Experimental drought and heat can delay phenological development and reduce foliar and shoot growth in semiarid trees

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Abstract

Higher temperatures associated with climate change are anticipated to trigger an earlier start to the growing season, which could increase the terrestrial C sink strength. Greater variability in the amount and timing of precipitation is also expected with higher temperatures, bringing increased drought stress to many ecosystems. We experimentally assessed the effects of higher temperature and drought on the foliar phenology and shoot growth of mature trees of two semiarid conifer species. We exposed field-grown trees to a -45% reduction in precipitation with a rain-out structure (‘drought’), a -4.8 °C temperature increase with open-top chambers (‘heat’), and a combination of both simultaneously (‘drought + heat’). Over the 2013 growing season, drought, heat, and drought + heat treatments reduced shoot and needle growth in piñon pine (Pinus edulis) by ≥39%, while juniper (Juniperus monosperma) had low growth and little response to these treatments. Needle emergence on primary axis branches of piñon pine was delayed in heat, drought, and drought + heat treatments by 19–57 days, while secondary axis branches were less likely to produce needles in the heat treatment, and produced no needles at all in the drought + heat treatment. Growth of shoots and needles, and the timing of needle emergence correlated inversely with xylem water tension and positively with nonstructural carbohydrate concentrations. Our findings demonstrate the potential for delayed phenological development and reduced growth with higher temperatures and drought in tree species that are vulnerable to drought and reveal potential mechanistic links to physiological stress responses. Climate change projections of an earlier and longer growing season with higher temperatures, and consequent increases in terrestrial C sink strength, may be incorrect for regions where plants will face increased drought stress with climate change.

Keywords: climate change, Juniper, Juniperus monosperma, nonstructural carbohydrate, phenology, piñon pine, Pinus edulis, water potential

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Introduction

The phenology of plants, that is, the timing of development and growth, has a strong influence on the atmospheric concentration of CO₂ at both short and long timescales. Interannual fluctuations of atmospheric CO₂ concentrations illustrate the dominance of terrestrial phenology on earth’s atmosphere (Keeling, 1960; Keeling et al., 1996). At longer timescales, changes in the phenology of the terrestrial biosphere could affect planetary climate regulation, as lengthening of the growing season and an associated increase in terrestrial primary production are an important feedback to climate change (Rennenberg et al., 2006; Richardson et al., 2010, 2012, 2013; Keenan et al., 2014). The potential effects of climate change on growing season shifts, typically estimated as a negative feedback on warming, are represented in the global vegetation components of earth system models used in climate change projection (Richardson et al., 2012; Oleoson et al., 2013; Keenan et al., 2014).

Studies of long-term records have consistently found that increased temperatures are linked to an earlier start and longer duration of the growing season in temperate (Aono & Kazui, 2008; Miller-Rushing & Primack, 2008; Primack et al., 2009) and Mediterranean ecosystems (Peñuelas et al., 2002; Ogaya & Peñuelas, 2004; Gordo & Sanz, 2009). Warming experiments have often shown the same effect of earlier growth and an extended growing season in trees (Norby et al., 2003; Morin et al., 2010; Han et al., 2014). There is also evidence from experiments that increased temperatures do not always produce the typical response but can
have no effect on phenological timing (Guak et al., 1998; Kuster et al., 2014). Increased temperatures can also reduce growth and increase tree physiological stress, for example, via an inability to meet chilling requirements for bud vernalization (Morin et al., 2009) and damage to chloroplasts that reduces photosynthetic rates (Larcher, 2003). Trees in relatively warm environments are potentially more vulnerable to high temperature stress and growth reduction (Adams & Kolb, 2005; Williams et al., 2013). An additional explanation for such atypical growth and phenology response to temperature could be that temperature increases are often associated with an increase in drought stress (Overpeck & Udall, 2010; Williams et al., 2013). Observations of reduced growth, decreased branching rate, and delayed leaf development have been reported in trees subjected to rainfall exclusion experiments (Borghetti et al., 1998; Ogaya & Peñuelas, 2004; Peñuelas et al., 2004; Girard et al., 2011, 2012; Limousin et al., 2012).

