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PHYSIOLOGICAL AND STRUCTURAL RESPONSES OF LOW SAGEBRUSH  
(*ARTEMISIA ARBUSCULA*) TO WARMING AND SNOWPACK REMOVAL  
IN A MONTANE MEADOW

by

Lindsay M. Curran

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## Committee Approval

To the Graduate Faculty:

The members of the committee appointed to examine the thesis of Lindsay M. Curran find it satisfactory and recommend that it be accepted.

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## CHAPTER 1. GENERAL INTRODUCTION

Climate change is an important driving force on natural systems (Parmesan and Yohe 2003). Recent changes in climate have been shown to affect the phenology and distribution of organisms, in addition to community dynamics, across multiple biomes (Walther et al. 2002, Walker et al. 2006). As a result of climate warming, montane plants have shifted distribution, upward in elevation (Grabherr et al. 1994). As global change progresses, we are faced with the challenge of predicting future effects of climate on distribution, adaptation, phenology, and physiology of organisms (Hughes 2000).

Our research focuses on change in western North America, with emphasis on both temperature and snowpack. In that geographical region, climate change is predicted to result in 2-5° C of warming, accompanied by changes in precipitation seasonality and snowpack by 2050 (IPCC 2007). Historically, snowpack in the western United States has been declining since the 1950s, and is expected to continue declining (Barnett et al. 2008). Those substantial decreases in snowpack have coincided with increases in temperature (Mote 2003, Hamlet et al. 2005) and decreases in westerly winds during winter (Luce et al. 2013). Snowpack losses are especially influential in mountain regions in western North America, because precipitation is winter-dominant, and snowmelt is the primary source of water during summer (Hamlet et al. 2005).

Future increases in temperature and changes in precipitation may affect plant communities, but that is debated in the literature (Lambrecht et al. 2007, McDowell et al. 2008, Chmura et al. 2011, Lloret et al. 2013). It is difficult to predict impacts of climate change on vegetation, because the mechanisms that influence plant survival are poorly understood (McDowell et al. 2008). In addition, few experiments have manipulated both

temperature and precipitation to investigate potential effects (Wu et al. 2011). To better predict plant responses to climate, it is necessary to examine responses of individual species (Lambrecht et al. 2007), and it is critical to observe plant physiology, which is the foundation of acclimation and adaptation to climate (Chmura et al. 2011).

We used an ongoing climate experiment to investigate warming and snowpack effects on the physiology and structure of low sagebrush (*Artemisia arbuscula*) in a montane meadow. We focused on *A. arbuscula* because it is a widespread shrub, inhabiting 112,000 km<sup>2</sup> in the Intermountain and Rocky Mountain regions (Beetle 1960). Low sagebrush is distributed in the Intermountain and Rocky Mountain regions- generally on ridges, mountain slopes, high valleys, and basins at high elevations (1,500–3,800 m) with rocky soils (Shultz 2009, Tilley and St. John 2012). The shrubs at our study site generally represent the mid-range distribution for both temperature and precipitation for *A. arbuscula* in western North America. *A. arbuscula* is known to reduce soil erosion and add structural and biological richness to communities. It also provides habitat and valuable, high-quality forage for animals, including: *Antilocapra americana*, *Cervus canadensis*, *Odocoileus hemionus*, *Ovis canadensis*, as well as many species of grouse (Sheehy and Winward 1981, Tilley and St. John 2012). In general, sagebrush has the ability to recharge soil water via roots by hydraulic redistribution, which is likely important for soil-water dynamics (Ryel et al. 2003). Sagebrush also influences the vegetation community within its range, supporting the presence of native vegetation (Beck et al. 2012).

Though *Artemisia arbuscula* is a critical species, it, along with other vegetation in subalpine meadows, is thought to be especially vulnerable to climate change, due to

sensitivity to small changes in environmental conditions (Harte and Shaw 1995, Debinski et al. 2000, Shaw et al. 2000). In particular, *A. arbuscula* is expected to be quite sensitive to changes in hydroclimate (snowpack amount and timing, and snowpack melt timing), because it relies heavily on winter precipitation (Miller et al. 2011). In general, sagebrush occupies an ecohydrological niche that is dependent on deep soil water, recharged from winter precipitation (Nielson et al. 2005, Schlaepfer et al. 2012). That soil water is required to supply demands of transpiration in the summer, which requires sufficient snowpack in semi-arid montane ecosystems. Despite its wide distribution throughout subalpine zones, little ecophysiological research has focused specifically on *A. arbuscula*, and montane ecosystems are generally little understood. Thus, it is unknown how climate change will impact *A. arbuscula* in subalpine meadows, at least to ecosystem scales. Such future responses to changes in climate may be predictable by understanding how resources, such as snowpack and temperature, affect major species. (de Valpine and Harte 2001).

In this study, we manipulated nighttime temperatures of *A. arbuscula* and surrounding soils, as well as decreased the amount of snowpack in the spring, prior to natural snowmelt. Those treatments were intended to mimic natural climate warming and a decrease in winter snowpack totals. We investigated the effects of those treatments on the physiology, growth, and structure of *A. arbuscula* at our study site to determine whether climate treatments had marked effects. Such research is critical to predict future changes in structure, function, and distribution of *A. arbuscula* in an important, vulnerable, and little-studied species of sagebrush in western North America.

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**CHAPTER 2. PHYSIOLOGICAL AND STRUCTURAL RESPONSES OF LOW  
SAGEBRUSH (*ARTEMISIA ARBUSCULA*) TO WARMING AND SNOWPACK  
REMOVAL IN A MONTANE MEADOW**

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## Abstract

In western North America, climate change is predicted to result in 2-5° C warming with declines in snowpack. Those changes may influence the vegetation in high-elevation communities, which are sensitive to environmental conditions. Using an ongoing experiment in a montane meadow, we investigated the effects of warming and decreased snowpack on the physiology and structure of *Artemisia arbuscula*. Results indicated that gas exchange, hydraulic conductivity, and intrinsic water-use efficiency were not affected by treatments, while water potentials were improved by warming. Treatments led to increased density of xylem vessels, suggesting adjustments in hydraulic architecture. Stem elongation and biomass were generally not affected, while inflorescence density increased. Overall, there were occasional changes in growth, despite few changes in physiology. Collectively, our findings indicate minimal changes in response to warming and decreased snowpack, suggesting that *A. arbuscula* occurring at mid-temperature and precipitation ranges may be somewhat resistant to small changes in climate.

## Introduction

Plant species at high elevations are considered to be quite sensitive to changes in environmental conditions. Plants in montane ecosystems have limited resources, which are generally available only during a short period in the growing season (Walker et al. 1995). In turn, plant functioning in high-elevation communities is tightly linked to a narrow range of temperature and water, and is sensitive to changes in those conditions (Knight 1994, Debinski et al. 2000). In addition, plants at high altitudes experience colder air temperatures and lower levels of CO<sub>2</sub>, which may result in more drastic responses to environmental change (Smith et al. 2009). In total, changing climate is likely to cause high rates of loss in montane species (Thuiller et al. 2005). Short-term changes in environment may affect the condition of montane vegetation, while long-term changes may result in directional adjustments in community structure (Harte and Shaw 1995). However, in semi-arid ecosystems that are characterized by a more variable climate, plants may have higher phenotypic plasticity and may be adapted to that heterogeneous climate (Lázaro-Nogal 2015). Such adaptations may result in lower susceptibility to changes. Nevertheless, much climate-change research has focused on alpine and tundra ecosystems (e.g. Van Wijk et al. 2003, Walker et al. 2006, Harsch et al. 2009). Less research has focused on mid-elevation forests and subalpine ecosystems (2000-3000 m), which is problematic because snowpack in those ecosystems is particularly sensitive to temperature changes, and thus warming results in a significant decrease in snowpack totals (Grundstein and Mote 2013). Understanding physiological and structural responses to changes in snowpack and temperature is critical for predicting susceptibility of montane vegetation at mid-elevations.

Plants may exhibit changes in physiology in response to changes in environmental conditions. For example, Lambrecht et al. (2007) reported that photosynthesis, stomatal conductance, and xylem water potentials of alpine forbs were reduced due to warming. Soil water has also been shown to affect the physiology of plants. For example, Kolb and Sperry (1999) found differences in hydraulic conductivity between species of sagebrush, which coincided with water availability in their individual habitats (where species from dry ecosystems were more resistant to hydraulic failure). In addition, Saleska et al. (1999) reported that decreased soil water likely caused lower photosynthesis in montane plants. Similarly, Moyes et al. (2013) demonstrated that low soil water corresponded with lower photosynthesis and respiration, plus more negative xylem water potentials in subalpine *Pinus flexilis*.

Plants may also exhibit changes in structure and growth in response to changes in environmental conditions. For example, de Valpine and Harte (2011) reported that six individual species of montane forbs responded to experimental warming- with changes in size, flowering, or overall abundance. Similarly, the growth of *Artemisia tridentata* increased in response to warming (Perfors et al. 2003). In contrast, *Artemisia tridentata* Nutt spp. *vaseyana* exhibited narrower growth rings due to increased temperatures during summer (Poore et al. 2009). Higher temperatures have also been shown to disrupt montane flowering abundance (Aldridge et al. 2011), sometimes resulting in flower reduction (Lambrecht et al. 2007). Warming treatments have also resulted in lower leaf area for subalpine forbs (Lambrecht et al. 2007). Montane plants have also exhibited structural or growth responses due to soil water. For example, Franklin (2013) reported that growth ring size of the alpine shrub *Linanthus pungens* coincided with snowpack soil

water equivalence in April, where wider growth rings were correlated with lower soil water values. Alternatively, Poore et al. (2009) demonstrated that *Artemisia tridentata* Nutt spp. *vaseyana* exhibited larger growth rings in conjunction with more snowpack. Similarly, Loik et al. (2013) reported that ring widths were wider in high-elevation *Pinus jeffreyi* with more snowpack. In Wyoming, deep snowpack behind snow fences has resulted in decreased biomass and cover of herbaceous plants (Perryman et al. 2000). In contrast, in a climate experiment in Colorado, increased snowpack translated to less mortality of buds, and therefore, greater abundance of forb flowering (Inouye 2008), and decreased soil water led to increased biomass of *Artemisia tridentata* (Harte and Shaw 1995).

We used an ongoing climate experiment to investigate warming and snowpack effects on the physiology and structure of low sagebrush (*Artemisia arbuscula*) in a montane meadow. We focused on *A. arbuscula* because it is a widespread and locally dominant shrub, inhabiting 112,000 km<sup>2</sup> in the Intermountain and Rocky Mountain regions, and it is a keystone species (Beetle 1960, Sheehy and Winward 1981, Shultz 2009, Tilley and St. John 2012). We also chose this high-elevation shrub because it lies at elevations in which plants are sensitive to small changes in environmental conditions (Harte and Shaw 1995, Debinski et al. 2000, Shaw et al. 2000). In particular, sagebrush has been shown to be sensitive to hydroclimate (snowpack amount and timing, and snowpack melt timing), because it relies heavily on winter precipitation (Nielson et al. 2005, Miller et al. 2011, Schlaepfer et al. 2012). Our goal was to quantify changes in physiology and structure of *A. arbuscula* subjected to warming and snowpack removal manipulations over the 2014 growing season. We hypothesized that these treatments would cause marginal

physiological responses in *A. arbuscula*, as has been reported previously for sagebrush species at high elevation warming experiments. We further hypothesized that snowpack removal and experimental warming would lead to increased growth, as the treatments would alleviate thermal limitations to growth in this energy-limited system.

## Materials and Methods

### *Study site and treatments*

Our research was conducted from May through October 2014. We used an existing research site, established in 2009, in a montane meadow in Grand Teton National Park, Wyoming, USA (43°54' N, 110°34' W). The research site is located at 2,120 m in elevation, which is characterized by a mean annual temperature of 1.7 ° C, a mean maximum temperature of 18.6 ° C in July, and a mean minimum temperature of -19 ° C in January. The site receives an average of 630 mm of precipitation per year. The majority of annual precipitation falls as snow generally between November and April. Snowpack averages 3,600 mm depth, where ~550 mm persists until April each year. The vegetation of the meadow consists of *Artemisia arbuscula* (low sagebrush), as well as flowering forbs, including: *Balsamorhiza sagittata*, *Eriogonum umbellatum*, and *Senecio integerrimus*, and is intermixed with bare ground. Soils consist of fine to gravelly sandy loam with 1–4% organic matter (Soil Survey Staff 2013).

### *Experimental design*

The experimental design consisted of 12 plots, each  $2.5 \times 2.5$  m, with a replicated  $2 \times 2$  factorial design of the following treatments: control; snowpack removal; experimental passive warming; and both treatments simultaneously. These climate manipulations represent treatments for current and future potential climates, because snowpack is forecasted to decrease, and temperatures are postulated to increase  $2\text{--}5^\circ\text{C}$ , mostly at night (IPCC 2007). Removal is accomplished by manual excavation from treatment plots-prior to natural snowmelt in late April or early May (Supplemental Figure 1). Passive warming is achieved with open-sided chambers. Those chambers are constructed with  $2.5 \times 2.5$  m wooden frames, with plexiglass louver panels, attached to the frames at a  $45^\circ$  angle (Supplemental Figure 1). The louvers trap longwave radiation that is re-radiated from the ground, which increases the temperature of the ground and vegetation by  $\sim 1\text{--}4^\circ\text{C}$ . The louver minimally impede ambient precipitation and sunlight. The warming effect occurs mostly at night, thereby increasing the minimum night-time temperature (Germino and Demshar 2008). That experimental warming is similar to natural regimes of climate-warming (Germino and Demshar 2008).

### *Treatment application in 2014*

In early May 2014, we excavated approximately 150 mm depth of snow from snow removal plots. We placed a warming chamber on each warming plot, covering the entire  $2.5 \times 2.5$ -m area. Those chambers were deployed concurrently with snow removal and remained on plots for the duration of the experiment (until late October). We established a buffer zone, which spanned 30 cm around each plot, from the outer perimeter towards

the center. That buffer was defined to avoid the possibility of sampling a plant that was not completely inside the treatment area. All measurements were conducted on the inside boundary of the buffer zone, with the exception of one plot that did not contain *A. arbuscula* in the interior of the plot. Measurement campaigns were on May 29, June 11, June 25, July 9, July 23, August 16, September 20, and October 11, 2014.

#### *Leaf temperature, soil water, and soil temperature*

We used a non-contact infrared digital temperature gun (Etekcity Co., Anaheim, California USA) to record the temperature of *A. arbuscula* leaves and the surface of soils on all measurement days ( $n \geq 9$ ). We measured those temperatures during pre-dawn (between 500 and 700 h MDT) and mid-day (between 1000 and 1400 MDT). We installed one Decagon 5TM soil moisture–temperature probe (Decagon Devices, Inc, Pullman, Washington USA) in the center of each plot at 25-cm depth, and volumetric water content and temperature measurements were collected at 1 h intervals and stored with Em50 data loggers (Decagon Devices, Inc., Pullman, Washington USA).

#### *Gas exchange*

We measured instantaneous gas exchange with a portable photosynthesis system (LI-COR model LI-6400) (LI-COR Inc., Lincoln, Nebraska, USA), equipped with a CO<sub>2</sub> controller and LED leaf chamber. We measured dark respiration during pre-dawn- between 500 and 700 h MDT- at least once per month from late-May to mid-October. We also measured light-saturated photosynthesis during mid-day- between 1000 and 1400 MDT. In addition, we measured stomatal conductance simultaneously during other

gas-exchange measurements, with the portable photosynthesis system. At each date, variables were measured on current-year shoots of one randomly selected shrub, at the north-facing side of mid-canopy. We maintained the natural orientation of shoots during measurements.

During all measurements, we set the LI-6400 reference CO<sub>2</sub> concentration at 385 ppm. We also matched the reference air temperature and humidity to ambient conditions. During mid-day measurements, the LED light source was set to 1,200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . During dark respiration measurements, the chamber was darkened (0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). We expressed all gas exchange variables on a silhouette leaf-area basis (Smith et al. 1991). Silhouette leaf-area was determined by photographing leaves with an object of known size, and quantifying the area with ImageJ software (1.46r, Scion Co., Fredrick, MD, USA).

We also measured gas exchange of two forbs, *Eriogonum umbellatum* and *Balsamorhiza sagittata*, which were located in all plots with *A. arbuscula*. Those measurements were conducted at mid-day on June 25, July 8, and July 22, 2014. We used the same protocol to measure gas exchange for forbs as we used for *A. arbuscula*.

