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On *Baccharis pilularis* DC. (coyote brush, Asteraceae) Water Relations During Succession Into Coastal Grasslands in a Changing Climate

By

Allison Green Kidder

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Environmental Science, Policy, and Management

in the

Graduate Division

of

University of California, Berkeley

Committee in Charge:

Professor Todd. E. Dawson, Co-Chair
Professor Joe R. McBride, Co-Chair
Professor Wayne P. Sousa

Fall 2015

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Abstract

On *Baccharis pilularis* DC. (coyote brush, Asteraceae) Water Relations During Succession Into Coastal Grasslands in a Changing Climate

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Doctor of Philosophy in Environmental Science, Policy, and Management

University of California, Berkeley

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In California, the common native shrub, *Baccharis pilularis* DC. (coyote brush, Asteraceae) readily invades grasslands in the absence of fire and grazing. Research shows that water availability is important in regulating *B. pilularis* seedling establishment in grasslands, with late spring rainfall providing a timely source of moisture that helps *B. pilularis* roots compete with neighboring grassland plants. The ecohydrology of *B. pilularis*, particularly during invasion into grasslands, remains largely unexplored. For this research I investigated 1) intraspecific variation in water relations between the prostrate *B. pilularis* ssp. *pilularis* and erect *B. pilularis* ssp. *consanguinea* (herein *B. pilularis*) in a mature common garden, 2) seasonal water relations and use of experimentally planted *B. pilularis* seedlings with and without neighboring plants in cool, fog-prone, perennial-dominated and warm, dry annual-dominated coastal grasslands, and 3) seasonal water relations between experimental *B. pilularis* seedlings and naturally-occurring adults in the two contrasting grassland types. For these studies I measured a suite of water relations traits including stomatal conductance, stem water potentials, leaf stable carbon isotope composition, tissue-level parameters that reflect cell hydration, stable isotope composition of xylem water, and whole stem hydraulic conductance. First, I found that despite the decreasing water availability during the rainless summer, both *B. pilularis* morphotypes increased rates of stomatal conductance and photosynthesis. These increases are likely a strategy to meet increased carbon demands before *B. pilularis*' fall blooming period, so the plants can produce adequate numbers of wind-pollinated and wind-dispersed flowers to ensure successful dispersal and establishment into neighboring grasslands. Second, a manipulative neighbor removal treatment demonstrated that grassland neighbors harvest fog for use by *B. pilularis* seedlings when fog water is present but not in the warm, dry annual grassland with very little fog where seedlings grew significantly better. Third, as expected, adults showed greater tolerance for water deficit and seedlings readily used fog water when fog was present. As in Chapter One, plants in both age classes show the same increased demand for carbon before the onset of the blooming period as demonstrated by increased stomatal conductance, osmotic adjustment, bulk leaf $\delta^{13}C$ during the rainless summer. These results provide evidence that *B. pilularis* is well adapted to withstand water deficits yet plant water status can benefit from neighbors in fog-prone areas. In a warmer and drier climate, this shrub will likely become an increasingly dominant presence throughout the California Floristic Province.

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I dedicate my thesis to
my family and friends

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ACKNOWLEDGEMENTS

INTRODUCTION

On *Baccharis pilularis* DC. (coyote brush, Asteraceae) Water Relations During Succession Into Coastal Grasslands in a Changing Climate

Since Theophrastus (c. 371 – c. 287 BC), botanists have explored why certain types of plants live in certain climates, setting the stage for the field of modern plant ecology. Eighteenth and nineteenth century European biogeographers such as Alexander von Humboldt (1769-1859), Johannes Eugenius Bülow Warming (1841-1924), Andreas Schimper (1856-1901), and several others produced some of the first detailed descriptions of global plant distributions and classifications based on the correlation between climate and soil and that of plant growth form. The field rapidly progressed to recognize the importance of species interactions, with Augustin-Pyramus de Candolle (1778-1841), fittingly the botanical author of *Baccharis pilularis* DC., being one of the first botanists to consider the dynamic nature of plant competition in his 1820 article titled "Géographie botanique" in *Dictionaire des sciences naturelles* (Candolle 1820, Egerton 2010). It was not until late in the 19th century and early 20th century that ecologists began to consider the role that time plays in the succession of vegetation from one type of landscape to another (Cowles 1899, Clements 1907, Warming 1909). This early research on vegetation change over time described the sensitivity of plant-plant interactions to environmental conditions, and why some plants eventually persisted on a landscape while others did not. By synthesizing how plant morphology and adaptions to different climate and soil conditions influence vegetation change over time, early ecologists such as Frederic Clements, Henry Gleason helped push vegetation succession into the subfield of ecology that is still actively researched today.

One of the most active fields of modern vegetation secondary succession research is woody plant encroachment into grasslands, which occurs around the world in a variety of landscape types, from arid deserts to temperate and tundra climates (McBride 1968, Grover and Musick 1990, Sturm et al. 2001). Vegetation succession from one ecosystem type to another, such as the shift from grassland to shrubland, has been shown to alter the availability of water and nutrients as well as ecosystem function (Belsky et al. 1989, Schlesinger et al. 1990, Joffre and Rambal 1993, Scholes and Archer 1997, Chapin et al. 2000, Russell and McBride 2003, Huxman et al. 2005). In California's Coast Range, the common dioecious native shrub *Baccharis pilularis* DC. (coyote brush, Asteraceae) readily invades grassland habitats in the absence of fire and grazing (McBride 1968), resulting in increased fire hazard as a result of continued succession to Douglas-fir (*Pseudotsuga menziesii*) along the coast (Ford and Hayes 2007) and coast live oak (*Quercus agrifolia*) and California bay (*Umbellulari californica*) woodland in inland areas (McBride 1974, Russell and McBride 2003). Previous research has demonstrated the importance of water availability in the successful establishment of *B. pilularis* seedlings in neighboring grasslands (da Silva and Bartolome 1984, Williams et al. 1987, Williams and Hobbs 1989) but the physiological mechanisms behind its significance remain unknown. In light of documented long-term decreases in coastal fog (Johnstone and Dawson 2010) and a warmer climate (Cayan et al. 2008) in California, further research on the importance of fog deposition on *B. pilularis* succession is needed to help predict the future of Californian coastal grassland-shrubland landscapes.

The overarching goal of this dissertation research is to broadly explore *B. pilularis* water relations with a particular focus on seasonal water relations and water use of *B. pilularis* during

establishment in coastal grasslands. Water relations physiology involves measuring stem- and leaf-level parameters to evaluate how efficiently water is transported through the soil-plant-atmosphere continuum so the plant can maintain adequate cell hydration that is required to maximize rates of photosynthesis. For this research the water relations physiological parameters I measured include stomatal conductance (water use), stem water potentials (water status), leaf stable carbon isotope composition (long-term water use and photosynthetic rates), tissue-level parameters that reflect levels of cell hydration (by creating pressure-volume curves), stable isotope composition of xylem water (to compare to seasonal water sources available to the plants), and whole stem hydraulic conductance (efficiency of water movement through stem tissues).

In Chapter One, I compared intraspecific variation in seasonal water relations physiology of the two *B. pilularis* subspecies, the prostrate *B. pilularis* subsp. *pilularis* (10-15 cm high, 1-4 m across) and erect *B. pilularis* subsp. *consanguinea* (1-4 m high), in a mature common garden in Bodega, California. Contrary to typical plant water relations patterns, *B. pilularis* stomatal conductance for both morphotypes increased from wet season into the dry season, peaking at the end of the rainless summer. During this time, leaf stable carbon isotope composition and nitrogen use efficiency also increased, while calculated time-integrated intercellular CO_2 levels inside the leaf decreased, reflecting an increase in demand for CO_2 inside the leaf for both morphotypes. These trends indicate that both *B. pilularis* subspecies' carboxylation rates demanded more CO_2 than could be diffused into the leaf, even at the high rates of stomatal conductance observed. This high seasonal demand for carbon could likely be a result of *B. pilularis* preparing for its blooming period in early fall when it produces large numbers of wind-pollinated flowers and wind-dispersed seeds.

Given the influence that shifts in vegetation type have on ecosystem function and biogeochemistry, Chapter Two explores the water relations of *B. pilularis* subsp. *consanguinea* (herein *B. pilularis*) seedlings once they are established in coastal grasslands. After raising 400 seedlings in the greenhouse on the University of California Berkeley campus for seven months, 200 seedlings were planted in cool, fog-prone perennial-dominated grassland and 200 seedlings were planted in warm, dry, annual-dominated grassland in Mt. Tamalpais State Park. Using a subset of forty of these seedlings, I performed a competition experiment wherein we removed above-ground biomass of neighboring grassland plants within a 0.5 m diameter circle around 10 seedlings in each grassland type (ten were left as controls, so there were twenty experimental plants in each grassland type). *B. pilularis* seedlings generally performed better without neighbors and had significantly better growth in the warm, dry grassland. By comparing the stable water isotope composition of *B. pilularis* xylem water to that of water sources available to the plants (i.e., fog, rain, ground, soil waters), I found that experimental seedlings with neighbors present in the fog-prone grassland used fog water when it was available. The results from this experiment suggest that 1) neighbors may facilitate *B. pilularis* seedling establishment in fog-prone, perennial dominated coastal grasslands by harvesting fog water for use by seedlings, and 2) that *B. pilularis* seedlings have water relations traits that result in superior growth and establishment in warm, dry coastal grassland habitats.

In Chapter Three I investigated ontogenetic and seasonal variation of *B. pilularis* water relations physiology to see how water availability and use might shift between earlier and later stages of grassland-shrubland succession. By measuring water relations physiology and stable water isotope composition of experimentally planted seedlings and nearby naturally-occurring adults in the two contrasting types of coastal grassland, I found that *B. pilularis* plants of both

ages in both grassland types increased stomatal conductance rates and adjusted osmolytic solutes in their cells to lower turgor loss points from wet season to dry season, as observed in Chapter One. Similar to Chapter Two, seedlings in this study readily used fog water when summertime fog deposition occurred in the fog-prone grassland while seedlings in the warm, dry grassland used predominately groundwater sources similar to adults. As in Chapter One, both seedlings and adults increased leaf stable carbon isotope values as seasonal water availability waned.

Results from these three experiments provide an important piece of evidence in what drives *B. pilularis'* success in invading grasslands. These findings suggest that *B. pilularis* adults and seedlings optimize available water sources, especially during the rainless summer months, to achieve maximum levels of CO_2 diffusion into the leaf and subsequent carbon gain to meet reproductive costs incurred in early fall. The high rates of stomatal conductance and carboxylation allow the plant to afford the costly production of an abundance of wind-dispersed pollen and flowers into adjacent grasslands during a time when annual grassland plants have senesced, making it more likely the wind-dispersed *B. pilularis* seeds reach the soil surface and successfully germinate. *B. pilularis* is often the first shrub to invade neighboring coastal grasslands (Ford and Hayes 2007), and as such it shares many characteristics typical of pioneer species (e.g., thrives in areas with high light availability and warm temperatures, is wind-pollinated and a prolific seeder, and has several water relations and biochemical traits that enable it to maintain high rates of stomatal conductance and carboxylation in the face of water deficit (Bazzaz 1979)). However, *B. pilularis* is an atypical pioneer species because it is long-lived, woody, and a superior competitor in coast grassland environments, all of which leads to its dominance on the landscape.

Future directions for B. pilularis research

Future B. pilularis research could take several important directions. Given the morphological and ecophysiological differences between *B. pilularis* subsp. *consanguinea* and *B. pilularis* subsp. *pilularis* and the differential effects these two moprhotypes have on the immediate abiotic environment and the surrounding community (Crutsinger et al. 2013, Crutsinger et al. 2014), focused investigations on whether these differences have a genetic basis should be performed. These investigations could take a variety of forms, but it would be most interesting to perform a reciprocal transplant experiment using both plant cuttings and seed in costal and inland locations. Because *B. pilularis* is dioecious, the spatial distribution patterns and sex ratios, affiliation with habitat type, competitive ability, and physiology of male and female plants is also important to examine, particularly because dioecy is associated with pioneer species in secondary succession (Falińksi 1980).

The results presented here call for further research on how *B. pilularis* is able to maintain such high levels of stomatal conductance and photosynthetic rates in times of reduced water availability. More specifically, investigations into the xylem structure and of both morphotypes and sexes might elucidate how this plant can optimize water flow during low water availability and minimize xylem vulnerability to cavitation. Further, research on sap flow rates and *B. pilularis'* capacity for foliar uptake would also inform the high stomatal conductance rates. These investigations could be focused on the most extreme edges of *B. pilularis* distribution, such as where water deficits might be most intense. Finally, where appropriate, these physiological studies should incorporate ontogenetic variation and its dioecy to help identify age- and sex-specific physiological thresholds that might influence its persistence on California's landscape. In the meantime, we know that *B. pilularis* is a plant species that is perfectly poised to

greatly increase its distribution in California's predicted warmer and drier climate.

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CHAPTER 1

Intraspecific variation in plant architecture is linked to divergent biochemical and physiological water relations strategies

Abstract:

Environmental gradients can influence evolutionary processes, resulting in morphological differences within plant species. To this end, the dimorphic California shrub, *Baccharis pilularis* (Asteraceae), exists in a prostrate form found along the coast and a more common erect form found in both coastal and inland areas. Previous research suggests these differences in plant stature and their resulting canopy architecture have cascading effects at the landscape level and above- and below-ground community levels. With this as background, we hypothesized that the morphological variation in *B. pilularis* shrubs would be correlated with differences at the physiological level. We tested this hypothesis in a common garden setting by quantifying seasonal responses of several water relations traits in both *B. pilularis* morphotypes. There were minimal differences between the two morphotypes for stomatal conductance (water use), water potential (water status), Huber value, and whole stem leaf area specific hydraulic conductance. However, the two morphotypes differed in seasonal bulk leaf $\delta^{13}C$ values, and the bulk tissue water relations parameters of osmotic potential at full turgor, bulk modulus of elasticity, and mass-based capacitance before turgor loss point. These differences provide evidence of divergent water relations strategies between the two morphotypes. Prostrate plants exhibited a more mesic strategy reflective of their coastal environment and erect plants exhibited a more xeric strategy typical of their warmer inland location. Our results suggest that differences in biochemical and physiological water relations strategies are indeed linked with differences in plant architecture and should help inform *B. pilularis* evolutionary history.

Introduction

When species are distributed along environmental gradients, natural selection can favor certain traits and trait values at different points along the gradient. This in turn can result in divergence among populations within a species and the development of ecotypes or subspecies. These divergences, whether adaptive or not, can result in morphological differences within a species (Turesson 1922, Clausen et al. 1940). One such example of a divergent population is *Baccharis pilularis* DC (coyote brush, Asteraceae), a dioecious, perennial California native found throughout the California Floristic Province. *B. pilularis* is a C_3 plant that grows in two architectural morphotypes that have been intermittently classified into subspecies: the prostrate *B. pilularis* ssp. *pilularis* (10-15 cm high, 1-4 m across) that is found along the immediate, wind- and fog-prone coast and the more widespread erect *B. pilularis* ssp. *consanguinea* (1-4 m high) that is found both along the coast and at warm inland sites that receive only a weak maritime influence of slightly cooler temperatures (Munz and Keck 1973, Baldwin et al. 2012, Calflora 2014). Both forms of *B. pilularis* bloom September through January and depend on wind for pollination and achene dispersal.

Four lines of evidence have been used to suggest that prostrate and erect *B. pilularis* plants are genetically distinct: (1) all plants have retained their original architecture (i.e., prostrate or erect) in the common garden setting since it was established using clippings from adult plants on the site in 1998 (Rudgers and Whitney 2006), (2) the production of prostrate and erect F1 progeny when prostrate *B. pilularis* were hybridized with upright desert-native congener

B. sarathroides (Thompson et al. 1995), (3) prostrate *B. pilularis* are commonly used in landscaping yet retain their prostrate architecture (Ehler 1982), and (4) the progeny from seeds grown from one morphotype produce both erect and prostrate plants (Rudgers and Whitney 2006).

These morphological differences in canopy architecture can have cascading consequences at the landscape and community level. For example, at the landscape level, the prostrate morphotype facilitates the establishment of nitrogen-fixing shrub, *Lupinus arboreus* (Rudgers and Maron 2003). At the community level, the two *B. pilularis* morphotypes attract different gall-forming insects at different rates due to their architectural differences. The midge *Rhopalomyia californica* Felt (Diptera, Cecidomyiidae) creates galls on meristematic tissue at terminal buds on prostrate morphs more often than on erect morphs, while the moth *Gnorimoschema baccharisella* Busck (Gelechiidae) creates galls within internodes of woody stems on erect morphs more often than on prostrate morphs (Rudgers and Whitney 2006). Further, erect morphs have lower richness and abundance of canopy and litter arthropod assemblages compared to prostrate morphs (Crutsinger et al. 2014).

Because of the large impact these morphological differences have on processes at other scales, we hypothesized that the variation in canopy architecture will be linked to different water relations strategies in ways that typify coastal and inland environments. More specifically, we anticipated water relations parameters of prostrate plants would reflect their native maritime climate by having more negative $\delta^{13}C$ values, higher rates of stomatal conductance to water vapor (g_s), more negative water potentials (Ψ) during periods of low water availability, higher turgor loss point (Ψ_{TLP}), lower bulk modulus of elasticity (ε), higher solute potential at full turgor (Ψ_{SFT}), lower leaf area specific whole stem hydraulic conductance ($k_{ws/la}$), and lower sapwood area to leaf area ratio (or Huber value; SA:LA) than erect plants. Our overall expectations are that the physiological characteristics in each morphotype reflect adaptations required to live in the plants' dominant habitat.

Methods

Site description

Experimental work was conducted within sand dunes at University of California's 362-hectare Bodega Marine Reserve (BMR), Sonoma County, CA, USA (38°19' N, 123° 04' W) where both morphotypes of *B. pilularis* naturally co-occur. The two morphotypes account for similar percent cover at the Reserve, with 13% cover of the prostrate morphotype and 16% cover of the erect morphotype (Rudgers & Whitney 2006). BMR has a Mediterranean type climate of hot, dry summers and cool, wet winters with strong maritime influence of mild temperatures (Fig. 1; Barbour et al. 1973). In order to control for environmental variables in our study, we took advantage of a 60 m X 60 m area common garden established in 1998 of prostrate and erect architectural morphotypes of *B. pilularis* (Rudgers and Whitney 2006). Cuttings of the same size of both sexes of adult *B. pilularis* occurring naturally on the BMR were raised in the greenhouse and planted in the dunes (see Rudgers and Whitney 2006, Crutsinger et al. 2010 for detailed descriptions of the common garden).

In the common garden, the erect morphs were taller, possessed larger leaves, had lower crown biomass, had less and moister leaf litter, lower carbon to nitrogen ratio of leaf litter, higher understory light availability, warmer understory temperatures, and higher understory vapor pressure deficit (VPD) compared to prostrate morphs (Rudgers and Whitney 2006,

Crutsinger et al. 2010, Crutsinger et al. 2014). In the common garden, blooming period for this dioecious plant varies by sex and morphotype (A. Kidder, personal observation). For example, male prostrate *B. pilularis* plants were the first to bud or bloom during mid-September 2011, followed by female prostrate plants one to two weeks later, with the erect male plants blooming approximately one month later and lastly followed by female erect plants. We did not track plant sex for this study and measured plants that were not in bud or in bloom, with the exception of two prostrate plants that had buds in September 2011. Further, we avoided sampling galled leaves and stems for all measurements in this study.

Underground water sources were measured May 6, 2010 in wells installed as part of the BMR's septic field system. The wells were located 314 m (1,030 feet) west and 715.5 m (2,347 feet) east from the center of the common garden and had water levels 5 feet to 15 feet below the soil surface, respectively. Underground water levels in these wells were closer to the surface during the wet season (Kitty Brown, unpublished data). A study at BMR in the same dune complex measuring seasonal variation in water table depth ranged from a high of about 2.5m deep during the wet season (January and March) to a low of about 3.5m deep in September (DeJong 1979). Water tables in dunes fluctuate based on the amount of precipitation, with the water table rising as it is recharged from rains (Frank 1978, DeJong 1979).
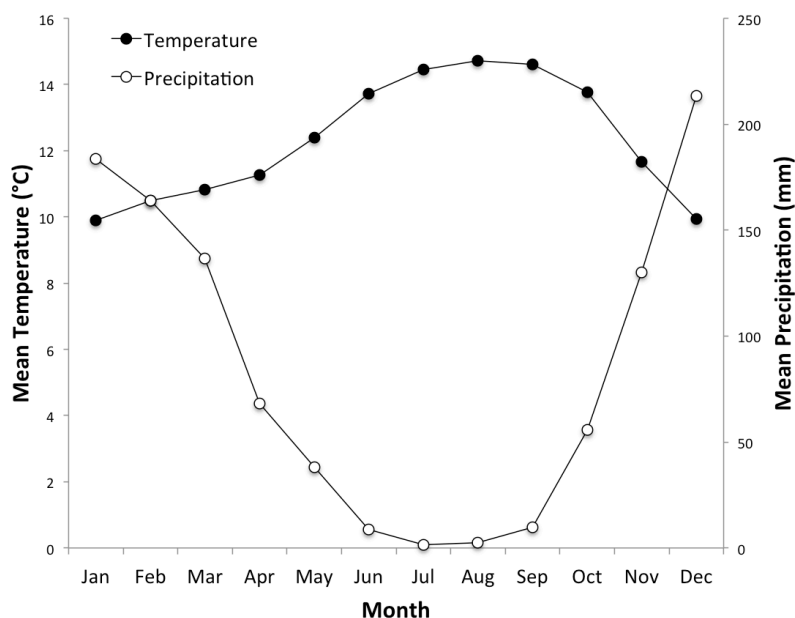
Figure 1. Mean monthly temperature (°C) and precipitation (mm) from 1981-2010 for Fort Ross, Sonoma County, California (38° 31' N, 123° 15'W), 16.7 miles from the *B. pilularis* common garden study site in Bodega Marine Reserve (Western Regional Climate Center 2013).

Environmental conditions

Temperature, relative humidity, photosynthetically active radiation (PAR), and rainfall were recorded by a weather station on the reserve, about 466 meters (0.29 miles) from the common garden from May 1, 2011 to May 31, 2012 (NOAA-Earth System Research Laboratory, Physical Sciences Division, Boulder, Colorado, USA from their website http://www.esrl.noaa.gov/psd/, and University of California, Davis, Bodega Ocean Observing Node, Bodega Marine Laboratory, Bodega Bay, California, USA from their website

). We did not record fog water deposition for this study. As a measure of evaporative demand, midday (1100-1500 hours local time) VPD was calculated using temperature and relative humidity measurements to obtain the difference between saturation vapor pressure and the water vapor pressure of the air as follows

$$VPD = e_{sat} - e_a \qquad\qquad\qquad\text{(Eq. 1)}$$

where

$$e_{sat} = (a)(e^{(b*T_{air})/(T_{air}+z)}) \qquad\qquad\qquad\text{(Eq. 2)}$$

where a is the coefficient 0.61121 (kPa), b is the coefficient 17.502, T_{air} is the air temperature (°C), and z is the coefficient 240.97 (°C), and

$$e_a = e_{sat}(\frac{RH}{100}) \qquad\qquad\qquad\text{(Eq. 3)}$$

where *RH* is the measured relative humidity (Jones 1992). Photosynthetically active radiation (PAR) was measured by a LI-190SA quantum sensor (LI-COR Biosciences, Lincoln, Nebraska, USA) placed 2m above ground level with an accuracy of 5%. PAR values were recorded via a 15-second running average that was integrated and logged hourly. The bulk of rainfall during the study occurred between October 2011 and May 2012 and was recorded by a TB4 tipping bucket rain gauge (Hydrological Services America, Lake Worth, Florida, USA).

Soil moisture was measured for the duration of the study. Twelve 20-cm dielectric soil moisture sensors (ECH$_2$O-20 probes, Decagon Devices, Pullman, WA, USA) were installed just below the soil surface and one meter deep under canopies of five prostrate and five erect shrubs. Measurements were recorded every 30 seconds and averaged every 30 minutes using a CR10X datalogger (Campbell Scientific, Logan, UT, USA). By the second month into the study, rodents had chewed through the wires of many of the soil moisture sensors reducing sensor replication.

Stable carbon isotope ratios and nitrogen use efficiency

In order to deepen our understanding of long-term trends in photosynthetic activity of the two morphotypes, we collected fully-formed *B. pilularis* leaves at the end of the wet season (May 11, 2011) and during peak period of seasonal drought (September 6, 2011) from the south-southwest quadrant of the same five prostrate and five erect morphs that were used for stomatal conductance to water vapor (gs) and pressure-volume curve measurements in the common garden. Leaves collected on May 11, 2011 were formed during an approximately 4-week period between early April to early May and leaves collected September 6, 2011 developed predominately during July, representing conditions during late wet season and mid-dry season, respectively. Both morphotypes of *B. pilularis* develop new leaves throughout the year, peaking in spring. Several same-aged, mature leaves from each plant were dried in a 70° C oven for more than seven days and ground to a fine powder in a grinding mill (Mini-Beadbeater-8TM, Biospec Products, Bartlesville, OK, USA). A 4-*µg* aliquot of ground leaf material from each plant was enclosed in tin capsules (Costech Analytical Technologies, Inc., Valencia, CA, USA) and analyzed for carbon stable isotope ratio using an elemental analyzer with long term precision of ±0.15‰ (Elementar vario ISOTOPE cube, Elementar, Hanau, Germany) coupled with a

continuous flow isotope ratio mass spectrometer with long term precision of ±0.10‰ (IsoPrime100, Isoprime Ltd., Cheadle Hulme, UK) using Vienna-Pee Dee Belamnite (V-PDB) as the standard. The reference material NIST SMR 1547 Peach Leaves was used as a calibration standard. All isotope analyses were conducted at the Center for Stable Isotope Biogeochemistry at the University of California Berkeley. Long-term external precision for C isotope analysis based on reference material NIST SMR 1577b Bovine Liver is $\pm$0.10‰. Carbon isotope ratios were calculated as

$$\delta^{13}C = \left(\frac{R_{plant}}{R_{V-PDB}}\right) - 1 \qquad\qquad\qquad (Eq.\ 4)$$

where R is the molar abundance ratio of heavy (^{13}C) to light (^{12}C) stable isotopes of carbon for the plant sample (R_{plant}) and the standard (V-PDB; R_{V-PDB}), respectively. Results are reported in per mil (‰) notation.

Here we use δ^{13}C for bulk leaf material to express overall trends and coarse temporal averages of carbon isotope discrimination at the seasonal level, with the material representing all metabolic isotopic effects of photosynthesis during the time of leaf development, including stomatal conductance, mesophyll conductance, photorespiration, respiration, and post-photosynthetic fractionation. $\delta^{13}C$ values represent both the isotopic composition of the source air CO_2 as well as how efficiently the carbon source is utilized by carboxylating enzymes within the leaf (Ehleringer 1993). Using the δ^{13}C value for each plant during each month, we followed a simplified model first presented by Farquhar et al. (1982) to calculate time-integrated intercellular partial pressure of CO_2 concentration ($\bar{C}_i$) in leaves of C_3 plants.

$$\bar{C}_i = c_a \frac{(\delta^{13}C_{air} - \delta^{13}C_{plant}) - a}{(b-a)} \qquad\qquad\qquad (Eq.\ 5)$$

where c_a is atmospheric CO_2 concentration (390ppm; Dlugokencky and Tans, NOAA/ESRL, accessed April 9, 2014), $\delta^{13}C_{air}$ is the δ^{13}C value of atmospheric CO_2 (-8.54‰ White & Vaughn), $\delta^{13}C_{plant}$ is the δ^{13}C value of the *B. pilularis* plant sample, a is the carbon isotope fractionation during diffusion through air (4.4‰), and b is the net fractionation during carboxylation, mainly by Rubisco (27‰) (Farquhar et al. 1982). $\delta^{13}C$ values and $\bar{C}_i$ levels can be used as an index of long-term temporal variation in stomatal sensitivity (which drives the supply of CO_2 to the chloroplasts) as well as the demand of CO_2 (Farquhar et al. 1982, Dawson and Ehleringer 1993). Further, plants adapted to dry areas typically have more responsive stomata when VPD is high and water availability is low and have a lower demand for CO_2 (Dawson and Ehleringer 1993). As technology improves to accurately measure specific photosynthetic contributions from respiration, photorespiration, mesophyll, stomata, and Rubisco, carbon discrimination models have become increasingly comprehensive (Cernusak et al. 2013, Ubierna and Farquhar 2014). The simplified model in Eq. 5 is appropriate for more coarse scale comparisons, such as seasonal comparisons in this study, as well as within-species comparisons such as the intraspecific variation in *B. pilularis* examined here (Ubierna and Farquhar 2014).

We also compared integrated nitrogen use efficiency (NUE), or the amount of carbon fixed in the leaf per amount of nitrogen, between the two morphotypes and between wet and dry seasons using percent carbon and nitrogen content in these same leaves.

Stomatal conductance

Stomatal conductance to water vapor (g_s) was used as a short-term, indicator of how the two *B. pilularis* morphotypes regulate leaf-level water flux. The seasonal progression of midday g_s was measured during the rainless summer on June 14, July 12, August 11, September 15, 2011 and the following spring on April 6, 2012 using a steady-state null balance porometer (Model LI-1600C, LI-COR Biosciences, Lincoln, Nebraska, USA) with a square chamber sensor head (aperture 4.0 cm^2) between 1130 and 1330 h PST on the same five prostrate and erect *B. pilularis* plants used for bulk $\delta^{13}C$ and pressure-volume curve measurements. In addition, diurnal g_s measurements were made on September 15, 2011 (peak dry season) and on April 6, 2012 (end of wet season) at 900, 1100, 1300, 1500, and 1800 hours. Stomatal conductance is calculated based on ambient relative humidity, and leaf and air (cuvette) temperature and the measured flow rate of dry air used to balance the relative humidity inside the sensor head of the LI-1600C (Li-Cor 1989). The porometer was calibrated before the study and the desiccant was changed before each use and periodically during diurnal measurements. At each time period measurements were made on five prostrate and five erect individuals of each morphotype on sunlit, first-year leaves at the end of stems on the south-facing side of each plant. Because of the small size of *B. pilularis* leaves, g_s measurements were made on leafy shoots and so include leaves and stems. If steady state conditions were not achieved within thirty seconds, measurements were stopped and made on another set of leaves. Due to the high relative humidity and morning and late afternoon coastal fog at the site on September 15, 2011, we were not able to sufficiently dry the leaves and stems for measurements at 900 and 1800 hours. As a result, adequate steady-state conditions were not met and these results are not reported. All leaves measured inside the porometer's square chamber were collected and scanned on a flatbed scanner within 24 hours to compute fresh leaf surface area in mm^2 using ImageJ software (Rasband 2014). Stomatal conductance measurements were corrected by leaf surface area and converted from velocity units (cm^2 s^{-1}) to molar units (mmol m^2 s^{-1}) using a conversion factor based on measured leaf temperature (Hall et al. 1976, Cowan and Farquhar 1977, Körner et al. 1979, McDermitt 1990). Values are reported on a two-sided leaf area basis as *B. pilularis* leaves are amphistomatous (A. Kidder, unpublished data).

Water Potentials

Similar to g_s, water potential (Ψ) measurements were used to monitor short-term responses to water deficit. Midday water potential measurements of five randomly selected individuals of each morphotype were taken June 14, July 12, and August 11, 2011 to measure peak water stress. In addition, daily (diurnal) courses of water potential measurements were taken September 15, 2011 (during peak drought) and April 6, 2012 (during the wet season) using a pressure chamber (PMS Instrument Company, Albany, OR, USA). On each of these days one similarly aged shoot from five randomly selected plants of each morphotype was measured within three minutes of collection at predawn, 800, 1000, 1200, 1400, 1600, and 1930 hours for a total of ten measurements at each time period.

Tissue water relations parameters

To quantify variation in the seasonal bulk leaf water relations parameters, five exposed, terminal, woody stems containing multiple years of growth were collected June 24, July 12, August 11, September 15, 2011 and April 6, 2012 from the same five prostrate and five erect *B. pilularis* individuals (10 total for each date) in the common garden and subjected to pressure-

volume curves (protocol followed Tyree and Hammel 1972, Tyree and Jarvis 1982, Koide et al. 1989). Stem samples were transported in a black plastic bag from the common garden, re-cut under distilled water within 30-60 minutes, and allowed to rehydrate in distilled water for 18-28 hours in dark, cool (14-20°C), humid conditions at room temperature to reach full turgor. Leaves were not in contact with the water during rehydration. Pressure-volume curves were conducted in the lab June 25, July 13, August 12, September 16, 2011 and April 7, 2012 on sub-shoots of similar size used for field water potential measurements were selected from the rehydrated stems. After the initial fully-hydrated shoot weight was measured we immediately measured the shoot water potential using a pressure chamber (Model 600, PMS, Instruments Albany, OR, USA; Scholander et al. 1965). Pressurization and depressurization rates inside the chamber were slow to minimize tissue disturbance and temperature changes during measurements. We allowed the stem samples to air dry on the laboratory bench (~50% relative humidity, ~20°C) in between water potential measurements. Because the samples tended to lose water quickly after cutting, water potential measurements were made every one to three minutes for about the first 30 minutes and about once every 10 to 20 minutes thereafter. We stopped measurements on each sample approximately four to five measurements after the stem reached the turgor loss point or between 60-75% of the rehydrated weight. Initial and post-water potential measurement weights were averaged and used to calculate turgor loss point (Ψ_{TLP}), solute (or osmotic) potential at full turgor (Ψ_{SFT}), bulk modulus of elasticity (ε), mass-specific capacitance before turgor loss point ($C_{T,mass}$).

