Response of Sagebrush Carbon Metabolism to Experimental Precipitation Pulses

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List of Symbols:

Abscisic acid concentration — ABA

Adenosine triphosphate — ATP

Air temperature — T_a

Ambient carbon dioxide concentration — C_a

Assimilated carbon (or Photosynthesis) — A_n

Assimilated carbon in the irrigated plots — A_i .

Assimilated carbon in the non-irrigated plots — A_{ni} .

Autotrophic Respiration — R_a

ΔA_n — $A_i - A_{ni}$

Bayes information criteria — BIC

Carbon dioxide concentration — CO_2

Day of year — DOY

Heterotrophic Respiration — R_h

Isotope ratio measurements — $\delta^{13}C$

Isotope measurement of leaf-respired CO_2 — $\delta^{13}C_{leaf}$

Isotope measurement of leaf-respired CO_2 in irrigated plot — $\delta^{13}C_{il}$

Isotope measurement of leaf-respired CO_2 in non-irrigated plot — $\delta^{13}C_{nil}$

$\Delta\delta^{13}C_l$ — $\delta^{13}C_{il} - \delta^{13}C_{nil}$

Isotope measurement of root-respired CO_2 — $\delta^{13}C_{root}$

Isotope measurement of root-respired CO_2 in irrigated plot — $\delta^{13}C_{ir}$

Isotope measurement of root-respired CO_2 in non-irrigated plot — $\delta^{13}C_{nir}$

$\Delta\delta^{13}C_r$ — $\delta^{13}C_{ir} - \delta^{13}C_{nir}$

Isotope measurement of root-respired CO₂ from trench plots — $\delta^{13}C_{\text{trench}}$

Leaf temperature — T_l

Predawn Water Potential — Ψ_{pd}

Photosynthetically active radiation — PAR

Quantum yield — Φ_{PSII}

Quantum efficiency for light adapted leaves — F_v'/F_m'

Relative Humidity — RH

Ribulose-1,5-bisphosphate — RuBP

Soil Respiration — R_s

Soil moisture in the respiration plots — θ_s

Soil moisture in the trench plots — θ_h

Stomatal conductance — g_s

Temperature of the respiration plots — T_s

Temperature of the trench plots — T_h

Terrestrial Regional Ecosystem Exchange Simulator — TREES

Vapor pressure deficit — D

Vienna Pee Dee Belemnite — V-PDB

WUE_i — Water Use Efficiency

Abstract

Effects of future large summer storm events due to climate change on vegetation carbon metabolism across western United States remains poorly understood. Canopy carbon metabolism of sagebrush (*Artemisia tridentata*) was evaluated for 7 days during the two driest months (July and August) by irrigating sagebrush plots with 20 mm precipitation pulses. Due to its dimorphic rooting system, we hypothesized sustained response to large precipitation events. Photosynthesis (A_n) and stomatal conductance (g_s) peaked within 2-3 days of irrigation and returned to pre-irrigation values by day 7. Predawn water potential (Ψ_{pd}) peaked within 1 day and returned to its pre-pulse value by day 3 while potential quantum efficiency for light adapted leaves (F_v'/F_m') as well as intrinsic water use efficiency (WUE_i) was unresponsive. Unlike leaves, fine roots in the top 30 cm of soil were not a carbon sink. Heterotrophic respiration (R_h) was the dominant contributor to total soil respiration (R_s), and peaked within 24 hours before it dropped to pre-pulse value by day 3. Different environmental drivers regulated R_s and R_h , highlighting different kinetics of carbon production. Our study suggests ephemeral response of cold desert vegetation to future large summer storm events with important implications for the overall carbon storage capacity.

Keywords: Photosynthesis, Soil Respiration, Heterotrophic Respiration, Fine Roots, Carbon Metabolism

1.0 Introduction

The Great Basin of western United States contains a large area of shrub cover (~ 60 million hectares, Anderson and Inouye, 2001), which stores a significant amount of carbon, especially belowground (de Graaff et al., 2014). Snow remains the predominant form of precipitation across these regions while summer rainfall events are sporadic. Soil moisture recharge during the snow season not only favors the growth and development of woody species, but also allows it to sustain physiological activities during the summer (Ehleringer et al., 1991; Schenk and Jackson 2002; Ogle and Reynolds 2004). Most summer rain pulses across these regions are $\leq 5\text{mm}$ (Loik et al., 2004) and they initiate microbial activity and photosynthetic response from shallow rooted plant species (Schwinning and Sala, 2004). Climate models predict dominant precipitation dynamics across these regions to shift from a winter to fall/spring regime, while summer precipitation is expected to show a reduction in storm frequency with a greater amount of rain per event under future scenarios of climate change (IPCC 2007) with potentially significant impacts on the overall carbon sequestration capacity across these regions.

Our overarching question is whether large summer storms ($>15\text{ mm}$, Schwinning et al., 2003) can trigger lasting physiological responses in the plant-soil system? The focus on the response of vegetation to large events is necessary for three reasons: 1) It will trigger physiological responses from both aboveground and belowground plant process ; 2) Responses to a large event can help us design experiments to analyze productivity of vegetation to smaller rainfall events (Snyder et al., 2004); 3) From the perspective of climate change, a few large rainfall events in the future could be the difference between ‘wet’ and ‘dry’ years (Schwinning et al., 2003). Predicting vegetation responses to large summer storm events across semi-arid ecosystems in general remains complex because threshold-type responses are modulated by plant

architecture, soil texture, and soil nutrient conditions (Huxman et al., 2004a; Potts et al., 2006a, b).