### *Water potential*

We measured pre-dawn water potential on samples of *A. arbuscula* in the field with a Scholander-type pressure bomb (Model 1000, PMS Instrument Co., Corvallis, Oregon, USA) on one randomly-selected shrub per plot. We conducted measurements between 500 and 700 h MDT, at least once per month from late-May until mid-October. We randomly chose and excised a ~5 cm stem of current-year growth from each shrub to be

sealed in the pressure chamber. We gradually applied compressed N<sub>2</sub> gas to the chamber, until the xylem sap was forced to exit the excised end of the stem. That amount of pressure (MPa) was equal to the negative tension in the shrub xylem. We expected that more negative water potential was indicative of increasing water stress and limitations.

#### *Isotopes, carbon discrimination and intrinsic water-use efficiency*

We quantified trade-offs between carbon gain and water loss with intrinsic water-use efficiency (WUE<sub>i</sub>). We used stable carbon isotopes on both reproductive and vegetative stems from one shrub per plot, which were harvested in October. The stems were randomly selected (n = 3 per treatment, for each stem type). All leaves were removed from the harvested stems. During photosynthesis, there is large fractionation that discriminates towards the lighter carbon isotope (<sup>12</sup>C), and plant biomass is depleted in <sup>13</sup>C. The carbon stable isotope composition (δ<sup>13</sup>C) of leaves depends on the ratio between the partial pressures of CO<sub>2</sub> in the chloroplast (at the carboxylation sites) and in the atmospheric air (Moreno-Gutiérrez et al. 2012). That ratio is a good proxy to determine leaf-level WUE<sub>i</sub>, which is the ratio between photosynthesis and stomatal conductance of water (Farquhar et al. 1989, Moreno-Gutiérrez et al. 2012).

Leaf samples were dried at 60° C for approximately 48 h. Leaves from each shrub were ground separately, and ~5 mg of each material was weighed into separate tin capsules for analysis. Leaf components of δ<sup>13</sup>C, δ<sup>15</sup>N, and %N were obtained with a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS), with a ConFlo IV interface and Costech Elemental Analyzer (EA) in the Stable Isotope Laboratory at the Center for Archaeology, Materials and Applied Spectroscopy (CAMAS) on the campus

of Idaho State University, Pocatello ID, USA. Precision of measurement was  $\pm 0.2\text{‰}$  for  $\delta^{13}\text{C}$  and  $\pm 0.2\text{‰}$  for  $\delta^{15}\text{N}$ . All  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes were reported relative to the Vienna Pee Dee Belemnite (VPDB) (carbon) and atmospheric air (nitrogen) standards as:  $\delta (\text{‰}) = 10^3[\text{R}_{\text{sample}} / \text{R}_{\text{standard}} - 1]$ . We calculated carbon isotope discrimination with equation (1),

$$\Delta(\text{‰}) = \frac{(\delta_{\text{air}} - \delta_{\text{plant}})}{(1000 + \delta_{\text{plant}})} \times 1000; \quad (1)$$

where  $\Delta$  is the discrimination against  $^{13}\text{C}$  during carbon fixation; and  $\delta$  is the  $^{13}\text{C}/^{12}\text{C}$  ratio (Duquesnay et al. 1998). We used -8.354 for  $\delta_{\text{air}}$  (Mauna Loa, 2012). We also calculated  $\text{WUE}_i$  (A/g) with equation (2),

$$\Delta(\text{‰}) = a + (b - a)\left(1 - \frac{1.6 A}{C_a g}\right); \quad (2)$$

where  $\Delta$  is the discrimination against  $^{13}\text{C}$  during carbon fixation;  $a = 4.4\text{‰}$ , the discrimination against  $^{13}\text{C}$ ;  $b = 27\text{‰}$ , the discrimination associated with carboxylation;  $C_a$  = the ambient  $\text{CO}_2$  concentration; and  $A/g$  is the intrinsic water-use efficiency (O'Leary 1981, Farquhar and Richards 1984, Duquesnay et al. 1998). We used 40 Pa for  $C_a$ , and fit a sigmoidal plot to data points. By conducting those measurements at the end of the experiment, we expected  $\delta^{13}\text{C}$  values to integrate measures of  $\text{WUE}_i$  across the growing season, which was informative about the inter-relationship between carbon gain and water loss in shrubs.

### *Leaf-specific hydraulic conductivity and vulnerability curves*

We measured xylem hydraulic conductivity in June and August. At each date, we harvested a ~15 cm main stem from one randomly-selected shrub from each plot. Stems were sealed in individual bags, and kept cool and damp while transported back to the laboratory. We removed the leaves and bark, and trimmed the edges of the stems under nanopure water (filtered to  $0.2\mu\text{m}$ ) (pH 2) with a fresh razor blade- prior to, and in-between hydraulic measurements. We measured hydraulic conductivity under native embolism (field conditions) by applying a hydrostatic pressure head ( $\sim 0.1\text{ MPa}$ ) to stem segments. We applied tubing and a pipette to the distal end of the stems, and timed the intervals necessary for filtered water to flow through stems, to successive gradations on the pipette (similar to Johnson et al. 2007). We measured total leaf area for each stem by scanning the one-sided area of the fresh leaves, and quantified total area with ImageJ software. We calculated leaf-specific hydraulic conductivity by dividing the flow rate of water by the pressure gradient, by the total leaf area ( $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ). In addition, we measured total sapwood area for each stem by measuring cross-sections with an AMScope microscope digital camera and provided software (AMScope, Irvine CA, USA). We calculated specific hydraulic conductivity by dividing the flow rate of water by the pressure gradient, by the total sapwood area ( $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ). We then submerged stems in a vacuum chamber with nanopure water, acidified with HCl (pH 2) for ~12 h (similar to McCulloh et al. 2011). The vacuum removed embolisms inside the stems, which allowed us to measure maximum hydraulic conductivity, which was sampled with the same procedure as we used to measure hydraulic conductivity under native embolism.

We used the air injection method to obtain xylem vulnerability curves (Sperry and Saliendra 1994) for three stems in control treatments, and three stems in snowpack removal + warming treatments. We used a pressure bomb (Model 1000, PMS Instrument Co., Corvallis, Oregon, USA) equipped with an external stem sleeve to add four levels of pressure (1MPa, 2 MPa, 3 MPa, 4 MPa) to stems. Following each pressure application, stems were submerged under nanopure water until air bubbles stopped flowing. We determined leaf-specific hydraulic conductivity for each level of pressure, and calculated the percent loss of conductivity (PLC) at each level of pressure with equation (3),

$$PLC = 100 \times \left( \frac{k_{l\ max} - k_l}{k_{l\ max}} \right) \quad (3)$$

where  $k_{l\ max}$  = maximum leaf-specific hydraulic conductivity, and  $k_l$  = leaf-specific hydraulic conductivity at a given pressure.

#### *Annual xylem growth, vessel density, and vessel diameter*

Because sagebrush forms annual growth rings (Ferguson 1960), we were able to quantify the hydraulic architecture of shrubs from each treatment. We used a hand microtome to make cross-sections of one stem per experimental plot (which were harvested for hydraulic conductivity measurements), one cross-section per each end of a stem. Cross-sections were dyed with Toluidine blue stain and mounted onto microscope slides with glycerol. We used an AMScope microscope digital camera and provided software (AMScope, Irvine CA, USA) to measure xylem features. We created three random transects along each cross-section. Within each transect, we measured annual growth rings from current-year growth and the previous three years (when shrubs were subjected to the climate experiment). We also quantified the diameter of xylem vessel

elements along each transect ( $n \geq 5$ ). In addition, we calculated the xylem vessel density as the total number of vessels along each transect, divided by the total growth ring width.

#### *Stem elongation*

In late-September, we measured total stem growth of *A. arbuscula* from the 2014 growing season. We evaluated both vegetative stems and reproductive stems (inflorescences) ( $n \geq 5$  per stem type, per plot). Stem elongation was evident by color and textural differences from previous-year growth, in addition to an absence of fully-developed bark.

#### *Specific leaf area*

We measured specific leaf area ( $\text{cm}^2 \text{g}^{-1}$ ) with the leaves from hydraulic measurements, which had been harvested in mid-August. After quantifying the one-sided, total leaf area from fresh leaves (also used for leaf-specific hydraulic conductivity), we dried the leaves from each stem, separately at  $60^\circ \text{C}$  for approximately 48 h. After the drying period, we used a microbalance to quantify the total dry mass (g) for the leaves from each stem. We calculated specific leaf area as the total one-sided, fresh area, divided by the total dry mass.

#### *Inflorescence density and mass, and flower density*

In mid-October, we counted the total number of inflorescences per experimental plot. We then randomly selected and harvested at least three whole inflorescences from each plot. We sealed the stems in individual bags, and kept them damp and cool while

transported. We dried the individual inflorescences separately at 60° C for approximately 48 h. After the drying period, we used a microbalance to quantify the dry mass of each stem. We also quantified the total number of flowers for each inflorescence.

#### *Plot-level plant cover*

We digitally photographed each plot from approximately 2.5m above ground level, at each campaign. We analyzed the photos with ImageJ software to determine the total percent cover of *A. arbuscula*, all vegetation including *A. arbuscula*, and bare ground. We determined that the peak greenness of all vegetation, across all plots, occurred in late-July, and compared the percent cover of vegetation among treatments from that date.

#### *Statistical analyses*

We conducted data analyses in JMP® (Version 11.0.0. SAS Institute Inc., Cary, NC, USA). We assessed effects of snowpack removal and warming, and the interactions between those two variables, on leaf temperature, soil moisture, photosynthesis, respiration, stomatal conductance, and water potential, using a repeated-measures 2×2 factorial analysis of variance (ANOVA) with significance set at  $\alpha = 0.05$ . In addition, we assessed treatment effects on leaf-area specific conductivity, intrinsic water-use efficiency, xylem ring width, vessel density and diameter, stem elongation, specific-leaf area, inflorescence density and mass, flower density, and plant cover using a 2×2 factorial analysis of variance (ANOVA) with significance set at  $\alpha = 0.05$ . For all analyses, we used Post-hoc Tukey tests to assess effects within subject treatments. The unit of replication was each experimental plot.

## Results

### *Microclimate and leaf temperatures*

The study site received approximately 985 mm of precipitation during 2014 (including the snowpack), compared to a historical average of 640 mm. When we removed snowpack in May, snow depths were ~15 cm, resulting in a ~15.2% decrease in annual soil water inputs. In addition, multiple precipitation events occurred during the summer, which contributed to the above-average precipitation value for 2014.

Soil moisture at 25 cm depth declined from maximum values in May to mid-June in all treatments (Figure 2). Water content generally declined until September, at which point fall rain and snow increased soil water. The magnitudes of change in soil moisture were not consistent across treatments, but snowpack removal in May did not have a significant effect on soil volumetric water content among plots throughout the summer of 2014 (Figure 2, Table 1). Furthermore, passive warming did not have a significant effect on soil water, and there was no interaction between warming and snowpack removal. Post-hoc Tukey tests indicated that there were no significant differences in soil water means at any date ( $P > 0.05$ ).

From May to November 2014, mean leaf temperatures of *A. arbuscula* in warmed plots during pre-dawn were an average of  $5.40 \pm 3.59$  °C, while shrubs that were not warmed had mean leaf temperatures of  $4.06 \pm 3.75$  °C (Figure 1a, Table 1). Warming led to the greatest increases in leaf temperature in mid-August and September (Figure 1a). Post-hoc Tukey tests indicated that warmed plots had significantly higher temperatures than non-warmed plots on six of eight occasions ( $P > 0.05$ ) (Figure 1a). Snowpack removal did not affect leaf temperatures during pre-dawn, and there was no significant interaction

between warming and snow removal (Table 1). Leaf temperatures during mid-day ranged from 18 to 32° C between May and November (Figure 1c). Neither warming nor snowpack removal had any significant effects on leaf temperatures at mid-day in *A. arbuscula* (Table 1, Figure 1c, Figure 1d). There were no significant differences in leaf temperature means during mid-day at any date (Tukey  $P > 0.05$ ).

Soil surface temperatures at pre-dawn were strongly influenced by warming (Figure 1a, Table 1). On average, the soil surface of warmed plots was  $8.87 \pm 4.37^\circ \text{C}$  at pre-dawn, compared to  $6.98 \pm 4.67^\circ \text{C}$  in plots that were not warmed (Figure 1a). Tukey post-hoc tests indicated that warmed plots had significantly higher temperatures than non-warmed plots on all eight occasions (Figure 1b). Warming had the greatest effect on soil surface temperatures ( $> 3^\circ \text{C}$ ) in May, June, August, September, October, and November (Figure 1b). Snowpack removal did not have a significant effect on soil surface temperatures at pre-dawn (Figure 1b, Table 1). During mid-day, warming treatments did not have an effect on soil surface temperatures. Similarly, snowpack removal did not have an effect on soil surface temperatures during mid-day (Figure 1b, Figure 1d, Table 1). Post-hoc Tukey tests indicated that there were no significant differences in soil temperature means during mid-day at any date ( $P > 0.05$ ). Soil temperatures at 25 cm were not significantly influenced by either snowpack removal or warming (Table 1, Figure 2a).

### *Gas exchange*

Photosynthetic gas exchange of current-year stems of *A. arbuscula* was greatest in May and June in all treatments, and decreased sharply in late-June (Figure 3a), after which there was little variation. Neither warming nor snowpack removal had significant

effects on photosynthesis of shrubs (Figure 3a, Table 2). Post-hoc tests indicated that there were no differences in photosynthesis among any treatments at any given date. Similarly, warming and snowpack removal did not lead to differences in photosynthesis for *Balsamorhiza sagittata* (Supplemental Figure 3a) or *Eriogonum umbellatum* (Supplemental Figure 5a) across the growing season (Supplemental Table 2).

Respiration of *A. arbuscula* was low in June and July in all treatments (Figure 3b). Between late-June and early July, respiration increased in shrubs in all treatments. Experimental warming did not have a significant influence on respiration (Figure 3b, Table 2). Similarly, snowpack removal did not significantly affect respiration, and there was no interaction between those two treatments (Figure 3b, Table 1). Post-hoc tests specified that there were no significant differences in respiration among any treatments at any given date. In addition, treatments did not significantly affect the respiration of *B. sagittata* (Supplemental Figure 3b) or *E. umbellatum* (Supplemental Figure 5b, Supplemental Table 2).

Stomatal conductance of water decreased from May to mid-June, increased from mid-June until mid-August, and slightly declined through October in all treatments (Figure 4a). Stomatal conductance was not significantly influenced by either snowpack removal, or by warming (Table 2). There was no interaction between those two treatments (Figure 4a, Table 2). Post-hoc tests indicated that there were no significant differences in stomatal conductance of water among treatments at any given date. Similarly, snowpack removal and warming did not lead to significant differences in stomatal conductance of water for *E. umbellatum* (Supplemental Figure 6) or for *B. sagittata* (Supplemental Figure 4, Supplemental Table 2).

### *Water potentials*

Water potentials (water status) in xylem of *A. arbuscula* at pre-dawn became increasingly negative from late-May until late-July in all treatments (Figure 4b). Water potentials were less negative for the remainder of the experiment (until mid-October). Warming significantly improved shrub water potential ( $-0.574 \pm 0.255$  MPa), compared with shrubs that were not warmed ( $-0.689 \pm 0.351$  MPa) (Figure 4b, Table 2). In contrast, snowpack removal did not influence shrub water status, and there was no interaction between snowpack removal and warming. Post-hoc tests indicated that there were no significant differences between individual treatment means at any date.

### *Isotopes, carbon discrimination and intrinsic water-use efficiency*

Carbon isotope discrimination for leaves on reproductive stems of *A. arbuscula* averaged  $22.2 \pm 0.4$  ‰, while leaves from vegetative stems averaged  $20.0 \pm 0.7$  ‰ (Figure 5a). Warming had no effect on the discrimination of leaves from vegetative stems, or leaves from reproductive stems (Figure 5a, Table 2). Similarly, snowpack removal did not lead to significant differences in the carbon isotope discrimination of vegetative leaves, or for reproductive leaves. There were no significant interactions between warming and snowpack removal for either leaf type (Figure 5a, Table 2). Tukey post-hoc tests indicated that there were no significant differences among treatment means. Similarly, snowpack removal and warming did not lead to significant differences in percent nitrogen (Supplemental Figure 2a) or in the nitrogen isotope ratio of *A. arbuscula* (Supplemental Table 1).

