

2018

NUTRIENT CONSERVATION TRAIT RESPONSES OF LOW RESOURCE ADAPTED CHAPARRAL SHRUBS TO INCREASED RESOURCE AVAILABILITY

Reina Lee Nielsen

John Carroll University, rnielsen19@jcu.eduFollow this and additional works at: <https://collected.jcu.edu/masterstheses>Part of the [Biology Commons](#), and the [Plant Biology Commons](#)

Recommended Citation

Nielsen, Reina Lee, "NUTRIENT CONSERVATION TRAIT RESPONSES OF LOW RESOURCE ADAPTED CHAPARRAL SHRUBS TO INCREASED RESOURCE AVAILABILITY" (2018). *Masters Theses*. 36.
<https://collected.jcu.edu/masterstheses/36>

This Thesis is brought to you for free and open access by the Master's Theses and Essays at Carroll Collected. It has been accepted for inclusion in Masters Theses by an authorized administrator of Carroll Collected. For more information, please contact mchercourt@jcu.edu.

NUTRIENT CONSERVATION TRAIT RESPONSES OF LOW RESOURCE ADAPTED
CHAPARRAL SHRUBS TO INCREASED RESOURCE AVAILABILITY

A Thesis Submitted to the
Office of Graduate Studies
College of Arts & Sciences of
John Carroll University
in Partial Fulfillment of the Requirements
for the Degree of
Master of Science

By
Reina Lee Nielsen
2018

The thesis of Reina Lee Nielsen is hereby accepted:



Reader – Dr. Christopher A. Sheil

26 July 2018

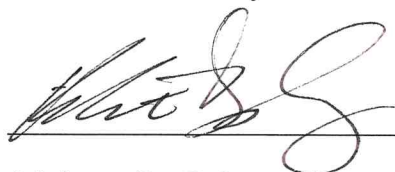
Date



Reader – Dr. Jeremy J. James

26 July 2018

Date

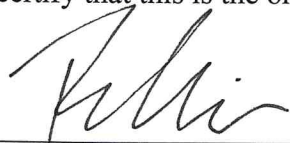


Advisor – Dr. Rebecca E. Drenovsky

26 July 18

Date

I certify that this is the original document.



Author – Reina Lee Nielsen

7/26/2018

Date

Table of Contents.....	3
Abstract.....	5
1. Introduction.....	6
2. Materials and Methods.....	10
2.1 Site and Species Descriptions.....	10
2.2 Greenhouse Experiment.....	11
2.2.1 Initial Conditions.....	11
2.2.2 Experimental Design.....	13
2.2.3 Measurements.....	14
2.2.4 Harvest.....	15
2.3 Field Experiment.....	16
2.4 Analysis.....	18
2.4.1 Nutrient use and conservation trait calculations.....	18
2.4.2 Statistical Analysis.....	18
3. Results.....	19
3.1 Greenhouse Experiment.....	19
3.1.1 Physiological measurements.....	19
3.1.2 Biomass measurements.....	20
3.1.3 Tissue chemistry.....	21
3.1.4 Plasticity.....	21
3.1.5 Tradeoffs.....	22
3.2 Field Observations.....	22
3.2.1 Physiological measurements.....	22
3.2.2 Tissue chemistry.....	23
4. Discussion.....	24
4.1 Species response to water and nutrient additions.....	24

4.2 Plasticity responses to water and nutrient additions.....	25
4.3 Effects of phylogeny on trait responses.....	26
4.4 Trade-offs in nutrient conservation mechanisms.....	27
4.5 Relation to field trait responses.....	27
4.6 Conclusions.....	28
Acknowledgements.....	29
References.....	30
Tables.....	39
Table 1.....	39
Table 2.....	39
Table 3.....	40
Figures.....	41
Figure 1.....	41
Figure 2.....	42
Figure 3.....	43
Figure 4.....	44
Figure 5.....	45
Figure 6.....	46
Figure 7.....	47

Nutrient conservation trait responses of low resource adapted chaparral shrubs to increased resource availability

Abstract

Traits that increase mean nutrient retention times are essential to plant performance in low resource environments, where multiple stressors (low water and nutrients) are present. Although physiological responses to either water or nutrient stress are well understood, fewer studies have investigated the interaction of these stressors from a phylogenetically-controlled, whole-plant perspective. This research focused on three, phylogenetically-controlled pairs of shrubby evergreen species from the California chaparral that either grow on or off serpentine soils, which differ in nutrient availability. Using greenhouse and field studies, the responses of these plants to altered water and nutrient availability were evaluated. The greenhouse study addressed trade-offs among nutrient conservation traits, trait plasticity in response to resource availability, and instantaneous measures of plant stress to increased water and nutrients. The responses of the greenhouse-grown juvenile plants were then compared to juvenile plants growing in the field. I hypothesized that: 1) all species would respond positively to increased water and nutrients by increasing biomass production and having higher rates of gas exchange and nutrient use; 2) faster growing species would exhibit a larger degree of plasticity; and 3) there would be an effect of phylogeny among congener pairs. Juvenile species' trait responses were negatively affected by increased water but not by increased nutrients, and faster growing species generally exhibited higher plasticity. Additionally, phylogeny constrained how these traits respond to environmental changes. Future research will be

crucial to the California chaparral and other low-resource ecosystems as anthropogenic environmental changes continue to accelerate their impact potential, especially near biodiversity hotspots.

1. Introduction

In low-resource systems, soil aridity can exacerbate and amplify soil nutrient stresses due to its negative impacts on soil organic matter decomposition and mineralization (Austin et al., 2004) and plant nutrient uptake (Nye and Tinker, 1977). As a result, plants adapted to these environments possess a suite of traits that promote conservative resource use (Chapin, 1980). Trade-offs in traits that increase mean nutrient retention times are key to plant growth and survival in these environments (Aerts, 1999; Reich et al., 2003; Kou et al., 2016), as high mean retention times promote nutrient conservation (Aerts, 1990; Wright and Westoby, 2003). However, these systems are exposed increasingly to anthropogenic stressors, such as altered precipitation patterns due to climate change (Cayan et al., 2008; Trenberth, 2011) and atmospheric nutrient deposition (Aerts and Chapin, 2000). Given that their plasticity is typically low (Lambers and Poorter, 1992), low-resource adapted plants may be unable to alter trait responses under environmental change scenarios to maintain fitness and survivorship. However, most studies on low-resource adapted plants have focused on single stressors (Funk, 2008; O'Dell et al., 2006), and therefore, it is unknown how they may respond to concurrent changes in water and nutrient availability.

Plants adapted to low-nutrient, arid systems typically are slow growing species that produce lower overall biomass, thicker, long-lived leaves, and higher root mass

ratios than those that are adapted to high resource availability (Grime, 1977; Aerts and Chapin, 2000; Wright et al., 2001; Lambers et al., 2008; Rao et al., 2016). Leaf longevity increases when plants invest more heavily in leaf thickness and defense over metabolic components, and plant nutrient absorption and storage potential is augmented as root biomass allocation is increased (Wright et al., 2001; Rao et al., 2016). Together, these traits can increase mean nutrient residence time but come at a cost to future carbon gain (Wright et al., 2001, 2002; Drenovsky et al., 2010).

Investing more in leaf structural components decreases the amount of carbon that can be acquired by the plant. As leaf thickness increases and more nitrogen is allocated to cell wall proteins (Lambers and Poorter, 1992), less nitrogen is allocated to photosynthesis, decreasing nitrogen use efficiency (PNUE: the ratio of carbon gain to leaf nitrogen; Reich et al., 1998; Wright et al., 2003). As PNUE decreases, less water is lost to transpiration, increasing water use efficiency (WUE: the ratio of the amount of carbon gained per water lost; Lambers et al., 2008). Maintaining a high WUE in order to limit water loss is especially important for low-nutrient adapted plants growing in dry conditions (Vaitkus and McLeod, 1995; Xu et al., 2007). In addition, phosphorus storage in vacuoles, supporting luxury consumption (nutrients taken up from the soil and stored until needed under limiting soil nutrient conditions; Chapin, 1980), can impact carbon gain and thus decrease photosynthetic phosphorus use efficiency (PPUE: the ratio of carbon gain to leaf phosphorus; Hidaka and Kitayama, 2009).

Furthermore, plants from low-resource environments tend to exhibit proficient resorption (moving nutrients from senescing leaves to storage tissues; Chapin 1980; Killingbeck, 2004), which increases mean nutrient residence time (Wright and Westoby,

2003). By internally recycling nutrients through resorption, fewer nutrients need to be absorbed from the soil but requires investment in nutrient mobilization and storage (Silla and Escudero, 2003). Based on these trade-offs, either root nutrient absorption or leaf nutrient resorption may be the best strategy for increasing mean nutrient retention time, depending on soil nutrient availability (Wright and Westoby, 2003). Thus, multiple traits influence mean nutrient residence time, but they may come at a cost if investment in one trait decreases dependence on another—i.e., the traits may be compensatory.

