12.2	75.3±7.4	74.4±10.8
RWR	0.54±0.009	0.55±0.009	0.52±0.013	0.52±0.019	0.54±0.01	0.53±0.021
SRL (cm/mg)	17.8±0.6	18.7±0.6	18.5±0.8	20.2±1.1	17.8±0.7	19±1.2
Fine Root Proportion	0.75±0.009	0.75±0.006	0.73±0.016	0.74±0.014	0.76±0.01	0.79±0.014
Intermediate Root Proportion	0.2±0.006	0.2±0.005	0.21±0.01	0.21±0.01	0.2±0.007	0.17±0.01
Coarse Root Proportion	0.05±0.004	0.05±0.003	0.05±0.007	0.04±0.007	0.04±0.004	0.04±0.005

	Competition		N Treatment		
	No Comp.	Comp.	L	M	H
<i>A. salsola</i>					
n	586	551	367	387	383
Shoot Length (mm)	20.1±0.5	4.9±0.1	11.4±0.5	12.8±0.6	14±0.6
Leaf number	14.3±0.1	6±0.1	9.9±0.2	10.5±0.2	10.4±0.3
Biomass (mg)	88±2	20±0	48±2	58±2	59±2
RWR	0.6±0.003	0.61±0.004	0.61±0.005	0.61±0.005	0.61±0.005
SRL (cm/mg)	4.97±0.07	4.18±0.09	4.66±0.1	4.54±0.09	4.57±0.1
Fine Root Proportion	0.56±0.004	0.43±0.007	0.51±0.008	0.49±0.007	0.49±0.008
Intermediate Root Proportion	0.36±0.004	0.48±0.007	0.41±0.008	0.42±0.008	0.42±0.007
Coarse Root Proportion	0.08±0.002	0.09±0.003	0.08±0.003	0.09±0.003	0.09±0.004
<i>H. rigida</i>					
n	412	314	370	-	356
Shoot Length (mm)	104.5±1.7	48.5±0.8	79.1±2.1	-	81.5±2.1
Leaf number	12.7±0.2	5.1±0.1	9.4±0.3	-	9.4±0.2
Biomass (mg)	140.5±2.8	27.1±0.8	91±3.8	-	91.8±3.7
RWR	0.54±0.004	0.51±0.006	0.52±0.005	-	0.53±0.005
SRL (cm/mg)	10.1±0.2	10.5±0.3	10.3±0.2	-	10.2±0.3
Fine Root Proportion	0.66±0.004	0.6±0.007	0.64±0.006	-	0.63±0.006
Intermediate Root Proportion	0.22±0.003	0.34±0.006	0.27±0.006	-	0.27±0.005
Coarse Root Proportion	0.11±0.003	0.05±0.002	0.08±0.003	-	0.09±0.003
<i>S. speciosa</i>					
n	194	168	172	-	190
Shoot Length (mm)	65.7±1	47.2±0.8	54.9±1.3	-	59.1±1.1
Leaf number	20±0.3	4.5±0.1	12.4±0.6	-	13.1±0.6
Biomass (mg)	127.7±1.9	16.3±0.5	71.7±4.3	-	79.9±4.4
RWR	0.58±0.004	0.5±0.008	0.54±0.007	-	0.54±0.007
SRL (cm/mg)	15.3±0.3	21.8±0.5	18.5±0.5	-	18.2±0.4
Fine Root Proportion	0.71±0.004	0.81±0.005	0.76±0.006	-	0.75±0.005
Intermediate Root Proportion	0.22±0.003	0.17±0.005	0.2±0.004	-	0.2±0.004
Coarse Root Proportion	0.07±0.002	0.02±0.001	0.04±0.003	-	0.05±0.002

	N Site X Competition					
	L,No Comp.	L,Comp.	M,No Comp.	M,Comp.	H,No Comp.	H,Comp.