  The pressure-volume curve parameters were calculated from the relationship between the negative reciprocal of the water potential (MPa) and one minus relative water content (RWC; %) (Tyree and Jarvis 1982, Koide et al. 1989, Lenz et al. 2006), where RWC = (fresh weight – dry weight)/(saturated weight – dry weight) (Robichaux 1984). In order to minimize the plateau effect from rehydrated stems and obtain the estimated saturated water content of the sample shoot, we excluded points in the regression of stem water mass (g) and xylem pressure potential (MPa) where xylem pressure remained stable or decreased only slightly relative to simultaneous losses in water (Parker & Pallardy 1987, Kubiski & Abrams 1990). This condition suggests excess water was being stored in the intercellular apoplast making the stem resistant to changes in xylem pressure potential. Overhydrating stem samples can cause an artifact of increased osmotic potential and increased cell wall elasticity (low ε) by artificially diluting the percentage of osmolytes and stretching the cell walls, both of which could lower turgor loss point (Meinzer 1986).

  In order to then optimize the number of data points included in the calculation of RWC, we used the pressure-volume curve analysis tool provided by Sack and Pasquet-Kok (2010) to maximize the standard major axis Model II regression R^2 values between the relationship of water potential (MPa) and mass of stem water content (g), and of the negative inverse of the water potential (MPa) and 1-RWC (%). Due to the unlikelihood of measuring water potential exactly at turgor loss point, we used the modeled relationship between 1-RWC (%) and the negative inverse of the water potential (MPa) in the pressure-volume curve fitting spreadsheet provided by Tu (2008) to model the turgor loss point. For modeling the turgor loss point, this tool allows for both modeled and manual designation of points in the linear portion of the pressure-volume curve, uses a quadratic equation to estimate changes in turgor pressure in the non-linear portion of the curve, and objectively assesses the turgor loss point by the x-intercept of the turgor pressure versus 1-RWC relationship and then fitting these into the pressure-volume curve using a default of one standard error in the turgor loss point calculation (Tu 2008). The

bulk tissue water relations parameters calculated from this pressure-volume curve fitting process using the two spreadsheets include turgor loss point (Ψ_{TLP}, MPa; the theoretical point at which cells lost positive pressure and become flaccid), osmotic potential at full turgor (Ψ_{SFT}; MPa; measure of solutes comprised of simple sugars and amino acids in the cell), bulk modulus of elasticity (ε; MPa; measure of cell rigidity), and mass based capacitance before turgor loss point ($C_{T,mass}$; g g^{-1} MPa^{-1}; the change in the amount of water volume per change in water potential per gram of dry shoot tissue before the turgor loss point).

Stem hydraulics and morphological traits

In order to compare the hydraulic efficiencies and capacities of shoots from the two morphotypes, we measured whole stem hydraulic conductance (k_{ws}) using a low-pressure flow meter (Kolb, Sperry, and Lamont 1996). Apical, branching shoots of similar exposure and aspect ranging 7-22 cm long were collected May 13, 2013 from seven randomly selected plants of each morphotype (14 stems total) in the common garden. Within 45 minutes of collection, ends were cut under distilled water. Cut ends were submerged for approximately 24 hours and allowed to rehydrate. During this time, shoots were defoliated and fresh leaf surface area (LA) for each stem was determined using a flatbed scanner and measured using ImageJ (Rasband 2014). Stems were connected by a compression fitting to partially degassed 0.10 M KCl solution sitting on a balance with resolution to 0.01 mg (CP225D, Sartorius AG, Göttingen, Germany). Stems were placed in a vacuum chamber and flow rates were measured consecutively under -15, -25, -35, -50, and -60 kPa below ambient. k_{ws} was calculated as the slope of the linear regression of flow rate (kg s^{-1}) as a function of vacuum pressure (kPa), and subsequently normalized by leaf area to provide leaf area specific whole stem hydraulic conductance ($k_{ws/la}$; mmol s^{-1} m^{-2} MPa^{-1}). In order to further quantify the relationship between the sapwood area supplying water to the leaves and the total living leaf area we obtained the Huber value for each stem, which is the cross-sectional area of stem sapwood (sapwood area, SA) and branch leaf area to sapwood area ratio (SA:LA). As a metric of branch ramification, we normalized the number of active apical meristems by sapwood area. Sapwood area was determined by taking the mean of three diameters on dried stems with the bark removed and pith area subtracted from total cross-sectional area.

Data Analyses

Due to the uneven replication of soil moisture sensors during the study, overall trends in soil moisture values were analyzed qualitatively. However, we could statistically analyze the differences in soil moisture between morphotype and soil depth by pooling remaining sensor data together, calculating daily means for each sensor, and normalizing all values by the mean daily values to account for the expected variation in soil moisture between days. This process enabled us to remove the effect of time from the analysis and provided a general comparison of soil moisture at different depths between the two morphotypes. We performed a two-way ANOVA on the normalized data with morphotype, soil depth, and their interaction as fixed effects.

Stable carbon isotope ratios, $\bar{C}_i$, and log-transformed NUE were analyzed using a two-way ANOVA with time of year and morphotype as fixed effects and a Tukey HSD test was performed for a posteriori comparisons of means for significant interaction effects. In order to evaluate differences between morphotype, time of year, and time of day for the stomatal conductance and water potential data, we required a higher resolution of interpretation than a

linear mixed effect model allow. Therefore, in the case of diurnal measurement days we performed two-tailed t-tests between morphotypes for individual dates and time periods when we expected the plants to respond to environmental conditions differently. For g_s these time periods were the midday time periods of 1100 and 1300 on both diurnal measurement days (September 15, 2011 and April 6, 2012) when VPD would be at its highest. For Ψ, we tested for differences between morphotypes at 1400, 1600, and 1930 hours during peak drought on September 15, 2011 and at 1930 hours during spring on April 6, 2012. As a result, we were careful to restrict our interpretation to these targeted comparisons. Bulk tissue water relations parameters (Ψ_{TLP}, Ψ_{SFT}, RWC_{TLP}, and ε) were analyzed with two-way ANOVAs with morphotype and time of year as fixed effects and Tukey HSD tests were performed for a posteriori comparisons of means when needed. Mean $k_{ws/la}$ and the ratio of the number of apical meristems to sapwood area of prostrate and erect plants were analyzed with Student's t-tests.

All analyses were performed in JMP Pro 11.0 (SAS Institute Inc., Cary, North Carolina, USA) except soil moisture, which was performed in R (R Core Team 2012). Data were log-transformed if they did not meet the assumptions of homoscedasticity using Levene's Test and normality using the Shapiro-Wilk W test. An outlier datum was removed from $\delta^{13}C$ values for the erect morphotype measurements in April 2012 based on a significant Grubb's test ($p = 0.05$) using the extreme studentized deviate method from an online outlier calculator (GraphPad Software, Inc., La Jolla, California, USA, http://graphpad.com/quickcalcs/Grubbs1.cfm, accessed February 25, 2014). All statistical tests were considered to be significant at $p \leq 0.05$.

Results

Environmental conditions

Mean midday (1100-1500 hours) temperatures at BMR from May 1, 2011 to May 31, 2012 reflected its coastal location, ranging from a low of 7.5°C in early spring to a high of 18.9°C in early fall. Relative humidity was consistently higher in the summer months of June, July, August, and September 2011 and lower in the following spring in April 2012. Further, the presence of sustain summertime advective fog events resulted in RH measuring at or near 100% for the entire day several times in 2011. Relative humidity ranged from 32.6% during the presence of seasonal high pressure systems that bring dry easterly winds (e.g., November through February) to 100% during rainy periods and sustained spring and summer fog events. Mean midday VPD was consistently low, with an overall annual mean of 0.22 ($\pm$0.18 standard deviation) kPa and a range of a minimum value of zero (during rain and fog events) to a maximum value of 1.26 kPa (Figs. 2 and 5). Mean midday (1100-1500 hours Pacific Daylight Time) PAR for BMR was 1842.5 ($\pm$106.5) µmols m^{-2} s^{-1} on June 14, 2011, 1580.8 ($\pm$271.9) µmols m^{-2} s^{-1} on July 12, 2011, 1279.5 ($\pm$210.4) µmols m^{-2} s^{-1} on August 11, 2011, 1265.7 ($\pm$411.3) µmols m^{-2} s^{-1} on September 15, 2011, and 1618.7 ($\pm$116.2) µmols m^{-2} s^{-1} on April 6, 2012.
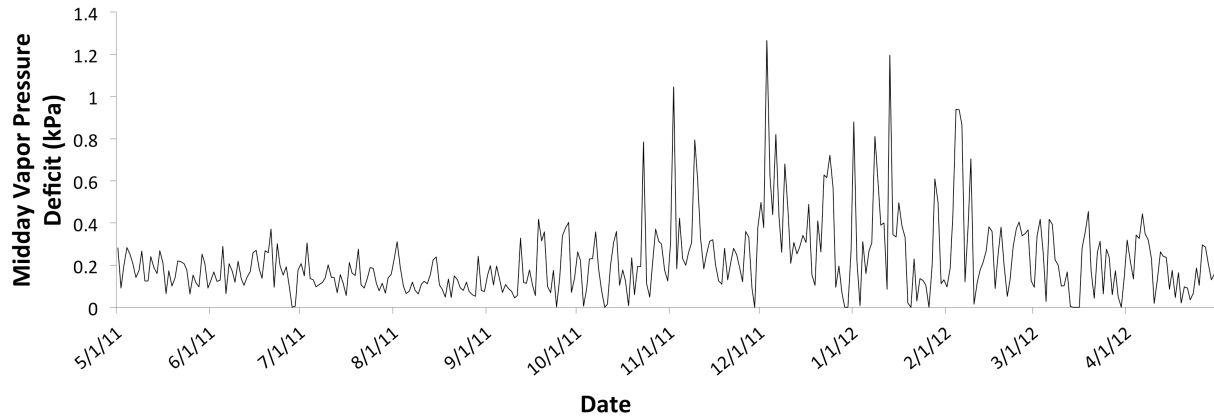
Figure 2. Mean midday vapor pressure deficit (VPD) from 1100-1500 hours local time from May 1, 2011 to April 30, 2012.

The study site received a total annual rainfall of 750 mm (29.53 in) of rain from May 1, 2011 to April 30, 2012, and with meaningful amounts during late spring (Fig. 3). For all of May 2011 rainfall totaled 68.1 mm (2.68 in); on June 4, 2011 (ten days before our June 14 measurements) the study site received 34.5 mm (1.36 in) of rain; between June 28 and July 12 (two weeks before our July 12 measurements) the site received 28.2 mm (1.11 in); and from March 29, 2012 to April 5, 2012, the week before our late wet season measurement date of April 6, 2012, the site received a total of 15.2 mm (0.60 in) of rain. There were 15 days during August 2011 when 0.01 inches of precipitation were recorded, for a total of 0.15 inches. September 2011 was similar to August, with a total of 0.21 inches recorded precipitation.
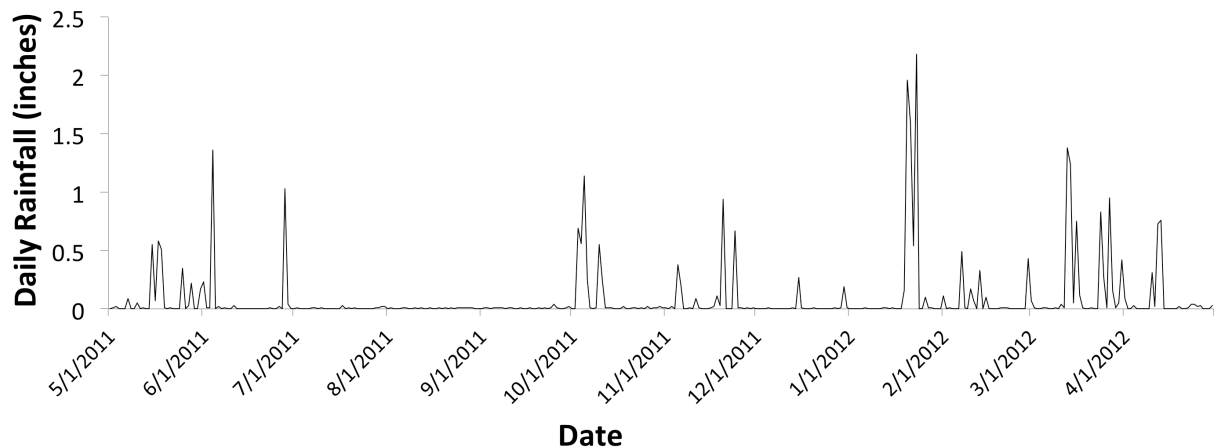
Figure 3. Daily rainfall (inches) for May 1, 2011 through April 30, 2012.

The soil under prostrate plants had higher soil moisture at shallow depths and lower soil moisture deeper in the soil profile than erect plants for most of the months recorded, particularly during late spring. In contrast, the soil under erect plants had relatively less water in the shallow soil layers and more water in deep soil layers. To this end, two-way ANOVA using data normalized by daily mean values indicates there was a significant interaction effect between morphotype and soil depth ($F_{1, 9} = 76.14$, $p < 0.0001$; Fig. 4).

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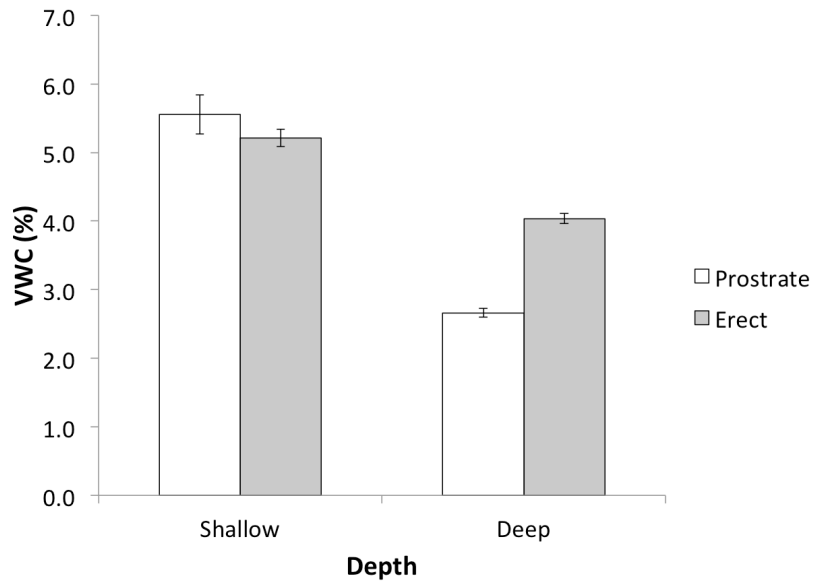
Figure 4. Volumetric water content in shallow (just below surface) and deep (100 cm deep) soils underneath prostrate and erect morphotypes. ($\pm$1 SE)

Stable carbon isotope ratios and NUE

Results of the two-way ANOVA for δ^{13}C values indicate the main fixed effects of morphotype and time of year were both significantly different ($F_{1,14} = 5.5531$, $p = 0.0335$ and $F_{1,14} = 4.7158$, $p = 0.0476$, respectively; Fig. 5a). The interaction effect between morphotype and time of year was not significant. The δ^{13}C values of prostrate morphotypes were significantly more negative than erect morphotypes for both times of year and the δ^{13}C values for both morphotypes combined were significantly different between May and September. Mean δ^{13}C values for prostrate plants were -28.2‰ ($\pm$0.3‰ SD) during May 2011 and -27.7‰ ($\pm$0.8‰ SD) during September 2011, and mean δ^{13}C values for erect plants were -27.6‰ ($\pm$0.2‰ SD) during May 2011 and -26.8‰ ($\pm$0.9‰ SD) during September. Overall, while δ^{13}C values for both morphotypes changed from May 2011 to September 2011, erect plants exhibited a larger percent change during this time period than prostrate plants.

As expected based on the δ^{13}C values, time-integrated intercellular CO_2 concentration ($\bar{C}_i$) mirrors these trends. $\bar{C}_i$ was significantly higher during the wet season in May 2011 than during the dry season in September 2011 ($F_{1,14} = 4.6319$, $p = 0.0493$), with a mean $\bar{C}_i$ in May of 258.5 ppm ($\pm$6.5 SE) and 245.4 ppm ($\pm$5.5 SE) in September (Fig. 5b). $\bar{C}_i$ was also significantly higher in prostrate plants for both seasons ($F_{1,14} = 5.1526$, $p = 0.0395$), with an overall mean of 258.9 ppm ($\pm$3.5 SE) for prostrate plants and an overall mean of 245.0 ppm ($\pm$4.6 SE) for erect plants. Similar to the δ^{13}C ANOVA results, the interaction term of plant morphotype and season for $\bar{C}_i$ was not significant.

The ANOVA on log-transformed NUE data indicates NUE was significantly lower for both morphotypes during the wet season and higher during the dry season ($F_{1,15} = 170.0527$, $p <0.0001$; Fig. 5c).
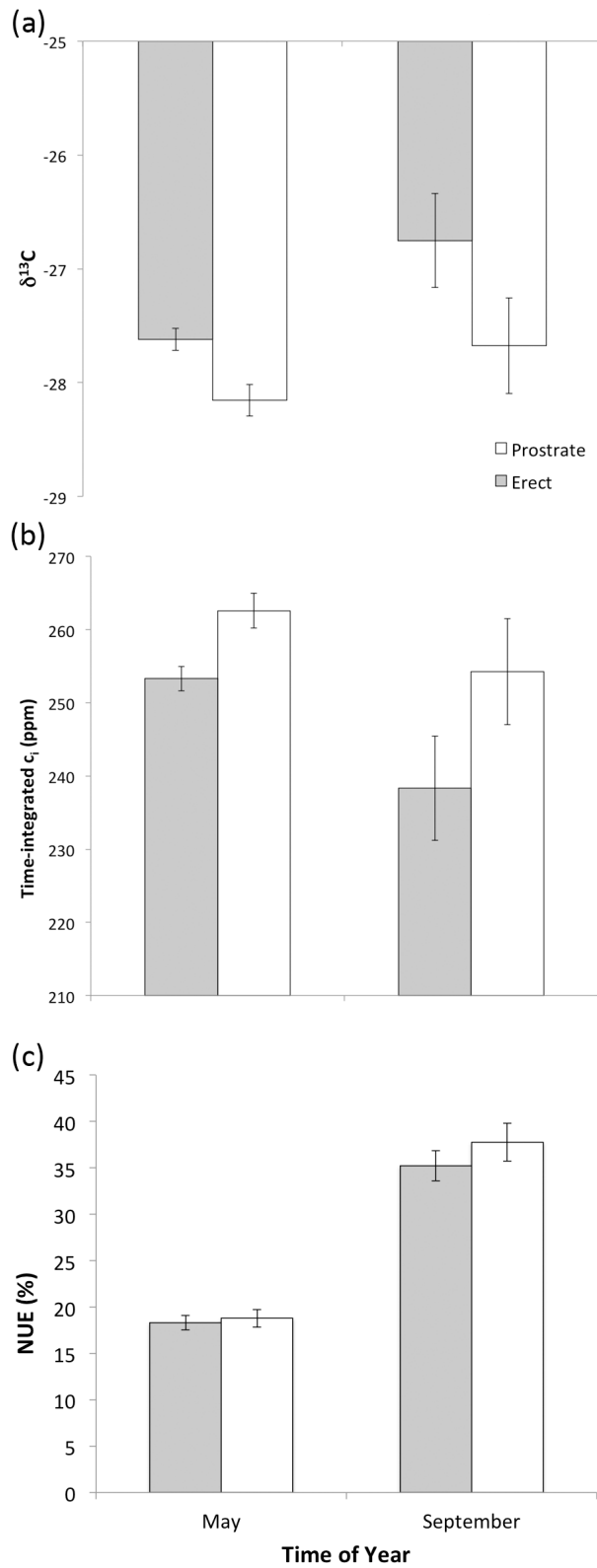
Figure 5. Stable carbon isotope ratios ($\delta^{13}C$, (a), time-integrated intercellular CO_2 ($\bar{C_i}$), (b), and time-integrated NUE, (c), of prostrate morphotypes (open bars) and erect morphotypes (solid bars) of *B. pilularis* in May and September 2011. (± 1 SE)

Stomatal conductance

Stomatal conductance to water vapor was not significantly different between prostrate and erect morphotypes for midday g_s (June 14, July 12, August 11, and September 15, 2011 and April 6, 2012). There was also no significant difference in g_s between the two morphotypes during seasonal diurnal measurements (September 15, 2011 and April 6, 2012; Fig. 6 and Table 1). Both morphotypes responded to conditions similarly by gradually increasing midday conductance rates as the rainless summer progressed, with the highest g_s occurring at peak drought in September, and decreasing the following spring in early April near the end of the wet season. Midday g_s for prostrate plants increased 36.6% from June to July, 30.5% from July to August, 34.0% from August to September, with an overall percentage increase from June to September of 70.9%. The percentage increase in midday g_s for erect morphotypes was 33.8% from June to July, 35.0% from July to August, 23.4% from August to September, with an overall percentage increase of 67.0%.
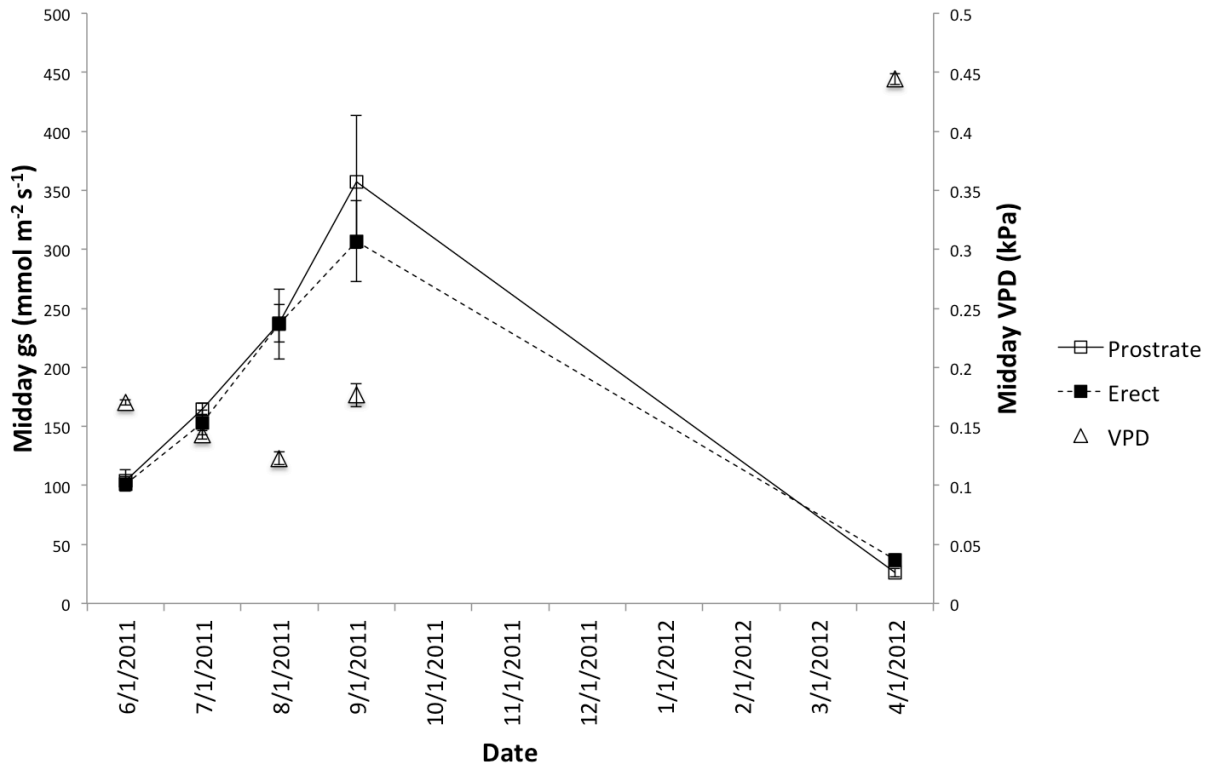
Figure 6. Midday (1300 hours) stomatal conductance (g_s) for prostrate (open squares) and erect (solid squares) *B. pilularis* morphotypes and mean midday (1100-1500 hours) vapor pressure deficit (open triangles) for June 14, July 12, August 11, September 15, 2011 and April 6, 2012. (± 1 SE)

Water potentials

Indicative of the mild coastal climate location, diurnal courses of water potentials for both *B. pilularis* morphotypes remained above -2 MPa, with water potentials during peak drought (September 15, 2011) ranging from -1.75 to -0.5 MPa and spring (April 6, 2012) ranging

from -1.45 to -0.1 MPa. Further, diurnal water potentials showed a similar overall pattern for both morphotypes during both peak drought (September 15, 2011) and spring (April 6, 2012), with the most negative values observed during peak sunlight hours after the morning marine layer cleared (Fig. 7). Both morphs showed signs of the dry conditions at summer's end in September by having slightly more negative diurnal water potentials and not recovering as well at the end of the day as during April's diurnal recovery. The among-groups recovery (last measurement time point of each day) was significantly more negative during September than April ($F_{1,16} = 24.9601$, $p = 0.0001$). While there was little difference between morphotype during spring, during September the two morphotypes diverged toward the end of the day, with the 4pm water potentials being the only time point at which the two morphotypes differed significantly (t-test, $p = 0.0082$). The 1930 hours water potentials showed a trend toward being significantly different (t-test, $p = 0.0629$; Fig. 7). Mean midday water potentials were not significantly different between seasons, reflecting the consistently mild maritime climate of the coastal common garden setting and suggesting no difference in how stressed both morphotypes were between summer and the following spring (Fig. 8). The variability between seasonal midday water potentials for both morphotypes might be related to intermittent rainfall received before the June and July 2011 and April 2012 measurements.
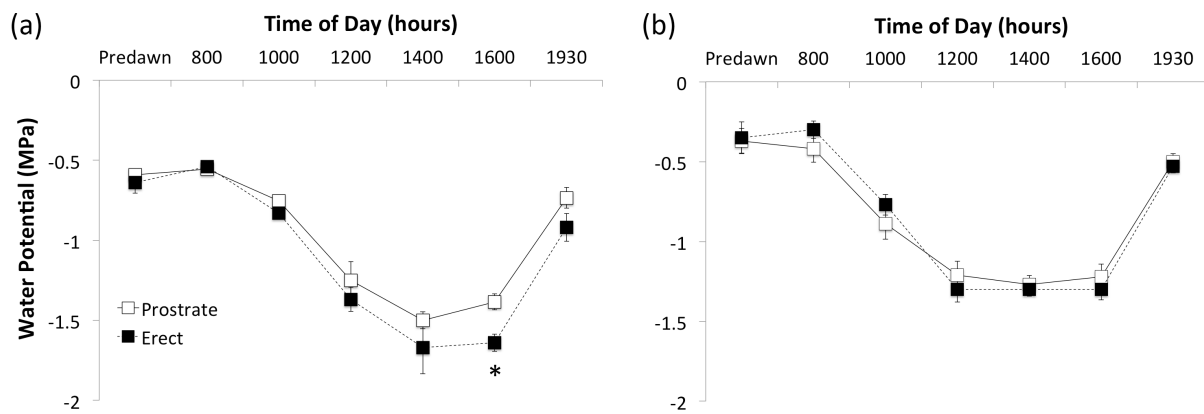
Figure 7. Diurnal water potential measurements for prostrate (open squares) and erect (solid squares) *B. pilularis* during (a) September 2011 and (b) April 2012. (± 1 SE; * = significantly different)

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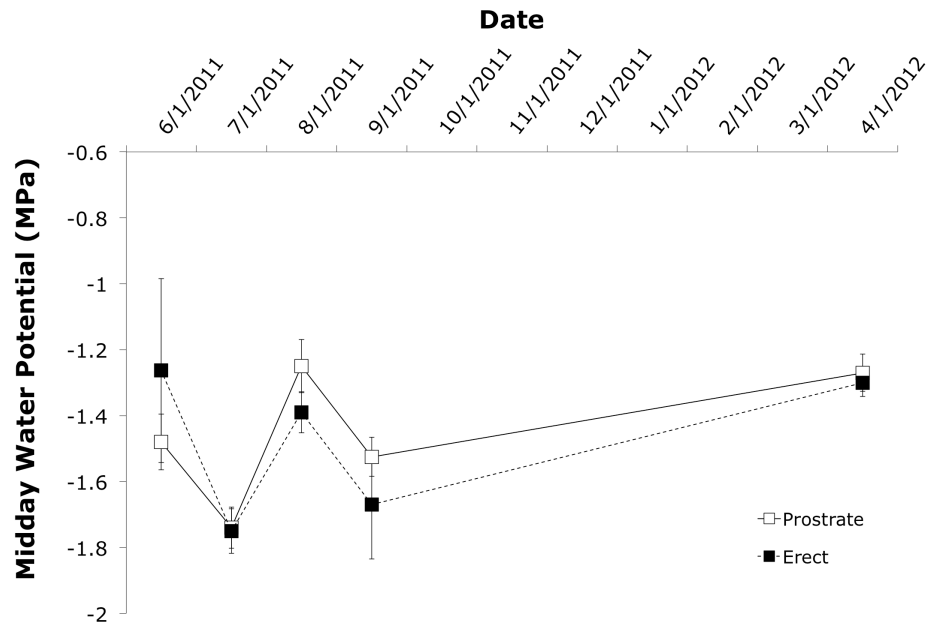
Figure 8. Midday water potentials for prostrate (open squares) and erect (solid squares) *B. pilularis* morphotypes on June 14, July 12, August 11, September 15, 2011, and April 6, 2012. (± 1 SE)

Table 1. Seasonal progression of mean (±SD) stomatal conductance (g_s) of prostrate and erect *B. pilularis* morphotypes and environmental parameters from measurements with LI-1600 steady state porometer. N=5 for each measurement time period. Stomatal conductance is presented in both *mmol m^{-2} s^{-1}* and *cm s^{-1}* for reference.

		June	July	August	September			April (2012)				
					11:00	13:00	15:00	9:00	11:00	13:00	15:00	18:00
g_s (mmol m^{-2} s^{-1})*	Prostrate	104.1 (19.9)	164.7 (10.7)	237.4 (35.8)	347.0 (71.0)	316.5 (112.7)	213.1 (53.3)	38.0 (18.9)	42.6 (16.1)	26.3 (8.1)	46.3 (13.4)	23.9 (12.1)
	Erect	101.3 (13.9)	153.3 (23.6)	236.7 (66.7)	406.3 (52.3)	270.5 (67.4)	247.6 (76.2)	38.1 (7.1)	41.1 (10.3)	37.1 (10.1)	44.0 (16.3)	28.4 (10.7)
g_s (cm s^{-1})	Prostrate	0.25 (0.05)	0.40 (0.03)	0.57 (0.09)	0.83 (0.17)	0.77 (0.27)	0.52 (0.13)	0.09 (0.05)	0.10 (0.04)	0.07 (0.02)	0.11 (0.03)	0.06 (0.03)
	Erect	0.25 (0.03)	0.37 (0.06)	0.57 (0.16)	0.97 (0.12)	0.65 (0.16)	0.60 (0.18)	0.09 (0.02)	0.10 (0.02)	0.09 (0.02)	0.11 (0.04)	0.07 (0.03)
Leaf Temp (°C)	Prostrate	22.8 (0.69)	21.9 (1.1)	21.2 (0.25)	17.8 (0.36)	23.2 (0.81)	23.8 (0.89)	19.3 (1.9)	25.8 (1.7)	28.4 (2.1)	23.2 (0.91)	14.9 (0.36)
	Erect	21.9 (0.78)	21.9 (1.3)	20.9 (0.65)	18.0 (0.38)	22.8 (0.82)	23.7 (0.70)	18.0 (0.79)	23.7 (0.77)	27.3 (2.2)	22.3 (1.17)	14.7 (0.53)
Cuvette Temp (°C)	Prostrate	21.7 (0.61)	21.0 (0.89)	20.4 (0.26)	17.6 (0.26)	22.0 (0.57)	22.6 (0.80)	16.0 (0.98)	21.5 (1.34)	25.6 (1.29)	19.8 (0.49)	15.2 (1.03)
	Erect	21.3 (0.78)	21.0 (0.92)	20.4 (0.55)	17.7 (0.73)	21.6 (0.73)	22.4 (0.48)	15.5 (0.64)	20.0 (1.7)	23.8 (2.2)	19.5 (1.0)	15.2 (1.2)
RH (%)	Prostrate	57.8 (0.96)	60.1 (0.66)	72.0 (1.6)	84.5 (4.7)	71.2 (5.4)	66.3 (3.4)	59.8 (3.0)	74.4 (0.85)	71.3 (5.3)	57.4 (7.3)	62.8 (1.3)
	Erect	57.9 (1.6)	60.3 (0.59)	71.2 (1.4)	86.2 (4.2)	69.4 (3.4)	64.6 (2.3)	58.3 (3.7)	74.4 (6.5)	70.4 (7.5)	58.4 (6.3)	63.4 (1.6)
PAR (µmol s^{-1} m^{-2})	Prostrate	1844 (80.2)	1282 (0.66)	1002 (173.2)	386 (70.2)	1276 (124.6)	1150 (213.9)	1496 (148.8)	1614 (306.5)	1274 (436.0)	1308 (213.94)	304.2 (229.3)
	Erect	1898 (65.3)	1454 (317.2)	990 (171.8)	458 (100.3)	1392 (152.5)	1240 (133.2)	1186 (160.4)	1514 (72.0)	1530 (266.6)	1390 (133.2)	262.6 (186.5)

*Converted from cm s^{-1} to mmol m^{-2} s^{-1} using linear relationship of $y = -1.3667x + 444.5$, where x is the leaf temperature measured by the LI-1600 (Hall et al. 1976, Körner et al. 1979).