However, it is rare for resorption processes to be placed within studies of whole-plant nutrient budgets, so little is understood about how multiple plant traits (e.g., growth rate, allocation, and resorption) may interact. Moreover, slow-growing species, which are typically less plastic, and fast-growing species, which are generally more plastic, differ in their ability to respond to increased nutrients (Campbell and Grime, 1989; Lambers and Poorter, 1992; Grime and Mackey, 2002). Therefore, the potential for compensatory action in response to resource amendment may depend on trait plasticity. For example, low water and nutrient availability should increase plant root allocation, but have negative effects on nutrient resorption. Therefore, because differences in plasticity exist, fast and slow growing species may differ in their ability to compensate for poor resorption with greater root allocation.

Although most physiological responses to either water or nutrient stress are well understood (e.g., Field et al., 1983; Wright et al., 2003; Hidaka and Kitayama, 2009), fewer studies have comprehensively investigated the interaction of nutrient and water stress on low-nutrient adapted species from a phylogenetically-controlled, whole-plant perspective. Evaluating the interacting effects of multiple stressors is necessary to

understanding plant responses to anthropogenic environmental change. Likewise, accounting for evolutionary history can help determine whether trait responses are the result of adaptation, a consequence of shared common ancestor (Felsenstein, 1985), or both.

This research evaluates how related species from differing nutrient availabilities respond to altered water and nutrient availability, particularly with respect to potential compensatory nutrient conservation mechanisms. This research focused on a suite of related, shrubby species from the California chaparral. Within the chaparral, areas of serpentine soils (those derived from ultramafic bedrock and low in nutrients; Harrison and Rajakaruna, 2011) host shrub species adapted to low water and nutrients; many of these species are endemic to California's serpentine soils. These serpentine soils often form mosaics with interspersed non-serpentine soils, and these soil mosaics are home to congener species growing only on or only off serpentine soils, providing an ideal study system to study related species adapted to different soil types (different levels of nutrients), but within the same climate conditions. Furthermore, California chaparral systems are predicted to experience change in precipitation frequency and intensity (Cayan et al., 2008) and an overall increase in temperature as a result of climate change (Lenihan et al., 2003; Bachelet et al., 2016). Likewise, urban and agricultural pollution threaten the California chaparral with atmospheric nutrient deposition (Bobbink et al., 2010; Fenn et al., 2003, 2010).

The goal of this study was to compare how phylogenetically-controlled pairs of plant species adapted to contrasting resource environments would respond to differing water and nutrient availabilities in a common garden greenhouse experiment, and to

compare greenhouse trait responses to those expressed by mature and immature plants in the field. This study addressed: (1) trade-offs among nutrient conservation traits; (2) overall trait plasticity; and (3) instantaneous measures of plant stress. It is hypothesized that: (1) all species would respond positively to increased water and nutrients by increasing biomass production and having higher rates of gas exchange and nutrient use; (2) faster growing species would exhibit a larger degree of plasticity; and (3) congener pairs would respond similarly to resource amendment as a consequence of their shared evolutionary history. As a result, the higher nutrient adapted species are predicted to have greater compensation among nutrient conservation traits than the lower nutrient adapted species.

2. Materials & Methods

2.1 Site and Species Description

The University of California Donald and Sylvia McLaughlin Natural Reserve, Lower Lake, California, USA is characterized by serpentine and non-serpentine soils that are dominated by chaparral shrublands, grasslands, and seeps. The climate is Mediterranean, with dry, hot summers and cool, wet winters with temperatures ranging from 40° C in the summer to below 0° C in the winter (University of California Davis, 2009). Annual precipitation is ≈ 75 cm (30-year average; US Climate Data, 2017). The chaparral ecosystem at the Reserve is downwind from agricultural areas in the California Coast Range, making it a target for anthropogenically-caused atmospheric nutrient deposition (Fenn et al., 1998).

Three congeneric pairs of evergreen chaparral shrubs were used in this study: *Arctostaphylos manzanita* Parry, *Ceanothus cuneatus* (Hook) Nutt., and *Quercus berberidifolia* Liebm. grow on non-serpentine soil, whereas *A. viscida* Parry, *C. jepsonii* Greene, and *Q. durata* Jepson grow on serpentine soil. *Ceanothus* and *Arctostaphylos* species require specific cues for germination. *Ceanothus* species germinate in response to fire cues, whereas species of *Arctostaphylos* germinate in response to a combination of fire cues and scarification by acid in a mammalian gut. However, *Quercus* readily germinates and requires no special germination cues.

2.2 Greenhouse Experiment

2.2.1 Initial conditions

Fruits of all species were collected from multiple populations at McLaughlin Natural Reserve from at least 10 maternal plants per species. *Arctostaphylos* spp. and *Ceanothus* spp. fruits were collected in 2012 and stored at 28° C to promote after-ripening and maintain desiccated conditions, whereas *Quercus* spp. were collected in winter 2016 and stored at 4° C to prevent germination prior to planting. Because there are inherent differences in seed viability, germination requirements, germination percentages, and seed or fruit size, seed pre-treatments and germination conditions differed by species. Following protocol developed in our lab, *Arctostaphylos* spp. were germinated in fall of 2016, whereas *Ceanothus* spp. and *Quercus* spp. were germinated in January 2017. *Arctostaphylos* and *Ceanothus* seeds were removed from fruits by hand prior to treatments. *Arctostaphylos* spp. were scarified by soaking in concentrated sulfuric acid for 6 hrs. Seeds were rinsed in deionized water until pH paper indicated the rinse solution

was neutral. Seeds were then soaked in < 2% liquid smoke treatments for 12 hours to imitate chemical cues found in wildfire smoke. *Ceanothus* spp. seeds were placed in boiling water for 6 min. To promote germination, seeds of *Arctostaphylos* spp. and *Ceanothus* spp. were germinated in nutrient agar under full spectrum growth lights (PPFD: 100 $\mu\text{mol/s-m}^2$) in a laboratory at John Carroll University, University Heights, Ohio, USA. Upon first appearance of the cotyledons, seedlings were transferred into 4 X 14 cm deep seedling tubes (SC7R Ray Leach Cone-tainer, Stuewe & Sons, Inc.) with a mix of 1:1 sand and fritted clay mixture that contained 1 g of water storing crystals (Miracle-Gro Lawn Products, Inc) homogenized throughout the growing medium. *Quercus* spp were planted immediately into 7 X 25cm deep tree tubes (D40H Deepot, Stuewe & Sons, Inc.) containing sand and fritted clay and placed under full-spectrum Na halide growth lights (PPFD: 350 $\mu\text{mol/s-m}^2$) in the greenhouse. When the specimens of *Arctostaphylos* and *Ceanothus* had at least three sets of true leaves and were able to withstand a higher PPFD without risk of rapid desiccation (approx. 3 months of growth), these species were moved to the greenhouse.

Within one week of planting, all species were watered with a 10% modified Hoagland's solution (Epstein, 1972) and a 10% Bonide Captan Fungicide solution to minimize fungal growth. A modified Hoagland's solution was supplied twice more within the first month of growth. Fungicide was reapplied twice more after approximately one and two months of growth. Once the plants were well-established (April 13, 2017), all species were transplanted into deep, 2.83 L pots (TP414 Tall One Treepot, Stuewe & Sons, Inc.) to ensure enough rooting space for the duration of the study, and plants were allowed to adjust to these pots for two weeks prior to initial treatment.

2.2.2 Experimental Design

Treatment initiation began on May 8, 2017. Species were assigned to treatments using a randomized complete block design with each species having 10 replicates for each of four treatments that were represented once per block. Treatments were: (1) high nutrients (N, P, K), high water; (2) low nutrients, high water; (3) high nutrient, low water; and (4) low nutrients, low water. High nutrient treatments consisted of 2 grams of 10-10-10 slow release NPK fertilizer (0.2 g total N and 0.2g P₂O₅; Miracle-Gro Lawn Products, Inc), whereas low nutrient treatments did not have any added fertilizer. Fertilizer was applied once to the high nutrient treatments at treatment initiation to represent a natural spring nutrient pulse. High water represented a soil water capacity $\geq 18\%$, and low water treatments maintained a soil water capacity of $\approx 9\%$. These water treatments were chosen to mimic a very wet season that could be observed under climate change scenarios and a typical dry season. Soil water availability was monitored three times a week using a Campbell Scientific Hydrosense II probe (Campbell Scientific Inc., North Logan, Utah). Initially for high water treatments, 250 mL of water was added if soil moisture was $< 18\%$, (see Khasanova et al., 2013). For low water treatments, no water was added if soil moisture was $> 9\%$, but 100 mL of water was added if the soil moisture was $< 9\%$. After three weeks of treatment, plant water demand had increased as a consequence of plant growth and longer day lengths; therefore, high water plants received 500 mL every day unless soil moisture capacity was $> 18\%$. This watering regime was followed until October 23, 2017, at which point all plants were allowed to slowly dry-down, mimicking end-of-season field conditions encouraging leaf senescence. Seasonal dry-down was encouraged by decreasing water addition treatments and maintaining the soil water

capacity at lower levels. The high water treatment was maintained at 11 %, and plants were given 100 mL of water when soil moisture levels were not met. The low water treatment was maintained at 9 % soil moisture capacity, and plants were given 50 mL of water when soil moisture levels were not maintained. Some plants in the low water treatment were large enough to need 100 mL of water in order to maintain a 9 % soil moisture capacity (Table 1). Also at this time, the greenhouse was set to cooler conditions similar to winter months at the field site (daytime: 13–18° C; night: down to 7° C).

