University of Nevada, Reno

Phenotypic plasticity within the native perennial grass, *Poa secunda* (Sandberg bluegrass), an important component of the Western United States sagebrush grassland vegetation

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in Natural Resources and Environmental Science

By

Allison J. Phillips

Dr. Elizabeth A. Leger/Thesis Advisor

May 2012

University of Nevada, Reno
Statewide • Worldwide

THE GRADUATE SCHOOL

We recommend that the thesis
prepared under our supervision by

ALLISON J. PHILLIPS

entitled

Phenotypic Plasticity Within The Native Perennial Grass, Poa Secunda (Sandberg Bluegrass), An Important Component Of The Western United States Sagebrush Grassland Vegetation

be accepted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

Elizabeth Leger, Ph. D., Advisor

Marjorie Matocq, Ph.D., Committee Member

Thomas Albright, Ph.D, Graduate School Representative

Marsha H. Read, Ph. D., Dean, Graduate School

May, 2012

ABSTRACT

Native plant communities of the Nevada Great Basin are experiencing a rapid shift from native perennial grasses to invasive annual grasses. The ability to respond to competition through root plasticity may be an important trait for survival of native plants in invaded systems. I investigated the following questions about *Poa secunda*, a common native perennial grass in sagebrush ecosystems: 1) Can *P. secunda* respond plastically to directly-manipulated nutrient availability? 2) Does *P. secunda* respond to the presence of *B. tectorum*? and 3) Are these plastic responses adaptive? For the nutrient experiment, ten seeds from twenty families were sown individually into greenhouse pots, where one *P. secunda* family is defined as one individual plant. Within each family, five individuals received a low nutrient treatment, and five a high nutrient treatment. Plants were harvested fifty days after emergence. Roots were rinsed, clipped from leaf mass, and digitally scanned. For the competition experiment, twenty seeds from forty-eight families were sown individually into small and large greenhouse pots and one seed of *B. tectorum* was added to half of the pots. Plants were harvested sixty-seven days after emergence (early harvest), and after one growing season (late harvest) for the competition experiment. Roots were rinsed, clipped from their leaf mass and digitally scanned in a similar manner as the nutrient experiment. For both the nutrient and the competition experiments, measurements included total biomass, root to shoot ratio, leaf number, specific root length (SRL), percent allocation to different root diameter size classes, and plasticity in allocation to these same root diameter classes. In addition change in percent allocation to different root diameter size classes was calculated for plants in response to both low nutrients and competition from *B. tectorum*. Measures were correlated with plant performance measured as CPI (competitive performance index) and total biomass to determine if they were adaptive. Finally, to determine if plastic changes were simply a result of plant size or represented a change in strategy due to different growing environments, traits were assessed for “active” or “passive” plasticity by accounting for ontogenetic drift.

In response to low nutrient treatment, *P. secunda* showed a 27% decrease in biomass (P<0.0001), and a higher root to shoot ratio (P<0.0001). Plants showed an increase in specific root length (P<0.0001) and a decrease in leaf number under low nutrients (P<0.0001). Percent allocation to different root diameter size classes changed in response to low nutrients, with significant increases in allocation to fine root diameter classes (0.0mm through 0.2mm), and decreased allocation to larger root categories (0.3mm through 0.6mm, and >2.0mm; P=0.04). Families differed in their response to low nutrients, and the nutrient by family interaction was significant for total biomass (P=0.0059). The nutrient experiment results demonstrated that *P. secunda* has the ability to allocate biomass to the production of more fine roots in response to low resource environments.

In response to competition with *B. tectorum* for early harvest plants, *P. secunda* showed a 46% decrease in total biomass (P<0.0001) and an increase in root to shoot ratio of 14% (P<0.0001). Plants had a higher specific root length and a decrease in leaf number under competition. Percent allocation to different root diameter size classes changed with competition status, with significant increases in allocation to fine root diameter classes (0.0-0.1mm, and 0.2mm through 0.4mm) when grown with *B. tectorum*, and significant decreases in allocation to course root diameter classes (0.4mm through >2.0mm; P<0.0001). Families differed in their response to traits measured, and the treatment by family interaction was significant for leaf number (P=0.0071) and percent allocation to different root diameter size classes (P=0.0001). The shift towards the production of finer root class 0.2-0.3mm and a higher root to shoot ratio may be an adaptively plastic response to competition with *B. tectorum*.

For late harvest plants, *P. secunda* produced 88% less biomass (P<0.0001) and had 44% higher root to shoot ratio (P<0.0001) in response to competition. Families different in their root to shoot ratio (P<0.0001) and in their percent allocation to different root diameter size classes (P<0.0001). The treatment by family interaction, and the location by treatment interaction was significant for percent allocation to different root diameter size classes (P<0.0001). Plants allocated overall more biomass to fine root diameter size classes (0.1mm through 0.4mm) under competition (P<0.0001). In contrast to early harvest, allocation to coarse roots was adaptive in the late harvest. When ontogenetic drift was assessed, results show that *P. secunda* had a higher root to shoot ratio and allocated more resources to coarse roots relative to their size for some traits measured. This shift in allocation from producing less fine to more coarse roots may suggest a shift towards an adaptively plastic response to competition with *B. tectorum.* These results also suggest that *P. secunda* may have the ability to adaptively respond to invasion from *B. tectorum* through phenotypic plasticity.

ACKNOWLEDGEMENTS

I would like to thank Beth Leger, my academic advisor, for allowing me to further my passion for science by providing me with the opportunity to be her graduate student, and for her continuous support over the past three years. Beth possesses the rare combination of intelligence and positive interpersonal skills that make her an exceptional advisor. I would also like to thank Erin Goergen, a post-doc for the majority of time I was a student in Beth's lab. Erin was a strong role model and I would especially like to thank her for her continuous guidance in answering any question, aiding in the physically demanding work of setting up my greenhouse experiment and providing feedback on my thesis, of which, exceeded her job description. I would like to thank Sarah Kulpa for her positive encouragement throughout my graduate studies and for sharing her passion of Botany with me. I would also like to thank Donna Withers for allowing me the opportunity to serve as a STEP with the U.S. Fish & Wildlife. Also, thank you to my committee members Tom Albright and Marjorie Matocq for offering continued guidance and editorial suggestions for my thesis. I would also like to thank several lab technicians and fellow graduate students, Kevin Badik, Owen Baughman, Jamie Greer, Tiffany Larrieu, Sandra Li, Bryce Wehan and Ashlee Younie who offered their greenhouse skills and data collection, which involved many hours of washing, scanning and weighing roots. Finally, thank you to my husband, Scott Phillips, for providing emotional support and being a wonderful dad by dedicating time to watching Greta, which allowed me enough necessary time to complete my thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

BACKGROUND

Thesis summary

This thesis addresses the ecology of the native perennial grass, *Poa secunda* (Sandberg bluegrass), an important component of the sagebrush grasslands in the Western United States, focusing on variability found within wild *P. secunda* populations. Ultimately, this information can be used to infer which traits are beneficial to natives in invaded systems and determine if native plant communities may be becoming locally adapted in the face of disturbance from invasion. Specifically, this thesis addresses the following research objectives: to measure the phenotypic plasticity of root traits (i.e. fine root production, allocation to roots, specific root length) that allow *P. secunda* to persist in the face of invasion, determine if plasticity is adaptive, and determine if these traits vary by family. The following chapter describes three separate experiments: nutrients, early harvest and late harvest. For the nutrient experiment, *P. secunda* was first assessed for variability in response to low nutrients. This was a pilot experiment in that results from this experiment led to further investigation of *P. secunda* in response to competition with *B. tectorum* and was an important first step in determining the ability for *P. secunda* populations to exhibit plasticity given its mating strategy, facultative apomixis. I addressed whether *P. secunda* could respond plastically to changes in nutrient addition, whether responses to growing with invaders were similar, and whether these changes may be adaptive.

Study species

Poa secunda is a small to medium sized, long-lived bunchgrass. It contains soft basal leaves and few to many naked flowering stalks. Typical to bluegrass, the leaves have a prow-shaped tip and the upper leave surface has a double grove down the center, while the seeds are glabrous. Flowers occur in narrow panicles that spread during anthesis (USDA, NRES 2009). Growth begins in the early spring, before most other native grasses and with persistent moisture, *P. secunda* can remain green throughout the summer, though it usually senesces earlier than other native grasses (USDA, NRCS 2009). It is therefore possible for this species to have the highest abundance on the landscape among grass species (USDA, NRCS 2009). *P. secunda* grows in small tufts, usually less than thirty centimeters in height. It is drought and grazing tolerant and is considered to be fire adapted due to its relatively low biomass and resprouting ability, which allows it to persist through fire and stabilize disturbed areas quickly (USDA, NRCS 2009). It outperforms other native species in performance under dry conditions and in shallow soil (Goergen et al. 2011). Although it is adapted to a wide variety of soil, it prefers medium textures soils. Additionally, it is known to be effective at suppressing weed growth due to its course, fibrous and deep-penetrating roots which can occasionally develop short rhizomes (USDA, NRCS 2009; Goergen et al.2011). Specifically, *P. secunda* is known for its competitive ability against *B. tectorum* due to its early spring growth and quick maturity (Goergen et al. 2011).

Mating Strategy

Since plant variation in phenotypic plasticity relies on genomes exhibiting a certain degree of variability, is important to understand the underlying mechanisms that create genetic diversity among plants. Genetic variability within plant populations changes predictably among various mating strategies. In general, inbreeding populations tend to be highly homozygous and low in genetic diversity (Silverton 2001). On the other hand, outcrossing populations tend to have high genetic diversity due to gene flow among different populations and among individuals within populations (Silverton 2001). As such, we might expect genetic variability to be apportioned among individuals within populations of species that have high rates of gene flow.

Apomixis is a unique form of asexual reproduction in which plants reproduce asexually without fertilization. Flowering plants produce cells that contain the same genotype as their maternal parent which develop into an embryo. Apomictic plants will therefore contain seeds that have the same genotype as their mother. In many apomictic flowering plants, fertilization of the endosperm, called pseudogamy, is common. This may suggest that pseudogamy evolved after sexual reproduction (Silverton 2001).

There can be instances when plants exhibit both sexual and asexual mating systems (i.e. facultative apomixis). The genus *Poa* encompasses a large diversity of reproductive strategies in which both sexual and asexual reproduction is found. The primary mating strategy among *Poa* is facultative apomixis, exhibiting gametophytic apomixis in which non-reduced (i.e. cells that have not undergone meiosis), asexual embryo sacs develop from differentiated cells of the nucellus following differentiation of the megaspore mother cell (Koltunow 1993). However, asexual and sexual reproduction occur simultaneously, in individual plants, so that they are not considered obligately asexual. Aposproric development, or the production of progeny identical to the female genotype, occurs along with pseudogamous apomixis, in which fertilization of the polar nuclei for the creation endosperm development, happens simultaneously during seed development (Kellogg 1987). As a result, pollen is produced which has the potential to fertilize other plants and thus retain gene flow (Kellogg 1990).

Although *P. secunda* is primarily regarded as a clonal species, sexual reproduction has been demonstrated to produce higher amounts of genetic diversity among some populations (Jones 2005). The percent of asexual ovules within one population of *P. secunda* varied as much as 40% between plants (Kellogg 1987). This percentage also varied within the same plant grown in different environments (Kellogg 1987). Additional studies along these same lines have concluded that factors such as temperature, length of day and latitude all play a key role in determining the degree of apomixis within a population. It should be noted, therefore, that external environmental factors, and not merely the species itself, affect the degree the apomixis and it is this degree of apomixis that directly affects gene flow and genetic diversity within populations.

REFERENCES

Ghalambor CK, McKay JK, Carroll SP and Reznick DN. 2007. Adaptive verses non adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* **21**: 394-407.

Goergen, E.M., E.L. Leger & E.K. Espeland. 2011. Native perennial grasses show evolutionary response to *Bromus tectorum* (cheatgrass) invasion. PLoS ONE 6(3): e18145.

James JJ, Mangold JM, Sheley RL and Svejcar T. 2009. Root plasticity of native and invasive Great Basin species in response to soil nitrogen heterogeneity. *Plant Ecology* **202**: 211-220.

Jones, T.A. and S.R. Larson. 2005. Status and use of important native grasses adapted to sagebrush communities. USDA Forest Service Proceedings RMRS-P 38: 49-55.

Kellogg, E.A. 1987. Apomixis in the Poa secunda complex. American Journal of Botany 74(9): 1431-1437.

Kellogg, E.A. 1990. Variation and species limits in agamospermous grasses. Systemic Botany 15(1): 112-123.

Koltunow, A.M. 1993. Apomixis: Embryo sacs and embryos formed without meiosis or fertilization in ovules. The Plant Cell. 5(10): 1425-1437.

Leger, E.A. 2008. The adaptive value of remnant native plants in invaded communities: an example from the Great Basin. Ecological Applications 18(5): 1226-1235.

Silverton, J. and D. Charlesworth. 2001. Introduction to Plant Population Biology. Fourth ed. Blackwell Publishing. Malden, MA.

Sun, G.-L., O. Diaz, B. Solomon, and R.V. Bothmer. 1998. Microsatellite variation and its comparison with allozyme and RAPD variation in *Elymus fibrosus* (Schrenk) Tzvel. (*Poaceae*). Hereditas 129: 275-282.

USDA NRCS. 2009. The PLANTS Database. National Plant Data Center, Baton Rouge, LA. Available at: http://plants.usda.gov. Last accessed: 24 November 2011.

Varshney, R. K., A. Graner, and M.E. Sorrells. 2004. Genetic microsatellite markers in plants: features and applications. Trends in Biotechnology 23: 48-55.

Assessing phenotypic plasticity of root traits that allow for a competitive advantage of *Poa secunda* (Sandberg bluegrass) in response to competition with cheatgrass

A.J. Phillips[1], E.A. Leger[2]

Authors are [1] graduate student, [2] Assistant Professor of Plant Ecology, Department of Natural Resources and Environmental Science, University of Nevada, Reno

This research was funded by the University of Nevada, Reno, and the USDA-Hatch.

Correspondence: Allison J. Phillips, Department of Natural Resources and Environmental Science, University of Nevada, Reno.

Email: allisonphillips18@gmail.com

ABSTRACT

Native plant communities of the Nevada Great Basin are experiencing a rapid shift from native perennial grasses to invasive annual grasses. The ability to respond to competition through root plasticity may be an important trait for survival of native plants in invaded systems. I investigated the following questions about *Poa secunda*, a common native perennial grass in sagebrush ecosystems: 1) Can *P. secunda* respond plastically to directly-manipulated nutrient availability? 2) Does *P. secunda* respond to the presence of *B. tectorum*? and 3) Are these plastic responses adaptive? For the nutrient experiment, ten seeds from twenty families were sown individually into greenhouse pots, where one *P. secunda* family is defined as one individual plant. Within each family, five individuals received a low nutrient treatment, and five a high nutrient treatment. Plants were harvested fifty days after emergence. Roots were rinsed, clipped from leaf mass, and digitally scanned. For the competition experiment, twenty seeds from forty-eight families were sown individually into small and large greenhouse pots and one seed of *B. tectorum* was added to half of the pots. Plants were harvested sixty-seven days after emergence (early harvest), and after one growing season (late harvest) for the competition experiment. Roots were rinsed, clipped from their leaf mass and digitally scanned in a similar manner as the nutrient experiment. For both the nutrient and the competition experiments, measurements included total biomass, root to shoot ratio, leaf number, specific root length (SRL), percent allocation to different root diameter size classes, and plasticity in allocation to these same root diameter classes. In addition change in percent allocation to different root diameter size classes was calculated for

plants in response to both low nutrients and competition from *B. tectorum*. Measures were correlated with plant performance measured as CPI (competitive performance index) and total biomass to determine if they were adaptive. Finally, to determine if plastic changes were simply a result of plant size or represented a change in strategy due to different growing environments, traits were assessed for “active” or “passive” plasticity by accounting for ontogenetic drift.

In response to low nutrients and competition with *B. tectorum*, plants were significantly smaller in biomass ($P<0.0001$), had a higher root to shoot ratio ($P<0.0001$), a higher specific root length ($P<0.0001$), and allocated more biomass to the production of fine roots. Overall, families significantly varied from one another for traits measured, which demonstrates that populations are genetically diverse. Correlations with CPI and total biomass for early harvest plants showed allocation to fine roots was positively correlated with plant performance, whereas late harvest plants showed allocation to coarse roots was positively correlated with plant performance. For early harvest plants, seven out of twelve correlations were actively plastic, showing a shift in allocation of coarse to fine roots and increased root to shoot ratio under competition. Late harvest plants showed ten out of twelve correlations to be actively plastic, with some shifts towards allocation to coarse roots. These shifts in allocation may suggest an adaptively plastic response to competition. Ultimately, long-term persistence of *P. secunda* populations on the landscape depends on adaptive traits to withstand competition from invasives.

INTRODUCTION

Native plant communities of the Great Basin are experiencing a rapid shift from native perennial vegetation to invasive annual grasses, namely cheatgrass, *Bromus tectorum.* This winter annual grass is currently found in all 50 states including most of Canada and parts of Mexico. In the western states, *B. tectorum* has been well established since the 1930s and is now estimated to have invaded more than 20,000 km^2, making this shift in flora the most dramatic invasion in all of North America (Mack 1981; Bradley & Mustard 2004; USDA, NRCS 2009). Intensive grazing and a short fire return have created a window of opportunity for *B. tectorum* invasion. When initial colonization is followed by fire, a positive feedback can result in increasing dominance of *B. tectorum* (D'Antonio & Vitousek 1992). Numerous studies demonstrate the competitive superiority of *B. tectorum* over natives in the Great Basin. For example, under greenhouse conditions *B. tectorum* seeded at 609 and 2,760 plants per m^2 drastically inhibited the root and shoot growth of *Agropyron cristatus* (crested wheatgrass, Evans 1961). Likewise, in a field competition experiment, *B. tectorum* significantly reduced first-year relative growth rates and biomass of *Elymus elymoides* (squirreltail), which led to further reductions in second-year biomass and flowering (Humphrey & Schupp 2003). In *B. tectorum* invaded systems, native perennials are likely to experience a decrease in recruitment and seed production immediately and over the long-term from early seedling competition with *B. tectorum*.

Despite the success of *B. tectorum*, some native perennial species can still be seen in invaded landscapes. In many places throughout the Great Basin, competition between natives and *B. tectorum* has yielded coexisting populations (Arredondo et al. 1998;

Gurevitch and Padilla 2004; Sax and Gaines 2008; Leger & Espeland 2010). Although native populations have decreased in abundance in invaded areas, studies have shown that certain traits in native perennial populations may allow for native plant persistence (Leger 2008; Rowe & Leger 2010, Goergen et al. 2011). For instance, *E. multisetus* seeds collected from *B. tectorum* invaded areas and grown in a common environment showed, on average, smaller biomass and allocated greater production to fine roots compared to the same species from uninvaded areas, and were more competitive with *B. tectorum* (Rowe & Leger 2010). Likewise, adult perennial grasses from invaded areas displayed earlier initial re-growth, which led, in some cases, to a competitive advantage over *B. tectorum* (Leger 2008, Goergen et al. 2011). A study with *E. elymoides* indicated that second-year growth was more tolerant of *B. tectorum*, suggesting that mature perennials are less vulnerable to *B. tectorum* relative to seedlings (Humphrey & Schupp 2003). Identifying traits that allow native populations to persist in the face of *B. tectorum* invasion should be a top priority for land managers whose goal it is to restore and maintain native plant communities (Knapp 1996; Leger 2008). Seedling traits are particularly important because successful seedling establishment is central to the maintenance of populations.

The Great Basin includes five states including parts of California, Oregon, Idaho, Nevada and Utah, where Nevada comprises most of the approximately 390,000 km ^{2} area. Over 300 distinct mountain ranges have been recorded with average elevations greater than 1500m (Knapp 1996). In Nevada alone, the sagebrush vegetative zone compromises more of Nevada than any other vegetative zone and is dominated by *Artemisia tridentata* (big sagebrush). Ecologically important species in this area also include several species of perennial bunchgrasses such as *Poa secunda* (Sandberg bluegrass), *Elymus multisetus* (big squirrletail), *Hesperostipa comata* (needle-and-thread) and *Achnatherum speciosum* (desert needlegrass) (Knapp 1996; Charlet 1998). Plants in the Great Basin are well adapted to exploit soil nutrients in short-duration pulses when the nutrient influx from early spring freeze-thaw snowmelt is greatest (Chapin et al. 1990). The arid climate allows for approximately 250mm of precipitation annually (Knapp 1996), usually occurring from November through May, with ample sunlight year round. Therefore, limited access to water (Knapp 1996) creates a competitive environment for plant communities where most population level competition occurs belowground.

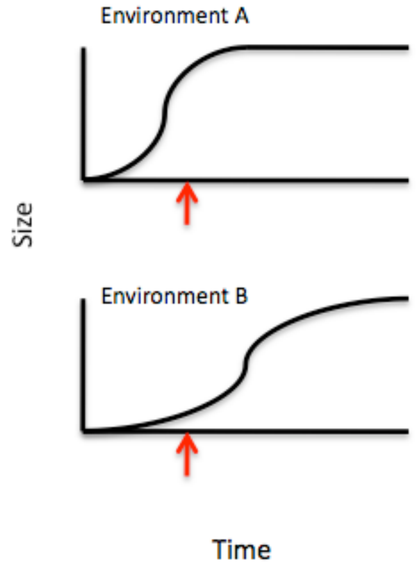
In general terms, plants tend to respond to low resource environments by allocating a larger portion of their biomass to organs involved in capturing the limiting resource (Bloom et al. 1985; Gedroc et al. 1996; Wahl et al. 2001; Hodge 2009). Therefore, plants must be able to exhibit phenotypic plasticity in response to environmental factors (Grime & Mackey 2002). Phenotypic plasticity, which is defined as an environmentally-induced morphological or physiological response to changes in the environment (Bradshaw 1965), can allow species to have a higher tolerance to a diverse array of environmental conditions therefore increasing individual fitness. Plasticity is considered to be adaptive if fitness is increased by a change in phenotype (Ghalambor et al. 2007). For example, genetic replicates of *Polygonum cespitosum* (Oriental lady's thumb) expressed dramatically different phenotypes in response to growing in contrasting greenhouse environments, in which plants grown in dry soil and full sun developed multiple branches and reproductive axes, narrow leaves, and allocated greater biomass to roots. In contrast, plants grown in moist soil and shade developed fewer branches, had a more upright form with large, broad leaves, and allocated greater biomass to leaves, which allowed for maximum photosynthetic surface area despite lower total biomass (Sultan 2010). These plastic changes maximized fitness under contrasting growing conditions.

When assessing a plastic response, it is important to consider that traits can change dramatically over the course of plant growth and development, a process referred to as ontogenetic drift (Evans 1972). For example, some plants exhibit an initial high root to shoot ratio during early seedling establishment, which eventually decreases after a few weeks of growth (Gedroc et al. 1996). This change is not is not a response to an environmental stimulus, but a consequence of increased growth and plant size. Therefore, when measuring changes in root allocation, it is important to consider differences in plant size in these calculations (more detail provided below).

In addition to changes in root to shoot ratio, plants have the ability to alter their root architecture, or the spatial structure of root systems in soil, without changing their overall root biomass, where the ability to capture limiting resources belowground is not determined by root biomass alone (Ryser 1998; Wahl et al. 2001; Hodge 2004; Gregory 2006). Rather, research suggests that the ability to capture limiting resources may be directly related to specific root length (SRL), which is defined as the root length per unit biomass (Eissenstat 1991). Root proliferation, which is defined as a plants' ability to respond to fertile soil patches by increasing their local root length within fertile areas, has been well documented as a mechanism for capturing limiting resources (Jackson et al. 1989; Caldwell et al. 1991; Hodge 2004, 2008). For example, exposure of part of the main lateral roots of the crop plant *Hordeum vulgare* (barley) to increased levels of nutrients resulted in increased root production within the nutrient zone (Drew 1975). Root proliferation is particularly important for nutrient capture when plants are in direct competition with one another and when the nutrient resource is limiting (Hodge et al. 1999, 2004, 2009). In the Great Basin, for example, the non-native grass *Agropyron desertorum* (desert wheatgrass) responded dramatically to increased nutrients by rapidly proliferating more roots relative to native species, which may be an important factor for its success on the landscape (Jackson et al. 1989). Likewise, Jackson et al. (1990) demonstrated that root proliferation in localized nutrients patches can be rapid for two other perennial species, *Artemisia tridentata* (big sagebrush) and *Pseudoroegneria spicatum* (bluebunch wheatgrass), common to the Great Basin. Similar studies have also shown that increased root proliferation of fine roots in nutrient patches depends largely on timing and the duration of the nutrient supply (Pregitzer et al. 1993).

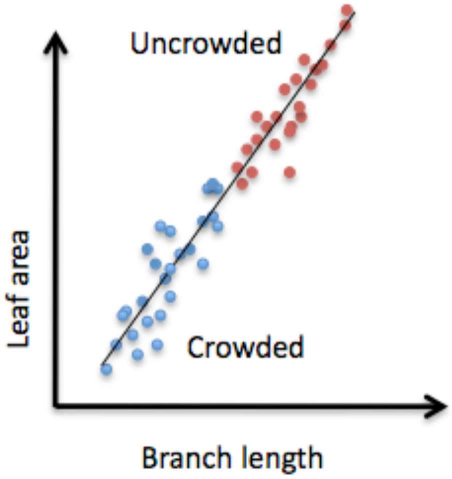
As mentioned above, it is well known that many plant traits, such as allocations in biomass to roots, can change in predictable ways over the course of growth and development and that growth rates themselves are also plastic (Evans 1972; Coleman et al. 1994; Wright & McConnaughay 2002; Weiner 2004). Because most of plant growth and development follows predictable ontogenetic trajectories, any change in measured traits from novel stimulus may simply be a result of plant size. Therefore, it is important to distinguish whether plasticity in traits is a result of ontogenetic changes during growth and development, or, rather, a result of changes in the ontogenetic trajectory of a trait in response to environmental variation (Coleman et al 1994; Weiner 2004). Any changes in measured traits resulting solely from changes in growth rate, or ontogenetic drift, is referred to a "passive plasticity," whereas "active plasticity" refers to environmentally-induced variation in the ontogenetic trajectory of a trait (Wright & McConnaughay 2002).