<i>A. salsola</i>						
n	216	199	181	175	189	177
Shoot Length (mm)	19.9±0.7	4.6±0.2	21.3±0.9	4.7±0.3	19.1±0.8	5.5±0.2
Leaf number	14.4±0.2	6.2±0.2	14.7±0.2	5.8±0.2	13.7±0.2	6±0.2
Biomass (mg)	95±3	21±1	82±3	18±1	87±3	23±1
RWR	0.61±0.005	0.61±0.007	0.6±0.007	0.62±0.009	0.6±0.005	0.61±0.007
SRL (cm/mg)	4.85±0.1	4.13±0.14	5.16±0.13	4.38±0.17	4.93±0.13	4.04±0.14
Fine Root Proportion	0.57±0.006	0.42±0.011	0.54±0.007	0.39±0.013	0.56±0.007	0.46±0.012
Intermediate Root Proportion	0.34±0.006	0.49±0.01	0.38±0.007	0.53±0.014	0.35±0.006	0.43±0.011
Coarse Root Proportion	0.08±0.003	0.09±0.005	0.08±0.003	0.07±0.005	0.09±0.003	0.11±0.007
<i>H. rigida</i>						
n	96	78	132	108	184	128
Shoot Length (mm)	102.9±3.3	48±1.7	112.2±3.3	52±1.2	99.9±2.3	45.8±1.3
Leaf number	13.2±0.4	5.2±0.2	12.6±0.3	5.2±0.2	12.4±0.3	5±0.1
Biomass (mg)	143.1±5.3	27.1±1.4	146.7±5.5	29.3±1.6	134.7±4.1	25.2±1.3
RWR	0.53±0.009	0.5±0.012	0.53±0.008	0.51±0.01	0.55±0.007	0.52±0.01
SRL (cm/mg)	10±0.4	10.4±0.6	8.9±0.3	10.7±0.4	10.9±0.4	10.4±0.4
Fine Root Proportion	0.68±0.009	0.61±0.014	0.64±0.008	0.6±0.013	0.67±0.006	0.6±0.012
Intermediate Root Proportion	0.2±0.006	0.33±0.012	0.23±0.005	0.34±0.011	0.23±0.005	0.34±0.01
Coarse Root Proportion	0.11±0.005	0.05±0.005	0.13±0.005	0.06±0.004	0.1±0.003	0.05±0.003
<i>S. speciosa</i>						
n	115	105	28	17	51	46
Shoot Length (mm)	67.7±1.1	48.5±1	65.5±2.9	41.5±2.5	61.5±2.1	46.3±1.7
Leaf number	20.1±0.4	4.6±0.2	17.2±0.7	4±0.2	21.2±0.8	4.5±0.1
Biomass (mg)	132.9±2	16.6±0.7	106.3±4.7	13.2±1	127.6±4.2	16.7±1
RWR	0.58±0.006	0.5±0.011	0.55±0.009	0.47±0.02	0.58±0.01	0.49±0.014
SRL (cm/mg)	14.9±0.3	21.9±0.7	18.4±0.8	20.4±1.2	14.5±0.5	22.2±0.8
Fine Root Proportion	0.7±0.005	0.8±0.007	0.71±0.012	0.79±0.015	0.71±0.008	0.83±0.009
Intermediate Root Proportion	0.22±0.003	0.17±0.006	0.22±0.007	0.19±0.014	0.22±0.006	0.16±0.008
Coarse Root Proportion	0.07±0.002	0.02±0.001	0.07±0.005	0.02±0.002	0.06±0.003	0.01±0.001

	N Site X N treatment								
<i>A. salsola</i>	L,L	L,M	L,H	M,L	M,M	M,H	H,L	H,M	H,H
n	138	142	135	111	126	119	118	119	129
Shoot Length (mm)	11.8±0.9	13±1	12.9±1	10.7±1	13.8±1.1	14.8±1.2	11.5±0.9	11.7±0.8	14.3±1.2