During the duration of this experiment, fungal growth and powdery mildew were evident on some plants. To combat fungal diseases, 100 mL of Bonide Captan Fungicide was applied four times throughout the experiment. When fungicide was applied, a 100 mL of water normally allocated for each daily treatment was substituted with the 100 mL of fungicide. If a sample did not need watering that day, fungicide was applied the next time water was required. When powdery mildew was observed, the affected leaf was treated with soapy water and 1 % Rose Rx 3 in 1 solution, alternating when one was no longer effective, and then rinsed with small amounts of water.

2.2.3 Measurements

Physiological and morphological measurements were made on a subset of plants from each treatment. Gas exchange was measured on the youngest fully mature leaf using a LI-COR 6400XT Portable Photosynthesis System to assess instantaneous plant nutrient use efficiency and water use efficiency (LI-COR Inc., Lincoln, Nebraska, USA). Following measurement, the leaf was harvested and used to measure projected leaf area via image analysis (WinRhizo, etc.) in order to correct area-based gas exchange rates.

Gas exchange measurements were measured midday on July 17, 2017 (24° C; sunny) and again on August 25, 2017 (22° C; sunny) when the plants were experiencing maximal seasonal growth. CO₂ flow was set to 400 μmol s⁻¹, the CO₂ mixer to 400 μmol mol⁻¹, and the light level in the LED chamber to a PPFD slightly above ambient conditions (1300 μmol m⁻²s⁻¹). Three subsamples were taken at 10-seconds intervals for each replicate plant. IRGAs were matched every 3 plants. All leaves harvested at this time were dried and weighed to be included in further biomass analyses.

Stem height was measured four times throughout the experiment. Initial stem height was measured during treatment initiation. Stem height was measured from soil level to apical meristem. As soil levels shifted throughout the experiment, nail polish was used to mark the soil level on plant stems at the initial stem height reading. Stem height was also recorded on 8 July, 11 September, and 1 December 2017 and was measured from the nail polish marking to the apical meristem. These measurements were used to determine relative growth rates (increases in stem height over time; mm/day; Hunt, 1982) of each sample. Senescent leaves were collected throughout the fall drawdown treatment phase, and the date of collection was recorded.

2.2.4 Harvest

A destructive harvest was performed on 1 and 2 December, 2017, at which point remaining senescent leaves were collected to determine nutrient resorption proficiency (*sensu* Killingbeck, 1996); these leaves were set aside for later analysis. Belowground biomass was separated from aboveground biomass at the soil level. Soil was removed from the belowground biomass, and all biomass was dried at 65°C until constant mass

was achieved. Once dried, roots, stems, and leaves were rinsed to remove excess soil or dust, allowed to dry for another 48 hours at 65° C, and weighed separately. The leaves previously harvested for physiological measurements were included in the final leaf masses at this time. A soil sample from each replicate was taken in order to analyze total nitrogen and extractable phosphorus at the University of California Davis on a later date. Total nitrogen and phosphorus were analyzed for green leaves collected during the harvest and for senescent leaves. Prior to analyses, all biomass was ground using a Wiley mill and a #40 mesh screen. Samples too small to be ground using a Wiley Mill were hand ground using a stainless steel mortar and pestle. Total leaf nitrogen concentration was analyzed via a CN analyzer (ECS 4010; Costech Analytical, Valencia, California, USA). Total leaf tissue phosphorus was analyzed via ICP-OES (Plasma 400; Perkin-Elmer, Waltham, Massachusetts, USA), following dry-ashing and acid dissolution. Phosphorus resorption proficiency was not determined due to low senescent leaf sample sizes. Senesced leaf N and P represent resorption proficiency of these nutrients (*sensu* Killingbeck, 1996).

2.3 Field Experiment

In late June 2017, field measurements were made on 10 mature and 10 immature plants of each species of interest at the University of California Donald and Sylvia McLaughlin Natural Reserve. The non-serpentine sampling site was located along an access road and adjacent hill (N 38 52.747, W 122 26.625). The immature specimens were found atop this hill and along the roadside. The serpentine site was located on a steep hill (N 38 51.999, W 122 24.148) that had been partially burned by a wildfire in

2016. The mature specimens were all located on the unburnt section, whereas most of the immature specimens were found in the burnt areas. Plants were selected based on size similarity to one another in order to measure plants that were roughly the same age. Immature species selected for study were similar in size (< 75 cm) to those grown in the greenhouse experiment at the time of physiological measurements. Midday gas exchange, leaf nitrogen, and leaf phosphorus were measured following the same methods as in the greenhouse experiment. For gas exchange measurements, CO_2 flow was set to $400 \mu\text{mol s}^{-1}$, the CO_2 mixer to $400 \mu\text{mol mol}^{-1}$, and the light level in the LED chamber to a PPFD slightly above ambient conditions ($1800 \mu\text{mol m}^{-2}\text{s}^{-1}$). Fully mature green leaves for nitrogen and phosphorus analysis were harvested from each plant, photographed for later SLA analysis, and dried until constant weight. Using a model 1000 pressure bomb chamber (PMS Instruments, Albany, Oregon, USA), midday stem water potential was measured on a subset of samples. A plastic bag was placed over the shoot before removing it from the plant to ensure transpiration did not continue. The sample was placed on ice until measured. Bark was removed prior to measurement to prevent phloem sap from obscuring water potential measurements. These measurements were used to compare plants growing in natural conditions to the plants grown in the greenhouse. Five soil samples were taken at a depth of 15–20 cm from areas within each site where the majority of our sample mature and immature species were located. The soil was sieved to determine percent gravel ($< 2\text{mm}$), and the rest of the soil was used to analyze soil nitrogen and extractable phosphorus concentration.

2.4 Analysis

2.4.1 Nutrient use and conservation trait calculations

Plant relative growth rate (RGR) was calculated using the following equation:

$$\text{RGR} = \frac{\text{final height/starting height}}{\text{days since initial stem height measurement}}$$

Gas exchange and leaf nutrient data were used to calculate instantaneous water use efficiency (ratio of the amount of carbon gained per water loss; A/g_s , $\mu\text{mol mol}^{-1}$), photosynthetic phosphorus use efficiency (ratio of carbon gain to leaf phosphorus; $\mu\text{mol CO}_2 \text{ mol P}^{-1} \text{ s}^{-1}$), and photosynthetic nitrogen use efficiency (ratio of carbon gain to leaf nitrogen; $\mu\text{mol CO}_2 \text{ mol N}^{-1} \text{ s}^{-1}$).

$$\begin{aligned}\text{WUE} &= \frac{\text{photosynthetic rate}}{\text{conductance}} \\ \text{PPUE} &= \frac{\text{photosynthetic rate}}{(\text{P g kg}^{-1} * \text{SLA kg/m}^2 * \text{mol P})} \\ \text{PNUE} &= \frac{\text{photosynthetic rate}}{(\text{N g kg}^{-1} * \text{SLA kg/m}^2 * \text{mol N})}\end{aligned}$$

Trait plasticity on RGR, total biomass, A , PPUE, PNUE, WUE, and senesced leaf nitrogen was determined using a PI_v analysis (see Valladares et al., 2006).

2.4.2 Statistical Analysis

To identify factors influencing physiological and morphological response variables, mixed-model MANOVAs were used. Greenhouse data were analyzed using MANOVAs that included congener pair (random effect), species (random effect), block (random effect), water treatment (fixed effect), nutrient treatment (fixed effect), and origin (serpentine or non-serpentine; fixed effect) as the main effects (see Funk et al.

2015). The interaction effect was the two-way interactions of water and nutrients, nutrients and origin, and water and origin, and a three-way interaction of water, nutrients, and origin. Field data were analyzed using MANOVAs that included congener pair (random effect), species (random effect), soil (serpentine or non-serpentine; fixed effect), and age (fixed effect) as the main effects. The interaction effect was the two-way interaction of soil and age. Physiological traits (A, WUE, PNUE, and PPUE) for the greenhouse data and the field data, biomass related traits (RMR, RGR, and total biomass), tissue chemistry (green leaf N and P) and soil components (N, P, K, electrical conductivity (E.C.), and pH) were grouped together for separate MANOVA models. Instantaneous physiological traits were averaged between the two dates of measurements for the greenhouse data. Assumptions of MANOVA (equal variance and normal distribution) were tested with a Shapiro-Wilks test and a Bartlett's test, and corrected when possible before a MANOVA model was run. For the soil and greenhouse physiological MANOVA's, data were transformed using a log10 transformation to better meet these assumptions. Because of low sample sizes, ANOVAs were run for field water potential and greenhouse nitrogen resorption. All analyses were run using the R statistical program (R coding team, 2016) using version 3.3.1 (2016-06-21).

3. Results

3.1 Greenhouse Experiment

3.1.1 Physiological measurements

Physiological responses depended on the water and nutrient treatment the species received ($F_{4,78} = 6.137$; Pillai = 0.239; $p < 0.001$). In general, the low water, high nutrient

treatment tended to have higher rates of A and PPUE and lower rates of PNUE than other treatments (Figure 1), whereas the high water, low nutrient plants had low rates of A, WUE, and PPUE, and moderate rates PNUE.