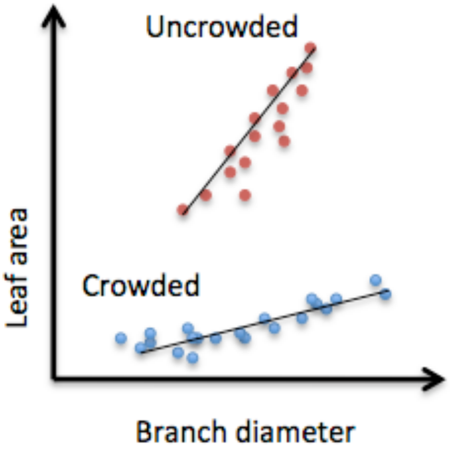
It is possible to mistakenly conclude that plants are exhibiting active plasticity in response to varying environmental conditions when traits are measured at a common point in time, rather than at a common size. The illustration below exemplifies how rate of growth and development can be drastically different for two individual plants harvested at the same point in time (arrow), because of differences in plant size at a given time in the different environments (re-illustrated from Weiner 2004). Environment A is more favorable, and the plant reaches a larger size sooner, while in environment B, plants are growing much slower.



For example, the figure presented below illustrates how the relationship between plant traits of *Kochia scoparia* (ragweed) in response to crowding can be drastically altered when plants are compared at the same size (re-illustrated from Weiner 2004; Weiner &

Fishman 1994). In the left graph below, for instance, plants show a non-plastic response to crowding in the relationship between traits measured, in which there is a continuous ontogenetic trajectory between crowded and uncrowded stands. This result shows, for instance, that crowding does not have an effect on the relationship between branch length and leaf area of *K. scoparia*. In contrast, the right graph below shows an example of active plasticity in which the ontogenetic trajectory, or slope, of traits measured differs between crowded and uncrowded plants, where crowded plants have much less surface area at a given stem diameter compared to uncrowded plants. Overall, this study shows the importance of plant strategies in response to crowding, where plants can plastically respond to crowding for some traits.





Because plants alter percent allocation to different organs according to size, then environmentally-induced responses that affect plant growth rates will also, in turn, affect the percent allocation to different organs (Weiner 2004). In another example, Gunn et al. (1999) reported increased allocation to leaves and shoots for three herbaceous plants grown at enriched CO_2 atmospheric levels compared to control plants that were not grown with enriched CO_2, when plants were compared at a common age. However, when compared again at a common size, these differences did not exist, suggesting that passive plasticity was responsible for observed differences due to an accelerated growth rate under enriched CO_2. Therefore, not accounting for ontogenetic differences while measuring the plasticity of plants at different sizes may have profound effects on the interpretation of results.

Although plants can exhibit active phenotypic plasticity, not all plasticity leads to increased fitness, and phenotypic plasticity may either be adaptive or maladaptive. Phenotypic plasticity is most likely to be adaptive when variable environmental conditions give reliable cues to individuals and when different phenotypes are favored in novel environments, and where no single phenotype is more advantageous than another phenotype across all environments (Ghalambor et al. 2007). In contrast, maladaptive plasticity results when the new environmentally-induced phenotype gives rise to a reduction in fitness (Ghalambor et al. 2007). The most common form of maladaptive plasticity is seen when individuals experience a failure to develop or function properly due to environmental stress. For example, Grether (2005) found that plants may fail to mature properly or produce an optimal number of seeds when grown in resource limiting soil. Another type of maladaptive plasticity occurs when environmental cues produce a novel trait that reduces fitness. In a study conducted by Langerhans and DeWitt (2002), freshwater snails raised with either molluscivorous or non-molluscivorous sunfish species responded unnecessarily to non-molluscivorous species by reducing growth rates which led to a lowered fecundity and by producing rotund shells which increased vulnerability to shell-entry predators. Plant species confronted with novel cues from competition may also be at risk of maladaptive responses (van Kleunen & Fisher 2005; Aphalo et al. 1999; Schwinning & Weiner 1998)).

To date, the plastic response of Great Basin native plant populations to invasion has received relatively little attention. Rather, focus has been on key traits of invasive species that allow invasives, such as *B. tectorum*, to outperform native perennial grasses and fixed traits of natives that increase tolerance (James 2008; Leger 2008; Leger & Rowe 2010, Goergen et al. 2011). The goals of this study were threefold: 1) determine if *P. secunda* responds plastically to directly-manipulated nutrient availability, 2) determine if *P. secunda* responds to the presence of *B. tectorum*, and 3) determine if any changes are adaptive. Since competition with *B. tectorum* is known to greatly reduce available nutrients, I focused on root responses to both nutrient availability as well as to competition with *B. tectorum* during early seedling establishment so that any similarities between responses could aid in determining advantageous traits in invaded systems. I also focused on root responses to competition with *B. tectorum* after one growing season since the ability for *P. secunda* to survive to full maturity is an important trait for continued establishment in invaded systems. Additionally, I assessed family-level variation to see if root traits and plastic responses to environmental variation were inherited, which is important for identifying families that may be used for restoration of invaded systems.

METHODS

Seed Collection

During November and December 2008, 40 individual adult plants of *P. secunda* were collected from two locations: McClellan Peak, Nevada (39 14 21.30N 119 44 34.70W, 1750m elevation) and Bedell Flat (39 49 58.10N 119 45 56.10W, 1513m elevation) and brought to the University of Nevada, Reno agricultural experimental station greenhouse complex. From each original location, 20 individual plants from invaded areas and 20 individual plants from uninvaded areas were transplanted, representing 40 total families from each location. Plants were grown in a common environment for one season and seeds were collected as they matured, from May-June 2009. Seeds were stored at room temperature until planting.

Nutrient Study

Seeds from 20 McClellan Peak families were used to measure plant response to variable nutrients. Ten seeds from each family were grown in small greenhouse pots (Stuewe & Sons RLC4 66mL, 2.5cm diameter, 16cm depth). Pots were filled with coarse sand and sown over the course of five days from 25 January 2010 to 30 January 2010 in order to stagger harvesting of 200 pots. One seed was directly sown in the center of each pot and seeds were held at controlled greenhouse conditions (4.4-26°C, 5-25% relative humidity, full daylight). Pots were immediately watered upon sowing with a solution of one of two treatments: low or high nutrients (Miracle-Gro all purpose water soluble plant food; 15% nitrogen, 30% phosphate, 15% potash). Emergence date was recorded for all 200 plants and seeds were allowed to grow for 50 days after emergence, at which point they were harvested.

For the high nutrient treatment, one tablespoon of nutrients was mixed with one gallon of water. For the low nutrient treatment, 0.25 of a tablespoon of nutrients was mixed with one gallon of water for the first 30 days after sowing, with no further nutrients provided for an additional 20 days.

Harvest occurred over a 15-day period from 15 March 2010 to 30 March 2010. To extract roots at harvest, pots were submerged underwater and planting media was manually removed from pots. Roots were gently rinsed, clipped from leaf mass, refrigerated (<24 hours), then digitally scanned for analysis. Number of leaves were recorded and shoots were immediately dried (7 days at 60°C) before weighing. WinRhizo root scanning software (Regents Instruments Inc, Siante-Foy, Canada) was used to analyze scanned root images. From this, total root length (cm) and the root diameter for 10 size classes (0.0-0.1mm, 0.1-0.2mm, 0.3-0.4mm, 0.4-0.5mm, 0.5-0.6mm, 0.6-0.8mm, 0.8-1.2mm, 1.2-2.0mm, >2.0mm) were quantified for each sample scanned. Root and shoot biomass measurements were recorded separately by weighing roots and shoots individually after drying (10 days at 60°C). Root to shoot ratio (R:S) was calculated as root weight ÷ shoot weight. Specific root length (SRL) was calculated as total root length (cm) ÷ root mass (mg). Total biomass for both *P. secunda* was calculated as root weight + shoot weight. Additionally, percent allocation to the 10 root diameter size classes was calculated as [total length of a root diameter category (i.e. 0.0-0.1) ÷ total root length] x 100 for plants under low and high nutrients.

Analysis for Nutrient Study

Three types of analysis were performed using JMP 9.0.2 (SAS Institute 2010). (1) Analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were conducted to compare a) changes in means between nutrient treatments, to determine if treatments had an effect on traits measured, b) differences in means between families to determine genetic variability, and c) to test the interaction between families and treatments to determine if all families had similar responses to the nutrient treatments. For our second analysis, (2) change in family-level means for all measured traits were calculated to determine plasticity in response to nutrient treatments. (3) Finally, to determine whether plasticity was ontogenetic, both ANCOVA (analysis of covariance) and regressions were performed to test for different trajectories of plastic traits for both low and high nutrients (explained further below).

(1) ANOVA was used to test the effects of nutrients and family on the following *P. secunda* traits: total biomass, root to shoot ratio, leaf number, specific root length (SRL) and percent allocation to 10 root diameter size classes. The ANOVA model included the following factors: nutrient status (fixed factor, either low or high), family (random factor) and the nutrient status by family interaction. Several growth traits did not display normal residual distributions and standard transformations did not improve this or change model significance, thus they were not transformed. SRL was log transformed to improve normal distribution. Significant results ($P<0.05$) are presented in figures with raw means and standards errors. Shifts in root to shoot ratio family means in response to nutrients were compared *a posteriori* using Tukey HSD. For the MANOVA analysis, the Wilk's lambda method was used to determine significant affects of nutrients status and family on percent allocation to all root diameter size classes; significant MANOVA results were followed with ANOVA to determine significant differences within individual root size classes.

(2) Using the percent allocation calculations for each individual under low and high nutrients, we used JMP statistical software to calculate low and high nutrient means for

each family. Percent allocation to different size classes of low and high nutrient family means were then used to quantify the change in family level plasticity for each root diameter size class as follows: percent allocation for high nutrients – percent allocation for low nutrients (Valladares et al. 2006). Additionally, the sum of the total change in percent allocation of all root diameter size classes was calculated for each family. Low and high nutrient family means were also calculated for SRL. Specific root length (SRL) of low and high nutrient family means were then used to quantify the change in family level plasticity as follows: SRL_{high} - SRL_{low}. Root to shoot ratio of low and high nutrient family means were also calculated and used to quantify the change in family level plasticity as follows: Root to shoot $_{high}$ – root to shoot $_{low}$. Nutrient performance index (NPI) was used to quantify the ability of *P. secunda* to tolerate low nutrient availability, where NPI is the percent decrease in plant performance when grown with low nutrients compared to plant performance when grown in high nutrients (Rowe & Leger 2010; Goergen et al. 2011). NPI is different from strict biomass measures where any decrease in plant size is compared relative to the original plant size at the starting point of nutrient treatments. NPI is calculated as follows: (total $biomass_{high}$ – total $biomass_{low}$) ÷ total $biomass_{high}$. Family means under high and low nutrients were used to calculate NPI.

(3) Lastly, ANCOVA was used to determine if results were based on active plasticity or passive plasticity. We first located the top ten root diameter size classes that had the strongest negative correlations with one another using individual plant data. Next, correlated root diameter size classes were analyzed to determine whether allocation to these different root diameter size classes was actively plastic. ANCOVA was used with one class as the response and the other class as a model factor. “Treatment” was included

as a factor, and the factor by size class interaction was used to determine if slopes of lines were on different trajectories in different treatments. When interactions were significant, regressions were then used to analyze the relationship between fine roots and coarse roots under different nutrient treatments. An increase in the slope of the ratio of fine to coarse roots under low nutrients compared to high nutrients would indicate that fine root production is increasing in a plastic way, with plants producing more fine roots than expected based on their size. In a similar manner, ANCOVA was also used to indicate if root to shoot ratio and specific root length (SRL) results were based on active or passive plasticity, where *P. secunda* total biomass was used as the response and root traits (root to shoot ratio; specific root length) was used as a model factor. “Treatment” was included as a factor, and the factor by root trait (root to shoot ratio; specific root length (SRL)) interaction was used to determine if slopes of lines were on different trajectories in different treatments. When interactions were significant, regressions were then used to analyze the relationship between total biomass and root traits under different nutrient treatments. An increase in the slope of root traits (root to shoot, specific root length) to total biomass under low nutrients compared to high nutrients would indicate that root traits are increasing in a plastic way, with plants producing more roots than shoots and that plants are producing a greater amount of fine roots (i.e. higher specific root length).

Competition Experiment

In order to address whether phenotypic plasticity in response to *B. tectorum* is similar to that in response to low nutrients, and to determine if plasticity of certain traits allows for the survival of *P. secunda* in the face of invasion by *B. tectorum*, we conducted a competition experiment under controlled greenhouse conditions. Soil was provided by

Moana Nursery located in Reno, NV and laboratory analysis yielded low amounts of nitrogen (NH4 ppm=2.36, NO3 ppm=9.52, %N=0.04), and high alkalinity (pH=8.08). Perlite was added to the soil in a one to three ratio and placed in a cement mixer in order to homogenize the soil mixture. Both small and large pot sizes were used to allow for maximum rooting depth for both early seedling establishment and establishment after one growing season (Stuewe & Sons SC10 super 164mL, 3.8 diameter, 21cm depth for early and TP49 10cm width, 24cm height, 1.65L for late). Pots were fitted with polyester fiber squares in the bottom to prevent soil loss, filled with soil mixture and immediately watered. For our competition experiment, seeds were used from 48 families: 25 families from McClellan Peak and 23 from Bedell Flat. Out of the 25 families from McClellan Peak, 12 were originally from invaded areas and 13 were originally from uninvaded areas. Out of the 23 families from Bedell Flat, 10 were from invaded areas and 13 were from uninvaded areas. The community type distinction (plants originally from invaded and uninvaded areas) did not affect results, thus it is not included in the analysis presented here.

Poa secunda and *B. tectorum* seeds were sown over the course of four days from 6 December 2010 to 9 December 2010, in a complete random design. In each pot, one seed of *P. secunda* was placed directly into the soil using forceps (n=960 for both small and large pots). One seed of *B. tectorum* was sown directly adjacent to the *P. secunda* seed in half of the pots (n=480 for both small and large pots) in the competition treatments. Pots were misted once a day for the first two weeks following planting and thereafter small pots were watered once every four to seven days until time of harvest, while large pots were watered once a week until time of harvest, allowing soil to dry between waterings. No supplemental fertilizer was added for the entire duration of this experiment.

Two harvest times were conducted that align with natural growth phases of *P. secunda*: early seedling establishment and end of growing season, when plants showed signs of senescence. Early harvest of all small pots (n =960) occurred 67 days after date of emergence, taking place from 15 February 2011 through 24 March 2011, with harvest timed to correspond with the emergence date of each pot. Late harvest of large pots began approximately six month after date of emergence on 31 May 2011, at which time, *B. tectorum* had already produced seeds and *P. secunda* was becoming senescent. Due to the large number of late harvest pots and the time-consuming nature of the harvest, we could not harvest all of the plants, and we harvested above and below ground biomass for 778 pots out of 960 between 31 May and 25 June. We prioritized families for harvest based on families that showed variation in their plastic response to percent allocation of fine roots during the early harvest experiment and total root length from the previous nutrient experiment, including families with a range of plasticity values in the harvest group. The remaining 182 late harvest pots were collected for above ground biomass only between 26 June and 29 June. Plants that were harvested for roots and shoots of *P. secunda* were manually separated from *B. tectorum* based on root and leaf characteristics; *P. secunda* and *B. tectorum* roots were refrigerated and digitally scanned as in the previous nutrient experiment. Root measurements of all *P. secunda* from the early harvest were conducted along with all but 182 pots from the late harvest.

Analysis for Competition Experiment

As described in the nutrient experiment, ANOVA, MANOVA (1), and plasticity calculations (2) were used for both early and late harvest. Additionally, Spearman's non-parametric equations were used to determine whether traits might be adaptive (3). Finally, as in the nutrients study, we used ANCOVA to determine if results were based on active plasticity or passive plasticity (4).

(1) Our first analysis was conducted using analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) to ask a) if differences in original location of plant families (either Bedell Flat or McClellan Peak) had an effect on traits measured, b) if there were changes in means between treatments to determine if competition with *B. tectorum* had an effect on traits measured, c) if there were differences in means between families to determine genetic variability of populations with respect to traits measured, and d) to test the interaction between families and treatments to determine if families had similar responses to competition with *B. tectorum.* Additionally, the interactions between original location, treatment, and family and treatment were also tested using ANOVA and AMOVA to determine if plants from different populations or families differed in response to competition with *B. tectorum*. For the ANOVA analysis, the following *P. secunda* traits for both early and late harvest were used to test the effects of competition with *B. tectorum*, location, and family: total biomass, root to shoot ratio, leaf number (for early harvest only), specific root length (SRL), and percent allocation to root diameter size classes. The ANOVA model included the following factors: original location (fixed factor), family nested within original location (random factor), competition status (fixed factor, with or without competition with *B. tectorum*), the competition status by family interaction, and the competition status by location interaction. Although the competition

status by location interaction was included in our model, it was only significant for percent allocation of root diameter size classes, and further results of this interaction are not presented here. Several growth traits did not display a normal residual distribution for both early and late harvest analysis and standard transformations did not improve this. Total biomass was log transformed to improve distribution of residuals for early harvest analysis. Additionally, total biomass was transformed using the Box-Cox method and root to shoot ratio was log transformed for the late harvest analysis. For the MANOVA analysis, the Wilk's lambda method was used to determine significant affects of competition status, family, and the family by competition status interaction on percent allocation to all root diameter size classes. Significant MANOVA were followed by individual ANOVAs for each root diameter size class.

(2) Relative competitive performance index (CPI) was used to quantify the ability of *P. secunda* to tolerate *B. tectorum* in a competitive environment (Rowe & Leger 2010; Goergen et al. 2011). CPI is the percent decrease in plant performance when grown with competition compared to plant performance when grown without competition, and is different from strict biomass measurements of fitness because any decrease in plant size is compared relative to the original plant size at the starting point of competition. CPI is calculated as follows: [total $\text{biomass}_{\text{control}}$ – total $\text{biomass}_{\text{competition}}$] ÷ total $\text{biomass}_{\text{competition}}$. We used family means with and without competition to calculate total biomass for CPI for both early and late harvest. Additionally, percent allocation to the 10 root diameter size classes were calculated as [total length of a root diameter category (i.e. 0.0-0.1) ÷ total root length] x 100 for both early and late harvest, similar to the previous nutrient experiment. Using percent allocation calculation for each individual from the

early harvest, we calculated competition and control means for each family. Percent allocation of competition and control family means were then used to quantify the change in family-level plasticity for each root diameter size class as follows: percent $\text{allocation}_{\text{control}}$ – percent $\text{allocation}_{\text{competition}}$. Additionally, the sum of the total change in percent allocation of all root diameter size classes was also calculated for each family. Family means for specific root length (SRL) and root to shoot ratio were also calculated, and plasticity was determined as described above.

(3) For both early and late harvest analysis, Spearman's non-parametric correlations were used to compare the relationship between individual means of the following traits and plant performance (total biomass, CPI): percent allocation to each root diameter size class, root to shoot ratio, and specific root length (SRL). Spearman's non-parametric correlations were also used to test the effect of a plastic response of *P. secunda* families to competition by correlating family-level means of the following traits with plant performance (total biomass and CPI): change in percent allocation to each root diameter size class, sum of change in percent allocation to each root diameter size class, change in percent allocation of specific root length (SRL), and change in percent allocation of root to shoot ratio.

(4) To determine whether plasticity was ontogenetic or active, both ANCOVA (analysis of covariance) and regressions were performed between different plastic traits both with and without competition using the same methods as the nutrient experiment described above. Individual data was used to determine the top 10 strongest correlations between root diameter size classes. Additionally, in order to determine whether plasticity may be adaptive, we used the relationship between individual traits and performance

(Table 3) to compare the shift in allocation of root diameter (i.e. fine versus coarse root production) between control and competition plants to plant performance (i.e. total biomass) for both early and late harvest plants. Similar to the nutrient methods, ANCOVA was used to indicate if root to shoot ratio and specific root length (SRL) results were based on active or passive plasticity, where *P. secunda* total biomass was used as the response and root traits (root to shoot ratio; specific root length) was used as a model factor. "Treatment" was included as a factor, and the factor by root trait (root to shoot ratio; specific root length) interaction was used to determine if slopes of lines were on different trajectories in different treatments. When interactions were significant, regressions were then used to analyze the relationship between total biomass and root traits under competition status (with or without competition with *B. tectorum*). An increase in the slope of root traits (root to shoot, specific root length) to total biomass under competition with *B. tectorum* compared to control plants would indicate that root traits are increasing in a plastic way, with plants producing more roots than shoots and that plants are producing a greater amount of fine roots (i.e. higher specific root length). In a similar manner, in order to determine whether plasticity may be adaptive, we used the relationship between individual traits and plant performance (total biomass, CPI) (Table 3) to compare the shift in root traits (root to shoot ratio, specific root length) between control and competition plants to plant performance (i.e. total biomass) for both early and late harvest plants.

Lastly, to see if early traits could predict plant performance of *P. secunda* plants after one growing season with *B. tectorum*, early harvest plant traits for percent allocation to all root diameter size classes, root to shoot ratio, and specific root length (SRL) were correlated with final performance using Spearman's non-parametric correlations with late harvest total biomass of *P. secunda* under competition with *B. tectorum*. Similarly, early harvest plant traits for change in percent allocation of all root diameter size classes, sum of the change in percent allocation to all root diameter size classes, change in specific root length (SRL), and change in root to shoot ratio were correlated with final plant performance (total biomass, CPI) using Spearman's non-parametric correlations with total biomass of *P. secunda* for late harvest plants under competition with *B. tectorum*.

RESULTS

Nutrient Experiment Results: Effects of treatments

A wide range of emergence dates were recorded for all 200 seeded plants. Time to emergence ranged from as few as 9 days to as many as 31 days from date planted, with the majority of seedling emergence (approximately 70%) ranging from 11 to 17 days. Out of the 300 total seeds planted, twenty-five did not emerge at all.

Nutrient treatments significantly affected all response variables. In response to low nutrient treatment, *P. secunda* showed an approximately 27% decrease in biomass (Figure 1, Table 1), and a higher root to shoot ratio (Figure 2, Table 1). *P. secunda* had approximately 25% fewer leaves in the low treatment compared to the high treatment (Table 1); mean leaf number for families under low nutrients was 5.6 (SE±0.12), and 7.5 (SE±0.18) under high nutrients. Plants in the low nutrient treatment had a higher specific root length (SRL), meaning more fine roots (F=89.5, P<0.0001; 26.6±0.06 under low nutrients and 20.4±0.50 under high nutrients). In addition, percent allocation to root diameter size classes changed in response to low nutrients, with significant increases in allocation to fine root diameter classes 0.0-0.1mm and 0.1-0.2mm, and decreased allocation in larger root categories 0.3-0.4mm, 0.4-0.5mm, 0.5-0.6mm, and >2.0mm. (Figure 3, Table 1). Total biomass, leaf number, specific root length (SRL), percent allocation to root diameter size classes, and root to shoot ratio differed by family (Figure 4, Table 1), along with change in percent allocation (Table 1, Figure 5). The family by nutrient treatment interaction was not significant for most variables with plants from different families responding similarly to treatments (i.e. Figure 4). However, there was an almost significant response in total biomass, with some families increasing performance by almost 60% under high nutrients, and others showing slight decreases. This resulted in a wide range of values for the nutrient performance index (NPI) (Figure 6).

Plasticity and ontogenetic drift results

Plants produced more fine roots in response to low nutrients overall (Figure 5), and we assessed whether this production of fine roots was due to passive plasticity, where reduction of biomass under low nutrients results in the production of more fine roots due to small plant size alone. Ten root diameter size classes showed the highest correlations (ρ =-0.7270 through -0.4475, P<0.0001): 0.1-0.2mm and 0.3-0.4mm, 0.0-0.1mm and 0.3-0.4mm, 0.1-0.2mm and 0.5-0.6mm, 0.10.2mm and 0.4-0.5mm, 0.0-0.1mm and 0.4-0.5mm, 0.1-0.2mm and 0.6-0.8mm, 0.0-0.1mm and 0.2-0.3mm, 0.0-0.1mm and 0.5-0.5mm, 0.1-0.2mm and 0.8-1.2mm, 0.1-0.2mm and 1.2-2.0mm. ANCOVA showed that the slopes between low and high nutrients for five out of ten correlations were different and therefore changes in allocation were a result of active plasticity. For all significant differences in slopes, plants produced a greater amount of fine roots under low nutrients compared to high nutrients relative to their size, indicated by steep slopes (Figure 7). The five actively plastic correlations include the following: 0.0-0.1mm and 0.3-0.4mm ($F=4.9_{(1, 175)}$, P=0.0277), 0.0-0.1mm and 0.5-0.6mm ($F=5.9_{(1, 175)}$, P=0.0161, Figure 7a), 0.1-0.2mm and 0.5-0.6mm ($F=4.2_{(1, 175)}$, P=0.0412, Figure 7b), 0.1-0.2mm and 0.6mm-0.8mm ($F=5.3_{(1, 175)}$, P=0.0223, Figure 7c), and 0.1-0.2mm and 0.8-1.2mm ($F=13.5_{(1, 175)}$, P=0.0003, Figure 7d). ANCOVA showed that the slopes between high and low nutrients for both root to shoot ratio and specific root length (SRL) were on the same trajectory, which resulted in passive plasticity. This means that increased root to shoot ratio and increased specific root length (SRL) under low nutrients was simply a function of small plant size.