Leaf number	10.4±0.4	10.8±0.4	10.3±0.4	9.4±0.5	10.8±0.5	10.8±0.5	9.7±0.4	10±0.4	10.2±0.4
Biomass (mg)	55±4	62±4	61±4	41±3	52±4	57±4	48±3	60±4	59±4
RWR	0.62±0.008	0.6±0.008	0.61±0.006	0.62±0.011	0.61±0.009	0.6±0.01	0.6±0.007	0.62±0.007	0.6±0.008
SRL (cm/mg)	4.59±0.16	4.46±0.13	4.46±0.16	4.77±0.22	4.9±0.18	4.66±0.18	4.64±0.17	4.26±0.14	4.59±0.19
Fine Root Proportion	0.51±0.012	0.5±0.011	0.49±0.013	0.47±0.016	0.46±0.015	0.47±0.013	0.53±0.011	0.5±0.012	0.51±0.013
Intermediate Root Proportion	0.4±0.012	0.41±0.012	0.42±0.012	0.46±0.017	0.46±0.016	0.44±0.013	0.37±0.011	0.4±0.012	0.4±0.012
Coarse Root Proportion	0.08±0.004	0.09±0.004	0.09±0.006	0.07±0.004	0.07±0.005	0.08±0.006	0.1±0.006	0.1±0.006	0.09±0.007
<i>H. rigida</i>									
n	82	-	92	128	-	112	160	-	152
Shoot Length (mm)	76.6±4.2	-	79.8±3.9	82.7±3.8	-	87.9±3.9	77.5±2.9	-	77.8±3
Leaf number	9.6±0.6	-	9.6±0.5	9.4±0.4	-	9.1±0.4	9.4±0.4	-	9.4±0.4
Biomass (mg)	89.8±7.8	-	92.2±7.3	94.3±6.9	-	92.8±6.9	88.9±5.6	-	90.7±5.5
RWR	0.51±0.01	-	0.53±0.011	0.52±0.009	-	0.52±0.009	0.53±0.008	-	0.54±0.008
SRL (cm/mg)	10.3±0.4	-	10.1±0.5	9.8±0.4	-	9.6±0.4	10.7±0.3	-	10.7±0.4
Fine Root Proportion	0.66±0.011	-	0.64±0.013	0.62±0.011	-	0.62±0.01	0.64±0.009	-	0.64±0.009
Intermediate Root Proportion	0.26±0.012	-	0.27±0.011	0.28±0.01	-	0.28±0.01	0.28±0.009	-	0.27±0.008
Coarse Root Proportion	0.08±0.006	-	0.09±0.006	0.1±0.006	-	0.09±0.006	0.07±0.004	-	0.08±0.004
<i>S. speciosa</i>									
n	107	-	113	19	-	26	46	-	51
Shoot Length (mm)	56.3±1.5	-	60.6±1.4	58.2±4.9	-	55.1±3	50.3±2.4	-	57.9±2
Leaf number	12.5±0.8	-	12.9±0.8	13±1.6	-	11.7±1.4	12.1±1.4	-	14.4±1.3
Biomass (mg)	73.7±5.7	-	80.9±5.9	74.2±11	-	68.9±10.2	65.9±8.4	-	83.3±8.7
RWR	0.54±0.01	-	0.55±0.009	0.49±0.013	-	0.53±0.015	0.53±0.013	-	0.55±0.014
SRL (cm/mg)	18.3±0.7	-	18.2±0.5	19.4±1	-	19±0.9	18.6±0.8	-	17.7±0.9
Fine Root Proportion	0.75±0.008	-	0.75±0.007	0.74±0.016	-	0.73±0.015	0.78±0.012	-	0.76±0.011
Intermediate Root Proportion	0.2±0.006	-	0.2±0.005	0.21±0.012	-	0.21±0.009	0.18±0.009	-	0.2±0.008
Coarse Root Proportion	0.05±0.003	-	0.05±0.003	0.05±0.007	-	0.05±0.007	0.04±0.005	-	0.04±0.004

	N treat X Competition					
<i>A. salsola</i>	L,No Comp.	L,Comp.	M,No Comp.	M,Comp.	H,No Comp.	H,Comp.