Extensive analyses at plant scale (e.g., Lauenroth et al., 1987; Cui and Caldwell 1997; Dougherty et al., 1996; Golluscio et al., 1998; BassiriRad et al., 1999; Gebauer and Ehleringer 2000; Schwinning et al., 2002) have provided insight with regard to the response of vegetation across semi-arid ecosystem to large summer experimental pulses. These studies have either used a combination of isotope analysis, sap flow, gas exchange measurements, soil water content or respiration to analyze the impact of simulated rainfall events on vegetation productivity. But simultaneous measurements of the three key processes – carbon assimilation, allocation and respiration – that regulate productivity at plant scale remain scarce. Measuring these processes together is important because the timing of photosynthesis and respiration responses to large precipitation pulses during summer has implications for the carbon storage capacity of dryland ecosystems as well as overall impact on plant community dynamics. Recent assessment of carbon exchange across cold-desert shrub ecosystems has found high intensity summer storm events lead to disproportionate increase in ecosystem respiration compared to photosynthesis, while small storm events facilitate the carbon sink behavior of the biome (Xie et al., 2015), both of which may be modulated by the spring moisture received by deep roots (Kwon et al., 2008). Ecological manipulation experiments that analyze the vulnerability of these key processes to large precipitation pulses can provide additional mechanistic insights into arid land carbon metabolism (Jentsch et al., 2007; Chen et al., 2009).

A_n is a robust metric to quantify carbon assimilation as it encompasses a broad range of a plant's physiological activities. For pulse-driven ecosystems, however, extensive periods of dry conditions during summer may impair the carbon uptake capacity of the plant in response to

large precipitation (Flexas et al., 2002; Grassi and Magnani, 2005; Flexas et al., 2006). Therefore, apart from A_n , a mosaic of metrics, including Ψ_{pd} , g_s , and quantum efficiency for light-acclimated leaves (F_v'/F_m') need to be analyzed to provide a comprehensive understanding of the role of *a priori* drought stress in regulating the carbon assimilation capacity of the vegetation including stomatal and non-stomatal regulation of A_n (Davis and Mooney, 1986; Maxwell and Johnson, 2000; Yan et al., 2000; Maestre et al., 2005). Another important metric to quantify change in vegetation productivity in response to variation in precipitation pulses is intrinsic water use efficiency (WUE_i). WUE_i is a function of plant genetics as well as the environment (Garten and Taylor, 1992; Zhang et al., 1993; Lauteri et al., 1997). While the metric can be measured at several scales, WUE_i , measured at leaf level and defined as the ratio of A_n / g_s , is of particular interest to quantify the physiological acclimation to changes in water stress and is strongly induced by stomatal control (Bierhuizen & Slatyer, 1965; Galmes et al., 2011; Zegada-Lizarazu et al., 2011).

For dryland vegetation to benefit from a precipitation pulse either it must have active roots, or it must quickly reestablish root activity (BassiriRad et al., 1999). Previous studies on shrubs in the Great Basin have found a decline in root activity during the late summer (Holthausen and Caldwell, 1980; Carbone and Trumbore, 2007). For non-forest ecosystems in general, dynamics of fine root biomass (< 2mm in diameter) in the upper 30 cm of soil is critical in modulating the overall productivity (Barbosa et al., 2012). An improved understanding of fine root turnover remains a hindrance for predicting vegetation productivity in a changing climate (Norby and Jackson, 2000). Hence, linking carbon allocation to fine roots will be key to understanding the response of vegetation to large precipitation pulses. A strong link between photosynthesis and root activity has already been established for grassland (Craine et al., 1998;

Johnson et al., 2002) and forest ecosystems (Hogberg et al., 2001; Irvine et al., 2005; Liu et al., 2006), but semi-arid shrublands remain understudied.

Total soil respiration (R_s), another critical component of the carbon balance, originates from roots (R_a , autotrophic) and microbes (R_h , heterotrophic). Separate analysis of the response of R_h , as well as its sensitivity to moisture and temperature is crucial as this provides information about the response of microbial decomposition processes to an altered precipitation regime. R_h should be analyzed separately because it has different responses to temperature, moisture and substrate availability compared to autotrophic respiration (Bond-Lamberty et al., 2004). Improving scientific understanding of the response of heterotrophic respiration to soil moisture and temperature is also critical to improving forecasts of soil respiration responses in dryland ecosystems to climate change (Lavigne et al., 2004).

Drought tolerant sagebrush (*Artemisia tridentata*), one of the most common Great Basin perennial shrubs, has key traits that allow it to survive in arid ecosystems (Flanagan et al., 1992). Sagebrush has both active shallow and deep roots that extend at least to 2-m depth (Richards and Caldwell, 1987). Extensive movement of soil water between the shallow and deep layers, also known as hydraulic redistribution (Richards and Caldwell, 1987) allows sagebrush to sustain physiological activities during the summer. On account of its active shallow root distribution as well as active foliage area we anticipate that sagebrush will have the capacity to utilize large summertime precipitation pulses. As precipitation pulses greater than 15 mm are categorized as large precipitation pulse across the Great Basin of Western United States (Ivans et al., 2003), the following hypotheses were tested to analyze the response of aboveground and belowground plant processes to a 20 mm pulse:

- Hypothesis 1 – Large precipitation events during summer result in sustained

increases in photosynthesis in sagebrush.

- Hypothesis 2 – Fine roots of sagebrush carry the isotopic signature of recently assimilated carbon.
- Hypothesis 3 - Different environmental drivers regulate soil (R_s) and heterotrophic respiration (R_h) during different times across the summer season in response to a large precipitation pulse.

2.0 Methods and Analysis

Study Site

An irrigation experiment was conducted near Saratoga, Wyoming (Latitude = 41.45°, Longitude = -106.81°, Elevation = 2173 m asl) in July and August in 2009. The research site had near-level topography (<1% slope) and sandy loam soils. Mean annual temperature and precipitation were 7.1°C and 32.7 cm at Rawlins (~70km from Saratoga and at the same elevation; 30-year means from 1980-2009) (www.wrcc.dri.edu). Mean temperature in 2009 was 4.9°C and precipitation was 25.8 cm. July and August are historically the driest months and this was also true in 2009 (www.wrcc.dri.edu) (Figure Appendix A1A & A1B). Over 95% of July-August precipitation events were less than 5 mm (Figure Appendix A1C). Total vegetation cover at the study area near Saratoga was about 37% with relative vegetation cover of Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) of 17.6% (Mitra et al., 2014). The site also supported perennial grasses (e.g., *Festuca idahoensis* and *Poa secunda*, *Koeleria macrantha*) and forbs (e.g., *Eriogonum umbellatum*, *Phlox* spp.).