Competition Experiment Results: Effects of treatments

For the early harvest competition experiment, *P. secunda* plants emerged between 10 days to 37 days after date planted with approximately 70% of plants emerging 10-21 days from planting. Out of the 960 pots, thirty-eight *P. secunda* plants did not emerge.

Competition status, original location, and family differed significantly for most responses in the early harvest along with treatment by family interaction and location by treatment interaction. *P. secunda* showed an approximately 46% decrease in total biomass when grown with *B. tectorum* (Figure 8, Table 2). In a similar response to low nutrients, *P. secunda* showed an increase in root to shoot ratio of 14% when grown with *B. tectorum* (Figure 9, Table 2). There was an approximately 30% decrease in leaf number when *P. secunda* was grown in competition with *B. tectorum* (Table 2). Also similar to results from the low nutrient treatment, there was an increase in specific root length (SRL), indicating greater production of fine roots, of approximately 10%

(28.8±0.4 control, 31.6±0.5 competition; Table 2). Percent allocation to root diameter size classes changed with competition status in a similar manner as the response to low nutrients, with significant increases in allocation to fine root diameter classes 0.0-0.1mm, 0.2-0.3 and 0.3-0.4mm when grown with *B. tectorum*, and significant decreases in allocation to coarse root diameter classes 0.4 through 2.0mm (Figure 10, Table 2). Plants also differed in their allocation to root diameter sizes by location, family, and there was a location by treatment interaction. Plants from Bedell Flat had approximately 35% less biomass (3.6mg±0.2 for Bedell Flat and 5.2mg±0.2 for McClellan Peak; F=58.6, P<0.0001), and had approximately 6% fewer leaves (3.8±0.1 for Bedell Flat and 4.06±0.1 for McClellan Peak; F=5.6, P=0.0219) overall compared to plants from McClellan Peak. Plants from Bedell Flat also had greater specific root length (31.5±0.5 for Bedell Flat, 28.8±0.4 for McClellan Peak; F=11.0, P=0.0017). The location by treatment interaction was also significant for total biomass (F=5.9, P=0.0191), specific root length (F=12.5, P=0.0009), and percent allocation of root diameter size classes (F=3.5, P<0.0002, Table 2). Plants from McClellan Peak invaded areas were approximately 10% larger in biomass compared to plants from Bedell Flat invaded areas. Plants from Bedell Flat invaded areas also had an approximately 14% higher specific root length, and allocated more biomass to root diameter size classes 0.3mm through 1.2mm, compared to plants from McClellan Peak invaded areas.

In addition, family-level variation was also seen for most traits including total biomass, root to shoot ratio (Figure 11), and specific root length (SRL); however, no significant effects were seen between families in their leaf number (Table 2). Families also differed from one another in their change in percent allocation to root diameter size classes. While all families tended to shift allocation in a similar way, families showed an overall shift in allocation towards fine root classes 0.1 through 0.4mm, and a shift away from coarse root diameter classes 0.4-2.0mm under competition with *B. tectorum,* with some families more plastic than others (significant treatment by family interaction, Figure 12, Table 2). The treatment by family interaction was also significant for leaf number, where some families had a greater decrease in amount of leaves when grown in competition compared to control than other families (Table 2).

Correlation between traits and performance

Many traits were correlated with individual plant size (Table 3). The strongest correlations for early harvest were between total biomass (mg) and percent allocation of root diameter size classes 0.1-0.2mm, 0.2-0.3mm, 0.3-0.4mm, and 1.2-2.0mm with competition (Figure 13). Plasticity in allocation was also correlated with total biomass (Table 4) with the strongest relationships seen in change in percent allocation to root diameter size classes 0.3-0.4mm and 1.2-2.0mm with competition, along with total biomass (mg) and total change in percent allocation with competition (Figure 14). Allocating biomass to fine root diameter size classes 0.1-0.2mm and 0.2-0.3mm was positively correlated with total biomass when grown in competition with *B. tectorum,* and not significantly related to biomass in the control (Figure 13a, Table 3). Allocating roots to the next diameter size class up, 0.3-0.4mm, was correlated with decreased plant biomass under competition status and control (Figure 13b, Table 3). Allocating biomass to coarse root classes 0.6 through >2.0mm was positively correlated with increased biomass for both competition and control plants. In the control, there was a significantly negative correlation between allocation to 0.4-0.5mm and total biomass and a significantly positive correlation between 0.5-0.6mm and total biomass, which was not seen when plants were grown in competition (Table 3). In addition, increased root to shoot ratio was positively correlated with size when grown with *B. tectorum* but not in the control (Table 3). There was a negative correlation between total biomass and increasing specific root length (SRL) under competition status and control (Table 3).

P. secunda plasticity for root diameter class 0.3-0.4mm was correlated with a decrease in total biomass, whereas plasticity in more coarse root diameter classes 0.6-0.8mm through 1.2-2.0mm was correlated with an increase in total biomass (Figure 14a & 14b, Table 4). A similar relationship was seen with competitive performance index (CPI), where plasticity in root class 0.3-0.4mm was correlated with a decrease plant performance and plasticity in coarse root diameter classes 0.6-0.8mm through 2.0mm was correlated with an increased plant performance. Overall plasticity in root diameter size and plasticity in specific root length (SRL) were negatively correlated with plant size under competition (Figure 14c, Table 4). However, plasticity in root to shoot ratio was correlated with larger plant size and better competitive ability (CPI) under competition with *B. tectorum* (Table 4).

Plasticity and ontogenetic drift

The following ten root diameter size class pairs showed the highest negative correlations for the early harvest experiment (ρ =-0.8509 through -0.3869, P<0.0001):

0.2-0.3mm and 0.4-0.5mm, 0.2-0.3mm and 0.5-0.6mm, 0.1-0.2mm and 0.4-0.5mm, 0.2-0.3mm and 0.6-0.8mm, 0.1-0.2mm and 0.5-0.6mm, 0.2-0.3mm and 0.8-1.2mm, 0.1-0.2mm and 0.6-0.8mm, 0.3-0.4mm and 0.8-1.2mm, 0.3-0.4mm and 1.2-2.0mm, 0.3-

0.4mm and 0.6-0.8mm. ANCOVA showed that the slopes between control and competition for five out of ten correlations were different and therefore a result of active plasticity. In all cases, plants produced a greater amount of fine roots under competition compared to control relative to their size (Figure 15); illustrated by a greater slope. The five actively plastic correlations include the following: 0.2-0.3mm and 0.5-0.6mm ($F=14.7_{(1, 808)}$, P=0.0001, Figure 17), 0.2-0.3mm and 0.6-0.8mm ($F=39.5_{(1, 808)}$, P <0.0001), 0.2-0.3mm and 0.8-1.2mm ($F=6.2_{(1, 808)}$, P=0.0128), 0.1-0.2mm and 0.6-0.8mm ($F=12.0_{(1, 808)}$, P=0.0006), 0.3-0.4mm and 1.2-2.0mm ($F=4.7_{(1, 808)}$, P=0.0299). Plants produced more fine roots in response to competition overall (Figure 12), and regressions between root diameter size classes showed that the slopes between control and competition plants differed, and therefore were a result of active plasticity. Correlations between percent allocation to 0.1 through 0.3mm and total biomass for *P. secunda* under competition were positively correlated (Table 3), and thus the increase in the ratio of 0.1 through 0.3mm fine root class to 0.5 through 1.2mm coarse root class relative to control plants may be a shift towards adaptive plasticity. In contrast, correlations between 0.3-0.4mm and total biomass under competition were negative (Table 3) thus the increase in the ratio of 0.3-0.4mm fine root class relative to control plants may be a maladaptive shift. Additionally, ANCOVA showed that the slopes between control and competition for root to shoot ratio ($F=4.5_{(1, 800)}$, P=0.0340) and specific root length ($F=17.0_{(1, 803)}$, P<0.0001) correlations were different and therefore a result of active plasticity. Comparisons with correlations between root to shoot and total biomass for *P. secunda* under competition were positively correlated (Table 3), and this the increase in the ratio of roots to shoots relative to control plants may be a shift towards adaptive plasticity. Comparison with correlations between specific root length (SRL) and total biomass for *P. secunda* under competition was negatively correlated (Table 3), and thus the increase in the ratio of specific root length (SRL) relative to control may be a shift towards maladaptive plasticity.

Effects of treatment-late harvest

For the late harvest competition experiment, *P. secunda* emergence dates spanned a twenty-three day period, with most plants emerging 10-21 days from date planted. Out of the 960 pots total, fifty-six *P. secunda* plants did not emerge.

Competition status affected all response variables for late harvest plants (Table 5). Only percent allocation to root diameter classes differed significantly by location. Plants produced approximately 88% less biomass (Figure 16), had a higher specific root length (11.4±1.0 control, 20.2±0.3 invaded), had a 44% higher root to shoot ratio (Figure 17), and changed in overall allocation in response to competition (Figure 18, Table 5). As in the early harvest, plants allocated more biomass to fine root diameter size classes 0.1-0.4mm under competition and significantly less to 0.5mm through >2.0mm (Figure 18, Table 5). *P. secunda* plants also showed differences in percent allocation to all root diameter sizes by original location, where plants from McClellan Peak showed greater allocation to root diameter classes 0.0-0.1mm and 0.3 through 0.8mm and Bedell Flat showed greater allocation to 0.1-0.3mm and 0.8 through >2.0mm. Families differed from one another in their root to shoot ratio (Figure 19, Table 5). Competitive performance index (CPI) was consistent among families, with strong reduction in response to competition in all families (Figure 20). Families showed variation in their plastic response to percent allocation of root diameter size classes (significant family by treatment interaction) with some families exhibiting greater plasticity than others in response to *B. tectorum* (Figure 21, Table 5). Though they differed in degree, all families showed a shift toward fine root production of 0.1-0.4mm in root diameter under competition and shift away from producing coarse roots 0.6->2.0mm (Figure 21, Table 5). A location by treatment interaction was also seen for percent allocation, where plants from Bedell Flat invaded areas allocated more biomass to fine root diameter size classes 0.0mm through 0.3mm and to coarse roots 0.6mm through 2.0mm compared to McClellan Peak in which plants from invaded areas allocated more biomass to root diameter size classes 0.3-0.6mm and >2.0mm (Table 5).

Correlations between traits and performance

Analysis from Spearman's correlations between root traits and performance for the late harvest competition experiment yielded similar results to the early harvest and nutrients experiment for coarse (>0.6) roots (Table 3). However, for the early harvest, allocating roots to size diameter class 0.1-0.2mm and 0.2-0.3mm was correlated with increased biomass when exposed to competition, whereas in the late harvest, allocation to fine size diameter classes 0.0-0.1mm through 0.2-0.3mm was correlated with decreased plant biomass in competition (Figure 22a). Similar to early harvest results, allocation of roots to coarse diameter size classes 0.6-0.8mm through >2.0mm was significantly correlated with increased total biomass (Figure 22, Table 3). Likewise, having a high specific root length (SRL), which indicates producing a greater amount of fine roots, was correlated with decreased total biomass under competition with *B. tectorum* for early and late harvest plants (Table 3).

Plasticity for the finest root diameter class 0.0-0.1mm was correlated with a decrease in total biomass for the late harvest (Figure 23a, Table 4). Consistent with results for the early harvest, plasticity for coarse root diameter classes 0.6-2.0mm for late harvest plants under competition was correlated with an increase in plant performance (CPI) (Table 4). Plasticity for specific root length (SRL) under competition was also correlated with a decrease in plant performance for CPI in both early and late harvest experiments, whereas overall plasticity to root diameter sizes under competition was correlated with a decrease in plant performance for CPI in the late harvest (Figure 23b, Table 4). In contrast, in the control treatment, plasticity for root diameter size class 0.2-0.3mm was correlated with increased plant biomass (Table 4). Additionally, plasticity in root coarse root diameter size classes 0.4 through >2.0mm were correlated with decrease in plant biomass under control, while under competition root diameter size classes 0.5 through 2.0mm were correlated with increased biomass (Table 4).

Plasticity and ontogenetic drift

For the late harvest experiment, ANCOVA showed that the slopes between control and competition for nine out of ten correlations were different and therefore a result of active plasticity. Plants produced a greater amount of fine roots for six correlations and more coarse roots for three correlations under competition compared to control relative to their size (Figure 24). The nine actively plastic correlations include the following: 0.1-0.2mm and 0.5-0.6mm ($F=63.2_{(1,672)}$, $P<0.0001$), 0.1-0.2mm and 0.6-0.8mm ($F=18.1_{(1,672)}$, $P<0.0001$), 0.1-0.2mm and 0.8-1.2mm ($F=28.7_{1,672)}$, $P<0.0001$), 0.1-0.2mm and 1.2-2.0mm ($F=83.2_{(1,672)}$, $P<0.0001$), 0.1-0.2mm and >2.0mm ($F=75.0_{(1,672)}$, $P<0.0001$), 0.2-0.3mm and 0.5-0.6mm ($F=10.9_{(1,672)}$, $P=0.0010$), 0.2-0.3mm and

0.6-0.8mm ($F=59.9_{(1, 672)}$, $P<0.0001$, Figure 24a), 0.2-0.3mm and 0.8-1.2mm ($F=67.4_{(1,672)}$, $P<0.0001$, Figure 24b), 0.2-0.3mm and >2.0 ($F=72.8_{(1, 653)}$, $P<0.0001$). Although plants produced more fine roots in response to competition overall (Figure 21), correlations between root diameter size classes show that the slopes between control and competition plants differ for most traits measured and are therefore a result of active plasticity. Out of the nine actively plastic traits measured, six exhibit a shift towards maladaptive plasticity and three exhibit a shift towards adaptive plasticity. The six correlations that exhibit a shift towards maladaptive plasticity all show that *P. secunda* produced more fine to coarse roots (Figure 24). Comparisons with correlations between fine root diameter classes 0.1mm through 0.3mm and total biomass for *P. secunda* under late harvest competition (Table 3) suggest that the increase in the ratio of 0.1 through 0.3mm fine root classes and decrease in 0.5mm through >2.0mm coarse root classes relative to control plants may be a shift towards maladaptive plasticity (Table 3). In contrast, the three plastic responses that exhibit a shift towards adaptive plasticity show that *P. secunda* increased allocation to coarse roots and decreased allocation to fine roots under competition. Plants that produced a lower amount of fine roots in competition with *B. tectorum* compared to control plants relative to their size include correlations between 0.2-0.3mm and 0.5-0.6mm, 0.2-0.3mm and 0.6-0.8mm, and 0.2-0.3mm and 0.8-1.2mm (Figure 24a,b). Comparisons with correlations between fine root diameter classes 0.2-0.3mm and total biomass for *P. secunda* under late harvest competition (Table 3) suggest that the decrease in the ratio of 0.2-0.3mm fine root classes and increase in 0.5mm through 1.2mm coarse root classes relative to control plants may be a shift towards adaptive plasticity (Table 3). Additionally, ANCOVA showed that the slopes between control and competition for root to shoot ratio ($F=121.1_{(1, 670)}$, $P<0.0001$) correlation was different and therefore a result of active plasticity, while the slopes between control and competition for specific root length (SRL) was the same and therefore a result of passive plasticity. Comparisons with correlations between root to shoot and total biomass for *P. secunda* for control plants was positively correlated (Table 3), and thus the increase in the ratio of roots to shoots relative to plants under competition may be a shift towards adaptive plasticity. Comparisons with correlations between root to shoot ratio and total biomass for *P. secunda* under competition was positively correlated (although this finding is not significant; Table 3), and thus the decrease in the ratio of roots to shoots relative to control may be a shift towards maladaptive plasticity.

Predicting late harvest plant performance

Early harvest plant traits for percent allocation to all root diameter size classes, root to shoot ratio, and specific root length (SRL) were examined for correlations with late harvest total biomass of *P. secunda* under competition with *B. tectorum*. Results show that early harvest percent allocation to fine roots classes 0.0-0.3mm was negatively correlated with total biomass for late harvest plants under competition, which suggests that allocation to fine roots may be a predictor for decreased plant performance under competition with *B. tectorum* after one growing season (Table 6). In addition, early harvest plants that allocated biomass to coarse root diameter size classes 0.3 through >2.0mm showed a positive correlation with total biomass of *P. secunda* late harvest plants under competition with *B. tectorum* (Table 6). Lastly, early harvest root to shoot ratio, and specific root length (SRL) showed a negative correlation with late harvest total biomass of *P. secunda* under competition with *B. tectorum* (Table 6).

Early harvest plant traits for change in percent allocation of all root diameter size classes, sum of the change in percent allocation to all root diameter size classes, change in specific root length (SRL), and change in root to shoot ratio were correlated with total biomass of *P. secunda* for late harvest plants under competition with *B. tectorum*. Significant results show that only change in root to shoot ratio of early harvest plants was negatively correlated with total biomass of *P. secunda* under competition with *B. tectorum* (Table 7).

DISCUSSION

Although environmental change is ubiquitous, no genetic trait has evolved plasticity so perfect that it is capable of increasing fitness in every instance. Rather, lack of reliable environmental cues, constraints that act directly on plasticity, and the fact that some traits can be highly correlated allow for maladaptive phenotypes to persist in populations (Schlichting 1986; Pigliucci 2005; Valladardes et al. 2007; DeWitt et al. 2008). In the Great Basin the primary invader, *B. tectorum,* is a very strong competitor for limited belowground resources (Mack 1981; Chambers et al. 2007). The ability for natives to respond to belowground competition for limited resources through adaptive root plasticity may be an important trait for survival in invaded systems. Specifically, investment in fine roots in invaded systems may allow plants to gain greater access to limited resources, since fine roots serve as the primary foraging mechanism for plants in competition (Caldwell et al. 1996; Hodge 2004; Gregory 2006). I investigated whether *P. secunda*, a common native perennial grass in sagebrush ecosystems, could 1) respond plastically to directly-manipulated nutrient availability, 2) respond in a similar way to the presence of *B. tectorum*, and 3) adaptively respond to this change. The experiment was conducted in a controlled greenhouse environment and focused on how root traits affected performance during seedling establishment and after one growing season for plants in competition, and I examined family-level variation to see if traits are inherited. Because root traits can change over time as plants get larger, I also determined if changes observed were due to active plasticity or passive plasticity due to ontogenetic drift.

Results from the experiment showed that plants under low nutrients and in competition with *B. tectorum* responded similarly: they were smaller, allocated a greater percentage of biomass to the production of fine roots, and families varied in their overall response to nutrients and competition. Plants grown in competition with *B. tectorum* showed that increased allocation to fine roots was positively correlated with increased plant performance in the early seedling stage, but not after one growing season. However, since small plants tend to produce more fine roots as a function of size alone, I also assessed whether the observed increase in fine roots under low nutrients and in competition with *B. tectorum* was simply due to a reduction in growth rate, or passive plasticity, or if this response was a result of active plasticity, where plants allocated a greater portion of biomass to fine roots in the presence of *B. tectorum*. Results showed that after one full growing season, plants exhibited a greater degree of active plasticity in their percent allocation to different root diameter size classes overall compared to early seedlings. For instance, correlations for late harvest plants between fine to coarse roots and plant performance show that plants allocated a greater amount of biomass to fine roots relative to their reduced size for six out of nine correlations tested. Additionally, late harvest plants also showed a higher root to shoot ratio and allocated a greater amount of biomass to the production of coarse roots for three out of nine correlations tested.

Moreover, late harvest plants that showed a shift towards producing a greater amount of coarse roots under competition (a trait positively correlated with an increase in total biomass) may demonstrate a shift towards adaptive plasticity. Similar to late harvest plants, early harvest plants also showed a higher root to shoot ratio under competition. However, in contrast to late harvest plants, early seedlings had a higher specific root length (SRL) and produced a greater amount of fine roots in all correlations tested relative to their reduced size. For early harvest plants, increased production of fine roots was positively correlated with an increase in total biomass and better competitive ability (CPI). This may demonstrate a shift towards adaptive plasticity for early harvest plants, where increased production of fine roots may have played a critical role in allowing for greater resource acquisition when grown in competition with *B. tectorum* in early interactions.

For our first research question, we assessed whether *P. secunda* could respond plastically to directly-manipulated nutrient availability and whether this response varied by family. We found that individuals did respond plastically to nutrient availability, where under low nutrients, plants were smaller and produced a fewer number of leaves. In general, large plant size has long been recognized as playing a large role in competition for resources, where a larger below and aboveground root and shoot systems ensures greater access limited resources compared to a smaller root system (Caldwell et al. 1996). However, recent studies have shown that fine roots may be more important than large size alone, especially in low resource environments where fine roots can help exploit localized nutrient zones (Caldwell et al. 1996; Hodge 2003; Aarssen et al. 2006; Rowe & Leger 2010). Results from our study show that *P. secunda* produced more fine roots (i.e. higher root to shoot ratio, and a higher specific root length) and allocated a greater amount of biomass to the production of fine root diameter size classes 0.0mm through 0.2mm under low nutrients. These results are in alignment with studies that show that plants can allocate biomass to the production of fine roots and even change their architecture in response to limited resources. For instance, changes in fine and coarse roots have been observed with root branching patterns where some roots can take on a herringbone appearance with greater abundance of fine roots in response to heterogeneous soils in order to gain greater access to limited resources that may be located deeper within soil profiles (Fitter 1982; Gregor 2006). In contrast, some roots can take on a dichotomous structure in response to ample resources in which shallow, coarse roots allow for numerous root tips that increase soil exploration (Fitter 1982; Wright & McConnaughay 2002; Gregor 2006). Additional studies suggest that fine root production in low resource environments allows for greater success, especially when resources are limited due to competition with invasives (Caldwell et al 1996; Arrendondo & Johnson 1999; Hodge 2003; Rowe & Leger 2010). For instance, populations of *E. multisetus* developed more fine roots in invaded systems compared to field controls and these fine roots had a negative impact on *B. tectorum* (Rowe & Leger 2010; Leger & Espeland 2010).

Since environments exert a strong influence on growth and development, we also assessed whether root plasticity in allocation to fine roots was simply a result of ontogenetic drift, due to reduced growth rate under low nutrients. Evans (1972) originally described ontogenetic drift as a trait that changes in a predictable way throughout growth and development. In this view, “passive plasticity” can be described as any change in measured traits that result from environmentally-induced changes in growth rate, where traits vary as function of position along a single ontogenetic program (Wright & McConnaughay 2002). Passive plasticity can be exemplified with the typical increased production of fine roots during early plant growth, which allows for firm anchoring and resource acquisition required for successful seedling establishment (Aguirre & Johnson 1991). In contrast, "active plasticity" occurs when the environment induces a change in the ontogenetic trajectory of a trait, where traits change, not as a function of their size at a single point in time, but rather, as a result of an environmental cue which when sensed by the organism, induces a novel phenotypic response (Wright & McConnaughay 2002, Weiner 2004). Results from the nutrient experiment show that seedlings exhibited active plasticity for percent allocation to root diameter size classes for five out of ten correlations tested. This means that plants showed an increase in the ratio of fine to coarse roots in response to low nutrients relative to their small size compared to plants under high nutrients, and that this occurred for half of the correlations tested.

In addition to assessing *P. secunda* response to nutrient treatments, we also assessed whether families varied in response to overall nutrients. This was an important assessment since the primary mating strategy is facultative apomixis and, therefore, populations have the potential to consist of entirely all clonal individuals (Kellogg 1990). Results showed that families differed in their overall response to nutrients for traits measured. Specifically, families varied in their total biomass, root to shoot ratio, leaf number, specific root length (SRL), and in their percent allocation to different root diameter size classes, where some families exhibited greater plasticity than other families for traits measured. These results demonstrate that families were not all clones and plasticity for traits is inherited. This particular finding was pivotal in allowing us to further investigate whether the *P. secunda* produced more fine roots when grown with *B. tectorum* and whether this response was adaptive.

For our second question, we investigated whether *P. secunda* responded in a similar way to the presence of *B. tectorum*. Results from both early and late harvest show similar results to the nutrient experiment, where *P. secunda* plants were smaller and produced a fewer number of leaves in response to *B tectorum*. In addition, *P. secunda* produced more fine roots in response to *B. tectorum*, where plants had a higher root to shoot ratio, a higher specific root length (SRL) and a greater percent allocation to fine root diameter size classes. Specifically, both early and late harvest plants produced more fine root diameter classes 0.1mm through 0.4mm in response to competition. This result is comparable to low nutrient plants, which shifted their allocation to producing more 0.0mm through 0.2mm fine roots. The increased production of fine roots in response to competition again demonstrates that plants perceive the presence of an invader in a similar manner as a nutrient deficiency.

In a similar manner to the nutrient experiment described above, I was also interested in assessing whether the increase in fine root production was caused by a reduction growth rate alone, or ontogenetic drift (Coleman et al. 1994; Weiner 2004). Results from the early harvest were similar to that of the nutrient experiment, where seedlings grown under low nutrients and in competition with *B. tectorum* showed an actively plastic response in their percent allocation to root diameter size classes for half of the correlations between fine and coarse root diameter size classes. This means that *P. secunda* showed an increase in the ratio of fine to coarse roots relative to their reduced size compared to control plants for half of the root diameter size class correlations tested. In contrast to the nutrient experiment, early harvest plants showed an actively plastic response to specific root length (SRL) and both early and late harvest plants showed an actively plastic response in their root to shoot ratio compared to control plants. Overall, late harvest plants exhibited a greater degree of active plasticity overall compared to seedlings from the early harvest and nutrient experiment, where in ten out of the twelve correlations tested between fine and coarse root diameter size classes, root to shoot ratio, and specific root length (SRL) showed differences in their ontogenetic trajectory, or slope, between plants grown with and without competition. Unlike seedlings from the early harvest and nutrient experiment, results for late harvest plants showed that out of the nine actively plastic correlations between fine and coarse root diameter size classes, six of these correlations showed an increase in the ratio of fine to coarse roots, while the remaining three correlations showed an increase in the ratio of coarse to fine roots. This means most of the observed differences were not simply a result of reduced growth rate, but rather, *P. secunda* made more fine roots in response to competition with *B. tectorum* relative to their small size for some root classes, but also produced more coarse roots of other root classes in response to competition.

