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Changes in structure and physiological functioning due to experimentally enhanced precipitation seasonality in a widespread shrub species

Keith Reinhardt · Kathryn McAbee · Matthew J. Germino

Abstract Semi-arid shrub steppe occupies a vast geographic range that is characterized in part by distinct seasonal patterns in precipitation. Few studies have evaluated how variability in both the amount and timing of precipitation affect the structure and physiology of shrubs in these systems. We quantified changes in foliar crown parameters, xylem anatomy, gas exchange, and hydraulic transport capacity in deep-rooted *Artemisia tridentata* shrubs following 20 years of experimental manipulations in amount and seasonal timing of precipitation. We hypothesized that shrub growth (total leaf area per shrub and cover of shrub community), hydraulic transport efficiency, and gas exchange would increase in shrubs in irrigated plots compared to non-irrigated control plots, especially for irrigation applied in winter rather than summer. We also predicted similar changes in xylem anatomy (ring width, vessel size and frequency). Most treatment responses entailed changes in plant structure, and were generally consistent with our hypotheses: total-shrub leaf area, shrub basal area, canopy cover, and maximum sapwood-specific branch hydraulic conductivity were more than $2\times$ greater in shrubs in winter-irrigated compared to control plots, while summer irrigation had few effects on these variables. Irrigation in either season did not affect xylem vessel size, but did increase xylem ring width by $\sim 2\times$ and decreased xylem vessel frequency by about half. Anatomical, morphological, and stand-level abundance of A. *tridentata* appeared much more responsive to irrigation than state changes in gas exchange, particularly when the extra water is received during winter. Thus, it appears for sagebrush that seasonal timing is at least as important as the amount of precipitation, and that responses to changes in precipitation timing occur through changes in carbon allocation more so than changes in leaf-level carbon gain.

Keywords *Artemisia tridentata* · Ecohydrology · Hydraulics · Photosynthesis · Xylem

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Introduction

Global and regional precipitation patterns are predicted to shift (IPCC 2014), and understanding which aspects of plant communities are likely to be more or less responsive to the changes needs to be assessed and

ecosystem's vulnerability should also be monitored. In many regions of the world, such as the semi-arid Great Basin of the U.S., climate models project changes in precipitation seasonality more so than changes in mean annual precipitation (Abatzoglou and Kolden 2011; Mote and Salathé Jr. 2010). How changes in precipitation seasonality will affect productivity and overall physiological functioning of plants adapted to long-term precipitation patterns is thus an important question in plant ecology. This is especially so in water-limited systems, where changes in either the amount or the timing of precipitation might drive plant responses, which little research has addressed (Bates et al. 2006; Patrick et al. 2007), especially at long time scales (> 10–20 years; Germino and Reinhardt 2014; McAbee et al. 2017).

Previous ecophysiological studies on the responses of semi-arid plants to changes in the amount or timing of precipitation (and thus water availability) highlight that there are species-specific differences, some of which may be related to what soil layers (i.e., shallow vs. deep) plants primarily access soil–water from (e.g., Walter's two-layer hypothesis; Walter 1973). For example, Loik et al. (2015) showed that experimental manipulations of wintertime snowpack significantly impacted summertime photosynthesis in *Artemisia tridentata* (which has shallow and deep roots) but not *Purshia tridentata* (which has mostly deep roots) species that span large climatic and geographic ranges. Others have reported little to no changes in physiology or productivity of several species of adult shrubs with summertime irrigations (which wet only shallow soil layers) in both warm and cold deserts (Evans and Black 1993; Snyder et al. 2004; McAbee et al. 2017), perhaps due to co-limitation by nutrients or "phenological canalization" based in part of the evolutionary history of these species (Snyder et al. 2004). Similarly, McAbee et al. (2017) found no differences in leaf-level photosynthesis in *A. tridentata* shrubs in summer-irrigated versus control plots. Finally, in a sotol grassland in Texas, supplemental watering during wet periods decreased photosynthesis in the subshrub *Dasylirion leiophyllum*, possibly due to nutrient limitation (Patrick et al. 2007). Overall, few studies have investigated how the timing of precipitation impacts plants and plant communities using controlled experiments, and even fewer evaluate the ecophysiological responses or adjustments underlying the species' changes.

Another way plants respond to altered precipitation and/or water availability is through changes in plant structure or community assembly (McDowell et al. 2006; Germino and Reinhardt 2014). At the whole-plant scale, a conceptual framework to explain such adjustments was proposed by Whitehead and Jarvis (1981 for conifers), whereby changes in biomass allocation and xylem/sapwood transport capacity act to maintain plant water-relations and fluxes from the plant-to-stand level (e.g., Whitehead et al. 1984; McDowell et al. 2006). Their model predicts that leaf area-to-sapwood area ratios should decrease with decreasing water availability (assuming little stomatal adjustments), or that changes in xylem architecture (e.g., vessel wall thickness, conduit size and frequency) should occur to regulate water fluxes. In general, this theoretical framework has been supported by numerous field studies, but with less examples for desert plants. Carter and White (2009) reported that changes in leaf area-to-sapwood-area ratios maintained leaf water status and cell water relations in *Eucalyptus kochii* trees growing in sites with varying water availability in Australia. Also, the ratio of leaf area to sapwood area decreased in *P. ponderosa* growing in drier microsites relative to mesic microsites in Nevada, USA, resulting in relatively greater branch hydraulic conductivity (Maherali and DeLucia 2000). At the population scale, Germino and Reinhardt (2014) reported that crown and canopy size, but not shrub density (#shrubs/area), increased for *A. tridentata* shrubs growing in winter-irrigated plots with greater spring and early summer soil moisture availability relative to control plots, but summer-irrigation had little effect.