Investigation of the physiological causes of phenological responses may be a useful approach for improving climate change predictions, given the variation in temperature and drought effects on tree growth and development (Schaber & Badeck, 2003). Growth and development are highly sensitive to stress from water availability (Körner, 2003; Fatichi et al., 2014). Following the sink limitation hypothesis, reduced growth from drought is thought to be caused by the direct effects on turgor necessary for cell wall expansion at active meristems, not through any source limitation of C assimilation or availability of stored C resources (Körner, 2003; Würth et al., 2005; Fatichi et al., 2014; Palacio et al., 2014). This hypothesis is supported by much work demonstrating that drought, reflected in high xylem water tensions, limits growth more than photosynthesis and that nonstructural carbohydrates (NSC) tend to increase in response to stress and are reduced when environmental conditions favor growth, consistent with change in C sink demand (Tissue & Wright, 1995; Körner, 2003; Würth et al., 2005; Oberhuber et al., 2011; Gruber et al., 2012; Deslauriers et al., 2014; Fatichi et al., 2014; Dickman et al., 2015). Changes in carbon supply through reduced photosynthesis serve to exacerbate these impacts (McDowell, 2011; Adams et al., 2013; Sevanto et al., 2014; Dickman et al., 2015; Hartmann et al., 2015).

Few studies have examined the combined effects of increased temperature and drought on phenological responses in mature trees, despite the need for improved phenology models in global change assessments (Richardson et al., 2012). Even fewer have measured physiological and phenological effects simultaneously to investigate the processes behind the development and growth responses to these environmental stresses in the same study. In this study, we measured phenological, growth, and physiological responses to an experimental field manipulation of temperature and drought in mature trees of two semiarid conifer species, Juniperus monosperma and Pinus edulis. These tree species are widespread across the southwest USA, a semiarid region where tree phenological responses to climate have not been well studied. Our objectives were to determine the effects of temperature and drought, separately and in combination, on tree phenology and to investigate the causal links between physiological and phenological responses to these treatments. We tested the following hypotheses:

1. Increased temperature will cause earlier phenological development and increased growth.
2. Drought will delay development and reduce growth.
3. In combination, the effects of drought will counteract any heat effects, resulting in no change in development and growth relative to ambient conditions.
4. Growth and development will be constrained primarily by xylem tension and will be either unrelated or negatively correlated with NSC concentration.

**Materials and methods**

**Site description and experimental design**

The Los Alamos Survival–Mortality experiment (SUMO) is located on Frijoles Mesa near Los Alamos, New Mexico, USA, at an elevation of 2150 m (Garcia-Forner et al., 2015). The experiment is located in a piñon-juniper woodland near the ponderosa pine (Pinus ponderosa) forest ecotone at 35°49’5”N, 106°18’19”W. The tree community at SUMO is dominated by piñon pine (Pinus edulis Engelm.) and one-seed juniper (Juniperus monosperma (Engelm.) Sarg.) with Gambel oak (Quercus gambelli Nutt.), and the occasional ponderosa pine (Pinus ponderosa Douglas ex C.Lawson), alligator juniper (Juniperus deppeana Steud.), and rocky mountain juniper (Juniperus scopulorum Sarg.) are also found. Soils are Hackley clay loam and range in depth from 40 to 80 cm above a parent material of volcanic tuff.

The SUMO experiment includes a below canopy rain-out structure that diverts ~45% of precipitation and 18 transparent, open-top chambers (OTCs) regulated by heating and cooling units (RJPL Package Heat Pump and RLPL Package Air Conditioner; Rheem Manufacturing Company, Atlanta, GA, USA) to enable temperature control (Garcia-Forner et al., 2015). The rainout structure is similar in design to that described by Pangle et al. (2012), but with troughs at ~1.3 m above the ground. Pición pine and one-seed juniper trees were assigned to five treatments: drought (~45% rain-out), heat (~4.8 °C OTC), drought + heat (~45% rain-out and ~4.8 °C OTC), ambient (ambient precipitation and
temperature), and ambient chamber control (ambient precipitation and \( \sim +0^\circ \mathrm{C} \) OTC; Fig. S1). OTCs maintained at ambient temperatures allowed testing for any chamber effects independent of heating. Temperature was monitored by two weather stations at the site and in each OTC at two height locations (1 m and 2/3 tree height; CS215 Temperature and Relative Humidity Probe and CR1000 data logger; Campbell Scientific, Logan, UT, USA). This system was used as a thermostat to set desired temperature conditions in chambers.