Similar to the nutrient experiment, I assessed whether families varied in response to competition for both early and late harvest plants. Results showed that families differed from one another for traits measured, including total biomass (significant for early harvest only), root to shoot ratio, specific root length (significant for early harvest only), and percent allocation to different root diameter size classes. This again demonstrates genetic variation in these populations, as well as that families differ in their ability to be plastic for traits measured, where some families are more plastic than others, and that plasticity is inherited.

I also assessed whether the two sample populations of *P. secunda* varied by original location (Bedell Flat and McClellan Peak) for traits measured. For early harvest plants, results showed that the two populations of *P. secunda* varied significantly, where plants from Bedell Flat experienced a greater reduction of total biomass, had fewer leaves and had an overall increase in fine roots (i.e. higher SRL). Conversely, plants from McClellan Peak showed an overall greater competitive performance (decreased CPI) compared to plants from Bedell Flat (although this was not found to be significant). This finding is consistent with studies showing that *P. secunda* populations from McClellan Peak are more tolerant of *B. tectorum* overall compared to *P. secunda* plants from Bedell Flat (Goergen et al. 2011).

Lastly, we investigated whether plasticity was adaptive for *P. secunda* plants grown in competition with *B. tectorum* for both early seedlings and after one growing season. For early harvest plants in competition, results showed that change in percent allocation to the production of more fine root diameter classes 0.1mm through 0.3mm was positively correlated with increased total biomass, and that these plants were more competitive overall (i.e. decreased CPI). Likewise, having a higher root to shoot ratio during early seedling establishment was positively correlated with total biomass for plants in competition with *B. tectorum*. Even after ontogenetic affects were taken into account, early harvest plants consistently showed that exhibiting a higher root to shoot ratio and allocating biomass to fine root diameter classes 0.1mm through 0.3mm may be an adaptive response to competition. In contrast, initial correlations between root to shoot ratio and plant performance for late harvest plants showed a positive relationship between increased root to shoot ratio and total biomass (although this finding was not significant). After ontogenetic effects were taken into account, plants continued to show a decrease in root to shoot ratio under competition, which may suggest a maladaptive response. This contradicts previous studies that consistently show that having a higher root to shoot ratio in invaded systems increases plant performance (Leger 2008; Rowe & Leger 2010; Goergen et al. 2011). In addition, initial correlations between change in percent allocation of root diameter size classes and plant performance for late harvest plants showed a negative relationship between increased fine root production and total biomass, and that allocating biomass to the production of fine roots was less competitive overall (i.e. increased CPI). Likewise, after ontogenetic affects were taken into account, six out of the nine correlations tested showed an increase in the ratio of fine root diameter size classes 0.1mm through 0.3mm and a decreased in the ratio of coarse root diameter size classes 0.5mm through >2.0mm, which may be maladaptive.

In general, there may be several reasons why the increased ratio of fine to coarse roots proved to be maladaptive for late harvest plants. For instance, traits can respond simultaneously to various environmental stressors and traits may be highly correlated to important functions that are under the influence of natural selection. Since fine root production is highly associated with the function of increased resource uptake in low resource environments, then producing more fine roots in response to stress from competition may have proven to be the best strategy in the absence of *B. tectorum*, since in the past, natural selection has favored this response as being the optimal phenotype, even though this was not the case in this experiment. In a similar manner, maladaptive responses can also occur when plants are initially correct in sensing their environment, but then the environment changes and organisms are then faced with coping with novel stimuli (DeWitt et al. 1998). For example, stem elongation in plants is more advantageous under competition since longer stems allow for greater access to light, but long stems may be maladaptive for changes in environment such as high winds or freezing temperatures (Gedroc et al. 1996). Our results suggest that although allocating a greater percent of biomass to fine roots during early growth and development was beneficial for successful seedling establishment in limited resource environments, as plants matured and competition between *P. secunda* and *B. tectorum* intensified, continued production of fine roots over more coarse roots proved to be a maladaptive response to competition. Rather, allocating biomass to the production of more coarse roots was seen as a more advantageous strategy in that coarse root diameter size classes were positively correlated with increased plant size. In general, coarse roots serve as the primary energy storage organs of plants, where the products of photosynthesis can temporarily reside until a later time when these products are relocated to above ground structures responsible for reproduction (Gregory 2006). Lastly, associated plasticity costs may also be responsible for lack of more coarse root production, which implies that use of limited resources (i.e. carbon energy reserves, time) ensures that there is a trade-off between producing more fine roots versus coarse roots (DeWitt et al. 2008).

However, not all correlations between fine and coarse root diameter size classes tested suggested a shift towards a maladaptive response for late harvest plants in competition with *B. tectorum*. Results also show that three out of nine actively plastic responses suggest a shift towards adaptively plastic. Specifically, these plastic responses included an increase in production of coarse root diameter classes 0.5mm through 1.2mm and a decrease in production of fine root diameter class 0.2-0.3mm. Moreover, this shift was correlated with increased total biomass. This shift may indicate that *P. secunda* plants were just beginning to adjust their allocation in an adaptive manner in response to competition at the end of one growing season, in which the shift from away from fine root production towards more coarse root production may require additional seasonal growth as well as continuous environmental stimulation from competition with *B. tectorum* (DeWitt et al. 2008; Pigliucci 2005; Valladares et al. 2007).

Again, while it may be beneficial for plants to produce more fine roots early in ontogeny, results suggest that it may be more advantageous for plants to shift their allocation to produce a greater amount of coarse roots during later stages of growth, which allows for increased resource storage and increased size. In general, differences in plasticity between early and late growth demonstrate how some plants can adjust their plasticity at different times throughout their growth and development (i.e. early versus late growth phases). For instance, *P. secunda* early harvest plants shifted their ontogenetic growth to produce more fine roots, whereas some late harvest plants that may have demonstrated adaptive plasticity shifted their allocation to produce less fine roots in response to competition. Additionally, *P. secunda* plants after one growing season demonstrated a higher degree of active plasticity compared to early seedlings for traits measured. Interestingly, this result is contradictory to some studies, which suggest that active plasticity in plants tends to decrease later in development compared to early development (Wright & McConnaughay 2002). Additionally, studies have shown that plants may plastically respond to heterogeneous environments only during a certain period in their growth and development, after which time, ontogeny may become fixed (Wright & McConnaughay 2002). However, the question of whether *P. secunda* can vary in their expression of ontogenetic plasticity relative to their growth phase and in response to the changing environment was not directly tested. Rather, each plant was tested in response to discrete treatments, including nutrients (either low or high) and competition status (with or without *B. tectorum*). Therefore, further investigation into how *P. secunda* responds to a continuum of novel stimuli over the course of growth and development is still needed and may shed light on the resiliency of native populations to heterogeneous environments on the landscape (i.e. nutrient patches, climate change). This could be done by repeat harvests, which would facilitate the comparisons of multiple treatments throughout growth and development as well as throughout the growing season (Coleman et al. 1994).

An assessment of *P. secunda* populations in the field after a second growing season in invaded systems would give further insight into how native populations are able to persist on the landscape. While *B. tectorum,* an annual species, senesces at the end of the growing season, *P. secunda* a perennial species, does not. Rather, dormant *P. secunda* plants in invaded systems tend to remain established after one full growing season. *P. secunda* plants may therefore have an increased size advantage over *B. tectorum* beginning at the onset of the second growing season with these invasive annuals. Over the long-term, *P. secunda* plants in invaded systems may have a competitive advantage over *B. tectorum* as each additional year brings increased biomass and potentially greater fecundity.

Ultimately, long-term persistence of *P. secunda* populations on the landscape depends heavily on adaptive traits to withstand competition from invasives (Leger 2008, Leger & Espeland 2010 Rowe & Leger 2010, Goergen et al. 2011). Since natural selection favors traits that increase fitness, the ability to be plastic for maladaptive traits may impede the evolution of adaptive plasticity in invaded systems (Ghalambor et al. 2007). Moreover, if plasticity for a trait is maladaptive, then selection may favor fixed traits or genetic variation of non-plastic traits that induce no costs (Callahan et al. 2008). If this is the case, then variation in the phenotypes expressed by plasticity for maladaptive traits will likely decrease in the population. Conversely, if organisms harbor the ability to express an adaptively plastic trait, but in doing so, individuals incur costs that decrease overall fitness, then populations should also favor fixed traits over the ability to be plastic. In both cases, local adaptation of fixed traits may then be favored by natural selection in invaded systems and populations may experience differentiation from one another (Pigliucci & Murren 2003; Lande 2009). Based on our results, the ability for *P. secunda* to produce more fine roots during early growth and more coarse roots after one growing season in competition with *B. tectorum* may demonstrate a shift towards adaptive plasticity. However, the ability to be plastic overall for percent allocation to different diameter size classes did not exhibit perfect plasticity when grown with *B. tectorum.* This may be due to differences among families in their ability to express the optimal phenotype, where some families produced more optimal phenotypes than others. Over time, family-level variation in the expression of plasticity for this trait may become greatly reduced through genetic assimilation resulting in the canalization of the optimal phenotype (Lande 2009). In this case, canalization will allow individuals to express this trait without great cost to fitness (Pigliucci & Murren 2003; Callahan et al. 2008; Lande 2009). Additionally, directional selection may enhance the optimal phenotype for the populations tested, which would reduce variation for this trait and, again, lead to local adaptation (Ghalambor et al. 2007). Ultimately, in order to accurately assess the outcome of Great Basin native plant populations in invaded systems, it is important to consider further testing of additional wild populations on a much broader scale. In doing so, any *P. secunda* populations that exhibit increased fitness due to the ability to express adaptively plastic traits may serve as a source of restoration material in invaded systems.

CONCLUSION

Native plant communities of the Nevada Great Basin are experiencing a rapid shift from native perennial grasses to invasive annual grasses. However, remnant populations of natives are still able to persist on the landscape even in the face of invasion (Leger 2008, Leger & Espeland 2010 Rowe & Leger 2010, Goergen et al. 2011). The ability to respond to below ground competition for limited resources through adaptive root plasticity may be an important trait for survival of native plants in invaded systems. To date, the ability of native perennial grasses to plastically respond to invasion has received relatively little attention. Rather, much research has focused on the ability for invasive species to plastically adapt to novel environments (Arredondo et al. 1998; Evans et al. 2001; Alpert et al. 2002; Gurevitch et al. 2004; Bradley et al. 2004; Bassdorf et al. 2005; Callaway et al. 2005). In order to improve restoration efforts in the Great Basin, more research needs to focus on identifying key adaptive traits that allow natives to be success in the face of invasion, and plasticity in response to invasion may be one such trait.

The nutrient experiment served as pilot study for the focal species, *P. secunda* in assessing the adaptive plasticity of traits to low resource environments. Determining whether *P. secunda* would respond to nutrient availability through greater resource allocation to fine roots served as the first step in showing that these populations do respond plastically to environmental stress. This result is especially important when considering the primary mating strategy of *P. secunda* is facultative apomixis. In general, populations that exhibit facultative apomixis may hypothetically be comprised of as few as one genotype due to cloning. Therefore, our results showing phenotypic plasticity in response to nutrient availability suggests possible underlying genetic variation.

Moreover, this research has important implications for improving current restoration practices in the Nevada Great Basin. An increased demand for use of local native grasses in restoration has led restorationists to consider the genetic integrity of native seed sources (Jones and Johnson 1998). Currently, restoration in the Nevada Great Basin is implemented using agricultural seed that is produced for a wide range of locations. Plants that have been produced commercially are provided ample amounts of nutrients and water, all of which do not reflect the natural environmental conditions (precipitation, soil composition, climate, etc.) of the various locations where the seed will eventually end up for restoration. These and other “unnatural pressures” (harvest techniques, etc.) create genotypes that may not be adapted to their environmental restoration locations. Several studies indicate that using nonlocal genotypes in restoration may be detrimental for two important reasons: 1) nonlocal genotypes may be unable to establish, and 2) outbreeeding depression may occur caused by crossing any pre-existing native populations with seed adapted to different environments (McKay et al. 2005). Furthermore, agriculturally grown seed may lack the genetic diversity needed to create locally adapted populations. Genetic diversity allows for natural selection to act upon a diverse array of inherited traits, thereby allowing the evolutionary process to occur and locally adapted populations to establish. Over time, the result is a more sustainable native population. Lack of genetic variation within populations can therefore constrain local adaptation (Leimu & Fischer 2008). Federal and state agencies are now beginning to recognize the importance of the evolutionary potential found in genetically diverse restoration material (McKay et al. 2005). Native populations that harbor a significant level of genetic diversity, unlike that of agriculturally grown seed, will allow native populations to be more resilient in the face of greater ecological disturbances (i.e. invasion by exotics) (Lau 2008). This research shed light on how native populations of *P. secunda* can adaptively respond to novel environmental stimuli, such as invasion from *B. tectorum*, via phenotypic plasticity. In turn, this will allow restorationsists to identify native plant populations that may become locally adapted to persist in the face of disturbance from *B. tectorum*. Ultimately, the results of this research will better inform restorationists of where to look for such locally adapted genotypes as plants from these locations can serve as a source of seed production and collection and thus the improvement of current restoration practices.

REFERENCES

Aarssen, L.W., B.S. Schamp, & J. Pither. 2006. Why are there so many small plants? Implications for species coexistence. Journal of Ecology 94: 569-580.

Aguirre, L. & D.A. Johnson. 1991. Root morphological development in relation to shoot growth in seedlings of four range grasses. Journal of Range Management 44(4): 341-346.

Alpert P & E.L. Simms. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? Evolutionary Ecology 16: 285-297.

Aphalo. P.J., C.L. Ballare & A.L. Scopel. 1999. Plant-plant signaling, the shade avoidance response and competition. Journal of Experimental Biology 50(340): 1629-1634.

Arrendondo, J.T. & D.A. Johnson. 1999. Root architecture and biomass allocation of three range grasses in response to nonuniform supply of nutrients and shoot defoliation. New Phytology 143: 373-385.

Arredondo, J.T., T.A. Jones & D.A. Johnson. 1998. Seedling growth of intermountain perennial and weedy annual grasses. Journal of Range Management 51: 584-589.

Bates, T.R. & J.P. Lynch. 1996. Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. Plant, Cell and Environment 19: 529-538.

Bilbrough, C.J. & M.M. Caldwell. 1997. Exploitation of springtime ephemeral N pulses by six Great Basin plant species. Ecology 87(1): 231-243.

Bloom, A.J., F. S. Chapin, III & H.A. Mooney. 1985. Resource limitation—an economic analogy. Annual Review of Ecology and Systematics 16: 363-392.

Bossdorf, O., H. Auge, L. Lafuma, W.E. Rogers, E. Siemann & D. Prati. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144: 1-11.

Bradley, B.A. and J.F. Mustard. 2004. Identifying land cover variability distinct from land cover change: cheatgrass in the Great Basin. Remote Sensing of Environment 94: 204-213.

Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13: 115-155.

Caldwell, M.M., J.H. Manwaring & R.B. Jackson. 1991. Exploitation of phosphate from fertile soil microsites by three Great Basin perennials when in competition. Functional Ecology 5: 757-764.

Caldwell, M.M., J.H. Manwaring & S.L. Durham. 1996. Species interactions at the level of fine roots in the field: influence of soil nutrient heterogeneity and plant size. Oecologia 106: 440-447.

Callahan, H.S., H. Maughan & U.K. Steiner. 2008. Phenotypic plasticity, costs of phenotypes, and costs of plasticity. Annals of the New York Academy of Sciences 1133: 44-66.

Callaway, R.M., W.M. Ridenour, T. Laboski, T. Weir, and J. M. Vivanco. 2005. Natural selection for resistance to the allelopathic effects of invasive plants. Journal of Ecology 93: 576-583.

Campbell, B.D., J.P. Grime & J.M.L. Mackey. 1991. A trade-off between scale and precision in resource foraging. Oecologia 87: 532-538.

Canadell. J., R.B. Jackson, J.R. Ehleringer, H.A. Mooney, O.E. Sala & E-D. Schulze. 1996. Maximum rooting depth of vegetation types at the global scale. Oecologia 108(4): 583-595.

Casper, B. & R. B. Jackson. 1997. Plant competition underground. Annual Review of Ecology and Systematics 28: 545-570.

Chambers, J.C., B.A. Roundy, R.R. Blank, S.E. Meyer & A. Whittaker. 2007. What makes Great Basin sagebrush ecosystems invasible by *Bromus tectorum*? Ecological Monographs 77(1): 117-145.

Chapin, III, F. S., E-D Schulze & H.A. Mooney. 1990. The Ecology and Economics of storage in plants. Annual Review of Ecology and Systematics 21: 423-447.

Charlet, D.A. 1998. Atlas of Nevada Mountain Ranges: Vegetation. Biological Resources Research Center, University of Nevada, Reno. Available at: http://www.csn.edu/science/Charlet/daczone.html. Last accessed: 04 January 2012.

Chun, Y.J., M. L. Collyer, K.A. Moloney & J.D. Nason. 2007. Phenotypic plasticity of native vs. invasive purple loosestrife: a two-state multivariate. Ecology 88(6): 1499-1512.

Cipollini D., C.B. Purrington & J. Bergelson. 2003. Costs of induced responses in plants. Basic and Applied Ecology 4: 79-85.

Coleman, J.S, K.N.D. McConnaughay & D.D. Ackerly. 1994. Interpreting phenotypic variation in plants. Trends in Ecology and Evolution 9(5): 187-191.

Craine, J.M., D.A. Wedin, F.S. Chapin, III & P.B. Reich. 2002. Relationship between the structure of root systems and resource use for 11 North American grassland plants. Plant Ecology 165: 85-100.

Crick, J.C. & Grime, J.P. 1987. Morphological plasticity and mineral nutrient capture in two herbaceous species of contrast ecology. The New Phytologist 107: 403-414.

Crispo, E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. Journal of Evolutionary Biology 21: 1460-1469.

D'Antonio C.M & P.M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics 23: 63-87.

deKroons, H., H. Huber, J.F. Stuefer & J.M. van Groenendael. 2005. A modular concept of plasticity in plants. New Phytologist 166: 73-82.

deKroons, H. & M.J. Hutchings. 1995. Morphological plasticity in clonal plants: the foraging concept. Journal of Ecology 83(1): 143-152.

Derner, J.D & D.D. Briske. 2001. Below-ground carbon and nitrogen accumulation in perennial: a comparison of caespitose and rhizomatous forms. Plant and Soil 237: 117-127.

Des Marais, D.L. & T.E. Juenger. 2010. Pleitropy, plasticity, and the evolution of plant abiotic stress to tolerance. Annals of the New York Academy of Sciences 1206: 56-79.

DeWitt, T.J. 1998. Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. Journal of Evolutionary Biology 11: 465-480.

DeWitt, T.J. 1998. Costs and limits to plasticity. TREE 13(2): 77-81.

Donohue, K. & J. Schmitt. 1999. The genetic architecture of plasticity in Impatiens capensis. Society for the Study of Evolution. 53(5): 1377-1386.

Drew, M.C. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot in barley. New Phytology 75: 479-490.

Eissenstat, D.M. 1991. On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstocks. New Phytology 118: 63-68.

Evans, R.D., R. Rimer, L. Sperry & J. Belnap. 2001. Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecological Applications 11(5): 1301-1310.

Franse, B., H. deKroon & F. Berendse. 1998. Root morphological plasticity and nutrient acquisition of perennial grass species from habitat of different nutrient availability. Oecologia 115: 351-358.

Gedroc, J.J., K.D.M. McConnaughay & J.S. Coleman. 1996. Plasticity in root/shoot partitioning: optimal, ontogenetic, or both? Functional Ecology 10: 44-50.

Ghalambor, C.K., J.K. McKay, S.P. Carroll and D.N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology 21: 394-407.

Gilroy, S. & D.L. Jones. 2000. Through form to function: root hair development and nutrient uptake. Trends in Plant Science 5(2): 56-60.

Goergen, E.M., E.L. Leger & E.K. Espeland. 2011. Native perennial grasses show evolutionary response to *Bromus tectorum* (cheatgrass) invasion. PLoS ONE 6(3): e18145.

Gregory, P. 2006. Plant Roots, Growth, activity and interaction with soils. Blackwell Publishing. Oxford, UK. 318 p.

Grether, G.F. 2005. Environmental change, phenotypic plasticity and genetic compensation. The American Naturalist 166(4): E115-E123.

Grime, J.P. & J.M.L. Mackey. 2002. The role of plasticity in resource capture by plants. Evolutionary Ecology 16: 299-307.

Gurevitch J. & Padilla D.K. 2004. Are invasive species a major cause of extinctions? Trends in Ecology and Evolution 19(9): 470-474.

Harris, G.A. 1967. Some competitive relationships between *Agropyron spicatum* and *Bromus tectorum*. Ecology Monographs 37: 89-111.

Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. The American Naturalist 173(5): 579-588.

Hodge, A., D. Robinson, B.S. Griffiths & A.H. Fitter. 1999. Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. Plant, Cell and Environment 22: 811-820.

Hodge, A. 2009. Root decisions. Plant, Cell and Environment 32: 628-640.

Hodge, A. 2004. The plastic plant: root responses to heterogenous supplies of nutrients. New Phytologist 162: 9-24.

Huber-Sannwald, E., D.A. Payke & M.M. Caldwell. 1996. Morphological plasticity following species-specific recognition and competition in two perennial grasses. American Journal of Botany 83(7): 919-931.

Hulbert, L.C. 1995. Ecological studies of *Bromus tectorum* and other annual bromegrasses. Ecology Monographs 25: 181-213.

Humphrey, L.D. & E. W. Schupp. 2004. Competition as a barrier to establishment of a native perennial grass (*Elymus elymoides*) in alien annual grass (*Bromus tectorum*) communities. Journal of Arid Environments 58: 405-422.

Jackson, R.B., J. Canadell, J.R. Ehleringer, H.A. Mooney, O.E. Sala & E.D. Schulze. 1996. A global analysis of root distributions for terrestrial biomes. Oecologia 108(3): 389-411.

Jackson, R.B., L.H. Manwaring & M.M. Caldwell. 1990. Rapid physiological adjustment of roots to localized soil enrichment. Nature 344: 58-60.

Jackson, R.B. & M.M. Caldwell. 1989. The timing of root proliferation in fertile-soil microsites for three cold-desert perennials. Oecologia 81: 149-153.

James, J.J. 2008. Leaf nitrogen productivity as a mechanism driving the success of invasive annual grasses under low and high nitrogen supply. Journal of Arid Environments 72: 1775-1784.

James, J.J., J.M. Mangold, R.L. Sheley and T. Svejcar. 2009. Root plasticity of native and invasive Great Basin species in response to soil nitrogen heterogeneity. Plant Ecology 202: 211-220.

Jones, T. A. and D.A. Johnson. 1998. Integrating genetic concepts into planning rangeland seedlings. Journal of Range Management. 51: 594-606.

Kellogg, E.A. 1990. Variation and species limits in agamospermous grasses. Systamic Botany 15(1): 112-123.

Klemmendson, J.O. and J.G. Smith. 1964. Cheatgrass (*Bromus tectorum* L.) The

Botany Review 30: 226-262.

Knapp, P.A. 1996. Cheatgrass (*Bromus tectorum*) dominance in the Great Basin Desert: History, persistence, and influence to human activities. Global Environmental Change 6: 37-52.

Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. European Society for Evolutionary Biology 22: 1435-1446.

Langerhans, R. B. & T.J. DeWitt. 2002. Plasticity constrained: over-generalized induction cues cause maladaptive phenotypes. Evolutionary Ecology Research 4: 857-870.

Lau, J.A. 2006. Evolutionary responses of native plants to novel community members. Evolution 60(1): 56-63.

Lau, J.A. 2008. Beyond the ecological: biological invasions alter natural selection on a native plant species. Ecology 89(4): 1023-1031.

Lee, C.E. 2002. Evolutionary genetics of invasive species. Trends in Ecology & Evolution 17(8): 386-391.

Leger, E.A. 2008. The adaptive value of remnant native plants in invaded communities: an example from the Great Basin. Ecological Applications 18(5): 1226-1235.

Leger, E.A. & E. K. Espeland. 2010. Coevolution between native and invasive plant competitors: implications for invasive species management. Evolutionary Applications ISSN 1752-4571: 169-178.

Leimu, R. and M. Fischer. 2008. A meta-analysis of local adaptation in plants. Plosone 12: e4010 (1-8).

Lynch, J. 1995. Root architecture and plant productivity. Plant Physiology 109: 7-13.

Mack, R.N. 1981. Invasion of *Bromus Techorum* into western North American: an ecological chronicle. Agro-Ecosystems 7: 145-165.

McConnaughay, K.D.M. & J.S. Coleman. 1999. Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. Ecology 80(8): 2581-2593.

McCully, M. 1995. How do real roots work? Plant Physiology. 109: 1-6.

McKay, J.K, C.E. Christian, S. Harrison, and K.J. Rice. 2005. "How local is local?"- A review of practical conceptual Issues in the Genetics of Restoration. Restoration Ecology 13: 432-440.

Monaco, T.A., C.T. Mackown, D.A. Johnson, T.A. Jones, J.M. Norton, J.B. Norton & M.G. Redinbaugh. 2003. Nitrogen effects on seed germination and seedling growth. Journal of Range Management 56: 646-653.

Mealor, B.A. and A.L. Hild. 2007. Post-invasion evolution of native plant populations: attest of biological resilience. Oikos 116: 1493-1500.