The mean intrinsic water-use efficiency of leaves on reproductive stems was  $5.36 \pm 0.442 \mu\text{mol mol}^{-1}$ , while the mean for leaves on vegetative stems was  $6.75 \pm 0.754 \mu\text{mol mol}^{-1}$  (Figure 5b). For each leaf type, intrinsic water-use efficiency of *A. arbuscula* was similar between treatments through 2014 (Figure 5b). Passive warming did not have a significant effect on intrinsic water-use efficiency for leaves on reproductive stems, or for leaves on vegetative stems (Figure 5b, Table 2). There was no significant difference in intrinsic water-use efficiency for leaves on reproductive stems, or for leaves on vegetative stems due to snowpack removal. For both leaf types, there were no significant interactions between warming and snowpack removal (Figure 5b, Table 2). There were no significant differences among treatment means for either leaf type.

#### *Hydraulic conductivity and vulnerability to embolism*

Leaf-specific hydraulic conductivity of *A. arbuscula* stems in June averaged  $7 \times 10^{-5} \pm 5 \times 10^{-5} \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$  under native embolism, and  $6.9 \times 10^{-5} \pm 5.6 \times 10^{-5} \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$  under maximum conductivity (Figure 6 a,b). In June, warming did not significantly affect leaf-specific conductivity in stems for maximum or native embolism (Figure 6a, Table 2). Similarly, warming did not have a significant influence on conductivity for stems in August for native embolism or for maximum conductivity. Leaf-specific conductivity was not affected by snowpack removal in June for native embolism or for maximum conductivity (Figure 6a, Table 2). Snowpack removal did not have a significant effect on conductivity under native embolism or under maximum conductivity in August (Figure 6b, Table 2). There were no significant interactions among treatments for either type of leaf-specific conductivity means during June or August (Table 2).

Similarly, warming or snowpack removal did not have an effect on the specific conductivity of stems in June or August for either native or maximum conductivity. (Table 2, Figure 7). Regardless of month or type of conductivity, there were no interactions among treatments (Table 2).

The xylem vulnerability curves indicated that all *A. arbuscula* stems lost > 90% leaf-specific hydraulic conductivity by a xylem pressure of -4 MPa (Figure 8). The MPa that corresponded with a 50% loss in conductivity ( $P_{50}$ ) was -1.6 for stems from the snowpack removal + warming treatment. The stems from the control treatment had an average  $P_{50}$  that corresponded to -1.4 MPa (Figure 8).

#### *Annual xylem growth, vessel diameter, and vessel density*

The width of xylem growth rings of *A. arbuscula* in 2014 was not affected by snowpack removal or warming (Figure 9a, Table 3). However, mean ring widths from 2011-2014 were significantly affected by an interaction between warming and snowpack removal treatments, but were not significantly influenced by warming or snowpack removal alone (Figure 9b, Table 3). Post-hoc Tukey tests indicated that mean ring widths were wider in plots with only snowpack removal, and plots with only warming, compared to the control (Figure 9b).

The diameter of xylem vessels in 2014 was not significantly affected by warming or snowpack removal only, but the combination of warming and snowpack removal led to a significant difference in vessel diameter (Figure 9c, Table 3). Post-hoc tests indicated that there was no significant difference among individual treatment means (Figure 9c). Alternatively, the mean vessel diameter in xylem rings from 2011-2014 was significantly

greater in snowpack removal plots, with an interaction between warming and snowpack removal, but were not significantly different due to warming alone (Figure 9d, Table 3). Post-hoc tests indicated that mean vessel diameters were greater in warming and snowpack removal plots, compared to control plots.

Warming had a strong effect on vessel density in 2014 and from 2011-2014. Vessel density was also significantly influenced by snowpack removal in all years (Figure 9e, 9f, Table 3). There was a significant interaction between snowpack removal and warming in all years. Tukey post-hoc tests indicated that vessel density was significantly greater (at least two-fold) in snow removal + warming plots for both time intervals (Figure 9e, 9f).

#### *Stem elongation*

Elongation of *A. arbuscula* stems from May to mid-September 2014 was largely different between reproductive stems and vegetative stems (Figure 10a, 10b). The mean length of reproductive stems was  $20.79 \pm 4.56$  cm, while the mean length of vegetative stems was  $4.66 \pm 1.49$  cm. The growth of reproductive stems was not significantly affected by warming, snowpack removal, or by an interaction between treatments (Figure 10b, Table 3). Post-hoc Tukey tests indicated that reproductive stem length in all treatments were not significantly different. The growth of vegetative stems in 2014 was not significantly influenced by warming. In contrast, snowpack removal had a significant effect on elongation, while there was no interaction between treatments (Figure 10a, Table 3). Post-hoc tests indicated that individual treatment means were not significant different.

### *Specific leaf area*

Leaf-specific area from May to mid-August was significantly affected by warming (Figure 11, Table 3). Shrubs in warmed plots had a mean specific leaf area of  $76.37 \pm 3.83 \text{ cm}^2 \text{ g}^{-1}$ , while shrubs in warmed plots averaged  $84.26 \pm 5.57 \text{ cm}^2 \text{ g}^{-1}$  (Figure 10). In contrast, snowpack removal did not have an effect on specific leaf area, and there was no interaction between treatments (Table 3). Tukey post-hoc tests indicated that there was no significant difference between individual treatment means (Figure 11).

### *Inflorescence density and mass, and flower density*

The number of inflorescences per shrub was strongly influenced by warming, snowpack removal, and the interaction between the two treatments (Figure 12c, Table 3). Shrubs in snowpack removal + warming plots had at least two-fold more inflorescences than shrubs in any other treatment (Figure 12c). However, the mass of individual inflorescences was not significantly different among treatments. Inflorescence mass was not influenced by snowpack removal, by warming, or by an interaction between the two treatments (Figure 12b, Table 3). Similarly, the number of flowers on individual inflorescences was not affected by warming, by snowpack removal, or by an interaction between those treatments (Figure 11a, Table 3). Tukey post-hoc tests indicated that there were no significant differences among treatment means.

### *Plot-level plant cover*

Vegetation cover was similar among all treatments at peak-greenness in mid-July (Figure 12a). Tukey post-hoc tests indicated that there were no significant differences among treatment means. Total cover of all vegetation was not significantly affected by snowpack removal or by warming (Figure 13a, Table 3). However, there was a significant interaction between warming and snowpack removal (Table 3). Alternatively, total cover of *A. arbuscula* was significantly influenced by snowpack removal (Figure 13b, Table 3). There was roughly twice as much sagebrush in snow removal plots ( $16.79 \pm 8.79$  %) compared to non- snow removal plots ( $8.620 \pm 3.96$  %) (Figure 13b). However, warming did not lead to significant differences in sagebrush cover, and there was no interaction between treatments. Tukey post-hoc tests indicated that there were no significant differences among treatment means.

## Discussion

Future changes in climate for western North America are predicted to result in increased temperatures and losses in snowpack. Many species at high elevations have been shown to be sensitive to changes in temperature and soil moisture. In our experiment, we increased soil and leaf temperatures  $\sim 1\text{--}4^\circ\text{C}$ , and removed  $\sim 15\%$  soil water. Those changes had few effects on the physiology, structure, and growth of *Artemisia arbuscula*. We expected that increased nighttime temperatures and decreased spring snowpack would promote growth and structural changes in *Artemisia arbuscula*, while leading to few changes in physiology, as has been reported by others (e.g. Lambrecht 2007). Under the background ambient conditions during our study, experimental warming caused increased leaf and soil surface temperatures ( $\sim 1\text{--}4^\circ\text{C}$ ) during predawn, but had no effect on soil water. As a result, warming had occasional effects on structure and growth, and marginal to no effects on physiology. Snowpack removal caused small or no biological impacts due to soil water, as volumetric water content was not significantly different among treatment plots. However, the spring snowpack removal treatment did lead to several changes in structure and growth of *A. arbuscula* from May to October 2014.

We found few adjustments in physiology of *Artemisia arbuscula* due to increased leaf and soil temperatures, which generally supported our hypothesis. That absence of treatment effects occurred despite differences in leaf temperature, even in earlier and later “shoulder season” months (e.g. May, June, October and November), when ambient air temperatures were relatively low for the season and plant physiology may have been thermally-limited. These results are similar to findings of Loik et al. (2000), which indicated that  $>1^\circ\text{C}$  increases in temperature did not have marked effects on the

physiology of *Artemisia tridentata*, a similar species of sagebrush. However, we did find differences in water relations of *A. arbuscula* stems, as passive warming increased (improved) xylem water potentials (Figure 4b). That result is contrary to the findings of Loik et al. (2000), which indicated that warming did not affect the water potential *Artemisia tridentata*. Our results are also opposite from the work of Shaw et al. (2000), which demonstrated that water potentials of *A. arbuscula* decreased with warming. We suspect that several mechanisms caused passive warming frames to improve water status of shrubs. Because we observed moisture condensation on frames at night throughout the season, we suspect that humidity was higher in warmed plots, however, we do not have data to support. We also suspect that warming at night lessened cavitation in xylem, which can occur due to drought and freezing, which was marginally supported by less decline in conductivity in stems that were subjected to warming and snowpack removal, compared to control (Figure 8). In total, our data demonstrate that photosynthesis, respiration, stomatal conductance, hydraulic conductivity, and intrinsic water-use efficiency in *A. arbuscula* were not sensitive to increases in temperature at our study site, while water potential and hydraulic vulnerability may be slightly improved by temperature increases. However, we acknowledge that our overall interpretations were limited by our small sample sizes, and thus we can only make general statements relative to the environmental conditions at the study site, during the experiment.

We did not find any significant differences in physiology due to snowpack removal, which generally supported our hypothesis. There were no differences despite the fact that the shrubs were likely uncovered from snowpack for at least one week earlier in the growing season. All gas exchange and water-relation variables showed no response to

snowpack removal. That lack of response was also true for intrinsic water-use efficiency and hydraulic conductivity, which are integrated measures of physiology across a longer time-scale. Pratt and Mooney (2013) also reported that intrinsic water-use efficiency did not change in *Artemisia californica* that was subjected to a fourfold increase in precipitation. However, it is noteworthy that intrinsic water-use efficiency was greater for vegetative stems than for reproductive stems. That relationship is the opposite from that of *Artemisia tridentata* (Evans and Black 1993), where reproductive stems had greater water-use efficiency than vegetative stems. However, shrubs in that study became more water stressed during summer ( $> -3$  MPa) and thus altered physiology. Importantly, water potentials during our study remained relatively high ( $-1.8$  MPa maximum), compared to Drivas and Everett (1988), who reported water potentials for *A. arbuscula* as low as  $-5.5$  MPa. Thus, *A. arbuscula* in our study was not as water-stressed as it may occur in other regions. Therefore, in future studies, we might expect differences in physiology among treatments, if more snowpack had been removed or if it was a drier year. Those conditions would cause soil water to become more limiting and potentially reach a threshold of water stress. In addition, we speculate that above-average rain events during the summer prevented differences in soil water among snowpack removal treatments. Taken together, those data demonstrate that physiological performance in *A. arbuscula* was generally not affected by a  $\sim 15\%$  reduction in spring soil water.

Despite few differences in physiology, our study did reveal occasional changes in the structure of *A. arbuscula* due to changes in temperature and soil water, which generally supported our hypothesis. There were no adjustments in xylem growth rings in response

to temperature increases during 2014. Nonetheless, growth rings were wider from 2011-2014 in snowpack removal and warmed plots (Figure 9), highlighting that the snowpack removal treatment in previous years may have had greater impacts on snowpack inputs so soil moisture. That result is contrary to the findings of Poore et al. (2009), which demonstrated that growth ring size of *Artemisia tridentata vaseyana* was negatively correlated with increased temperatures. The diameter of xylem vessels in 2014 and 2011-2014 were wider in snowpack removal and warming plots. In addition, there were increases (more than two-fold) in vessel density for both time periods. That increase was a result of an interaction with snowpack removal, and occurred with no changes in stomatal conductance or hydraulic conductivity. That result is contrary to another study in Argentina (1,700 m) that reported vessel density of *Prosopis flexuosa* was negatively correlated with temperature (Giantomasi et al. 2009). It is also contrary to findings of Villar-Salvador et al. (1997), which indicated that vessel density of evergreen species along a climate gradient decreased with more precipitation, and was not correlated with temperature. Those results suggest that the architecture of xylem in *A. arbuscula* generally demonstrated plasticity to small changes in temperature and snowpack at our study site, which may have allowed it to maintain physiological performance.

We found several differences in the growth of *A. arbuscula* due to changes in temperature or precipitation, which generally supported our hypothesis. There was no difference in stem elongation for reproductive stems (Figure 10b). However, vegetative stems were significantly longer due to snowpack removal (Figure 10a). That may reflect the seasonal development of the shrubs, because vegetative stems elongate earlier in the year than reproductive stems do, when soil moisture is higher (Evans and Black 1993).

However, warming led to decreased specific leaf area, suggesting morphological adjustments in response to temperature stress (Figure 11). There were no changes in inflorescence mass or the number of flowers per inflorescence, however, warming and snowpack removal led to drastic increases in the number of inflorescences per shrub. That increase suggests resource allocation to reproductive structures in *A. arbuscula*, but is opposite to the findings of Lambrecht et al. (2006), which revealed that flowering was decreased by warming in some montane species. Our data also indicated that total cover of *A. arbuscula* was increased by snowpack removal (Figure 13b). We hypothesize that may be due to earlier emergence from snowpack, or due to cumulative growth effects from treatments since 2011. In contrast, total plant cover in plots was dependent upon the interaction between snowpack removal and warming, despite significant differences in soil water. Our plant cover data contradict that of Price and Waser (2000), who did not find differences in total plant cover due to warming. In total, our data representing morphology and growth suggest that *A. arbuscula* is occasionally affected by snowpack removal and warming at our study site, and that some gained carbon may be allocated to reproductive, vegetative, and hydraulic structures.

Though our small sample sizes may have reduced the statistical power to detect differences in biological impacts, we did observe differences in structure and growth, and few differences in the physiology of montane *A. arbuscula*. Because we did not find robust or consistent responses due to climate treatments, we do not report a directional change, i.e., there did not appear to be enhanced or detrimental treatment effects. In addition, it may be possible that *A. arbuscula* did not show strong plasticity due to an ability to resist small changes in environmental conditions. Nonetheless, there are also

other factors that may have been driving physiology and growth, including: soil-nutrient dynamics, soil texture, and nutrient limitations, which we did not analyze. It is possible that patches of nutrients may exist in the soils at the study site.

*A. arbuscula* at our treatment site may not have been driven to their hydraulic or thermal thresholds where physiological changes must be made. Stronger treatments may be necessary to lead to more robust changes in this particular climate. For example, Pratt and Mooney (2013) used a four-fold increase in precipitation, and revealed some differences in leaf-level traits and growth traits of shrubs. In addition, Loik et al. (2000) and Harte and Shaw (1995) used a greater degree of warming (2° C), which was strong enough to dry soils and show treatment differences. Though our treatments were less vigorous, they were consistent with climate prediction models, though our snowpack removal treatment was likely diminished by the substantial rain events that occurred during the summer months. Because we identified more robust changes in xylem characteristics from previous treatment years, it is likely that there is interannual variability in this system, and a potential for more change. It is also likely that *A. arbuscula* in other geographical regions may experience greater or lesser changes in structure and physiology than at our site. That degree of change will depend on distribution, as *A. arbuscula* occupies a wide climate gradient, characterized by different amounts of temperature and precipitation (Thompson et al. 2015). That gradient spans between 10-30° C maximum summer temperatures, with between 100-1000 mm in annual precipitation. Thus, the shrubs at our study site generally occupy mid-range for both climate variables (19° C maximum temperature and 630 mm of precipitation per year). Therefore, it is likely that shrubs which occupy other climate gradients may

respond more or less to changes in climate. For example, shrubs at a drier site may be more sensitive to declines in precipitation or increases in temperature.

We contend that further investigation, with larger sample sizes, may reveal more adjustments in physiological performance and structure- unless *A. arbuscula* is somewhat resistant to change. It will also be important to study *A. arbuscula* across a larger geographical region, which includes communities of shrubs that are distributed across many different temperature and precipitation regimes. In addition, future work should investigate effects of climate treatments on seed production and viability. That data would demonstrate whether additional adjustments in reproductive output are being made, and whether climate treatments may have a population-level impact. Similarly, future work should incorporate surveys on naturally-occurring *A. arbuscula* seedlings and on seed sources in a common garden experiment to better inform about reproduction under current and potential, future changes in climate.