Experiment Overview

Physiological responses of sagebrush shrubs and associated fluxes of R_s and R_h to pulse precipitation events were analyzed for 7 days following each of two experimental irrigations, one

in July and one in August. There were two treatments (irrigated & non-irrigated) with three replications for each treatment. The irrigation treatments were conducted on 1 m² plots that encompassed a single sagebrush plant, separated by more than 2 m to prevent spatial autocorrelation and interaction between plots (Ewers and Pendall, 2008; Mitra et al., 2014).

We employed the trench method to analyze R_h (Hanson et al., 2000), whereby a trench 0.5 m deep was excavated outside of the plot (1 m²) in the third week of June. At 0.5 m depth, the trenches were below the main rooting zone (Cleary et al., 2010). A layer of water permeable, root barrier fabric was installed and the trenches were backfilled. In order to prevent the flow of aboveground carbon to the roots and rhizosphere, we sprayed herbicidal treatment (2% Glyphosate) on the leaves. After the plants died, they were cut at the root crowns and then removed. This treatment was done three weeks prior to the first irrigation experiment.

We exposed the plants to large summer precipitation event by simulating two 20-mm rainfall events, one in July and one in August, in order to trigger physiological response from plants, soil and microbes. Water was applied evenly and slowly above the irrigated sagebrush and trench plots in order to ensure there was no surface runoff. Our method of water addition was similar to the method used in previous irrigation experiments (Gebauer et al., 2002; Ivans et al., 2003; Loik 2007). Predawn water potential, photosynthesis, soil and heterotrophic respiration and carbon isotope ratio measurements were initiated one day prior to the irrigation experiment (day -1) and then repeated on days one, three, five and seven following the irrigation treatment (Table 1). Measurement time period for the sagebrush and trench plots have been summarized in Table 1 and DOY for the irrigation in July and August have been highlighted in Figure A2 (Appendix).

Leaf Level Measurements

Ψ_{pd} quantifies antecedent water stress and was measured using a pressure chamber (PMS Instruments, Albany, OR, USA) on two stems (2-3 mm diameter) in each of the six plots. We measured g_s , A_n , quantum yield (Φ_{PSII}), and F_v'/F_m' with an open path gas exchange system (LI-6400XT, LI-COR, Inc., Lincoln, NE) integrated with a fluorescence chamber head (LI-6400-40 leaf chamber fluorometer; LI-COR, Inc., Lincoln, NE). The measurements were conducted between 9.00 – 12.00 local time one day prior to the irrigation experiment (day -1) and then repeated on days one, three, five and seven following irrigation (Table 1). A red-blue light source attached to the gas exchange system was used to illuminate the leaves, while controlling for vapor pressure deficit (D), air temperature (T_a), photosynthetically active radiation (PAR), and ambient carbon dioxide concentration (C_a) concentration in the chamber. Prior to gas exchange measurements, leaves kept inside the chamber were allowed to reach steady-state photosynthesis at ambient C_a (360 ppm), leaf temperature (T_l) (26°C) and at saturating PAR conditions (1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The range of D was 1-3 kPa to replicate the changing environmental conditions in the field. All measurements were conducted on a minimum of 3 leaves per plot. After the measurements were conducted, leaves were brought back to the laboratory for leaf area analysis. The leaves were first scanned and then leaf areas were calculated using Image-J software (<http://rsb.info.nih.gov/ij>).

Soil Respiration Measurement

To measure soil respiration, an EGM-4 IRGA with an SRC-1 soil respiration chamber (PP Systems International, Inc., Amesbury, MA, USA) was used. At each of 12 plots (3 irrigated shrub, 3 non irrigated shrub, 3 irrigated trench and 3 non irrigated trench), respiration was measured at the center and periphery of the plot. R_s was measured on intact plots while R_h was

measured on trenched plots. Adjacent to the respiration measurement points, soil temperature (°C) (HI 9053, Hanna Instruments, Smithfield, RI, USA) and volumetric soil moisture (HH2 Moisture Meter; delta – T Devices, Cambridge, England) were measured at 0.06 m depth.

Isotope Measurements

Field sampling of the leaf respired CO₂, $\delta^{13}C_{\text{leaf}}$, and root respired CO₂, $\delta^{13}C_{\text{root}}$, for the sagebrush plot and root respired CO₂ for the trench plot, $\delta^{13}C_{\text{trench}}$, were conducted between 13.00-15.00 hours on all experimental days. Carbon isotope analyses of the leaves were conducted in order to examine whether leaves were strong carbohydrate sinks and whether they competed with roots for carbon allocation. Large-bore syringes of 60 ml capacity were used to collect leaf and root respired CO₂ from the sagebrush plots and roots from the trench plots. Fine roots (diameter < 2mm) were collected and washed with water to remove soil particles. For each plot, root samples were composited into a single sample of about 0.5g fresh weight and placed inside the syringe barrel and flushed with CO₂-free air. A similar procedure was applied for the leaf samples. The syringe samples containing the leaves were covered with aluminum foil to prevent photosynthesis. CO₂ inside the syringe barrels was allowed to accumulate for 40 minutes in the dark at ambient temperature and then 15 ml of the air was collected in pre-evacuated vials, which were sent to the University of Wyoming Stable Isotope facility (www.uwyo.edu/sif) for $\delta^{13}C$ analysis by Thermo Finnigan Gas Bench in line with a Finnigan Delta Plus XP (Thermo Finnigan, Bremen, Germany). $\delta^{13}C$ values were reported in ‰ with respect to Vienna Pee Dee Belemnite (V-PDB). Instead of reporting absolute values, change in $\delta^{13}C_{\text{leaf}}$, $\delta^{13}C_{\text{root}}$ and $\delta^{13}C_{\text{trench}}$ with respect to corresponding $\delta^{13}C$ value on day - 1 (Table 1) have been used for analysis. Measurement time periods for the sagebrush and trench plots are summarized in Table

1, and day of year (DOY) for the irrigation in July (DOYs 194 – 202) and August (DOYs 229 – 238) are shown in relation to natural precipitation in Figure A2 (Appendix).