Ostonen, I., U. Puttsepp, C. Biel, O. Alberton, M.R. Bakker, K. Lohmus, H. Majdi, D. Metcalfe, A.F.M. Olsthoorn, A. Pronk, E. Vanguelova, M. Weih & I. Brunner. 2007. Specific root length as an indicator of environmental change. Plant Biosystems 141(3): 426-442.

Padilla, D.K & S.C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. Evolutionary Ecology 10: 105-117.

Pellant, M., B. Abbey & S. Karl. 2004. Restoring the Great Basin desert, U.S.A.:

integrating science, management, and people. Environmental Monitoring and Assessment 99: 169-179.

Pigliucci, M. & C.J. Murren. 2003. Perspective: Genetic assimilation and a possible evolutionary paradox: can macroevolution sometimes be so fast as to pass us by? Evolution 57(7): 1455-1464.

Pregitzer, K.S., R.L. Hendrick & R. Fogel. 1993. The demography of fine roots in response to patches of water and nitrogen. New Phytologist 125(3): 575-580.

Reynolds, H.L. & C. D'Antonio. 1996. The ecological significance in root weight ratio in response to nitrogen. Plant and Soil 185: 75-97.

Rossiter, M.C. 1996. Incidence and consequences of inherited environmental effects. Annual Review Ecology & Systematics 27: 451-476.

Rowe, C.L.J. & E.A. Leger. 2010. Competitive seedlings and inherited traits: a test of rapid evolution of *Elymus multisetus* (big squirreltail) in response to cheatgrass invasion. Evolutionary Applications ISSN 1752-4571: 1-14.

Ryel, R.J., M.M. Caldwell & J.H. Manwaring. 1996. Temporal dynamics of soil spatial heterogeneity in sagebrush-wheatgrass steppe during a growing season. Plant and Soil 184: 299-309.

Ryser, P. 2006. The mysterious root length. Plant Soil 286: 1-6.

Sax. D.F. & Gaines S.D. 2008. Species invasions and extinction: the future of native biodiversity on islands. Proceedings of the National Academy of Sciences of the United States of America 105(1): 11490-11497.

Scheiner, S.M. 1993. Genetics and evolution of phenotypic plasticity. Annual Review of Ecology and Systematics 24: 35-68.

Schilesinger, W. H. 1996. On the spatial pattern of soil nutrients in desert ecosystems. Ecology 77(2): 364-374.

Schlichting, C.D. 1986. The evolution of phenotypic plasticity in plants. Annual Review of Ecology and Systematics 17: 667-693.

Schlichting, C.D. 2008. Hidden reaction norms, cryptic genetic variation, and evolvability. Annals of the New York Academy of Sciences 1133: 187-203.

Schmid, B. & F.A. Bazzaz. 1992. Growth responses of rhizomatous plants to fertilizer application and interference. Oikos 65(1): 13-24.

Schmitt, J., S.A. Dudley & M. Pigliucci. 1999. Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade-avoidance responses in plants. The American Naturalist154: S43-S54.

Silander Jr., J.A. & S.W. Pacala. 2009. Neighborhood predictors of plant performance. Oecologia 66(2): 256-263.

Strauss, S.Y., J.A Lau, and S.P. Carroll. 2006. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? Ecology Letters 9: 357-374.

Sultan, S.E. 2010. Plant development responses to the environment: eco-devo insights. Current Opinion in Plant Biology 13: 69-101.

Sultan, S.E. 2005. An emerging focus on plant ecological development. New Phytologist 166(1): 1-5.

Sultan, S.E. 2003. Phenotypic plasticity in plants: a case study in ecological development. Evolution & Development 5(1): 25-33.

Schwinning, S. & J. Weiner. 1998. Mechanisms determining the degree of size asymmetry in competition among plants. Oecologia 113: 447-455.

USDA NRCS. 2009. The PLANTS Database. National Plant Data Center, Baton Rouge, LA. Available at: http://plants.usda.gov. Last accessed: 24 November 2011.

Valladares, F., E. Gianole & J.M. Gomez. 2007. Ecological limits to plant phenotypic plasticity. New Phytologist 176: 749-763.

Valladares, F., D. Sanches-Gomez, M.A. Zavala. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. Journal of Ecology 94: 1103-1116.

Van Kleunen, M & M. Fisher. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. New Phytologist 166(1): 49-60.

Via, S. & R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39(3): 505-522.

Wahl, S., P. Ryser & P.J. Edwards. 2001. Phenotypic plasticity of grass root anatomy in response to light intensity and nutrients supply. Annals of Botany 88: 1071-1078.

Wahl, S. & Ryser P. 2000. Root tissue structure is linked to ecological strategies of grasses. New Phytology 148: 459-471.

Weinig, C. & L.F. Delph. 2001. Phenotypic plasticity early in life constrains developmental responses later. Evolution 55(5): 930-936.

Weiner, J. 2004. Allocation, plasticity and allometry in plants. Perspectives in Plant Ecology, Evolution and Systematics 6(4): 207-215.

S.D. Wright & K.D.M. McConnaughay. 2002. Interpreting phenotypic plasticity: the importance of ontogeny. Plant Species Biology 17: 119-131.

Young, J.A., R. A. Evans & J. Major. 1972. Alien plants in the Great Basin. Journal of Range Management 25(3): 194-201.

FIGURE LEGENDS

Figure 1. *Poa secunda* biomass response to low and high nutrients (***P<0.0001).

Figure 2. *Poa secunda* root to shoot ratio response to low and high nutrients (***P<0.0001).

Figure 3. *Poa secunda* percent allocation of overall root diameter size classes (mm) in response to low and high nutrients (MANOVA for overall shift P=0.0390, ***P<0.0001 for individual size class comparisons). Percent allocation to each to root diameter size classes are based individual plant means. Significant differences in larger root categories are shown in inset bar graphs for clarity.

Figure 4. *Poa secunda* family level differences in root to shoot ratio in response to low and high nutrients (P=0.0038). Letters refer to Tukeys HSD comparisons among families for root to shoot ratio averaged between low and nutrients.

Figure 5. *Poa secunda* family level differences in change in percent allocation to mean root diameter size classes (mm) in response to nutrients. Negative values indicate reduced allocation to that size class, and positive values indicate increased allocation to that size class under low nutrients.

Figure 6. *Poa secunda* family level differences in nutrient performance index (NPI) in response to nutrients, calculated as the difference in family means under low and high nutrient treatments. Positive values indicate decreased plant performance (percent reduction in biomass) and negative values indicate increased plant performance in response to low nutrients.

Figure 7. Relationship between a) percent allocation of root diameter size classes 0.0-0.1mm and 0.5-0.6mm, b) percent allocation of root diameter size classes 0.1-0.2mm and 0.5-0.6mm, c) percent allocation of root diameter size classes 0.1-0.2mm and 0.6-0.8mm, d) percent allocation of root diameter size classes 0.1-0.2mm and 0.8-1.2mm under low (left column) and high nutrients (right column). Points represent individual plant data. Regression equations are shown, with slopes in bold.

Figure 8. *Poa secunda* biomass response to competition with *Bromus tectorum* for early harvest (***$P<0.0001$).

Figure 9. *Poa secunda* root to shoot ratio response to competition with *Bromus tectorum* for early harvest (***$P<0.0001$).

Figure 10. Change in *Poa secunda* percent allocation of root diameter size classes (mm) in response to competition with *Bromus tectorum* for early harvest (***$P<0.0001$). Significant differences in root classes 0.0 -0.1, 1.2-2.0 and >2.0 are shown in inset bar graphs for clarity.

Figure 11. *Poa secunda* family level differences in root to shoot ratio in response to competition with *Bromus tectorum* for early harvest (***P<0.0001).

Figure 12*.* *Poa secunda* family level differences in percent allocation to mean root diameter size classes (mm) in response to competition with *Bromus tectorum* for early harvest. Negative values indicate reduced allocation to that size class, and positive values indicate increased allocation to that size class under competition with *Bromus tectorum*.

Figure 13. Relationship between a) total biomass and percent allocation to root diameter size class 0.1-0.2mm, b) total biomass and percent allocation to root diameter size class 0.3-0.4, and c) total biomass and percent allocation to root diameter size class 1.2-2.0mm under competition with *Bromus tectorum* for early harvest. Points represent individual plant data.

Figure 14. Relationship between a) total biomass and change in percent allocation to root diameter size class 0.3-0.4mm, b) total biomass and change in percent allocation to root diameter size class 1.2-2.0, and c) total biomass and total plasticity in percent allocation to all root diameter classes under competition with *Bromus tectorum* for early harvest. Points represent family means.

Figure 15. Relationship between percent allocation of root diameter size classes 0.2-0.3mm and 0.5-0.6mm with and without competition with *Bromus tectorum* for early harvest. Points represent individual plant data. Regression equations are shown, with slopes in bold.

Figure 16. *Poa secunda* biomass response to competition with *Bromus tectorum* for late harvest (***P<0.0001).

Figure 17. *Poa secunda* root to shoot ratio response to competition with *Bromus tectorum* for late harvest (***P<0.0001).

Figure 18. *Poa secunda* percent allocation of root diameter size classes (mm) in response to *Bromus tectorum* for late harvest (***P<0.0001).

Figure 19. *Poa secunda* family level differences in root to shoot ratio in response to competition with *Bromus tectorum* for late harvest (***P<0.0001).

Figure 20. *Poa secunda* family level differences in competitive performance index (CPI) in response to competition with *Bromus tectorum* for late harvest. Positive values indicate decreased plant performance and negative values indicate increased plant performance.

Figure 21. *Poa secunda* family level differences in change in percent allocation of mean root diameter size classes (mm) in response to competition with *Bromus tectorum* for late harvest. Negative values indicate reduced allocation to that size class, and positive values indicate increased allocation to that size class under competition with *Bromus tectorum*.

Figure 22. Relationship between a) total biomass and percent allocation to root diameter size class 0.1-0.2mm, and b) total biomass and percent allocation to root diameter size class 1.2-2.0mm under competition with *Bromus tectorum* for late harvest.

Figure 23. Relationship between a) total biomass and change in percent allocation to root diameter size class 0.0-0.1mm, and b) competitive performance index (CPI) and total plasticity in percent allocation to all root diameter classes under competition with *Bromus tectorum* for late harvest, where positive values indicate decreased plant performance and negative values indicate increased plant performance.

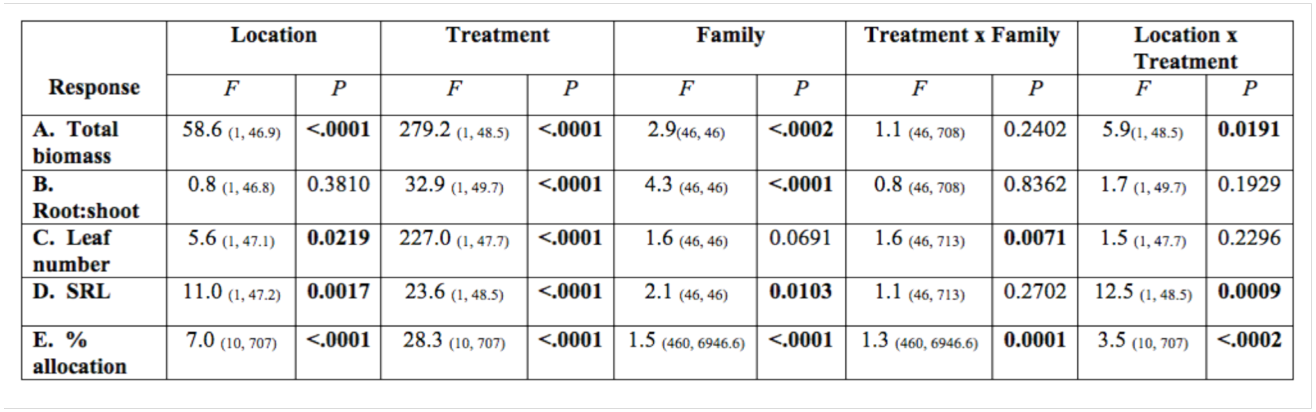
Figure 24. Relationship between a) percent allocation of root diameter size classes 0.2-0.3mm and 0.6-0.8mm, b) percent allocation of root diameter size classes 0.2-0.3mm and 0.8-1.2mm with and without competition with *Bromus tectorum* for late harvest. Points represent individual plant data.

TABLES

Table 1. ANOVA results of *Poa secunda* response to low and high nutrient treatments for (A) total biomass, (B) root to shoot, (C) leaf number, (D) specific root length (SRL) and MANOVA results for (E) change in percent allocation to all root diameter size classes. Numbers in parentheses are numerator degrees of freedom, denominator degrees of freedom. Data were not transformed, except for SRL, which was log transformed. Analyses were conducted using individual plant data.

Response	**Nutrients**		**Family**		**Nutrient x Family**	
	F	*P*	*F*	*P*	*F*	*P*
A. Total biomass	$27.6_{(1, 20.7)}$	**<.0001**	$3.1_{(19.19)}$	**0.0082**	$1.6_{(19, 139)}$	0.0559
B. Root:shoot	$263.0_{(1, 20.81)}$	**<.0001**	$3.6_{(19,19)}$	**0.0038**	$1.49_{(19,139)}$	0.0973
C. Leaf number	$135.0_{(1, 23.5)}$	**<.0001**	$3.9_{(19,19)}$	**0.0023**	$0.6_{(19, 139)}$	0.8855
D. SRL	$89.5_{(1, 22.4)}$	**<.0001**	$5.7_{(19, 19)}$	**0.0002**	$0.8_{(19, 139)}$	0.6871
E. % allocation	$1.2_{(190, 1178.3)}$	**0.0390**	$2.9_{(190, 1178.3)}$	**<0.0001**	——	——

Table 2. ANOVA results of *Poa secunda* early harvest (67 days after emergence) in response to competition with *Bromus tectorum* for (A) total biomass, (B) root to shoot ratio, (C) leaf number, (D) specific root length (SRL) and MANOVA results for (E) change in percent allocation to all root diameter size classes. Numbers in parentheses are numerator degrees of freedom, denominator degrees of freedom. Data were not transformed for analysis, except for total biomass, which was log transformed. Analyses were conducted using individual plant data.

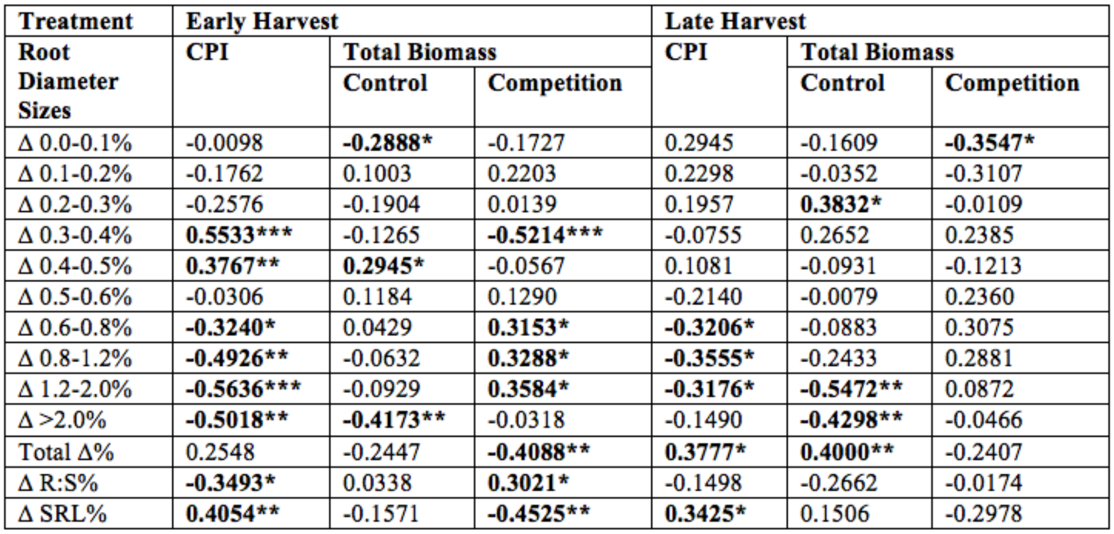


Response	**Location**		**Treatment**		**Family**		**Treatment x Family**		**Location x Treatment**	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
A. Total biomass	58.6 (1, 46.9)	**<.0001**	279.2 (1, 48.5)	**<.0001**	2.9(46, 46)	**<.0002**	1.1 (46, 708)	0.2402	5.9(1, 48.5)	**0.0191**
B. Root:shoot	0.8 (1, 46.8)	0.3810	32.9 (1, 49.7)	**<.0001**	4.3 (46, 46)	**<.0001**	0.8 (46, 708)	0.8362	1.7 (1, 49.7)	0.1929
C. Leaf number	5.6 (1, 47.1)	**0.0219**	227.0 (1, 47.7)	**<.0001**	1.6 (46, 46)	0.0691	1.6 (46, 713)	**0.0071**	1.5 (1, 47.7)	0.2296
D. SRL	11.0 (1, 47.2)	**0.0017**	23.6 (1, 48.5)	**<.0001**	2.1 (46, 46)	**0.0103**	1.1 (46, 713)	0.2702	12.5 (1, 48.5)	**0.0009**
E. % allocation	7.0 (10, 707)	**<.0001**	28.3 (10, 707)	**<.0001**	1.5 (460, 6946.6)	**<.0001**	1.3 (460, 6946.6)	**0.0001**	3.5 (10, 707)	**<.0002**

Table 3. Spearman's ρ correlations between percent allocation of individual *Poa secunda* plants to each root diameter size class (mm), root to shoot ratio, and specific root length (SRL), and total biomass for early harvest competition and late harvest competition treatments. Analyses were conducted using individual plant data. Asterisks represent significant difference between competition status, where *P<0.05, **P<0.01, ***P<0.0001.

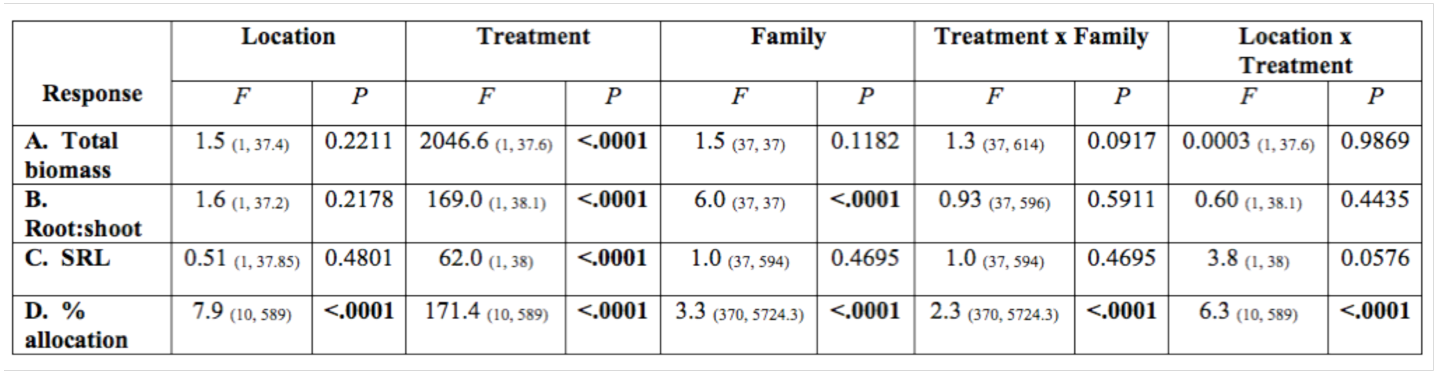
Treatment	**Competition: Early Harvest**		**Competition: Late Harvest**	
	Total Biomass		**Total Biomass**	
Root Diameter Sizes	**Control**	**Competition**	**Control**	**Competition**
% Allocation 0.0-0.1	0.0184	0.0428	-0.0829	**-0.2738*** **
% Allocation 0.1-0.2	0.0777	**0.2134*** **	**-0.1979** **	**-0.2590*** **
% Allocation 0.2-0.3	0.0666	**0.2165*** **	**-0.2995*** **	**-0.3226*** **
% Allocation 0.3-0.4	**-0.3260*** **	**-0.3300*** **	**-0.2897*** **	0.0056
% Allocation 0.4-0.5	**-0.1533** **	-0.0491	-0.0200	**0.2061*** **
% Allocation 0.5-0.6	**0.1596** **	0.0684	**-0.1139* **	**0.3559*** **
% Allocation 0.6-0.8	**0.2804*** **	**0.1247* **	**0.1485** **	**0.3996*** **
% Allocation 0.8-1.2	**0.3773*** **	**0.1574** **	**0.4155*** **	**0.4614*** **
% Allocation 1.2-2.0	**0.4663*** **	**0.3302*** **	**0.5156*** **	**0.4994*** **
% Allocation > 2.0	**0.4838*** **	**0.2856*** **	**0.5188*** **	**0.5372*** **
R:S	0.0199	**0.1452** **	**0.4165*** **	0.0376
SRL	**-0.4858*** **	**-0.4446*** **	**-0.7040*** **	**-0.4787*** **

Table 4. Spearman's ρ correlations between change in percent allocation of *Poa secunda* mean root diameter size classes (mm), change in overall percent allocation of mean root diameter size classes (mm), change in overall specific root length (SRL), and change in overall root to shoot ratio and plant performance (CPI and total biomass) for early harvest, and late harvest competition treatments with *Bromus tectorum*. CPI = competitive performance index, where positive values indicate a decrease in plant performance, and negative values indicate an increase in plant performance. Analyses were conducted using family means. Asterisks represent significant difference between competition status, where *P<0.05, **P<0.01, ***P<0.0001.



Treatment	**Early Harvest**			**Late Harvest**		
Root Diameter Sizes	**CPI**	**Total Biomass**		**CPI**	**Total Biomass**	
		Control	**Competition**		**Control**	**Competition**
Δ 0.0-0.1%	-0.0098	**-0.2888***	-0.1727	0.2945	-0.1609	**-0.3547***
Δ 0.1-0.2%	-0.1762	0.1003	0.2203	0.2298	-0.0352	-0.3107
Δ 0.2-0.3%	-0.2576	-0.1904	0.0139	0.1957	**0.3832***	-0.0109
Δ 0.3-0.4%	**0.5533*****	-0.1265	**-0.5214*****	-0.0755	0.2652	0.2385
Δ 0.4-0.5%	**0.3767****	**0.2945***	-0.0567	0.1081	-0.0931	-0.1213
Δ 0.5-0.6%	-0.0306	0.1184	0.1290	-0.2140	-0.0079	0.2360
Δ 0.6-0.8%	**-0.3240***	0.0429	**0.3153***	**-0.3206***	-0.0883	0.3075
Δ 0.8-1.2%	**-0.4926****	-0.0632	**0.3288***	**-0.3555***	-0.2433	0.2881
Δ 1.2-2.0%	**-0.5636*****	-0.0929	**0.3584***	**-0.3176***	**-0.5472****	0.0872
Δ >2.0%	**-0.5018****	**-0.4173****	-0.0318	-0.1490	**-0.4298****	-0.0466
Total Δ%	0.2548	-0.2447	**-0.4088****	**0.3777***	**0.4000****	-0.2407
Δ R:S%	**-0.3493***	0.0338	**0.3021***	-0.1498	-0.2662	-0.0174
Δ SRL%	**0.4054****	-0.1571	**-0.4525****	**0.3425***	0.1506	-0.2978

Table 5. ANOVA results of *Poa secunda* late harvest (after one growing season) in response to competition with *Bromus tectorum* for (A) total biomass, (B) root to shoot ratio, (C) specific root length (SRL) and MANOVA results for (D) change in percent allocation to all root diameter size classes (mm). Numbers in parentheses are numerator degrees of freedom, denominator degrees of freedom. Data were transformed using Box-Cox for total biomass and log transformed for R:S. No transformation were performed for SRL. Analyses were conducted using individual data.



Response	**Location**		**Treatment**		**Family**		**Treatment x Family**		**Location x Treatment**	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
A. Total biomass	1.5 (1, 37.4)	0.2211	2046.6 (1, 37.6)	**<.0001**	1.5 (37, 37)	0.1182	1.3 (37, 614)	0.0917	0.0003 (1, 37.6)	0.9869
B. Root:shoot	1.6 (1, 37.2)	0.2178	169.0 (1, 38.1)	**<.0001**	6.0 (37, 37)	**<.0001**	0.93 (37, 596)	0.5911	0.60 (1, 38.1)	0.4435
C. SRL	0.51 (1, 37.85)	0.4801	62.0 (1, 38)	**<.0001**	1.0 (37, 594)	0.4695	1.0 (37, 594)	0.4695	3.8 (1, 38)	0.0576
D. % allocation	7.9 (10, 589)	**<.0001**	171.4 (10, 589)	**<.0001**	3.3 (370, 5724.3)	**<.0001**	2.3 (370, 5724.3)	**<.0001**	6.3 (10, 589)	**<.0001**

Table 6. Spearman's ρ correlations between percent allocation of individual *Poa secunda* plants to each root diameter size class (mm), root to shoot ratio, and specific root length (SRL) at early harvest, and total biomass for late harvest under competition with *Bromus tectorum*. Analyses were conducted using individual data. Asterisks represent significant difference, where *P<0.05, **P<0.01, ***P<0.0001.

Poa secunda **Early Harvest: Traits**	**Late Harvest: Total Biomass Competition**
% Allocation 0.0-0.1	**-0.2850***
% Allocation 0.1-0.2	**-0.4417****
% Allocation 0.2-0.3	-0.1498
% Allocation 0.3-0.4	0.0949
% Allocation 0.4-0.5	**0.3729***
% Allocation 0.5-0.6	0.0421
% Allocation 0.6-0.8	0.0516
% Allocation 0.8-1.2	0.1545
% Allocation 1.2-2.0	**0.3437***
% Allocation > 2.0	0.1769
R:S	**-0.2121***
SRL	**-0.4791****

Table 7. Spearman's ρ correlations between change in percent allocation of *Poa secunda* mean root diameter size classes (mm), change in overall percent allocation of mean root diameter size classes (mm), change in overall specific root length (SRL), and change in overall root to shoot ratio at early harvest and plant performance (CPI and total biomass) for late harvest competition with *Bromus tectorum*, where CPI = competitive performance index. Analyses were conducted using family means. Asterisks represent significant difference, where *P<0.05, **P<0.01, ***P<0.0001.