In total, our study revealed few differences in physiological performance of *A. arbuscula*, and occasional adjustments in structure and growth. Taken together, our data demonstrate that the structure and function of montane *A. arbuscula* may be somewhat resistant, or potentially improved by small changes in nighttime warming and decreases in spring snowpack removal, which are predicted for the future in western North America. This research will be important for managing sagebrush ecosystems, as a number of species, including *Centrocercus urophasianus* and *Brachylagus idahoensis*, rely largely on sagebrush for habitat and forage (Himes & Drohan 2007, Dzialak et al. 2012).

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## Tables for Chapter 2

Table 1. Summary ANOVA table showing the effects of snowpack removal and warming on microclimate variables of *A. arbuscula* in experimental plots. (pd) indicates measurements taken during pre-dawn hours; (md) indicates measurements taken during mid-day hours.

|                               | Warming |       |                 | Snowpack Removal |       |       | Warming × Snowpack Removal |       |       |
|-------------------------------|---------|-------|-----------------|------------------|-------|-------|----------------------------|-------|-------|
|                               | d.f.    | F     | P               | d.f.             | F     | P     | d.f.                       | F     | P     |
| Leaf temperature (pd)         | 7, 8    | 82.42 | < <b>0.0001</b> | 7, 8             | 1.228 | 0.300 | 7, 8                       | 0.871 | 0.378 |
| Leaf temperature (md)         | 7, 8    | 1.023 | 0.342           | 7, 8             | 0.004 | 0.950 | 7, 8                       | 0.018 | 0.897 |
| Soil surface temperature (pd) | 7, 8    | 141.2 | < <b>0.0001</b> | 7, 8             | 0.052 | 0.825 | 7, 8                       | 0.070 | 0.798 |
| Soil surface temperature (md) | 7, 8    | 0.326 | 0.584           | 7, 8             | 0.041 | 0.844 | 7, 8                       | 0.013 | 0.912 |
| Soil temperature at 25 cm     | 6, 8    | 1.706 | 0.228           | 6, 8             | 0.030 | 0.866 | 6, 8                       | 0.000 | 0.996 |
| Soil moisture at 25 cm        | 6, 8    | 2.834 | 0.131           | 6, 8             | 1.485 | 0.258 | 6, 8                       | 1.525 | 0.252 |

Table 2. Summary ANOVA table showing the effects of snowpack removal and warming on physiology of *A. arbuscula* in experimental plots.

|                                   | Warming |        |              | Snowpack Removal |        |       | Warming × Snowpack Removal |       |       |
|-----------------------------------|---------|--------|--------------|------------------|--------|-------|----------------------------|-------|-------|
|                                   | d.f.    | F      | P            | d.f.             | F      | P     | d.f.                       | F     | P     |
| Photosynthesis                    | 7, 8    | 1.722  | 0.226        | 7, 8             | 1.788  | 0.218 | 7, 8                       | 0.181 | 0.682 |
| Respiration                       | 7, 8    | 0.809  | 0.395        | 7, 8             | 0.598  | 0.462 | 7, 8                       | 1.602 | 0.241 |
| Stomatal conductance (md)         | 7, 8    | 3.095  | 0.117        | 7, 8             | 0.747  | 0.413 | 7, 8                       | 1.213 | 0.303 |
| Water potential                   | 7, 8    | 16.04  | <b>0.004</b> | 7, 8             | 3.158  | 0.114 | 7, 8                       | 0.962 | 0.356 |
| Discrimination ‰ (reproductive)   | 1, 8    | 0.0017 | 0.968        | 1, 8             | 0.934  | 0.362 | 1, 8                       | 1.044 | 0.337 |
| Discrimination ‰ (vegetative)     | 1, 8    | 0.085  | 0.778        | 1, 8             | 1.664  | 0.233 | 1, 8                       | 0.018 | 0.897 |
| WUEi (reproductive)               | 1, 8    | 0.002  | 0.968        | 1, 8             | 0.923  | 0.365 | 1, 8                       | 1.067 | 0.332 |
| WUEi (vegetative)                 | 1, 8    | 0.086  | 0.777        | 1, 8             | 1.669  | 0.232 | 1, 8                       | 0.019 | 0.894 |
| Ks maximum, June                  | 1, 8    | 0.077  | 0.789        | 1, 8             | 0.248  | 0.632 | 1, 8                       | 0.950 | 0.358 |
| Ks native, June                   | 1, 8    | 0.988  | 0.349        | 1, 8             | 0.004  | 0.954 | 1, 8                       | 0.923 | 0.365 |
| Ks maximum, August                | 1, 8    | 1.434  | 0.270        | 1, 8             | 0.019  | 0.893 | 1, 8                       | 0.092 | 0.771 |
| Ks native, August                 | 1, 8    | 0.018  | 0.898        | 1, 8             | 0.001  | 0.971 | 1, 8                       | 0.054 | 0.823 |
| K <sub>leaf</sub> maximum, June   | 1, 8    | 0.146  | 0.713        | 1, 8             | 0.0002 | 0.991 | 1, 8                       | 0.657 | 0.441 |
| K <sub>leaf</sub> native, June    | 1, 8    | 0.202  | 0.665        | 1, 8             | 0.102  | 0.758 | 1, 8                       | 1.174 | 0.310 |
| K <sub>leaf</sub> maximum, August | 1, 8    | 0.856  | 0.386        | 1, 8             | 0.145  | 0.715 | 1, 8                       | 1.002 | 0.350 |
| K <sub>leaf</sub> native, August  | 1, 8    | 0.249  | 0.633        | 1, 8             | 0.513  | 0.497 | 1, 8                       | 0.621 | 0.457 |

Table 3. Summary ANOVA table showing the effects of snowpack removal and warming on structure and growth of *A. arbuscula* in experimental plots, in addition to plot-level plant cover.

|                            | Warming |        |                   | Snowpack Removal |       |               | Warming × Snowpack Removal |       |                   |
|----------------------------|---------|--------|-------------------|------------------|-------|---------------|----------------------------|-------|-------------------|
|                            | d.f.    | F      | P                 | d.f.             | F     | P             | d.f.                       | F     | P                 |
| Ring width 2014            | 1, 68   | 0.395  | 0.532             | 1, 68            | 3.101 | 0.083         | 1, 68                      | 1.451 | 0.233             |
| Vessel diameter 2014       | 1, 356  | 0.916  | 0.339             | 1, 356           | 0.435 | 0.510         | 1, 356                     | 5.880 | <b>0.016</b>      |
| Vessel density 2014        | 1, 68   | 8.182  | <b>0.006</b>      | 1, 68            | 4.234 | <b>0.044</b>  | 1, 68                      | 4.074 | <b>0.048</b>      |
| Ring width 2011-2014       | 1, 272  | 0.839  | 0.360             | 1, 272           | 2.285 | 0.132         | 1, 272                     | 12.57 | <b>0.0005</b>     |
| Vessel diameter 2011-2014  | 1, 1346 | 0.0002 | 0.989             | 1, 1346          | 10.59 | <b>0.001</b>  | 1, 1346                    | 4.074 | <b>0.044</b>      |
| Vessel density 2011-2014   | 1, 269  | 17.57  | <b>&lt;0.0001</b> | 1, 269           | 13.07 | <b>0.0004</b> | 1, 269                     | 21.86 | <b>&lt;0.0001</b> |
| Stem growth (reproductive) | 1, 51   | 0.766  | 0.386             | 1, 51            | 2.983 | 0.090         | 1, 51                      | 2.761 | 0.103             |
| Stem growth (vegetative)   | 1, 56   | 1.374  | 0.246             | 1, 56            | 6.155 | <b>0.016</b>  | 1, 56                      | 1.092 | 0.301             |
| Specific leaf area         | 1, 8    | 8.784  | <b>0.018</b>      | 1, 8             | 0.770 | 0.406         | 1, 8                       | 1.99  | 0.196             |
| Inflorescences per shrub   | 1, 51   | 7.055  | <b>0.011</b>      | 1, 51            | 14.98 | <b>0.0003</b> | 1, 51                      | 7.780 | <b>0.007</b>      |
| Inflorescence mass         | 1, 35   | 0.045  | 0.834             | 1, 35            | 0.059 | 0.810         | 1, 35                      | 0.028 | 0.867             |
| Flowers per tiller         | 1, 34   | 0.202  | 0.657             | 1, 34            | 0.031 | 0.862         | 1, 34                      | 0.894 | 0.351             |
| Plant cover (sagebrush)    | 1, 18   | 0.412  | 0.529             | 1, 18            | 7.856 | <b>0.012</b>  | 1, 18                      | 1.256 | 0.277             |
| Plant cover (all plants)   | 1, 18   | 0.024  | 0.878             | 1, 18            | 0.855 | 0.368         | 1, 18                      | 5.054 | <b>0.037</b>      |

## Figures for Chapter 2

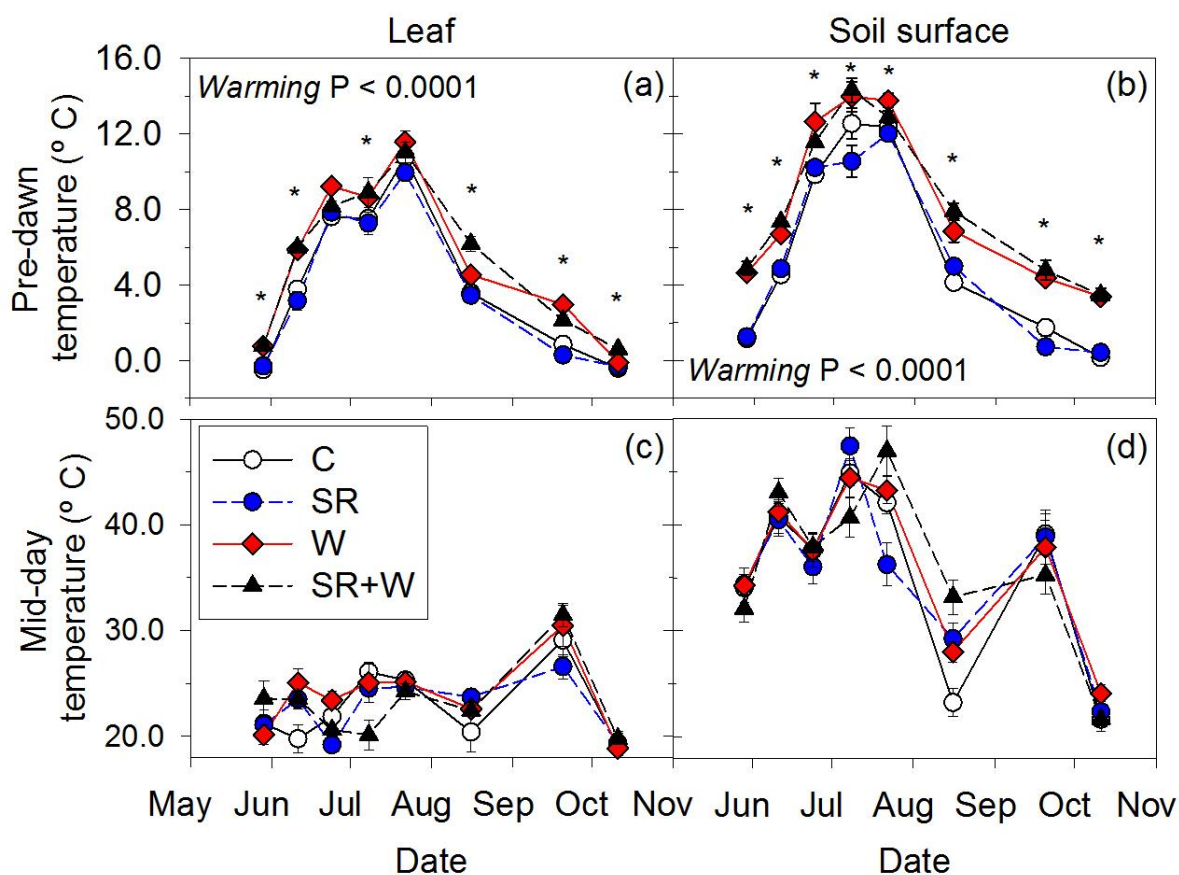


Figure 1. Mean leaf temperatures of *Artemisia arbuscula* at (a) pre-dawn and (c) mid-day, and soil surface temperatures at (b) pre-dawn and at (d) mid-day ( $^{\circ}\text{C}$ ) in experimental climate plots. C = control, SR = snowpack removal, W = warming, and SR+W = both snow removal and warming. Tick marks by date indicate the first day of the month. Error bars are  $\pm 1$  SE from the mean. Asterisks indicate dates when mean temperatures were significantly different ( $P > 0.05$ ).

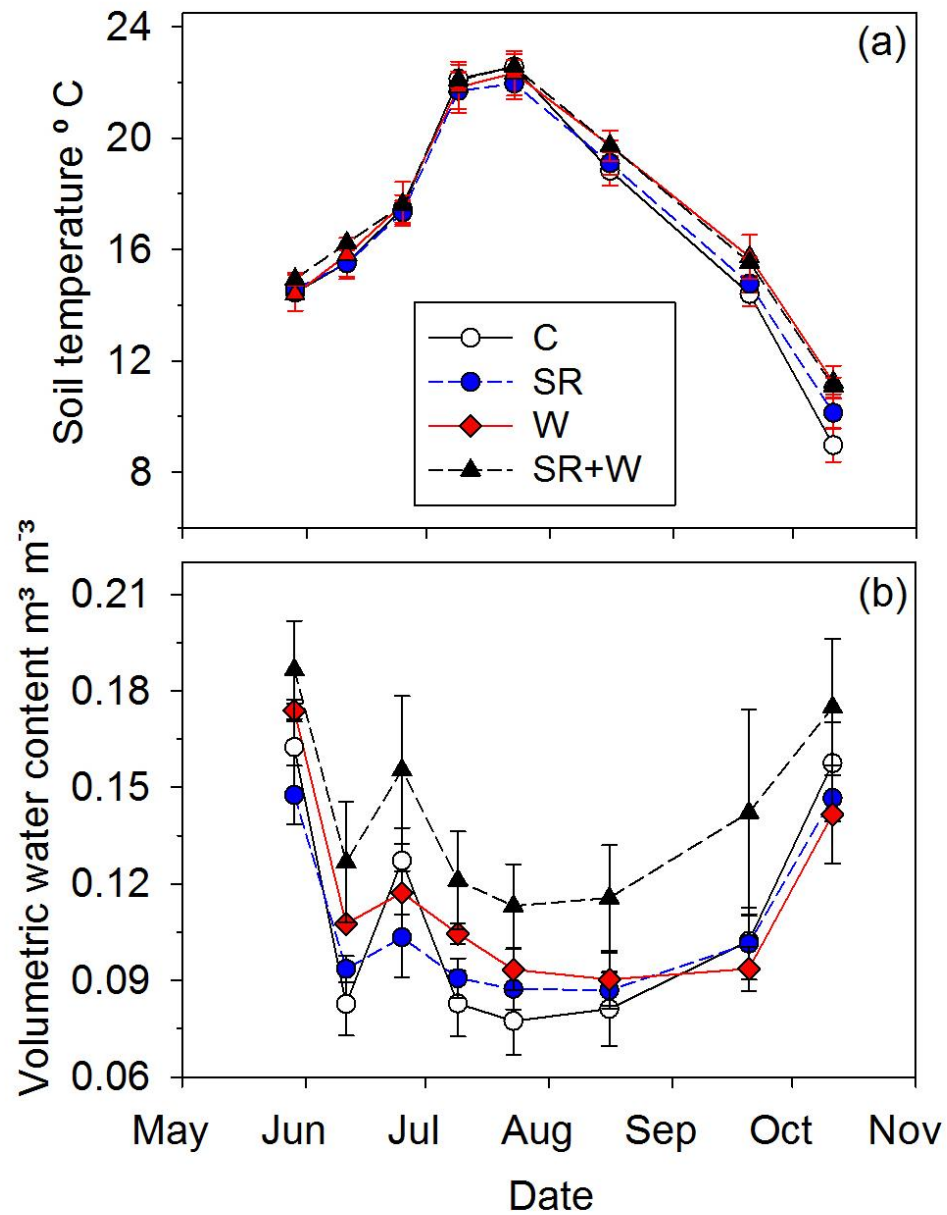


Figure 2. Mean soil temperature (a) and volumetric water content (b) at 25 cm depth in experimental climate plots. C = control, SR = snowpack removal, W = warming, and SR+W = both snow removal and warming. Tick marks by date indicate the first day of the month. Values are means for treatment plots at 0300 h each day ( $n = 3$ ). Error bars represent one standard error from the mean.