3.1.2 Biomass measurements

There was a significant interaction between water and nutrients ($F_{3,195} = 13.563$; Pillai = 0.173; $p < 0.001$), water and origin ($F_{3,195} = 4.091$; Pillai = 0.059; $p < 0.01$), and nutrients and origin ($F_{3,195} = 8.240$; Pillai = 0.113; $p < 0.001$) for growth and biomass allocation measures (RMR, RGR, and total biomass). In general, plants receiving the low water, high nutrient treatment had faster growth rates and a larger overall biomass (Figure 2) than plants receiving all other treatments. Plants treated with high water and no added nutrients had the slowest RGR and lowest total biomass. RMR was lowest under the low water, high nutrient treatments and generally was highest in the high water, low nutrient treatment. Non-serpentine species under low water treatments had the fastest growth rate and largest total biomass. When given additional water, both serpentine and non-serpentine species decreased in RGR, total biomass, and increased slightly in belowground allocation. However, the serpentine species always had a slightly lower RGR and total biomass when given water than the non-serpentine species. When only observing the nutrient treatments, non-serpentine species had a higher RGR and larger total biomass in the high nutrient treatment as compared to the low nutrient treatments of all species. RMR was lowest in the serpentine species in the high nutrient treatments and low water treatments.

3.1.3 Tissue chemistry

There was a significant interaction between water and nutrients ($F_{2,156} = 24.208$; Pillai = 0.237; $p < 0.001$) and between nutrients and origin ($F_{2,156} = 6.394$; Pillai = 0.076; $p < 0.01$) for green leaf nitrogen and phosphorus. For all species, the low water, high nutrient treatment resulted in the highest green leaf nitrogen, whereas the lowest concentration of nitrogen were in the plants under the high water, low nutrient treatment, as well as the high water, high nutrient treatments (Figure 3). Green leaf phosphorus concentration was the lowest in the low water, high nutrient treatment. In the low nutrient treatments, the serpentine species tended to have lower green leaf nitrogen and phosphorus concentrations, whereas the high nutrient treatments resulted in similar green leaf nitrogen and phosphorus concentrations between the serpentine and non-serpentine species.

There was a significant two-way interaction between water and nutrients ($F_{1,79} = 4.236$; $p < 0.05$) for senesced leaf nitrogen. Generally, the low water, high nutrient and high water, high nutrient treatments had the least amount of nitrogen resorbed, whereas the high and low water treatments that also had low nutrients resorbed the most nitrogen from their leaves. In general, plants receiving high nutrients began senescing leaves sooner than plants in the low nutrient treatments, with high nutrient plants beginning leaf senescence up to 23 days earlier than plants in other treatments ($F_{1,115} = 6.948$; $p < 0.01$).

3.1.4 Plasticity

Overall, non-serpentine species were generally more plastic than the serpentine species, and traits associated with growth rate and biomass accumulation were more plastic than those associated with instantaneous physiological measures or tissue chemistry. Additionally, although not true for every trait, there was some evidence that congener pairs were similarly plastic, particularly for senesced leaf nitrogen and RGR (Table 3).

3.1.5 Tradeoffs

Based on the PCA visualization of trait responses, species responded more to environmental pressures than phylogenetic influences, and most resource conservation traits tended to group closely together (Figure 4). Whereas the *Quercus* species tended to group close together regardless of treatment, all other species responses were driven by the water and nutrient treatments. Those plants that received the low water, high nutrient treatment generally also expressed resource conservation traits more strongly than plants in other treatments. Most resource conservation traits were complementary to one another as the vectors representing these traits were close to one another. However, there was a direct tradeoff between WUE and PNUE. Contrary to expectations, there was no direct tradeoff between RMR and senesced leaf nitrogen.

3.2 Field Observations

3.2.1 Physiological measurements

Origin ($F_{4,103} = 9.490$; Pillai = 0.269; $p < 0.001$), age ($F_{4,103} = 5.074$; Pillai = 0.165; $p < 0.001$) and species ($F_{16,424} = 6.948$; Pillai = 0.831; $p < 0.001$) were the main

drivers of physiological function for plants growing under field conditions. Serpentine species had higher rates of A, PNUE, and PPUE and lower WUE than non-serpentine species. Mature species had higher rates of A and PPUE but lower PNUE than the immature species. WUE did not differ by age. *Ceanothus jepsonii* had the highest rates of A ($8.15 \mu\text{mol m}^{-2} \text{s}^{-1}$) and PPUE ($152.55 \mu\text{mol CO}_2 \text{mol P}^{-1} \text{s}^{-1}$), whereas *A. manzanita* had the highest water use efficiency ($87.99 \mu\text{mol mol}^{-1}$). The *Arctostaphylos* species had the highest PNUE. The *Quercus* species were the lowest in A, WUE, and PNUE, and *Q. berberidifolia* the lowest in PNUE ($9.1 \mu\text{mol CO}_2 \text{mol N}^{-1} \text{s}^{-1}$; Figure 5). Water potential in these plants was dependent on species ($F_{4,51} = 25.668$; $p \leq 0.001$) and age ($F_{1,51} = 169.026$; $p \leq 0.001$). *Arctostaphylos Manzanita* (-3.05 MPa) and *C. cuneatus* (-3.05 MPa) had the lowest water potentials, whereas all other species were similar in their water potential values. The juveniles had slightly lower water potentials than the adults (Figure 6).

3.2.2 Tissue chemistry

Origin ($F_{2,105} = 27.421$; Pillai = 0.343; $p < 0.001$), age ($F_{2,105} = 14.428$; Pillai = 0.216; $p < 0.001$), and species ($F_{8,212} = 21.612$; Pillai = 0.898, $p < 0.001$) were the main drivers of green leaf tissue chemistry. Non-serpentine species had slightly higher green leaf nitrogen concentrations and much higher leaf phosphorus concentrations than serpentine species. Adults had higher green leaf nitrogen and phosphorus than the juvenile species. *Arctostaphylos* species had the lowest concentrations of green leaf nitrogen, whereas all other species were similar. *Quercus berberidifolia* had the highest

green leaf phosphorus concentration (1.85 g kg^{-1}), whereas *C. jepsonii* had the lowest green leaf phosphorus concentration (0.80 g kg^{-1} ; Figure 7).

4. Discussion

4.1 Species response to water and nutrient additions

The hypothesis that all species would respond positively to increased water and nutrients was not supported. Instead, plant performance was often greatest in the low-water, high-nutrient treatment, and lowest in high-water only treatment. Plants in the low-water, high-nutrient treatment invested less resources in root biomass and achieved the highest RGR and total biomass, contrary to what we might expect under droughted conditions (Chapin, 1980). Allocation aboveground, rather than into roots, promotes future carbon gain and thus a greater return on investment (Drenovsky and James, 2010), which likely supports higher biomass accumulation in this treatment. If biomass is an approximate proxy for fitness in these species, our data suggest that these chaparral species could respond positively to future nutrient deposition, but only if precipitation patterns support dry soil conditions. In contrast, high-water treatments had negative impacts on plant performance, particularly under low nutrient conditions and for the serpentine species, suggesting that increased precipitation may be a greater threat to plant success under future environmental change scenarios than nutrient deposition.

Compared to other treatments, plants had higher green leaf nitrogen and lower green leaf phosphorus in the low water, high nutrient treatment, which could be due to the mobility of these nutrients. Nitrogen, which has a high mobility, can easily be taken up from the soil, as transpiration in these species was maintained even under droughted

conditions (data not shown). In contrast, phosphorus is relatively immobile, thus limiting the amount of phosphorus that could be absorbed, especially in low-water conditions (Marschner et al., 2012). It is also possible that the low green leaf phosphorus observed in the low-water, high-nutrient treatment could be the result of biomass dilution (Jarrell and Beverly, 1981), as these plants also had the largest overall biomass. Not only did the treatments affect green leaf tissue chemistry but also resorption proficiencies of these species. The species given the low-nutrient treatments, particularly the high-water, low-nutrient treatment, tended to be very proficient resorbers, often exhibiting complete resorption (Killingbeck, 1996). However, the species in high nutrient treatments tended to resorb less, implying that these species rely less on resorption as a nutrient conservation strategy when soil nutrients are high. Because resorption is an energy intensive process (Chapin and Kedrowski, 1983), plants exhibiting intermediate or incomplete resorption will have more energy available to spend on other processes, such as growth, as was observed in all our species.

4.2 Plasticity responses to water and nutrient additions

The hypothesis that faster growing, non-serpentine species would be more plastic in functional traits than the slower growing, serpentine species was supported for all traits measured. Higher overall plasticity in fast-growing species is to be expected, as plants from higher resource areas are better adapted to take up nutrients when they become available (Funk, 2008), unlike slow-growing plants, which tend to have a steady rate of uptake regardless of nutrient availability (Chapin, 1980). Species were the most plastic in traits related to growth and biomass allocation rather than physiological traits or tissue

chemistry. Physiological traits, particularly WUE, had only slight differences in plasticity, implying that these traits respond similarly between all species. However, gas exchange data were highly variable within species and treatment, which could have masked underlying physiological plasticity. Unpublished data collected in 2016 on *C. cuneatus* and *C. jepsonii* rarely had values of A exceeding $20 \mu\text{mol m}^{-2} \text{s}^{-1}$, whereas data for *C. cuneatus* almost always exceeded $20 \mu\text{mol m}^{-2} \text{s}^{-1}$, except for the high-water, low-nutrient treatment, suggesting that the values for this species were high. In contrast to physiological traits, biomass and RGR were highly plastic, increasing in response to nutrient addition and decreasing in response to soil moisture. These responses indicate that plasticity, particularly in non-serpentine species, may help these species respond positively to nutrient deposition and negatively to any increases in precipitation.