Poa secunda **Early Harvest: Traits**	**Late Harvest:**	
	CPI	**Total Biomass**
Δ 0.0-0.1%	0.0302	-0.2668
Δ 0.1-0.2%	0.1747	0.1081
Δ 0.2-0.3%	0.2014	-0.0051
Δ 0.3-0.4%	-0.1538	-0.0939
Δ 0.4-0.5%	-0.0154	0.1067
Δ 0.5-0.6%	0.0310	0.1200
Δ 0.6-0.8%	-0.1862	0.0223
Δ 0.8-1.2%	0.0115	-0.0087
Δ 1.2-2.0%	0.0494	0.0405
Δ >2.0%	0.0696	0.0132
Sum Δ%	-0.0107	-0.0623
Δ SRL%	0.1775	-0.1879
Δ R:S%	-0.2638	**-0.3538***

Figure 1.

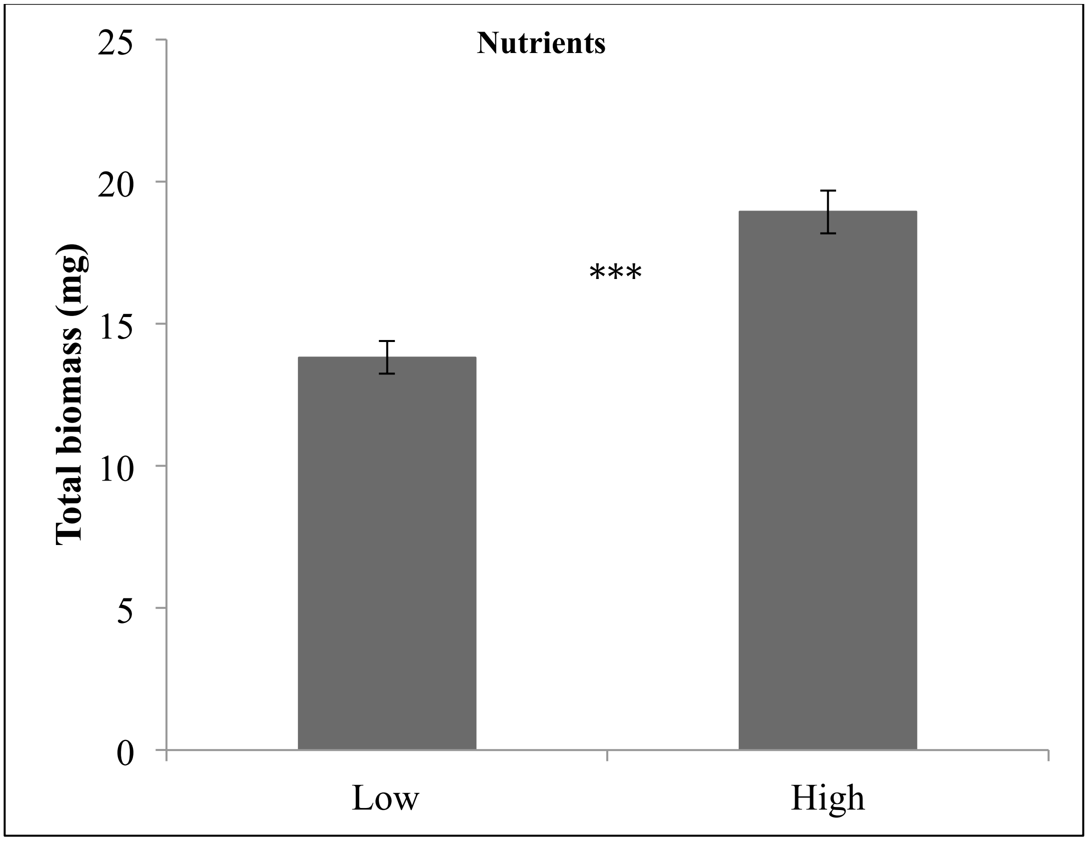


Figure 2.

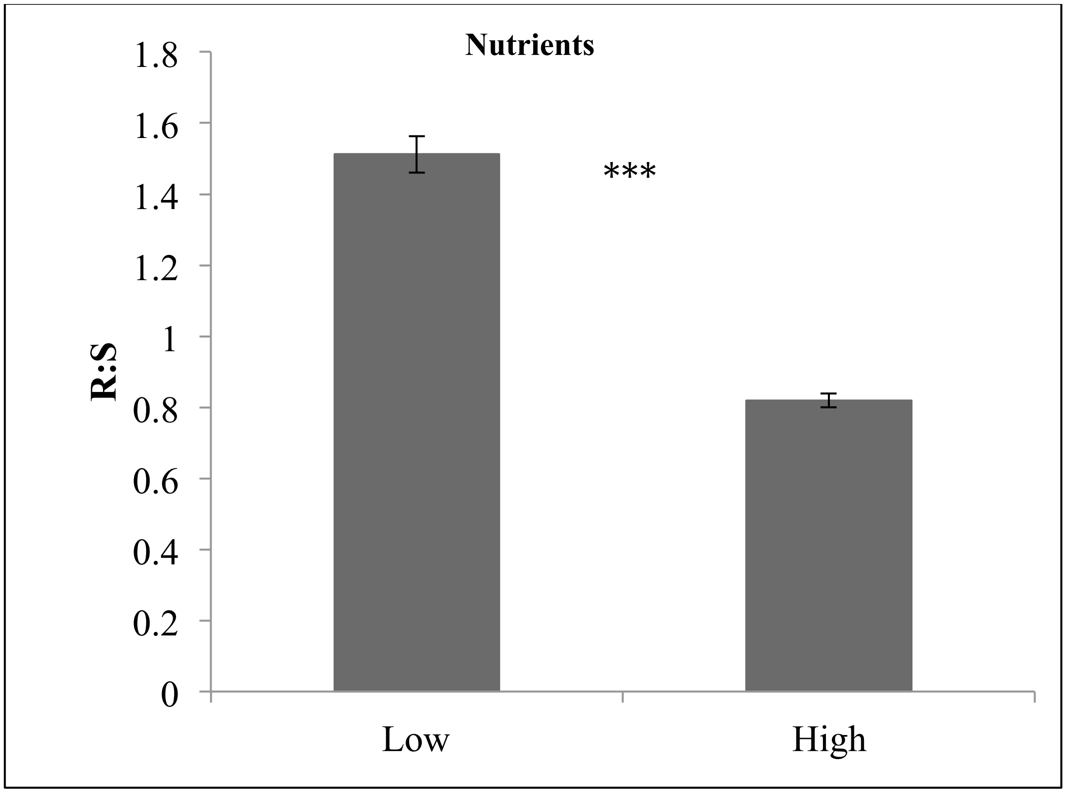


Figure 3.

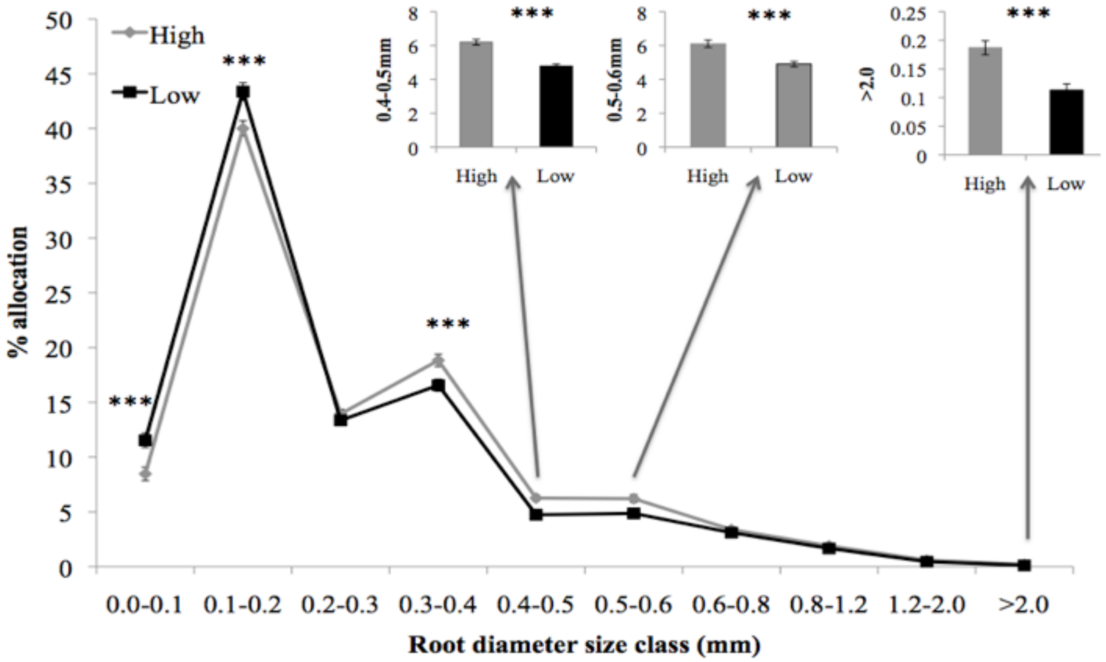

High
Low
% allocation
Root diameter size class (mm)
0.0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.8
0.8-1.2
1.2-2.0
>2.0
0
5
10
15
20
25
30
35
40
45
50
***
***
***
0.4-0.5mm
0
2
4
6
8
High
Low
***
0.5-0.6mm
0
2
4
6
8
High
Low
***
>2.0
0
0.05
0.1
0.15
0.2
0.25
High
Low
***

Figure 4.

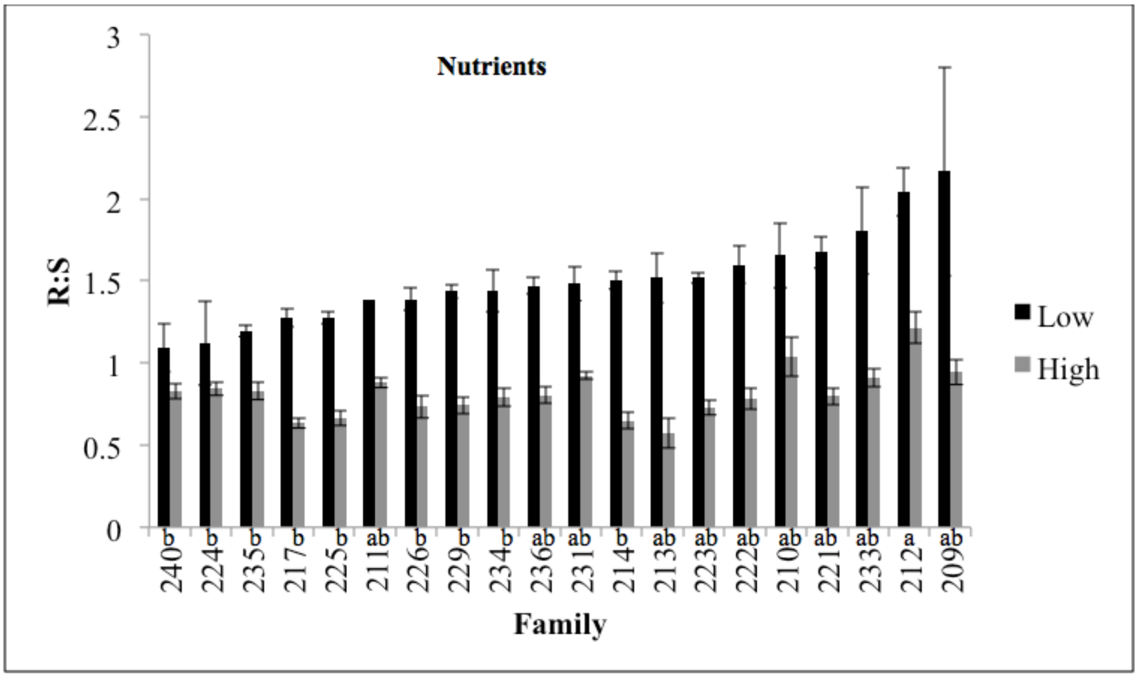

Nutrients
R:S
3
2.5
2
1.5
1
0.5
0
Low
High
b
b
b
b
b
ab
b
b
b
ab
ab
b
ab
ab
ab
ab
ab
ab
a
ab
240
224
235
217
225
211
226
229
234
236
231
214
213
223
222
210
221
233
212
209
Family

Figure 5.

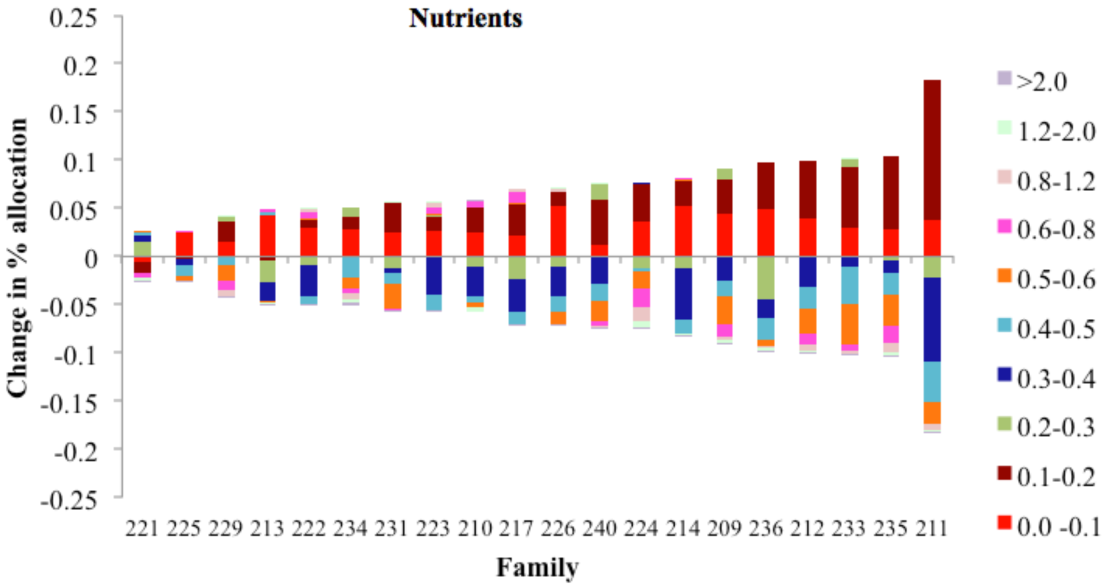

Nutrients
Change in % allocation
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
221
225
229
213
222
234
231
223
210
217
226
240
224
214
209
236
212
233
235
211
Family
>2.0
1.2-2.0
0.8-1.2
0.6-0.8
0.5-0.6
0.4-0.5
0.3-0.4
0.2-0.3
0.1-0.2
0.0 -0.1

Figure 6.

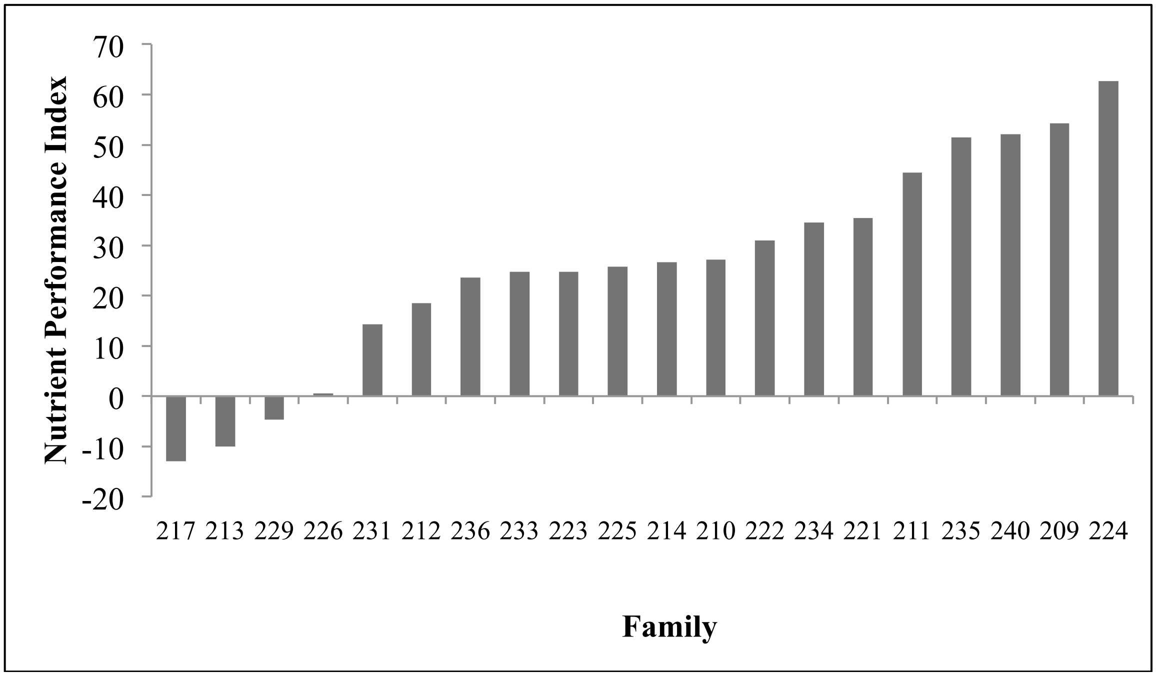

Nutrient Performance Index
70
60
50
40
30
20
10
0
-10
-20
217 213 229 226 231 212 236 233 223 225 214 210 222 234 221 211 235 240 209 224
Family

Figure 7.

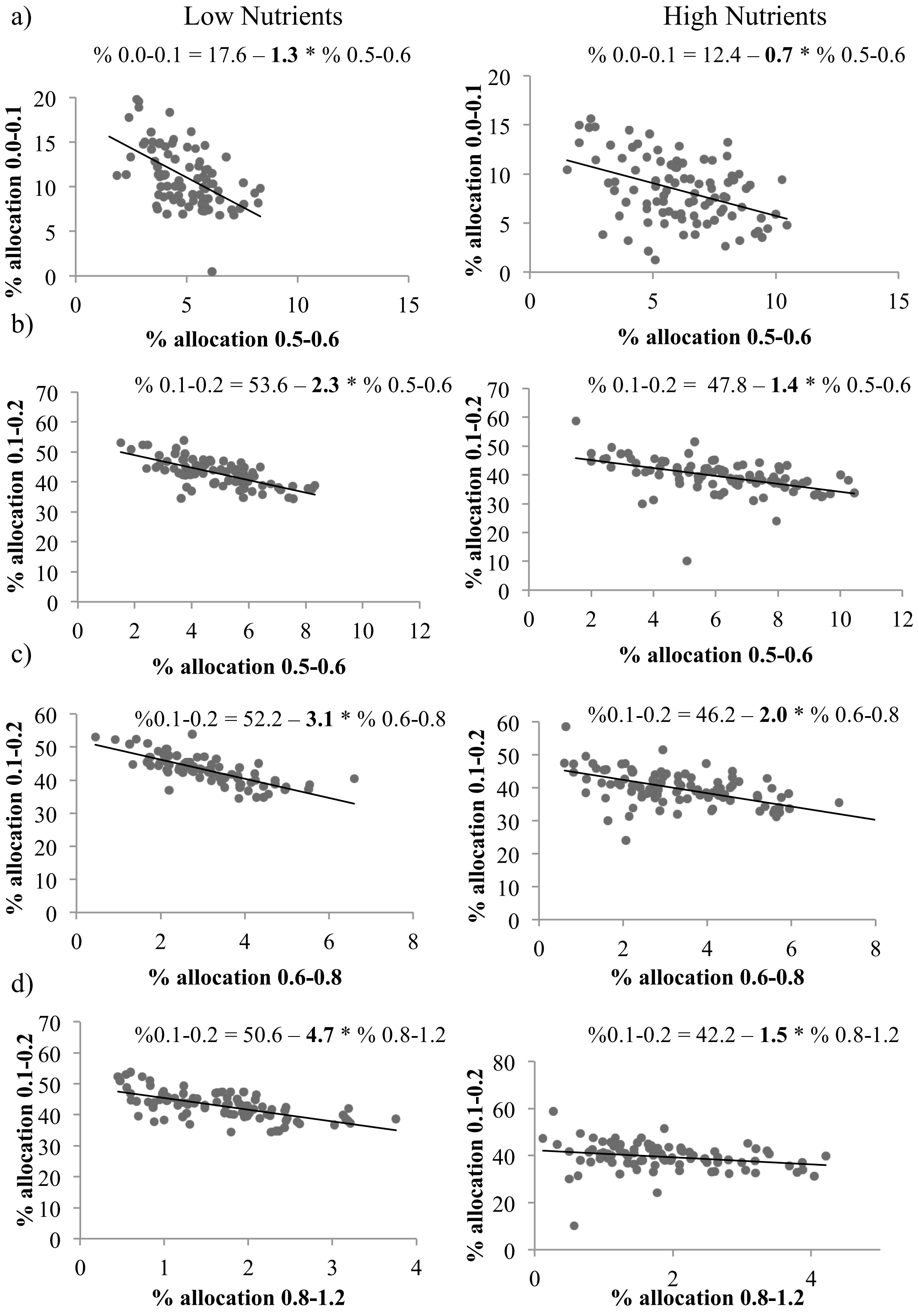

a)
Low Nutrients
High Nutrients
% 0.0-0.1 = 17.6 – 1.3 * % 0.5-0.6
% 0.0-0.1 = 12.4 – 0.7 * % 0.5-0.6
% allocation 0.0-0.1
% allocation 0.5-0.6
0
5
10
15
20
b)
% 0.1-0.2 = 53.6 – 2.3 * % 0.5-0.6
% 0.1-0.2 = 47.8 – 1.4 * % 0.5-0.6
% allocation 0.1-0.2
% allocation 0.5-0.6
0
10
20
30
40
50
60
70
2
4
6
8
12
c)
%0.1-0.2 = 52.2 – 3.1 * % 0.6-0.8
%0.1-0.2 = 46.2 – 2.0 * % 0.6-0.8
% allocation 0.1-0.2
% allocation 0.6-0.8
d)
%0.1-0.2 = 50.6 – 4.7 * % 0.8-1.2
%0.1-0.2 = 42.2 – 1.5 * % 0.8-1.2
% allocation 0.1-0.2
% allocation 0.8-1.2
1
3
80

Figure 8.

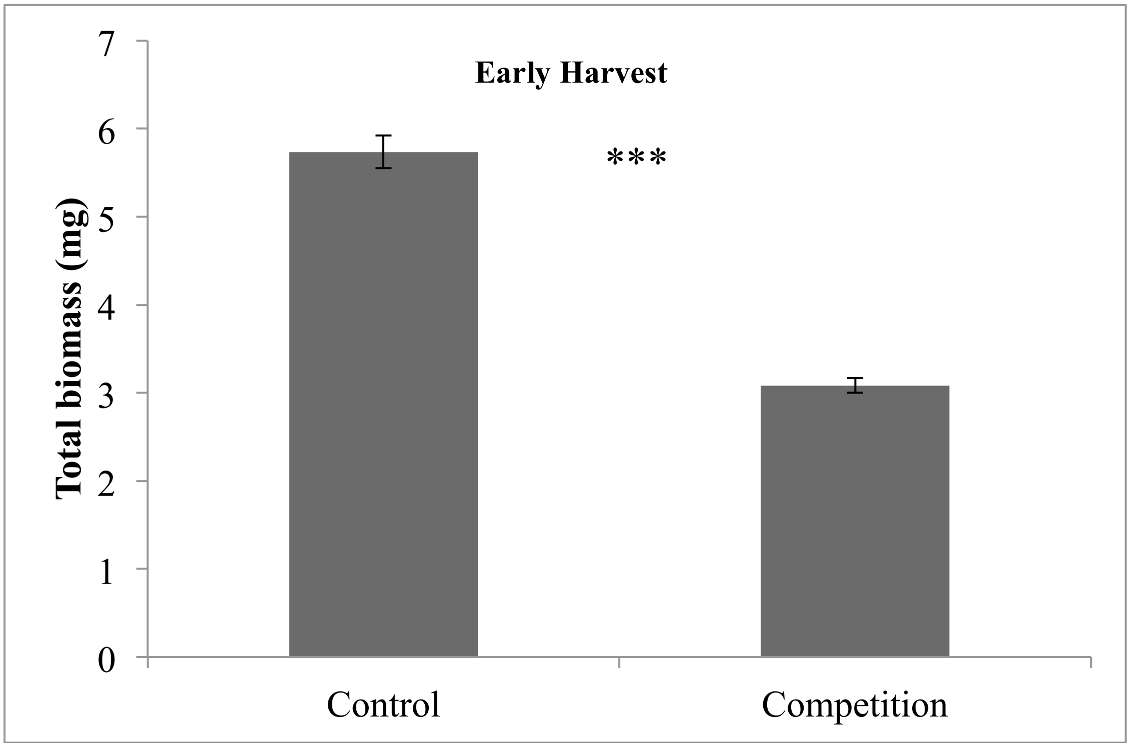

Early Harvest
Total biomass (mg)
7
6
5
4
3
2
1
0
***
Control
Competition

Figure 9.