n	180	187	209	178	197	186
Shoot Length (mm)	18.3±0.8	4.7±0.2	19.4±0.8	5±0.3	22.4±0.9	5±0.2
Leaf number	14±0.2	5.9±0.1	14.2±0.2	6.1±0.2	14.5±0.2	6.1±0.2
Biomass (mg)	78±3	20±1	90±3	21±1	96±3	21±1
RWR	0.6±0.006	0.62±0.008	0.61±0.005	0.61±0.008	0.61±0.006	0.61±0.007
SRL (cm/mg)	5.1±0.12	4.24±0.16	4.92±0.11	4.1±0.14	4.91±0.13	4.21±0.15
Fine Root Proportion	0.57±0.007	0.44±0.012	0.55±0.007	0.42±0.012	0.55±0.007	0.42±0.012
Intermediate Root Proportion	0.34±0.007	0.47±0.012	0.36±0.007	0.5±0.013	0.36±0.006	0.48±0.012
Coarse Root Proportion	0.08±0.003	0.08±0.005	0.08±0.003	0.09±0.005	0.08±0.002	0.1±0.007
<i>H. rigida</i>						
n	206	164	-	-	206	150
Shoot Length (mm)	104.1±2.5	47.7±1.1	-	-	105±2.3	49.3±1.3
Leaf number	12.9±0.3	5.1±0.1	-	-	12.4±0.3	5.2±0.1
Biomass (mg)	142.6±4	26.4±1.2	-	-	138.4±3.9	27.8±1.2
RWR	0.54±0.006	0.5±0.008	-	-	0.54±0.006	0.52±0.009
SRL (cm/mg)	10±0.3	10.6±0.3	-	-	10.1±0.4	10.4±0.4
Fine Root Proportion	0.67±0.006	0.6±0.01	-	-	0.66±0.006	0.6±0.011
Intermediate Root Proportion	0.22±0.004	0.34±0.009	-	-	0.23±0.004	0.34±0.009
Coarse Root Proportion	0.11±0.003	0.05±0.003	-	-	0.11±0.004	0.05±0.003
<i>S. speciosa</i>						
n	91	81	-	-	103	87
Shoot Length (mm)	64.1±1.6	44.5±1.2	-	-	67.2±1.2	49.6±1.1
Leaf number	19.6±0.5	4.4±0.1	-	-	20.3±0.5	4.6±0.2
Biomass (mg)	122.4±2.6	14.7±0.6	-	-	132.3±2.6	17.8±0.8
RWR	0.57±0.007	0.49±0.011	-	-	0.58±0.006	0.5±0.011
SRL (cm/mg)	15.5±0.4	22±0.8	-	-	15.2±0.3	21.7±0.6
Fine Root Proportion	0.71±0.006	0.81±0.008	-	-	0.71±0.005	0.81±0.007
Intermediate Root Proportion	0.22±0.004	0.17±0.007	-	-	0.22±0.003	0.17±0.006
Coarse Root Proportion	0.07±0.003	0.02±0.001	-	-	0.07±0.002	0.02±0.001

	N site X Competition X N treatment							
<i>A. salsola</i>	L,No Comp.,L	L,No Comp.,M	L,No Comp.,H	L,Comp.,L	L,Comp., M	L,Comp., H	M,No Comp.,L	M,No Comp.,M
n	73	78	65	65	64	70	49	68
Shoot Length (mm)	18.2±1.2	20.1±1.3	21.7±1.3	4.5±0.4	4.4±0.5	4.8±0.4	18.7±1.6	21.2±1.6