**Environmental conditions**

From 1987 to 2012, mean annual temperature at SUMO was 10.5 °C, varying on average from \(-0.6^\circ \mathrm{C}\) in December to 21.6 °C in July, with a mean annual precipitation total of 414 mm (http://environweb.lanl.gov/weathermachine/). Precipitation at the site is influenced by the North American Monsoon season and is highest from July to September with a relatively even distribution throughout the rest of the year. The timing of the growing season for this ecosystem is variable and dependent on soil moisture, but is typically from April through October (Herman, 1956; McDowell et al., 2010). Mean annual temperature in 2012, the year prior to the study, was 12.2 °C, and total precipitation was 198 mm. Mean annual temperature in 2013, the year of this study, was 9.7 °C, and total precipitation was 426 mm. However, 45% of annual precipitation in 2013 (194 mm) fell in September (Fig. S2), such that the majority of the 2013 growing season and study measurements occurred during a period of drought continuing from 2011 (Williams et al., 2014).

Treatments were initiated in June 2012 (Garcia-Forner et al., 2015), approximately 9 months prior to initial phenology and growth measurements in March of 2013. Over the course of this study, the mean effect in the heated treatments (heat and drought + heat) was +4.77 °C, but trees in the drought + heat treatment had a slightly higher difference in temperature from ambient (+5.05 °C) than trees in the heat treatment (+4.50 °C; Fig. S2). The temperature difference from ambient site conditions was \(-0.13^\circ \mathrm{C}\) in the ambient chamber control treatment and \(+0.26^\circ \mathrm{C}\) in the drought treatment. Mean vapor pressure deficit differences from ambient site conditions were +0.04 kPa for control chamber, +0.36 kPa for heat, +0.01 kPa for drought, and +0.69 kPa for drought + heat treatments over the course of the study (Fig. S2). We measured soil water content from 10 to 40 cm in depth periodically with a Diviner 2000 (Sentek Sensor Technologies, Stepney, SA, Australia). Mean treatment soil water content between varied from 0.1 to 0.37 among treatments and measurements (Fig. S2).

**Phenology and growth**

For each treatment, 11 branches were selected among four piñon pine and four juniper trees (two to three per tree) for repeated phenology and growth measurements. On each branch, we measured measurements on the primary axis of growth and also on a secondary axis that had three to 5 years of growth (measured in piñon pine, estimated in juniper) following the methods of Girard et al. (2011, 2012). After branch selection, initial measurements were made on March 16, 2013 (day of year (DOY) 75) and measurements were repeated periodically (every 9–29 days, mean sampling interval was \( \sim 18 \) days) throughout the 2013 growing season until November 19, 2013 (DOY 323). In branches of piñon pine, we noted phenophase (developmental stage) and measured the length of any new needles with a digital caliper. We described shoot phenophase using a numerical classification scheme adapted for piñon pine from the methods of Girard et al. (2011, 2012; Table 1, Fig. S3). This scheme included the following stages: (i) bud dormant and unchanged in size, (ii) bud swelling or growth observed, (iii) needle scales open (budbreak), (iv) new needle emergence and growth, (v) needle pairs separate. From these measurements, we determined the day of needle emergence for each branch (phenophase 4) and calculated the proportion of branches which had needle growth for each piñon pine tree for each sampling date.

Concurrently, measurements of shoot length were also made with a digital caliper. For piñon pine, we measured shoot length from the visible boundary that can be easily discerned in this species between the previous year’s growth and the base of the bud, to the tip of the growing shoot. As prior annual shoot growth cannot be discerned visually in juniper, we marked the transition between green and brown shoot tissue with ink at the start of the study and measured from this mark to the tip of the growing shoot. In both species, we also noted the presence or absence of male and female reproductive structures and calculated the percentage of measured branches with these structures. In juniper, male cones grow in late summer for pollen release the following early spring, and we quantified the presence of both 2013 and 2014 pollen cones in 2013. Branches of both species were also photographed approximately each month.