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Tissue water relations parameters

Prostrate and erect *B. pilularis* stems diverged in several bulk tissue water relations parameters during peak drought in September 2011 while the morphotypes exhibited similar strategies during the following spring in April 2012 (Fig. 9). The two-way ANOVA for Ψ_{TLP} indicated no significant differences between morphotype, time of year, and the interaction of morphotype and time of year. It is worth noting, however, that in September erect morphs had a mean Ψ_{TLP} of -2.49 ($\pm$ 0.19 SD) and prostrate morphs had a mean Ψ_{TLP} of -1.80 ($\pm$ 0.31 SD). For osmotic potential at full turgor (Ψ_{SFT}) the interaction between the effects of morphotype and time of year ($F_{1,12} = 4.7801$, $p = 0.493$) was significant, with Ψ_{SFT} higher (less negative) for prostrate morphs than erect morphs during September and no difference between morphs during April. Bulk modulus of elasticity (ε) mirrored this pattern, with prostrate morphs having significantly lower ε (more elastic cell walls) than erect morphs during September and no differences in ε between morphotype during April ($F_{1,1} = 6.6057$, $p = 0.0245$). The interaction of time of year and morphotype was significant for $C_{T,mass}$ values ($F_{1,12} = 6.3814$, $p = 0.0266$), with the largest difference being prostrate plants having lower mass-based capacitance than erect plants during April. There were no differences in relative water content at turgor loss point (RWC_{TLP}) between the two morphotypes but both morphs were significantly different between September and April, with ($F_{1,12} = 7.4031$, $p = 0.0186$).
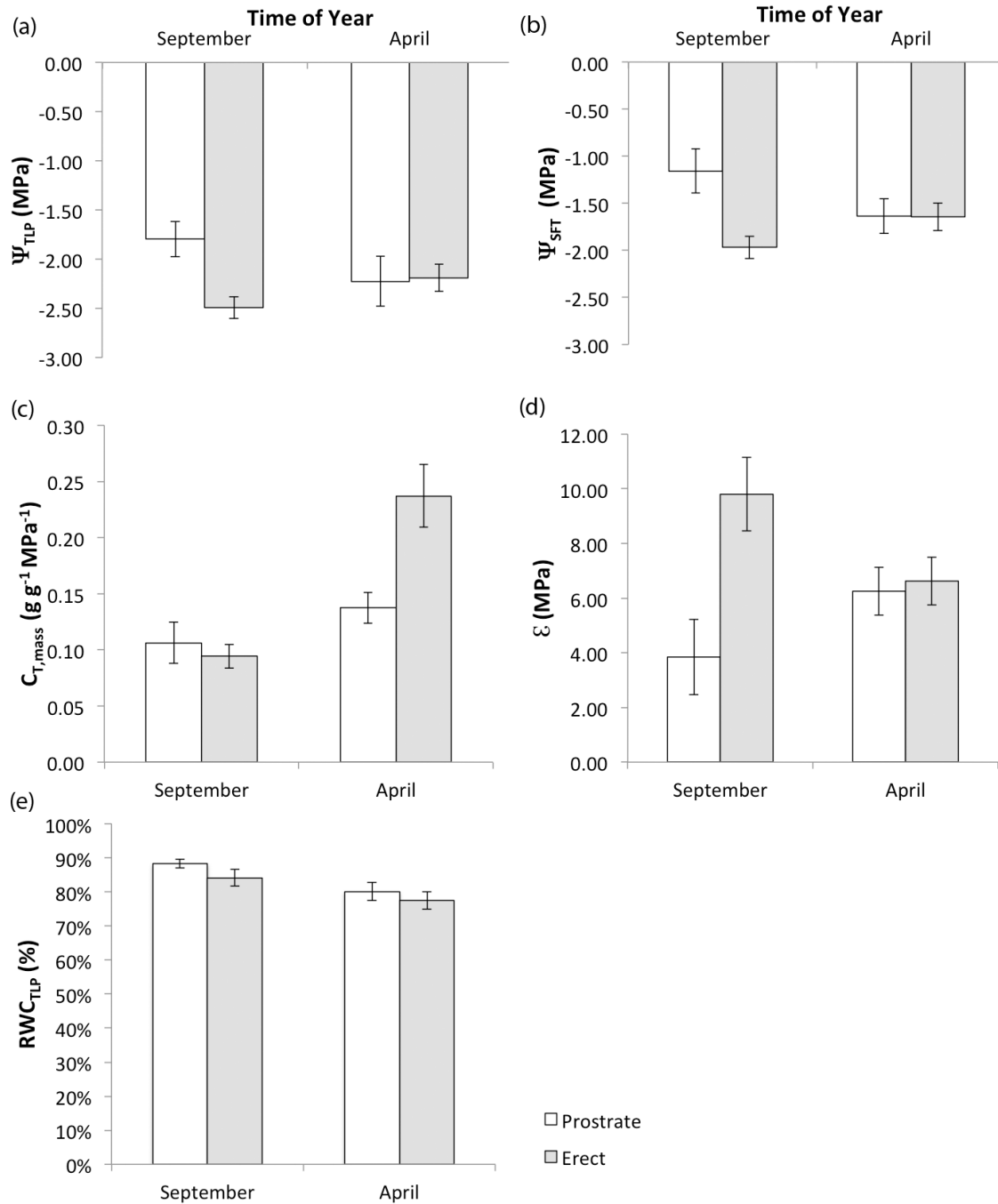
Figure 9. Bulk tissue water relations parameters for prostrate (open bars) and erect (solid bars) *Baccharis pilularis* stem samples in September 2011 and April 2012. Parameters measured and calculated include (a) water potential at turgor loss point (Ψ_{TLP}), (b) osmotic potential at full turgor (Ψ_{SFT}), (c) mass-based capacitance before turgor loss point ($C_{T,mass}$), (d) bulk modulus of elasticity (ε), and (e) relative water content at turgor loss point (RWC_{TLP}). ($\pm$ 1 SE)

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Whole-stem hydraulic conductance and morphological traits

      Although leaf area specific whole stem hydraulic conductance ($k_{ws/la}$) did not significantly differ between the two *B. pilularis* morphotypes, there was a trend of prostrate morphs having significantly lower $k_{ws/la}$ than erect morphs (t-test: $p = 0.0939$; Table 2). As expected, the ratio of the number of apical meristems per shoot to sapwood area was significantly different between prostrate morphotypes (mean = 64.3 $\pm$49.5 SD) and erect morphotypes (mean = 10.3, $\pm$3.2 SD; t-test, $p = 0.0003$; Fig. 10). Although $k_{ws/la}$ was not significantly different between the two morphotypes, the same trend of erect plants having slightly higher $k_{ws/la}$ than prostrate plants was seen when presented as a function of branch ramification, with higher conductance with fewer branch ramifications (Fig. 11). There was no difference in SA:LA between morphs and no correlation between SA:LA and $k_{ws/la}$.
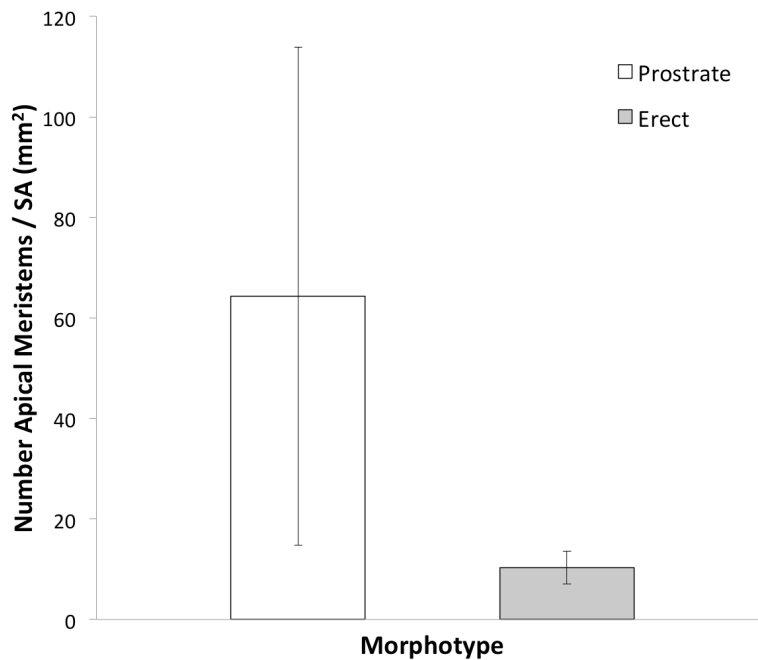
Figure 10. Ratio of number of apical meristems to sapwood area for prostrate (open bar) and erect (solid bar) *B. pilularis* morphotypes. Original data and variances are displayed and log-transformed data were used in T-test. ($\pm$ 1 SE; N=7 for each morphotype)
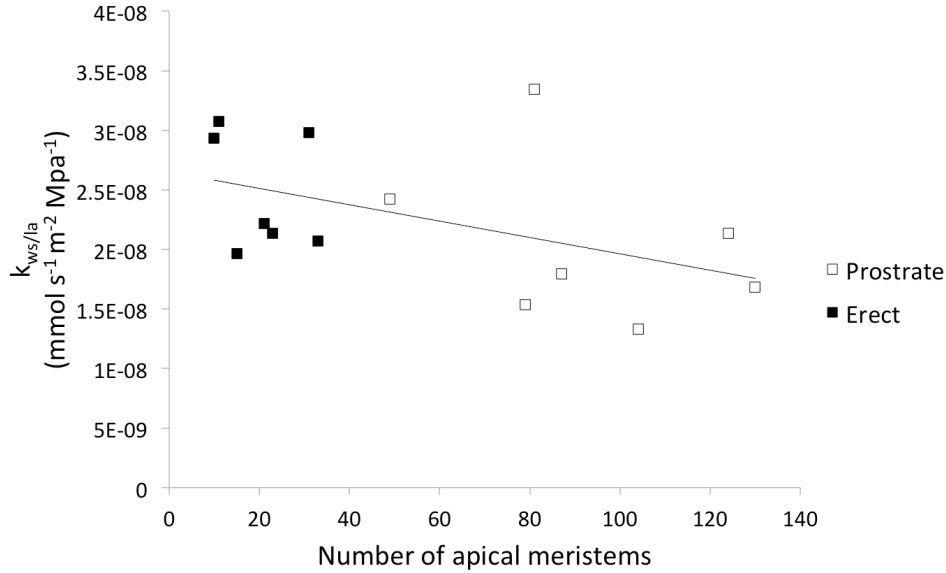
Figure 11. Leaf area specific whole stem hydraulic conductance ($k_{ws/la}$) as a function of the number of apical meristems for prostrate (open squares) and erect (solid squares) *B. pilularis* morphotypes.

Table 2. Mean functional traits for each morphotype ($\pm$SD). N=7 stems

| Trait | Abbr. | Units | Morphotype | |
			Prostrate	Erect
Leaf area specific whole stem hydraulic conductance	$k_{ws/la}$	mmol s^{-1} m^{-2} Mpa^{-1}	2.04×10^{-08} (6.82×10^{-09})	2.48×10^{-08} (4.89×10^{-09})
Total stem leaf area	LA	cm^2	77.2 (29.2)	94.2 (38.3)
Sapwood area	SA	mm^2	1.95 (0.95)	2.07 (1.0)
Huber value (sapwood area to leaf area ratio)	SA:LA	cm^2 m^{-2}	2.75 (1.74)	2.30 (0.78)
Number of apical meristems			93.4 (28.2)	20.6 (9.2)
Ratio of number of apical meristems to sapwood area		SA is in mm^2	64.3 (49.5)	10.3 (3.2)

Discussion

Our data suggest that prostrate and erect *B. pilularis* morphotypes display traits that reflect an adaptive suite of physiological and morphological characteristics in line with their predominate distributions. Erect plants mostly live inland and experience hot, dry summers and high leaf to air vapor pressure deficits, and prostrate plants mostly live along the immediate coast where vapor pressure deficits are modulated by the maritime climate and the presence of advective fog. Even though the plants in this study coexisted in the coastal setting of the Bodega Marine Reserve, the two morphotypes differed in some diurnal Ψ patterns during peak drought, tissue water relations parameters of Ψ_{SFT}, $C_{T,mass}$, and ε, and the longer-term, seasonal responses of bulk leaf $\delta^{13}C$ and $\bar{C}_i$ values, but

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showed little to no differences in Ψ_{TLP}, RWC_{TLP}, $k_{ws/la}$, Huber value, mean midday Ψ, and diurnal and seasonal midday g_s.

Stomatal conductance and stable carbon isotopes

Contrary to our hypothesis that g_s would steadily decrease during the rainless summer months and be higher near the end of the wet season (Mooney et al. 1975, Ehleringer 1985, Kramer and Boyer 1995), g_s instead increased during the summer and was lower the following spring during the wet season. Further, g_s was unexpectedly decoupled from VPD. Results from a companion study in a coastal grassland in Central California with a similar climate show *B. pilularis* seedlings and adults possessing a similar increase in g_s during summer of 2011 (Kidder 2015). Although the seasonal increase in stomatal conductance may be counterintuitive, it likely occurred because of a confluence of multiple interacting factors including, 1) sufficient water availability from the combination of low vapor pressure deficit resulting from the cool and moist maritime atmosphere and advective fog brought ashore by prevailing westerly winds and 2) a relatively high underground water levels at the study site, and 3) an increased demand for carbon during the onset of the plants' reproductive season in fall.

The maritime climate and relatively high underground water sources of this coastal study site contribute to low evapotranspiration and vapor pressure deficit, which could favor higher g_s rates. Advective fog along California's coast creates a "fog belt" that serves as a water subsidy and drought stress relief important for plant growth during dry summer for many plant and plant communities (Jacobs et al. 1985, Dawson 1998, Limm et al. 2009, Corbin et al. 2005, Cole 2005, Fischer et al. 2009, Carbone et al. 2013), and also influences landscape dynamics among ecotones (Kennedy and Sousa 2006). Further, growth and establishment of *B. pilularis* are highest in the presence of coastal fog (i.e., high humidity), late spring rains, and elevated mean rainfall (Williams et al. 1987, Ford and Hayes 2007). The combination of *B. pilularis*' deep root system and the relatively high water table at the study site could mean the roots of the study plants reach the water table (but see Cole and Mahall 2006) or a deep source of water in the subsurface, possibly providing a steady water supply during the rainless summer months. By tapping such a consistent water source, these *B. pilularis* plants would be able to fix carbon without facing stomatal closure (but still face biochemical limitations to photosynthetic rates), maintaining high stomatal conductance rates (Kramer and Boyer 1995).

The seasonal increase in g_s could also reflect an increased need for carbon as plants approached their reproductive phase, from the end of September through January (Calflora 2014). During the pre-flowering stage, stems of both male and female *B. pilularis* elongate terminal rosette-like leaf structures, lengthening the internodes to eventually form flower buds in leaf axils so that the leaves from the original rosette ultimately subtend the flowers. Although identifying the many plant nutrients and mechanisms involved in resource allocation to flower development in plants is complex, carbon has been shown to be a useful metric to gauge plant growth during this period (Obeso 2002). Because the carbon used in the production of flowers and fruits most often originates in the leaves nearest them (Mooney 1984), our stomatal conductance measurements made on the terminal ends of stems where fruits and flowers were being developed could reflect the plants' increased demand for carbon as the diffusion of CO_2

into the leaf and the diffusion of water vapor out of the leaf are both controlled by stomatal aperture (Hsiao 1973, Kramer and Boyer 1995, Obeso 2002). Stem elongation and flower production result in construction costs that create a demand for carbon late in the dry season when water is typically least available to these plants.

Further evidence of an increased carbon demand can be seen in the changes observed in leaf carbon isotope composition and their relationship to $\bar{C}_i$ and NUE. For both morphotypes, as g_s increased from wet season to dry season, $\delta^{13}C$ values increased, $\bar{C}_i$ decreased, and NUE increased. This trend suggests that carboxylation rates increased to the point of demanding more CO_2 than could adequately be diffused into the leaf during stomatal conductance. Lower $\bar{C}_i$ resulted in less negative $\delta^{13}C$ values as ribulose-1,5-bisphosphate carboxylase/oxygenase was forced to discriminate less against larger ^{13}C molecules. Erect plants, which had the lowest $\bar{C}_i$ and high gs rates (similar to prostrate plants) therefore fixed more carbon for a given amount of water lost as water vapor during stomatal conductance, directly reflecting this morphotype's adaptation to its warmer, inland location. However, the higher $\delta^{13}C$ values of erect morphotypes could be intensified by the taller stature and less dense canopies of these plants, making these plants more vulnerable to wind and increased evapotranspiration, both possible sources of water loss.

Water potentials

Relatively high water potentials of both morphotypes suggest that plants did not experience large water deficits during the course of this study. Both morphotypes had less negative water potentials than erect *B. pilularis* plants in warm, inland conditions (Ackerly 2004). September's significantly more negative late afternoon water potentials exhibited by the erect morphotype coupled with its high stomatal conductance values indicate erect plants are able to tolerate seasonal drought better than prostrate plants by fixing more carbon in the presence of low water availability.

Tissue water relations parameters

Results from the pressure-volume curve analyses suggest that prostrate and erect morphotypes use different tissue-level strategies that again reflect their coastal and inland habitats, respectively. The two strategies are seen mainly during higher water deficit in September. For example, during peak drought in September, in a response typical of plants adapted to xeric habitats (Zimmermann 1978b), erect plants had an increase in cell solutes that lowered osmotic potential at full turgor, a seasonal decrease in cell wall elasticity (increase in ε), and a slightly lower turgor loss point. The higher ε in erect plants during peak drought allows these plants to retain more water for a given loss of turgor pressure. Prostrate plants displayed a more mesic strategy, with a significant increase in cell wall elasticity (decrease in ε), a less negative osmotic potential at full turgor than in April, and an increase in turgor loss point. The low ε value of the coastal prostrate plants allows these plants to respond quickly to abrupt changes in environment in their otherwise stable climate, such as when VPD spikes during seasonal high pressure systems. Both of these strategies help them maintain adequately hydrated leaf and stem tissue for carbon fixation, as indicated by both morphotypes having similar Ψ_{TLP} and RWC_{TLP}.

Osmoregulation also varied significantly between the two morphotypes, with erect plants having a lower (more negative) osmotic potential at full turgor in September than in April and prostrate plants have a higher (less negative) osmotic potential at full turgor in September than in April. This likely led to the slightly lower turgor loss point by erect plants during September, as osmotic adjustment has been suggested as the main driver of differences in turgor loss point (Bartlett et al. 2012). The lower osmotic potential in erect plants reflects an adaptation for increased drought avoidance that is favored in this morphotype's native warm, dry habitat (Dawson and Bliss 1989). It is important to note whether or not these seasonal differences in osmotic potential are due to active solute accumulation or passive solute concentration from the loss of symplastic water (Morgan 1984). Additionally, osmotic adjustment can be influenced by the hydration of stem samples prior to pressure-volume curve measurements (Dawson and Bliss 1989). Although Pavlik (1984) advises considering symplastic water fraction (SWF) to determine active (SWF stays the same or increases) or passive solute accumulation (SWF decreases), our method did not provide enough resolution in the calculation of symplastic water fraction to resolve this issue.

Morphotypes also differed in their $C_{T,mass}$, or the amount of water released for a given change in water potential per gram of dried biomass, during the end of the wet season in April, with erect plants having a significantly higher $C_{T,mass}$ than prostrate plants. The higher $C_{T,mass}$ of erect plants in April suggests that erect plants may depend on the ability to store water in their tissues when it is available during the wet season to serve as a buffer against the intense water deficit experienced during hot inland summer droughts. Conversely, coastal prostrate plants, which do not experience as intense of a seasonal water deficit, appear not to rely as much on wet season water storage. During September, $C_{T,mass}$ is similar for both morphotypes, indicating the plants may adjust other tissue properties such as osmotic concentrations and cell wall elasticity to maintain water potentials above the Ψ_{TLP} when the plants experience a decrease in water availability.

Whole-stem hydraulic conductance and morphological traits

The lack of significant differences in $k_{ws/la}$ mirrors the very similar stomatal conductance rates in the two morphs. However, the marginally-significant trend of erect morphs having a higher $k_{ws/la}$ than prostrate morphs closely supports our hypothesis that plants adapted to warmer and drier habitats would demand a higher rate of stem water transport to their distal leaves. Results from another study using the same method suggest that leaves in low versus higher rainfall areas in a tropical forest have higher whole stem hydraulic conductance (Brenes-Arguedas et al. 2013). Further, due to the larger number of apical meristems in prostrate plants than in erect plants (Fig. 8, Rudgers and Whitney 2006), we expected erect plants to have higher $k_{ws/la}$, because more branching points would increase the number of parallel conducting pathways, creating a more meandering path for water flow. In woody plants, hydraulic conductivity of the more distal, higher-order branches is lower than the more basal, lower-order branches or stems (Zimmermann 1978a, Tyree and Zimmermann 2002). Additionally, the higher ratio of apical meristems per functional xylem area (SA) in prostrate morphs would also explain these plants' lower $k_{ws/la}$.

The lack of difference of the Huber value between the two morphotypes was surprising, as we expected erect plants to require a larger sapwood area to provide a

greater water transport capacity. Results from other studies suggest plants adapted to drier habitats have higher Huber values (Tyree and Zimmermann 2002, Carter and White 2009, Gotsch et al. 2010). We were also surprised by the similar total distal leaf area in the two morphotypes because mean leaf area of these same common garden plants were found to be significantly different (Rudgers and Whitney 2006). Measuring other hydraulic architectural traits like vessel size and root and leaf hydraulic conductance might help explain the similarity between the two morphotypes in Huber Values and $k_{ws/la}$ as well as the differences in $\delta^{13}C$ values and bulk tissue water relations parameters.

Conclusion

   The variation in plant architecture in *B. pilularis* is linked to suites of coordinated adaptive traits unique to each morphotype. By using a common garden setting, we can conclude that several biochemical and physiological water relations characteristics differ between two morphotypes. For the traits where prostrate and erect *B. pilularis* morphotypes differed (bulk leaf $\delta^{13}C$ values, $\bar{C}_i$, afternoon and recovery Ψ during peak drought, Ψ_{SFT}, $C_{T,mass}$, and ε), prostrate plants exhibited responses associated with more mesic coastal habitats while erect plants responded as would be expected by a plant from more xeric habitats. Moreover, tissue-level differences of Ψ_{SFT}, $C_{T,mass}$, and ε were only present during the dry season when water availability was lowest. Contrary to our hypothesis, the two morphotypes did not differ in seasonal and diurnal gs, Ψ_{TLP}, RWC_{TLP}, and $k_{ws/la}$, and Huber value. The distinct sets of adaptive strategies enable erect and prostrate morphotypes to meet the same goal of adequate hydration and plant function while reflecting each morphotype's evolutionary history.

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CHAPTER 2

Water relations of *Baccharis pilularis* during seedling establishment in a changing climate

Abstract

Californian plant communities within the state's coastal fog belt are of growing conservation concern due to projected temperature increases and the high probability of continued decline in coastal fog. Coastal grasslands are already becoming increasingly rare due to urbanization pressures as well as altered grazing and fire regimes and resulting woody plant encroachment. *Baccharis pilularis* DC. (coyote brush, Asteraceae), a ubiquitous native woody shrub, is often the first to invade both annual-dominated and perennial-dominated coastal grasslands along California's central coast. In order to gain insights into this pivotal stage of landscape change, this study examined the influence of neighboring grassland species on *B. pilularis* seedlings by performing a manipulative clipping experiment in both warmer, annual-dominated and cooler, fog-prone, perennial-dominated coastal grasslands. In areas with and without neighboring grassland plants, we tracked *B. pilularis* seedling performance (height, growth, leaf number, stem length, above-ground biomass, and basal stem diameter), water relations physiology (water potentials and stable carbon isotopes of bulk leaf tissue), and water use (stomatal conductance, and comparing stable water isotopes of rain, fog, soil, and ground waters to plant xylem water). *B. pilularis* seedlings grew significantly larger in the warmer annual-dominated grassland compared to the cooler perennial-dominated grassland, and in areas where neighbors were removed. In both grassland types, *B. pilularis* water potentials were significantly lower with neighbors present during the wet season when neighboring grasses were actively growing. Dry season water source use reflected the contrasting coastal grassland types, with deuterium values in plant xylem water significantly higher in the warmer annual grassland, where evaporative enrichment of near-surface moisture sources is more likely to occur. Seedlings in the annual grassland used a mixture of ground water and shallow soil waters (10-40 cm), where in contrast, seedlings in fog-prone perennial grasslands used a combination of fog and shallow soil waters, suggesting shallower rooting depth. Overall, our results suggest that *B. pilularis* seedlings thrive in dry, annual-dominated coastal grasslands with growth and water relations parameters that reflect minimal water stress, indicating these plants are well-adapted to persist in a warming climate. As such, it will be important to abate *B. pilularis* establishment in order to preserve California's unique coastal grasslands.

Introduction

Grasslands along California's central coast have become increasingly rare due to habitat conversion, agriculture, and succession to woody plant dominated communities (Keeler-Wolf, et al. 2007). As such, there is a need for land managers to preserve remnant coastal grasslands, particularly coastal prairie grasslands that contain some of the last vestiges of perennial native grasses and a suite of rare and endangered grasses and forbs (Ford and Hayes 2007). Northern coastal scrub is a woody plant community that readily encroaches into both non-native annual- and native perennial-dominated coastal grasslands along California's central coast when fire and grazing are removed (McBride and Heady 1968, McBride 1974, Elliot and Wehausen 1974, Russell and McBride 2003, Ford and Hayes 2007). *Baccharis pilularis* DC. (coyote brush,

Asteraceae) is a dominant plant of northern coastal scrub and is typically the first woody plant to invade (McBride and Heady 1968, Ford and Hayes 2007). Deliberate management actions are required for grassland habitat preservation to prevent *B. pilularis* encroachment.

Water availability has been shown to be important to *B. pilularis* establishment in coastal grasslands. Late spring rainfall facilitates the survival of *B. pilularis* seedlings because it promotes essential early tap root development when competing with grasses for dwindling soil moisture as winter precipitation wanes (Williams et al. 1987, Williams and Hobbs 1989). Further, in controlled greenhouse conditions, *B. pilularis* is a better competitor in moister conditions when competing with a non-native annual grass (Da Silva and Bartolome 1984). Despite the ubiquity of *B. pilularis* throughout the California Floristic Province, there is a dearth of physiological information about this plant. A better understanding of coyote brush physiology could prove useful in understanding why it is such a successful invader of coastal grasslands.

Seasonal fog deposition during rainless summer months provides a significant and often crucial moisture subsidy for plants along California's coastal fog belt (Jacobs et al. 1985, Dawson 1998, Limm et al. 2009, Corbin et al. 2005, Fischer et al. 2009, Carbone et al. 2013). Further, advective coastal fog is a major driver of ecosystem function in these coastal environments, helping to regulate fire regimes, species composition, hydrological mechanisms, and biogeochemical cycles (Azevedo and Morgan 1974, Jacobs et al. 1985, Weathers et al. 1995, Ingraham and Matthews 1995, Dawson 1998, Weathers 1999, Corbin et al. 2005, Kennedy and Sousa 2006, Fischer and Still 2007, Ewing et al. 2009, Limm et al. 2009, Carbone et al. 2013, Templer et al. 2015). Fog ameliorates plant water stress during the rainless summer months by reducing temperatures, evaporation, and the vapor pressure deficit between leaves and the surrounding air, thereby easing transpiration during carbon fixation (Burgess and Dawson 2004, Limm et al. 2009, Carbone et al. 2013). Distribution of fog across the landscape is spatially heterogeneous, particularly in areas with variable topography and abrupt edges in vegetation structure (Oberlander 1956, Weathers et al. 1995, Ewing et al. 2009). Further, coastward topographic ridges receive more fog than inland ridges (Jacobs et al. 1985, Corbin et al. 2005). In coastal grasslands, the use of fog water during the summer drought by perennial grasses is positively correlated with distance to the coast and fog water inputs, directly influencing grassland community composition (Corbin et al. 2005).

Changing precipitation and cloud and fog patterns that have been linked to global warming are being documented in biomes around the world (Pounds et al. 1999, Still et al. 1999). On a more local scale, climate models project changes in California's upwelling regime (Snyder et al. 2003) which, when combined with projected temperature increases (Hayhoe et al. 2004, Cayan et al. 2008), may produce shifts in the spring/summer fog regime (Diffenbaugh et al. 2004). Indeed, fog frequency has declined 33 percent since the early 20th century (Johnstone and Dawson 2010), increasing evaporative demand on California's coastal plant communities.

Given the aforementioned projections of changing water availability in California's coastal environments, we investigated *B. pilularis* water relations physiology, growth, and water use in two contrasting coastal grassland habitats. Our overarching research question for this study is does the presence of neighboring grassland plants affect the water relations physiology, growth, and water use of *B. pilularis* seedlings as they become established in annual- and perennial-dominated coastal grasslands? We hypothesized that 1) in both grassland types, *B. pilularis* seedlings would have higher levels of all growth-related parameters in areas without neighbors than with neighbors, 2) seedlings would have higher rates of stomatal conductance (higher water use) and less negative water potentials (better water status) in areas without

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neighbors than with neighbors in both types of grasslands due to less competition for water, and lower rates of stomatal conductance and lower water potentials in warmer, drier annual-dominated grassland that does not have the fog subsidy that occurs in the perennial-dominated grassland, with largest differences occurring during the dry season, 3) in areas of lower water availability *B. pilularis* seedlings would have higher (less negative) $\delta^{13}C$ values that reflect an overall down-regulation of physiology than seedlings with more water available, and 4) during the summer dry season seedlings in the more fog-prone perennial-dominated grassland would use more fog water than seedlings warmer annual-dominated grassland with less fog, and that seedlings with neighbors present would use deeper water sources than plants without neighbors.

Methods

Study site

Experimental work was conducted within Mount Tamalpais State Park, Marin County, California, USA at two grassland sites on the southwestern slopes of Mt. Tamalpais. One site was a low elevation site at 500 feet (152 m) elevation (37° 52' 25.87" N, 122° 35' 29.58" W) and one was a high elevation site at 1500 feet (457 m) elevation (37° 53' 50.22" N, 122° 36' 14.21" W); both sites had similar west-southwest aspects. We took advantage of a prominent elevational fog gradient. Along the gradient advective coastal fog clears at our high elevation site first but persists later into the day at low elevation (McBride and Barnhart 2002). Grassland plant assemblages at each site differed, with the low elevation site being a coastal prairie type of grassland that supports significantly more native and perennial grasses and forbs than the high elevation site, which is a typical California annual grassland (t-tests, t = 2.10, *p* = <0.0001 and t = 49.75, *p* <0.0001, respectively; Bartolome et al. 2007). Dominant plants at the low elevation site included the native perennial bunchgrasses *Festuca rubra* and *Stipa pulchra* and the non-native perennial forb *Plantago lanceolata*, and at the high elevation site non-native annual grasses such as *Avena barbata*, *Brachypodium distachyon*, and *Briza maxima* dominated. Grasslands in Mt. Tamalpais State Park were grazed by cattle from the mid-1800s to mid-1900s, during which time exotic annual grasses displaced many perennial grasses and forbs (Laćan et al. 2010). A comparison of aerial photographs of the south slopes of Mt. Tamalpais from 1954 and 2000 show succession of both types of grasslands to shrublands dominated by *B. pilularis* (McBride and Barnhart 2002).

Due to logistical constraints and the high cost of sample analyses, it was not feasible to replicate this study at multiple sites of each grassland type. While this limits our ability to make broad inferences from our findings (Hurlbert 1984), these two grassland types are common along the northern coast of California (Keeler-Wolf et al. 2007), so it is reasonable to expect that our results have relevance beyond our immediate sites.

The climate of the study site is Mediterranean with cool, wet winters and mild springs (approximately October through May) and warm, rainless summers (approximately June through September). Coastal fogs occur during the summer when moist ocean air is driven by prevailing westerly winds over cool, upwelled ocean water offshore, causing atmospheric moisture to condense into fog as it moves onshore. At the study site these summertime fogs range in ceiling height depending on large-scale pressure systems, resulting in coastal stratus with its higher ceiling to lower, ground-level wind-driven fogs. The soils at both elevations are Mollisols, the soil order typical of grassland environments characterized by a surface horizon derived from accumulated organic matter, have a pH close to neutral, and high levels of bioavailable cations

(Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture 2010). Soil at the high elevation site consists of the Cronkhite-Barnabe Complex, a moderately well-drained, heavy gravelly clay-loam formed from weathered sandstone and shale that is typical under grassland-coastal scrub mosaics (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture 2010). The soil at the low elevation site is a Tamalpais-Barnabe Variant, a well-drained gravelly clay-loam formed from weathered radiolarian chert and sandstone that is typical of cool foggy summers.

Study species

  Baccharis pilularis DC. is a dioecious, perennial shrub native to the California Floristic Province. *B. pilularis* grows in two morphotypes that are thought to be genetically distinct and are classified as subspecies, the prostrate *B. pilularis* ssp. *pilularis* DC. (10-15cm tall, 1-4m across) found along the coast, and the more widespread *B. pilularis* ssp. *consanguinea* (DC.) C.B. Wolf (1-4m tall) that is found along the coast as well as inland areas that receive a weak maritime influence (Munz and Keck 1973, Baldwin et al. 2012). This study focuses on the erect morphotype, herein termed *B. pilularis*, which grows at both high and low elevation sites.

  Seeds were collected from mature *B. pilularis* shrubs at both high and low elevations within Mt. Tamalpais State Park during October and November 2009, mixed together, sown July 11, 2010, and raised in a glasshouse on the University of California Berkeley campus. Seedlings were planted at the low and high elevation grassland sites in randomly selected locations during late February and early March 2011 when the seedlings were seven months old. All plants were of similar size when planted. *B. pilularis* seedlings acclimated in place at both elevations from February 2011 to February 2012 to simulate typical seasonal establishment of *B. pilularis* seedlings into coastal grasslands.