Leaf number	14±0.4	14.4±0.3	14.8±0.3	6.2±0.2	6.3±0.3	6.2±0.3	14.2±0.4	15±0.4
Biomass (mg)	86±4	96±4	105±5	20±1	22±2	21±1	71±5	81±5
RWR	0.61± 0.009	0.61± 0.009	0.61± 0.009	0.63± 0.013	0.6±0.014	0.62±0.01	0.6±0.012	0.6±0.01
SRL (cm/mg)	4.84±0.17	4.77±0.17	4.94±0.18	4.31±0.28	4.08±0.2	4.02±0.24	5.3±0.24	5.4±0.22
Fine Root Proportion	0.57± 0.011	0.56± 0.011	0.58± 0.012	0.44± 0.019	0.43± 0.018	0.4±0.018	0.57± 0.015	0.54± 0.012
Intermediate Root Proportion	0.34± 0.009	0.35± 0.011	0.34± 0.011	0.48± 0.019	0.48± 0.018	0.5±0.016	0.36± 0.015	0.38± 0.012
Coarse Root Proportion	0.08± 0.006	0.08± 0.004	0.08± 0.004	0.08± 0.006	0.09± 0.008	0.1±0.01	0.07± 0.006	0.08± 0.005
<i>H. rigida</i>								
n	46	-	50	36	-	42	69	-
Shoot Length (mm)	100.7±4.9	-	104.9±4.5	45.9±2.5	-	49.9±2.4	110.3±5	-
Leaf number	13.2±0.6	-	13.2±0.5	5.1±0.2	-	5.4±0.2	12.9±0.5	-
Biomass (mg)	140.1±8	-	145.8±7.1	25.6±2.2	-	28.4±1.7	150.8±7.8	-
RWR	0.53± 0.014	-	0.54± 0.012	0.48± 0.016	-	0.51± 0.018	0.53±0.01	-
SRL (cm/mg)	9.9±0.5	-	10.1±0.6	10.8±0.8	-	10.1±0.9	8.8±0.4	-
Fine Root Proportion	0.69± 0.013	-	0.67± 0.012	0.63± 0.018	-	0.6±0.022	0.63± 0.012	-
Intermediate Root Proportion	0.2±0.011	-	0.21± 0.007	0.32± 0.017	-	0.34± 0.018	0.23± 0.008	-
Coarse Root Proportion	0.11± 0.007	-	0.12± 0.008	0.04± 0.006	-	0.06± 0.008	0.13± 0.007	-
<i>S. speciosa</i>								
n	56	-	59	51	-	54	13	-
Shoot Length (mm)	65.3±1.7	-	69.9±1.5	46.4±1.5	-	50.5±1.3	66.4±5.7	-
Leaf number	19.7±0.5	-	20.4±0.5	4.5±0.2	-	4.7±0.3	17.1±1.1	-
Biomass (mg)	127.3±2.9	-	138.2±2.7	14.8±0.7	-	18.2±1.1	102.3±7.4	-
RWR	0.59± 0.008	-	0.58± 0.007	0.5±0.015	-	0.51± 0.015	0.52± 0.013	-
SRL (cm/mg)	14.7±0.5	-	15.2±0.4	22.3±1.1	-	21.6±0.7	19.4±1.3	-
Fine Root Proportion	0.7±0.007	-	0.71± 0.006	0.8±0.011	-	0.8±0.009	0.72± 0.014	-
Intermediate Root Proportion	0.22± 0.005	-	0.22± 0.004	0.18±0.01	-	0.17± 0.008	0.21±0.01	-
Coarse Root Proportion	0.07± 0.003	-	0.07± 0.003	0.02± 0.002	-	0.02± 0.002	0.06± 0.005	-

	N site X Competition X N treatment (cont.)								