**Water potential**

Plant water potential on each tree (four per treatment) was measured monthly during the 2013 growing season to assess xylem water tension. Two twig samples were excised from the south side of each tree before dawn and kept in a refrigerator at the site until measurement within 2 h of collection. Water potential was measured with a Scholander pressure chamber (PMS Instruments, Albany, OR, USA) and determined as the mean of the two samples for each tree. A growing season mean in predawn plant water potential was calculated from monthly measurements taken in March through October 2013.

**Table 1**  Phenophase descriptions of piñon pine shoot development. This classification scheme was adapted for piñon pine from Girard et al. (2011, 2012). Photographic examples of phenophases in piñon pine are shown in Fig S3

| Phenophase | Description                           |
|------------|--------------------------------------|
| 1          | Bud dormant and unchanged in size     |
| 2          | Bud swelling or growth observed       |
| 3          | Needle scales open (budbreak)         |
| 4          | Needle emergence                      |
| 5          | Needle pairs separate                 |
Nonstructural carbohydrates

On June 19, 2013 (DOY 170), approximately 1 year after experimental treatments were initiated, tissue samples were collected from all trees in the study for nonstructural carbohydrate (NSC) analysis. June NSC analysis enabled assessment of the influence of tree carbohydrate resources early in the growing season on phenology and growth. Prior research on piñon pine has found that the largest effects of a drought treatment on NSC are typically observed in June (Dickman et al., 2015), and we did not assess NSC in other months. Foliar and shoot (twig) samples were collected by clipping recent canopy tissue. Bole and large root samples were collected with an increment borer. Roots samples were not obtained for some trees when roots of sufficient size for coring could not be found. All NSC samples were placed in liquid N2 at collection, transported to the laboratory on dry ice, stored at −70 °C, microwaved for 5 min at 800 W, and then dried at 65 °C for 48 h. All samples were ground into a fine powder using a ball mill (VWR, Radnor, PA, USA), and woody samples were pregrounded with a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). NSC, defined as soluble sugars (glucose, fructose, and sucrose) and starch, were assayed with water extraction and enzymatic quantification following the protocol of Dickman et al. (2015), which was modified from the method of Hoch et al. (2002). Extraction was performed on ~12 mg of sample with 1.6 mL of deionized water for 1 h in a 100 °C water bath. Quantification was achieved via NAD-linked enzymatic assay and spectral assessment at 340 nm (Varian Cary 50 UV-Vis spectrophotometer, Palo Alto, CA, USA). Additional details of our NSC protocol are described in the online Supporting Information.

Data analysis

Prior to data analysis, time series of phenology and growth data for each branch were checked and inconsistent or missing data were corrected from branch photographs if possible, or removed. All statistical analyses were performed in SPSS Statistic 22.0 (IBM, Armonk, NY, USA) with an α of 0.05. Repeated measures ANOVA was used in time series analysis of proportion of branches with needles, needle growth, and shoot growth, with treatment (five categories) and time as main fixed factors, and with Fisher’s LSD for post hoc analysis. We used the Huynh-Feldt correction to determine significance of results when unequal variance assumptions were violated (as determined by Mauchly’s test for sphericity; Huynh & Feldt, 1976). Repeated measures ANOVA requires balanced inputs, for example, that no data were missing for any branch from any sampling date. To meet these conditions prior to analysis, missing data were imputed by repeating the measurement recorded at the previous sampling date on the same branch. On average, only 1.1% of data analyzed with repeated measures ANOVA were imputed, and this ranged from 0 to 2.2% among repeated measures analyses. This approach to missing data replacement was conservative, as retaining lower values from earlier sampling was more likely to result in a lack of significant changes. For a few cases where data were missing for more than three subsequent sampling dates, the entire series from that branch axis was removed from the analysis. For day of needle emergence, percent of branches with reproductive structures, water potential, and NSC, data were analyzed with ANOVA using treatments as factors and Fisher’s LSD for post hoc analysis. Data were analyzed using Kruskal–Wallis with Dunn’s test for post hoc analysis if a Levene’s test for unequal variance was significant. For analysis of the relationships of NSC and water potential with phenology and growth metrics, we calculated tree means of multiple branch data separately for the primary and secondary axes and used linear regression.