To gain greater understanding of how woody plants respond physiologically and anatomically to precipitation timing, and specifically how these responses relate to the population-level responses reported previously (Germino and Reinhardt 2014), we took advantage of an ecohydrological experiment in Idaho, USA in which the amount and timing (summer- or winter-irrigation) of precipitation has been manipulated for > 20 years in basin big sagebrush (*A. tridentata* ssp. *tridentata*) steppe plots. Sagebrush is a widespread, often locally dominant shrub, and is an ecosystem engineer that dominates carbon and water fluxes in communities where it is present (Prevéy et al. 2010). We compared differences in shrub allometry, xylem anatomy, leaf-level gas exchange, and

hydraulic transport capacity in years 20 and 21 of the experiment. Sagebrush has been shown to be responsive to changes in winter precipitation (total amount, and also the ratio of rain to snow during winter; Schlaepfer et al. 2012). Thus, we hypothesized that changes in structure and ecohydrological functioning would be strongest in winter-irrigated plots, with fewer changes observable in summer-irrigated plots, compared to control plots. We further hypothesized that we would observe more adjustments in structure rather than physiological responses, as physiological responses in sagebrush to manipulative treatments are often minimal (Evans and Black 1993; Loik et al. 2000, 2004; Pratt and Mooney 2013; McAbee et al. 2017).

Methods

Study site and experimental design

We conducted this study at an ongoing ecohydrological experiment located at the Idaho National Laboratory, which is situated in sagebrush steppe in the Snake River Plain (for details see Anderson and Forman 2002). Local climate is typical of sagebrush steppe communities: annual mean temperature is 5.5 °C, mean temperature of the warmest month is 20 °C, and mean temperature of the coldest month is − 8.8 °C (Clawson et al. 1989). Mean annual precipitation is 213 mm, with > 60% occurring during October–May. The experimental site was constructed in 1993 and consists of three replicated blocks of four soil profile configurations (only one was used in this study), three irrigation treatments, and two planted vegetation communities (only one used in this study— a mixture of native grass, forb, and grass species; for more information, see Germino and Reinhardt 2014). At the beginning of the study, sagebrush seedlings were transplanted from local sagebrush communities. Plots were planted with eight individuals of *A. tridentata* ssp *tridentata* and eight *A. tridentata* ssp *wyomingensis*, but only *A. tridentata* ssp *tridentata* (diploid) survived. In the nine 8 m × 8 m plots used in this study, there was an average of nine shrubs per plot at the time of this study, ∼ 1.5–2 m tall, with ∼ 0.5–2.0 m^3 crown volume per shrub (Germino and Reinhardt 2014). Temporal changes in shrub abundance and cover over the life of the experiment were reported in Germino and Reinhardt (2014).

In the plots used in the study, the soil depth was 2 m, consisting of a rock-free silty clay loam (mean texture 19% sand, 48% silt, and 33% clay) that was obtained from a nearby site at the Idaho National Laboratory, and mixed prior to filling plots. Each plot was lined with 0.1 m of gravel at 2 m depth, and then filled with soil in 0.2 m increments, with each increment compacted to a bulk density of 1.29 g cm^{-3}. Soil horizons were not reconstructed upon installation, but by 2001 depth profiles of soil organic carbon and phosphorous had reformed considerably relative to surrounding, undisturbed communities (McGonigle et al. 2005). The soils in plots are representative of soils throughout much of the Great Basin and particularly the loess-derived soils of the upper Snake River Plain (deep, well-drained, with little horizonation beyond the top few cm of soil).

The three irrigation treatments are: ambient precipitation with no supplemental irrigation ("AMB"; control plots), summer-only supplemental irrigation ("SUM"), and winter-only supplemental irrigation ("WIN"). The SUM irrigation treatment consists of four 50 mm irrigations was applied biweekly beginning in mid-June (200 mm total). These treatments are intended to simulate large summer-monsoon rainfall events that wet soils down to ∼ 0.4 m depth (boosting volumetric soil water content ∼ 2–4 percentage points relative to AMB plots), and this moisture is mostly evapotranspired within 2 weeks. The WIN irrigation treatment applied 200 mm of water as quickly as possible in October or early April each year (or 100 mm in both October or April in some years), increasing volumetric soil water content ∼ 4–7 percentage points throughout the entire soil profile (0–2 m) relative to AMB plots, with the increased soil water amount evident through July in most years. Irrigation was applied to the plots via a drip line and emitter system located on the ground. Both supplemental irrigation treatments increased "annual precipitation" by ∼ 100% (200 mm, i.e., a doubling of average annual precipitation at this site) in most years, resulting in maximum annual precipitation amounts observed for this community type.

Foliar and crown parameters

We measured specific leaf area (SLA, g dry mass cm^{-2} one-sided leaf area) and leaf area index (LAI, m^2 one-sided leaf area m^{-2} ground area) on five *A. tridentata* shrubs per plot ($N = 15$ total per irrigation treatment) in early July 2013 (approximately peak greenness in the 20th year of the experiment). For SLA, we harvested 5 shoot tips (one tip per shrub) per plot from south-facing branches at mid-shrub height with both ephemeral and perennial leaves. We placed shoot tips in plastic bags that were kept in a cooler during transport to the laboratory (same day). We measured projected leaf area using photographs of detached leaves laid flat on paper with a scale bar using image analysis software (ImageJ, U.S. N.I.H, Bethesda, MD, U.S.A.). We then dried leaves for 48 h at 70 °C in a drying oven, and weighed to ± 0.1 mg to obtain dry mass. We measured LAI of five individual shrubs per plot using a Sunfleck PAR Ceptometer (Decagon Devices, Pullman, WA). We made measurements between 1200 and 1300 h local time on a cloudless day. We made five measurements of photosynthetically active radiation (PAR) above shrub crowns in every plot with the 0.55 m bar of the Ceptometer, and then recorded three measurements of PAR (two orthogonal, one random diagonal) under each shrub. LAI was calculated using a leaf area index calculator spreadsheet utility (http://www.decagon.com/support/lai-calculator/). LAI calculations using this technique do not separate leaf area from woody stem area. Thus, our measurements more accurately reflect "plant area index", but we chose to represent the data using the conventional LAI term. LAI estimations from hand-held instruments that use light extinction equations ("light-bar" techniques) often overestimate true LAI in sagebrush (Finzel 2011). Thus, here we emphasized relative, and not absolute, changes in LAI among precipitation treatments. We estimated total-shrub biomass for each shrub using allometric equations determined on-site from shrubs outside of the treatment plots (McAbee et al. 2017). These equations required measurements of stem basal area ("A_b", in cm^2), which we calculated for 50 shrubs ($N = 16, 16, 18$ for AMB, SUM, WIN plots, respectively) by measuring the circumference of all individual stems of a shrub at ground level, summing for total circumference, and then converting to area. Because we could not destructively sample shrubs, we

could not directly measure sapwood area. We then calculated aboveground total-shrub leaf area ("A_l") for each shrub by multiplying A_l for each shrub by its respective SLA.