<i>A. salsola</i>	M,Comp. ,L	M,Comp. ,M	M,Comp. ,H	H,No Comp.,L	H,No Comp.,M	H,No Comp.,H	H,Comp. ,L	H,Comp. ,M	H,Comp., H
n	62	58	55	58	63	68	60	56	61
Shoot Length (mm)	4.2±0.4	4.9±0.6	4.9±0.4	18±1.4	16.8±1.1	22.2±1.6	5.3±0.4	5.7±0.5	5.4±0.4
Leaf number	5.6±0.2	5.8±0.3	6.2±0.3	13.8±0.4	13.2±0.4	14±0.4	5.9±0.3	6.3±0.3	5.9±0.3
Biomass (mg)	17±1	17±1	19±2	75±4	91±5	93±5	23±1	24±1	22±2
RWR	0.63± 0.016	0.63± 0.015	0.6± 0.014	0.59± 0.01	0.62± 0.008	0.6±0.01	0.61± 0.01	0.61± 0.011	0.6± 0.012
SRL (cm/mg)	4.35± 0.33	4.31± 0.28	4.5±0.29	5.25± 0.23	4.58± 0.15	4.98± 0.26	4.04± 0.23	3.91± 0.23	4.17± 0.28
Fine Root Proportion	0.4± 0.023	0.38± 0.025	0.41± 0.023	0.57± 0.012	0.55± 0.012	0.55± 0.011	0.49± 0.017	0.44± 0.019	0.46± 0.023
Intermediate Root Proportion	0.54± 0.023	0.55± 0.026	0.51± 0.023	0.34± 0.012	0.35± 0.011	0.36± 0.01	0.4± 0.018	0.45± 0.019	0.44± 0.021
Coarse Root Proportion	0.06± 0.005	0.07± 0.009	0.08± 0.012	0.08± 0.005	0.09± 0.005	0.08± 0.004	0.11± 0.011	0.1± 0.011	0.11± 0.014
<i>H. rigida</i>									
n	59	-	49	91	-	93	69	-	59
Shoot Length (mm)	50.4±1.7	-	53.9±1.8	101.1± 3.3	-	98.6±3.3	46.4±1.6	-	45.1±2.1
Leaf number	5.3±0.3	-	5.1±0.2	12.8±0.4	-	12.1±0.4	4.9±0.2	-	5.2±0.2
Biomass (mg)	29.3±2.6	-	29.2±1.8	137.8± 5.8	-	131.7± 5.7	24.4±1.5	-	26.1±2.2
RWR	0.5± 0.015	-	0.52± 0.014	0.55± 0.01	-	0.55± 0.01	0.52± 0.013	-	0.53± 0.015
SRL (cm/mg)	10.9±0.6	-	10.4±0.7	11±0.4	-	10.9±0.7	10.3±0.5	-	10.6±0.5
Fine Root Proportion	0.6± 0.019	-	0.59± 0.017	0.68± 0.007	-	0.65± 0.009	0.59± 0.017	-	0.62± 0.017
Intermediate Root Proportion	0.33± 0.016	-	0.35± 0.015	0.22± 0.006	-	0.24± 0.007	0.36± 0.014	-	0.33± 0.015
Coarse Root Proportion	0.06± 0.006	-	0.06± 0.006	0.09± 0.004	-	0.1± 0.005	0.05± 0.005	-	0.04± 0.004
<i>S. speciosa</i>									
n	6	-	11	22	-	29	24	-	22
Shoot Length (mm)	40.5±3.3	-	42±3.5	59.8±3.5	-	62.8±2.7	41.5±2.2	-	51.5±2.3
Leaf number	4.2±0.2	-	3.9±0.3	20.7±1.4	-	21.7±1	4.3±0.2	-	4.8±0.2
Biomass (mg)	13.3±1.3	-	13.1±1.5	121.7± 5.3	-	132.1±6	14.7±1	-	19±1.7
RWR	0.44± 0.021	-	0.48± 0.029	0.58± 0.013	-	0.59± 0.014	0.5± 0.019	-	0.48± 0.021
SRL (cm/mg)	19.3±1.4	-	21±1.6	15±0.8	-	14.2±0.6	22±1.1	-	22.4±1.3
Fine Root Proportion	0.79± 0.036	-	0.79± 0.014	0.72± 0.015	-	0.71± 0.01	0.83± 0.011	-	0.82± 0.013
Intermediate Root Proportion	0.2± 0.034	-	0.19± 0.012	0.21± 0.01	-	0.22± 0.007	0.15± 0.011	-	0.16± 0.012
Coarse Root Proportion	0.01± 0.002	-	0.02± 0.003	0.06± 0.005	-	0.07± 0.004	0.01± 0.001	-	0.01± 0.002