Environmental variables

  Fog water was measured from June 24 to August 19, 2012 by way of a multidirectional fog collector (after Fischer and Still 2007). Before placement in the field, fog collectors were calibrated in the same location to ensure they all measured consistently while taking into consideration the natural variability of fog water deposition. Each fog collector was rigged with a system of troughs that drain into a funnel attached to UV-resistant Tygon tubing that led to 64oz (1.9 L) UV-resistant polyethylene amber bottles with scintillated caps that stored collected fog water. Stoppers and air tubes were placed in the cap to allow air to escape while trapping evaporated vapor within the bottle between collection dates. Fog water was measured regularly in the field with graduated cylinders. Rainfall data was obtained by nearby weather station in Kentfield, California (37° 57' N, -122° 32' W; Kentfield, California Period of Record Monthly Climate Summary, Western Regional Climate Center, 2015). At each elevation, soil moisture was measured as volumetric water content (VWC), or the volume of liquid water in a certain volume of soil, by a dielectric permittivity probe sensor (HydroSense Soil Water Measurement System, CD620 and CS620, Campbell Scientific, Inc., Logan, Utah) on February 25, May 19, and August 2, 2012. Temperature and relative humidity were measured at each elevation using an HMP45C Temperature and Relative Humidity Probe (Campbell Scientific, Inc., Logan, Utah) that contains a platinum resistance temperature detector and a Vaisala HUMICAP® 180 capacitive relative humidity sensor (Vaisala, Inc., Boulder, Colorado) wired to a CR10X datalogger and AM16-32a Relay Multiplexer (both Campbell Scientific, Inc., Logan, Utah).

Using these temperature and relative humidity values, we calculated vapor pressure deficit as the difference between saturation vapor pressure and the water vapor pressure of the air as follows

$$VPD = e_{sat} - e_a$$

where

$$e_{sat} = (a)(e^{(b*T_{air})/(T_{air}+z)})$$

where a is the coefficient 0.61121 (kPa), b is the coefficient 17.502, T_{air} is the air temperature (°C), and z is the coefficient 240.97 (°C), and

$$e_a = e_{sat}(\frac{RH}{100})$$

where RH is the measured relative humidity (Jones 1992).

Experimental neighbor removal treatment

        n order to evaluate water relations characteristics of *B. pilularis* seedlings with and without grassland neighbors, we randomly assigned forty planted *B. pilularis* seedlings to a neighbor removal experiment. During February 2012, at each elevation, ten plants were assigned to control plots and ten plants were assigned to treatment plots where above-ground biomass of neighboring plants was clipped as close to the ground as possible in a half meter radius from the *B. pilularis* seedling. Clipped vegetation was returned to the plot and scattered in an even layer over the plot. Grasses and forbs were repeatedly clipped to ground level throughout the growing season until the end of the experiment on August 22, 2012, with clipping frequency ranging from once weekly to once monthly depending on how much the plants grew after the clipping treatment. At each clipping treatment, residual vegetation was removed, vegetation clipped, and clipped vegetation returned to the plot in an even layer. Perennial grasses at low elevation grew back readily and required the most treatments whereas the plots with annual grasses often only required one clipping treatment during the entire study. Repeated clipping of above-ground biomass of grasses and forbs removes photosynthesizing tillers, leaves, and stems, thereby reducing below-ground root growth and resource use (Crider 1955, Carman and Briske 1982, Caldwell et al. 1981, Caldwell et al. 1987).

Plant growth

        Growth parameters measured on the forty experimental seedlings at both elevations included the relative increase in plant height, the relative increase of leaf number, and relative increase in stem length of marked stems from February to August 2012. We found that plant height was significantly different between low elevation (mean = 41.1 cm, SE = 2.5) and high elevation (mean = 56.2 cm, SE = 2.4) at the beginning of the neighbor removal treatment in February 2012 due to the long acclimation period of the plants that began when they were planted in February and March 2011 (T-test, t = 4.36, p = <0.0001). In order to account for this initial size difference in comparing the growth of *B. pilularis* seedlings at both elevations in treatment and control plots, we calculated relative increase in plant height as

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$$Relative\ Height\ Increase = \frac{height_{final} - height_{initial}}{height_{initial}}$$

where the initial plant height was measured in February 2012 and the final plant height was measured in August 2012. We used a similar formula to calculate the relative increases in leaf number and stem length. In order to track the increase in leaf number and stem length, we marked three terminal stems on south, west, and east sides of each plant by lightly tying twine around the stem and counting the number of leaves and measuring stem length in February and again in August (30 marked stems in each elevation and for each treatment and control plants, for a total of 120 marked stems). For each plant, we calculated the mean leaf number and mean stem length of all living marked stems and used these values to calculate total means for each group (i.e., low elevation treatment, low elevation control, etc.). Plant sex was not tracked for this study.

At the end of the experiment in August 2012, we also measured basal stem diameter and above-ground biomass. Basal stem diameter was measured at ground level using calipers. To measure above-ground biomass we collected all experimental plant biomass at ground level and weighed plant material in grams after it was dried in a 70° C oven for between two and three weeks.

Physiology

To assess plant water use, midday stomatal conductance (g_s) was measured at the beginning of the experiment during the wet season (February 28, 2012) and peak dry season (August 17, 2012) using a steady-state porometer (LI-1600C, LI-COR, Inc., Lincoln, Nebraska, USA). The porometer was calibrated before the experiment began and desiccant was changed before each use. Due to the small size of *B. pilularis* leaves, we used a 1600-02 Square Chamber sensor head so that measurements included terminal, current-growth stem and leaves. At both elevations between 1130 and 1400 hours measurements were made on the south side of five randomly selected seedlings in each treatment group, for a total of twenty measurements per date. If steady state conditions were not achieved within thirty seconds, measurements were stopped and made on another set of leaves. All leaves measured inside the porometer's square chamber were collected and scanned on a flatbed scanner within 12 hours to compute fresh leaf surface area using ImageJ software (Rasband 2014). Stomatal conductance measurements were corrected by leaf surface area and converted from velocity units ($cm^2\ s^{-1}$) to molar units ($mmol\ m^2\ s^{-1}$) using a conversion factor based on measured leaf temperature (Hall et al. 1976, Cowan and Farquhar 1977, Körner et al. 1979, McDermitt 1990). Values are reported on a two-sided leaf area basis as *B. pilularis* leaves are amphistomatous (A. G. Kidder, personal observation). Measurements were made on stems without leaf or stem galls.

To determine responses of young *B. pilularis* plants to seasonal water availability, predawn and midday water potentials (Ψ) were measured on five randomly selected *B. pilularis* plants in both treatment and control groups at both elevations during the wet season (March 10, 2012) and peak dry season (August 21, 2012), for a total of twenty measurements each date. Ψ measurements were made using a pressure chamber (PMS Instrument Company, Albany, Oregon, USA) within 1 minute or less of collection.

Stable isotopes

Leaf Carbon Isotope Composition

        Several similarly aged, mature leaves were collected from the south side of randomly selected experimental *B. pilularis* seedlings at both elevations on August 7, 2012 to assess effects of the neighbor removal treatment and high- and low-fog conditions on seedling carbon isotope discrimination. Leaf samples for each plant were dried in a 70° C oven for more than three days and ground in a grinding mill (Mini-Beadbeater-8TM, Biospec Products, Bartlesville, OK, USA). A 4-μg aliquot of ground leaf material from each plant was enclosed in a tin capsule (Costech Analytical Technologies, Inc., Valencia, CA, USA) for bulk $\delta^{13}C$ analysis. Analyses were performed on a continuous flow isotope ratio mass spectrometer with a long-term precision of ±0.10‰ and an elemental analyzer with long term precision of ±0.15‰ (Elementar vario ISOTOPEcube interfaced with an IsoPrime100, Elementar Analysis, Mt. Laurel, NJ, USA) using Vienna-Pee Dee Belamnite (V-PDB) as the standard. Carbon isotope ratios for bulk leaf material were calculated as:

$$\delta^{13}C \ = \left(\frac{R_{sample}}{R_{standard}}\right) - 1$$

where R is the molar abundance ratio of heavy (^{13}C) to light (^{12}C) stable isotopes of carbon for the plant sample (R_{sample}) and the V-PDB standard ($R_{standard}$), respectively. Results are reported in per mil (‰). $\delta^{13}C$ values incorporate both the isotopic composition of the source air CO_2 as well as differential diffusion of $^{13}CO_2$ and $^{12}CO_2$ into the leaf and discrimination by the carboxylating enzymes within the leaf (Ehleringer 1993).

Hydrogen and Oxygen Isotopes of Water

        In order to evaluate the effect of neighbors on the kinds of water used by *B. pilularis* seedlings during the dry season, we compared pools of water sources (fog, ground, rain, and soil) to plant xylem water in stem samples of experimental *B. pilularis* seedlings. By associating the stable isotopes of hydrogen and oxygen composition of the water sources to that in the plant stems, we were able to obtain a "fingerprint" of which water sources the plants are using at the time of stem collection as well as gain insights into how deep the plants' roots are (Dawson 1993). We can do this because there is little to no fractionation in water stable isotopes as water during uptake by roots of plants and transported through suberized, woody stem tissue (see review by Dawson & Ehleringer 1998).

        For this study we collected rain, ground, and fog source waters and soil samples at multiple depths on several dates between May 2010 and August 2012 to compare to water in *B. pilularis* stem tissue collected on August 21, 2012. Rain was collected aluminum rain collection canisters by deploying three canisters at each elevation. In each canister we established a film of mineral oil to prevent evaporation until we collected rain samples within 24-48 hours of a rain event. Rain water samples were acquired using a large syringe to siphon the water beneath the layer of mineral oil and immediately deposit it into 4 oz. (118 mL) polypropylene scintillation vials with plug-style stoppers (VWR International, Radnor, Pennsylvania, USA), sealed with self-sealing plastic film (Parafilm, Bemis Company, Inc., Oshkosh, Wisconsin, USA), and placed in a subzero freezer in University of California Berkeley's Center for Stable Isotope Biogeochemistry at day's end until analysis. We calculated deuterium excess (*d*-excess) for rain

samples as $d = \delta^2H - 8*\delta^{18}O$ (Dansgaard 1964) in order to estimate the amount of intermixing and recirculation of meteoric water sources within clouds and the atmosphere at the two elevations. Ground water samples were obtained from untreated water sources at low and high elevation in a process similar to rain water samples. Low elevation ground water samples were obtained from a naturally occurring spring at approximately 270 feet altitude near the study site and high elevation ground water samples were obtained from a well in Mt. Tamalpais State Park at approximately 2000 feet altitude. During the summer fog seasons of 2011 and 2012 we obtained fog water samples from the collection bottles attached to each fog collector. There were a few collection dates when one or more collectors at high elevation had not collected any fog water due to lack of fog between measuring dates. Fog water samples were collected in scintillation vials in the same manner as the rain and ground water samples. The rainy season had ended by the time we began measuring and collecting fog deposition.

Plant stem tissue samples were collected August 21, 2012 from five randomly selected treatment and control *B. pilularis* plants at both elevations (twenty plants total) and prepared the same way as the rain, ground, and soil samples. In order to obtain samples of maximally woody and suberized stem tissue, some seedling samples were taken at or near ground level, in some cases killing the plant.

In order to estimate where in the soil profile the roots of *B. pilularis* seedlings were obtaining water, we collected soil samples to establish a soil water hydrogen isotope profile. High elevation soil profile samples were collected on May 14, 2011 (wet season) and August 16, 2012 (dry season) and low elevation samples were collected May 13, 2011 and March 15, 2012 (wet season) and August 27 and September 23, 2011 (dry season) from three holes centrally located to the *B. pilularis* seedlings randomly selected for plant xylem water sampling. Using a 3-inch (7.6 cm) diameter steel soil auger, samples were taken from depths of 10cm, 20cm, 40cm, 60cm, 80cm, and 100cm. At each depth we collected three vials of soil immediately upon bringing soil to a tarp, resulting in a total of 18 vials of soil per hole and a total of 54 vials of soil at each elevation.

In addition to soil profiles we collected soil samples to create soil evaporation lines following the methods in Corbin et al. (2005) in order to capture the stable isotope composition of shallow soil waters as they evaporate throughout the day and relate this to plant xylem water. Soil evaporation line samples were collected on April 9, 2012 at the low elevation site and May 19, 2012 at the high elevation site at randomly selected locations within each meadow at 0 cm to 2 cm and 3 cm to 5 cm depths during morning, midday, and late afternoon. All soil vials were likewise sealed and frozen at day's end.

We extracted water from plant and soil samples by using cryogenic vacuum distillation in University of California Berkeley's Center for Stable Isotope Biogeochemistry following the methods described in West et al. (2006). Extracted waters were then analyzed for hydrogen (δ^2H, or deuterium) and oxygen ($\delta^{18}O$) stable isotopes using isotope ratio mass spectrometry. Deuterium was analyzed using a dual inlet Thermo Delta Plus XL mass spectrometer interfaced with a hot chromium reactor unit (H/Device™, Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) that reduced the injected water sample to H_2 gas for isotopic analysis. Long-term external precision for δ^2H analyses is ±0.80‰. In order to analyze waters for $\delta^{18}O$, samples were pipetted into septum-capped 10mL glass vials (Exetainer®, Labco Ltd., Ceredigion, UK), purged with 0.2% CO_2, and equilibrated at room temperature for at least 48 hours. The $\delta^{18}O$ in the CO_2 gas in the glass vial was subsequently analyzed using continuous flow with the Thermo Delta Plus XL interfaced with a Thermo Gas Bench II (Thermo Fisher

Scientific, Inc., Waltham, Massachusetts, USA). Long-term external precision for the $\delta^{18}O$ analyses is ±0.12‰. δ^2H and $\delta^{18}O$ values are expressed in a similar stable isotope ratio notation as that described for $\delta^{13}C$ in bulk leaf tissue, where R is the molar abundance ratio of heavy to light stable isotopes of hydrogen ($^2H/H$) and oxygen ($^{18}O/^{16}O$), R_{sample} is the ratio for the plant xylem, soil, ground, rain, or fog water, and $R_{standard}$ is the ratio for the industry standard for waters, Vienna-Standard Mean Ocean Water (V-SMOW).

Statistical analyses

All data were tested for equal variances using Levene's Test and normality using Shapiro-Wilks W Test and transformed if needed. Tukey's Honestly Significant Difference orthogonal contrast post hoc test was performed for posteriori comparison of means. Figures depict untransformed data for clarity and significance was determined at the 0.05 level. Soil VWC data were log-transformed to meet the assumptions of ANOVA performed for February and August data. Stomatal conductance data met the assumptions of ANOVA and the model included the fixed factors of time of year (February and August), elevation (low and high), treatment (control and neighbor removal treatments), and all interaction terms. The absolute values of Ψ data were log transformed to meet the assumption of equal variances and analyzed using an ANOVA model that included time of year (March and August), time of day (predawn and midday), elevation (high and low), and treatment (control and neighbors removed) and all associated interaction terms. Stable carbon isotope values met ANOVA assumptions and the model consisted of elevation, treatment, and the elevation X treatment interaction term.

Plant growth variables included relative increase in plant height, relative increase in leaf number, and relative increase in stem length over the course of the experiment (February through August 2012). Relative height increase and relative increase in stem length data met assumptions of ANOVA and were analyzed using elevation and treatment as the main effects and elevation X treatment interaction term. Relative increase in leaf number values were log transformed due to unequal variances and analyzed using the same ANOVA model.

We tested for differences in final above-ground biomass between treatment and control plots by performing a separate ANCOVA for each elevation, using initial plant height as the covariate to adjust for the initial differences in plant height resulting from growing *in situ* for approximately one year before the neighbor removal treatment began. We elected to forgo performing the combined two-factor ANCOVA with elevation and treatment as main effects, initial plant height as the covariate, and biomass as the dependent variable because the significant difference in initial plant height between elevations resulted in heterogeneous slopes between initial plant height and biomass between the two elevations, violating the ANCOVA assumption of homogeneous slopes. Further, the Wilcox adjustment to the Johnson-Neyman post-hoc procedure that can be used to adjust for heterogeneous slopes in ANCOVA is designed for one-factor ANCOVA, not two-factor ANCOVA like our model (Quinn & Keough 2002, Engqvist 2005). We were not able to calculate relative increase in above-ground biomass because we did not have initial biomass measurements and the model developed by Russell and Tompkins (2005) was suitable for estimating biomass for adult *B. pilularis* plants, not seedlings. Final above-ground biomass data were log-transformed to obtain equal variances between treatment groups for the ANCOVA for each elevation. To analyze final basal stem diameter measured in August 2012, we applied a similar approach of separate ANCOVAs for each elevation due to heterogeneous slopes and because we did not measure initial basal stem diameter. Basal stem diameter data were log-transformed to meet the ANCOVA assumption of

equal variances. In order to statistically compare final above-ground biomass and basal stem diameter between elevations, we performed t-tests on the log-transformed data using only data from control plants at each elevation from plants planted in February 2011.

Using a dual-isotope approach, we constructed a Local Meteoric Water Line (LMWL) for Mount Tamalpais State Park using hydrogen (δ^2H) and oxygen (δ^{18}O) stable isotope values of rain and fog waters collected from 2010-2012. This LMWL was compared to the Global Meteoric Water Line (GMWL) and used to qualitatively compare the δ^2H and δ^{18}O composition of plant xylem water to that of all water sources (i.e., fog, rain, ground, soil waters). To see if δ^2H values of non-soil water sources (fog, rain, and ground waters) differed topographically we performed a two-way ANOVA with elevation and treatment as main effects as well as an elevation X treatment interaction term. The stable isotope values of hydrogen (δ^2H) of the water sources met assumptions of normality and equal variances. Soil evaporation lines were constructed using least squares regression between δ^2H and δ^{18}O. In order to test for differences between the slopes of the soil evaporation lines for each elevation, we performed an ANCOVA using log-transformed δ^2H and δ^{18}O values and with log(δ^2H) as the response variable and elevation and log(δ^{18}O) as the explanatory variables. Stem xylem deuterium values met assumptions for ANOVA and were analyzed with elevation and treatment as main effects and the elevation X treatment interaction term. δ^{18}O values of *B. pilularis* plant stems were log-transformed to meet the assumptions of normality and equal variances and those values were analyzed with an ANOVA with elevation and treatment as main effects and the elevation X treatment interaction term. Deuterium excess had equal variances but was log-transformed due to non-normal distributions. We analyzed *d*-excess using Wilcoxon Rank Sum Chi-Square Approximation because the log-transformed data did not conform to normal distributions. Stable isotopes for soil water were evaluated qualitatively and related to stable isotope values of plant xylem water.

Results

Environmental Conditions

As expected, the low elevation site received more advective fog than the high elevation site, with the fog "burning off" at high elevation before low elevation. By the end of the experiment the low elevation site had received just shy of 400% more fog water than the high elevation site during June, July and August 2012 (Fig. 1). The mean fog water input at the low elevation site was 8973 mL and at high elevation the mean fog input was 2253 mL. Both elevations increased in relatively equal amounts July 16-17, 2012 due to both a strong coastal drizzle event as well as light rain from remnants of tropical storm Fabio in the eastern Pacific Ocean. At both sites, our fog collectors were larger and taller than *B. pilularis* seedlings neighboring plant species so we did not use collected amounts to interpret throughfall of seedlings or grasses at either site. Daily rainfall for 2012 reflected typical Mediterranean climate weather patterns of rainfall from late fall through winter and into spring while little to no rain fell during summer into early fall (see Fig. 3).

Differences in VPD between low and high elevations were most pronounced during late summer in July through September (Fig. 2). The high elevation annual grassland site attained remarkably high VPDs while the presence of coastal fog considerably moderated the maximum temperatures and relative humidity levels at low elevation. In order to find out if the high VPDs observed at the high elevation site were an artifact or malfunction of the datalogger, we

compared the findings to a second temperature and relative humidity sensor deployed at each site and this device recorded similar VPDs for both sites (data not shown; EL-USB-2, Lascar Electronics, Inc., Erie, Pennsylvania, USA).

Soil VWC declined as anticipated from the wet season into the dry season, with the lowest soil moisture values occurring during August at the end of California's rainless summer (Fig. 4). During the wet season in February, soil VWC at low elevation was significantly higher than at high elevation but not different between the treatment and control plots ($F_{1,37} = 306.3$, $p < 0.0001$). In contrast, during the dry season in August, there was a significant interaction effect between elevation and treatment for soil VWC, with significantly lower soil VWC in high elevation control plots while soil VWC in high elevation treatment plots were similar to both treatment and control plots at low elevation ($F_{1,37} = 8.98$, $p = 0.0049$).
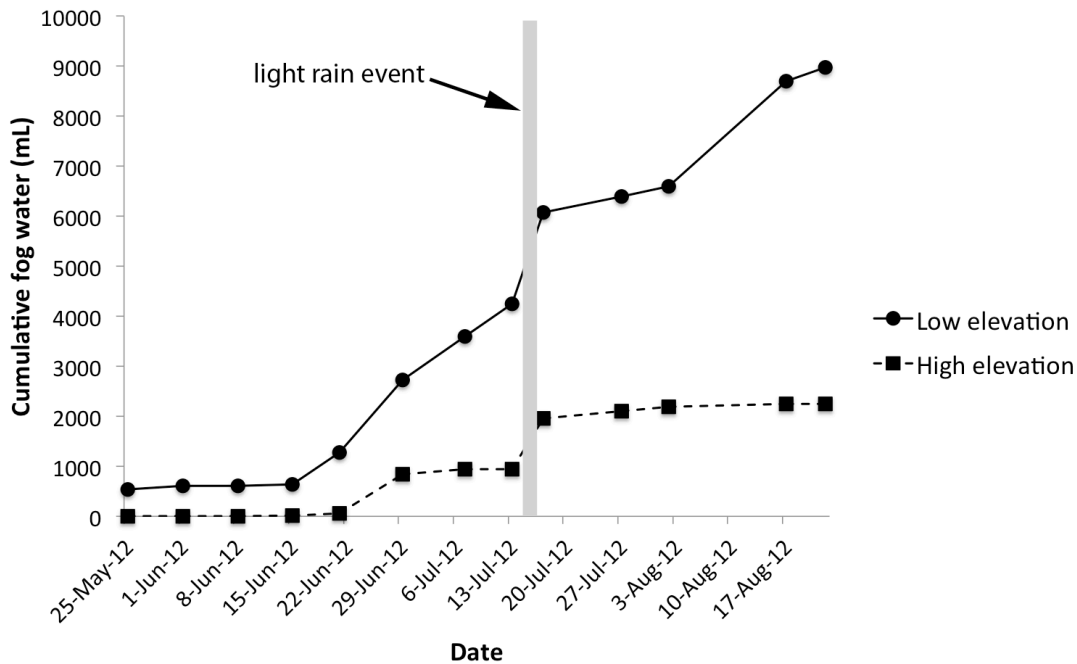
Figure 1. Cumulative fog water at low and high elevation study sites in Mt. Tamalpais State Park, Marin County, California, USA during summer 2012. Gray bar reflects light rain event. (±SD)
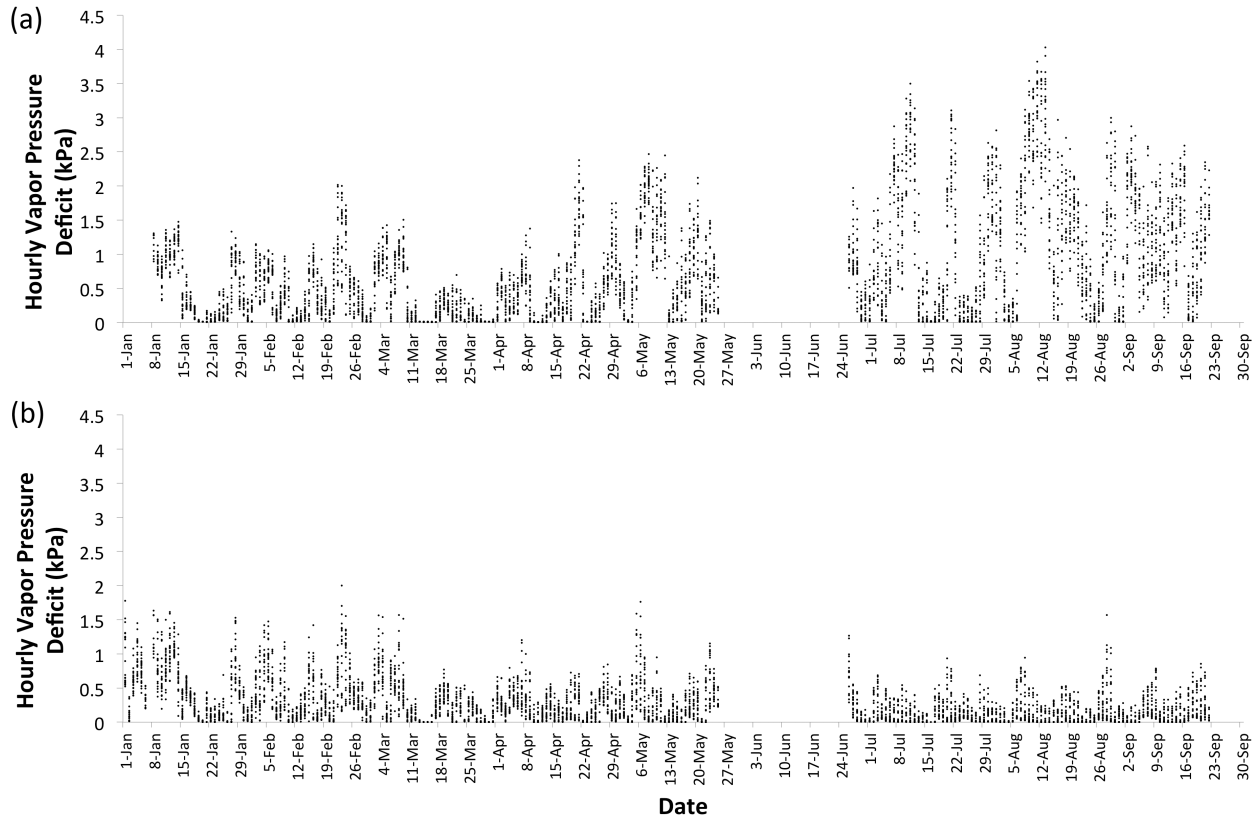
Figure 2. Hourly vapor pressure deficit (VPD) for high (a) and low (b) elevation sites at Mt. Tamalpais State Park, Marin County, California, USA from January 1, 2012 through September 22, 2012. Blank areas in graphs are due to missing data.
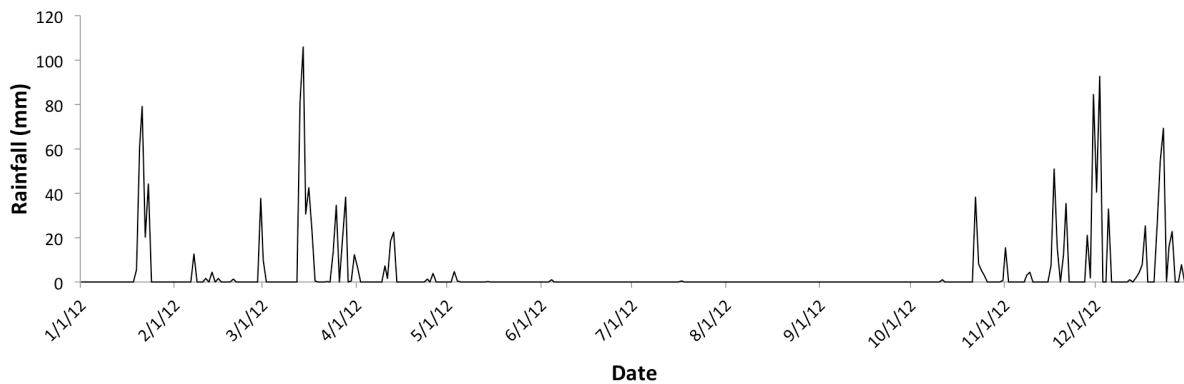
Figure 3. Daily rainfall during 2012 in Kentfield, California (37° 57' N, -122° 32' W) (Kentfield, California (044500) Period of Record Monthly Climate Summary, Western Regional Climate Center).

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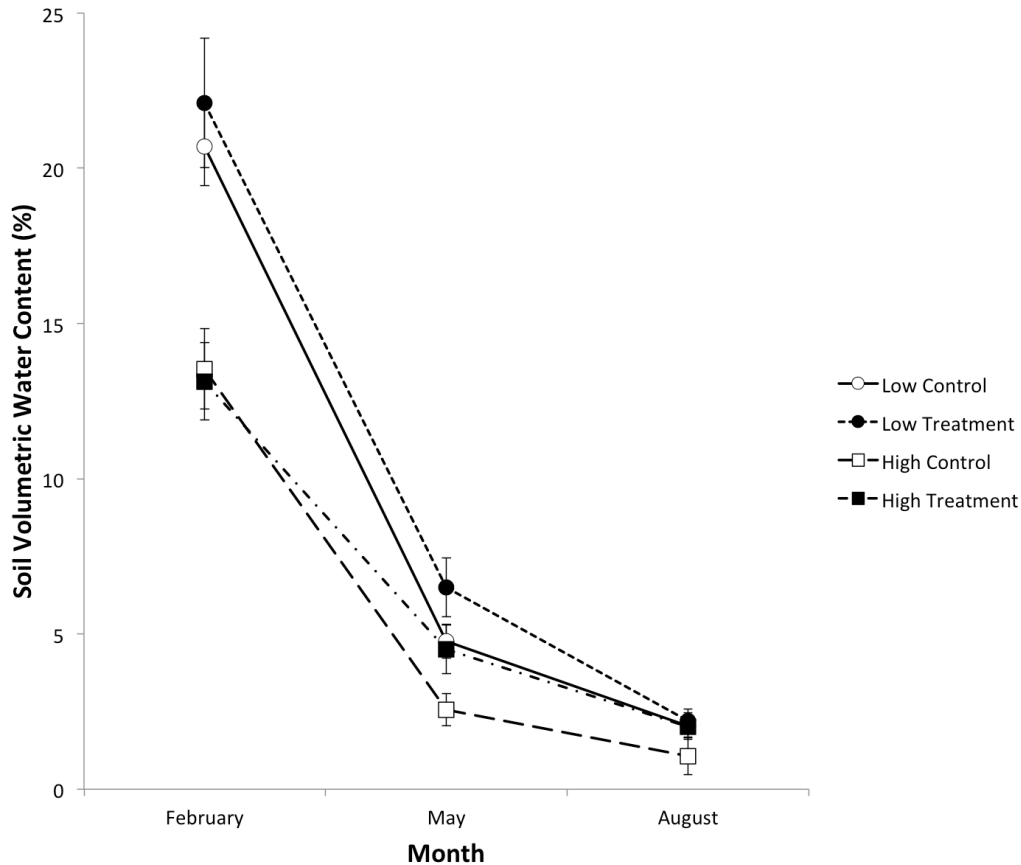
Figure 4. Growing season volumetric soil water content in low (circles) and high (squares) elevations in experimental treatment (solid) and control (open) plots, Mt. Tamalpais State Park, Marin County, California, USA. (±SD)

Growth

Experimental *B. pilularis* seedlings had significantly greater relative increase in height, leaf number, and stem length in treatment plots where neighbors were removed at both elevations (Figs. 5, 6, and 7, respectively; Table 1). The treatment effect on relative increase in height of *B. pilularis* seedlings was stronger at low elevation (0.15 cm difference between mean relative height increase in treatment and control plots) than at high elevation (0.08 cm difference between mean relative height increase in treatment and control plots). In contrast, the treatment effect was stronger at high elevation for relative increase in leaf number, with the differences between the mean relative increase in leaf number for treatment and control plots at high elevation with 11.24 leaves and at low elevation with 5.33 leaves. The treatment effect on relative increase in stem length was also stronger at high elevation, with difference in means between treatment and control plots of a 0.85 cm increase at high elevation and a 0.57 cm increase at low elevation. During the course of the study several of the 120 total monitored stems partially or completely died and were excluded from analyses. At high elevation, 63% of stems on control plants and 27% of stems on treatment plants were excluded while at low elevation, 73% of marked stems on control plants and 37% of stems on treatment plants were excluded. The fate of these stems could have resulted from marking the stems with twine, however we did

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not conduct a study to look at the effects of our experimental methods on stem survival. In their study where they tracked growth of marked *B. pilularis* stems, Hobbs and Mooney (1987) had 50% of seedling stems survive during the year-long study.

End-of-season above-ground biomass was significantly higher in plots without neighbors at both high ($F_{2,17} = 35.8905$, $p < 0.0001$) and low elevation sites ($F_{2,16} = 9.3434$, $p = 0.0075$; Fig. 8). ANCOVA revealed the initial height of experimental *B. pilularis* plants had a significant, positive effect on final above-ground biomass at high elevation ($F_{2,17} = 4.8009$, $p = 0.0427$) and marginally significant, positive effect on above-ground biomass at low elevation ($F_{1,16} = 4.2685$, $p = 0.0554$). The t-test comparing elevational differences in end-of-season above-ground biomass confirmed that *B. pilularis* plants in control plots at our low elevation site were significantly lower than plants at our high elevation site (low elevation mean dry biomass = 8.79g $\pm$4.38g SD; high elevation mean dry biomass = 32.13g $\pm$17.24g SD; t-test on log-transformed data, t = 5.06, $p < 0.0001$).