4.3 Effects of phylogeny on trait responses

The final hypothesis, that congener pairs would respond more similarly to each other than other congener species, was supported, especially in the *Quercus* species. The oaks, regardless of origin, responded very similarly to each other for all traits measured in the greenhouse experiment as seen in the PCA analysis, suggesting that phylogeny influences how these species respond to resource availability. If the oaks share a more recent common ancestor than the other congener pairs, they would have had less time for trait divergence. However, dated phylogenies on the suite of species studied here is needed to investigate this hypothesis. Although each congener pair of *Arctostaphylos* and *Cuneatus* were similar in the direction of their trait responses, the magnitude of their responses between species within a pair differed. For example, the serpentine species of

each pair had slower RGR and lower total biomass, irrespective of treatment, as is common among slow-growing species (Aerts and Chapin, 2000). These results are in agreement with other findings for traits exhibited by serpentine and non-serpentine species growing in the California chaparral, in which congener species behaved similarly in resorption proficiency (Drenovsky et al., 2013) and biomass and leaf nutrient concentrations (O'Dell et al., 2006).

4.4 Trade-offs in nutrient conservation mechanisms

Except for the expected trade-off observed between WUE and PNUE (Field et al., 1983), most resource conservation traits and traits associated with resource acquisition were complementary to each other. This result is surprising, as trade-offs between traits associated with obtaining resources versus retaining resources are predicted in the literature (Aerts, 1999). However, these data suggest that the high growth and biomass accumulation associated with the low water, high nutrient treatment was supported by complementary RGR and resource conservation traits. Additionally, an expected tradeoff between RMR and traits associated with resource retention (Aerts, 1999; Aerts and Chapin, 2000) was not observed. It is possible that under greenhouse conditions, plants still experienced sufficient water uptake to maintain plant function, without additional investment in root biomass. Alternatively, these data may suggest that investment in roots reflects greater need for storage under low resource conditions, as well as supporting resource uptake.

4.5 Relation to field trait responses

The field trait responses suggest that the greenhouse study may have provided more optimal growing conditions than what was observed in the field. At the time of field measurements, weather conditions were hot (35° C) and sunny, possibly causing significant stress on the plants we measured. The low water potentials observed indicated that these plants were at least under a high amount of water stress during this period. The stressful conditions observed in the field could account for the higher photosynthetic rates observed in the greenhouse compared to the low rates observed in the field, as the greenhouse measurements were made when it was 10° C cooler. Whereas field mature and immature species varied in their traits by species and age, gas exchange responses from all ages and species measured in the field were generally still lower than the responses to the same traits measured in the greenhouse. However, previously collected field data of senesced leaf nitrogen in the same species (Drenovsky et al., 2013) indicate that field individuals were equally proficient at resorbing nitrogen as those grown under low nutrient greenhouse conditions, but more proficient than greenhouse grown plants under high nutrient conditions. These data suggest that the immature species grown in the greenhouse under low nutrient concentrations may have been exposed to similar nutrient stress as the adults growing in the field and that age may not be a large driver of resorption proficiency in the focal taxa of this study.

4.6 Conclusions

This study suggests that combined anthropogenic effects of changing precipitation patterns and nutrient deposition may have strong impacts on physiological function and growth of low resource adapted plants, although trait responses to these stressors may be

evolutionarily constrained. Especially in the short-term for immature species, traits may be more sensitive to increased soil moisture than to nitrogen deposition. However, non-serpentine species may exhibit greater trait plasticity and likely be able to respond better to anthropogenic environmental stressors by increasing in biomass. However, as urban sprawl and nitrogen deposition increase (Bobbink et al., 2010; Fenn et al., 2003, 2010), further research will be needed to determine the potential limits of these plastic responses and to assess the nutrient loads that these species can tolerate. Additionally, plant responses to predicted precipitation scenarios (increased frequency, intensity, or duration) must be assessed, particularly in conjunction with increased nutrient pulses. Moreover, nitrogen deposition may promote growth of non-native species in the California chaparral, potentially increasing invasion success and yielding changes in community structure (Allen et al., 1998; Minnich and Dezzani, 1998; Vourlitis, 2017). It is unclear how these competitive pressures may influence shrub recruitment and success under various scenarios of environmental change. Future research will be crucial to the California chaparral and other low-resource ecosystems as anthropogenic environmental changes continue to accelerate their impact potential, especially near biodiversity hotspots.

Acknowledgements

The author would like to thank committee members Drs. Rebecca Drenovsky, Christopher Sheil, and Jeremy James for guidance and support. The author would like to thank Cathy Koehler for collecting fruits from the McLaughlin Natural Reserve for this study and Cathy Wheeler for help in germinating and maintaining seedlings. The author

extends thanks to labs members for helping process samples and providing support. Lastly, the author thanks Nels Foslien for continuous support and encouragement throughout this study. This research was supported through funding by John Carroll University.

References

- Aerts, R. (1990). Nutrient use efficiency in evergreen and deciduous species from heathlands. *Oecologia* 84, 391–397.
- Aerts, R. (1999). Interspecific competition in natural plant communities: mechanisms, trade-offs and plant–soil feedbacks. *Journal of Experimental Botany* 50, 29–37.
- Aerts, R., and Chapin, F. S. (2000). The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* 30, 1–67.
- Allen, E. B., Padgett, P. E., Bytnerowicz, A., and Minnich, R.. (1998). Nitrogen deposition effects on coastal sage vegetation of southern California. USDA Forest service General Technical Report 166, 131–140.
- Austin, A. T., Yahdjian, L., Stark, J. M., Belnap, J., Porporato, A., Norton, U., et al. (2004). Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia* 141, 221–235.

- Bachelet, D., Ferschweiler, K., Sheehan, T., and Strittholt, J. (2016). Climate change effects on southern California deserts. *Journal of Arid Environments* 127, 17–29.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., et al. (2010). Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* 20, 30–59
- Campbell, B. D., and Grime, J. P. (1989). A comparative study of plant responsiveness to the duration of episodes of mineral nutrient enrichment. *New Phytologist* 112, 261–267.
- Cayan, D. R., Maurer, E. P., Dettinger, M. D., Tyree, M., and Hayhoe, K. (2008). Climate change scenarios for the California region. *Climate Change* 87, S21–S42.
- Chapin, F. S. (1980). The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11, 233–260.
- Chapin, F. S. and Kedrowski, R. A. (1983). Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous taiga trees. *Ecology* 64, 376–391.
- Drenovsky, R.E., and James, J.J. (2010). Designing invasion resistant plant communities: The role of plant functional traits. *Rangelands* 32, 32–37.

Drenovsky, R. E., James, J. J., and Richards, J. H. (2010). Variation in nutrient resorption by desert shrubs. *Journal of Arid Environments* 74, 1564–1568.

Drenovsky, R. E., Koehler, C. E., Skelly, K., and Richards, J. H. (2013). Potential and realized nutrient resorption in serpentine and non-serpentine chaparral shrubs and trees. *Oecologia* 171, 39–50

Epstein, E. (1972). *Mineral nutrition of plants: principles and perspectives*. John Wiley, New York, New York, USA.

Felsenstein, F. (1985). Phylogenies and the comparative method. *The American Naturalist* 125, 1–15.

Fenn, M. E., Poth, M. A., Aber, J. D., Baron, J. S., Boremann, B. T., Johnson, D. W., et al. (1998). Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. *Ecological Applications* 8, 706–733.

- Fenn, M. E., Baron, J. S., Allan, E. B., Rueth, H. M., Nydick, K. R., Geiser, L., et al. (2003). Ecological effects of nitrogen deposition in the western United States. *BioScience* 53, 404–420.
- Fenn, M. E., Allen, E. B., Weiss, S. B., Jovan, S., Geiser, L. H., Tonnesen, G. S., et al. (2010). Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. *Journal of Environmental Management* 91, 2404–2423.
- Field, C., Merino, J., and Mooney, H. A. (1983). Compromises between water-use efficiency and nitrogen-use efficiency in five species. *Oecologia* 60, 384–389.
- Funk, J. L. (2008). Differences in plasticity between invasive and native plants from a low resource environment. *Journal of Ecology* 96, 1162–1173.
- Funk, J. L., Rakovski, C. S., and Schmid, J. M. M. (2015). On the analysis of phylogenetically paired designs. *Ecology and Evolution* 5, 940–947.
- Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist* 111, 1169–1194.
- Grime, J. P., and Mackey, J. M. L. (2002). The role of plasticity in resource capture by plants. *Evolutionary Ecology* 16, 299–307.