Results

Phenophase and needle emergence timing in piñon pine

The proportion of piñon trees that had needle emergence was highest in ambient and chamber control treatments for both primary and secondary branch axes (P < 0.001, Fig. 1). For the primary axis, this proportion was lower than ambient in the drought and drought + heat treatments (P < 0.05), but not in the heat treatment (P > 0.05, Fig. 1). For the secondary axis branches, the proportion of trees that had needle emergence was lower in the heat, drought, and drought + heat treatments than in ambient or chamber controls (P < 0.05). No needle emergence was observed in secondary axis branches on trees in the drought + heat treatment (Fig. 1). Needle emergence on primary axis branches occurred earliest in ambient and control chamber treatments, on average at DOY 196 (July 15) and 205 (July 24), respectively (Fig. 2). Relative to ambient, needle emergence in primary axis branches in the drought, heat, and drought + heat treatments was delayed on average by 19, 39, and 57 days, respectively (P < 0.05, Kruskal–Wallis and Dunn’s test, Fig. 2). There were no significant differences in the emergence timing of secondary axis branches among treatments, likely due to the large variability in responses and the absence of needle growth in the drought + heat treatment (P > 0.05). For both axes, standard error around needle emergence timing was higher in heat, drought, and drought + heat treatments relative to ambient, indicating increased variability with climate stress.

Needle and shoot growth

In primary axis branches, piñon needle growth in the ambient and chamber control treatments was greater than that in the drought, heat, and drought + heat treatments (P < 0.05, Fig. 3). For the secondary axis, there were no significant differences in piñon needle growth among ambient, heat, and drought trees (P > 0.05). However, no piñon needle growth was...
observed in drought + heat trees, and control chamber trees had higher needle growth than trees in the heat and drought treatments for the secondary axis \((P < 0.01, \text{Fig. 3})\). Shoot growth of piñon pine over the course of 2013 was higher, and increased earlier, in the ambient and chamber control treatments relative to the heat, drought, and drought + heat treatments for both primary and secondary axes \((P < 0.05, \text{Fig. 4})\). For juniper, there was no difference in shoot growth among treatments \((P > 0.05)\), and shoot growth was much lower than for piñon pine \((P < 0.001, \text{Fig. 4})\).

**Reproductive structures**

Observation of differences in the growth of reproductive structures among treatments in both species was minimal. We observed no female cone production for either species, and only one piñon branch in the control chamber treatment had male cones. We observed substantial production of male cones in juniper, both early (cones produced in 2012 which released pollen in early 2013) and later in the year (cones produced in 2013 for pollen release in early 2014, \text{Fig. S3}). There were no significant treatment effects on juniper male cone production for either year, likely due to the high variability among trees and branches. In the drought + heat treatment, male pollen cones were reduced from 2012 to 2013 on secondary axis branches \((P < 0.05, \text{Fig. S4, Kruskal–Wallis analysis with post hoc Dunn’s test})\).