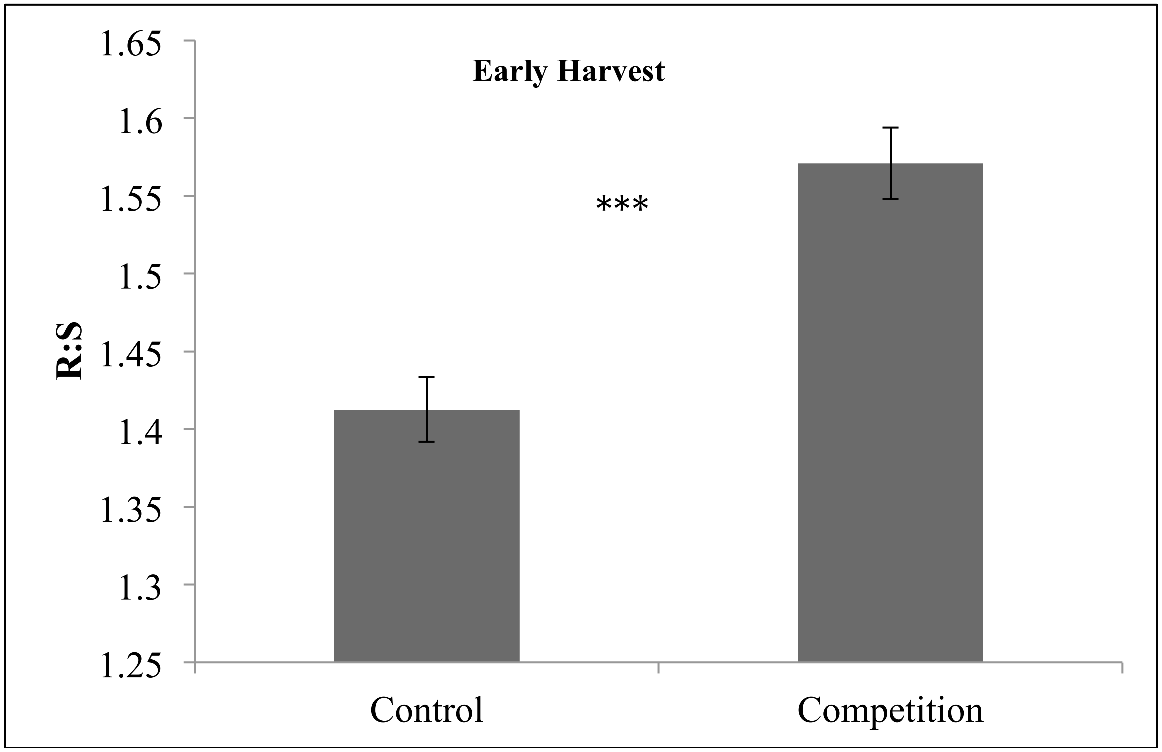

Early Harvest
R:S
1.65
1.6
1.55
1.5
1.45
1.4
1.35
1.3
1.25
***
Control
Competition

Figure 10.

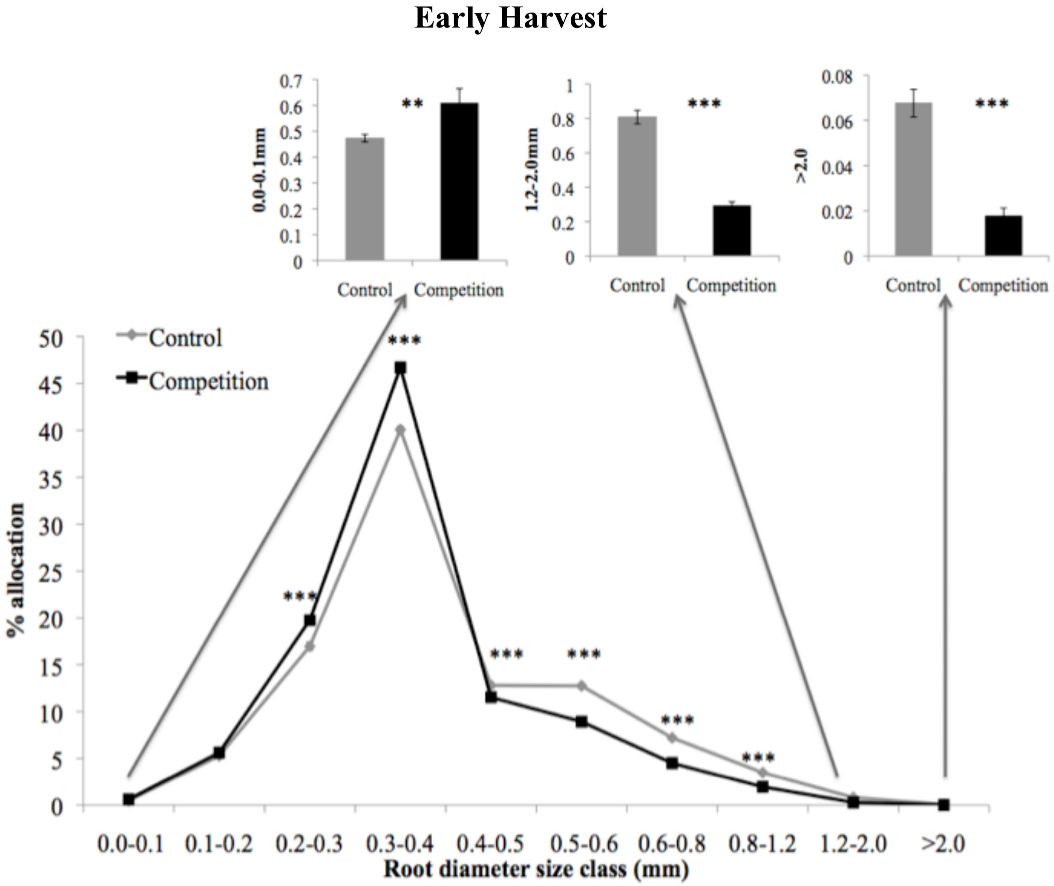

Early Harvest
0.0-0.1mm
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
Control
Competition
1.2-2.0mm
1
0.8
0.6
0.4
0.2
0
***
Control
Competition
>2.0
0.08
0.06
0.04
0.02
0
***
Control
Competition
Control
Competition
% allocation
50
45
40
35
30
25
20
15
10
5
0
***
***
***
***
***
***
0.0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.8
0.8-1.2
1.2-2.0
>2.0
Root diameter size class (mm)

Figure 11.

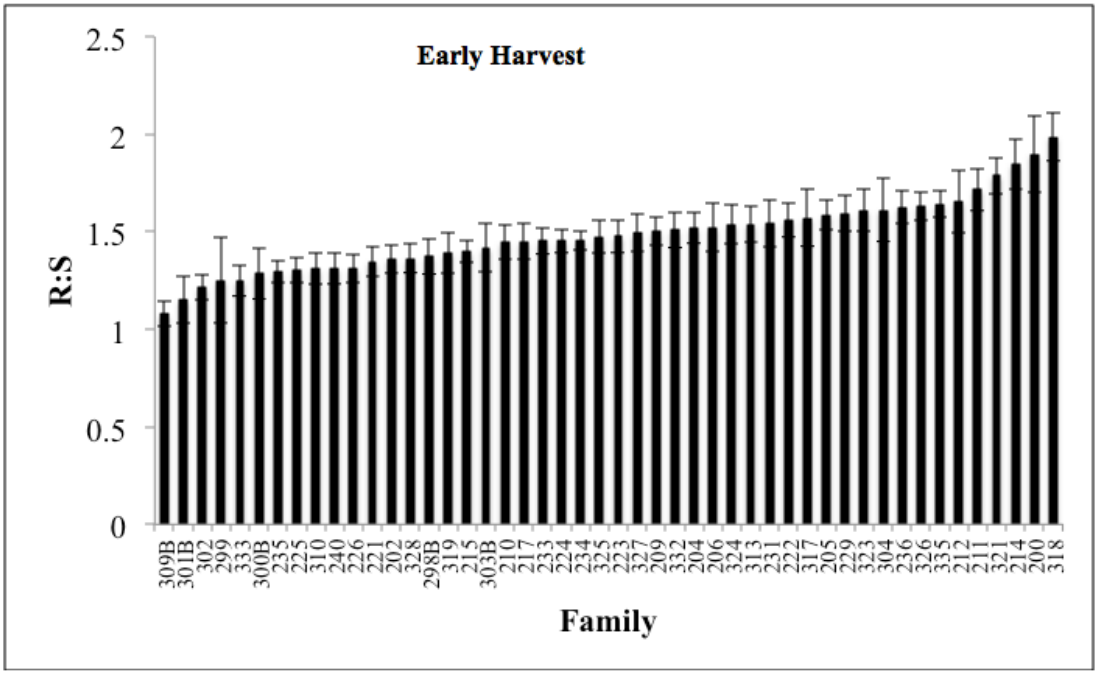

Early Harvest
R:S
2.5
2
1.5
1
0.5
0
309B
301B
302
299
333
300B
235
225
310
240
226
221
202
328
298B
319
215
303B
210
217
233
224
234
325
223
327
209
332
204
206
324
313
231
222
317
205
229
323
304
236
326
335
212
211
321
214
200
318
Family

Figure 12.

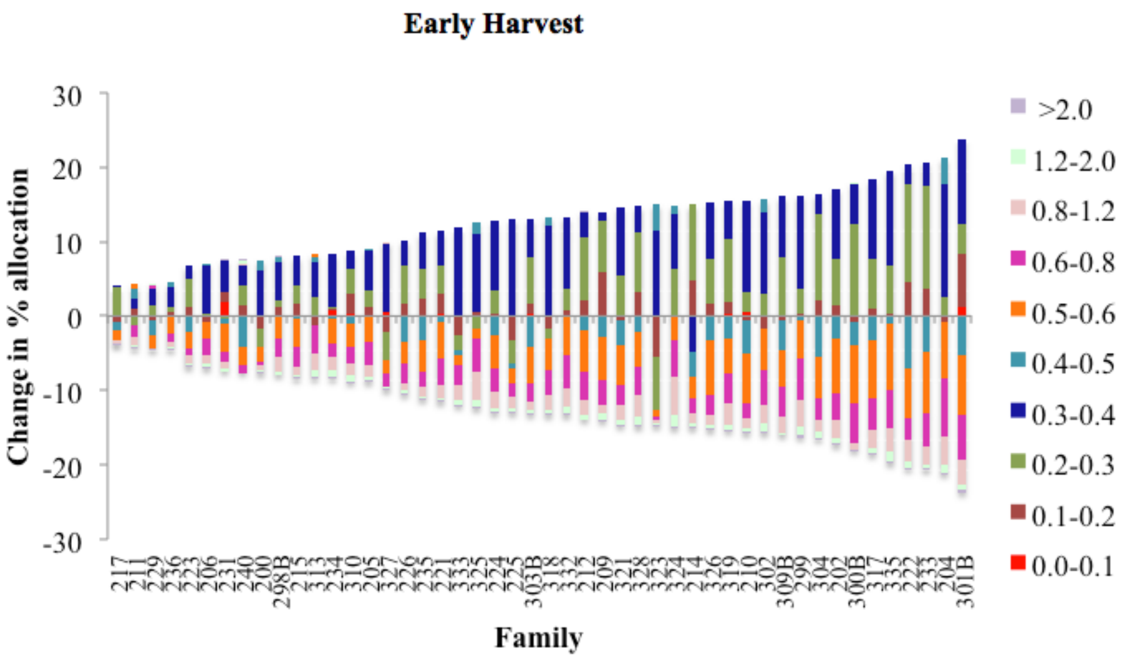

Early Harvest
Change in % allocation
30
20
10
0
-10
-20
-30
Family
217
211
229
236
223
206
231
240
200
298B
215
313
234
310
205
327
226
235
221
333
325
224
225
303B
318
332
212
209
321
328
323
324
214
326
319
210
302
309B
299
304
202
300B
317
335
222
233
204
301B
>2.0
1.2-2.0
0.8-1.2
0.6-0.8
0.5-0.6
0.4-0.5
0.3-0.4
0.2-0.3
0.1-0.2
0.0-0.1

Figure 13.

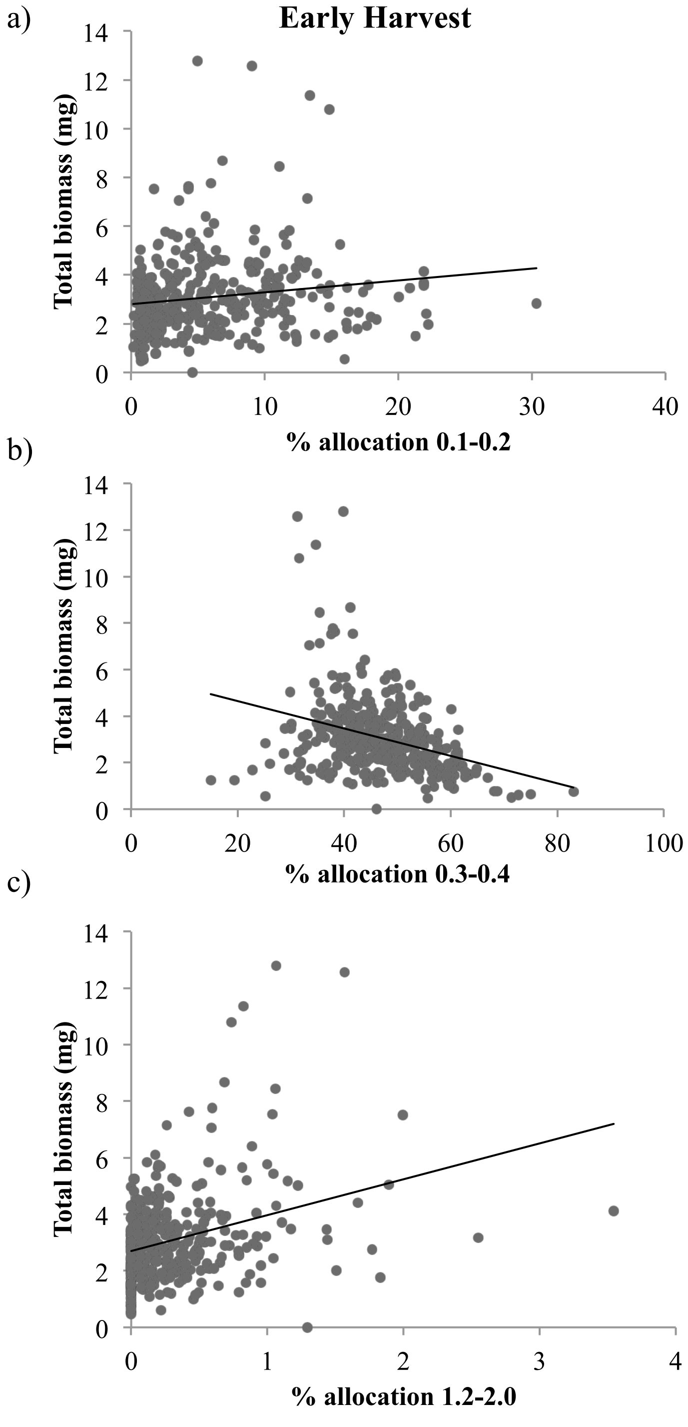

a)
Early Harvest
Total biomass (mg)
14
12
10
8
6
4
2
0
0
10
20
30
40
% allocation 0.1-0.2
b)
Total biomass (mg)
14
12
10
8
6
4
2
0
0
20
40
60
80
100
% allocation 0.3-0.4
c)
Total biomass (mg)
14
12
10
8
6
4
2
0
0
1
2
3
4
% allocation 1.2-2.0

Figure 14.

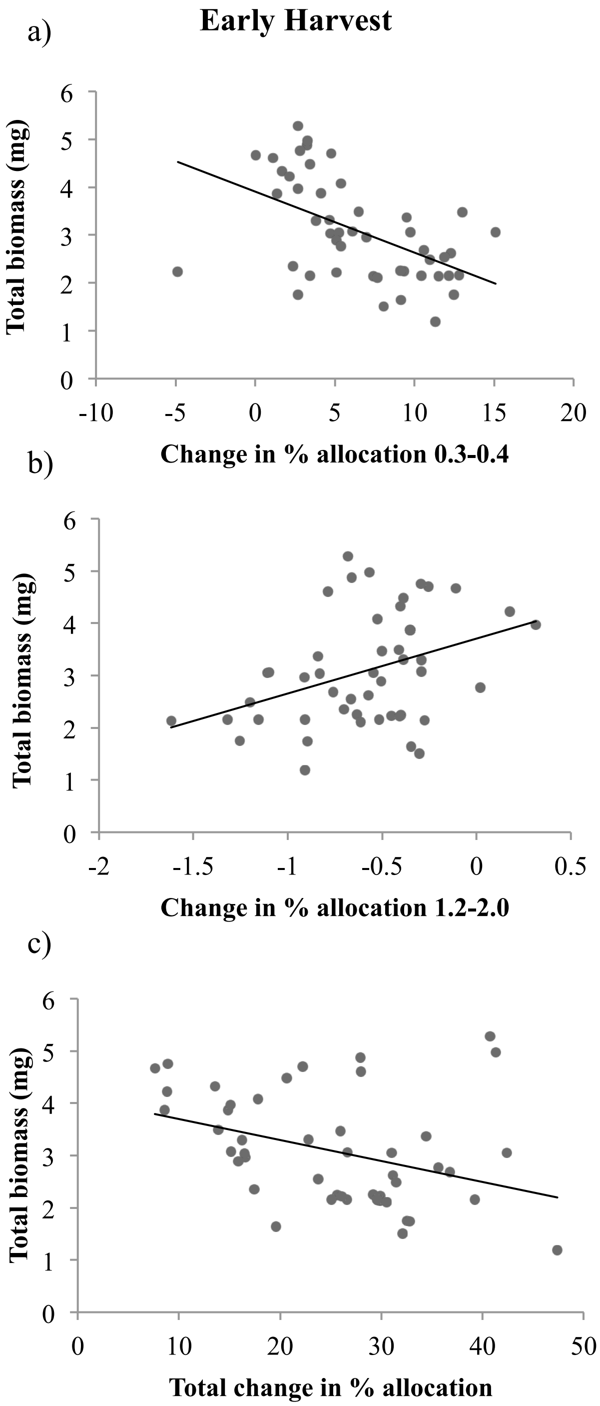

a)
Early Harvest
Total biomass (mg)
0
1
2
3
4
5
6
-10
-5
0
5
10
15
20
Change in % allocation 0.3-0.4
b)
Total biomass (mg)
0
1
2
3
4
5
6
-2
-1.5
-1
-0.5
0
0.5
Change in % allocation 1.2-2.0
c)
Total biomass (mg)
0
1
2
3
4
5
6
0
10
20
30
40
50
Total change in % allocation

Figure 15.

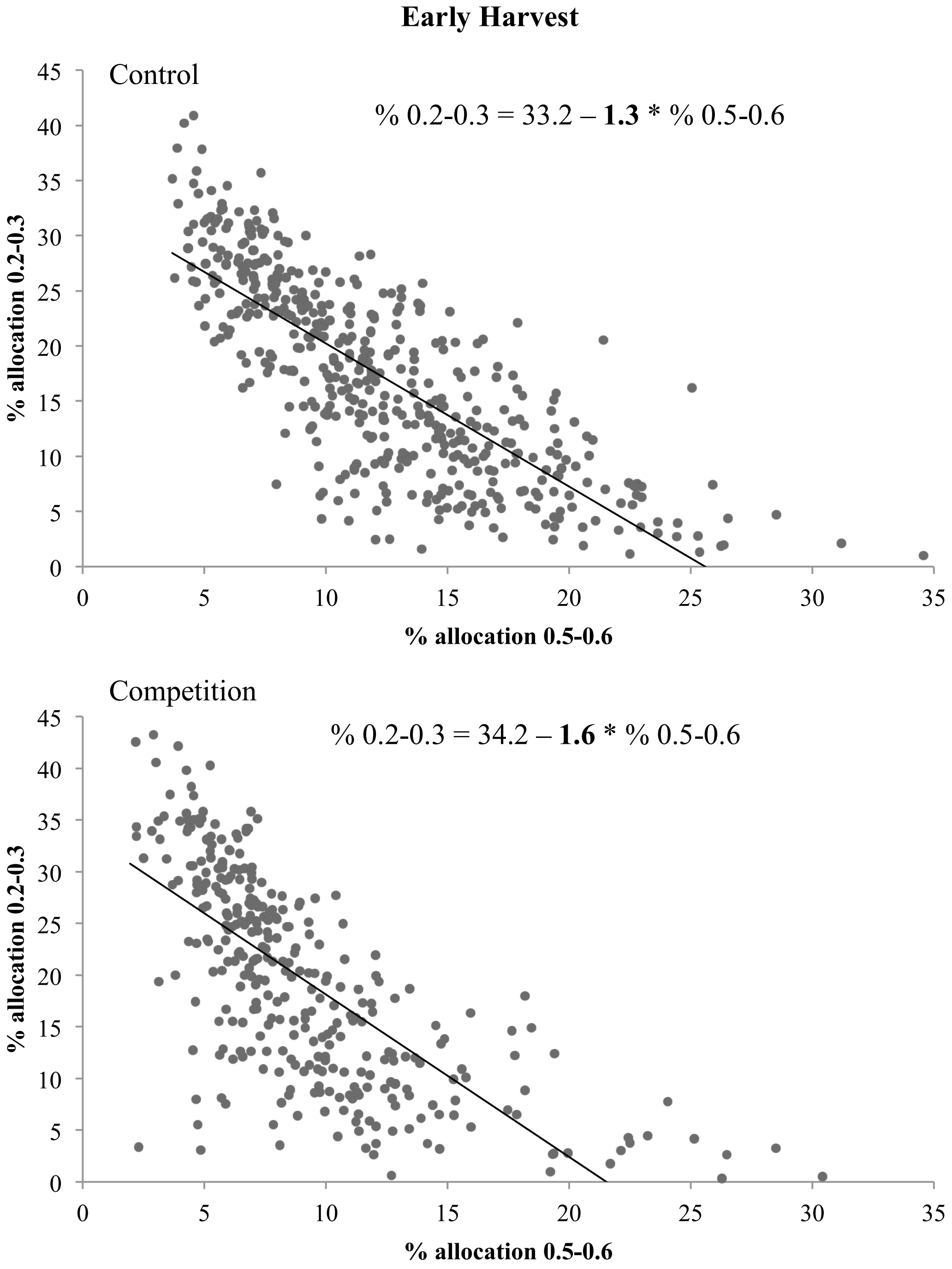

Early Harvest
Control
% 0.2-0.3 = 33.2 – **1.3** * % 0.5-0.6
% allocation 0.2-0.3
% allocation 0.5-0.6
0
5
10
15
20
25
30
35
40
45
Competition
% 0.2-0.3 = 34.2 – **1.6** * % 0.5-0.6
% allocation 0.2-0.3
% allocation 0.5-0.6

Figure 16.

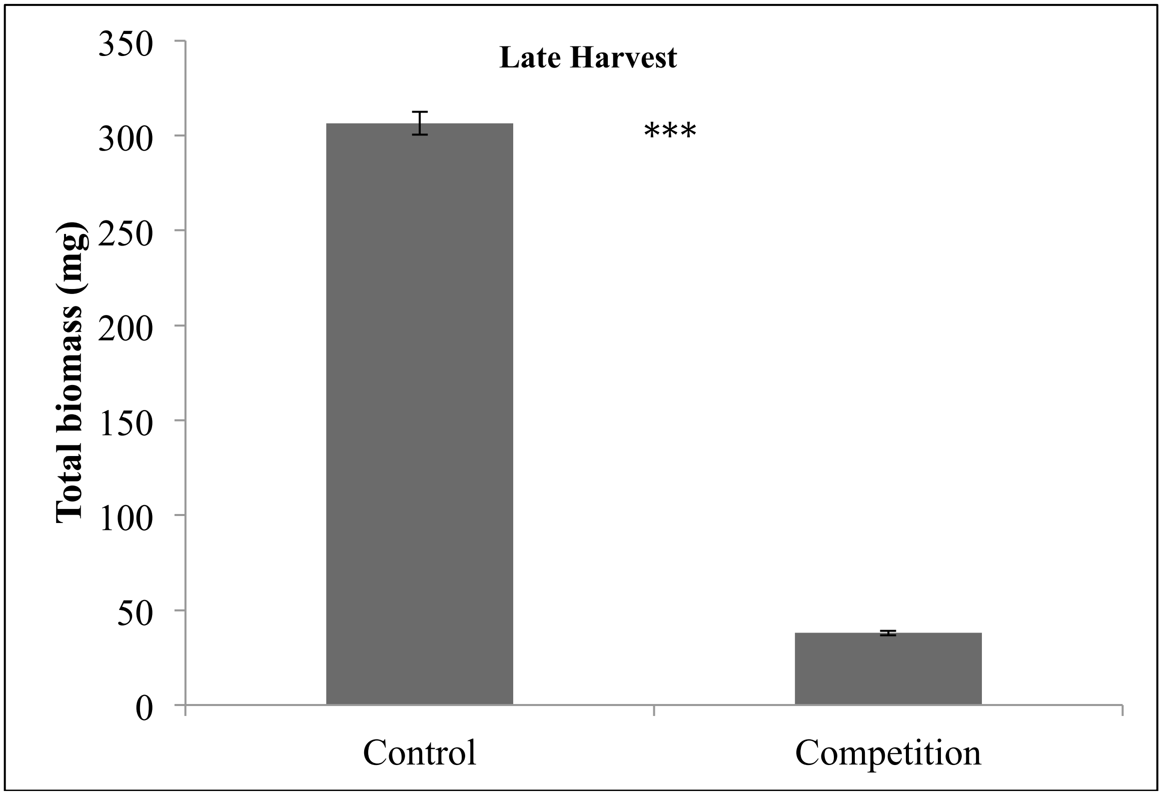

Late Harvest
***
Total biomass (mg)
350
300
250
200
150
100
50
0
Control
Competition

Figure 17.