- Harrison, S., and Rajakaruna, N. (2011). *Serpentine: the evolution and ecology of a model system*. University of California Press, Berkeley, California, USA.
- Hidaka, A., and Kitayama, K. (2009). Divergent patterns of photosynthetic phosphorus-use efficiency versus nitrogen-use efficiency of tree leaves along nutrient-availability gradients. *Journal of Ecology* 97, 984–991.
- Hunt, R. (1982). *Plant growth curves: the functional approach to plant growth analysis*. London: Edward Arnold Ltd.
- Jarrell, W. M., and Beverly, R. B. (1981). The dilution effect in plant nutrition studies. *Advances in Agronomy* 34, 197–224.
- Khasanova, A., James, J. J., and Drenovsky, R. E. (2013). Impacts of drought on plant water relations and nitrogen nutrition in dryland perennial grasses. *Plant and Soil* 372, 541–552.
- Killingbeck, K. T. (1996). Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. *Ecology* 77, 1716–1727.
- Killingbeck, K. T. (2004). Nutrient resorption *In* L. D. Nooden [Ed], *Plant cell death processes*, 215–226. Academic Press, San Diego, California, USA.

Kou, L., Wang, H., Gao, W., Chen, W., Yang, H., and Li, S. (2016). Nitrogen addition regulates tradeoff between root capture and foliar resorption of nitrogen and phosphorus in a subtropical pine plantation. *Trees* DOI 10.1007/s00468-016-1457-7.

Lambers, H., and Poorter, H. (1992). Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* 23, 187–261.

Lambers, H., Chapin III, F. S., and Pons, T. L. (2008). *Plant physiological ecology*, 2nd ed. Springer, New York, New York, USA.

Lenihan, J. M., Drapek, R., Bachelet, D., and Neilson, R. P. (2003). Climate change effects on vegetation distribution, carbon, and fire in California. *Ecological Applications* 13, 1667–1681.

Marschner, H. (2012). *Mineral nutrition of higher plants*. Amsterdam and Boston: Elsevier/Academic Press.

Minnich, R. A., and Dezzani, R. J. (1998). Historical decline of coastal sage shrub in the riverside-perris plain, California. *Western Birds* 29, 366–39.

Nye, P. H., and Tinker, P. B. (1977). Solute movement in the soil-root system. Berkeley and Los Angeles: University of California Press.

O'Dell, R. E., James, J. J., and Richards, J. H. (2006). Congeneric serpentine and non serpentine shrubs differ more in leaf Ca:Mg than in tolerance of low N, low P, or heavy metals. *Plant and Soil* 280, 49–64.

R Coding Team. (2016). R: A language and environment for statistical computing.

Rao, I. M., Miles, J. W., Beebe, S. E., and Horst, W. J. (2016). Root adaptations to soils with low fertility and aluminum toxicity. *Annals of Botany* 118, 593–605.

Reich, P. B., Ellsworth, D. S., and Walters, M. B. (1998). Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. *Functional Ecology* 12, 948–958.

Reich, P. B., Wright, I. J., Cavender-Bares, J., Craine, J. M., Oleksyn, J., Westoby, M., et al. (2003). The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences* 164, S143–S164.

Silla, F., and Escudero, A. (2003). Uptake, demand and internal cycling of nitrogen in saplings of Mediterranean *Quercus* species. *Oecologia* 136, 28–36.

Trenberth, K. E. (2011). Changes in precipitation with climate change. *Climate Research* 47, 123–138.

University of California-Davis. (2009). Plants. [online]. Website
<http://nrs.ucdavis.edu/mcl/natural/plants/index.html> [accessed 01 Jan. 2017].

US Climate Data. (2017). Climate Lower Lake - California. [online] Website
<http://www.usclimatedata.com> [accessed 25 Jan. 2017].

Wright, I. J., and Westoby, M. (2003). Nutrient concentration, resorption and lifespan: leaf traits of Australian sclerophyll species. *Functional Ecology* 17, 10–19.

Wright, I. J., Reich, P. B., and Westoby, M. (2001). Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Functional Ecology* 15, 423–434.

Wright, I. J., Westoby, M., and Reich, P. B. (2002). Convergence towards higher leaf mass per area in dry and nutrient-poor habitats has different consequences for leaf life span. *Journal of Ecology* 90, 534–543

Wright, I. J., Reich, P. B., and Westoby, M. (2003). Least cost input mixtures of water and nitrogen for photosynthesis. *The American Naturalist* 161, 98–111

- Valladares, F., Sanchez-Gomez, D., and Zavala, M. A. (2006). Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology* 94, 1103–1116
- Vaitkus, M. R., and McLeod, K. W. (1995). Photosynthesis and water-use efficiency of two sandhill oaks following additions of water and nutrients. *Bulletin of the Torrey Botanical Club* 122, 30–39.
- Vourlitis, G. L. (2017). Chronic N enrichment and drought alter plant cover and community composition in a Mediterranean-type semi-arid shrubland. *Oecologia* 184, 267–277.
- Xu, H., Li, Y., Xu, G., and Zou, T. (2007). Ecophysiological response and morphological adjustment of two Central Asian desert shrubs towards variation in summer precipitation. *Plant, Cell, and Environment* 30, 399–409.

Tables

Table 1. Average soil water capacity measurements of the three treatment watering regimes.

Treatment Phase	High water	Low water
Spring initiation	10.30% ± 1.53%	7.55% ± 1.04%
Summer water increase	14.05% ± 1.82%	7.97% ± 0.89%
Fall drawdown	12.78% ± 1.08%	8.46% ± 0.81%

Table 2. Average concentration of chemical elements, electrical conductivity (E.C.) and pH of field soils.

Site	Ca²⁺ g·kg⁻¹	Mg²⁺ g·kg⁻¹	K⁺ g·kg⁻¹	N g·kg⁻¹	E.C. ds m⁻¹	pH
Serpentine	0.002	0.018	0.018	1.783	0.251	7.37
Non-serpentine	0.001	0.001	0.001	0.885	0.079	6.07

Table 3. The PI_v values for plasticity for relative growth rate (RGR), total biomass (Total), photosynthetic rate (A), photosynthetic phosphorus use efficiency (PPUE), photosynthetic nitrogen use efficiency (PNUE), water use efficiency (WUE), and senesced leaf nitrogen (SLN). A value of 1 indicates that the trait is completely plastic.

Species	RGR mm·day	Total g	A $\mu\text{mol m}^{-2} \text{s}^{-1}$	PPUE $\mu\text{mol CO}_2 \text{mol N}^{-1} \text{s}^{-1}$	PNUE $\mu\text{mol CO}_2 \text{mol P}^{-1} \text{s}^{-1}$	WUE $\mu\text{mol mol}^{-1}$	SLN $\text{g} \cdot \text{kg}^{-1}$
<i>A. manzanita</i>	0.856	0.902	0.497	0.749	0.423	0.416	0.527
<i>A. viscida</i>	0.804	0.874	0.310	0.680	0.185	0.441	0.489
<i>C. cuneatus</i>	0.925	0.955	0.568	0.774	0.548	0.415	0.510
<i>C. jepsonii</i>	0.982	0.787	0.138	0.591	0.481	0.164	0.091
<i>Q. berberidifolia</i>	0.843	0.694	0.213	0.411	0.463	0.195	0.178
<i>Q. durata</i>	0.803	0.523	0.091	0.332	0.253	0.041	0.352

Figures

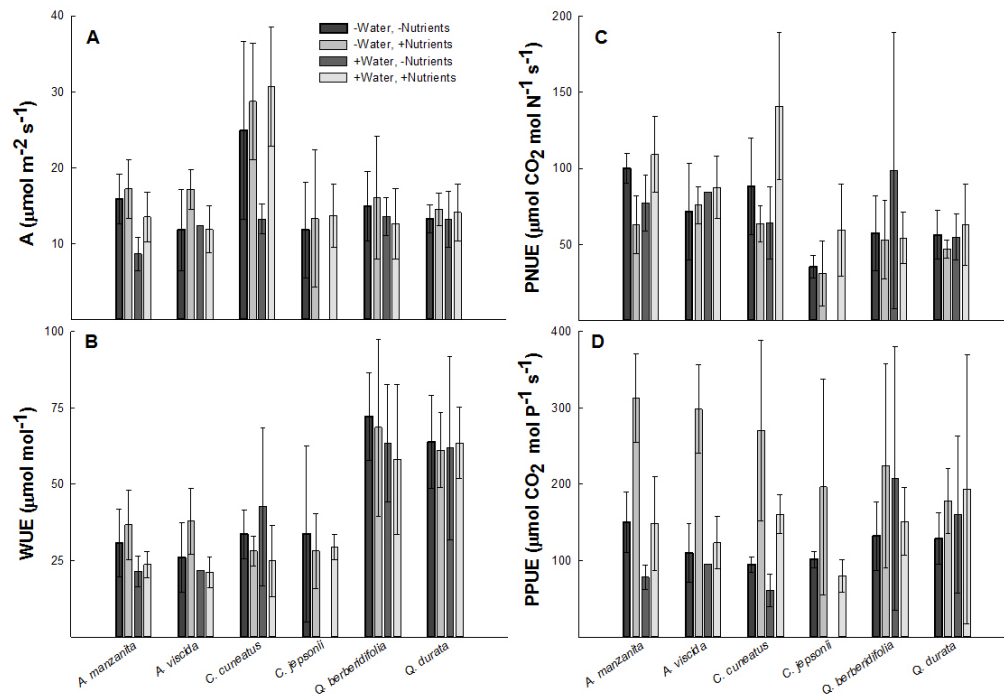


Figure 1. **A)** Photosynthetic rate, **B)** WUE, **C)** PNUE, and **D)** PPUE of the six species studied under varying water and nutrient treatments in the greenhouse experiment (n = 1–7).