Modeled Carbon Assimilation (A_n)

We modeled carbon assimilation in response to precipitation pulses with the Terrestrial Regional Ecosystem Exchange Simulator (TREES) (Mackay et al., 2003; Samanta et al., 2007; Lorant et al., 2010). The objective of modelling carbon assimilation from day -1 to day 7 was to assess the impacts of precipitation on the time-integrated carbon accumulation process. TREES solved for A_n for each of the experimental days at 30-minute time steps using the Farquhar model (Farquhar et al. 1980; Farquhar and von Caemmerer, 1982; de Pury and Farquhar, 1997) coupled to a simple plant hydraulic model (Katul et al., 2003). Environmental data to run TREES were obtained from an adjacent meteorological station. PAR (LI-190SZ, LI-COR Inc.), T_a and relative humidity (RH) (HMP45C, Vaisala, Helsinki, Finland) were monitored at a height of 2.5 m. g_s and Φ_{PSII} obtained from leaf-level measurements were used to constrain the photosynthesis subroutine of TREES. The remaining photosynthesis parameters were constrained based on light response curves measured on a similar sagebrush subspecies (*A. tridentata* var *vaseyana*) at Sierra Madre Mountain Range, Wyoming (Naithani 2009).

Data Analysis

Since each plot was an experimental unit, measurements were averaged by plot for analysis. A repeated measures ANOVA was used to test for effects of time and treatment on Ψ_{pd} , g_s , A_n , Φ_{PSII} , F_v'/F_m' , $\delta^{13}C_{leaf}$, $\delta^{13}C_{root}$, $\delta^{13}C_{trench}$, WUE_i , R_s , R_h , soil moisture of the respiration (θ_s) and trench plots (θ_h) as well as soil temperature of the respiration (T_s) and trench plots (T_h). Time was considered as a within-subjects factor, whereas treatment (irrigated versus non- irrigated) was a between subjects factor. Wherever we found a significant effect of

treatment, we used unpaired t-tests to compare the mean for any of the measured variables between the irrigated and non-irrigated plots. In repeated measures ANOVA, degrees of freedom were corrected whenever Mauchly's test indicated that the assumption of sphericity was violated. Deviation from the assumption of sphericity was estimated by epsilon (ϵ). Huynh-Feldt correction was applied when ϵ was > 0.75 and Greenhouse-Geisser correction was applied when ϵ was < 0.75 (Girden 1992).

We used a mixed modelling approach to analyze relationship between respiration, moisture, temperature, and time. We incorporated nonlinear effects as well as temperature-moisture interactions on account of the complex multiple pathways originating from roots, litter and rhizosphere that regulate respiration. Our mixed-modeling exercise involved a top-down strategy which can be summarized as follows: 1) Selected the fixed effects of the covariates including interactions; 2) Selected a structure of the random effects and 3) covariance structure of the model residuals. In steps 2 & 3, we used REML-based likelihood ratio tests to determine the optimum random as well as the model residual structure. 4) Finally we reduced the model by removing the fixed effects parameters which were not significant at $\alpha = 0.05$ level. We selected the model with the lowest Bayes information criteria (BIC).

Irrigation effects on the isotope values of the leaves and fine roots in both July and August were calculated as the difference between the isotope value of leaf (or roots) on irrigated and non-irrigated plots ($\Delta\delta^{13}\text{C}_l$ for leaves and $\Delta\delta^{13}\text{C}_r$ for roots). On each measurement day, we evaluated correlations of $\Delta\delta^{13}\text{C}_l$ and $\Delta\delta^{13}\text{C}_r$ with the net photosynthetic difference (ΔA_n) between the total assimilated carbon between the irrigated (A_i) and non-irrigated plots (A_{ni}). A_i and A_{ni} were calculated using the TREES model. The objective of correlation analysis was to assess whether change in isotope values of the roots and leaves bore the fingerprint of carbon

assimilated in response to the pulse of moisture. Since samples for $\delta^{13}C$ analysis were collected between 13.00-15.00 hours, ΔA_n was lagged by 0, +3, +6 and +9 hours with respect to 15:00 hours.

Unless otherwise noted, mean values were reported followed by standard errors. Statistical analyses were tested for significance at $p < 0.05$ level. All statistical analyses were conducted using R (version 2.15.2, R Development Core Team, 2007) and PASW Statistics 18 (formerly SPSS). Since we did not construct rain-out shelters, any precipitation event between the two irrigation experiments influenced both the irrigated and non-irrigated plots.

3.0 Results

Prior to irrigation, θ_s was lower in July (Figure 1A; $\sim 3\%$) compared to August (Figure 1B; $\sim 6\%$). With respect to soil moisture on day -1, θ_s increased by nearly 120% (Figure 1A) and 241% (Figure 1B) in irrigated plots on day 1 in July and August respectively. There were significant effects of treatment as well as time x treatment interaction in both months (Table 2). Significant differences between the two treatments were evident in both months on days 1 and 3 and only on day 5 in July (Figures 1A –B, t-test $p < 0.05$). Unlike soil moisture, we only found a significant effect of time for T_s for both months (Figures 1C-D, Table 2).

In the trench plots, significant effects of time, treatment and their interaction were observed for θ_h in both the months (Table 2), with θ_h on days 1, 3 and 5 being statistically different from each other (Figures 1E-F). T_h in the trench plots were significantly different between the two treatments only in July (Figure 1G, t-test $p < 0.05$).