We calculated total plot canopy cover (% areal cover per 64 m^2) by measuring the maximum width in two orthogonal directions for each shrub > 30 cm tall in plots, converting these measurements to two-dimensional area per shrub, and then summing all the individual shrub areas in each plot.

Gas exchange

We conducted diurnal measurements of leaf-level gas exchange ∼ biweekly from mid-June to mid-September. We made measurements on three shoot-tips (from three individual shrubs, one shrub per plot per irrigation treatment), at 6:00 a.m., 10:00 a.m., 2:00 p.m., and 6:00 p.m. local time, but we used only the 10:00 a.m. measurements in this study (diurnal curves are reported in McAbee et al. 2017). We define these as "midday" measurements, because the measurement interval occurred approximately from 10:00 to 11:30 a.m., and before any noticeable midday stomatal closure. We used a LI-6400 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA) equipped with a 2 cm × 3 cm clear-top chamber. We set chamber CO_2 concentration to 400 μmol/mol, and we matched conditions inside the chamber to outside conditions (< 10% difference from ambient conditions: mean air temperature, photosynthetically active radiation, and vapor pressure deficit were 32 °C, 1242 μmol m^{-2} s^{-1}, and 3.55 kPa, respectively). During all measurements, we oriented the clear chamber window towards the sun, and maintained natural orientation of the shoots. We report measurements of photosynthesis (A_{net}), transpiration (E), and stomatal conductance (g_s) on a sunlit (silhouette) leaf area basis, which is most appropriate for geometrically complex shoots (Carter and Smith 1985). We determined silhouette leaf area by photographing shoots from the direction of incident sunlight and then analyzing using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Plant carbon isotope ratios

In October 2013, we collected leaf samples from shrubs ($n = 9$ samples/irrigation treatment; three

subsamples per replicate plot), dried as at 60 °C until constant weight, ground to powder, and analyzed for $^{13}C/^{12}C$ at the Idaho State University Interdisciplinary Laboratory for Elemental and Isotopic Analysis using a Costech ECS 4010 elemental analyzer interfaced to a Thermo Delta V Advantage continuous-flow isotope-ratio mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Analytical precision, calculated from analysis of standards distributed throughout each run, was $\leq \pm 0.2‰$. We report isotopic values in the conventional δ-notation ($\delta = ([R_{sample}/R_{standard}] - 1) \times 1000$, where $R = {}^{13}C/{}^{12}C$) relative to the international standard Vienna Pee Dee Belemnite (VPDB) and expressed as per mil (‰). We estimated plant water use efficiency integrated across the growing season (WUEi) using the $\delta^{13}C$ data and equations in Farquhar et al. (1982), with 3.5 kPa being used as a representative value of leaf-to-air vapor pressure difference, as determined from measurements with the LI-6400 gas exchange instrument. We assumed that stomatal conductance scaled linearly with mesophyll conductance (which we did not measure) across all irrigation treatments. Thus, we acknowledge that our estimates of WUEi may be overestimated and instead emphasize relative differences in WUEi among treatments.

Branch hydraulic conductivity and xylem architecture

We collected 5–7 branches ~ 20 cm long and ~ 7–10 mm in diameter for each irrigation treatment (spread across the three replicate blocks) in both July 2013 and 2014, placed them in plastic bags in a cooler, and transported them back to the lab ($n = 5$–7 branches $\times 2$ years = 10–14 branches per irrigation treatment, total). We selected mid-canopy branches that were located on all sides of shrubs, because finding straight, unbranched branches on sagebrush is challenging, and did not allow us to harvest from only sun-exposed branches (to which the gas exchange measurements were confined). All tissue samples collected were between 5 and 8 years old, as determined by counting the number of growth rings. Once in the lab, we cut segments under water to ~ 15 cm length, and placed them in a vacuum chamber with filtered, distilled water at pH = 2 under vacuum overnight, to remove embolisms. The next day, we trimmed the ends of segments underwater using a

razor blade, and determined maximum stem hydraulic conductivity (k_{stem}) using a hydrostatic pressure head (~ 0.8 m) to induce flow through the branch segments. We attached tubing to the distal end of the branch and recorded the time it took for flow to reach gradations on a pipette to calculate volume flow rate. We then calculated k_{stem} by multiplying hydraulic conductance (volume flow rate of water/hydrostatic pressure gradient) by stem length. We then normalized k_{stem} by dividing it by cross-sectional xylem-sapwood area (k_{sap}) or leaf area of the branch segment (k_{leaf}). We determined total leaf area of the branch segment by detaching all leaves and placing them in a LI-3100 leaf area meter (LI-COR Biosciences, Lincoln, NE).

Using the same branches as for hydraulic conductivity measurements, we measured xylem ring width, individual-conduit diameter, hydraulic mean diameter (Pockman and Sperry 2000) of conduits, and vessel density (#conduits/length) for the outermost four rings in each branch (encompassing years 2010–2013 and 2011–2014 depending on the year of harvest). We made thin sections of branches by hand using a razor blade, stained them with toluidine blue dye, and placed them on microscope slides. We then took pictures of each thin section at $\times 10$ and $\times 40$ magnification using a microscope digital camera (model MU300, AmScope, Irvine, CA). We then analyzed the digital images using ImageJ software (National Institutes of Health, Bethesda, MD, USA). For each thin section, we established three transects from the center to the outer edge of the thin section. These transects were arranged around the entire thin section to capture the natural variation in the stem structure. Along each transect, we digitally measured ring width per year, and the number of vessels intersecting the transect/length within each individual ring. For all the vessels that intersected the transect, we also measured vessel width in two directions, and used the mean of these to calculate the area of each vessel.