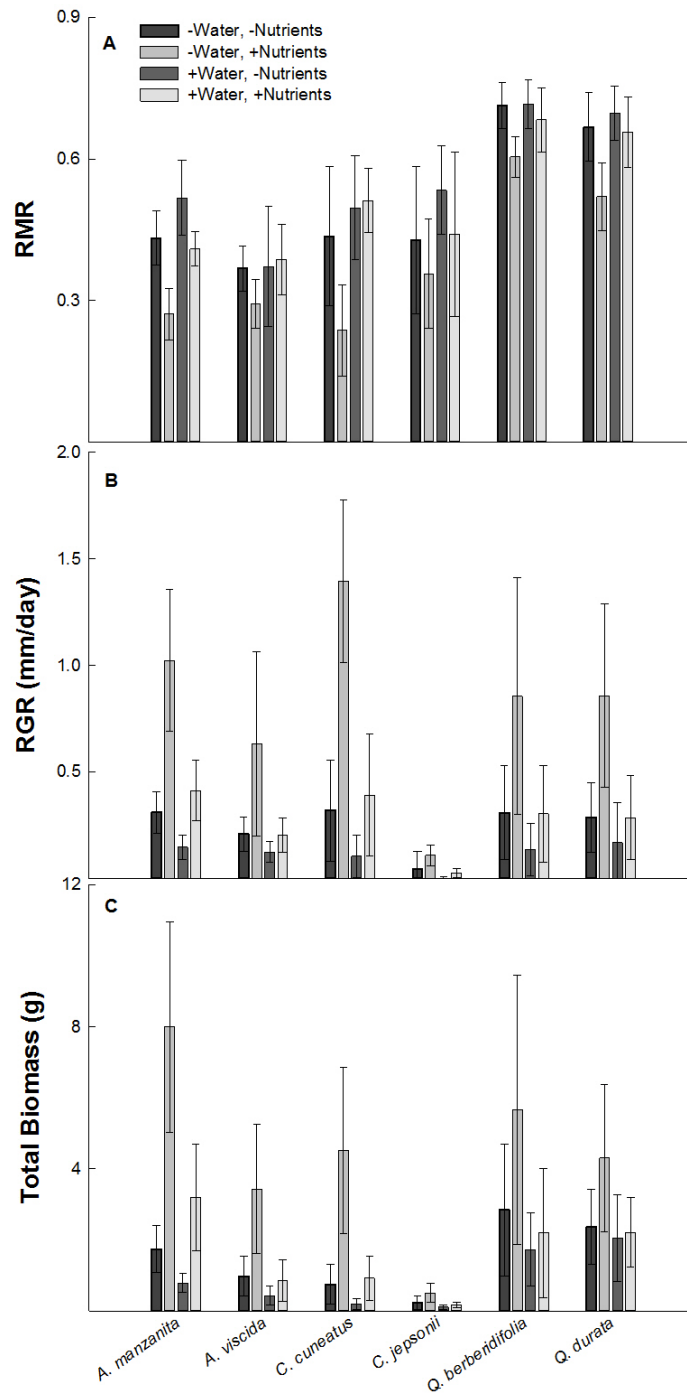


Figure 2. **A)** Total biomass **B)** root mass ratio, and **C)** relative growth rate of the six species studied under varying water and nutrient treatments in the greenhouse experiment (n = 7–10).

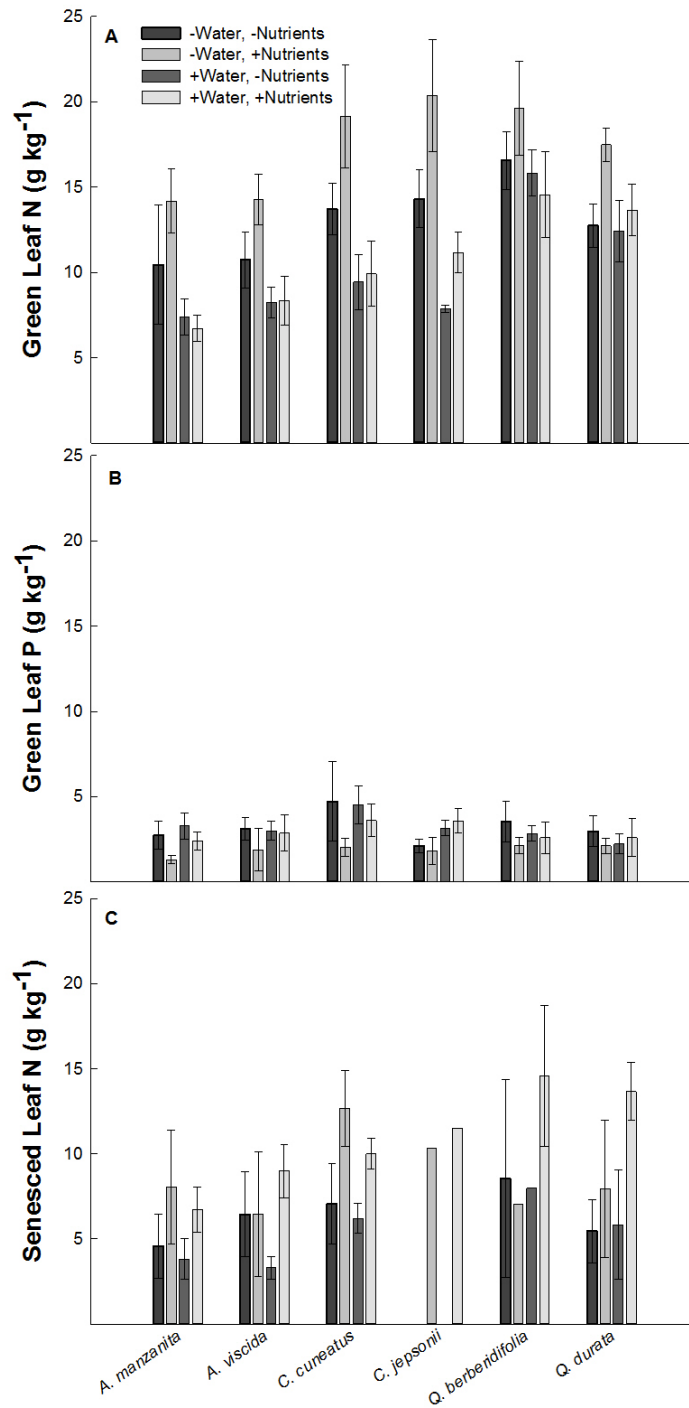


Figure 3. **A)** Green leaf nitrogen, **B)** green leaf phosphorus, and **C)** senesced leaf nitrogen concentrations for the six species studied under varying water and nutrient treatments in the greenhouse experiment (n = 1–10).

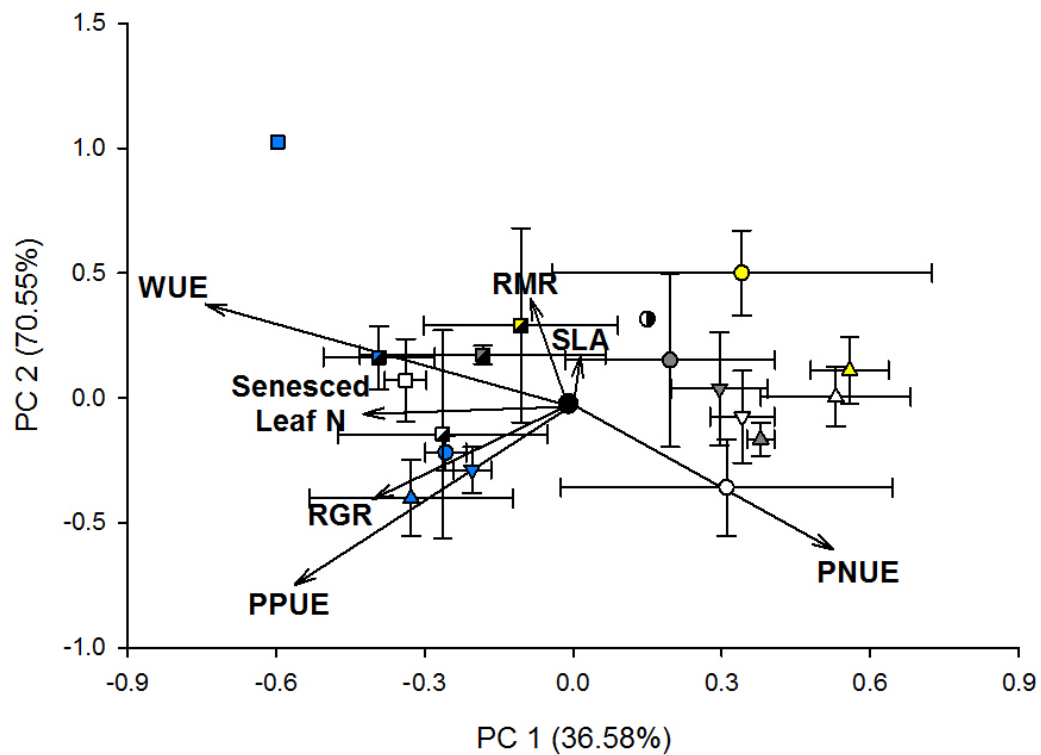


Figure 4. PCA of WUE, RMR, SLA, PNUE, PPUE, RGR, and senesced leaf nitrogen for species used in the greenhouse experiment (n = 1–5). *A. manzanita* = \triangle , *A. viscida* = ∇ , *C. cuneatus* = \bigcirc , *C. jepsonii* = \bullet , *Q. berberidifolia* = \square , and *Q. durata* = \blacksquare . Low water, low nutrients = gray, low water; high nutrients = blue, high water; low nutrients = yellow; and high water; high nutrients = white.