The response of A_n (Figures 2A-B) and g_s (Figures 2E-F) to irrigation was similar in both months. However, we found a significant effect of treatment only in August (Table 2), on days 1 (A_n & g_s), 3 (A_n & g_s) and 5 (g_s) (Figures 2B & 2F). Prior to pulse treatment (day -1), sagebrush

predawn water potential was lower in August ($\Psi_{pd} \sim -5$ MPa) compared to July ($\Psi_{pd} \sim 3.5$ MPa) (Figures 2C -D). In both months, Ψ_{pd} increased on day 1 compared to day -1, followed by a gradual decline. There were significant effects of time and time x treatment interaction on Ψ_{pd} in both July and August and of treatment in August (Table 2). Significant Ψ_{pd} differences in August were evident on days 1 and 3 (Figure 2D). We found significant time and time x treatment interactions in the responses of F_v'/F_m' (Figures 2G-H) and Φ_{PSII} (Figures not shown) to pulse inputs in both July and August (Table 2). Average WUE_i (shown as numbers) did not show any trend in response to irrigation (Figures 2E & 2F). R-ANOVA analysis also did not reveal any significant effects of time, treatment or both in July and August (Table 2). We also found no significant correlation between the absolute values of $\delta^{13}C_{leaf}$ (& $\delta^{13}C_{root}$) and WUE_i (not shown) in July or August. A_n was not significantly correlated with either Ψ_{pd} (Figures 3A-B) or F_v'/F_m' (Figures 3C - D) in July or August. However, A_n and g_s were strongly correlated in August ($r^2 = 0.93$, $p < 0.05$, Figure 3F).

No significant treatment or interactive effects were observed for the changes in $\delta^{13}C_{leaf}$ (Figures 4A-B), $\delta^{13}C_{root}$ (Figures 4C-D) or $\delta^{13}C_{trench}$ (Figures 4E-F) (Table 2). But in July there was a significant effect of time for changes in $\delta^{13}C_{leaf}$ (Table 2). Statistically significant correlation ($p < 0.05$) between ΔA_n and $\Delta \delta^{13}C$ were observed for leaves in August for lag periods corresponding to 0 and 3 hours (Table 3). No trend was observed for lag periods corresponding to 6 and 9 hours. In case of fine roots, we found no significant correlation in both the months (Table 3).

While R_s (Figures 5A & B) peaked within 24 hours after pulse application in both months, a significant difference between the two treatments existed only in August (Table 2). R_s values were significantly different between the two treatments on days 1, 3, 5 and 7 in August

(Figure 5B). While the response trend for R_h was similar to R_s , we found no significant time, treatment or interaction effects (Figures 5C & D, Table 2). We also found no significant relationship between R_s and Ψ_{pd} either in July or August (Figures 6A & B).

In July, R_s was negatively correlated with T_s but positively correlated with θ_s (Table 4). In August, both the main effects of T_s and θ_s negatively influenced respiration while the main effects of θ_s^2 and $T_s \times \theta_s$ interaction were weak positive drivers of soil respiration. θ_h was negatively correlated with R_h in both months (Table 4). θ_h^2 and $T_h \times \theta_h$ interaction were weak positive drivers of heterotrophic respiration in July and August respectively. R_h also significantly decreased with time in August (Table 4).

4.0 Discussion

Hypothesis 1 – Large precipitation events during summer will result in sustained increase in photosynthesis in sagebrush.

Contrary to our hypothesis, increase in A_n (Figures 2A – B) as well as g_s (Figures 2E – F) did not last beyond three days. A temporary increase in soil moisture (Figs. 1A-B) at 0-6 cm zone suggests that either there could have been rapid downward percolation of water to deeper soil layers for later use (30-50 cm), high fraction of moisture may have left the soil as evaporation or there could have been hydraulic redistribution by roots (Naithani et al., 2012). Isotopically labeled water was not applied to the shrubs to identify the extent of moisture utilization from the shallow and deep roots. We speculate that the increase in A_n was ephemeral because sagebrush primarily absorbed moisture from the shallow soil layer (0- 6 cm) during this time frame (Schwinning et al., 2003).

We believe lack of any response in the soil temperature in the irrigated plots (compared to non -irrigated plots) even on day 1 (Figs. 1C-D) may be linked to plant shade and not vegetation

productivity. The small duration of increase in Ψ_{pd} suggests lack of adequate fine roots for moisture uptake in response to application of precipitation (Figs 2C-D) (Flanagan et al., 1992). Ψ_{pd} reflects the amount of moisture available in the rhizosphere and has been widely used by researchers as an indicator to assess plant water stress including response to irrigation (Resco et al., 2008).

The delayed response of g_s (Figure 2E, peak value on day 3), can be attributed to the time required by water to reach photosynthetic chlorenchyma to initiate CO_2 assimilation. Probable secondary reasons include leakage of solute from guard cells (Ehret and Boyer, 1979; Yan et al., 2000) and increase in abscisic acid (ABA) concentration (Henson et al., 1984; Yan et al., 2000). We eliminate the possibility of fine root growth modulating the delayed response on account of the ephemeral increase in A_n as nutrients associated with increase in soil moisture does not appear to be available for plant acquisition.

Lack of significant changes (Table 2) in WUE_i between irrigated and non-irrigated plots was consistent with other studies (Miyashita et al., 2005; de Souza et al., 2005). WUE_i is strongly induced by changes in environmental conditions. Despite the application of 20 mm precipitation pulse, we found large changes in soil moisture conditions only for twenty-four hours. This probably accounted for the lack of significant time as well as treatment effect with regard to WUE_i . Moreover, our WUE_i estimation was based on instantaneous gas exchange measurements in the morning. On account of high spatial and temporal heterogeneity of A_n/g_s and lack of strong correlation (results not shown) between WUE_i and $\delta^{13}C_l$ (& $\delta^{13}C_r$), it remains uncertain the extent to which our measurement is representative of whole plant level WUE_i (Medrano et al., 2015). A time-integrated assessment of WUE_i can be used with greater confidence as a selection criterion to quantify response of plant to changes in precipitation intensity and can be better linked to isotopic discrimination (Guehl et al., 1995)