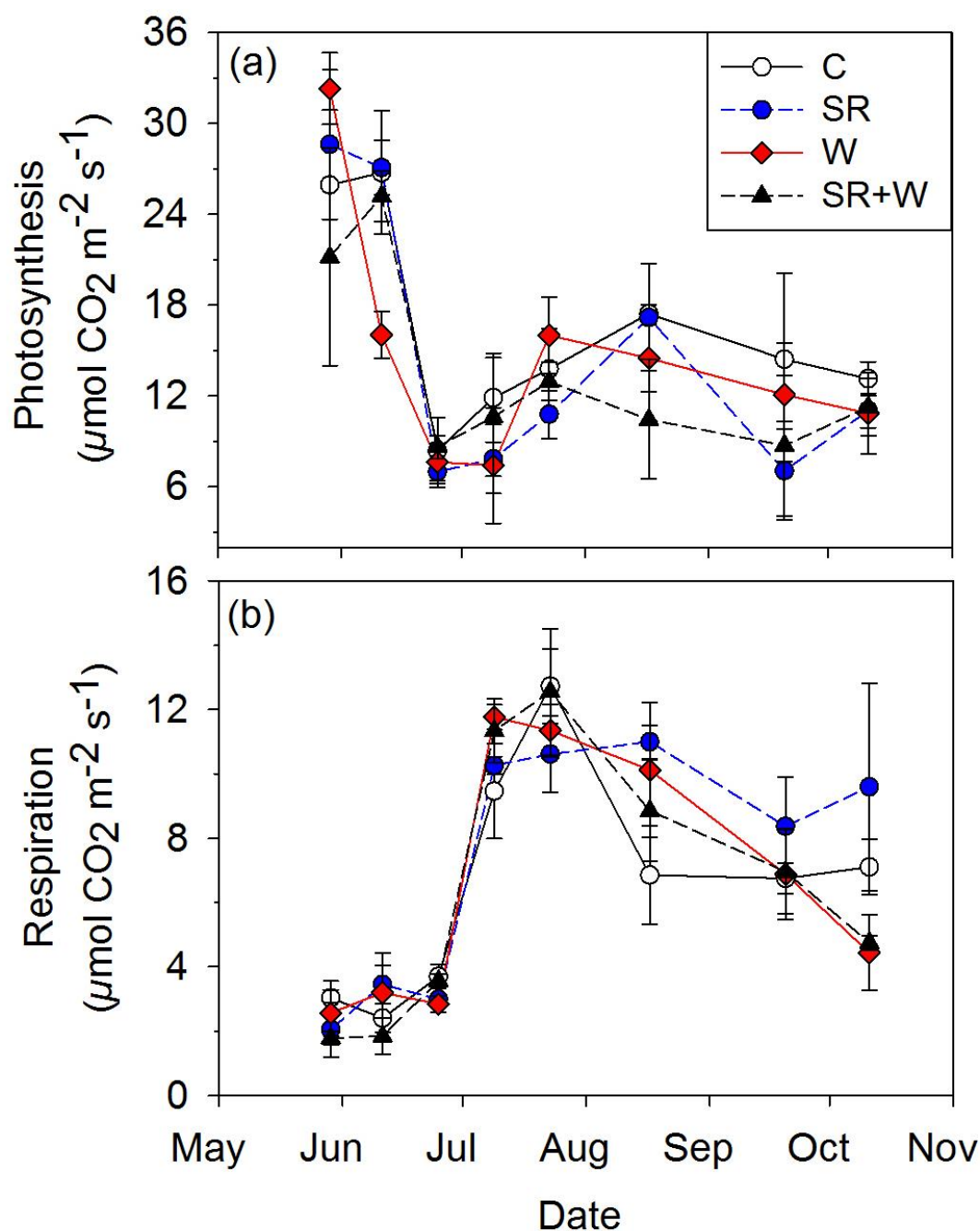


Figure 3. Mean mid-day photosynthetic gas exchange (a) and pre-dawn respiration (b) of vegetative leaves of *Artemisia arbuscula* from May through October, 2014. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Tick marks by date indicate the first day of the month. Measurements are reported on a silhouette leaf-area basis ( $n = 3$ ). Error bars are  $\pm 1$  SE from the mean.

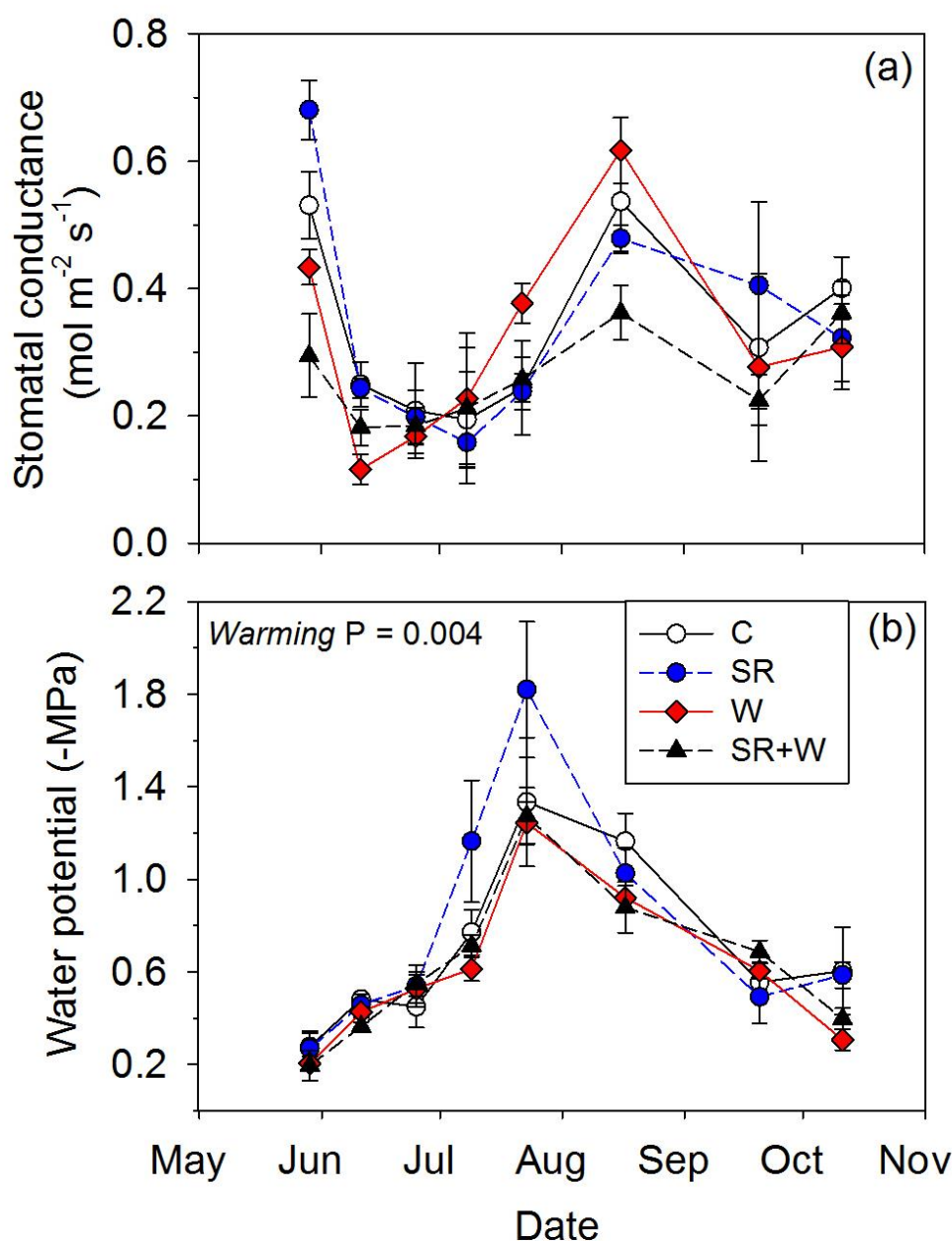


Figure 4. Mean stomatal conductance of water of vegetative leaves at mid-day (a) and xylem water potential at pre-dawn of *Artemisia arbuscula* from May through October, 2014. Tick marks by date indicate the first day of the month. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Stomatal conductance measurements are reported on a silhouette leaf-area basis ( $n = 3$ ). Water potential was measured with a pressure bomb between 0500 and 0700 h MDT ( $n = 3$ ). Error bars are  $\pm 1$  SE from the mean.

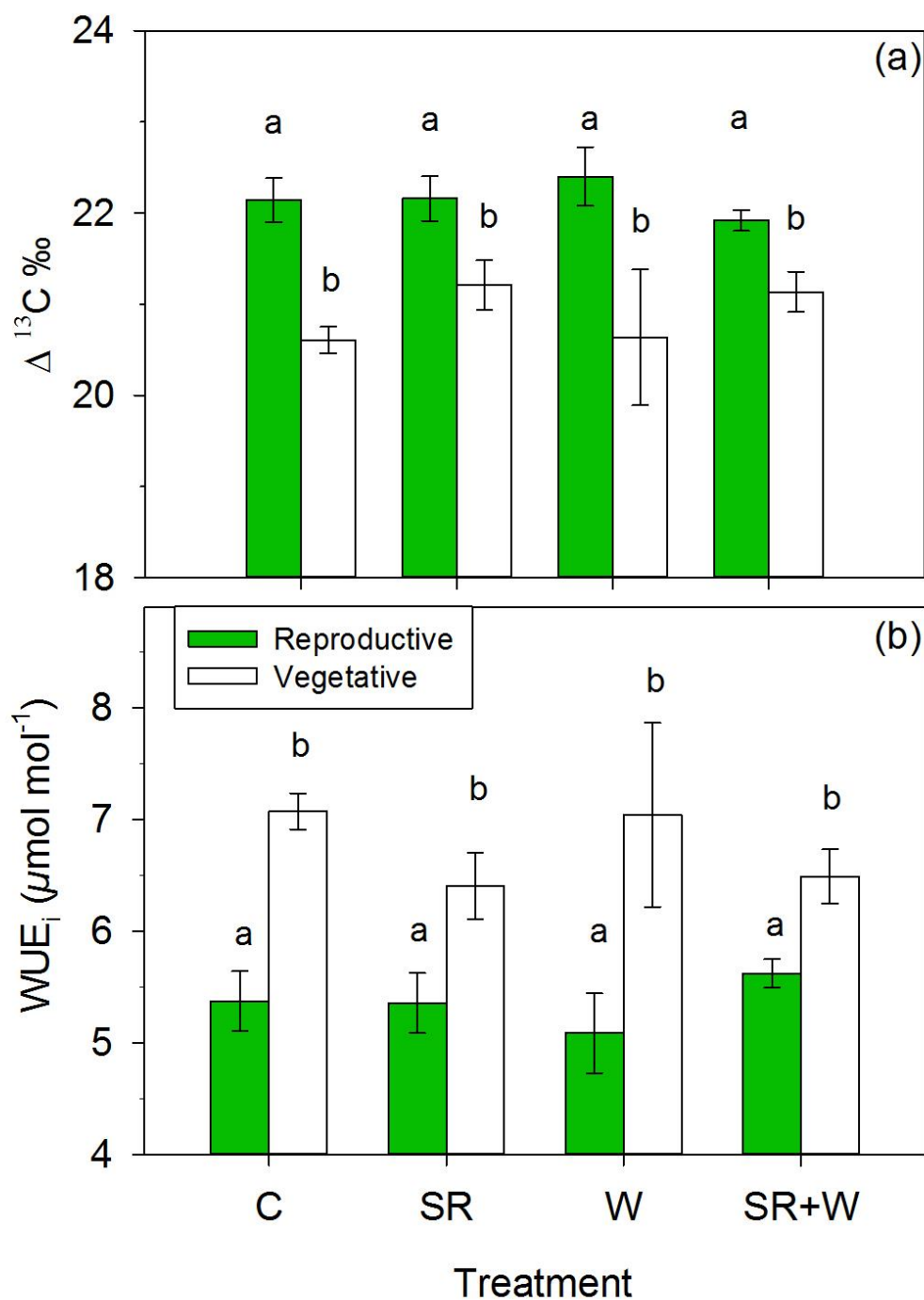


Figure 5. Effects of climate treatments on carbon isotope discrimination (‰) (a) and the calculated intrinsic water-use efficiency ( $\mu\text{mol mol}^{-1}$ ) of *Artemisia arbuscula* leaves from reproductive and vegetative stems. Treatments were in place from May through October 2014. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Error bars are  $\pm 1$  SE from the mean.

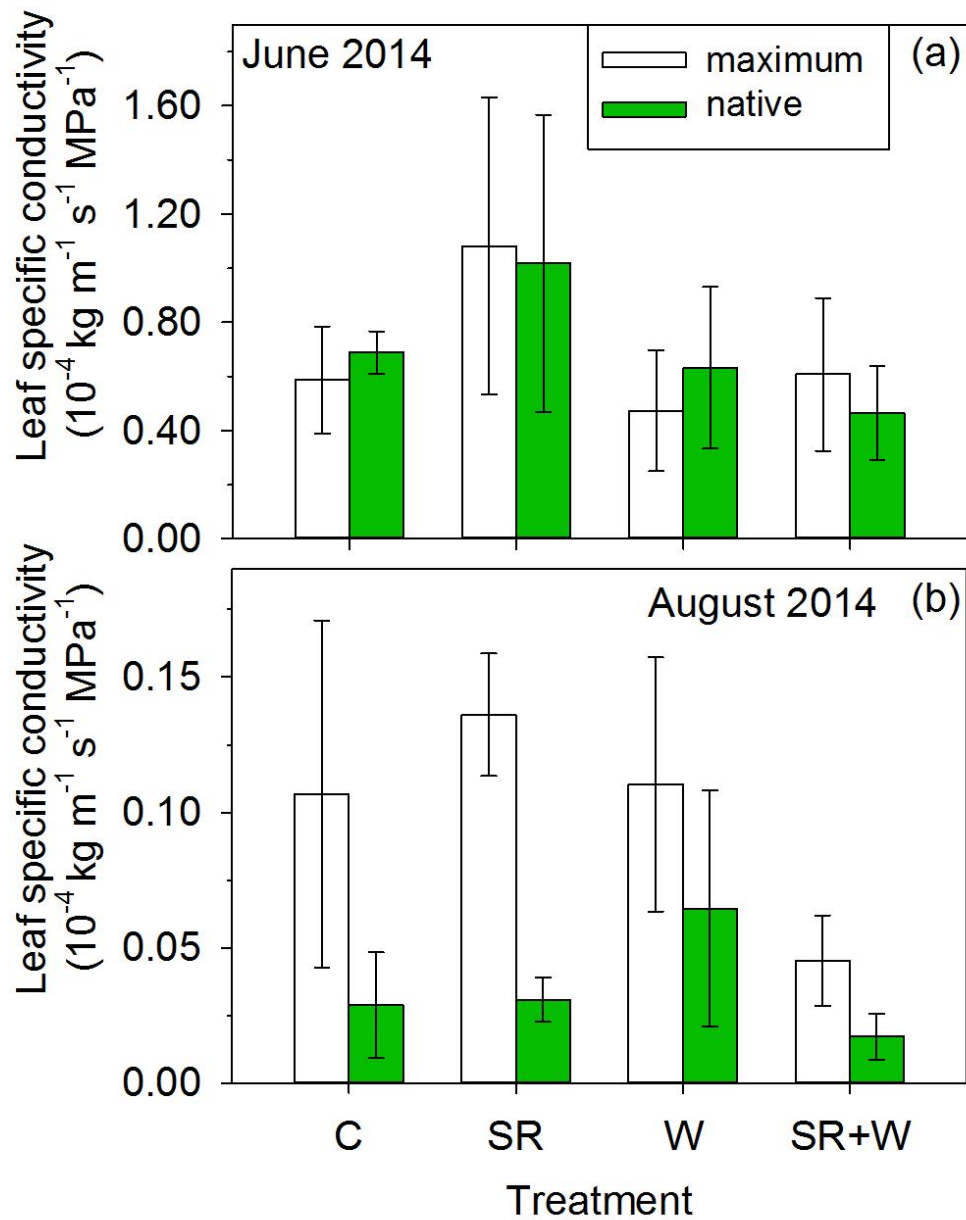


Figure 6. Leaf-area specific hydraulic conductivity of *Artemisia arbuscula* stems that were harvested in (a) June and (b) August 2014 from experimental climate plots. Green bars represent conductivity with native embolisms, and white bars represent conductivity after embolisms were removed with a vacuum. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Error bars are  $\pm 1$  SE from the mean.