At high elevation, end-of-season basal stem diameter was significantly larger without neighbors ($F_{2, 17} = 8.9109$, $p = 0.0083$) whereas at low elevation there was no significant difference in end-of-season basal stem diameter between treatment and control plants (Fig. 9). Further, basal stem diameters of the control plants were significantly narrower at low elevation (untransformed mean = 9.04mm $\pm$3.38mm SD) than high elevation (untransformed mean = 22.40mm $\pm$10.71mm SD; t-test on log-transformed data, t = 4.08, $p = 0.0007$).
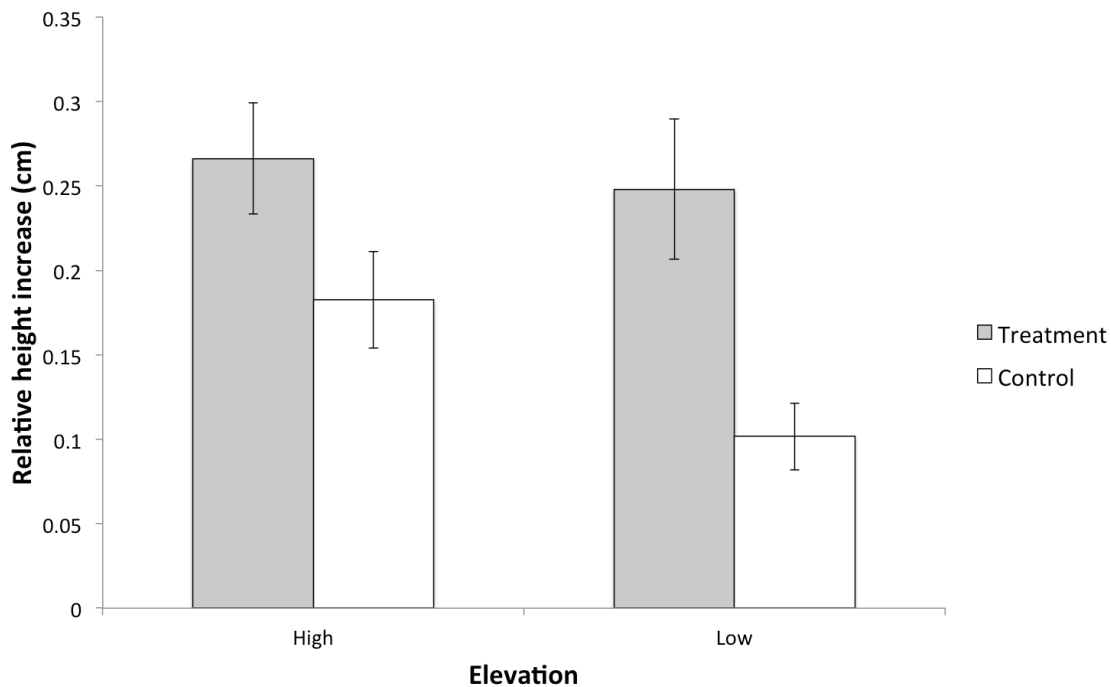
Figure 5. Relative increase in height of plants in treatment and control plots. ($\pm$ SE)
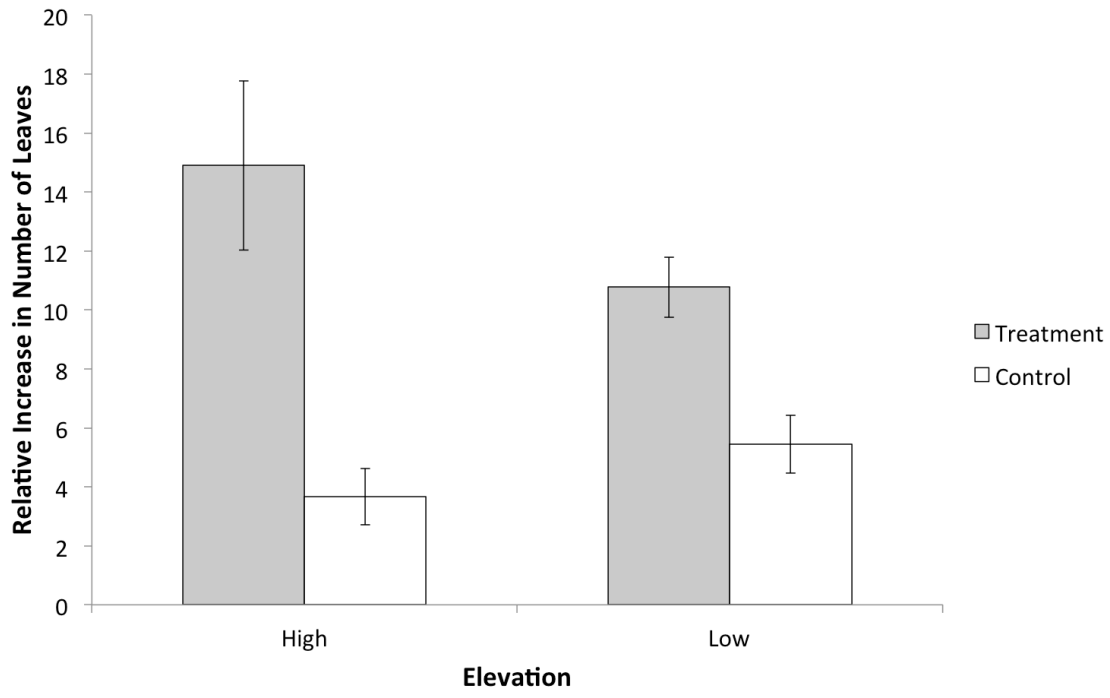
Figure 6. Relative increase in leaf number from February 25, 2012 to July 25, 2012. ($\pm$ SE)
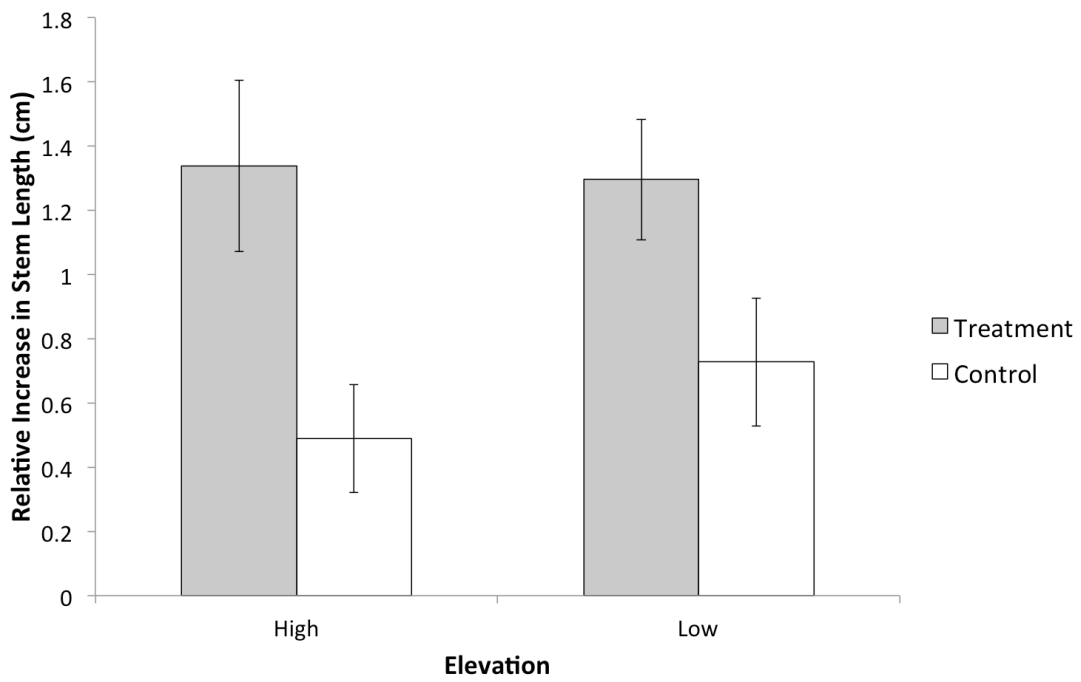
Figure 7. Relative increase in stem length from February 25, 2012 to July 25, 2012. ($\pm$ SE)

Table 1. ANOVA table for the effects of elevation (low or high), treatment (control or neighbor removal treatment), and the interaction of elevation x treatment for measured relative increase in height, relative increase in leaf number, and relative increase in stem length for experimental *B. pilularis* seedlings from February to July 2012.

Effect	Height (cm)				Leaf Number				Stem Length (cm)			
	df	SS	*F*	*P*	df	SS	*F*	*P*	df	SS	*F*	*P*
Elevation (E)	1	0.0232	2.51	0.123	1	0.100	1.05	0.3125	1	0.0734	0.180	0.675
Treatment (T)	1	0.125	13.5	0.0008	1	2.57	27.1	<0.0001	1	3.86	9.43	0.0047
E x T	1	0.00934	1.01	0.323	1	0.338	3.56	0.0680	1	0.151	0.370	0.548
Error	34	0.316			33	3.14			28	11.45		
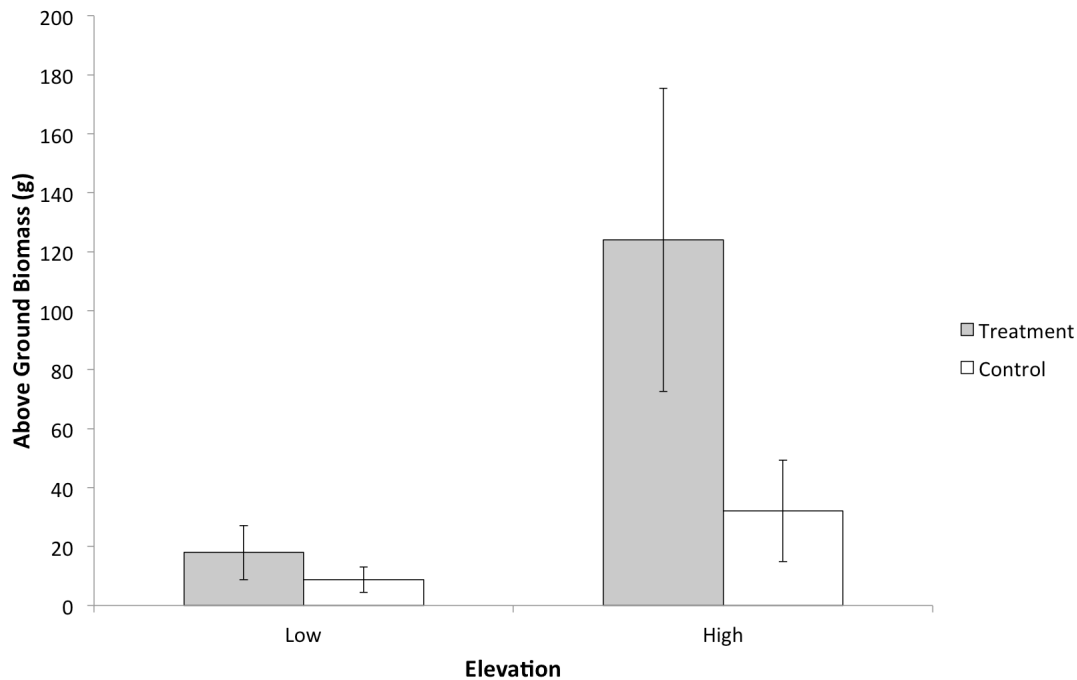
Figure 8. Final above-ground biomass for treatment and control *B. pilularis* plants at low and high elevations in Mt. Tamalpais State Park, Marin County, California, USA. ($\pm$ SE)
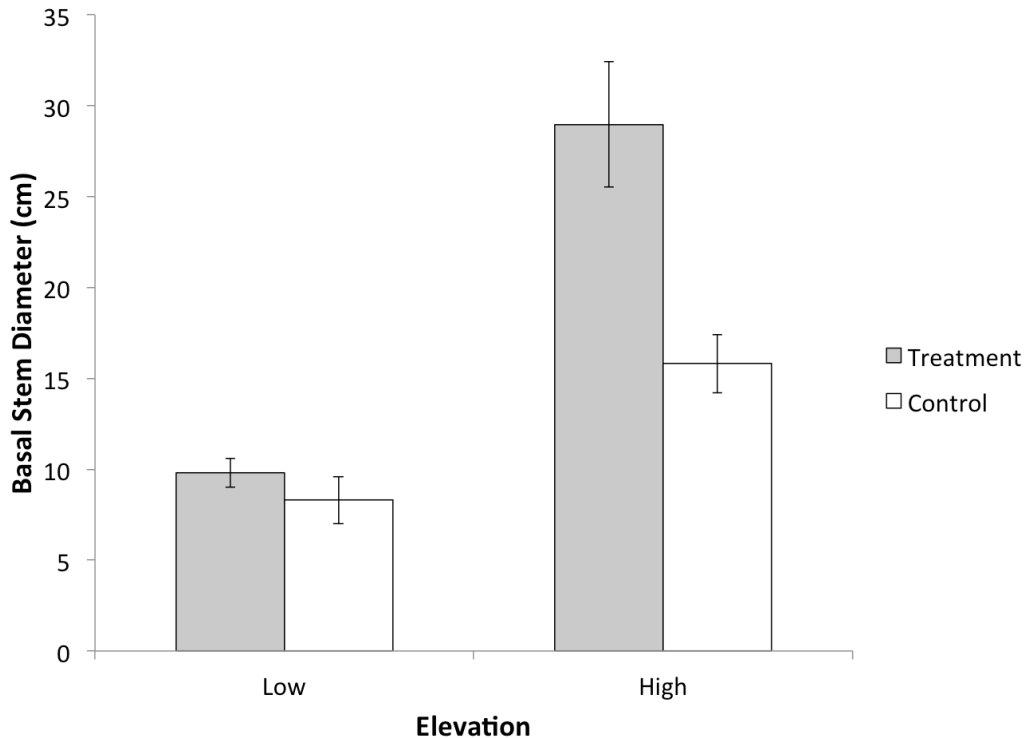
Figure 9. Basal stem diameter for treatment and control *B. pilularis* plants at low and high elevations in Mt. Tamalpais State Park, Marin County, California, USA. ($\pm$ SE)

Physiology

      There were no significant differences in stomatal conductance (g_s) between measurement dates (February and August), treatment and control plants, and low and high elevations. However, it is worth noting the marginally significant interaction term between time of year and neighbor removal treatment ($F_{1,32}$, $p = 0.0632$), indicating that for both elevations there was a trend for *B. pilularis* plants with neighbors (control) to have higher mean gs in February while plants without neighbors (treatment) had higher mean gs in August (Fig. 10).

    B. pilularis water potentials (Ψ) varied depending on clipping treatment, elevation, time of day, and time of year (Fig. 11, Table. 2). First, the significant elevation x treatment interaction (elevation x treatment, $F_{1,64} = 4.3571$, $p = 0.0408$) suggests the neighbor-removal treatment affected *B. pilularis* Ψ differently depending at which elevation the plants were living. At low elevation, *B. pilularis* seedlings in treatment plots without their neighbors experienced less of a water deficit (higher Ψ) than *B. pilularis* seedlings in control plots with their neighbors present. In contrast, at high elevation there was no significant difference in Ψ between treatment and control plots. Second, the significant interaction of elevation and time of day (elevation x time of day, $F_{1,64} = 9.2049$, $p = 0.0035$) is demonstrated by *B. pilularis* Ψ at high elevation having a larger difference between predawn and midday than plants at low elevation. As expected, at high elevation *B. pilularis* Ψ were at their lowest during midday in August (mean = -1.49 $\pm$ 0.34 SD) and highest during predawn hours in early March (mean = -0.55$\pm$ 0.32 SD). Third, the strength of the effect of the neighbor removal treatment on Ψ depended on the time of year (time of year x treatment, $F_{1,64} = 4.1842$, $p = 0.0449$), with seedlings experiencing a significantly stronger treatment effect during March than during August.

Figure 10. *B. pilularis* stomatal conductance (g_s; mmol m^{-2} s^{-1}) by month and neighbor removal treatment at low and high elevations. Values are offset for clarity. ($\pm$ SE)

Figure 11. *B. pilularis* predawn and midday water potentials (Ψ): (a) high elevation in March, (b) high elevation in August, (c) low elevation in March, and (d) low elevation in August. ($\pm$ SE)

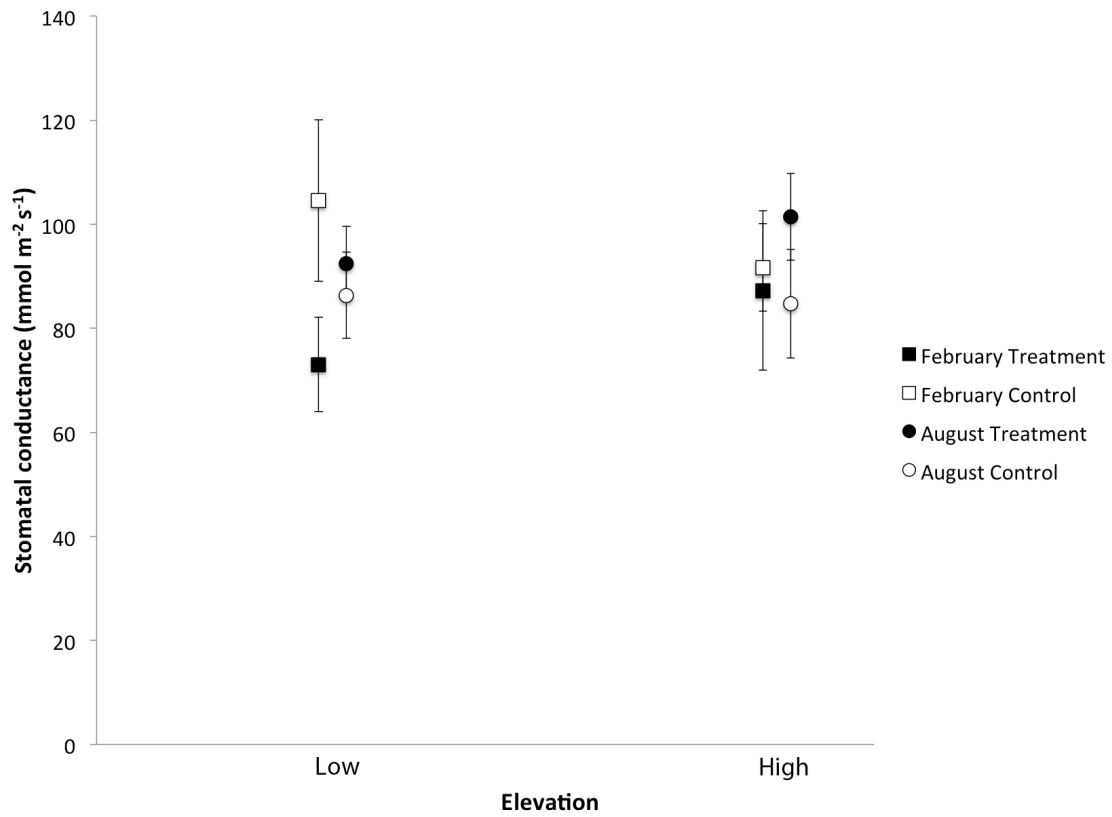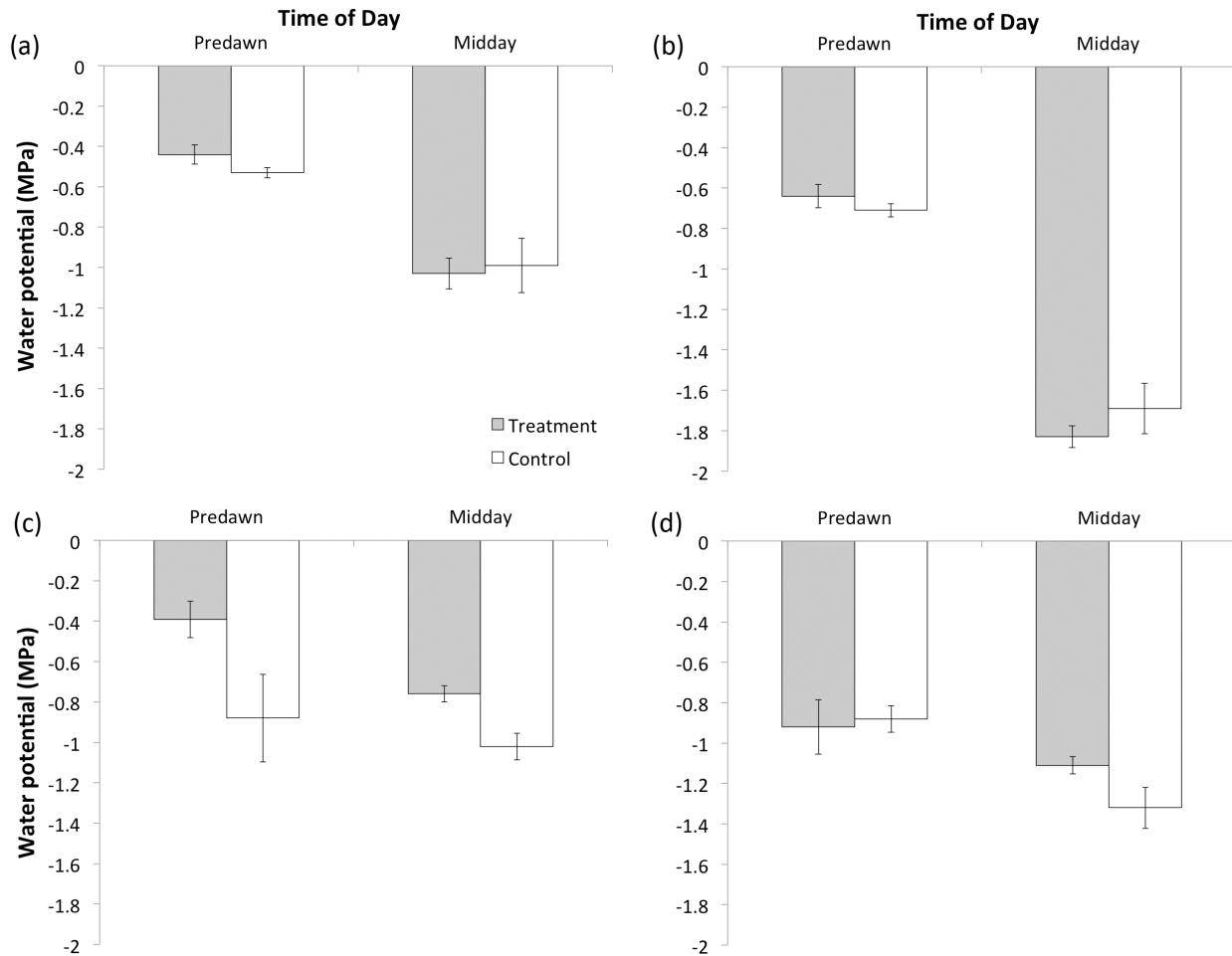Table 2. ANOVA results for *B. pilularis* seedling water potential (Ψ) measurements for the effects of time of year (March 10, 2012 (wet season) or August 21, 2012 (dry season)), time of day (predawn or midday), elevation (low or high), treatment (neighbor removal treatment or neighbors present control) and all interaction terms. Bold *p*-values indicate significant interaction terms. * after *p*-value indicates significant difference.

Effect	Height (cm)			
	df	SS	*F*	*P*
Time of Year	1	0.74	42.80	< 0.0001*
Elevation	1	0.007	0.40	0.5311
Time of Year * Elevation	1	0.0004	0.03	0.8763
Time of Day	1	1.54	89.16	< 0.0001*
Time of Year * Time of Day	1	0.00	0.00	0.9958
Elevation * Time of Day	1	0.16	9.21	**0.0035***
Time of Year * Elevation * Time of Day	1	0.05	2.88	0.0946
Treatment	1	0.13	7.53	0.0079*
Time of Year * Treatment	1	0.07	4.18	**0.0449***
Elevation * Treatment	1	0.08	4.36	**0.0408***
Time of Year * Elevation * Treatment	1	0.05	2.63	0.1099
Time of Day * Treatment	1	0.05	2.63	0.1098
Time of Year * Time of Day * Treatment	1	0.04	2.18	0.1443
Elevation * Time of Day * Treatment	1	0.00	0.02	0.8878
Time of Year * Elevation * Time of Day * Treatment	1	0.03	1.59	0.2128
Error	64	1.11		

Stable isotopes

Leaf Carbon Isotope Composition

Bulk leaf $\delta^{13}C$ values were significantly different between low and high elevations ($F_{1,36}$ = 23.4881, p < 0.0001) but not between treatment and control plots at either elevation, with all seedlings at high elevation having significantly less negative $\delta^{13}C$ values than seedlings at low elevation (Fig. 12).

Water Isotope Composition

We used ordinary least squares regression of hydrogen (δ^2H) and oxygen ($\delta^{18}O$) stable isotope composition of fog and rain samples to construct a Local Meteoric Water Line (LMWL) for Mount Tamalpais State Park. As expected, our LMWL, $\delta^2H = 6.88*\delta^{18}O + 2.6$ (‰)(n = 88, R^2 = 0.87), had a slightly lower slope and y-intercept than the Global Meteoric Water Line (GMWL; $\delta^2H = 8*\delta^{18}O + 10$ (‰); Craig 1961, Rozanski et al. 1993) with fog waters plotting below the GMWL and rain plotting both above and below the GMWL (Fig. 13).

Fog, rain, and ground waters plotted in distinct δ^2H *versus* $\delta^{18}O$ isotope space along the LMWL. δ^2H values for each water source were significantly different ($F_{1,36}$ = 12.1090, p = <0.0001) and all water sources were significantly more negative at high elevation than at low elevation ($F_{1,36}$ = 4.5680, p = 0.0394). The stable isotope composition for precipitation samples collected at low and high elevations for the same storms showed a similar trend, with δ^2H values for each storm at high elevation being consistently more negative than the δ^2H values for the same storm at the low elevation site. For example, a storm system on May 28, 2010 had a mean rain δ^2H value of -44.7‰ ±0.14‰ SD at low elevation and -47.3‰ ±0.21‰ SD high elevation.

Ranges of δ^2H values of the three types of waters varied by date and elevation, with fog water ranging from -44.1‰ (high elevation, July 7, 2011) to -10.2‰ (low elevation, July 19, 2011), ground water ranging from -34.3‰ (low elevation, November 24, 2011) to -32.7‰ (low elevation, September 23, 2011), and rain water ranging from -67.3‰ (high elevation, May 26, 2010) to -11.0‰ (low elevation, November 24, 2011). δ^{18}O values in fog waters ranged from -5.4‰ (high elevation, July 12, 2010) to -0.94‰ (high elevation, August 27, 2011), ground waters from -5.9‰ (high elevation, March 6, 2012) to -5.4‰ (low elevation, May 14, 2011), and rain from -10.3‰ (high elevation, March 21, 2011) to -3.5‰ (low elevation, March 5, 2012).

The slope of the soil evaporation line at high elevation (δ^2H = 2.19× δ^{18}O −35.9, $r^2 = 0.32$) was significantly lower than the soil evaporation line at low elevation (δ^2H = 6.81× δ^{18}O −6.36, $r^2 = 0.73$; $F_{1, 32}$ = 12.2492, p = 0.0014; Figs. 14 and 15). The low elevation slope of 6.81, while high, is within the range of those observed in very humid (non-evaporative) conditions or saturated soils (Ortega-Guerrero et al. 1997, Barnes and Turner 1998), and such higher slopes might be expected in this fog-prone, coastal location. The slopes for our low and high elevation sites bookend Corbin et al.'s (2005) reported slope of 3.5 for a soil evaporation line derived from pooled samples from two different coastal grassland habitats. The lower slope of the high elevation soil evaporation line is typical of drier, less humid locations.

Stable isotopes of hydrogen of *B. pilularis* seedling xylem water in August were significantly higher (less negative, more enriched in heavier isotopologues) at low elevation than plants at high elevation ($F_{1,15}$ = 19.4491, p = 0.0005), and treatment plants had marginally significantly lower deuterium values than control plants at both elevations ($F_{1, 15}$ = 4.2775, p = 0.0563; Fig. 16). The interaction effect of treatment and elevation was not significant. *B. pilularis* seedling xylem water did not significantly differ in its δ^{18}O composition between low and high elevations and neighbor removal treatment (p > 0.05 for all effects). Stable water isotope values of *B. pilularis* plants at low elevation plotted on the LMWL near fog and shallow soil waters (Fig. 14) while at high elevation *B. pilularis* plants plotted within a mix of groundwater and shallow soil waters (Fig. 15).

Deuterium excess of our precipitation samples was marginally significantly different between low and high elevations (Wilcoxon Rank Sum ChiSquare Approximation = 0.0662), with *d*-excess means for high elevation rainfall being 14.6‰ (± 6.1‰ SD) and low elevation being 11.4‰ (±7.7‰ SD).