Analysis

We analyzed differences in the response variables (foliar crown parameters, gas exchange, plant carbon isotope ratios, and xylem architecture) due to irrigation treatment using one-way ANOVA. For analysis of hydraulic data, we used two-way ANOVA for analysis because we used branch samples from 2 years for hydraulic measurements (with irrigation treatment and

year being the main effects). We determined that there were no significant year effects, and so we combined hydraulic data across the two study years and re-ran the analyses using one-way ANOVA. We performed all ANOVA analyses using JMP v. 12.0 (SAS Institute, Cary, NC) statistical software. For mechanistic comparisons among gas exchange variables, we used linear regression analysis using SigmaPlot v.13 (Systat Software, Inc., San Jose, CA). We tested all datasets for normal distribution and homoscedasticity prior to analysis. We used Tukey's HSD method for post hoc analyses of differences in response variables due to irrigation treatments.

Results

Plant structural parameters and whole-plot shrub cover

Leaf area index (LAI) of individual shrubs was not different among irrigation treatments, while SLA was slightly, but significantly, less in SUM compared to AMB and WIN shrubs (Fig. 1a; Table 1). Total-shrub leaf area (A_l) was $\sim 1.5 \times$ greater in WIN plots, and $\sim 1.2 \times$ greater in SUM plots, compared to AMB plots (Fig. 1b; Table 1). Basal area (A_b) was $\sim 3.4 \times$ and $\sim 1.9 \times$ greater in WIN and SUM plots, respectively, compared to AMB plots (Fig. 1b; Table 1). The ratio A_l:A_b significantly increased with irrigation, and was 0.359 ± 0.002, 0.363 ± 0.002, and $0.369 \pm 0.002 \times 10^2$ m^2/cm^2 in AMB, SUM, and WIN plots, respectively (Fig. 1b; Table 1). Total plot canopy cover of shrubs in WIN plots was nearly $2 \times$ greater than in AMB plots; shrub cover was not different between SUM and AMB plots (Fig. 1c; Table 1).

Gas exchange and plant carbon isotope ratios

There were no statistical differences in midday gas exchange, averaged across the summer. Photosynthesis (A_{net}) ranged between 8.01 ± 1.61 and 9.05 ± 1.95 μmol m^{-2} s^{-1} (Fig. 2a; Table 1). Transpiration (E) was slightly ($\sim 22\%$) greater in SUM plots compared to AMB plots, but again there were no statistically significant differences among irrigation treatments (Fig. 2b; Table 1). Stomatal conductance (g_s) ranged between 0.223 ± 0.29 and
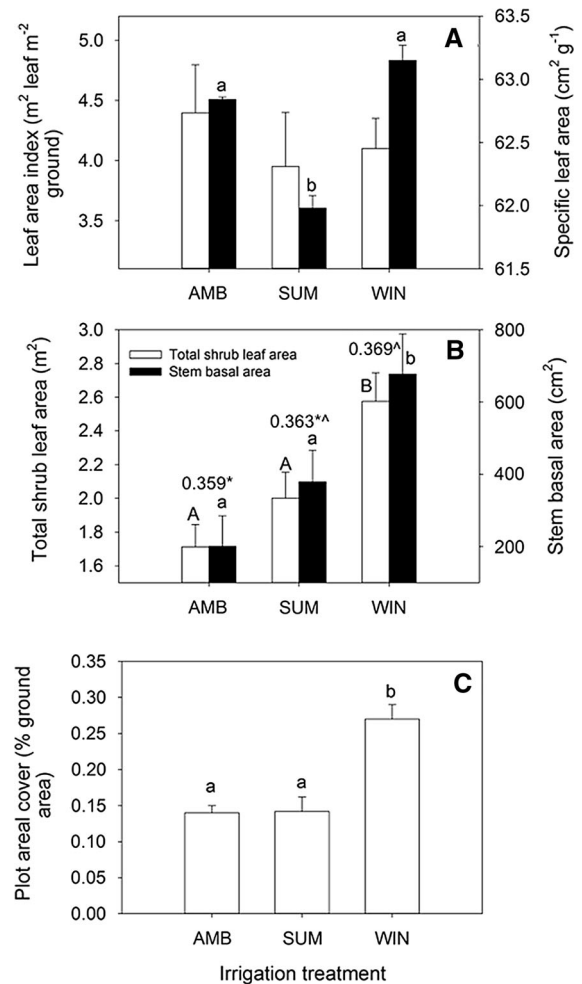
Fig. 1 Variation in leaf area index (white bars) and specific leaf area (black bars; **a**), total shrub leaf area, stem basal area, and the ratio of shrub leaf area to basal stem area (i.e., A_l:A_b; numbers above bars, in $m^2/cm^2 \times 10^2$) (**b**), and total canopy cover of shrubs per plot (**c**) due to irrigation treatment. *AMB* ambient (control), *SUM* summer irrigation, *WIN* winter irrigation. Different letters above bars (and different symbols for A_l:A_b) indicate statistically significant differences at the $p < 0.05$ level. Errors are ± 1 SE

0.247 ± 0.035 mol m^{-2} s^{-1} (Fig. 2c; Table 1). Both midday water-use efficiency (WUE; A_{net}/E) and intrinsic water-use efficiency (WUEi; A_{net}/g_s) were slightly, but significantly, greater in AMB plots compared to the other plots (Fig. 2d, e; Table 1). In WIN plots, both $\delta^{13}C$ and WUEi integrated across the growing season were marginally significantly different from $\delta^{13}C$ and integrated WUEi in AMB and SUM plots, which were not significantly different from each other (Fig. 2f; Table 1).