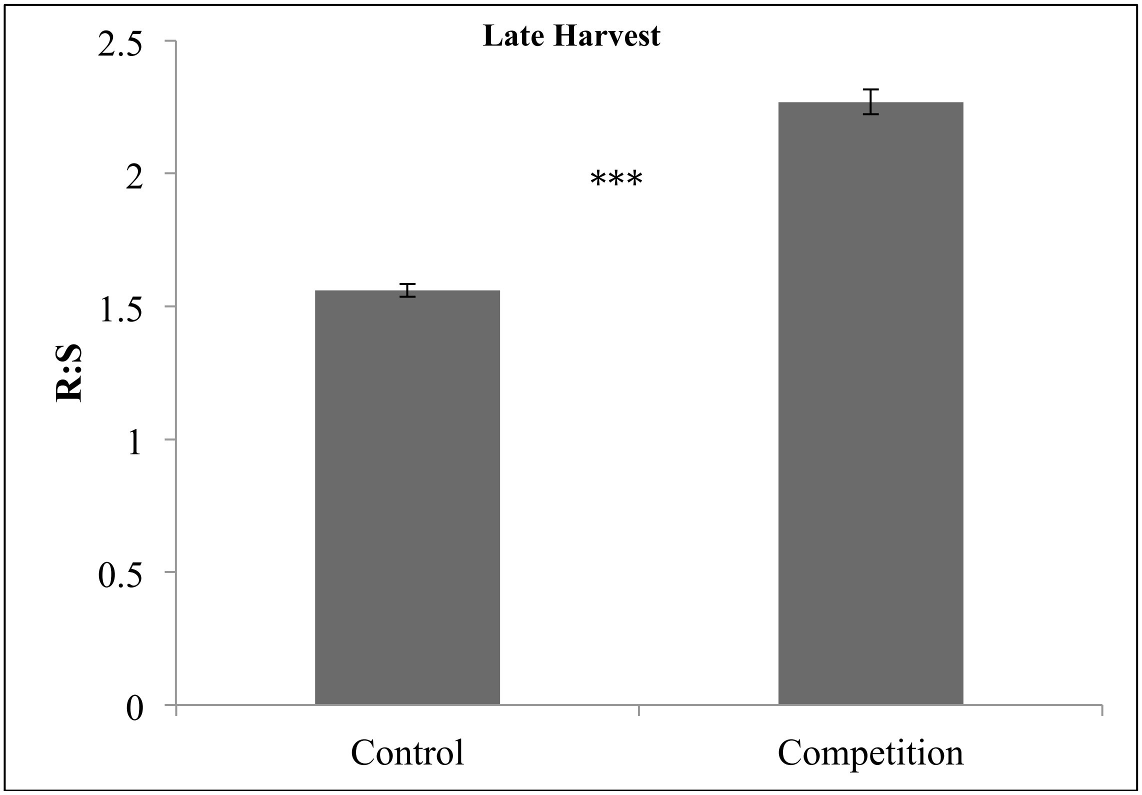


Figure 18.

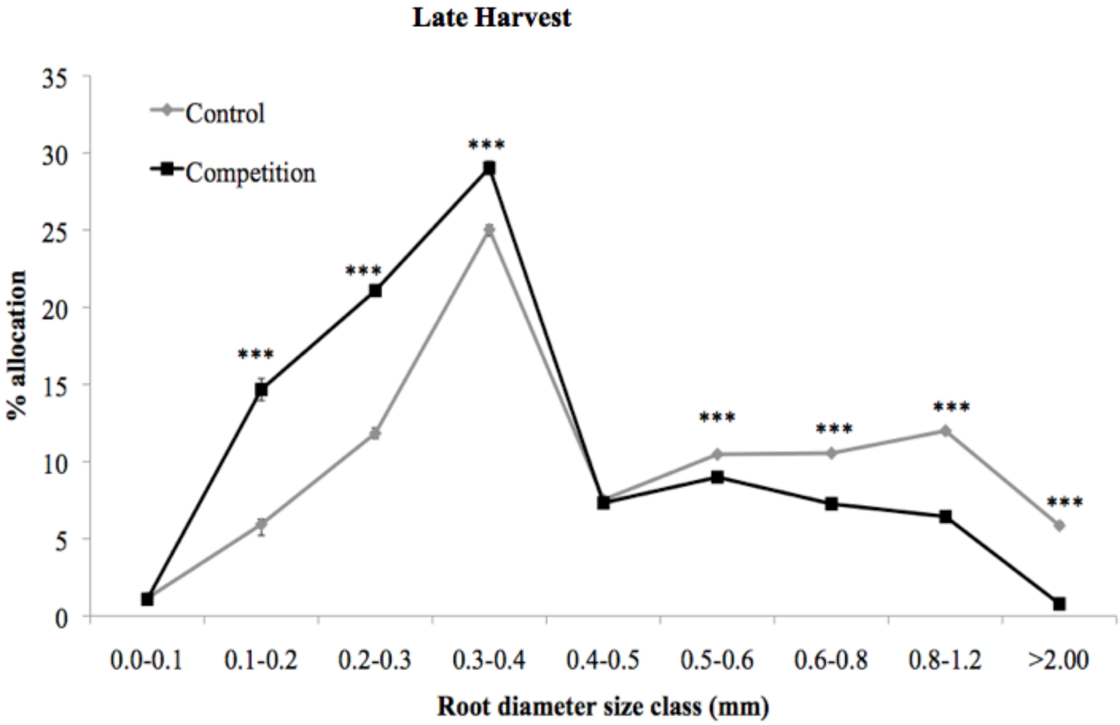

Late Harvest
Control
Competition
% allocation
35
30
25
20
15
10
5
0
***
***
***
***
***
***
***
***
0.0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.8
0.8-1.2
>2.00
Root diameter size class (mm)

Figure 19.

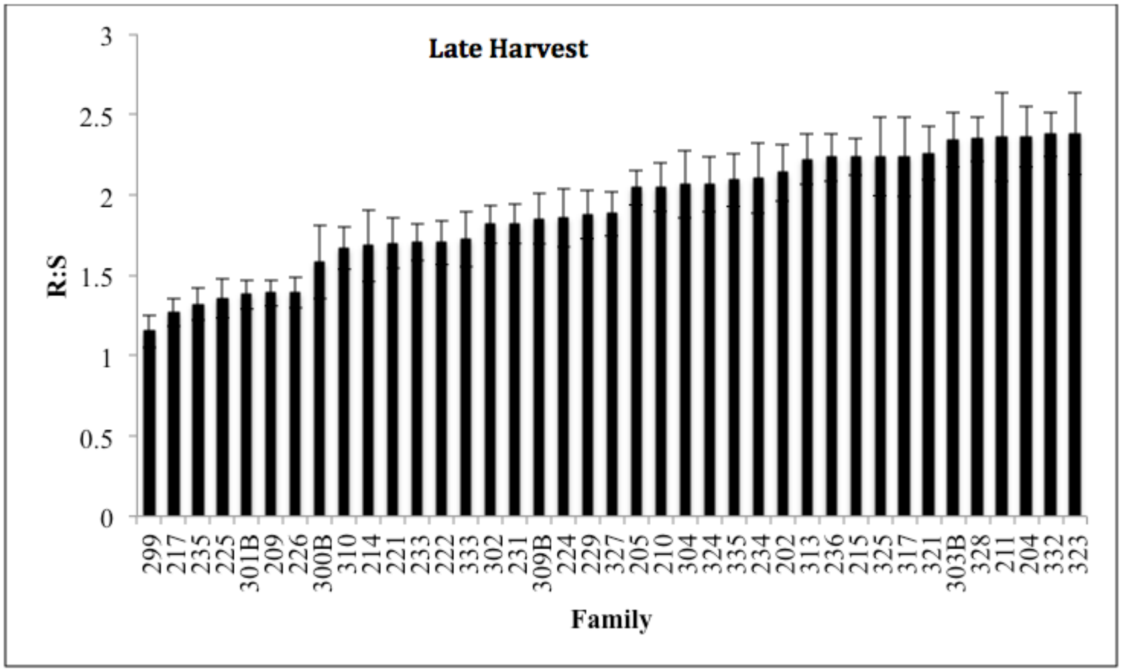

Late Harvest
R:S
3
2.5
2
1.5
1
0.5
0
299
217
235
225
301B
209
226
300B
310
214
221
233
222
333
302
231
309B
224
229
327
205
210
304
324
335
234
202
313
236
215
325
317
321
303B
328
211
204
332
323
Family

Figure 20.

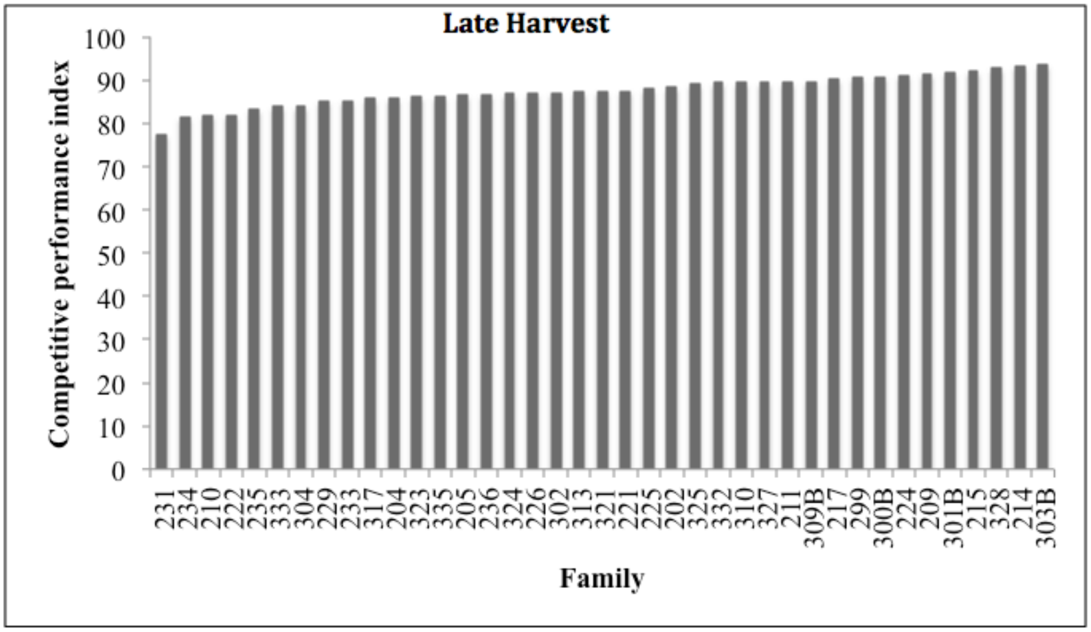

Late Harvest
Competitive performance index
100
90
80
70
60
50
40
30
20
10
0
231
234
210
222
235
333
304
229
233
317
204
323
335
205
236
324
226
302
313
321
221
225
202
325
332
310
327
211
309B
217
299
300B
224
209
301B
215
328
214
303B
Family

Figure 21.

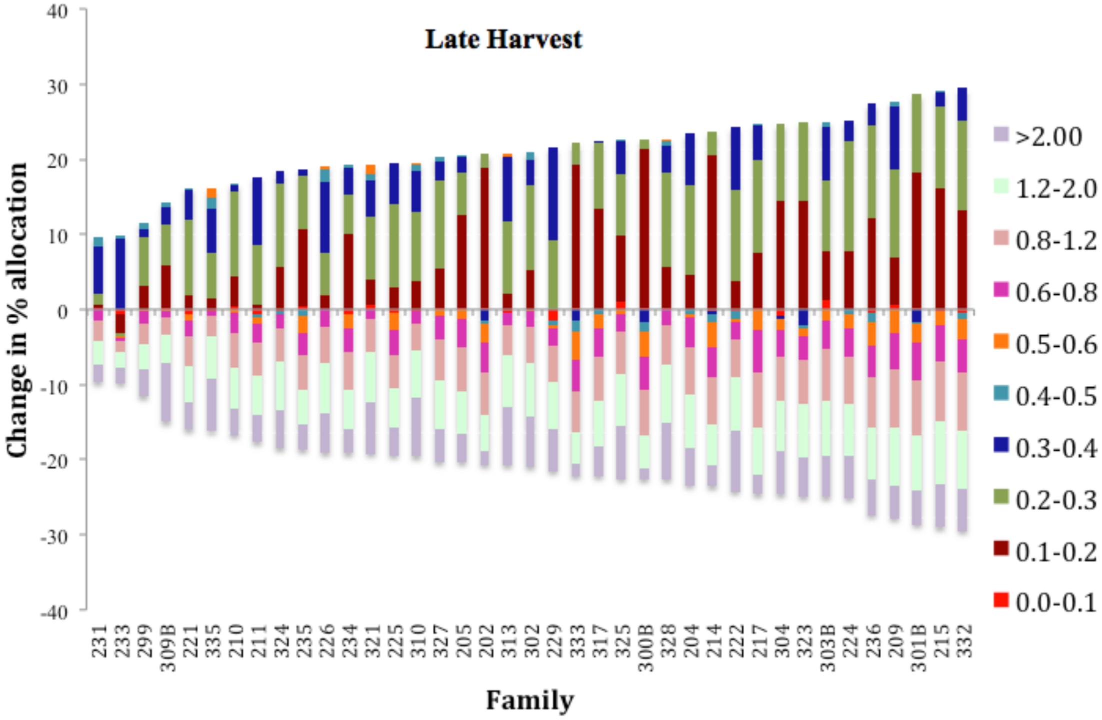

Late Harvest
Change in % allocation
40
30
20
10
0
-10
-20
-30
-40
Family
231
233
299
309B
221
335
210
211
324
235
226
234
321
225
310
327
205
202
313
302
229
333
317
325
300B
328
204
214
222
217
304
323
303B
224
236
209
301B
215
332
>2.00
1.2-2.0
0.8-1.2
0.6-0.8
0.5-0.6
0.4-0.5
0.3-0.4
0.2-0.3
0.1-0.2
0.0-0.1

Figure 22.

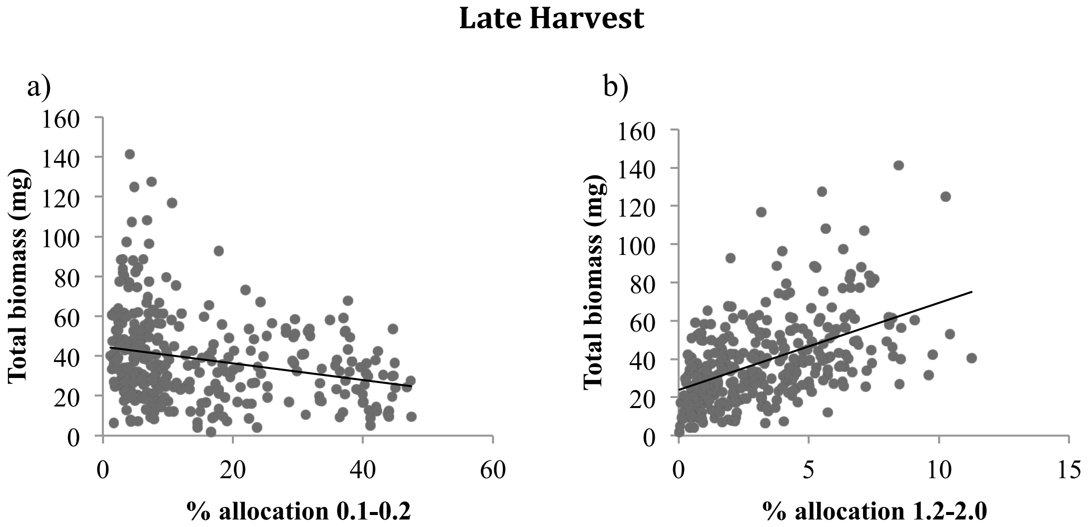

Late Harvest
a)
Total biomass (mg)
160
140
120
100
80
60
40
20
0
0
20
40
60
% allocation 0.1-0.2
b)
Total biomass (mg)
160
140
120
100
80
60
40
20
0
0
5
10
15
% allocation 1.2-2.0

Figure 23.

Late Harvest

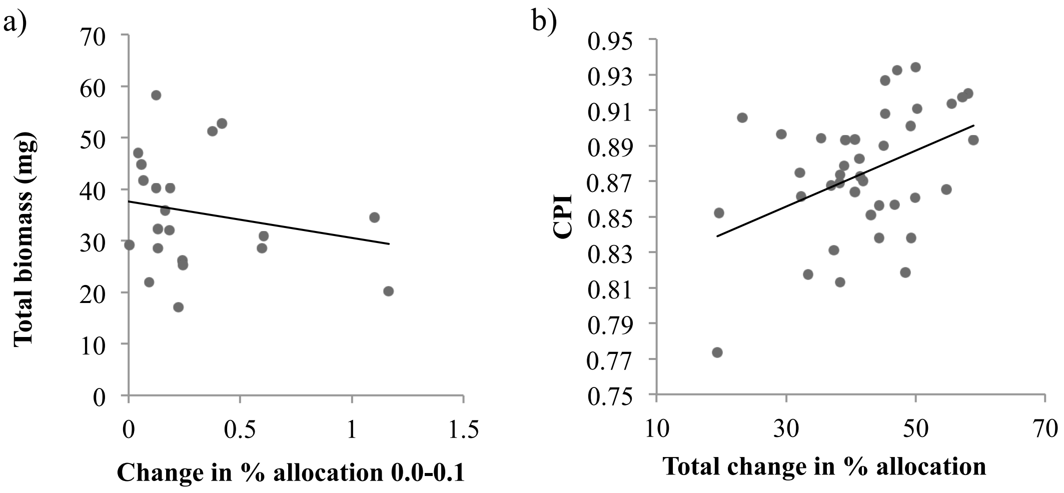
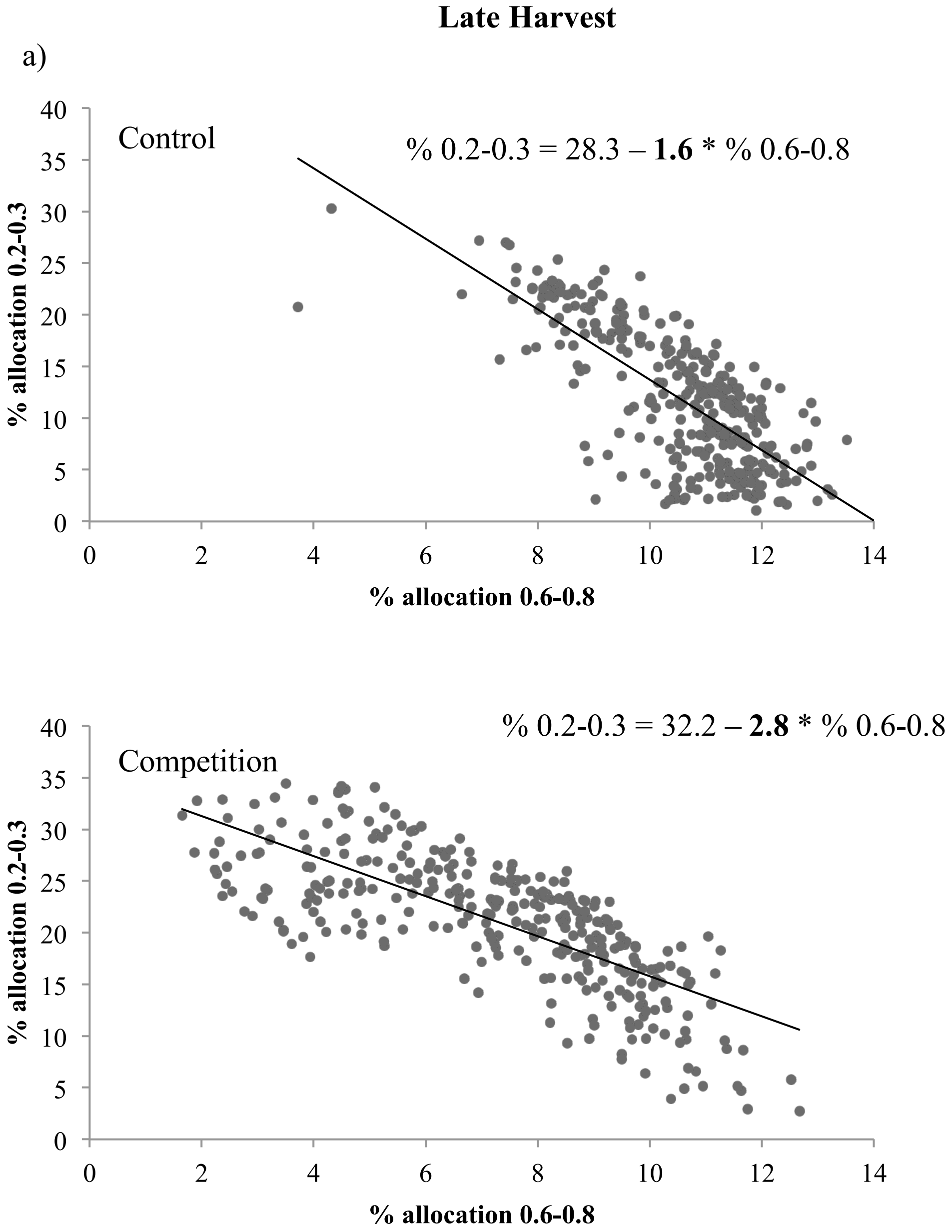
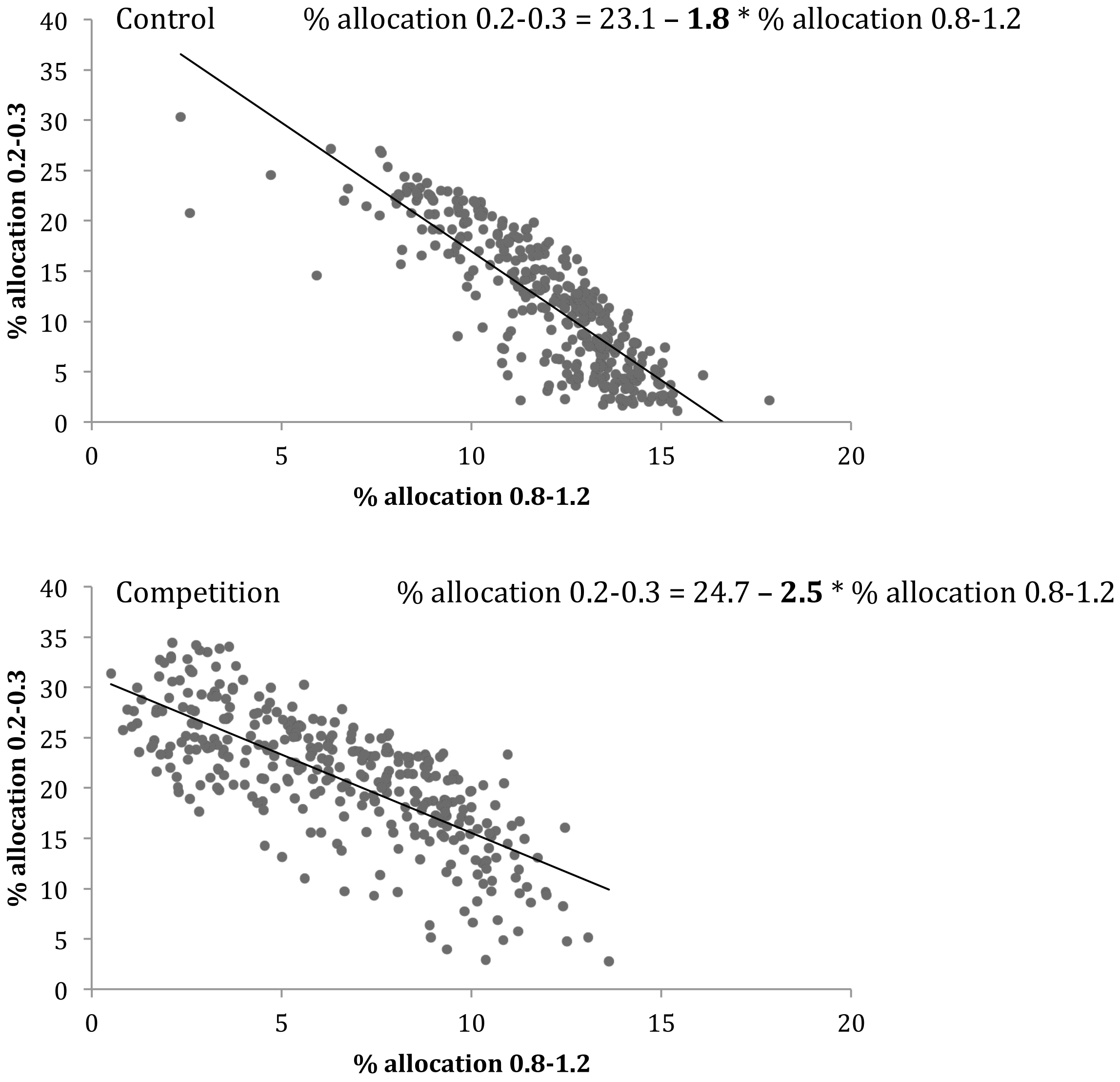


Figure 24.


Late Harvest
a)
Control
% 0.2-0.3 = 28.3 – 1.6 * % 0.6-0.8
% allocation 0.2-0.3
% allocation 0.6-0.8
0
5
10
15
20
25
30
35
40
0
2
4
6
8
10
12
14
% 0.2-0.3 = 32.2 – 2.8 * % 0.6-0.8
Competition
% allocation 0.2-0.3
% allocation 0.6-0.8

b)


Control
% allocation 0.2-0.3 = 23.1 – **1.8** * % allocation 0.8-1.2
% allocation 0.2-0.3
40
35
30
25
20
15
10
5
0
0
5
10
15
20
% allocation 0.8-1.2
Competition
% allocation 0.2-0.3 = 24.7 – **2.5** * % allocation 0.8-1.2
% allocation 0.2-0.3
40
35
30
25
20
15
10
5
0
0
5
10
15
20
% allocation 0.8-1.2