As expected, all soil water samples plotted below LMWL with shallow soils being more variable than deep soils at both elevations (Figs. 14 and 15). Shallow soils at both elevations plotted along the soil evaporation line established for each elevation (Figs. 14 and 15) and showed signs of evaporative enrichment during the wet season at low elevation and during both wet and dry seasons at high elevation (see Appendix).
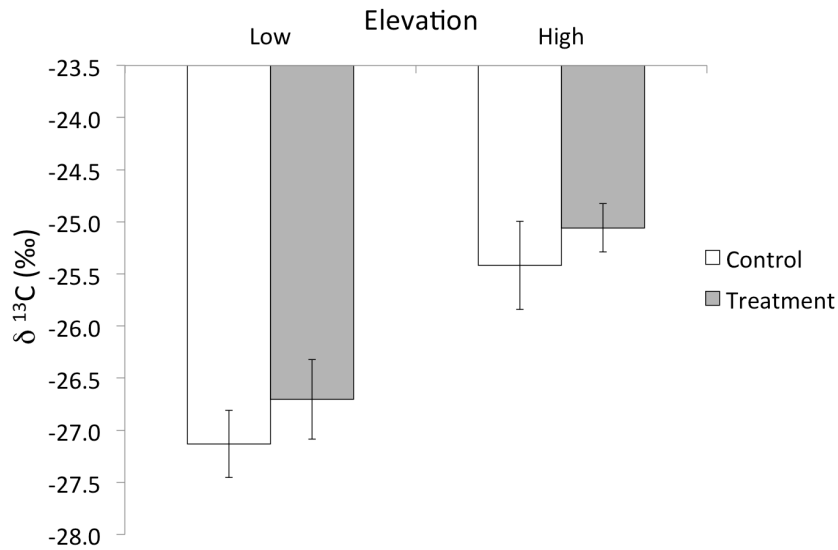
Figure 12. Bulk leaf $\delta^{13}C$ values for *B. pilularis* plants with (control plots) and without neighbors (treatment plots) at low and high elevations, Mt Tamalpais State Park, Marin County, California. ($\pm$ SE)
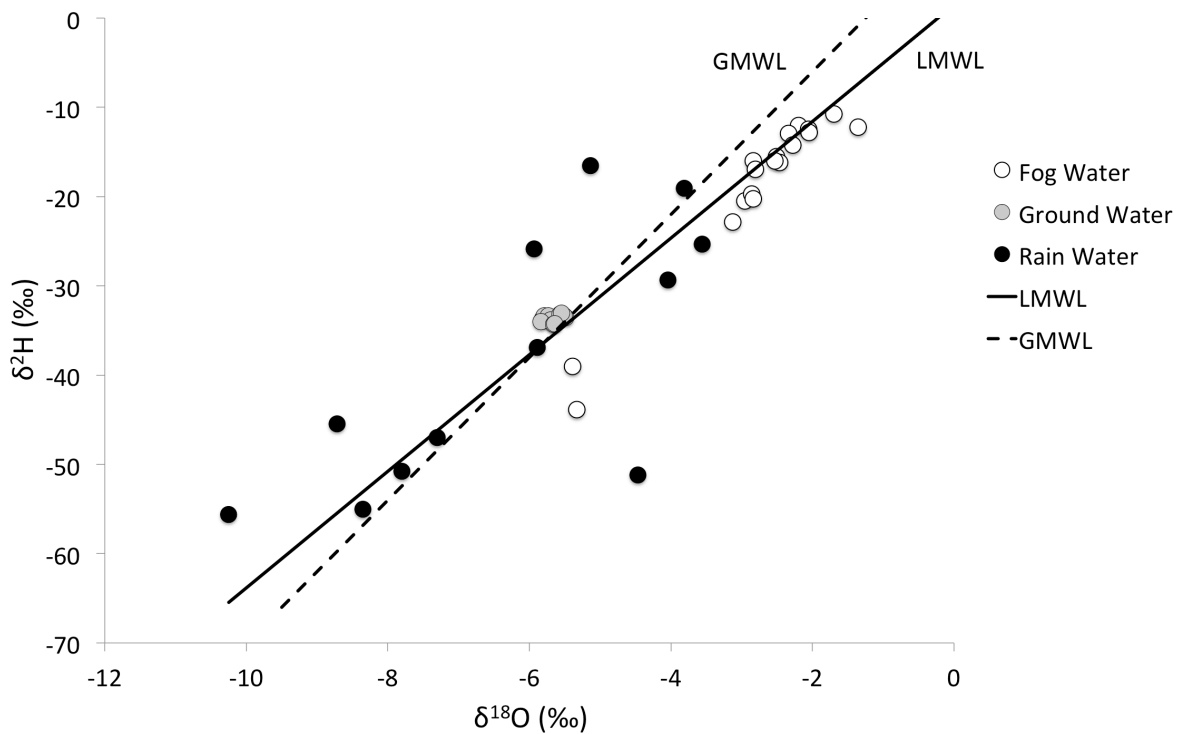
Figure 13. The local meteoric water line (LMWL) for Mt. Tamalpais State Park, Marin County, California, USA using least squares regression for δ^2H and $\delta^{18}O$ values for fog and rain waters is $\delta^2H = 6.88*\delta^{18}O + 2.6$ (‰). The global meteoric water line (GMWL), $\delta^2H = 8*\delta^{18}O + 10$ is included for reference (Craig 1961, Rozanski et al. 1993). Points are mean values by collection date. Error bars are excluded for clarity (see text for ranges and standard deviations).
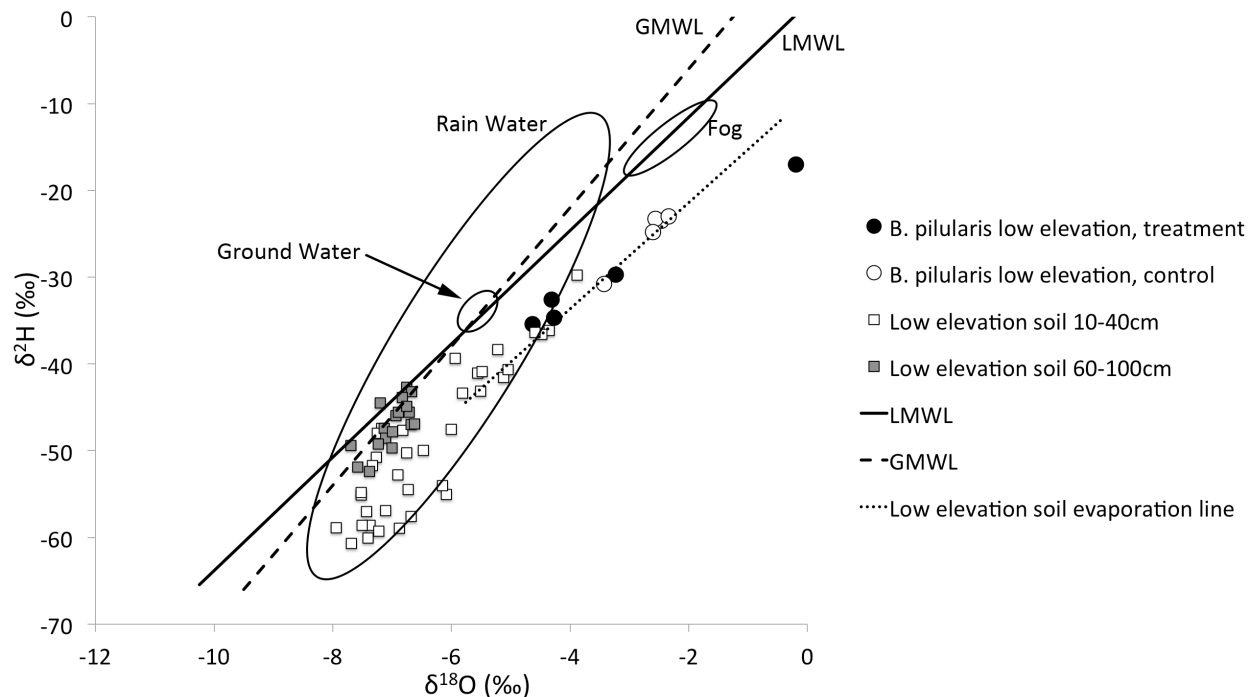
Figure 14. δ^2H and δ^{18}O values for low elevation meteoric water line (LMWL), the low elevation soil evaporation line (points not shown for clarity), *B. pilularis* treatment and control plant stem samples from low elevation, low elevation soil waters in shallow (10cm to 40cm) and deep (60cm to 100cm) soils at Mt. Tamalpais State Park, Marin County, California, USA. Labeled ellipses represent range of water sources for which points and error bars are not included for clarity (fog: δ^2H SD = $\pm$2.1‰, δ^{18}O SD = $\pm$0.35‰; ground water: δ^2H SD = $\pm$0.53‰, δ^{18}O SD = $\pm$0.07‰; rain δ^2H SD = $\pm$18.1‰, δ^{18}O SD = $\pm$1.72‰).
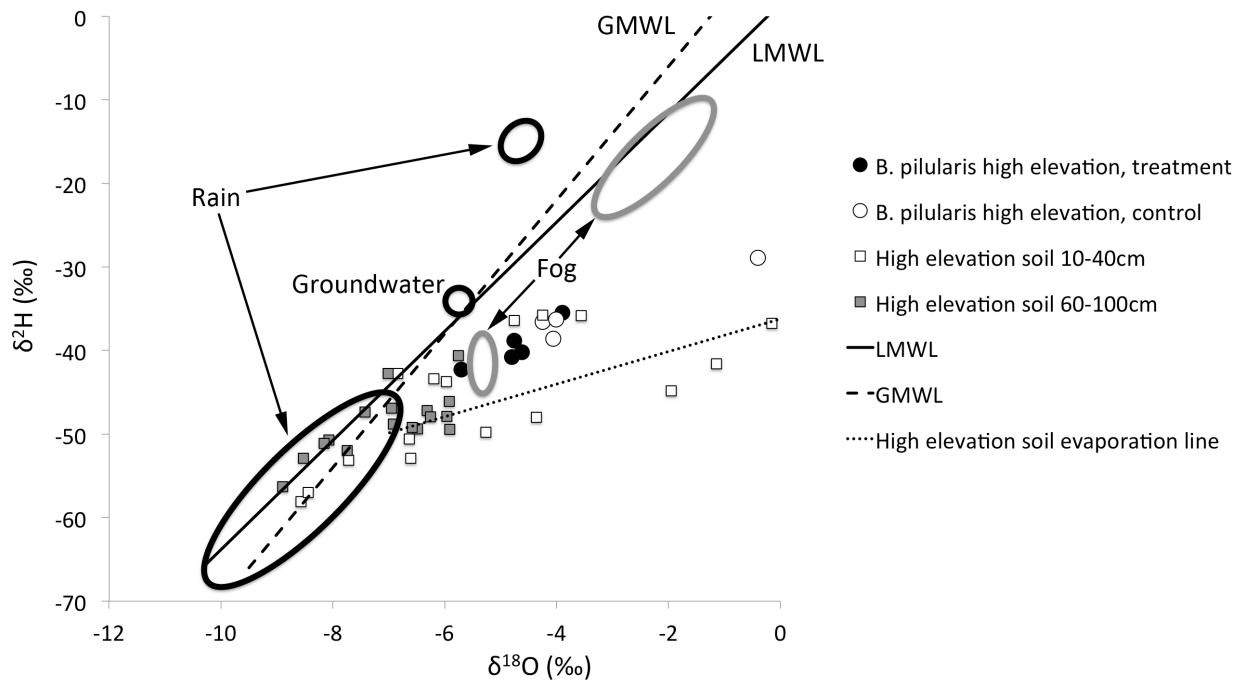
Figure 15. δ^2H and $\delta^{18}O$ values for high elevation meteoric water line (LMWL), the high elevation soil evaporation line (points not shows for clarity), *B. pilularis* treatment and control plant stem samples from high elevation, high elevation soil waters in shallow (10cm to 40cm) and deep (60cm to 100cm) soils at Mt. Tamalpais State Park, Marin County, California, USA. Labeled, shaded ellipses represent range of water sources for which points and error bars are not included for clarity (fog: δ^2H SD = ± 13.4‰, $\delta^{18}O$ SD = ± 1.78‰; ground water: δ^2H SD = ± 0.27‰, $\delta^{18}O$ SD = ± 0.05‰; rain: δ^2H SD = ± 23.7‰, $\delta^{18}O$ SD = ± 2.45‰).
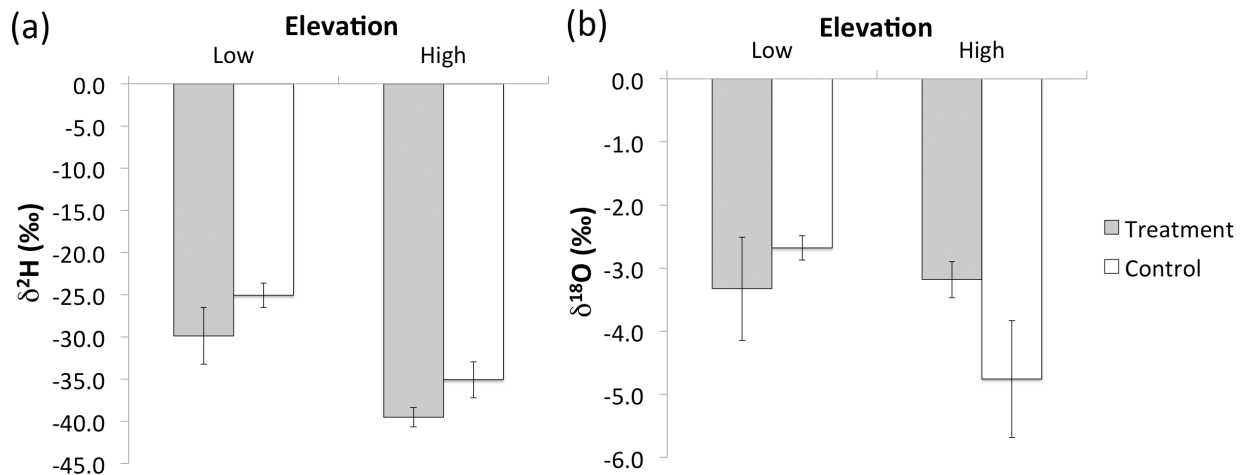
Figure 16. Stable isotopes of hydrogen (δ^2H) in xylem water in *B. pilularis* seedlings in (a) treatment (neighbors removed) and (b) control (neighbors present) plots. ($\pm$ SE)

Discussion

In this investigation, we found significant differences in *B. pilularis* seedling performance, water relations physiology, and water use between warm, dry annual-dominated coastal grasslands and cool, foggy perennial-dominated coastal grassland. In addition, the pattern and strength of competition between *B. pilularis* seedlings and neighboring grasses differed between the grassland types.

Growth

Plant growth was moderated by the presence of neighbors in both grassland types, providing evidence of a competitive effect. The higher relative increase in height, leaf number, and stem length of experimental *B. pilularis* seedlings when neighbors were removed reflects competitive effects when neighboring grassland species are present in both annual- and perennial-dominated grassland types. Competitive effects were also seen at high elevation for basal stem diameter, which was larger when neighbors were removed.

The higher relative height increase at low elevation is likely due to the seedlings growing upward instead of laterally, possibly resulting from the lower temperatures, lower light availability from to coastal stratus and fog, and coastal winds. The naturally-occurring adult *B. pilularis* plants at the low elevation site had typical shrub-like morphology so the morphology of the experimental seedlings could also be a residual effect from being raised in a glasshouse environment or perhaps not establishing adequate root growth. In contrast, high elevation seedlings had greater relative increase in leaf number and relative increase in stem length, which reflects the "bushier" nature of these plants with large amounts of lateral growth. The greater amount of lateral growth in the high elevation plants is also reflected in our measurements of final above-ground biomass and basal stem diameter, both of which were greater for high elevation plants. Indeed, Hobbs and Mooney (1987) found that most growth of *B. pilularis* plants occurs between April and September, suggesting that warm temperatures and higher light levels, such as those found at the high elevation annual-dominated grassland, are critical to *B. pilularis* growth.

The larger size of the high elevation *B. pilularis* plants unexpectedly influenced the rate of maturation as indicated by the difference in the percent of experimental plants that flowered. After we concluded experimental measurements we noted flowering and sex of the *B. pilularis* seedlings on October 28, 2012 at high elevation and on November 27, 2012 at low elevation because low elevation plants flowered later than plants at high elevation (A. G. Kidder, unpublished data). At high elevation, 51% of plants flowered and among these, 53.2% were males and 46.8% were females. In contrast, only one plant flowered at low elevation and it was male.

Water relations physiology

The effect of the neighbor removal treatment on midday g_s and Ψ suggests that plants displayed a combination of competitive and facilitative responses that depend on time of year and grassland type. Values of both g_s and Ψ reflected the lower soil moisture and higher evaporative demand at high elevation as well as differences in summertime fog deposition between the two elevations. The subtle differences in g_s between elevations indicate that *B. pilularis* seedlings do not strongly adjust water use rates based on water availability and temperature. The trend of higher g_s in August in treatment plots suggests *B. pilularis* seedlings

are able to grow more in areas of high VPD despite reduced water availability. Further, given the lack of significant differences in soil water availability between treatment and control plots at low elevation in February, the slightly higher g_s in control plots at this coastal prairie site suggests that perennial grasses and forbs might have a mild facilitative effect on *B. pilularis* seedling g_s during the wet season.

   B. pilularis Ψ varied depending on seasonal soil moisture availability, diurnal fluctuations in VPD, and the presence of neighbors. The higher *B. pilularis* Ψ in treatment plots at low elevation during the wet season and at midday during the dry season suggest seedlings are generally less water stressed with no neighbors present, despite the higher gs in low elevation control plots during the wet season. This decoupling of Ψ from gs could reflect the fact these measurements were taken on different dates that could have experienced different abiotic conditions. The greater difference between predawn and midday Ψ at high elevation compared to low elevation is expected given the high elevation site's more variable diurnal VPD and lower water availability and the low elevation site's more stable maritime climate and accompanying lower VPDs. The stronger treatment effect during the wet season at both elevations indicated competition for water was more intense when neighboring grasses were actively growing.

Stable isotopes
Leaf Carbon Isotope Composition
   The higher (less negative) bulk leaf $\delta^{13}C$ values of *B. pilularis* seedlings at high elevation in August were expected due to higher VPD and accompanying evapotranspiration rates as well as low fog deposition rates, resulting in lower summer water availability. The lack of difference in $\delta^{13}C$ values in seedlings with and without neighbors indicates presence of neighbors did not influence long-term photosynthesis rates. *B. pilularis* seedlings in control plots at low elevation had bulk leaf $\delta^{13}C$ values (-27.13‰ ±1.01 SD) within the range of *B. pilularis* adults in two other fog-prone environments, Point Reyes National Seashore and Tomales Bay State Park, where the values ranged from -27.4 to -27.9 (Langolletto et al. 2006). The combination of the higher $\delta^{13}C$ values and larger growth rates at high elevation suggest that despite the lower water availability *B. pilularis* seedlings flourish in these warm annual grasslands and is a dominant competitor. Indeed, over the past 75 years warm, inland grasslands have converted to *B. pilularis*-dominated shrublands in the greater San Francisco Bay region (Russell and McBride 2003).

Water Isotope Composition
   The LMWL for the study site had a slope lower than the GMWL that is typical of dry climates due to non-equilibrium evaporation of falling rain (Dansgaard 1964). The LMWL for Mt. Tamalpais State Park, $\delta^2H = 6.88*\delta^{18}O + 2.6$, had a similar slope of other LMWLs along California's coast (Dawson 1998, Fischer and Still 2007, Emery and Lesage 2015). The y-intercept was smaller than these other studies, suggesting fog-driven lower *d*-excess amounts.
   The significant difference between fog and rain was expected due to the differing origin of these two waters. Fog is produced immediately offshore and is regarded as an early stage condensate as it is blown onshore whereas rain originates from westward Pacific storms moving onshore (Ingraham 1998). These eastward-moving storms continuously condense as they travel oversea, losing a disproportionate amount of rain consisting of heavier water isotopologues (i.e., $^2H_2^{16}O$, $^2HH^{18}O$, $^2H_2^{18}O$) so that by the time rain falls on California's coast it is depleted in the heavier isotopologues of water and rain inputs to the ecosystem are depleted in them, especially

relative to fog or the rare summertime rain event (Gonfiantini and Longinelli 1962, Ingraham & Taylor 1986). We did not see the same seasonal enrichment in rain δ^2H values during summer as was observed at a site approximately 20 miles north in Point Reyes National Seashore, Marin County, California, USA, however δ^2H values of fog were in a similar range (-18‰ to -7‰; Ingraham and Matthews 1990). The more negative δ^2H values of fog water at our high elevation site might have occurred because the site is more inland at 2,580 meters (1.60 miles) from the coast than the low elevation site, which is 520 meters (0.32 miles). Ingraham and Matthews (1990) observed δ^2H values of fog water collected from their inland site in Point Reyes National Seashore that were more depleted than those collected at their coastal site. Similar trends of increasingly depleted deuterium values with increasing distance from the coast has been observed in surface, rain, and ground waters (Ingraham and Taylor 1991).

Different deuterium excess values were observed at each elevation. The marginally significantly higher *d*-excess at high elevation likely resulted from a combination of higher altitude and the higher temperatures and VPD at this site, which could lead to enhanced locally recycled evapotranspired plant or water vapor prior to subsequent rainfall and collection (Gat 2000, Gonfiantini et al. 2001, Frölich et al. 2002). The slightly lower *d*-excess values at low elevation suggest reduced evaporative recycling in precipitation, reflecting the lower VPDs at this site. For both elevations, rain samples plotted both above and below the LMWL. The rainfall values below the LMWL are more likely due to kinetic evaporation loss where evaporates were moved out of the system by wind and not recycled, whereas those values above the LMWL reflect recycled water vapor (Frölich et al. 2002). Deuterium excess for both elevations was higher than previously observed in California, with Rozanski et al. (1993) having reported a 30-year mean *d*-excess value of 7.3‰ in Santa Maria, California (34.90 N, 120.45W, 79m above sea level).

Plant water source use varied between grassland types. During the dry season, the significantly higher δ^2H values of low elevation seedling xylem water suggests that these plants were using δ^2H enriched water sources influenced by the shallow soils where both evaporation and fog water inputs are highest. One caveat is that the stems of the seedlings we sampled may not have been fully suberized and therefore susceptible to fractionation and subsequent enrichment through younger stem tissue. Despite this latter possibility, it is still very likely these low elevation plants were at least partially using fog water. For example, *B. pilularis* seedling xylem waters at low elevation plotted in a dual isotope space consistent with fog water use, with mean δ^2H values suggesting a mixture of fog and ground water sources. In contrast, at the high elevation site that had minimal fog water inputs *B. pilularis* seedlings used a mixture of ground and shallow soil waters and not fog water, as indicated by their location in dual isotope space.

Fog water use by plants has been observed worldwide to similarly help ameliorate water deficits and improve plant water status during dry seasons, either indirectly by wetting soil by throughfall (Dawson 1998, Kennedy and Sousa 2006, Carbone et al. 2013, Emery and Lesage 2015) or directly through foliar uptake of fog water (Burgess and Dawson 2004, Limm et al. 2009, West et al. 2012, Goldsmith et al. 2013). In a study looking at water used by perennial coastal grasses near our study sites during May-October, fog consisted of about forty percent of water used by the dominant neighboring perennial grass species at the low elevation site, *Festuca rubra* and *Nasella pulchra* (Corbin et al. 2005). In our study, it is likely that neighboring perennial grasses in our cool, fog-prone grassland site intercepted fog water and wetted shallow soil layers, facilitating the fog water use by *B. pilularis* seedlings.

The consistently lower stable water isotope values in seedling xylem water in treatment plots at both high and low elevations indicate that water source use was dependent on the presence of neighbors. Grassland plants in control plots might have preferentially used water with more negative stable water isotopes (isotopically depleted water), leaving *B. pilularis* seedlings in these plots to use less isotopically enriched water (water source partitioning). The opposite trend is observed in treatment plots at both elevations, where *B. pilularis* seedlings used isotopically more negative water. Such community-level neighbor-dependent species interactions were also observed in the lower water potentials in control plots during the wet season, which suggests that competition for water was more intense during the grassland's peak plant growth.

Overall, *B. pilularis* seedling growth, seasonal water relations physiology, and dry season water use was influenced by type of grassland habitat and presence of neighboring grassland species. At low elevation, coastal cloud stratus decreases VPD and fog deposition provides a moisture subsidy, both of which in turn decrease the need for *B. pilularis* seedlings to reach deeper water sources to compete with neighbors. Indeed, at the fog-prone perennial-dominated grassland at low elevation during peak dry season near the end of August, *B. pilularis* seedlings utilized more enriched soil water, reflecting shallow rooting depths. In contrast, at high elevation seedlings used water more similar to deeper soil water, reflecting the deeper root growth driven by higher VPDs at this annual-dominated grassland type. In addition to water use, other parameters were influenced by the warmer high elevation grassland habitat. Higher $\delta^{13}C$ values indicate seedlings were able to fix higher amounts of carbon and grow more biomass with moderate stomatal conductance rates and lower water potentials. Although seedlings were competing with neighboring grasses, they showed little signs of detrimental water deficit and were able to cope with atmospheric and soil dryness at this site. Our results suggest that *B. pilularis* seedlings will continue to successfully establish in coastal grasslands, particularly those that are warming with climate change.

Conclusions

This study investigated how competition, local climate, and edaphic characteristics interact to influence the growth, water relations physiology, and water use of *B. pilularis* seedlings as they become established in two contrasting types of coastal grasslands typical of central California's Coast Range. Our results suggest that when fog is present, such as at our cool, fog-prone, perennial-dominated site, *B. pilularis* seedlings use fog water as a significant portion of their water intake. The tradeoff for *B. pilularis* seedlings in fog-prone perennial-dominated grasslands that have lower VPDs and increased summer fog and cloud cover is to have improved leaf water status at the expense of sunlight and warmer temperatures, which are found in annual-dominated grasslands and appear to be important for plant growth. The effect of neighboring plants on *B. pilularis* seedling growth and water relations depended on grassland type and season. Although the moderation of growth and some water relations parameters by the presence of grassland neighbors indicates *B. pilularis* seedlings are mildly sensitive to competition, overall seedling water relations parameters and water use suggest this plant will continue to easily occupy coastal grasslands, especially warmer, annual-dominated grassland types. Our hope is that this study provides much-needed information to help land managers construct sound conservation measures for California's shrinking grassland habitats.

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Appendix

Stable water isotope soil profiles

The deuterium values in the isotopic soil water profiles varied between low and high elevations and wet and dry seasons and did not couple with *B. pilularis* seedling xylem water values, making it difficult to directly compare water use. Stable water isotope values of *B. pilularis* xylem water were consistently higher (less negative) than our soil samples. The higher values of plant xylem water could reflect that plants were using shallower water sources than we measured (i.e., shallower than 10cm deep). Alternatively, the discrepancy could reflect the different sampling dates for plant and soil samples.

The deuterium isotope profile in high elevation soils during both wet and dry seasons shows shallow soils above 40cm deep decreasing to a low mean value of -36.0‰ during the wet season and -51.1‰ during the dry season, and then increasing slightly again below 40cm before stabilizing. Low elevation deuterium values during the dry season were more negative than during the wet season, with shallow soils unexpectedly having the most negative values.

Stable water isotope profiles in each grassland type reflected seasonal abiotic conditions. Evaporative enrichment in shallow soil layers is expected in surface soils due to fractionation as the water with lower δ values is lost as water vapor during evaporation (Allison, Barnes, and Hughes 1983), particularly in areas of higher VPD and evapotranspiration such as the high elevation site. The weaker evaporative enrichment in the low elevation isotope soil water profile reflects the low VPD at this fog-prone, coastal location. Deuterium values of the soil water isotope profile at low elevation indicate that fog water did not penetrate as deep as 10cm. At high elevation the prominent decrease in δ^2H values at 40cm for both wet and dry seasons reflects what has been referred to as an "isotopic unstable zone," which can occur at 40cm deep (Dawson and Ehleringer 1998). This zone is thought to exist due to the presence of old water stored from past precipitation and condensation events (Mazor 1991). The trend of lower deuterium values at about 40cm deep indicates evaporative enrichment occurs in both shallower and deeper soils. The enrichment might be due to the increase in clay development in the A3 soil horizon that exists at about 40cm and a "moderate very fine [< 5mm diameter] subangular texture" (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture 2010), which would tend to have greater water adsorption, reducing evaporation. Below 40cm lies a Bt horizon with a coarser blocky structure and wider, more frequent cracks that might ease evaporation of water vapor upward in the soil profile until the thicker layer of clay is reached at approximately 40cm and more isotopically negative (lighter) water is stored. This zone could also be a result of legacy effects from previous storm fronts that require finer temporal and physical resolution than the sampling regime for this study. For example, the (unsampled) winter rains during 2010-2011 could have been cold and therefore more negative than those recorded during this study in 2011-2012, resulting in residual, more negative, soil water isotope profile that reflects these earlier values.

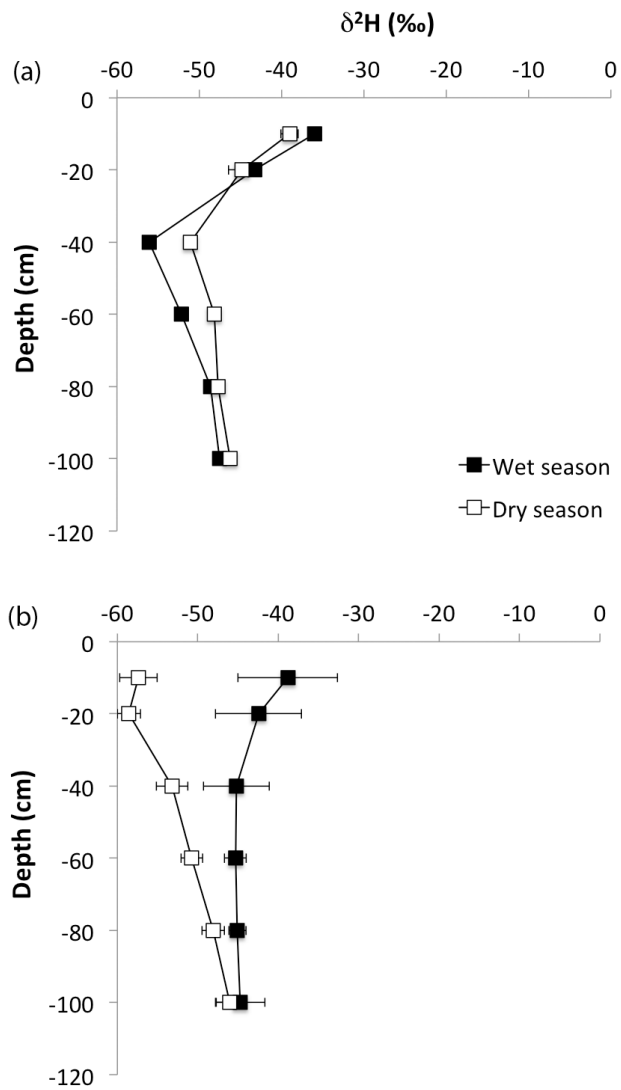**Figure 17**. Soil profiles of δ^2H values for high (a) and low (b) elevations during wet and dry seasons. High elevation samples are from May 14, 2011 (wet season) and August 16, 2012 (dry season) and low elevation samples are from May 13, 2011 and March 15, 2012 (wet season) and August 27 and September 23, 2011 (dry season). Values are means $\pm$SD (n=3).

CHAPTER 3

Ontogenetic variation in water relations physiology and water use by
***Baccharis pilularis* DC. (Asteraceae, coyote brush): implications for succession in coastal Californian grasslands**

Abstract

The California native shrub *Baccharis pilularis* (Asteraceae) routinely invades increasingly rare coastal grasslands along central California's fog belt. Incorporating ontogenetic, spatial, and temporal scales, we investigated *B. pilularis* water relations physiology during succession from grassland to shrubland. Several key water relations traits of seedling and adult plants were quantified in contrasting cool-mesic and warm-dry coastal grassland habitats during both the wet and dry seasons. We hypothesized that seasonal rates of water use, plant water status, tissue water relations, leaf stable carbon isotope composition and the water sources plants used would differ between age groups and reflect more conservative water use with increasing water deficits (drought). Our findings indicate that *B. pilularis* water relations strategies change as shrubs age and succession proceeds, with adults showing greater drought tolerance than seedlings as a result of lower turgor loss points, mass-based tissue capacitance, and seasonal midday water potentials in areas with low water availability and without a fog moisture subsidy. During the course of the dry season as plant water became increasingly scarce *B. pilularis* of both age classes and in both grassland types showed increased stomatal conductance rates and clear osmotic adjustments that lowered turgor loss points. By comparing stable water isotope composition of plant xylem water to water sources we found that seedlings readily used atmospheric fog water when available, allowing these plants to maintain higher rates of stomatal conductance, less negative water potentials, and tissue water relations characteristics typical of more mesic environments. *B. pilularis* readily withstands high vapor pressure deficits and low water availability, facilitating establishment into grasslands. This native shrub will therefore be successful in a warming and drying climate by continuing its dominant presence within the California Floristic Province.

Introduction

One of the more conspicuous changes in California's coastal landscape is the encroachment of the ubiquitous native shrub, *Baccharis pilularis* subsp. *consanguinea* (DC.) C.B. Wolf (Asteraceae, coyote brush, herein *B. pilularis*), into increasingly rare coastal grasslands, which results in a grassland-shrubland mosaic landscape found throughout much of California (Ford and Hayes 2007). For decades researchers have explored what drives *B. pilularis* to actively encroach in grasslands (McBride and Heady 1968, Bartholomew 1970, McBride 1974, Da Silva and Bartolome 1984, Davis and Mooney 1985, Hobbs and Mooney 1987, Williams et al. 1987, Williams and Hobbs 1989, Zavaleta 2006). While the main driver of succession from grassland to *B. pilularis*-dominated shrubland, when it occurs, is the removal of grazing and the natural fire regime, one of the dominant themes to emerge from these studies of shrub invasion is that timing of water availability appears to regulate *B. pilularis* seedling establishment in grasslands (Da Silva and Bartolome 1984, Williams et al. 1987, Williams and Hobbs 1989). Although these studies investigated the importance and timing of rainfall, they did

not consider the possible role of "occult precipitation" in the form of advection or coastal fog. Fog water deposition has been shown to be an essential moisture subsidy during California's rainless summers (Jacobs et al. 1985, Dawson 1998, Limm et al. 2009, Corbin et al. 2005, Fischer et al. 2009, Ewing et al. 2009, Kennedy and Sousa 2006, Carbone et al. 2013, Templer et al. 2015). It is important to consider ecohydrological implications of landscape changes such as succession and woody plant encroachment (Rodriguez-Iturbe 2000, Huxman et al. 2005).

Even though *B. pilularis* is one of the most common plant species in the California Floristic Province, physiological information on this plant is scarce. The few physiological studies that do include *B. pilularis* focus on adult shrubs (Ackerly 2004), leaving *B. pilularis* seedling and juvenile physiology unknown. When the importance of water availability to *B. pilularis* establishment during the early stages of succession is coupled with the influential role that coastal fog plays in landscape dynamics along California's coast, a study of *B. pilularis* water relations is clearly vital to fully understanding the dynamics of this species on the landscape. Here we present the results of an investigation of *B. pilularis'* water relations, which encompasses the maintenance of cell hydration and overall water status so the plant can collect water from the soil, photosynthesize, and transport water and photosynthates within the plant. A central goal was to conduct our investigation at several scales, 1) **ontogenetic**: from seedling to adult, as a way to place our ecophysiological findings into an early-to-late successional context, 2) **spatial:** from leaf and stem tissues to whole organisms to the landscape scales, and 3) **temporal:** from diurnal to seasonal scales. For this study we investigated ontogenetic variation in *B. pilularis* water relations during early (seedling) and late (adult) stages of succession from grassland to shrubland during the wet season and through the progression of the rainless summer dry season in two contrasting grassland types: a cool, fog-prone coastal prairie dominated by native perennial grasses and a warm, dry, low-fog grassland dominated by exotic annual grasses. We hypothesized that *B. pilularis* shrubs would shift water relations strategies as the plants aged during advancing successional stages, with adult plants being less vulnerable to drought than seedlings. Further, we expected plants of both ages to exhibit stomatal conductance rates, water potentials, tissue-level parameters, and water use patterns that are more conservative with water loss in the warmer, drier grasslands and during the dry season, and less conservative in cooler, mesic grasslands and during the wet season.

Methods

Site Description and Study Species

This study was conducted in the same low and high elevation sites in Mt. Tamalpais State Park, Marin County, California as reported in the previous chapter (Kidder 2015b). The low elevation study site was located in a cool, mesic coastal grassland while the high elevation study site was located in a warm, dry coastal grassland, with the two elevations serving as a proxy for contrasting climates. Due to logistical constraints and the high cost of sample analyses, it was not feasible to replicate this study at multiple sites of each grassland type. While this limits our ability to make broad inferences from our findings (Hurlbert 1984), these two grassland types are common along the northern coast of California (Keeler-Wolf et al. 2007), so it is reasonable to expect that our results have relevance beyond our immediate sites.

In order to compare adult and seedling water use in early successional stages of shrub encroachment, we planted 200 greenhouse-raised *B. pilularis* seedlings in coastal grassland meadows at each elevation (400 total) amidst existing adult *B. pilularis* shrubs. The seedlings

were sown in the greenhouse on July 11, 2010 and planted in the field during the late February and early March 2011 when seedlings were about seven months old. All seedlings were similar size when planted and numbered. Within a month of planting, seven plants at high elevation were eaten by a fossorial animal, likely a pocket gopher (*Thomomys bottae*), as evidence of gopher activity existed in the meadow and the entire seedlings, including roots, were gone with minimal to no sign of above-ground disturbance. We did not have the same herbivory problems that Zavaleta (2006) experienced in a grassland approximately 82 km (51 miles) from the study site, but this is likely because our seedlings were older and larger when planted in the field (30-40 cm (12-16 inches) tall above ground). Seedlings used in this study were distinct from those used in the manipulative neighbor-removal experiment in Kidder (2015b). Unlike other ecophysiological studies of dioecious plant species (Dawson and Bliss 1989, Dawson 1990, Dawson and Ehleringer 1993, Dawson and Bliss 1993, Dawson et al. 2004), a pilot study indicated no differences in water potentials and stomatal conductance measurements between male and female *B. pilularis* shrubs so plant gender was not considered in this study.

Environmental Conditions

Fog water, temperature, and relative humidity were measured using the same equipment as in the previous chapter (Kidder 2015b). Temperature and relative humidity data were used to calculate vapor pressure deficit (VPD) using the same methods as in the previous chapter (see Chapter Two). Precipitation data for 2011 and 2012 were obtained from the same nearby weather station in Kentfield, California as in the previous chapter (37° 57' N, -122° 32' W; Kentfield, California (044500) Period of Record Monthly Climate Summary, Western Regional Climate Center, 2015). We did not measure wind in this study.

Water relations physiology

We measured a suite of plant water relations characteristics for both adult and seedling *B. pilularis*, including seasonal midday stomatal conductance (g_s), seasonal diurnal water potentials, seasonal tissue water relations parameters from pressure volume curves. Midday g_s was measured five times during the course of the summer season (June 7, July 7, August 4, August 23, September 10, 2011) and the following wet season (March 10, 2012) using a steady state porometer (LI-1600, LI-COR, Lincoln, Nebraska, USA) and the same protocol as the previous chapter (Kidder 2015b). On each date, 5 adult and 5 seedling *B. pilularis* plants at each elevation were randomly selected for g_s measurements (10 plants at each elevation, 20 plants total on each date).