Table 1 Summary ANOVA table for the effects of irrigation treatment on physiological and structural response variables

Parameter	DF	F	p
LAI	2, 96	0.44	0.65
SLA	2, 24	1.45	0.25
A_l	2, 69	8.27	**0.0006**
A_b	2, 69	8.27	**0.0006**
A_l:A_b	2, 69	6.34	**0.003**
Areal cover	2, 69	4.9	**0.0007**
Xylem ring width	2, 356	16.68	**< 0.0001**
Xylem conduit density	2, 356	12.20	**< 0.0001**
Xylem conduit diameter	2, 1083	3.85	**0.0119**
A_{net}	2, 87	0.11	0.90
E	2, 87	0.538	0.22
g_s	2, 87	0.13	0.88
WUE	2, 87	6.23	**0.0032**
WUEi	2, 87	5.31	**0.0071**
$\delta^{13}C$ (integrated WUEi)	2, 3	3.88	*0.058*
k_{sap}	2, 26	3.55	**0.05**
k_{leaf}	2, 26	0.295	0.75

See text for abbreviations

Bold lettering indicates statistically significant differences at $p < 0.05$ level. Italicized lettering indicates marginally significant ($0.05 < p < 0.10$). See text and figures for pairwise comparisons between individual irrigation treatments

There were no differences in relationships among A_{net} with E, C_i (internal concentration of CO_2), and g_s, or between WUE and C_i (Fig. 3) due to irrigation treatment. Across all treatments, A_{net} appeared uncoupled to E and g_s, (Fig. 3a, c) while there were tight relationships for A_{net} and WUE with C_i (Fig. 3b, c).

Hydraulic conductivity and xylem architecture

The sapwood-specific maximum hydraulic conductivity (k_{sap}) of branches was $\sim 4 \times$ and $\sim 2 \times$ greater in shrubs in WIN and SUM plots, respectively, compared to shrubs in AMB plots (Fig. 4a; Table 1). However, the leaf area-specific maximum hydraulic conductivity (k_{leaf}) of branches was not different among irrigation treatments (Fig. 4b; Table 1). There were no significant relationships between k_{sap} or k_{leaf} with any xylem architectural parameters within or among all irrigation treatments, except for a marginally significant relationship ($p = 0.07$) between k_{sap}

and xylem ring width, lumped across all irrigation treatments (Fig. 5).

Annual xylem-ring width was generally greater in WIN and SUM plots compared to AMB plots in every year (Fig. 6a; Table 1). Averaged across 4 years, ring width in WIN plots was $\sim 1.5 \times$ greater, and $\sim 33\%$ greater in SUM plots, compared to AMB plots. Xylem conduit density was greatest in AMB plots and least in WIN plots, within any 1 year and averaged across the 4 years (Fig. 6b; Table 1). On average, vessel density in WIN plots was 70% of that in AMB plots. There was less, but still statistically significant variation in vessel diameter among irrigation treatments. Vessel diameter in SUM plots (grand mean: 9.25 ± 0.42 μm) was about 10% less than that in AMB and WIN plots (grand means: 9.38 ± 0.53 and 10.05 ± 0.36 μm, respectively; Fig. 6c; Table 1). The hydraulic mean diameter was 10% greater in WIN plots (11.35 ± 0.46 μm) compared to SUM (10.34 ± 0.61 μm) and AMB (10.25 ± 0.69 μm) plots, though this was not significantly different (Table 1).

Discussion

While it is generally known that plant productivity in water-limited environments increases with greater moisture availability, less is known about how plants in these environments respond to changes in the timing of moisture availability (i.e., when soil moisture is available), especially in environments with strongly seasonal precipitation regimes such as cold deserts. As hypothesized, the cold-desert adapted *A. tridentata* exhibited greater responses in the winter-irrigated plots, with fewer responses observed in the summer-irrigated plots. Other researchers have reported modest or no increases in plant productivity in cold desert shrubs with increased summertime precipitation (e.g., McAbee et al. 2017). This was attributed to poor metabolic efficiency of cold-desert plants in summers following drier springs (e.g., Evans et al. 2014); "phenological canalization" linked to the evolutionary history of these species, in which the species lack the flexibility to capitalize on pulses of water availability (discussed in Snyder et al. 2004), and/or reliance of cold desert shrubs on deeper water stores (e.g., Walter's two-layer hypothesis; Walter 1973; deep-soil water enhancement by winter but not
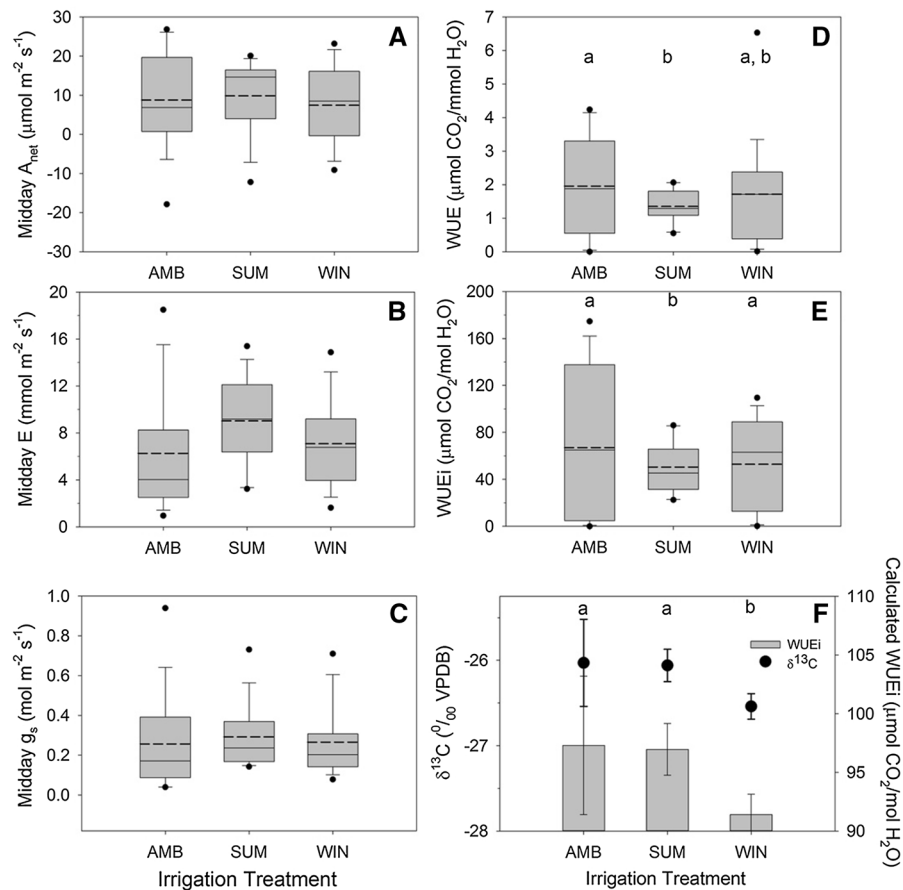
Fig. 2 Variation in mean midday photosynthesis ("A_{net}"; **a**), transpiration ("E"; **b**), stomatal conductance ("g_s"; **c**), water use efficiency ("WUE"; **d**) and intrinsic water use efficiency ("WUEi"; **e**) averaged across seven biweekly campaigns from mid-June to mid-September, due to irrigation treatment. Differences in $\delta^{13}C$ and calculated WUEi due to irrigation treatment determined from leaf samples collected in October are also shown (panel **f**). *AMB* ambient (control), *SUM* summer irrigation, *WIN* winter irrigation. In panels **a–c**, the boundaries of the boxes indicate 25th and 75th percentiles, the error bars above and below the boxes indicate 10th and 90th percentiles, and the dots outside the boxes indicate 5th and 95th percentiles. Inside the boxes, the solid horizontal line is the median, while the dashed horizontal line is the mean. In panel **f**, mean ± SE are reported. In panels **d–f**, different letters above columns indicate significant differences ($p = 0.05$ level)

summer irrigation). Moreover, most of the responses we did observe were changes in allocation, not physiological responses. Of all the physiological variables, only k_{sap} and WUE were significantly affected by the irrigations, and especially so in WIN plots (Figs. 2, 3, 4). This was mostly due to anatomical changes in leaf-to-basal area ratios and in the xylem ring width (discussed below; Figs. 1, 6), that allowed for greater water transport.

While we expected greater responses in winter-versus summer-irrigated shrubs, we were surprised that our irrigation treatments, both of which approximately doubled the annual amount of precipitation,

did not elicit stronger physiological responses in general. There may be several explanations for this, which our short-term (1–2 summers) data set cannot address. One is that transient responses to irrigations briefly occurred but were undetected. For example, Evans and Black (1993) and Loik (2007) reported temporary increases in photosynthesis in sagebrush immediately following summertime irrigations. Our gas exchange campaigns were timed to occur at least 6 days after any irrigation. This notion that we missed some brief physiological responses is supported by the fact that WUE and WUEi (instantaneous and integrated over the summer) were significantly lower in
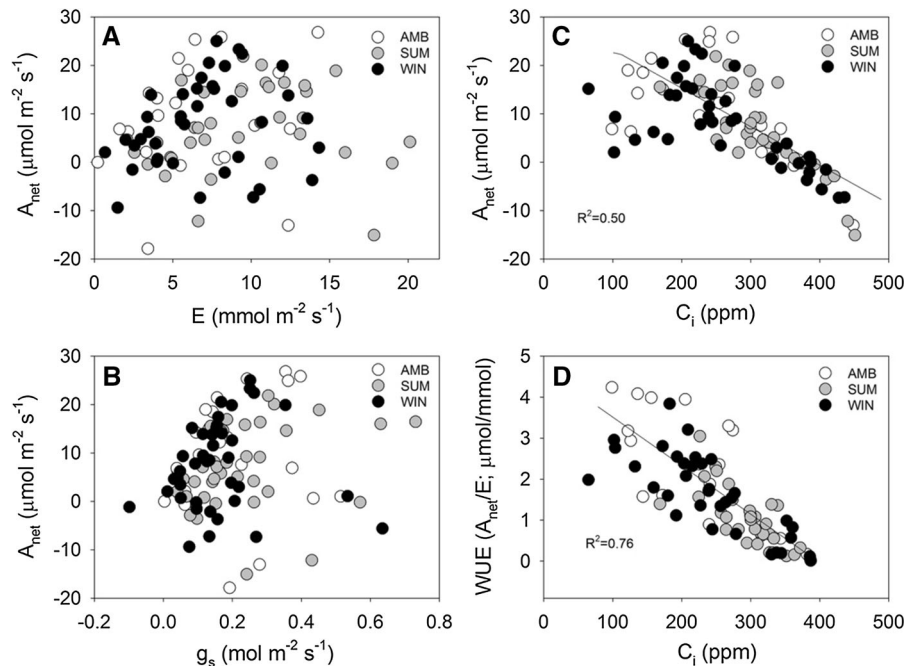
Fig. 3 Variation in photosynthesis with transpiration (A_{net} vs. E; panel **a**), photosynthesis with stomatal conductance (A_{net} vs. g; panel **b**), photosynthesis with internal concentration of CO_2 (A_{net} vs. C_i; panel **c**), and water use efficiency with internal concentration of CO_2 (WUE vs. C_i; panel **d**), due to irrigation treatment. Individual points represent midday measurements during diurnal campaigns in June–September. *AMB* ambient (control), *SUM* summer irrigation, *WIN* winter irrigation. In panels **a** and **b**, no regressions were statistically significant (all $p > 0.33$ and all $R^2 < 0.03$). In panels **c** and **d**, there were no significant differences in regressions among irrigation treatment (as determined by overlap of 95% confidence intervals of each regression), so the regression line for the group data is indicated ($p < 0.05$, and Pearson's correlation coefficients > 0.76 in both panels). In the panel **c** the relationship is defined by $y = -0.0732x + 28.243$, and in panel **d** the relationship is $y = -0.0142x + 5.1783$

irrigated plots compared to control plots (Figs. 2, 3). The declines in water use efficiency appeared to be due to increased non-stomatal limitations to photosynthesis, as photosynthesis did not vary with stomatal conductance, while both photosynthesis and WUE were tightly associated with internal-CO_2 concentration (C_i; Fig. 3). However, these relationships were not different among irrigation treatments.