**Physiology and Phenology**

Although no significant treatment differences in point measurements of soil moisture were observed at SUMO (\text{Fig. S2, Garcia-Forner et al., 2015}), differences in predawn tree water potentials (\text{Fig. S5}) indicate that the treatments affected available soil moisture in the rooting zones of these trees (\text{Pangle et al., 2012}). For piñon pine, mean growing season predawn water potentials were significantly lower in the heat, drought, and drought + heat treatments than in the ambient and chamber control treatments \((P < 0.05, \text{Fig. S5})\). Shoot and needle growth of piñon were positively correlated with predawn water potential while needle emergence timing was negatively correlated with water potential \((P < 0.05, \text{Fig. 5a,c, Table S1})\). Correlations between shoot growth, needle growth, and needle emergence timing were also found with soluble sugars, starch, and total NSC in bole, needle, and shoot tissues of piñon pine \((P < 0.05, \text{Fig. 5b,d, Table S2})\). Growth measurements were positively correlated with NSC components...
with one exception, an inverse relationship for primary axis shoot growth with shoot glucose and fructose ($r = -0.48, P < 0.05$). Needle emergence timing was negatively correlated with NSC variables in primary axis branches ($P < 0.05$), but this relationship was not significant for secondary axis branches ($P > 0.05$). Shoot growth, needle growth, and needle emergence timing were most consistently correlated with shoot starch content, and these relationships generally had the highest correlation coefficients (Table S2, Fig. 5c,d). Although the distribution of points in Fig. 5c,d suggests curvilinear relationships, curvilinear regression did not result in a statistical improvement over a straight linear trend. Significant differences among treatments in shoot starch concentrations of piñon pine were also observed (Fig. S6).

Despite differences among treatments in predawn water potential for juniper ($P < 0.01$, Fig. S5), there were no significant correlations for juniper between shoot growth and water potential and these data are not shown ($P > 0.05$). For juniper, there were no significant treatment differences among treatments in shoot starch ($P > 0.05$, Fig. S6), and only two correlations with NSC were found, shoot growth in secondary axis branches with shoot glucose and fructose ($r = -0.53, P < 0.05$) and with shoot total NSC ($r = -0.46, P < 0.05$; data not shown).

Monthly and mean growing season predawn water potentials were correlated with June NSC variables by tissue in piñon pine (Table S3). Soluble sugars were inversely correlated with water potential, but a greater number of tissue starch and total NSC variables were positively correlated with water potential ($P < 0.05$, Table S3). Mean concentrations of sugars, starch, and total NSC in bole, leaf, root, and shoot tissues are shown by treatment for piñon pine in Table S4 and for juniper in Table S5.

**Discussion**

Overall, we found that experimental drought and heat reduced foliar and shoot growth and delayed phenological development of piñon pine, while we did not observe treatment differences in juniper growth. We hypothesized that increased temperature would lead to earlier development and increased foliar and shoot growth. We found no support for this hypothesis in any measured phenological or growth variable. In contrast, the heat treatment (~+4.8 °C) reduced phenological development in secondary axis branches (Fig. 1), delayed needle emergence in primary axis branches (Fig. 2), reduced foliar growth (Fig. 3), and reduced shoot growth (Fig. 4) in piñon pine relative to the ambient treatment. These results contrast starkly with findings from much phenological research, which originates predominately from more mesic ecosystems where increased temperature was associated with earlier development and/or increased growth (Aono & Kazui, 2008; Miller-Rushing & Primack, 2008; Gordo & Sanz, 2009; Primack et al., 2009; Richardson et al., 2013; Keenan et al., 2014). Our results demonstrate that this paradigm may not apply for trees in drier regions, where the effects of increased temperature can cause physiological and growth stress directly through chloroplast damage, inhibition of vernalization, and increased respiratory demand (Larcher, 2003; Morin...
et al., 2009), and indirectly through increased vapor pressure deficit driving elevated xylem tensions and reduced stomatal conductance (Breshears et al., 2013).

In piñon pine, drought alone delayed phenological development and reduced foliar growth (primary axis) and shoot growth relative to the ambient treatment, consistent with our hypothesis (Figs 1–4). Moreover, there were no differences in any measured variable between the heat treatment and the drought treatment in either branch axis, indicating that these two stresses affected the growth and phenology of piñon pine similarly. Our results were consistent with the phenological and growth responses of several Mediterranean tree species to experimental and natural drought (Borghetti et al., 1998; Ogaya & Penuelas, 2004; Penuelas et al., 2004; Girard et al., 2011, 2012).

Given that the heat and drought treatments both delayed development and reduced foliar and shoot growth, it was surprising that the drought + heat treatment did not have more of a synergistic effect on piñon pine (Figs 1–4). The effects of heat and drought were most striking for foliar development, where heat delayed emergence by 19 days, drought by 39 days, and drought + heat by 57 days in the primary axis branches (Fig 2), while secondary axis branches in the drought + heat treatment had no needle growth at all in 2013 (Figs 1 and 2). An inability to grow foliar tissue could reduce vigor in these trees, as younger foliage can have higher photosynthetic efficiency in conifers (Ethier et al., 2006; Warren, 2006).