On the same dates we measured diurnal water potentials at each elevation, resulting in water potential measurements at predawn, 8am, 10am, 12pm, 2pm, 4pm, and early evening on 5 adult and 5 seedling randomly selected *B. pilularis* plants (for a total of 70 measurements at each elevation, and 140 measurements total, per date). Measuring water potentials at both fine (diurnal) and large (seasonal) timescales elucidated important temporal fluctuations in *B. pilularis* hydraulic status as the plant responds within a single day in the two contrasting grassland habitats as well as during the progression of California's rainless summer and during the wet season. Water potential measurements were made using a pressure chamber (PMS Instrument Company, Albany, Oregon, USA) within 1 minute or less of collection. Due to the nature of the measurements and the size of the seedlings, measurements often required destructive sampling of most seedlings. Different adult and seedling plants were selected for

measurements throughout one day's measurements when possible, but there were times when the same adult plant was sampled more than once throughout a day of diurnal measurements.

Tissue water relations parameters of osmotic (or solute) potential at full turgor (Ψ_{SFT} (MPa); a measure of solutes comprised of simple sugars and amino acids in the cell), turgor loss point (Ψ_{TLP} (MPa); the point at which cells lost positive pressure and become flaccid), bulk modulus of elasticity (ε (MPa); measure of cell rigidity), mass-based capacitance before turgor loss point ($C_{T,mass}$ (g g^{-1} MPa^{-1}); the change in the amount of tissue water volume per gram of dry shoot tissue per change in water potential), and relative water content at turgor loss point (RWC_{TLP} (decimal)) were obtained by creating pressure-volume curves. Five adult and five seedling *B. pilularis* stems at each elevation were randomly selected from both elevations (20 stems total) and subjected to the same protocol for pressure-volume curves as outlined in Kidder (2015a). Stems were collected on the same as the measurement dates for g_s and water potentials and the pressure-volume curves were conducted in the lab on June 11 and 12 (stems collected June 10), July 8, August 5, August 24, September 11, 2011 and March 11 and 12, 2012.

Stable isotopes

We obtained $\delta^{13}C$ values of bulk leaf tissue in order to compare carbon isotope discrimination of *B. pilularis* adults and seedlings in cool, moist, perennial dominated and warm, dry, annual-dominated grassland types. Several fully-developed leaves at the terminal ends of multiple stems were collected from the south side of five adult and five seedling randomly selected *B. pilularis* plants at each elevation on September 22, 2012. Leaves are estimated to have developed during the rainless summer between early July and mid-September, 2012. The collection protocol and calculation of carbon isotope composition follow Kidder (2015b).

We evaluated the effect of climate on seasonal ontogenetic variation in *B. pilularis* water use by comparing the stable isotope composition of water sources available to the plants to the stable isotope composition of the xylem water in adults and seedlings at both elevations during rainy and rainless (and foggy) times of the year. We collected stem samples of three to five randomly selected adult and seedling plants at both elevations (unless otherwise specified) on May 12, August 27 (low elevation only), and September 23, 2011 (low elevation only) and March 5 (low elevation only), May 19, August 17, and September 22, 2012. By May 2011 (about three months after planting in the field) the seedlings were nine months old and parts of the stems were not yet woody. Prior to extracting xylem water from these partially-suberized seedling stems, we carefully peeled the bark from the samples collected on May 12, 2011 (high and low elevations) and August 27, 2011 (low elevation). Sample collection and processing of water sources (fog, rain, soil, and ground waters), the extraction of plant and soil waters, and calculation of δ^{2}H and δ^{18}O values were the same as outlined in Kidder (2015). The same source water isotope data is presented here to qualitatively compare these source waters to seasonal differences in stable water isotope values in adult and seedling xylem waters. For all sample types (water sources, plant xylem waters), the amounts of stable isotopes of hydrogen (δ^{2}H, or deuterium) and oxygen (δ^{18}O) are reported relative to the internationally accepted standard of ocean water (V-SMOW, International Atomic Energy Agency, http://www.iaea.org), resulting in negative values. More negative values indicate the sample is depleted or lighter in the amounts of the heavier isotopes (e.g., ^{2}H or ^{18}O) while less negative values indicate the sample is enriched in the heavier isotopes. Water samples become enriched (or heavier) when evaporation (a one-way kinetic fractionation effect) causes the lighter isotopologues of water to evaporate first, leaving behind the heavier isotopologues in the sample. Very little to no fractionation occurs during

water uptake by plants, enabling the direct comparison of δ^2H and δ^{18}O in xylem water values to that of water sources (Dawson 1993).

Analyses

We used multiple least squares regression to estimate the variance explained for dependent variables gs, Ψ_{TLP}, Ψ_{SFT}, ε, $C_{T,mass}$, RWC_{TLP}, and seasonal predawn and midday water potentials, by predictor variables elevation (low and high), plant age (adult and seedling), and date using the following model,

$$y = \beta_0 + \beta_1(low) + \beta_2(adult) + \beta_3(date) + error$$

where β_0 is the intercept, $\beta_1(low)$ represents elevation, $\beta_2(adult)$ is plant age (adult or seedling), $\beta_3(date)$ is measurement dates, and $error$ is the remaining unexplained error in the model. Date was used as a rough proxy for seasonal water availability in order to compare plant performance during the wet season and during the rainless summer dry down. We coded the dependent variables using dummy variables, where low elevation = 1 and high elevation = 0; adult = 1 and seedling = 0; dates were June 7, 2011 = 1, July 7, 2011 = 2, August 4, 2011 = 3, August 23, 2011 = 4, September 10, 2011 = 5, and March 10, 2012 = 0. We did not perform a multiple regression to test for the difference between predawn and midday water potentials because we destructively sampled the majority of the seedlings and therefore did not have matched measurements for both time periods. We calculated expected geometric means for each predictor variable by taking inverse of the $\log_{10}$-transformed coefficient to obtain percentage difference predictions from the multiple regressions on log-transformed data.

All data were checked for normality and equal variances according to Shapiro Wilk's (Zar 1999) and Levene's (Quinn & Keough 2002) tests, respectively, and subsequently log-transformed using $\log_{10}(|X|)$. If warranted, once data met assumptions they were checked once for outliers at an alpha level of ≤ 0.05 using the extreme studentized deviate (Grubbs') test (GraphPad QuickCalcs, GraphPad Software, La Jolla, California, USA) and subsequently removed. Stomatal conductance data were log-transformed due to unequal variances and one result for one seedling at low elevation on March 10, 2012 was removed. Variances between groups for predawn water potentials were homogeneous but data were transformed to meet normality assumptions. Midday and each set of diurnal water potentials were log-transformed to achieve both normality and homogeneous variances. Each of the pressure-volume curve parameters was log-transformed to meet the assumption of normality (Ψ_{SFT}, Ψ_{TLP}, ε, $C_{T,mass}$, and RWC_{TLP}) and equal variances ($C_{T,mass}$).

Carbon stable isotope values met assumptions of normality and equal variances and were analyzed using a two-way analysis of variance with elevation and plant age as main effects and elevation*age as an interaction effect. For all meteoric waters (fog, rain, and ground waters), deuterium (δ^2H) values were plotted as a function of δ^{18}O values to derive the Local Meteoric Water Line (LMWL) for Mt Tamalpais State Park, which was compared to the Global Meteoric Water Line (Craig 1961, Rozanski et al. 1993; Kidder 2015). Deuterium excess, soil water isotope profiles, and soil evaporation lines used in this study are discussed and present in Chapter Two. Because some of the samples of water sources and plant stems were not collected on the same dates, we were not able to model the partitioning of plant water use. Instead, seasonal plant water use was analyzed qualitatively by comparing xylem water δ^2H values to soil water isotope profiles and the relative proximity to the various meteoric water sources in isotope space along

the LMWL. An ANOVA was used to test for differences in xylem δ^2H values, with age, season, and elevation as fixed main effects and all associated interaction effects. These deuterium data met assumptions of normality and homogeneity of variances and Tukey's Honestly Significant Difference (HSD) orthogonal contrast posthoc test was used for posteriori comparison of means. By using a dual isotope approach, we were able to increase accuracy in linking stable water isotopes in plant xylem to that in water sources.

Results

Environmental conditions

Environmental conditions measured were typical of California's Mediterranean climate with a largely distinct rainy season between October and early June, as well as a rainless summertime fog season between mid- to late June through September. Although the summer receives little to no rainfall, coastal fog provides a moisture subsidy so this time of year is not waterless. However, for ease of discussion the rainy season is referred to as the wet season and the rainless summer fog season as the dry season. The low elevation site had 128.3 percent more fog water than the high elevation site in 2011 and 119.7 percent more in 2012 (Fig. 1). On July 17, 2012 a small rain event resulted in 0.5mm rainfall and is reflected by a simultaneous increase at both elevations (Fig. 1b). In 2011 the area received 915.4 mm (36.0 inches) of rain and 1,460 mm (57.5 inches) of rain in 2012 (Fig. 2). Over 61 mm (2.4 inches) fell in June 2011, making it the wettest June in San Francisco Bay area since 1849, the time of the area's earliest rainfall records. Our wet season measurements on March 10, 2012 were preceded by a total of 64.8 mm (2.6 inches) of rainfall during two rain events, 48.5 mm (1.9 inches) of rain during March 2 and 3, 2012 and 16.3 mm (0.64 inches) of rain during March 6 and 7, 2012. For the dates we recorded temperature and relative humidity at both elevations, calculated VPD was most often higher at high elevation than at low elevation (Fig. 3). Indeed, maximum recorded VPDs during 2011 were 3.32 kPa (September 28) at high elevation and 2.76 kPa (October 14) at low elevation and during 2012, 4.55 kPa (August 13) at high elevation and 2.00 kPa (February 23) at low elevation. Minimum recorded VPDs at both elevations for both years were zero. The maximum VPDs occurring during fall through early spring are likely a result of warm easterly breezes brought on by high-pressure systems that are a common feature between storm systems. Temperature and relative humidity values measured by the Vaisala HUMICAP® 180 capacitive relative humidity sensor (Vaisala, Inc., Boulder, Colorado) were similar to those measured by another sensor deployed at each elevation (data not shown; EL-USB-2, Lascar Electronics, Inc., Erie, Pennsylvania, USA).
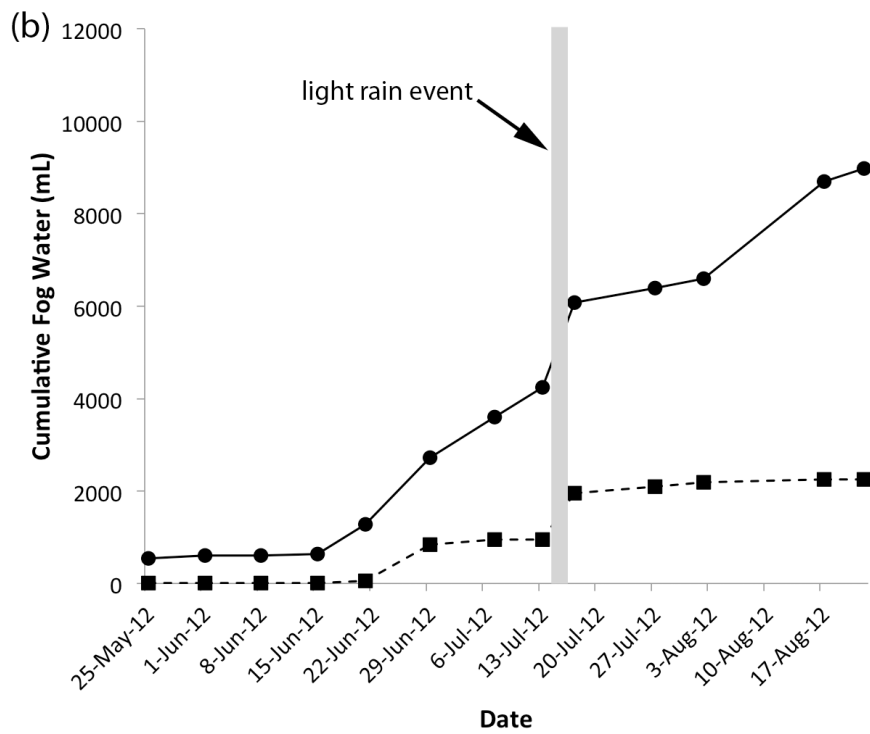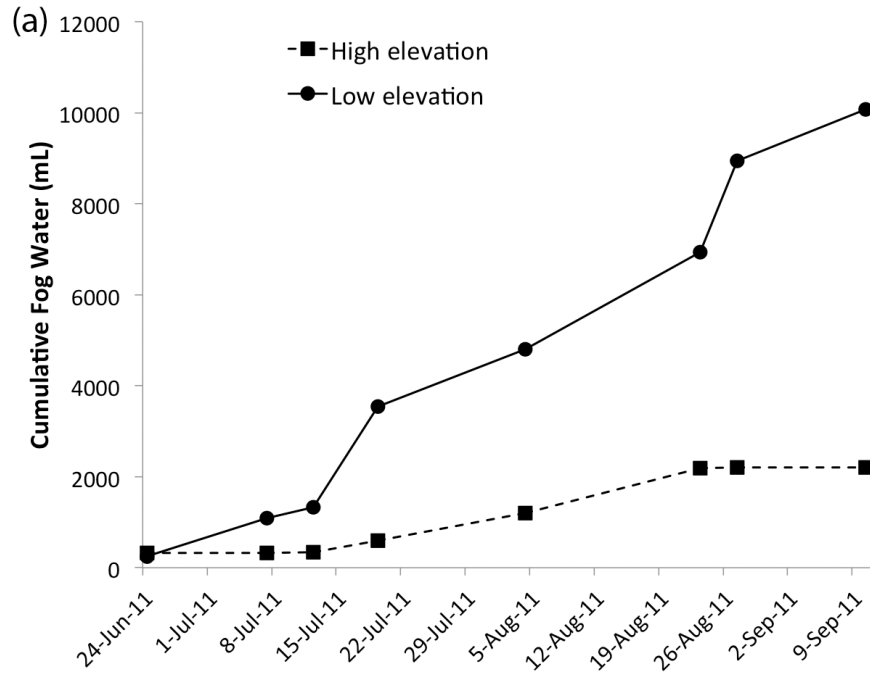
Figure 1. Cumulative fog water at low and high elevation study sites in Mt. Tamalpais State Park, Marin County, California, USA during 2011 (a) and 2012 (b).
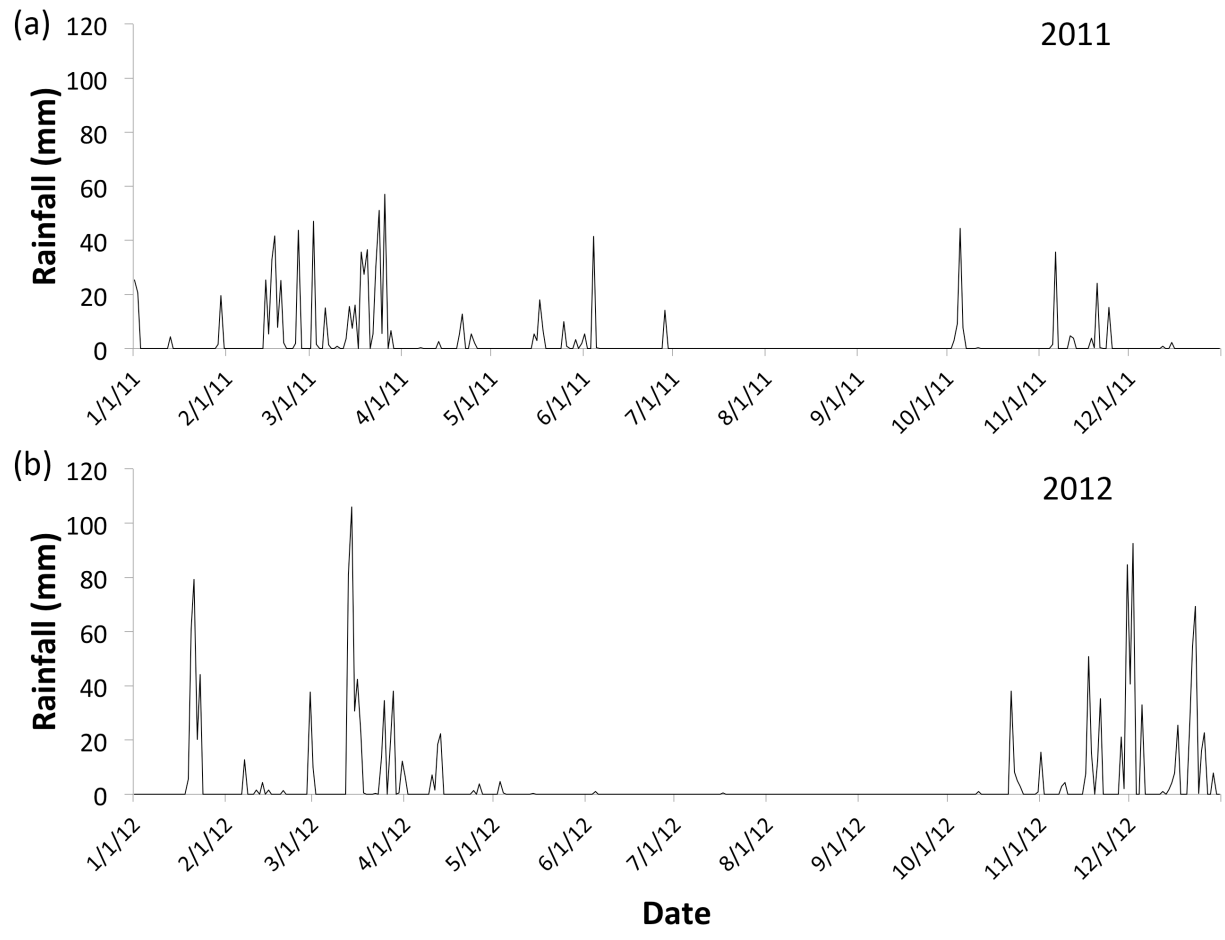
Figure 2. Rainfall for Kentfield, Marin County, California, USA for 2011 (a) and 2012 (b).
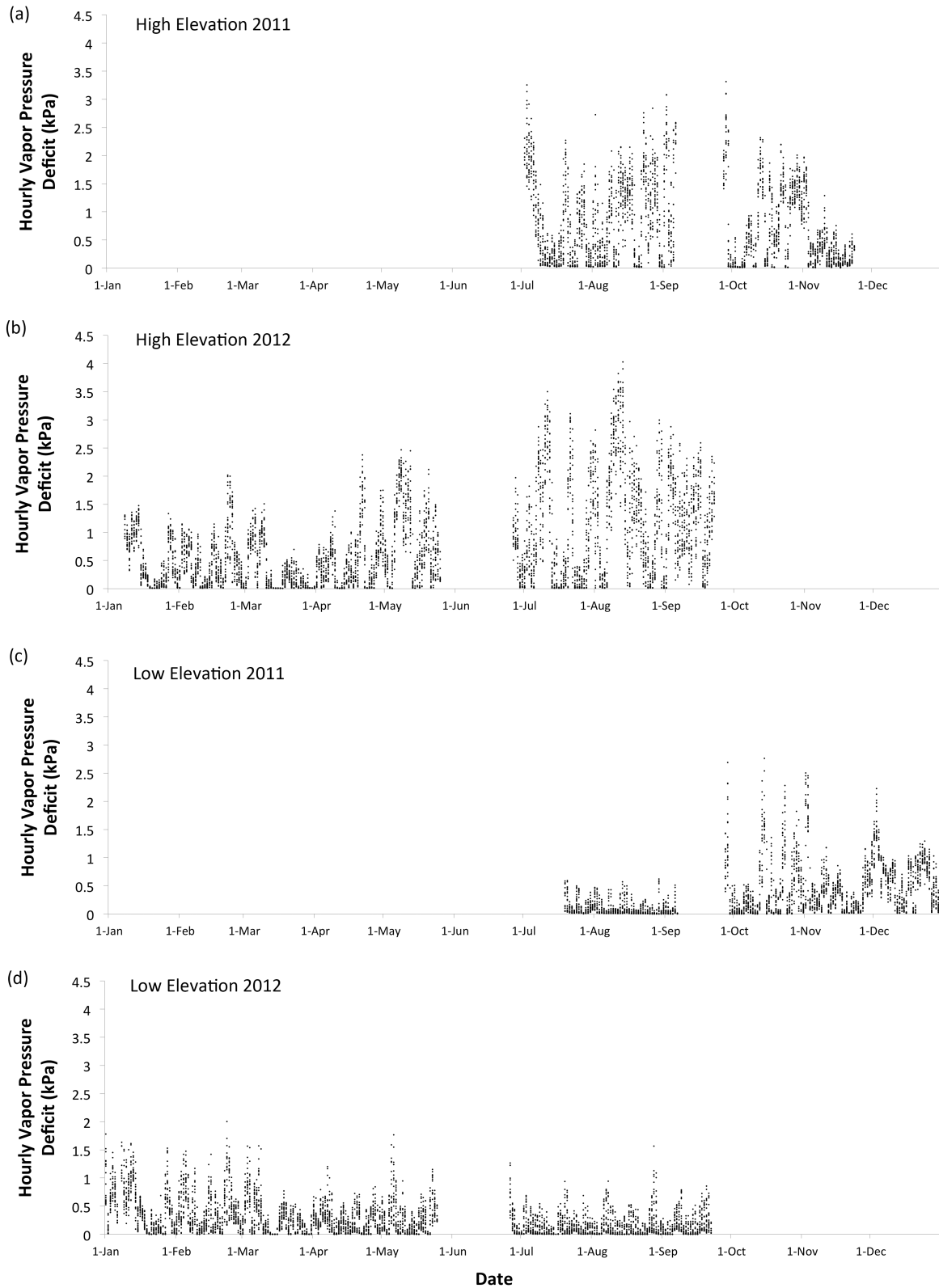
Figure 3. Vapor pressure deficit at high, (a) and (b), and low elevation, (c) and (d), study sites during 2011, (a) and (c), and 2012, (b) and (d), at Mt. Tamalpais State Park, Marin County, California, USA.

Water use and water potential

Contrary to our hypothesis, midday g_s for all plants at both elevations increased during the rainless summer and then decreased the following wet season (Fig. 4). Stomatal conductance was significantly different between elevations and the different measurement dates but not between adults and seedlings (Table 2). On average g_s was 54.8% higher for plants at low elevation compared to high elevation plants and increased 58.8% per measurement date from the rainy season to the end of the dry season (Tables 1 and 2). The decrease in g_s for both adults and seedlings at high elevation August 23, 2011 was likely due to high leaf temperatures, which ranged from 34.2-36.9 °C for adults and from 35.7-37.1 °C for seedlings as measured by the LI-1600. We did not include values for September 10, 2011 because the LI-1600 porometer could not accurately measure g_s in relative humidity greater than 80%, and relative humidity at low elevation ranged from 90.4-96.8% and at high elevation relative humidity ranged from 66.0-83.6%.

B. pilularis plants showed similar diurnal responses in their water potentials for each measurement date, decreasing (getting more negative) during peak sunshine and VPD and recovering to near predawn levels by early evening (Fig. 5). During summer plants at low elevation generally had less negative water potentials than plants at high elevation while during the wet season all plants had similar water potentials. There were several days where the diurnal decrease in water potentials was delayed due to overcast or foggy conditions, particularly at low elevation (Figs. 5b and 5d). Further, diurnal patterns in water potentials diverged for low and high elevation plants on September 10, 2011. On this day foggy, breezy, and otherwise cool conditions at low elevation kept plant water potentials high (less negative) while water potentials for plants at high elevation were lower, reflecting the mostly sunny conditions. Eight o'clock p.m. measurements on March 10, 2012 were not performed due to very light rain.

Trends for seasonal predawn water potentials were similar for plants of both ages at both elevations but diverged during midday hours (Figs. 5g and 5h). Seasonal predawn water potentials were similar with a statistically significant decrease from the rainy season through June and to September (Tables 1 and 2). In contrast, midday water potentials were significantly different between adults and seedlings, between low and high elevations, and between dates (Tables 1 and 2). Including both elevations and across all dates, adults had on average 23% higher (less negative) midday water potentials than seedlings. Moreover, all plants at low elevation had midday water potentials 30.5% higher than all plants at high elevation, and midday water potentials for plants for both elevations and ages decreased by 7% for each measurement date from the rainy season to the end of summer. There were larger differences in predawn and midday water potentials for plants at high elevation, with high elevation seedlings having the largest differences (Fig. 5i). Low elevation seedlings had less of a difference between predawn and midday, with seedlings showing the similar trend of having larger difference than adults except for the September 10, 2011 measurements when both low elevation adults and seedlings responded similarly. Differences between predawn and midday water potentials for all plants decreased from June through September.
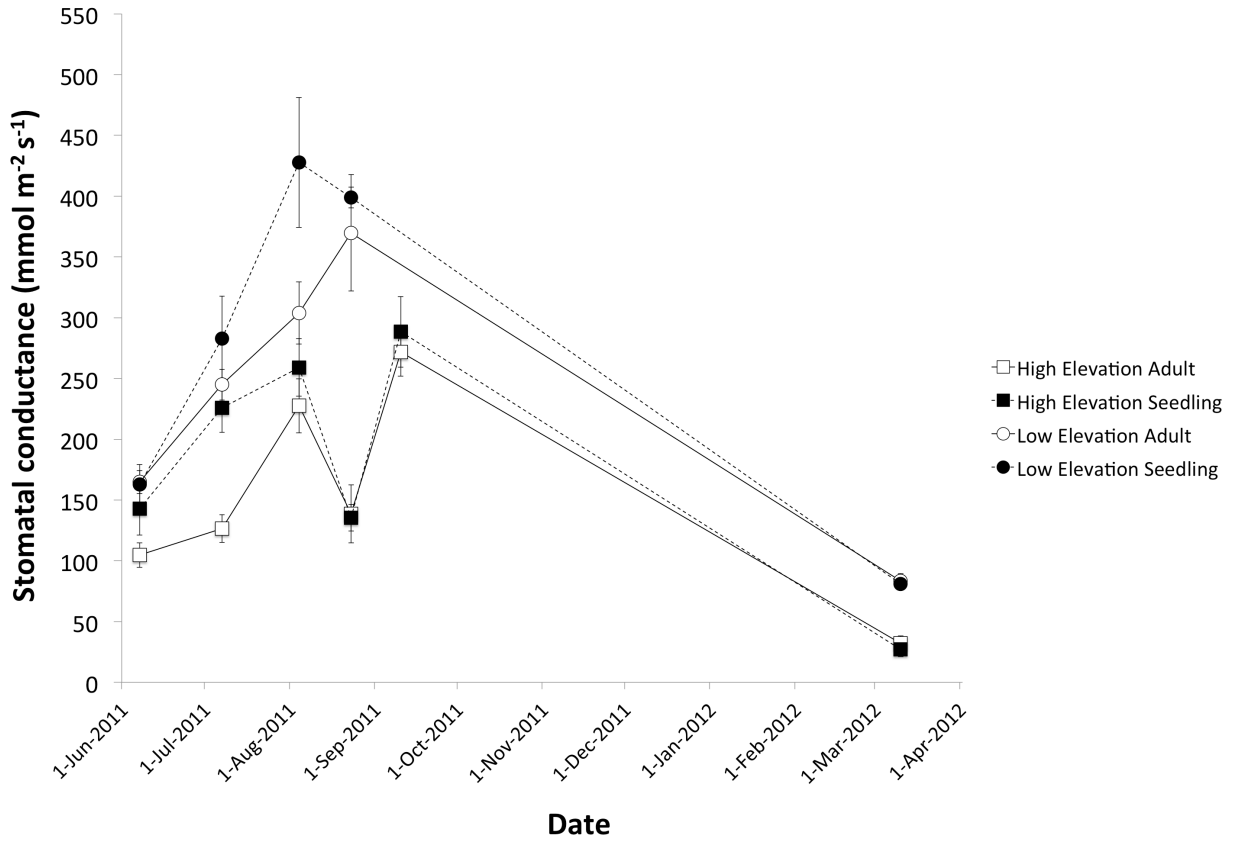
Figure 4. Stomatal conductance *Baccharis pilularis* adults (solid) and seedlings (open) at high (squares) and low (circles) elevations at Mt. Tamalpais State Park, Marin County, California, USA. (±SE).
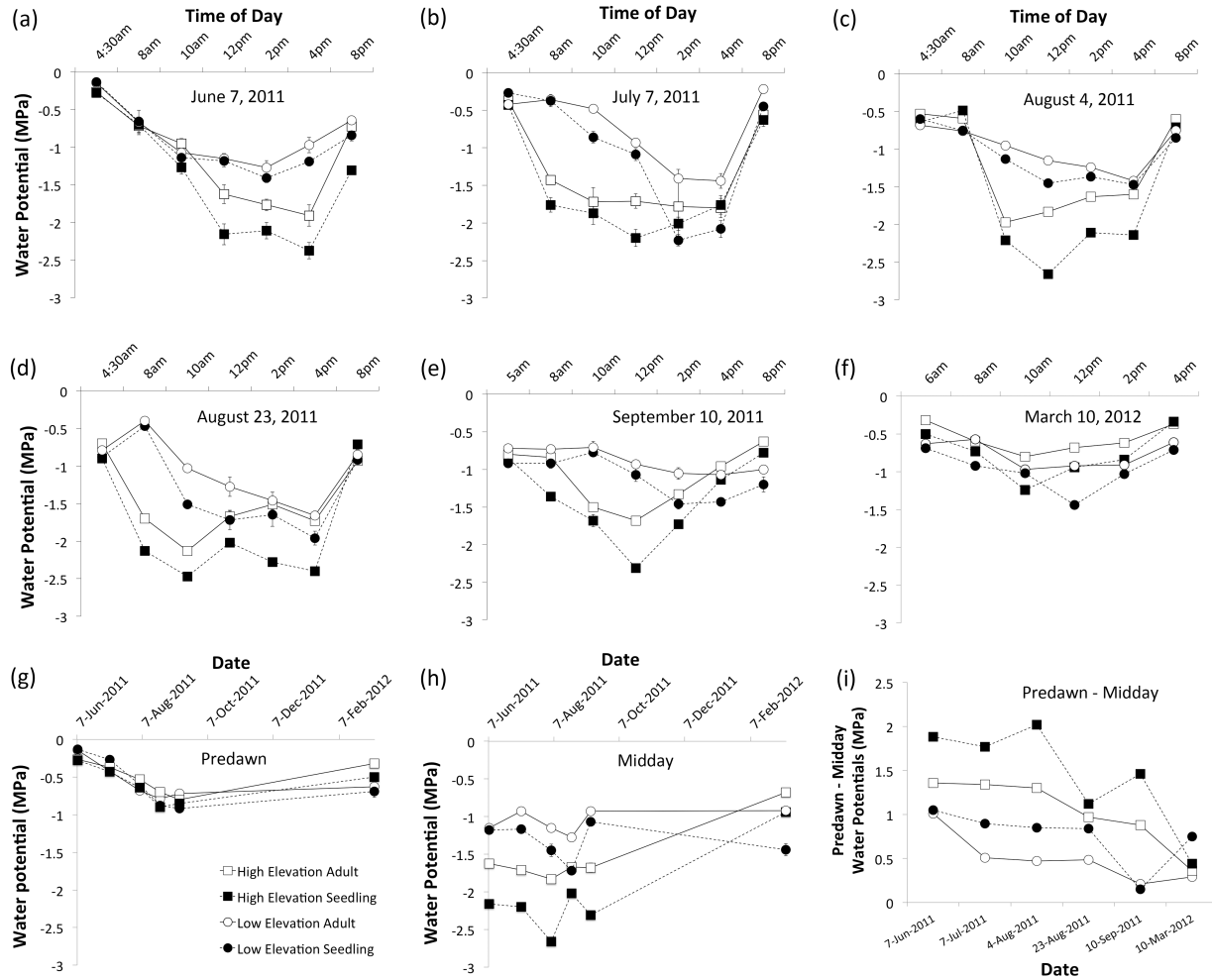
Figure 5. Seasonal diurnal, predawn, and midday water potential measurements. (a) June 7, 2011, (b) July 7, 2011, (c) August 4, 2011, (d) August 23, 2011, (e) September 10, 2011, (f) March 10, 2012, (g) seasonal predawns, (h) seasonal middays, (i) difference between mean predawn and mean midday water potentials (no error bars). ($\pm$ 1 SE)