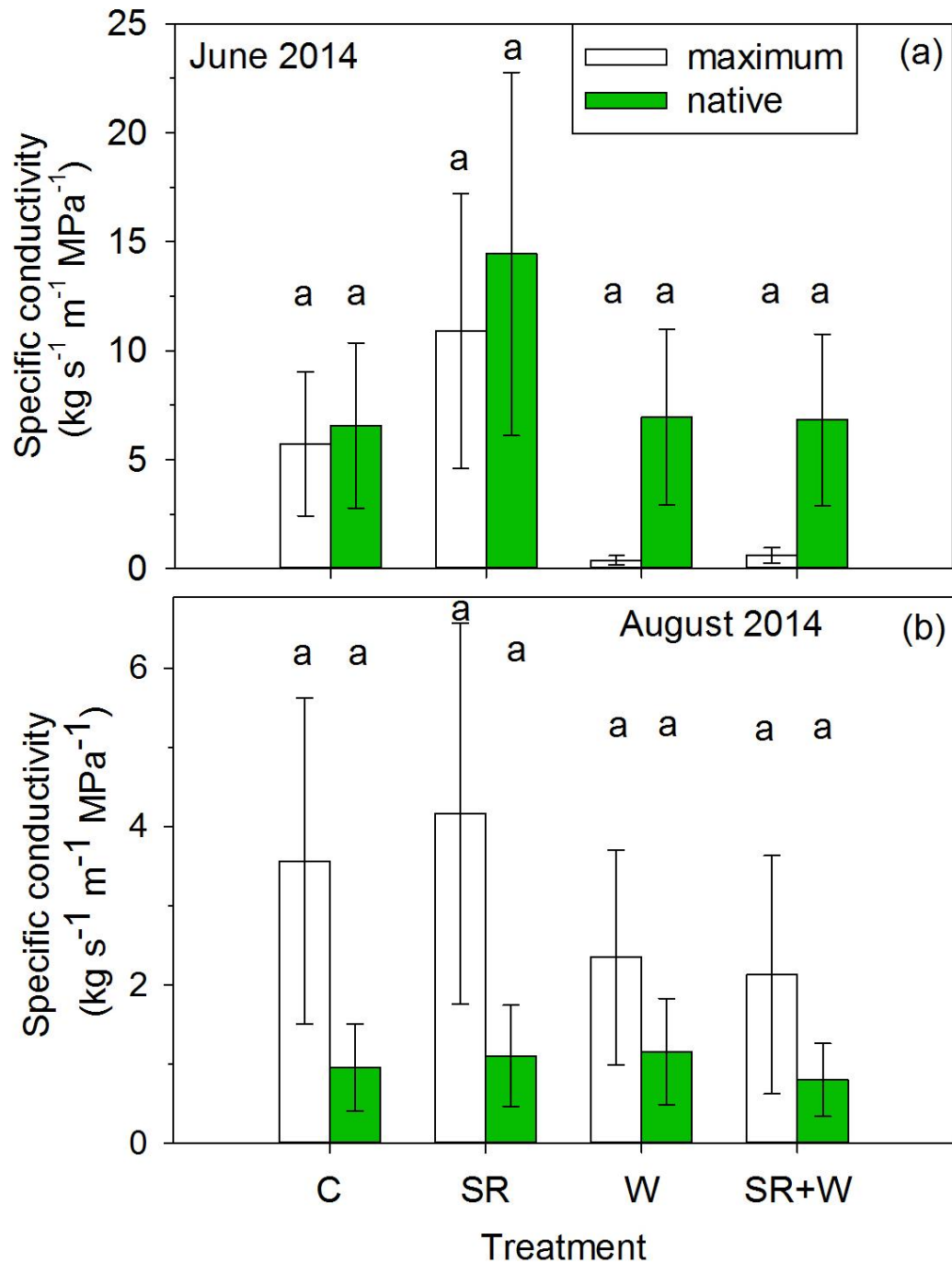


Figure 7. Sapwood-area specific hydraulic conductivity of *Artemisia arbuscula* stems that were harvested in (a) June and (b) August 2014 from experimental climate plots. Green bars represent conductivity with native embolism, and white bars represent conductivity after embolisms were removed with a vacuum. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Error bars are  $\pm 1$  SE from the mean.

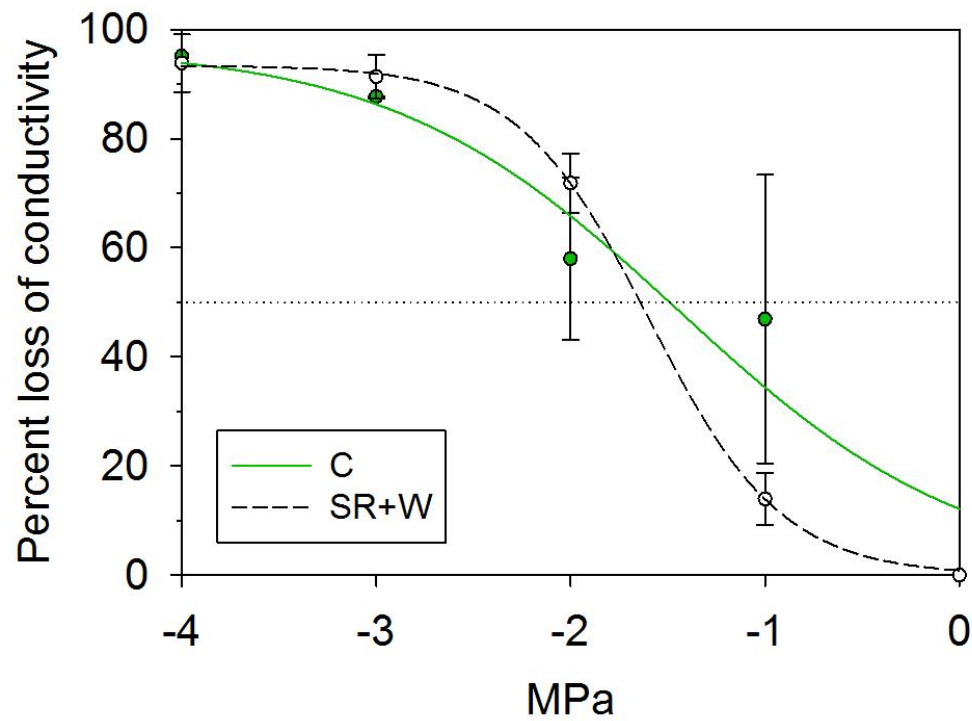


Figure 8. A xylem vulnerability curve for *Artemisia arbuscula*, showing the relationship between the percent loss of leaf-specific hydraulic conductivity and xylem pressure (-MPa). C = control, and SR+W = both snow removal and warming. Each individual curve represents one stem per treatment. The dotted line corresponds to a 50% loss in conductivity ( $P_{50}$ ).

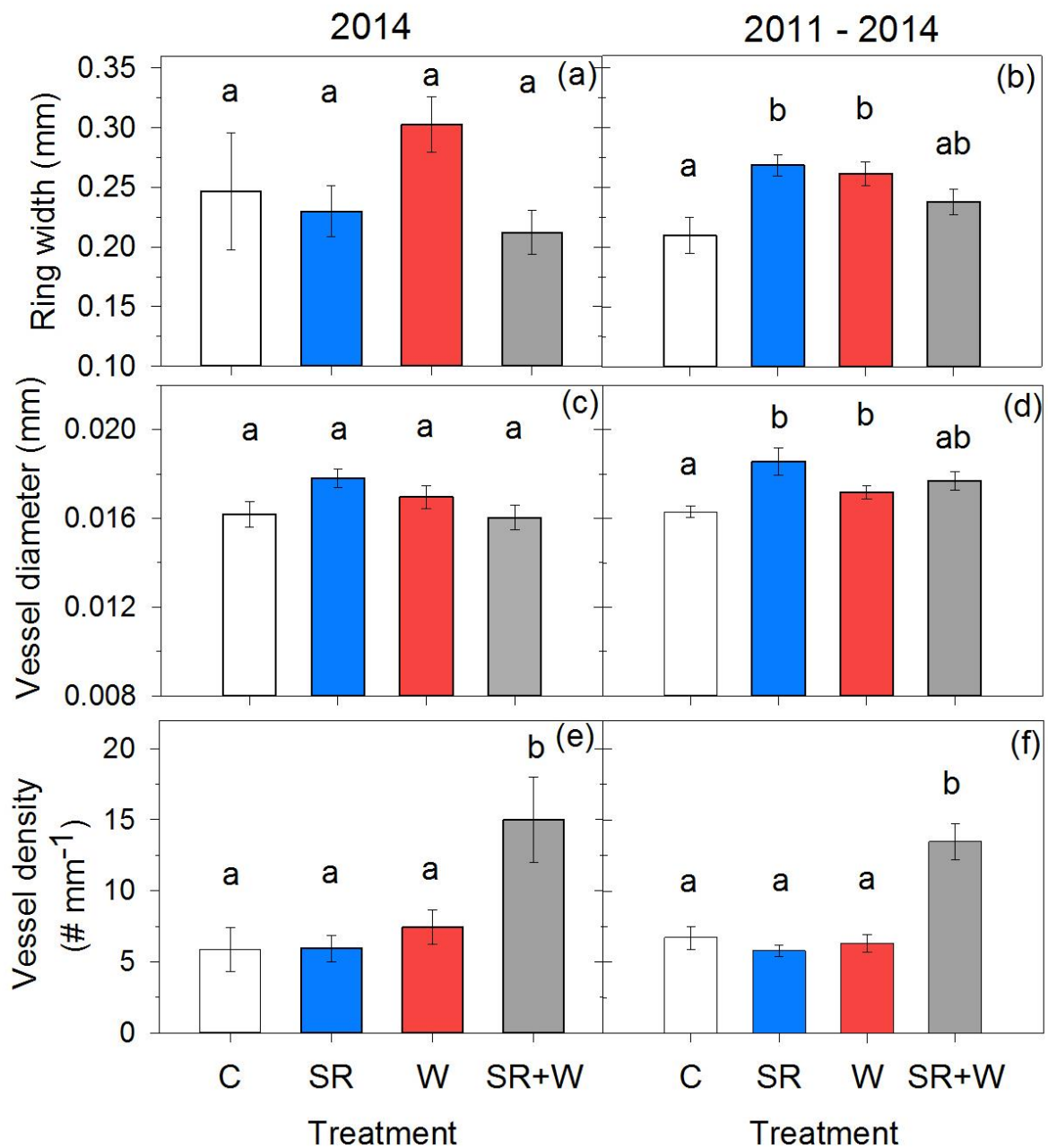


Figure 9. Structural responses in *Artemisia arbuscula* xylem to experimental climate treatments in 2014 only (a, c, e), and from 2011 to 2014 (b, d, f). C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Error bars are  $\pm 1$  SE from the mean.

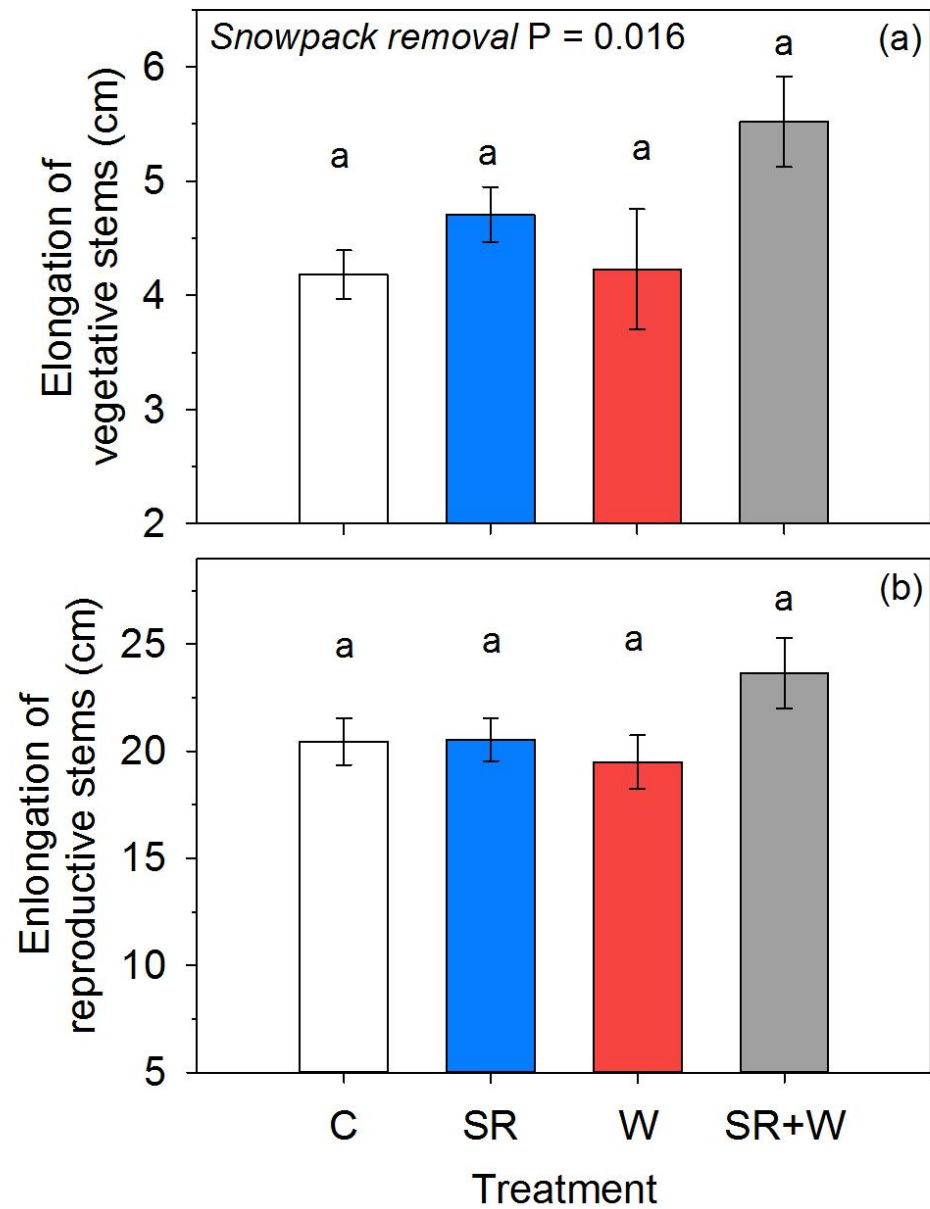


Figure 10. Elongation of vegetative (a) and reproductive (b) stems in *Artemisia arbuscula* from May to late-September 2014 in experimental climate treatments. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Error bars are  $\pm 1$  SE from the mean.

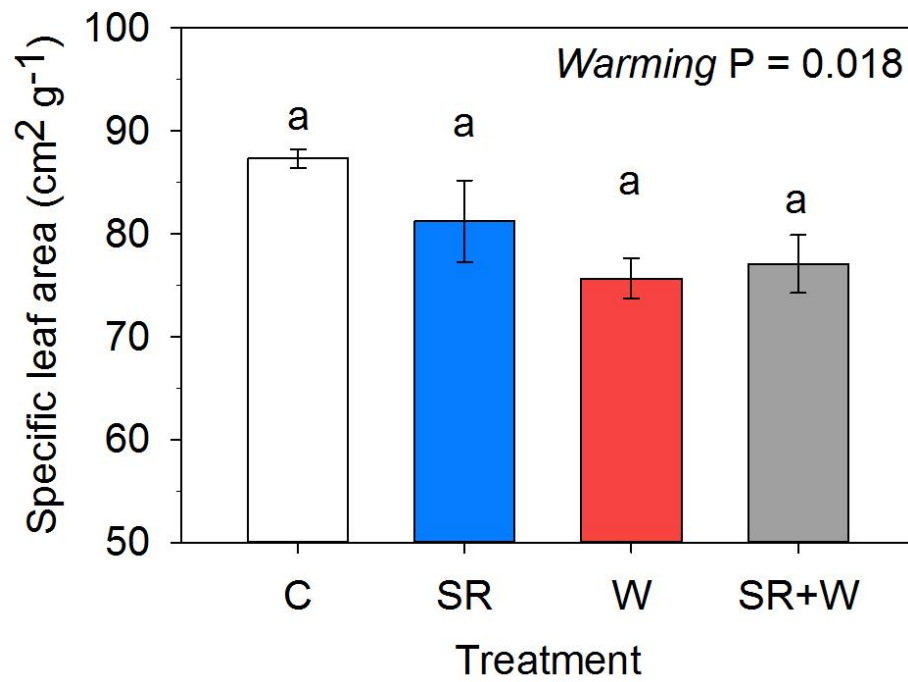


Figure 11. Effects of climate treatments on specific leaf area (cm<sup>2</sup> g<sup>-1</sup>) of *Artemisia arbuscula* leaves from reproductive and vegetative stems from May to mid-August. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Error bars are  $\pm 1$  SE from the mean.