Experiments have been found to underpredict advances in leaf-flushing from warming, perhaps due to treatment artifacts (Wolkovich et al., 2012). We found no differences in response between our ambient and chamber control treatment making this explanation for our results less likely. Previous research has shown that
advances in development from heating can be reduced as temperature increases from ~1 to ~3 °C (Repo et al., 1996; Morin et al., 2010; Kuster et al., 2014), and our increased temperature treatments were ~4.8 °C. Our result of reduced foliar and shoot growth in the heated treatment is consistent with previous research finding that that radial growth of piñon pine and other conifers in the southwest USA was negatively correlated with temperature, as increased temperatures are typically associated with drought stress in this region (Adams & Kolb, 2005; Williams et al., 2013).

Our phenology and growth results should be considered in the context of the unusually dry climate of 2011, 2012, and 2013 prior to September (Fig. S2; Williams et al., 2014). From January through August 2013, the SUMO experiment site received only 146 mm of precipitation, 51% of the 25-year mean of 284 mm for the same period. Prior to this, 2012 annual precipitation was only 48% of the 25-year mean. Thus for the majority of 2013, our heat and drought treatments were imposed on top of an already strong drought. Effects of this background drought on the ambient trees in our experiment may explain why we did not observe any earlier development or increased foliar or shoot growth in the heat treatment for piñon pine, relative to the ambient treatment. The lack of any reproductive development in ambient piñon, despite our observations that male pollen cones were common in 2012 in these trees, could also be explained by the dry start to 2013. The low precipitation in 2011–2013 may also explain the low shoot growth of juniper, relative to piñon pine, and also the lack of any treatment effect in juniper shoot growth as juniper is known for its highly conservative growth in dry years (Howell, 1941; Herman, 1956). This conservative growth strategy likely enables juniper’s survival at hotter and drier sites than piñon pine. Notably, the conditions in the drought + heat treatment during the already dry 2013 were not unrealistic, but analogous to...
those anticipated with future warming in megadrought climate conditions projected for later in the century under a ‘business-as-usual’ scenario (~6 °C in warm-season temperature and a Palmer Drought Severity Index of ~2.31 for 2100; Williams et al., 2013; Cook et al., 2015).

Consistent with our hypothesis, lower values of water potential appeared to constrain foliar and shoot growth and delay the timing of needle emergence in piñon pine (Fig. 5, Table S1). Correlation coefficients were typically highest for mean growing season water potential, and water potential values in the months of March, May, June, and August were consistently correlated with growth and phenology (Table S1). This result indicates that physiological drought stress associated with low plant water potential over the entire growing season influenced growth and development (Fig. 5, Table S1). In juniper, low observable variability in growth likely contributed to the lack of correlation with water potential.

We hypothesized that nonstructural carbohydrates (NSC) would be unrelated to foliar and shoot growth and phenology or that reduced growth and delayed development due to drought stress would be associated with increased NSC, consistent with a reduction in C sink demand preceding any C source limitation (Körner, 2003; Fatichi et al., 2014). However, low NSC in June was correlated with reduced growth and delayed needle emergence in piñon pine over the growing season (Table S2). Correlations were strongest and most consistent across growth and phenology variables for shoot starch (Fig. 5), indicating that trees with higher NSC reserves in shoots in the dry early growing season were more likely to show earlier needle emergences and greater growth over the rest of the season. This relationship, while unexpected given previous results from some species (e.g., Körner, 2003), is consistent with reduced NSC in piñon pine observed in response to seasonal and prolonged drought stress (Adams et al., 2013; Sevanto et al., 2014; Dickman et al., 2015). The response of piñon is not unique; Mediterranean Arbutus unedo with low lignotuber NSC following 14 years of experimental drought had reduced basal area increment growth (Rosas et al., 2013). Despite low variability in juniper growth and NSC content, we found two significant positive correlations of shoot growth with shoot glucose and fructose, and total NSC, in secondary axis branches, indicating that higher juniper growth was related to increased NSC. Glucose and fructose have been shown to increase in juniper in response to drought and may be important for osmotic adjustment that enables growth under drought conditions in this species (Dickman et al., 2015). The paucity of significant relationships in this species is consistent with our hypothesis that NSC would be primarily unrelated to growth.