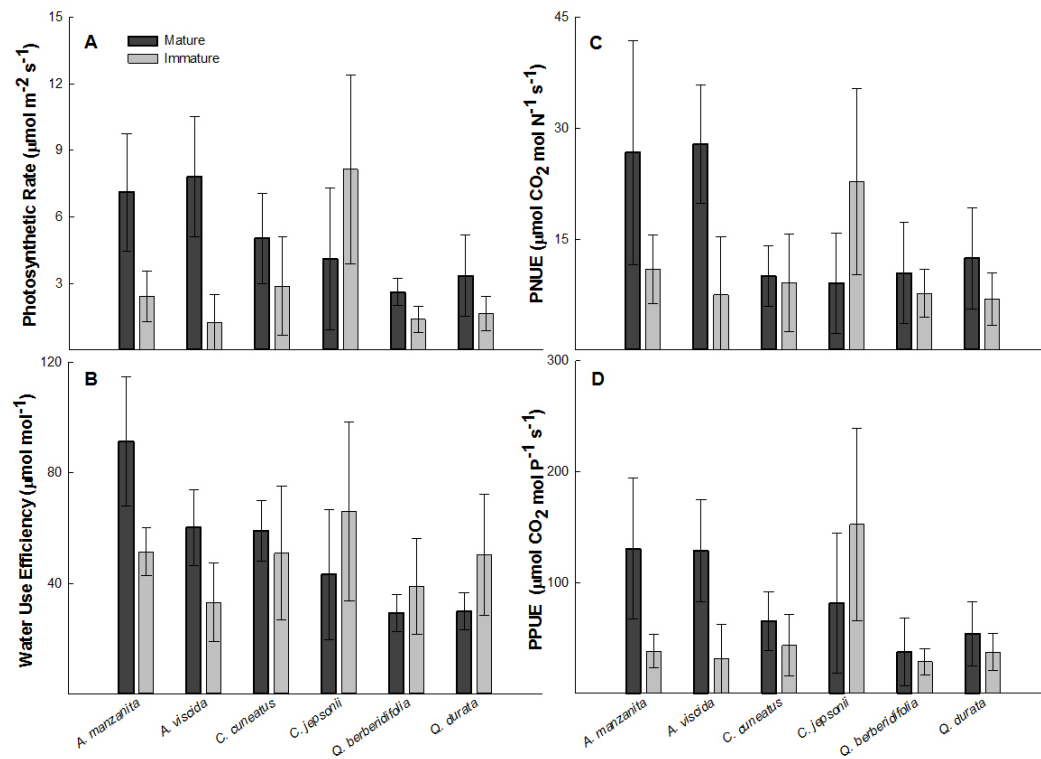


Figure 5. A) A, B) WUE, C) PNUE, and D) PPUE of the mature and immature species sampled at McLaughlin Natural Preserve (n = 10).

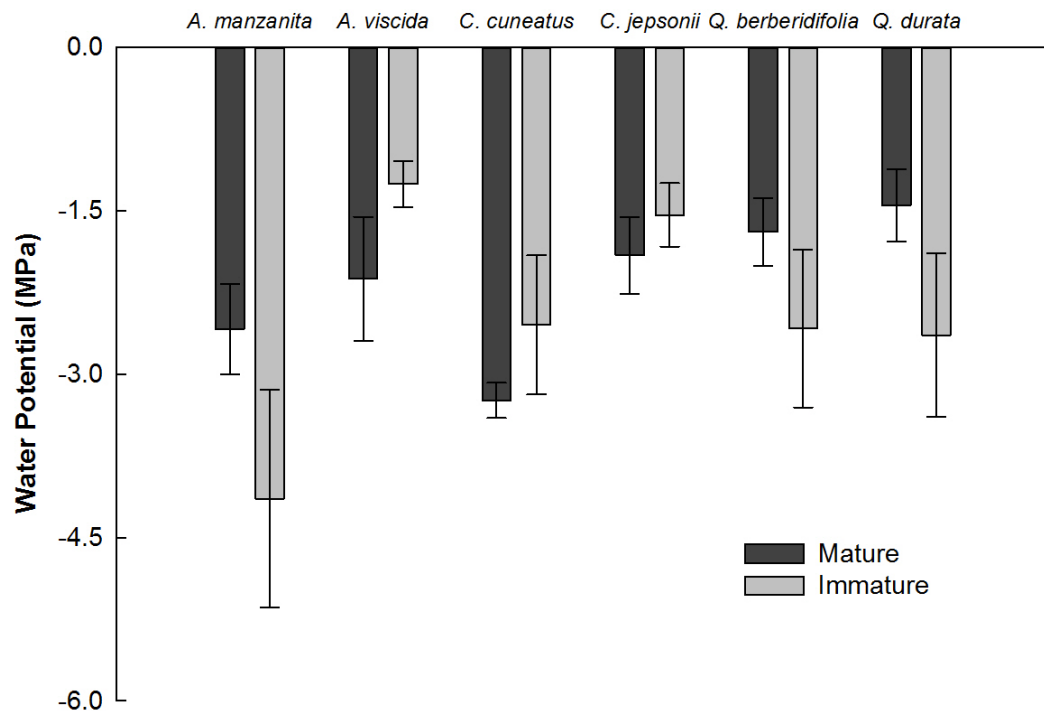


Figure 6. Water potentials of the mature and immature species sampled at McLaughlin Natural Preserve (n = 3–8).

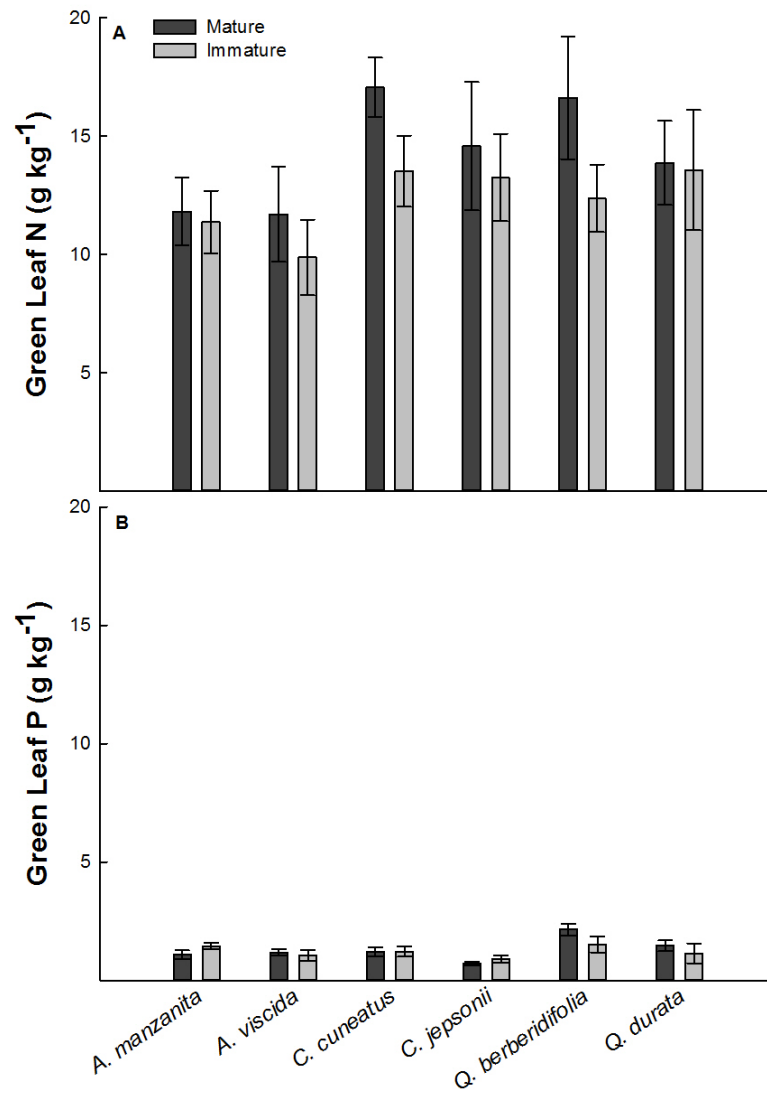


Figure 7. A) Green leaf nitrogen and **B)** phosphorus concentrations of the adult and juvenile species sampled at McLaughlin Natural Preserve ($n = 9-10$).