Lack of significant correlation between Ψ_{pd} and A_n indicates that wetting of the soil and microbes is a much faster process than carbon uptake (Figures 3A & B, Huxman et al., 2004b; Resco et al., 2008). Significant correlation between A_n and g_s (Figure 3F) indicates stomatal regulation of photosynthesis in sagebrush in August. Lack of correlation in July indicates possible biochemical limitations apart from stomatal regulation modulating the carbon assimilation process (Yan et al., 2000; Pockman and Small, 2010). On account of lack of photosynthesis versus intercellular CO_2 concentration measurements before and after irrigation treatment, the weak correlation between A_n and F_v'/F_m' (Figures 3C & D) itself does not provide any evidence of the nature of metabolic processes limiting carbon uptake. Possible multiple factors modulating the weak correlation between A_n and F_v'/F_m' include production of ascorbic acid, epoxidation of carotenoid pigments (Lu and Lu, 2004), increased dissipation of non-photochemical excitation energy (Maxwell and Johnson, 2000) and photoinhibitory damage at the photosystem II reaction centers (Valladares and Pearcy, 1997; Maxwell and Johnson, 2000). Researchers have argued that response of F_v'/F_m' across vegetation in semi-arid ecosystems is also a function of the intensity of precipitation pulse and as well as the duration of inter-pulse period (Resco et al., 2008). Weak correlation between A_n and F_v'/F_m' could therefore be attributed to the different time-lags in the response of A_n and F_v'/F_m' to large precipitation pulses.

Overall, the different time lags of Ψ_{pd} and A_n in response to irrigation and lack of any response of F_v'/F_m' highlights the need to exercise caution in using Ψ_{pd} and F_v'/F_m' as metrics of stress on biological activity in pulse-driven ecosystems (Resco et al., 2008). Our results are consistent with prior research showing that desert vegetation in Western North America is often unresponsive to large precipitation pulses (Schwinning et al., 2002; Schwinning et al., 2005). Researchers have attributed multiple factors including nutrient constraint (Snyder et al., 2004) as

well as root embolism in constraining the response to precipitation (Kolb and Sperry, 1999; Sperry and Hacke, 2002). Many researchers have argued that the conservative response of desert plants to summer precipitation should be viewed from the perspective of energetic requirements for vegetation (Ehleringer and Pearcy, 1983). From a cost-benefit analysis perspective, two 20 mm pulses were probably not sufficient to cross the threshold to allow sustained carbon investment (Comstock and Ehleringer, 1986; Schwinning et al., 2003).

It seems unlikely that a higher precipitation pulse (> 20 mm) would have induced a different response in photosynthesis or stomatal conductance. Rainfall manipulation experiments in sagebrush vegetation yielded similar response pattern in photosynthesis and stomatal conductance in response to a 46 mm precipitation pulse (Loik 2007). Researchers have attributed the suppressed carbon assimilation capacity of desert species to multiple complex interactions between root depth, soil nitrogen content, thermal stress, antecedent soil moisture conditions and general reliance on winter precipitation (Loik 2007; Kwon et al., 2008). Our study provides strong evidence to support this knowledge base.

Significant increase in photosynthesis and stomatal conductance of sagebrush in August may be attributed to changes in leaf morphology. Though we did not monitor phenological development as a function of DOY, previous research work have shown that sagebrush shed their ephemeral leaves in July and August and initiate growth of reproductive shoots which ultimately grow into stalks (Evans and Black 1993; Takahashi and Huntly 2010).

Hypothesis 2 – Fine roots of sagebrush carry the isotopic signature of recently assimilated carbon.

We expected to see a reduction in $\delta^{13}C$ of leaves in irrigated plots compared to controls. A lack of significant effect of treatment as well as across time for changes in $\delta^{13}C$ may be linked

to low sample size and large variance. Isotope ratio of leaves and roots were used as metrics to assess the impacts of precipitation on the total amount of carbon assimilated across time (Farquhar et al., 1988). Sagebrush leaves acted as carbon sinks following the August pulse, but we found no such evidence in fine roots (Table 3). Low nutrient content in cold desert ecosystems results in low root maintenance respiration, which in turn leads to increased lifespan of the shrub roots (Dobrowolski et al., 1990 ; Ryser, 1996), making them resilient to drought but potentially less responsive to precipitation pulses.

New carbon assimilates may have led to increased maintenance cost of existing roots (Ivans et al., 2003; Snyder et al., 2004) and investment of carbon in deeper soil (Caldwell 1985; Caldwell and Richards, 1986). Carbone and Trumbore (2007) attributed high winter precipitation and access to water table to modulate the low belowground carbon investment by sagebrush in the early and late growing season in Owens Valley, California.

Hypothesis 3 - Different environmental drivers regulate soil (R_s) and heterotrophic respiration (R_h) during different times across the summer season in response to a large precipitation pulse.

The high flux rates of R_s and R_h on day 1 (Figure 5) highlight the importance of carbon loss following irrigation treatment in arid and semi-arid ecosystems (Bachman et al., 2010). We hypothesize the primary abiotic factor which may have modulated respiration is displacement of carbon dioxide from soil pore spaces (Huxman et al., 2004b). Biotic factors that may have influenced carbon loss include initiation of microbial activity (Garcia-Pichel and Belnap, 1996), microbial oxidation of labile soil organic matter (Appel 1998), release of labile organic matter on account of osmotic shock (Kieft et al., 1987) and oxidation of cytoplasmic solutes (Fierer and Schimel, 2003). Decomposition of roots in the trenched plots may also have attributed to the high flux values (Lavigne et al., 2004). Glyphosate application could potentially bias the

heterotrophic respiration estimate. While glyphosate has been known to be harmless to microbes, excess application has been known to enhance carbon flux from microbes (Ratcliff et al., 2006). Increase in carbon flux values on day 7 can be attributed to natural rainfall event before day 7 (based on field observation). Since we did not find any change in Ψ_{pd} or θ_s , it appears that the precipitation only activated the microbes.