Tissue water relations

Separate multiple regressions were run with the absolute value of each $\log_{10}$-transformed tissue water relations parameter as the dependent variable. All multiple regressions explained a significant amount of variance in the data except for ε (Table 1). Solute potential was not significantly different between the different ages and elevations but was significantly different between dates, with a 4.4% decrease in Ψ_{SFT} for every measurement date from the wet season to the end of the dry season (Tables 1 and 2, Fig. 6a). Turgor loss point was significantly different between adults and seedlings, with adults estimated to have 14% more negative Ψ_{TLP} than seedlings (Tables 1 and 2). Further, the model predicted the Ψ_{TLP} to be 3.6% more negative for every measurement date as water availability decreased from the wet season through the dry season (Table 1, Fig. 6b). Bulk modulus of elasticity was not significantly different between ages, elevations, or dates (Fig. 6c). Multiple regression explained a significant amount of variation in $C_{T,mass}$ and elevation and age were each significant predictor variables (Tables 1 and 2). Date was not a significant predictor so it follows there was no seasonal trend (Fig. 6d). Low

elevation plants had an estimated overall 33% higher $C_{T,mass}$ than high elevation plants, although there was a large amount of variability, particularly in low elevation seedlings, that might have accounted for much of the estimated difference. High elevation adult plants consistently had the lowest $C_{T,mass}$ values. Although statistically significant, our multiple regression model did not strongly predict RWC_{TLP} (Tables 1 and 2). Low elevation plants, the only significant predictor variable for RWC_{TLP}, had an estimated 4% lower RWC_{TLP} than plants at high elevation (Fig. 6e). Adults at high elevation had consistently high RWC_{TLP} relative to other plant types while adults at low elevation generally lower RWC_{TLP}, and all types had similar RWC_{TLP} during the rainy season.
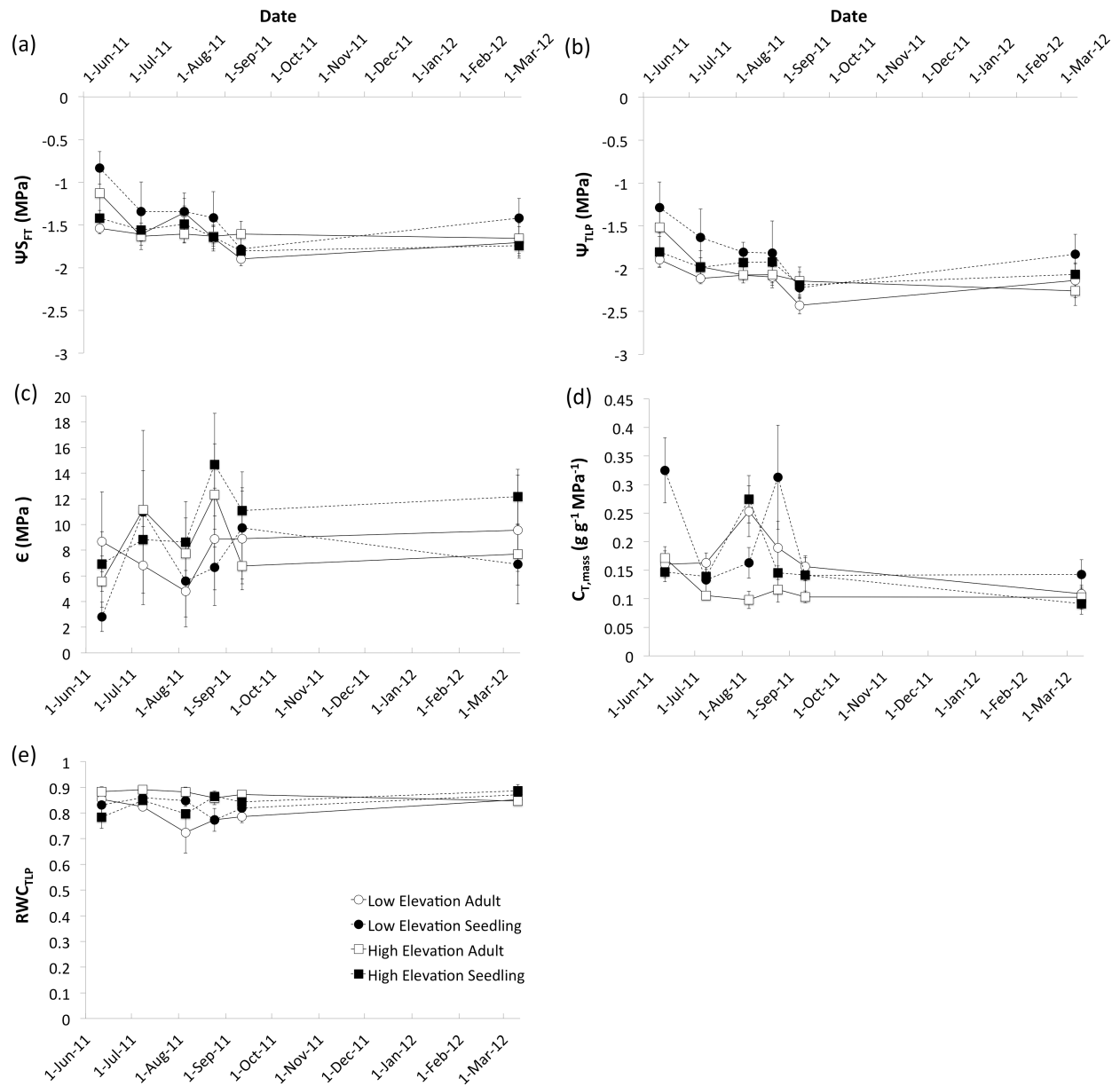
Figure. 6. Tissue water relations parameters derived from pressure-volume curves. (a) osmotic potential at full turgor (Ψ_{SFT}), (b) water potential at turgor loss point (Ψ_{TLP}), (c) bulk modulus of elasticity (ε), (d) mass-based capacitance before turgor loss point ($C_{T,mass}$), and (e) relative water content at turgor loss point (RWC_{TLP}). ($\pm$ 1 SE)

Table 1. Summary of multiple regressions for seasonal midday stomatal conductance (g_s), seasonal predawn and midday water potentials, and tissue water relations parameters (Ψ_{SFT}, Ψ_{TLP}, ε, $C_{T,mass}$, RWC_{TLP}). The absolute value of each dependent variable was log-transformed. Predictor variables of elevation, age, and time were coded with dummy variables as described in text. Coefficients in the model, $y = \beta_0 + \beta_1(low) + \beta_2(adult) + \beta_3(date) + error$, were considered significant if $p \le 0.05$.

Dep Variable	Estimated Model ($\log_{10}(|y|)$)	R^2	DF		F-Ratio	P-value
g_s	$1.672037 + 0.1897695(low) -0.064029(adult) + 0.2008018(date)$	0.637972	Model	3	55.8036	<0.0001
			Error	95		
Seasonal predawn water potentials	$-0.527323 - 0.025395(low) - 0.035586\ (adult) + 0.0047029(date)$	0.365302	Model	3	23.2139	<0.0001
			Error	121		
Seasonal midday water potentials	$0.2108628 - 0.15786(low) - 0.112824(adult) + 0.0293632(date)$	0.469415	Model	3	34.7987	<0.0001
			Error	118		
Ψ_{SFT}	$0.1114379 - 0.039172(low) + 0.0474298(adult) + 0.0188838(date)$	0.078284	Model	3	3.1708	0.0271
			Error	112		
Ψ_{TLP}	$0.2249211 - 0.026072(low) + 0.0567677(adult) + 0.015388(date)$	0.097853	Model	3	4.0494	0.0090
			Error	112		
ε	$0.8192382 - 0.105236(low) + 0.0203564(adult) + 0.0270444(date)$	0.057287	Model	3	2.2687	0.0844
			Error	112		
$C_{T,mass}$	$-0.884652 + 0.1252575(low) - 0.09157(adult) + 0.0114794(date)$	0.149462	Model	3	6.5605	0.0004
			Error	112		
RWC_{TLP}	$-0.06285 - 0.018217(low) + 0.0040058(adult) - 0.003751(date)$	0.091563	Model	3	3.7629	0.0128
			Error	112		

Table 2. Summary of multiple regression predictor variables for water relations physiology dependent variables. Significance at $p \leq 0.05$ (in bold, except for intercepts). All dependent variables were $\log_{10}$-transformed.

Dependent Variable	Predictor	t-ratio	P
g_s (mmol m^{-2} s^{-1})	Intercept	31.97	<0.0001
	low	4.07	**<0.0001**
	adult	-1.37	0.1726
	date	12.13	<0.0001
Seasonal predawn water potentials (MPa)	Intercept	-11.98	<0.0001
	low	-0.65	0.5137
	adult	-0.92	0.3607
	date	8.26	**<0.0001**
Seasonal midday water potentials (MPa)	Intercept	8.73	<0.0001
	low	-7.32	**<0.0001**
	adult	-5.23	**<0.0001**
	date	4.67	**<0.0001**
Ψ_{SFT} (MPa)	Intercept	3.47	0.0007
	low	-1.34	0.1823
	adult	1.63	0.1068
	date	2.26	**0.0258**
Ψ_{TLP} (MPa)	Intercept	8.65	<0.0001
	low	-1.10	0.2719
	adult	2.41	**0.0178**
	date	2.28	**0.0248**
$C_{T,mass}$ (g g$^-$1 MPa^{-1})	Intercept	-22.45	<0.0001
	low	3.50	**0.0007**
	adult	-2.56	**0.0119**
	date	1.12	0.2658
RWC_{TLP} (decimal)	Intercept	-8.44	<0.0001
	low	-2.69	**0.0081**
	adult	0.59	0.5546
	date	-1.94	0.0554

Stable isotopes

Carbon. $\delta^{13}C$ values for bulk leaf tissue collected September 22, 2012 during the rainless fog season were significantly different between low and high elevations ($F_{1,15} = 10.6533$, $p = 0.0052$) and between adults and seedlings ($F_{1,15} = 6.4446$, $p = 0.0227$), with no significant interaction effect between elevation and age (Fig. 7). Seedlings at both elevations had significantly lower $\delta^{13}C$ values than adults at both elevations, and low elevation plants of both ages had significantly lower $\delta^{13}C$ values than adults and seedlings at high elevation.

Water. A detailed description of the LMWL, deuterium excess, soil water isotope profiles, and soil evaporation lines are included in Kidder (2015b). In order to qualitatively compare adult and seedling *B. pilularis* xylem waters to water sources, we plotted adult and seedling xylem water δ^2H and $\delta^{18}O$ values with the LMWL for high (Fig. 8) and low elevations (Fig. 9).

At low elevation, deuterium and $\delta^{18}O$ values for deep (60-100cm) and shallow (10-40cm) soil waters, ground water, and fog plotted in largely separate isotope space (Fig. 9). Rain waters were more variable in their water isotope composition, which depended on the origin and temperature of incoming storm systems. During the dry season most *B. pilularis* seedlings used a

large amount of fog water, as seedlings plotted close to fog waters. Dry season xylem waters for adult *B. pilularis* plotted near groundwater, indicating these plants likely relied on deeper water sources. During the wet season, adults used deeper water sources than seedlings. Both adults and seedlings at low elevation plotted along the soil evaporation line, with seedling xylem water generally consisting of more enriched water than adults.

At high elevation, deep and shallow soils plotted with more overlap, with shallow soils plotting around the soil evaporation line. Ground waters plotted between rain and fog water sources and rain was as variable as low elevation rain. During the dry season adults and seedlings used similar water sources while during the wet season adults used ground water and seedlings used a mix of shallow and deep waters.

These results are reflected in the ANOVA of adult and seedling xylem water δ^2H values, which revealed significant interactions of elevation by age ($F_{1,67} = 6.1396$, $p = 0.0157$) and season by age ($F_{1,67} = 11.5190$, $p = 0.0012$; Figs. 10a and 10b, respectively), indicating differences in δ^2H composition between elevations and time of year indeed depended on plant age.
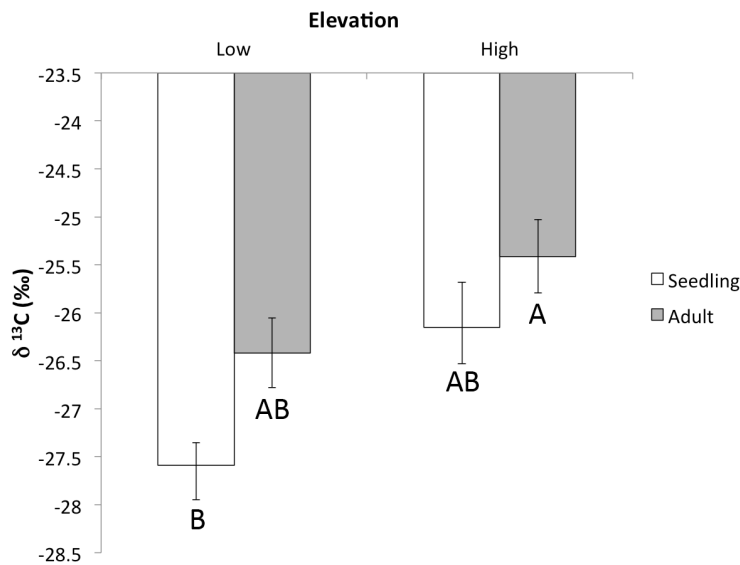

Figure 7. Stable isotopes of carbon for bulk leaf tissue of adults and seedlings at high and low elevations collected on September 22, 2012 in Mt. Tamalpais State Park, Marin County, California, USA. (±SE)

Table 3. Mean (SD) δ^2H and δ^{18}O for xylem water of *Baccharis pilularis* seedlings and adults.

Year	Date	Season	Elevation	Age	N	δ^2H (‰)	δ^{18}O (‰)
2011	May 12	Wet	Low	seedling	3	-37.63 (2.81)	-4.31 (1.00)
				adult	3	-34.73 (2.20)	-4.98 (0.48)
			High	seedling	3	-43.73 (3.93)	-3.39 (0.42)
				adult	3	-33.67 (1.67)	-4.73 (0.18)
	August 27	Dry	Low	seedling	4	-36.25 (5.75)	-4.05 (1.04)
				adult	4	-39.00 (2.15)	-4.92 (0.21)
	September 23	Dry	Low	seedling	3	-31.47 (5.22)	-3.62 (0.94)
				adult	3	-34.20 (3.50)	-4.59 (0.63)
2012	March 5	Wet	Low	seedling	2	-28.05 (1.06)	-3.88 (0.33)
				adult	3	-30.67 (1.16)	-4.23 (0.44)
	May 19	Wet	Low	seedling	3	-32.53 (3.14)	-4.37 (0.72)
				adult	3	-34.00 (1.80)	-4.83 (0.47)
			High	seedling	3	-38.17 (1.76)	-5.42 (0.32)
				adult	3	-34.90 (0.96)	-4.84 (0.19)
	August 17	Dry	Low	seedling	5	-36.22 (2.61)	-4.12 (0.63)
				adult	5	-39.14 (1.87)	-5.18 (0.35)
			High	seedling	5	-40.32 (2.59)	-5.22 (0.73)
				adult	5	-41.00 (1.38)	-5.18 (0.32)
	September 22	Dry	Low	seedling	3	-36.40 (2.55)	-4.72 (0.50)
				adult	3	-38.00 (0.69)	-5.35 (0.25)
			High	seedling	3	-39.17 (2.48)	-4.82 (0.39)
				adult	3	-41.37 (0.51)	-5.42 (0.18)

Table 4. ANOVA model δ^2H = season + elevation + (season*elevation) + age + (season*age) + (elevation*age) + (age*season*elevation). Significant parameters are in bold ($p < 0.05$).

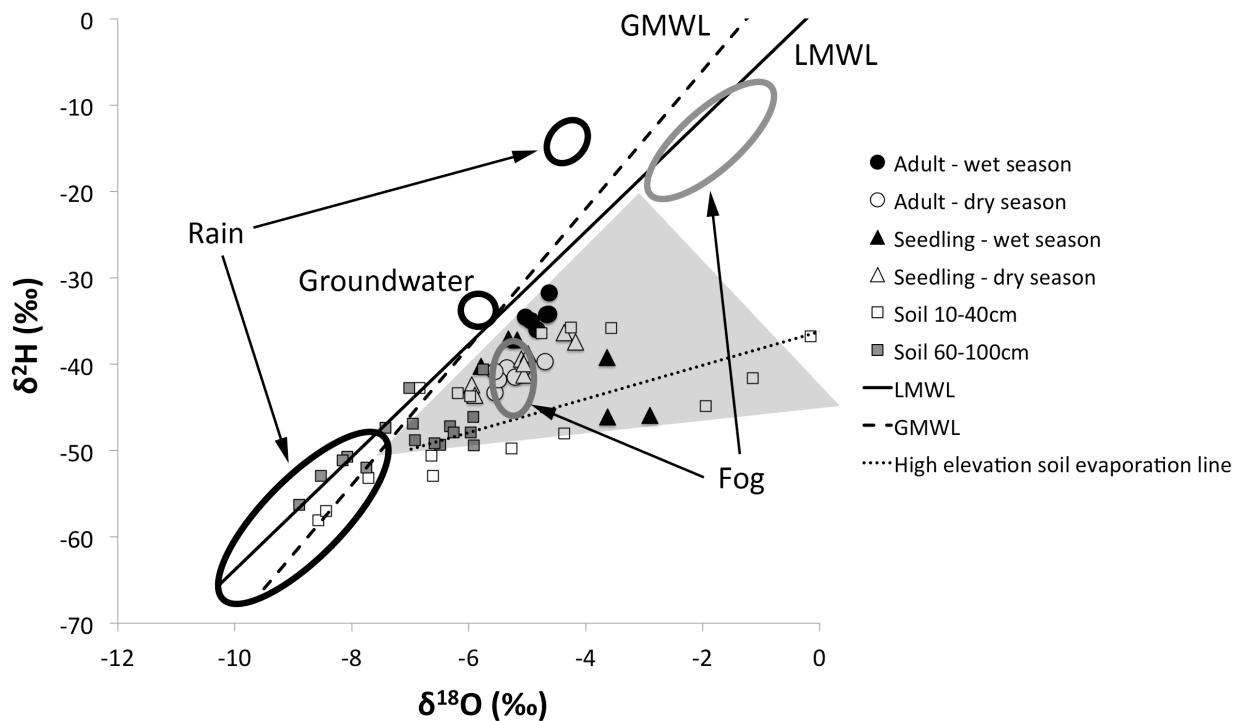|		δ^2H (‰)		
Effect	df	SS	F	P
season	1	164.87	15.8588	< 0.0002
elevation	1	289.22	27.8197	<0.0001
season*elevation	1	0.95	0.0911	0.7637
age	1	0.67	0.9299	0.3383
season*age	1	119.75	11.5190	**0.0012**
elevation*age	1	63.83	6.1396	**0.0157**
season*elevation*age	1	27.86	2.6794	0.1063
Error	67	1333.04		

Figure 8. δ^2H and δ^{18}O values for **high elevation** *B. pilularis* adult and seedling plant xylem waters during the wet and dry seasons, high elevation soil waters in shallow (10cm to 40cm) and deep (60cm to 100cm) soils, means of high elevation water sources (means calculated for each sampling date), and the high elevation soil evaporation line (δ^2H = 2.19× δ^{18}O −35.93; points not shown for clarity) for Mt. Tamalpais State Park, Marin County, California, USA. Local Meteoric Water Line (LMWL) derived from rain and fog waters collected from both elevations (δ^2H = 6.6208127*δ^{18}O + 2.1093331 (‰)) and the Global Meteoric Water Line (GMWL) is δ^2H = 8*δ^{18}O + 10 (‰) (Craig 1961, Rozanski et al. 1993). Large gray triangle represents estimated isotope space for *B. pilularis* described in Discussion. Error bars for high elevation water sources are not included for clarity (fog: δ^2H SD = ±13.4‰, δ^{18}O SD = ±1.78‰; ground water: δ^2H SD = ±0.27‰, δ^{18}O SD = ±0.05‰; rain: δ^2H SD = ±23.7‰, δ^{18}O SD = ±2.45‰).
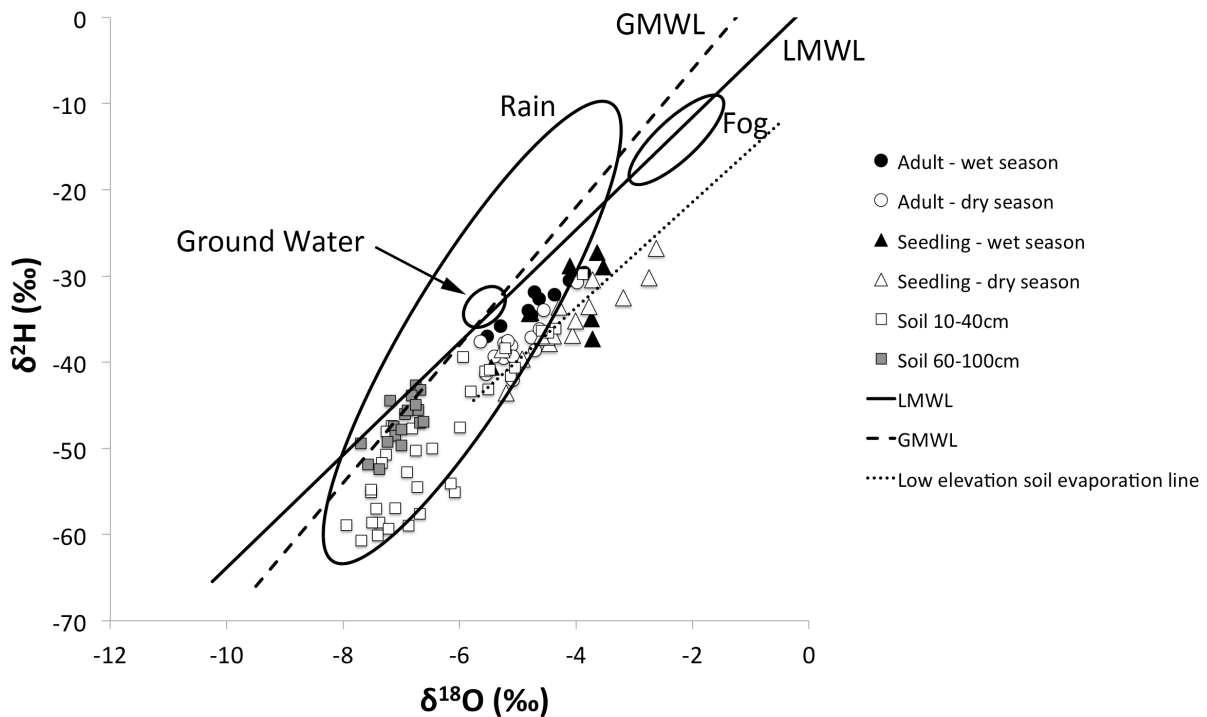
Figure 9. δ^2H and $\delta^{18}O$ values for **low elevation** *B. pilularis* adult and seedling plant xylem waters during the wet and dry seasons, low elevation soil waters in shallow (10cm to 40cm) and deep (60cm to 100cm) soils, means of low elevation water sources (means calculated for each sampling date), and the low elevation soil evaporation line ($\delta^2H = 6.81 \times \delta^{18}O -6.36$ (‰); points not shown for clarity) for Mt. Tamalpais State Park, Marin County, California, USA. Local Meteoric Water Line (LMWL) derived from rain and fog waters collected from both elevations ($\delta^2H = 6.6208127 * \delta^{18}O + 2.1093331$ (‰)) and the Global Meteoric Water Line (GMWL) is $\delta^2H = 8 * \delta^{18}O + 10$ (‰) (Craig 1961, Rozanski et al. 1993). Error bars for low elevation water sources are not included for clarity (fog: δ^2H SD = ± 2.1‰, $\delta^{18}O$ SD = ± 0.35‰; ground water: δ^2H SD = ± 0.53‰, $\delta^{18}O$ SD = ± 0.07‰; rain δ^2H SD = ± 18.1‰, $\delta^{18}O$ SD = ± 1.72‰).
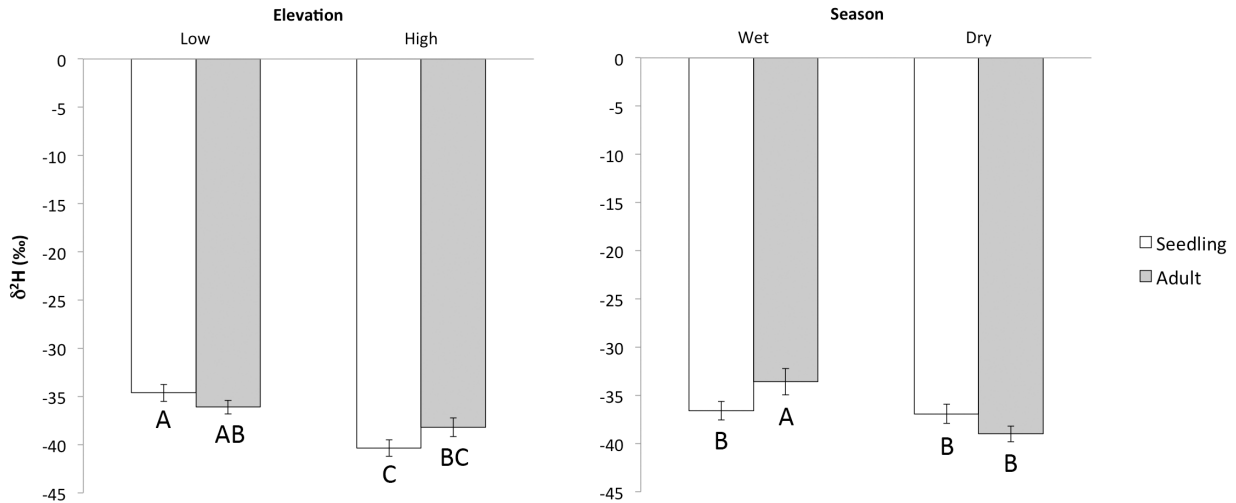
Figure 10. ANOVA results for δ^2H values of the significant interaction effects elevation*age and season*age. ($\pm$SE; letters indicate significant differences)

Discussion

Water use, water status and overall performance

Our measurements of fog water deposition, temperature, and relative humidity confirmed that the grassland habitats at the two elevations on Mt Tam were quite different. At low elevation, the moisture subsidy provided by atmospheric fog water combined with the lower vapor pressure deficits and the moderating effects of the site's close proximity to the ocean result in a habitat with an overall milder microclimate that includes less water lost through evapotranspiration and potentially more plant available water as also seen by Fischer et al. (2009) on Santa Cruz Island. These differences between the habitats likely allowed for higher rates of water use (stomatal conductance), overall better plant water status (less negative midday water potentials), and better integrated performance (more negative $\delta^{13}C$ values) for both adults and seedlings at low elevation when compared with our high elevation site.

While we predicted *B. pilularis* stomatal conductance (g_s) to decrease during the dry season and increase during the wet season based on other related work (Mooney et al. 1975, Ehleringer 1985, Kramer and Boyer 1995), *B. pilularis* of both ages at both elevations showed the same unexpected seasonal trend in g_s rates as exhibited by both morphotypes reported in Kidder (2015a); g_s steadily increased from wet season to peak dry season. It is well accepted that g_s rates are strongly correlated to photosynthetic rates (Wong et al. 1979), suggesting that higher conductance to water vapor often results in higher intake of CO_2 and therefore higher rates of carbon fixation (Körner et al. 1979). The higher g_s rates by low elevation plants are striking because one would therefore expect low elevation plants might also have high growth rates due to resulting higher levels of available carbon, especially low elevation seedlings, which had the highest measured g_s rates in our study. However, when we tracked the growth of experimentally-planted *B. pilularis* seedlings at both elevations (Kidder 2015b), we found that high elevation seedlings grew several times larger than low elevation seedlings. The study site's close proximity to the immediate coast suggests that wind may have prompted stomatal closure but this appears not to be the case as rates remained high during the summer months when westerly winds are typically strongest. High elevation seedlings also had the most negative midday water potentials, indicating these plants experienced greater water deficits and this is likely linked to this site's

high VPDs. The lower g_s rates, lower midday water potentials, and higher growth rates of high elevation plants suggest that *B. pilularis* g_s and carbon fixation rates might be decoupled. Our overall findings strongly support the assertion that plants at high elevation used water more efficiently (less water lost as water vapor per unit of carbon gained) than low elevation plants based on the higher $\delta^{13}C$ values of high elevation plants. Despite *B. pilularis*' ability to have high carboxylation rates during times of low water availability, the correlation of invasion success with late spring rainfall in previous research (Williams et al. 1987, Williams and Hobbs 1989) is not unfounded because such rainfall would still help *B. pilularis* seedlings compete with neighboring grasses, promoting succession from grassland to shrubland.

The pattern of increasing g_s during the rainless summer season coincides with decreasing predawn water potentials during the same timeframe. This suggests that plants were indeed experiencing higher water use and not able to recover at the end of the rainless summer in September as they were in early June. The lack of significant differences in seasonal predawn water potentials between plant age and elevation suggest that all plants recovered to similar levels overnight despite differing VPDs and moisture subsidies from fog water deposition. However, the larger differences between predawn and midday water potentials in high elevation plants reflects the more intense driving force of water movement at this site as opposed to the more moderate VPDs driven by the presence of fog water at low elevation where plants had smaller differences. The seasonal decrease in differences between predawn and midday water potentials suggests a concurring decrease in the driving force of water movement from soil to plant and decreasing soil water availability over the course of the rainless summer months.

Tissue water relations

For *B. pilularis* plants at both elevations it appears that they compensated for decreasing water availability by decreasing the turgor loss point over the course of the dry season. Lower turgor loss points and seasonal osmotic adjustment, such as in high elevation adults, are common in plants adapted to dry environments (Hinckley et al. 1983, Robichaux 1984, Dawson and Bliss 1989), and adult *B. pilularis* plants showed this same trend of seasonal osmotic adjustment to achieve lower turgor loss points as shown in Kidder (2015a). Turgor loss points are thought to be adjusted osmotically (Bartlett et al. 2012), although it is important to consider if this adjustment is passive or active (Morgan 1984), and if it is an artifact of aging of stem and leaf tissues over the course of the season as shown by Dawson & Bliss (1989). For *B. pilularis*, osmotic adjustment is likely active because both Ψ_{SFT} and Ψ_{TLP} significantly decreased in all plants (both elevations) equally over the course of the dry season, and they did so with the differences in seasonal fog water subsidy that could keep cells hydrated, preventing passive changes in cellular solute concentrations. By depressing seasonal Ψ_{TLP} via a decrease in Ψ_{SFT}, *B. pilularis* achieved higher water potentials and maintained higher tissue water content permitting increased g_s even as water availability waned. We were surprised to find that the lowest mean water potential of -2.66 MPa in this study measured in high elevation seedlings on August 4, 2011 was more negative than the turgor loss points for plants of both ages at both elevations measured from samples collected that same day. This would suggest that plants were living below their turgor loss point at least for some fraction of the day or season. Additional work is needed to more fully understand how these plants survive and thrive despite having what appears to be maladaptive water relations.

Water isotopes

In order to discern the location, movement, and plant use of different pools of water within an ecosystem, we can take advantage of our dual isotope approach by developing a conceptual ecohydrological model using δ^2H and $\delta^{18}O$ values of water sources and plant xylem water (Dawson and Simonin 2011, Goldsmith et al. 2012). The model represents the "universe" of plant-water interaction in an ecosystem that depends on the physical properties of the soil and the intensity and temperature of rain events. This area of isotope space is constructed using a site's LMWL, soil evaporation line, and δ^2H and $\delta^{18}O$ of plant xylem waters (Dawson and Simonin 2011). It is within this area that isotopic composition of source and plant waters varies based on when and how efficiently rain waters percolate into the soil profile, mix with rain waters from previous rain events, and evaporate into atmospheric water vapor.

At our high elevation site, δ^2H and $\delta^{18}O$ xylem water values place *B. pilularis* adults and seedlings within a triangular area that is bounded by the LMWL on the top, the soil evaporation line on the bottom, and on the right by the heaviest (least negative) *B. pilularis* xylem water values (Fig. 8). This bounded triangular isotope space represents the range of soil and meteoric waters sources used by *B. pilularis* adults and seedlings within this annual dominated grassland and serves as a conceptualization of the ecohydrological relationships within this grassland-shrubland ecotone. At high elevation ground water δ^2H and $\delta^{18}O$ values plot within the top edge of the triangle, indicating this was an important water source for *B. pilularis*. The soil, fog, and rain waters that plot outside of this conceptual triangle (beyond the area in which *B. pilularis* xylem waters are located) indicate these plants were not using these water sources. In contrast, the deep and shallow soil waters plotting within this triangle are "seen" by *B. pilularis* adults and seedlings and depict the mixing of meteoric and soil waters over time within this grassland ecosystem. High elevation seedlings plotted close to the LMWL amidst ground and rain water sources and near adults, especially during the dry season, suggesting these seedlings were able to grow deep roots and take in water sources similar to adults. The higher above-ground growth rates by seedlings at this elevation (Kidder 2015b) suggest corresponding below-ground root growth (Wright 1928), making it possible for seedlings to reach deep water sources. It also provides additional evidence *B. pilularis* seedlings easily withstand high VPDs and low water availability in order to acquire more light.

While this conceptual isotope space was well defined for samples collected at high elevation, it was not well defined at low elevation. This is largely because the soil evaporation line was parallel to that elevation's LMWL, likely a result of the moderate climatic conditions of this site (Fig. 9). Even so, it is still possible to estimate coarse-scale water use using dual isotope space and the LMWL. During both wet and dry seasons, the significantly more enriched δ^2H in low elevation plant xylem water is likely linked to the use of shallow water sources susceptible to evaporation (wet season) and fog water (summer fog season). This trend was strongest in seedlings, which had the most enriched mean δ^2H values of all plants (e.g. closest to those matching fog water). It follows that coastal fog helped low elevation seedlings maintain high g_s rates during the rainless summer months, thereby facilitating their successful establishment into the maritime-influenced coastal prairie.

Our stable water isotopes results revealed differences in seasonal and ontogenetic water use and water relations that in turn reflect the differences between the two grassland habitats. By integrating conceptual stable water isotope-based ecohydrological models with plant physiological measurements such as g_s, water potentials, and tissue-level water relations

parameters, we provide additional evidence for the importance of water to the establishment of *B. pilularis* into California's coastal grasslands.

Conclusion

       Overall, our results demonstrate ontogenetic differences in seasonal *B. pilularis* water relations physiology and water use and support our hypothesis that adults would exhibit water relations traits reflecting an adaptation to low water availability. These adaptations and different water relations strategies between adults and seedlings in contrasting coastal grassland habitats suggest this plant's performance is limited more by temperature or light than by water availability. However, when present, coastal fog plays a key role for successful establishment of *B. pilularis* seedlings into grasslands. The ability of *B. pilularis* to cope with water deficits as a young plant is expected in such an early-successional species that is a long-lived evergreen shrub. Our study provides crucial information on this widespread plant by helping us understand how *B. pilularis* succeeds in a range of diverse microclimates.

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