Another explanation is that after $20 +$ years of manipulations, responses to irrigations are manifested mostly at scales greater than the individual leaf, such as changes at the whole plant population, and thus community levels, and not in physiological acclimation at the tissue scale, as predicted by the hierarchical-response framework Smith et al. (2009). Although we do not have physiological measurements from early in the experiment to determine if this framework applies to *A. tridentata,* we can make comparisons across organizational scales. At the ecosystem scale, McAbee et al. (2017)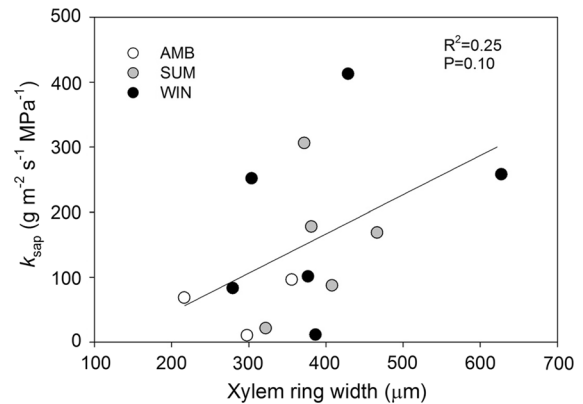 reported for the same study site and year that net ecosystem exchange (CO_2 uptake) increased due to both summer- and winter irrigation relative to ambient plots, indicating that ecosystem scale CO_2 fluxes were responsive to irrigations at whole-shrub and larger scales. At the plot (= stand) level, the sum total physiological responses of all the different tissues (e.g., leaves, wood, flowers, etc.) and taxa (e.g., woody shrubs, herbaceous plants, soil microbes, etc.) to irrigation treatments may become more apparent, which our sampling design could not test (but see McAbee et al. 2017).

Most of the responses that we observed in sagebrush to enhanced precipitation seasonality occurred more so through adjustments in structure (i.e., allocation) rather than in physiological responses. Indeed, significant differences among irrigation treatments occurred in only 25% (7/28) of the physiological comparisons we made were significant, while differences in 63% (17/27) of the structural comparisons we made were significant (Table 2). As observed in other
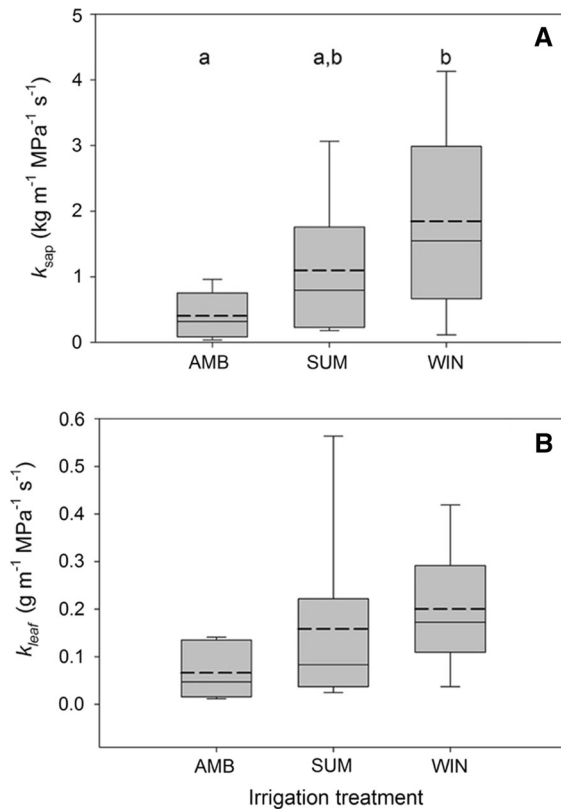
Fig. 4 Variation in mean maximum sapwood-area specific ("k_{sap}"; **a**) and leaf area-specific ("k_{leaf}"; **b**) hydraulic conductivity of branches due to irrigation treatment. *AMB* ambient (control), *SUM* summer irrigation, *WIN* winter irrigation. Different letters above bars indicate statistically significant differences at the $p < 0.05$ level. The boundaries of the boxes indicate 25th and 75th percentiles, the error bars above and below the boxes indicate 10th and 90th percentiles, and the dots outside the boxes indicate 5th and 95th percentiles. Inside the boxes, the solid horizontal line is the median, while the dashed horizontal line is the mean

Fig. 5 Relationship between maximum sapwood-specific hydraulic conductivity (k_{sap}) and xylem ring width for 2014 samples only, combined across all irrigation treatments. *AMB* ambient (control), *SUM* summer irrigation, *WIN* winter irrigation. Note differences in units (g vs. kg) compared to Fig. 3

studies examining woody-plant responses to altered soil moisture availability in water-limited environments, the structural adjustments expressed in response to irrigation would favor maximizing water transport capacity while minimizing exposure to xylem embolism (i.e., balancing the tradeoff of hydraulic efficiency and safety; Pockman and Sperry 2000). In WIN plots, which have greater and more-uniform soil moisture throughout shallow and deep soil depths that persists for several months during the early growing season (Germino and Reinhardt 2014), A_l, A_b, xylem ring width and hydraulic mean diameter were much greater compared to shrubs in both AMB

and SUM plots. In SUM plots (where soil moisture is boosted relative to AMB plots, but only episodically and temporarily, and only in shallow soil depths), only xylem ring width was greater than in shrubs in AMB plots, while conduit size was the same. Compared to shrubs in AMB plots, A_l:A_b was greater in shrubs in SUM plots, and greatest in shrubs in WIN plots (Fig. 1). Most likely, other traits that are linked with whole-plant water relations such as shoot:root ratios and root- and leaf-mass ratios also varied among the irrigation treatments, which we were unable to measure (e.g., Carter and White 2009). As with other studies (e.g., McDowell et al. 2006), these structural changes occurred with apparent homeostasis of gas exchange.

While the number of morphological adjustments in shrubs in WIN and SUM plots relative to AMB plots was about the same (seven and six traits, respectively), there were as many significant differences in morphological traits between winter- and summer-irrigated shrubs. This echoes our previous point that precipitation timing, and not just overall annual amounts of precipitation, is a key factor in sagebrush growth, allocation, and hydrological functioning, which models and future studies should take into consideration.

Implications

Regional climate models for the northern Great Basin predict wetter winters and drier summers in the next century (Abatzoglou and Kolden 2011). Combined
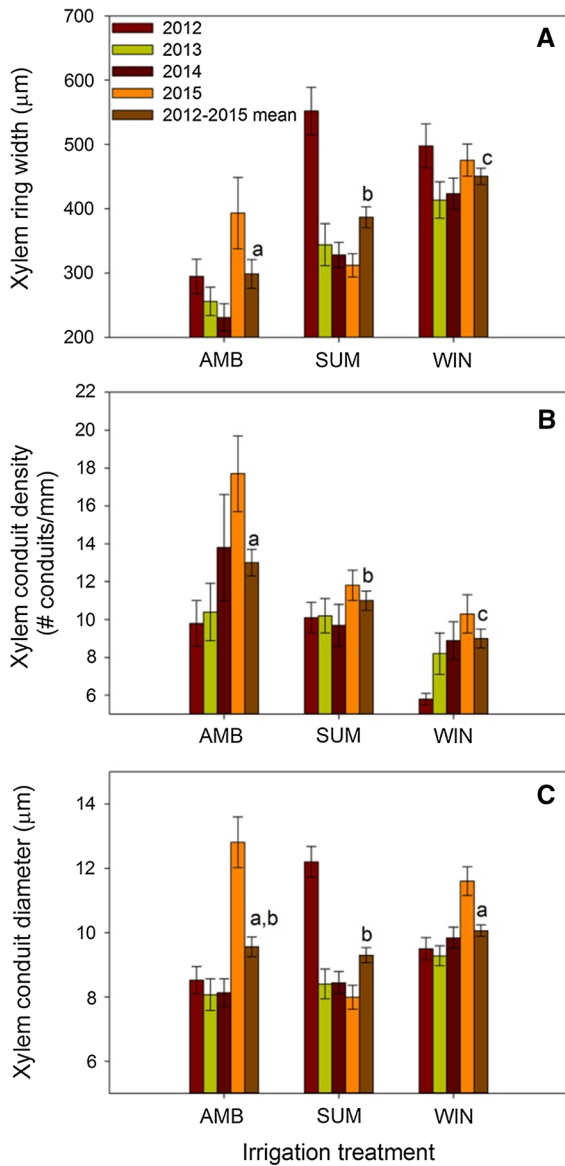
Fig. 6 Annual and grouped variation in xylem ring width (**a**), conduit density (**b**), and conduit diameter (**c**) due to irrigation treatment. *AMB* ambient (control), *SUM* summer irrigation, *WIN* winter irrigation. Different letters above grouped 2012–2015 bars indicate statistically significant differences at the $p < 0.05$ level. Errors are ± 1 SE

Table 2 Summary of responses in structure and physiological functioning to the irrigation treatments

	Parameter	SUM-AMB	WIN-AMB	WIN-SUM
Structure	LAI	–	–	–
	SLA	↓	–	↑
	A_l	–	↑	↑
	A_b	–	↑	↑
	A_l:A_b	∼ ↑	↑	∼ ↑
	Canopy cover	–	↑	↑
	Xylem ring width	↑	↑	↑
	Xylem conduit density	↑	–	–
	Xylem conduit diameter	↓	↑	–
	Xylem hydraulic mean diameter	–	–	–
Function	A_{net}	–	–	–
	E	–	–	–
	g_s	–	–	–
	WUE	↑	↑	–
	WUEi	–	↑	–
	$\delta^{13}C$ (integrated WUEi)	–	↑	↑
	k_{sap}	↑	↑	–
	k_{leaf}	–	–	–

Arrows up or down indicate statistically significant ($p < 0.05$) increases or decreases in a parameter between the treatments indicated in the column header. The order of the treatment listings within any single column header reflects the comparison, with the left-most treatment being compared to the right-most treatment (i.e., response of a parameter in SUM plots compared to in AMB plots for SUM-AMB column). A dash indicates no statistically significant difference, and a ∼ beside a symbol indicates marginal significance ($0.05 \geq p < 0.1$)

with the other studies from this same ecohydrological experimental site (Germino and Reinhardt 2014; McAbee et al. 2017), our results suggest that increased winter precipitation will result in larger *A. tridentata* shrubs, greater two-dimensional shrub %cover, and larger sapwood areas and greater A_l:A_b ratios in shrubs, yet with decreased WUE. The variability in xylem size, sapwood area, and A_l:A_b (i.e., increases with irrigation) among treatments represent tissue- and organismal-scale functional plasticity that may have led to the population scale (i.e., sagebrush cover) responses. This plasticity enabled more foliar area per unit plant or basal area with greater moisture, while the adjustments in WUE likely reduced differences in cover among treatments. One potential consequence for these responses could be more rapid transpirational water fluxes (or shifts in timing thereof) per unit ground area and time (but not on a per unit leaf area

basis). More studies in sagebrush steppe are needed that (1) quantify and link water losses across leaf-to-ecosystem scales and (2) investigate further the seemingly rather-limited plasticity in hydrological responses we observed in this widespread shrub species.

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