The contribution of carbon assimilates to R_s or R_h however remains unclear as we found no significant effect of treatment in either $\delta^{13}C_{root}$ (Figures 4C-D, Table 2) or $\delta^{13}C_{trench}$ (Figures 4E-F, Table 2). R_h was however higher than R_s , which we attribute to lack of canopy interception and higher plant water uptake in the trench plots. R_s increased significantly following the August pulse in a similar way to A_n . Most of this response can be attributed to increase in R_h , suggesting that root respiration did not strongly respond to irrigation within a span of 7 days. Lack of any significant relationship between Ψ_{pd} and R_s possibly implies different time lags in the response of root activity and water uptake to irrigation. We speculate that roots either had longer lags than observed or required greater background precipitation to respond to experimental pulses.

The different drivers of soil and heterotrophic respiration (Table 4) in July and August reflect upon the different kinetics of carbon dioxide production in roots and microbes across season. If seasonal controls of respiration are not taken into account in modeling respiration, we run the risk of overestimating the influence of environmental controls on respiration (Lavigne et al., 2004). The positive relationship between moisture and soil respiration in July confirms prior work indicating seasonal differences in the response of respiration to moisture availability (Bachman et al., 2010). Covariation of soil moisture and temperature (Davidson et al., 1998) probably accounted for the negative relationship with moisture but positive association with the multiplicative effects of moisture and temperature.

Limitations and Implications

Vertical and lateral root distribution are important aspects that influence the rate of carbon uptake. Root distribution studies across a similar site in Wyoming have found that the majority of the roots are in the top 50 cm or even at more shallow depths (Cleary et al., 2010). While sagebrush roots across our study site do extend up to 1m consistent with other studies (Richards and Caldwell, 1987), they do not contribute much of the total root mass by percentage (Cleary et al., 2010). The extent of soil moisture recharge at the deeper soil layers as well as that of hydraulic redistribution in inducing carbon gain and the overall plant water status remains unexplored. Empirical analyses from root sensors on a different sub-species of sagebrush in Wyoming demonstrated strong signals of hydraulic redistribution (Naithani et al., 2012). The influence of winter precipitation in modulating vegetation response to large rain event should also be investigated. Our inability to link precipitation to fine root turnover rate suggests photosynthetic carbon may either have been allocated belowground or been utilized for maintenance. With climate change predicting increase in intensity and frequency of large precipitation events, the fate of increase carbon uptake on the overall biomass accumulation represents an important knowledge gap. We did not extend our analysis beyond 7 days because most of our physiological metrics reached pre-irrigation values by day 7. The high degree of variation as well as sample space may have contributed to the lack of statistical significance in some of our analysis. However, we believe this sample size coupled with our random sampling procedure was representative of the population. Moreover, each measurement point was the average of six to nine measurement points. More importantly, measurements had to be conducted within a specific time-frame. For example, photosynthesis and respiration measurements had to be conducted within 9am – 12 noon in order to capture the peak responses. A larger sample size

would have pushed the measurement time frame beyond 1 pm. This would have incorporated time-dependent errors into our analyses. Moreover, in environmental studies, p-value alone should not serve as evidence against the pattern in our data or our underlying research objectives (Berger and Wolpert, 1988, Royall 1997, Lele 2004; Valpine et al., 2014). As observed by many ecologists, lack of statistical significance should not be dismissed as biologically/ecologically insignificant (Jones & Matloff, 1986; Gerrodette 2011). Compared to statistical significance, the evidence of the effect of large precipitation pulses on the carbon metabolism of shrubs is more informative. The parameters of biological interest derived from our research should be analyzed from the perspective of empirical reproducibility in future research work.

5.0 Conclusions

This short-term ecophysiological study highlights the conservative nature of sagebrush vegetation in response to large rain events with potential application to other shrubs in cold desert ecosystems. Different aspects of plant carbon budgets (assimilation, transport and respiration) display similarities in their response to increased resource availability. The increase in aboveground plant process as well as soil and heterotrophic respiration was ephemeral. While hydraulic limitation seems to be a critical factor in influencing the response of photosynthesis of sagebrush to pulse inputs, the role of non-stomatal factors in modulating the response of carbon assimilation could not be definitely established. Fine roots in the upper 30-cm of soil do not appear to be the sink for recently assimilated carbon, suggesting carbon could either been used up for maintenance or been transferred to the roots at depths greater than 30 cm. High contribution of heterotrophic respiration to total soil respiration demonstrates the importance of separate analysis of microbial respiration. The different drivers of soil and heterotrophic

respiration in July and August accentuate the importance of including seasonal variation for accurate estimation of respiration flux.

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Figure 6: Relationship between predawn water potential (Ψ_{pd}) and total soil respiration (R_s) for both the treatments in July (Figure 6A) and August (Figure 6B).

Figure Appendix A1: Time series of the A) 30 year (1980 – 2009) average of total precipitation (mm) as well as in total precipitation (mm) in 2009, B) 30 year (1980 – 2009) average monthly temperature ($^{\circ}\text{C}$) as well as in 2009 ($^{\circ}\text{C}$) as well as C) Frequency of 30-year average (1980 – 2009) precipitation bins for July and August. (www.wrcc.dri.edu).

Figure Appendix A2: Time series of the average daily precipitation (mm) in July and August and the duration of the two experiment period (-1, 1, 3, 5, 7). Precipitation data was obtained from Rawlins (< 45 miles from Saratoga and at same elevation) (www.wrcc.dri.edu).