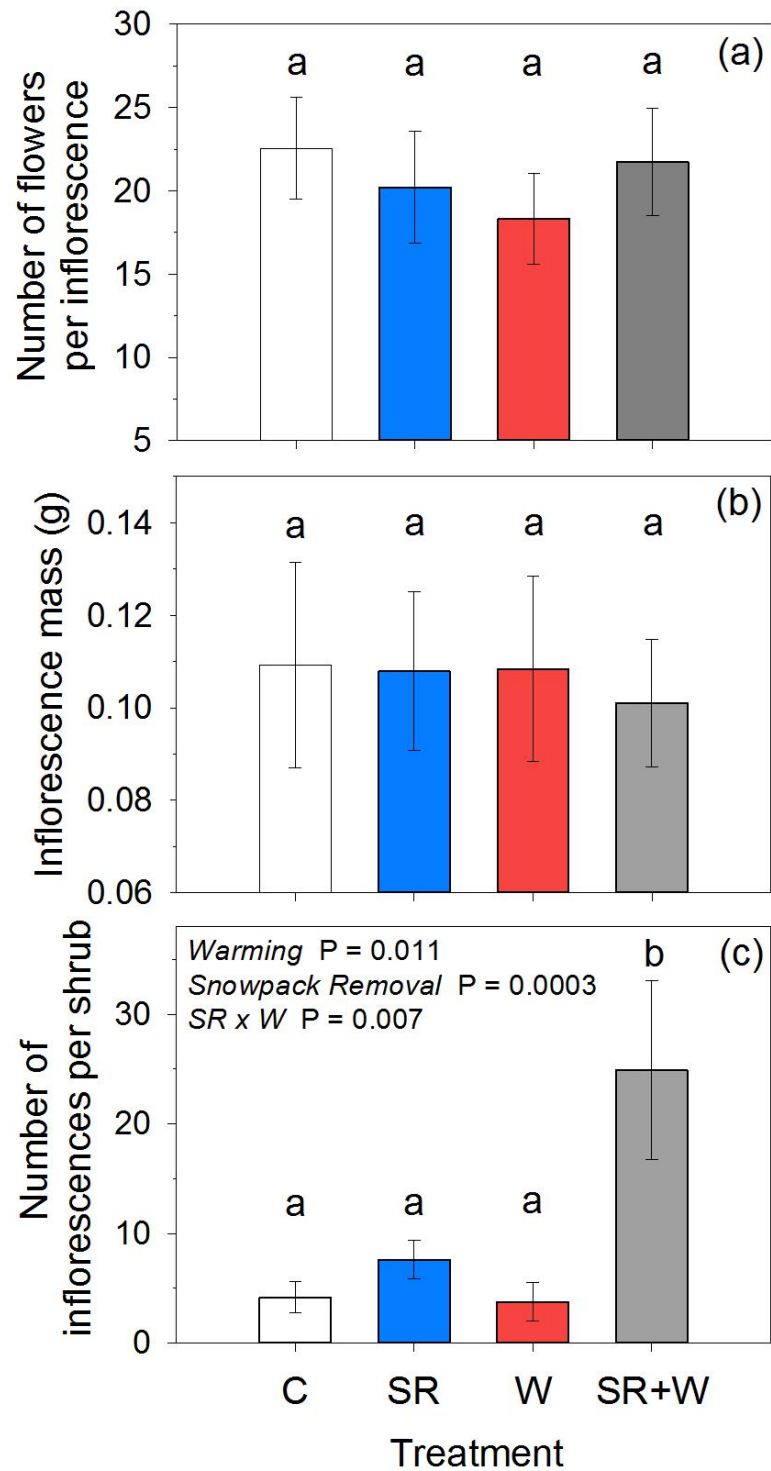


Figure 12. Mean number of flowers per individual inflorescence (a), mass of inflorescences (b), and number of inflorescences per *Artemisia arbuscula* that were subjected to climate treatments. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Error bars are  $\pm 1$  SE from the mean.

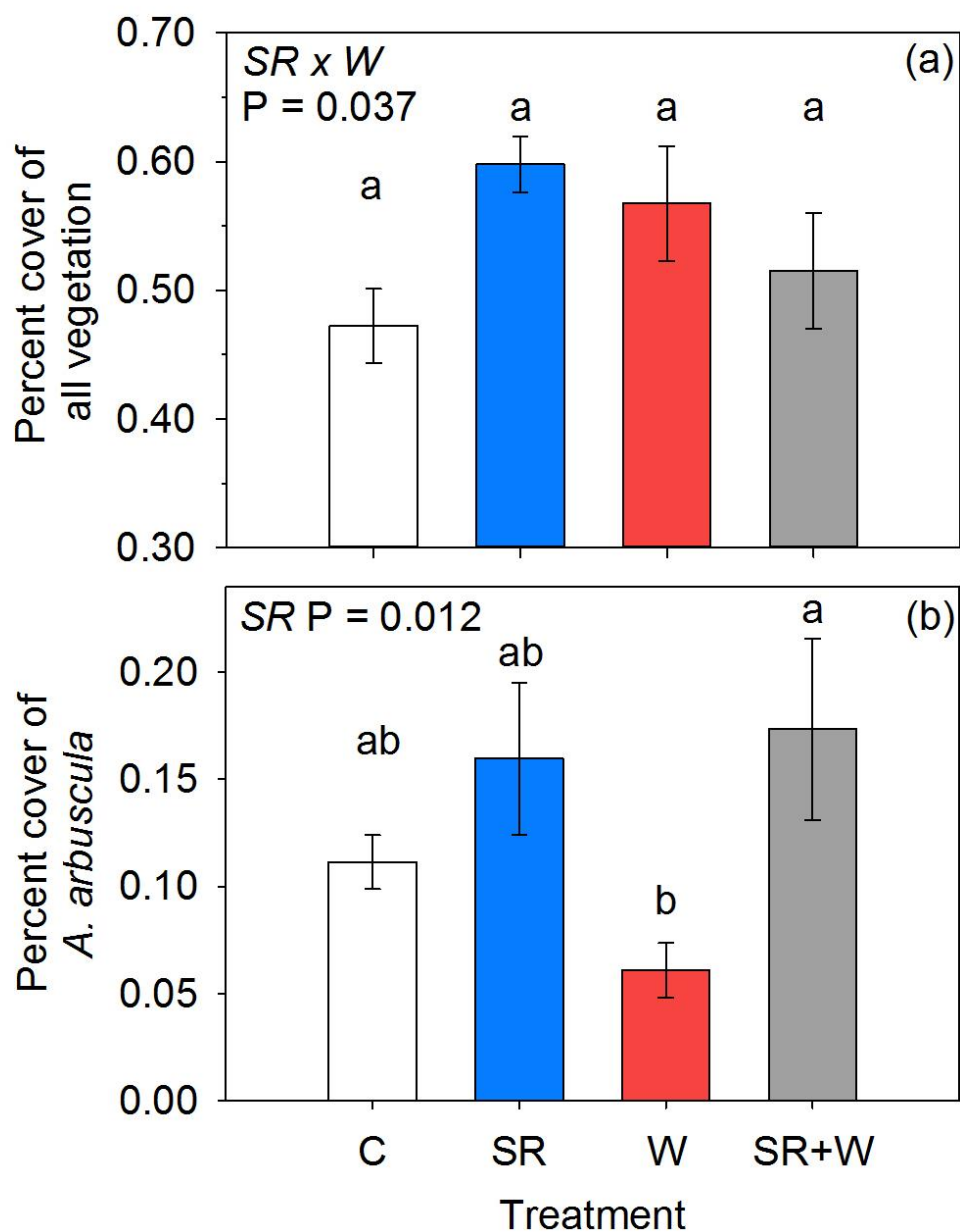


Figure 13. Mean percent cover of vegetation (a) and *Artemisia arbuscula* only (b) during peak greenness in July 2014 in climate plots. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Error bars are  $\pm 1$  SE from the mean.

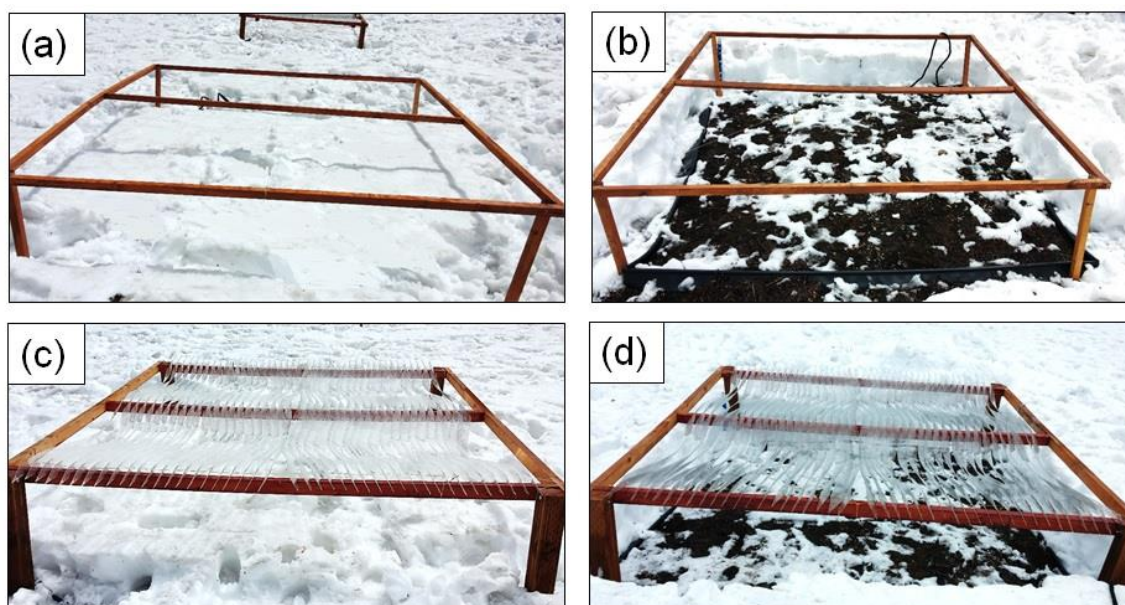
## Supplemental Tables and Figures

Supplemental Table 1. Summary ANOVA table showing the effects of snowpack removal and warming on percent nitrogen and the nitrogen isotope ratio of *Artemisia arbuscula* in experimental plots.

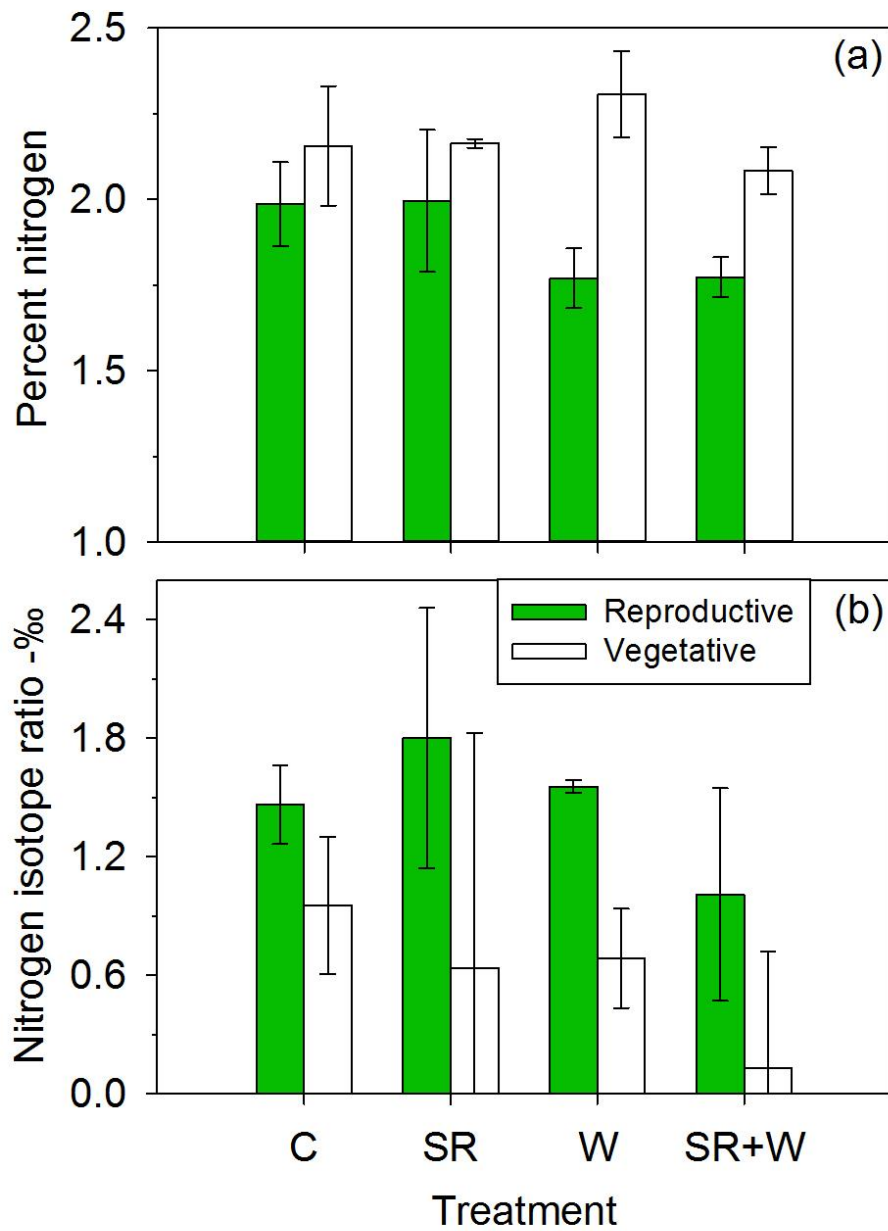
|                                       | Warming |       |       | Snowpack Removal |       |       | Warming $\times$ Snowpack Removal |       |       |
|---------------------------------------|---------|-------|-------|------------------|-------|-------|-----------------------------------|-------|-------|
|                                       | d.f.    | F     | P     | d.f.             | F     | P     | d.f.                              | F     | P     |
| Percent nitrogen (reproductive)       | 1, 8    | 2.809 | 0.132 | 1, 8             | 0.003 | 0.961 | 1, 8                              | 0.001 | 0.980 |
| Percent nitrogen (vegetative)         | 1, 8    | 0.078 | 0.787 | 1, 8             | 0.801 | 0.397 | 1, 8                              | 1.049 | 0.336 |
| Nitrogen isotope ratio (reproductive) | 1, 8    | 0.162 | 0.698 | 1, 8             | 2.544 | 0.149 | 1, 8                              | 0.099 | 0.761 |
| Nitrogen isotope ratio (vegetative)   | 1, 8    | 1.225 | 0.300 | 1, 8             | 0.600 | 0.461 | 1, 8                              | 0.117 | 0.741 |

Supplemental Table 2. Summary ANOVA table showing the effects of snowpack removal and warming on the physiology of *Balsamorhiza sagittata* and *Eriogonum umbellatum* in experimental plots.

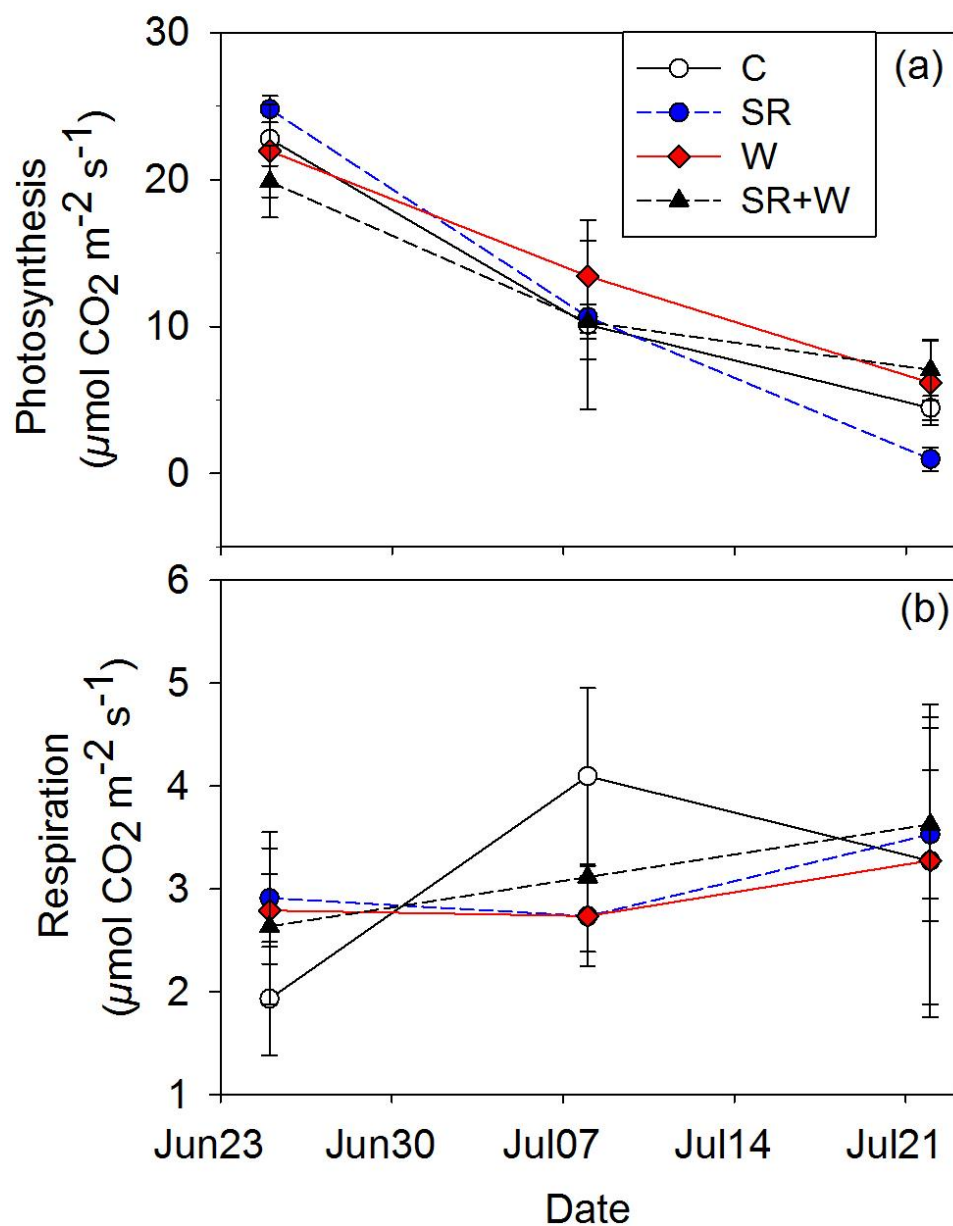
|                                           | Warming |       |       | Snowpack Removal |       |       | Warming × Snowpack Removal |       |       |
|-------------------------------------------|---------|-------|-------|------------------|-------|-------|----------------------------|-------|-------|
|                                           | d.f.    | F     | P     | d.f.             | F     | P     | d.f.                       | F     | P     |
| <i>B. sagittata</i> photosynthesis        | 2, 8    | 0.208 | 0.660 | 2, 8             | 0.228 | 0.646 | 2, 8                       | 0.097 | 0.764 |
| <i>B. sagittata</i> respiration           | 2, 8    | 0.006 | 0.940 | 2, 8             | 0.092 | 0.769 | 2, 8                       | 0.036 | 0.855 |
| <i>B. sagittata</i> stomatal conductance  | 2, 8    | 0.504 | 0.498 | 2, 8             | 0.007 | 0.936 | 2, 8                       | 0.128 | 0.730 |
| <i>E. umbellatum</i> photosynthesis       | 2, 8    | 4.347 | 0.071 | 2, 8             | 1.131 | 0.313 | 2, 8                       | 1.680 | 0.231 |
| <i>E. umbellatum</i> respiration          | 2, 8    | 3.499 | 0.098 | 2, 8             | 3.151 | 0.114 | 2, 8                       | 0.035 | 0.857 |
| <i>E. umbellatum</i> stomatal conductance | 2, 8    | 0.751 | 0.411 | 2, 8             | 0.422 | 0.534 | 2, 8                       | 0.016 | 0.903 |



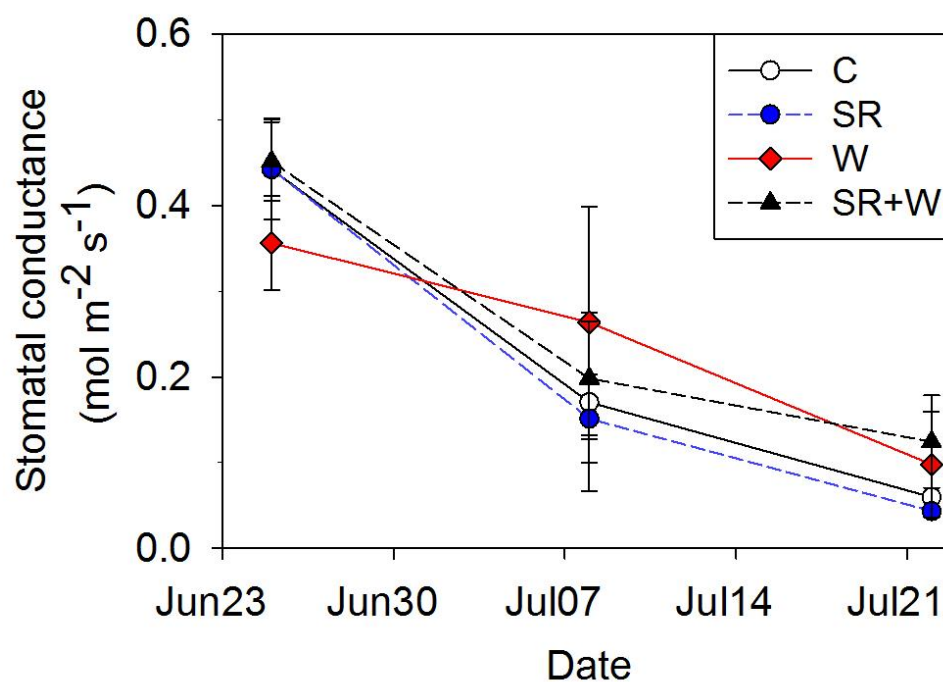
Supplemental Figure 1. Treatment applications of snowpack removal and passive warming on climate plots in early May, 2014. (a) shows control plots, (b) shows snowpack removal, (c) shows warming with open-sided passive warming frames, and (d) shows snowpack removal in conjunction with warming.



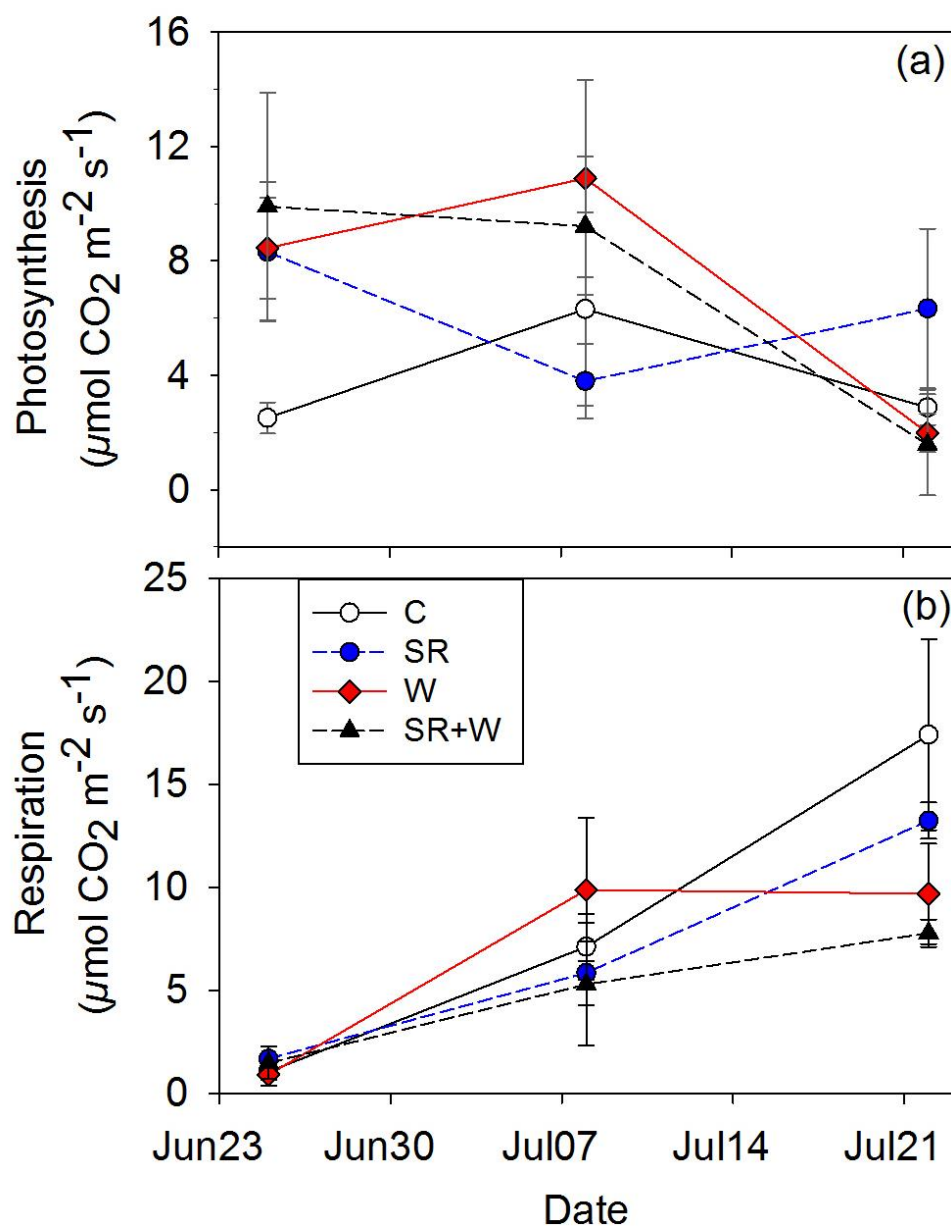
Supplemental Figure 2. Percent nitrogen (a) and nitrogen isotope ratio (b) of *Artemisia arbuscula* leaves from vegetative and reproductive stems. Treatments were in place from May through October 2014. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Error bars are  $\pm 1$  SE from the mean.



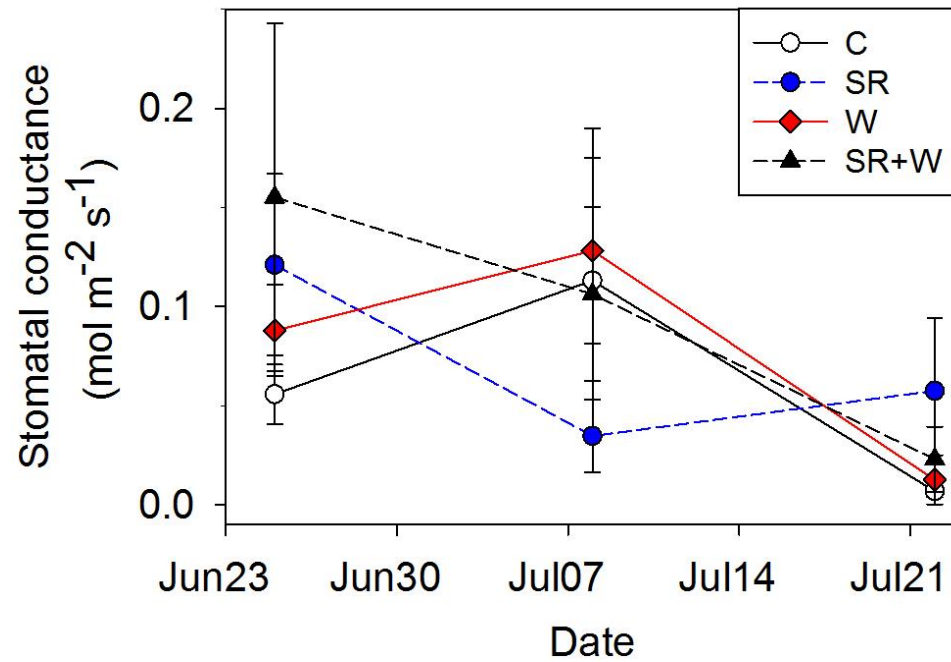
Supplemental Figure 3. Mean mid-day photosynthetic gas exchange (a) and pre-dawn respiration (b) of *Balsamorhiza sagittata* from June through July, 2014. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Values are reported on a silhouette leaf-area basis ( $n = 3$ ). Error bars are  $\pm 1$  SE from the mean.



Supplemental Figure 4. Mean mid-day stomatal conductance to water of *Balsamorhiza sagittata* from June through July, 2014. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Values are reported on a silhouette leaf-area basis ( $n = 3$ ). Error bars are  $\pm 1$  SE from the mean.



Supplemental Figure 5. Mean mid-day photosynthetic gas exchange (a) and pre-dawn respiration (b) of *Eriogonum umbellatum* from June through July, 2014. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Measurements are reported on a silhouette leaf-area basis ( $n=3$ ). Error bars are  $\pm 1$  SE from the mean.



Supplemental Figure 6. Mean mid-day stomatal conductance to water of *Eriogonum umbellatum* from June through July, 2014. C = control, SR = snowpack removal, W = warming, and SR+W = both warming and snow removal. Measurements are reported on a silhouette leaf-area basis ( $n = 3$ ). Error bars are  $\pm 1$  SE from the mean.

### CHAPTER 3: GENERAL CONCLUSIONS

Future effects of climate, including changes in snowpack and temperature, may have substantial effects on vegetation, but that is debated in the literature (Wu et al. 2011). Those environmental effects may potentially have implications for plant acclimation, and in the long-term, plant adaptation to changes in climate. Vegetation at high elevations are especially prone to changes in climate, and mid-elevation ecosystems are understudied.

We studied *Artemisia arbuscula*, a dominant and ecologically relevant shrub at 2,120 m in Grand Teton National Park, WY, USA. Our climate-change experiment allowed us to investigate potential changes in its physiology and structure in this particular geographic region. Our data demonstrated that the physiological performance of montane *Artemisia arbuscula* was generally not affected by an increase in temperature, or by reduced spring snowpack. We did not see instantaneous changes in photosynthesis, respiration, or stomatal conductance. We found small changes in plant water status, which requires further research, but may be important for *A. arbuscula*. Our data also indicated few changes in plant hydraulics throughout the growing season, in addition to long-term water use efficiency. In contrast, results indicated that *A. arbuscula* responded to treatments with some changes in structure and growth- including the addition of reproductive stems, elongation of vegetative stems, overall plant cover, and decreased specific leaf area. In addition, there were changes in xylem hydraulic architecture during the 2014 growing season. Because we were able to measure xylem from three previous treatment years, we revealed additional differences in xylem growth and structure that were more robust than our data from 2014 only. Taken together, those results may have

potential implications for adjustments in this distribution of montane sagebrush under environmental change.

Overall, we revealed occasional differences in sagebrush structure and function due to only slight changes in the environment (1° C of warming and minor snowpack removal, which did not cause significant changes in soil water). However, in total, the climate treatments were not detrimental to the shrubs. We used realistic treatments that mimic, or are less vigorous than the predicted climate in the future, which suggests that *A. arbuscula* may not be sensitive to small changes in climate in the future. We suspect that further investigation, with more robust treatments and larger sample sizes, may reveal more adjustments in physiological performance and structure- unless *A. arbuscula* is somewhat resistant to change. It is also likely that *A. arbuscula* in other geographical regions may experience greater or lesser changes in structure and physiology than at our site, depending on climate gradients. This research project was one of the first, to our knowledge, to investigate variations in the physiology and structure of *A. arbuscula* under different environmental conditions. That species is critical to study, because it, in addition to other montane species, is sensitive to environmental conditions, it may serve as an indicator of climate change (Debinski et al. 2000). Therefore, as changes in climate progresses, it will become increasingly essential to investigate the physiology of species in high and mid-elevation ecosystems, which are especially vulnerable to global change.

## References

Debinski, D. M., Jakubauskas, M. E., Kindscher, K. 2000. Montane meadows as indicators of environmental change. *Environmental monitoring and assessment*, 64(1), 213-225.

Wu, Z., Dijkstra, P., Koch, G. W., Penuelas, J., Hungate, B. A. 2011. Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biology*, 17(2), 927-942.