We urge caution in interpreting positive (inverse) relationships between growth (needle emergence timing) and NSC in piñon pine as a C source limitation. We have correlated foliar and shoot growth with NSC from relatively early in the growing season (June 19th, DOY 170). At this time for piñon pine, some shoot growth had been observed in the ambient and control chamber treatments, but needle emergence had not yet occurred in any measured branches. It is possible that NSC increased later in the growing season in trees which had reduced growth. June NSC and its components were correlated with shoot water potential across the growing season (Table S3), complicating the attribution of causation. For all cases where a significant relationship was found for both mean growing season water potential and shoot starch with growth or needle emergence (Fig. 5), the adjusted $r^2$ from a linear regression was always higher for the correlation with water potential than for that with shoot starch concentration. This indicates that direct drought stress on xylem water tension was a stronger constraint for growth and development than NSC, consistent with prior findings that water stress limits growth more than carbohydrate availability (Körner, 2003; Würth et al., 2005; Fatichi et al., 2014; Palacio et al., 2014).

Our results demonstrate that a 4.8 °C increase in temperature, a change consistent with business-as-usual emissions scenario projections of a 5–7 °C temperature increase by 2100 in northern New Mexico and many other regions of the world (IPCC 2013, Feng et al., 2014), can cause phenological delay and reduced foliar and shoot growth. Moreover, drought in combination with elevated temperature resulted in either a ~2-month delay in needle emergence or a complete lack of needle growth in piñon pine, which could cause a severe reduction in tree vigor over multiple growing seasons. These strong effects were observed after only 1 year of treatment, demonstrating that phenological and growth responses to temperature and drought stress can be rapid. Our results indicate that projections based on typical phenological responses of an earlier and longer growing season in response to higher temperatures, and consequent increased ecosystem C sink strength, may not be valid for regions where plants will face increased drought stress with future climate change (Weiss et al., 2012). Trees that experience growth reductions from drought and temperature stress have reduced vigor and are often more prone to drought-induced mortality (Manion, 1980; Waring, 1987; McDowell et al., 2010; Macalady & Bugmann, 2014). Outside of the Mediterranean region, phenological responses of semiarid tree species to drought and
temperature have not been well studied, and our results highlight the need to evaluate these tree responses in other dry regions, and in those regions which may become drought-prone with future climate change. The physiological link between xylem tension and phenological response demonstrates the mechanism by which drought stress influences growth and phenology. This relationship could provide a process-based modeling approach to account for drought- and temperature-induced developmental delay and reduced productivity in global vegetation models for drought-affected regions where phenological responses to climate are not well studied. Correctly representing such phenological feedbacks on the terrestrial C sink with a changing climate is necessary for robust projections of global climate change.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Additional Non-Structural Carbohydrate Methods. Details of the methods used to assess NSC.

Figure S1. Design of the Los Alamos Survival-Mortality experiment.

Figure S2. Temperature, vapor pressure deficit, precipitation, and soil water content data measured at the SUMO experiment.

Figure S3. An illustration of phenological phases in our classification scheme for pinon pine

Figure S4. Pollen cone production in one-seed juniper by treatment.

Figure S5. Mean growing season pre-dawn water potential for pinon pine and juniper trees by treatment.

Figure S6. Mean June starch concentration in shoots of pinon pine and juniper by treatment.

Table S1. Correlations between water potential and shoot growth, needle growth, and emergence timing.

Table S2. Correlations between NSC and components and shoot growth, needle growth, and emergence timing.

Table S3. Correlations between water potential and NSC components.

Table S4. Mean June concentrations (% of dry tissue mass) and standard error by treatment for NSC and components in tissues of pinon pine.

Table S5. Mean June concentrations (% of dry tissue mass) and standard error by treatment for NSC and components in tissues of juniper.