Table 1:

Day	-1	0	1	3	5	7
Sagebrush Plots						
03-05 hrs		Ψ_{pd}	Ψ_{pd}	Ψ_{pd}	Ψ_{pd}	Ψ_{pd}
09-12 hrs	$A_n \text{ \& } R$	Pulse	$A_n \text{ \& } R$	$A_n \text{ \& } R$	$A_n \text{ \& } R$	$A_n \text{ \& } R$
13-15 hrs	$\delta^{13}C$	Treatment	$\delta^{13}C$	$\delta^{13}C$	$\delta^{13}C$	$\delta^{13}C$
Trench Plots						
09-12 hrs	R	Pulse	R	R	R	R
13-15 hrs	$\delta^{13}C$	Treatment	$\delta^{13}C$	$\delta^{13}C$	$\delta^{13}C$	$\delta^{13}C$

Table 2:

Factors	df	F	Factors	df	F	Factors	df	F	Factors	df	F
Ψ_{pd}						g_s					
July			August			July			August		
Time	4,16	18.22*	Time	4,16	26.49*	Time	3.08,12.32	1.41	Time	2.24,17.91	1.72
Timex	4,16	3.21*	Timex	4,16	15.14*	Timex	3.08,12.32	1.32	Timex	2.24,17.91	2.17
Treat.			Treat.			Treat.			Treat.		
Treat.	1,4	1.76	Treat.	1,4	10.05*	Treat.	1,4	4.63	Treat.	1,8	18.89*
A_n						F_v/F_m					
July			August			July			August		
Time	3.19,31.92	0.49	Time	4,32	1.50	Time	4,16	3.55*	Time	3.51,28.09	6.18*
Timex	3.19,31.92	0.63	Timex	4,32	2.30	Timex	4,16	4.09*	Timex	3.51,28.09	5.42*
Treat.			Treat.			Treat.			Treat.		
Treat.	1,10	7.03	Treat.	1,8	0.02*	Treat.	1,4	2.59	Treat.	1,8	0.33
Φ_{PSII}						$\delta^{13}C_{leaf}$					
July			August			July			August		
Time	3.82,15.27	3.34*	Time	4,32	4.35*	Time	4,16	7.76*	Time	4,16	0.95
Timex	3.82,15.27	2.86**	Timex	4,32	3.64*	Timex	4,16	0.76	Timex	4,16	1.59
Treat.			Treat.			Treat.			Treat.		
Treat.	1,4	3.94	Treat.	1,8	1.64	Treat.	1,4	0.18	Treat.	1,4	1.84
$\delta^{13}C_{root}$						$\delta^{13}C_{trench}$					
July			August			July			August		
Time	4,16	0.41	Time	4,16	2.01	Time	4,16	0.45	Time	4,16	2.42
Timex	4,16	0.1	Timex	4,16	1.3	Timex	4,16	0.13	Timex	4,16	1.23
Treat.			Treat.			Treat.			Treat.		
Treat.	1,4	0.10	Treat.	1,4	3.36	Treat.	1,4	0.01	Treat.	1,4	3.18
R_s						R_h					
July			August			July			August		
Time	4,40	9.32*	Time	2.75,27.51	5.77*	Time	4,16	0.46	Time	4,16	2.42
Timex	4,40	5.7*	Timex	2.75,27.51	6.88*	Timex	4,16	0.13	Timex	4,16	1.20
Treat.			Treat.			Treat.			Treat.		
Treat.	1,10	2.8	Treat.	1,10	52.84*	Treat.	1,4	0.01	Treat.	1,4	3.18
θ_s						θ_h					
July			August			July			August		
Time	1.91,19.09	10.31*	Time	2.07,20.67	1.57	Time	1.22,12.17	8.96*	Time	4,40	4.71*
Timex		7.56*	Timex	2.07,20.67	5.58*	Timex	1.22,12.17	3.38**	Timex	4,40	5.65*
Treat.			Treat.			Treat.			Treat.		
Treat.	1,10	4.722**	Treat.	1,10	18.156*	Treat.	1,10	17.25*	Treat.	1,10	6.48*
T_s						T_h					
July			August			July			August		
Time	4,40	13.91*	Time	1.43,14.32	19.54*	Time	4,40	33.76*	Time	4,40	17.47*
Timex	4,40	.78	Timex	1.43,14.32	2.01	Timex	4,40	3.67*	Timex	4,40	0.47
Treat.			Treat.			Treat.			Treat.		
Treat.	1,10	.78	Treat.	1,10	.25	Treat.	1,10	8.50*	Treat.	1,10	0.3
$WUEi$											
July			August								
Time	4,16	0.96	Time	4,16	0.123						
Timex	4,16	1.63	Timex	4,16	0.652						
Treat.			Treat.								
Treat.	1,4	2.28	Treat.	1,4	0.79						

Table 3:

Plant Carbon	Lag Hours	0	-3	-6	-9
Leaves	July	0.05	0.27	0.16	0.01
	August	0.92*	0.86*	0.15	0.26
Fine Roots	July	0.00	0.65	0.17	0.08
	August	0.27	0.37	0.08	0.02

Table 4:

Time Period	Parameters	Estimate	SE	df	t	Sig
R_s						
July	Intercept	6.33	1.85	46.49	3.42	0.001
	T_s	-0.18	0.09	46.39	-1.98	0.053
	θ_s	0.22	0.04	39.21	5.11	0.000
August	Intercept	4.38	1.48	35.90	2.97	0.005
	T_s	-0.19	0.09	36.79	-2.26	0.030
	θ_s	-0.49	0.19	41.46	-2.53	0.015
	θ_s^2	0.013	0.01	33.97	2.18	0.036
	$T_s \times \theta_s$	0.033	0.01	45.05	4.16	0.000
R_h						
July	Intercept	4.14	0.32	44.98	12.88	0.000
	θ_h	-0.21	0.06	41.69	-3.49	0.001
	θ_h^2	0.01	0.01	26.01	3.85	0.001
August	Intercept	6.49	0.89	21.94	7.27	0.000
	Time	-1.12	0.24	26.80	-4.73	0.000
	θ_h	-0.32	0.14	36.96	-2.23	0.032
	$T_{h \cdot x} \theta_h$	0.02	0.01	41.88	2.24	0.031

Figure 1:

Figure 2:

Figure 3:

Figure 4:

Figure 5:

Figure 6:

Appendix Figure A1

Appendix Figure A2: