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Draft: Soil moisture controls at timberline

Soil moisture controls Engelmann Spruce (*Picea engelmannii*) seedling carbon balance and survivorship at timberline in Utah, USA

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Abstract

Most hypotheses about controls over high altitude forests, including treeline, the elevation for upright woody plants, or timberline, the upper elevation for aggregated forest, suggest that low temperature drives forest dynamics, either through effects on cell division and tree growth or indirectly through frost damage or nutrient availability. However, abiotic factors other than temperature, including water availability, may serve as other important controls at high elevations, particularly for seedlings. To test the hypothesis that the timing and amount of precipitation exerts a strong control over the high elevation forest boundary on the Wasatch Plateau in Central Utah, USA we conducted a field experiment that manipulated water availability and monitored photosynthesis, growth, and survivorship in *Picea engelmannii* seedlings. Survivorship increased from the driest to the wettest conditions while the timing of precipitation did not explain differences in survival. However, we found that large, infrequent rain events increased maximum photosynthetic flux density compared to frequent, small rain events. Our results highlight

Draft: Soil moisture controls at timberline

the potential role of growing season water availability in limiting timberline expansion below the low-temperature thermal limits of *Picea engelmannii*. As a consequence, the infilling of trees below treeline in this region in response to climate change is likely to be episodic and driven by multi-year periods of high water availability and frequency that overcome drought limitations.

Introduction

Renewed interest in upper treeline dynamics is in part driven by the hope that these systems will help us understand how ecosystems will respond to climate change (Harsch et al. 2009, Smith et al. 2009). Because these ecotones are closely correlated with temperature, and have low partial CO₂ pressure, they should be among the most responsive ecosystems to increased temperature and rising atmospheric CO₂ (Smith et al. 2009, Marshall 2014). Many have noted that upper treeline is often highly correlated with low or average temperature (Korner 2007) or seasonal thermal amplitude (Jobbagy and Jackson 2000). This correlation has proved useful to explain recent treeline advance that appears to be closely tied to increases in rising global temperatures, particularly at high altitudes and latitudes (Harsch et al. 2009). There continues to be debate over the mechanism that causes the abrupt transition from upright trees to dwarf trees and herbaceous vegetation as well as controls over forest infilling below treeline but above timberline (Lloyd and Fastie 2002, Hoch and Korner 2009, McNown and Sullivan 2013). Furthermore, only slightly more than half of treelines have advanced this past century while temperatures have increased, indicating that some treelines are sensitive to climate change and that for many treelines temperature alone is insufficient to explain patterns of treeline movement (Harsch et al. 2009).

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There are two principal alternative physiological mechanisms proposed to explain upper treeline: the carbon sink limitation and the carbon source limitation hypotheses. In the carbon sink limitation hypotheses, temperature directly impacts whole plant carbon status by restricting the growth potential of the plant. Photosynthesis is capable of fixing carbon beyond the immediate needs of the plant but low soil temperatures limit cell division and expansion, eliminating trees' ability to invest fixed carbon in growth (Korner 2003, 2007). Support for this hypothesis is found in the consistent accumulation of non-structural carbohydrates in needles of trees at treeline relative to lower elevation trees of the same species (Hoch and Korner 2003, Fajardo et al. 2012, Hoch and Korner 2012). In contrast to this, the carbon source limitation hypothesis contends that there are environmental conditions that preclude C fixation that ultimately lead to plant death. In these cases, temperature influences environmental factors such as needle freezing or ice abrasion (Hadley and Smith 1986), nitrogen availability (McNown and Sullivan 2013), or growing season drought (Lloyd and Fastie 2002), which indirectly influence plant C balance.

An emerging literature on tree seedling survival, physiology, and establishment shows how critical establishment and persistence is in explaining tree population dynamics (Germino et al. 2002, Smith et al. 2003, Smith et al. 2009, Bansal and Germino 2010, Bansal et al. 2011). One key finding of field-based seedling studies is that it is challenging to find large numbers of seedlings in the field indicating the pulsed nature of recruitment at forest boundaries (League and Veblen 2006). However, it is obvious that the seed dispersal, germination, and establishment phases of tree life history are key to understanding contemporary changes in distributional dynamics at treeline (Smith et al. 2003). There is

Draft: Soil moisture controls at timberline

substantial evidence that facilitation by vegetation and microtopography plays an important role in pre-establishment survivorship and physiology for new germinants and young seedlings (Germino et al. 2002, Johnson et al. 2004, Bansal and Germino 2009, Smith et al. 2009, Castanha et al. 2013). In the absence of available seedlings found near treeline, an alternative but less satisfactory approach includes the use of nursery-grown seedlings from seeds collected locally (Purdy et al. 2002, Carles et al. 2011).

Growing season soil moisture is a potentially important environmental factor that could limit seedling responsiveness to changes in temperature (Cui and Smith 1991, Lloyd and Fastie 2002, Johnson et al. 2004, Moyes et al. 2013). Harte et al. (1995) showed that warming in alpine meadows decreased seasonal snow cover and ultimately resulted in a 25% decrease in growing season soil moisture. Moyes et al. (2013) found that, in experimentally warmed plots, soil moisture content was consistently a strong predictor of stomatal conductance, photosynthesis, and respiration. This is a good example of how the carbon source hypothesis could work, with soil moisture deficits imposing a limit on the ability of seedlings to acquire C and persist between timberline and treeline. A number of scenarios have been proposed for future rainfall dynamics with more extreme climate (Heisler-White et al. 2008, Knapp et al. 2008, Heisler-White et al. 2009). Theoretically, in mesic systems such as subalpine forests we would see frequent, small to moderate rainfall events during the growing season. Under intensifying scenarios where events become less frequent and larger we would anticipate that the system would move from low water stress in ambient conditions to increased water stress because the event timing controls the probability of surface soils drying below a stress threshold (Knapp et al. 2008).

Draft: Soil moisture controls at timberline

On the Wasatch Plateau in central Utah, USA there is an abrupt transition from subalpine forest timberline to tall forb communities at approximately 3000-m (Ellison 1954, Morris et al. 2010). Within the tall forb communities there are tree islands composed of upright and occasionally dwarfed *Picea engelmannii*, *Abies lasiocarpa*, and *Pinus flexilis*. Along the highest ridgelines these species are found in the krummholz form (Smith et al. 2003). This area is a compelling region to study water controls over high elevation forest change because average growing season temperature at this timberline is approximately 8.5 °C, well above the 5.5-6.5 °C thermal average for typical treeline (Korner 2007) and water could be limiting because there is a pronounced dry period between snow melt and the arrival of monsoonal precipitation in late July and soils are shallow, due to erosion caused by overgrazing, and fine textured and dark which allows for rapid evaporation of water from the soil surface (Ellison 1949, 1954).

We hypothesized that growing season water availability is a dominant control over the establishment and persistence of *Picea engelmannii* seedlings at timberline. Furthermore, we anticipated that small, frequent water events would benefit *P. engelmannii* seedlings more than large, less-frequent precipitation by reducing cumulative water stress and increasing cumulative photosynthetic flux densities and growth (Knapp et al. 2008). We anticipated that water stress would manifest itself first in increased water use efficiency, manifest by increasing stomatal limitation to photosynthesis but ultimately resulting in seedling mortality (Bota et al. 2004, Johnson and Smith 2007). To test this hypothesis we used a three year field experiment to address two central questions about seedling physiology, growth, and survivorship: (i) how does the timing of rainfall, independent of total amount received, influence plant C balance and (ii) under what soil

Draft: Soil moisture controls at timberline

moisture conditions are we likely to see high photosynthesis flux densities or decreased stomatal limitation to photosynthesis and tree seedling persistence at treeline? To answer these questions we manipulated growing season precipitation by changing its timing or reducing the amount and then measured leaf-level gas exchange in field-grown seedlings and censused seedlings planted into field plots for three growing seasons where soil moisture was manipulated. By measuring gas exchange and mortality we can combine instantaneous measures of our treatments (water stress, C acquisition) with the long-term consequence of changes in these processes (survivorship).

Materials and methods

We used winter-hardened Engelmann Spruce (*Picea engelmannii*) seedlings that were germinated at the Nature High Nursery (Draper, UT). The seeds were collected near Electric Lake, Carbon County, UT, less than 45-km from our research site and on the same geological parent material. The collections were made at 2900-3000-m elevation, with a mean annual temperature of 2.3 °C and a growing season temperature of 8.5°C. Seedlings were moved to our research site during a time when the nursery was still experiencing freezing nighttime temperatures. At planting average seedling height was 6.4-cm with an average maximum root depth of 13.2-cm.

Field Experimental Design and Site Description

The Great Basin Experimental Range (GBER, 39° 17':-111° 30'; U.S. Department of Agriculture, USFS) on the Wasatch Plateau has been an important ecological research site since the early 1910s. The USDA Rocky Mountain Research Station-Shrub Science Laboratory in Provo, Utah currently manages this site. This work focuses on the subalpine, mountain meadow zone within the GBER. Mean annual precipitation on the windward side

Draft: Soil moisture controls at timberline

138 of the plateau for the past 75 years was 902 mm, with 81% of precipitation falling between
139 October and May (Prevedel et al. 2005). Mean annual temperature for the summit of the
140 Wasatch Plateau is 1.7 °C, with a July average of 14 °C and a December average of -7.7 °C.
141 During the snow-free period (June-October), average air temperature is 8.4°C. During
142 2010-2012, mean temperature between June and September was 11.7 °C in 2010, 11.6 °C in
143 2011, and 12.7 °C in 2012. It was driest during the 2010 growing season, with 102 mm of
144 rain and was wettest in 2011 with 160 mm of rain, with 2012 intermediate at 150-mm
145 (Figure 1 a-c). Soils are derived from the lacustrine limestone parent material. The soils are
146 classified as fine, mixed argic Cryoborolls with shallow (4-cm) A horizons and an average B
147 horizon of 52-cm (Klemmedson and Tiedemann 1998). Small islands of *Picea engelmanni*
148 Parry ex Engelm and *Abies lasiocarpa* (Hook.) Nutt.. are found within a broad matrix of
149 herbaceous vegetation. The herbaceous vegetation is a mixture of grasses (*Achnatherum*
150 *lettermanii* (Vasey) Barkworth, *Elymus trachycaulus* (Link) Gould, *Trisetum spicatum* (L.) K.
151 Richt.) and forbs (*Artemisia michauxiana* Besser, *Taraxacum officinale* F.H. Wigg., *Geranium*
152 *richardsonii* Fisch. & Trautv.).

153 In 2009 we constructed 4 rainout shelters per site on eight subalpine sites to
154 manipulate soil moisture. At each site five 2.5 X 2-m plots were established and the
155 boundary of each of the plots was trenched to bedrock (approximately 50-cm) and lined
156 with plastic to avoid lateral movement of water. On the plots that were on a slope, the
157 upslope edge of the plot had aluminum flashing that extended 10-cm aboveground to
158 prevent overland flow. One of the five treatments was randomly assigned to each of the
159 plots, with the treatments being control (no shelter), 30% ambient reduction, 70% ambient
160 reduction, 1-week application frequency, and 3-week application frequency.

Draft: Soil moisture controls at timberline

There were two styles of shelters used, depending on the treatment. The first were modeled after Heisler-White et al. (2008) and allow us to capture all incoming precipitation and reapply it at a specific frequency (*freq*), either 1 event per week or 1 event every 3 weeks. The second shelters, which reduce ambient precipitation (*-precip*), are modeled after those used by Yahdjian and Sala (2006) and had an aerial coverage of 30% or 70%. All plots were 2.5-m X 2-m. The *freq* shelters were constructed with four wooden corner posts buried to 50-cm and a wooden frame on which the roof was attached. The uphill end of the shelter was approximately 1.5-m tall and sloped to 1-m on the downhill side of the shelter. The roof on the *freq* shelters was constructed from furrowed polycarbonate sheeting (Green-Lite). The plastic used reduced photosynthetically active radiation (PAR) by approximately 20%, but in the sunny, high-elevation conditions at our site daytime PAR typically exceeded 1000- $\mu\text{mol m}^{-2}\text{sec}^{-1}$. Intercepted rain was guttered from the structure into 208-L (55-gallon) tanks and that water was used to irrigate the plots at 1 week or 3 week intervals. The *-precip* shelters were built using prefabricated aluminum frames that were placed onto permanent anchors and removed at the end of the growing season. The gutters on the *-precip* shelters were made from clear acrylic plastic extruded in a 270° angle to intercept incoming water and gutter it off from the plots. As expected there were unavoidable changes in microenvironment that were associated with the shelters. Average temperature changes were less than 1 °C daily, with changes slightly biased toward higher nighttime temperatures than daytime temperatures.

Experimental treatments and protocol

Shelters were installed between mid-June and the first week of July, depending on when we were able to access the research plots. For the *freq* treatments, accumulated water was

Draft: Soil moisture controls at timberline

reapplied using watering cans at a rate < 30 mm/h, which is consistent with the convective storms that provide most mid-summer rainfall in this region. The long-term average interval between events is 11-days. To ensure that there would be water available for application we added 75% of the accumulated water in the 1-week plots, leaving the remaining 25% in case there was no rain during the subsequent week. All accumulated water was ultimately applied. The one-week treatment had more frequent but smaller events than occurred in the control plots. Individual watering events differed in size based on ambient precipitation. Both *freq* treatments received the same amount of water during the growing season. In 2012, we did not match the *freq* treatments to ambient rainfall, instead applying the long-term average weekly precipitation to the 1-week plots and 3 times that amount on the 3-week plots.

In late June 2010, immediately after snowmelt, we planted five of our 3-year old site-acclimated *Picea engelmannii* seedlings into each treatment (5)*site (4) combination (n=100). We assessed mortality rates in 2010-2012 and leaf gas exchange rates in 2012. Seedlings were planted within the interspaces between perennial plants.

Soil microclimate and meteorological measurements

Volumetric soil moisture (VSM) at 5-cm and temperature were continuously monitored at four of our sites using 5TE probes and connected to Em5 dataloggers (Decagon Devices, Inc., Pullman, WA). This 5-cm depth is approximately the midpoint of the root zone of the planted seedlings. Probes were placed in bulk soil and not in the rhizosphere or within the root plug. At the other four sites, VSM and temperature were continuously monitored using GS3 sensors (Decagon Devices, Inc., Pullman, WA) connected to CR-1000 dataloggers (Campbell Scientific, Logan, UT; Figure 1d-f). Air temperature at

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0.5-m was measured inside and outside of the shelters at these four sites. We converted volumetric water content values to water potential using site-specific calibrations developed using a WP4C dewpoint potentiometer (Decagon Devices, Inc., Pullman, WA).

Plant Responses

We censused live trees at the beginning and end of each growing season beginning one week after they were planted in 2010, and occurring again in October 2010, June 2011, October 2011, May 2012, and continuing bi-weekly in 2012. A tree was considered alive if it possessed green needles.

In 2012, we measured photosynthesis twice weekly on surviving seedlings in our 1-Week and 3-Week *freq* plots between June 6 and July 11. This timing corresponded to two weeks following snowmelt and included one full dry-down, rewetting cycle for the 3-week *freq* plots. We measured A_{max} at saturating light intensity, $400 \mu\text{mol mol}^{-1} \text{CO}_2$, and at ambient temperature using a LiCOR LI-6400XT with a standard leaf chamber equipped with a 6400-02B LED light source (LiCOR, Lincoln, NE). The sampling was scheduled to correspond to the day of watering, with physiological measurements taken prior to irrigation, as well as measurements on the day after watering. For seedlings in the 3-week *freq* plots, measurements were made weekly at during the same time that the 1-week *freq* plots were measured. Two branches of each seedling were marked and all measurements were made on these branches. Leaf area was calculated using images collected and scanned using leaf area was calculated using ImageJ 1.43u (NIH, Bethesda, MD, USA). We estimated relative stomatal limitation to photosynthesis (l_g) using according to Jones (1985) and Johnson and Smith (2007)

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$$l_g = 100 \times \frac{(c_a - c_i)}{c_a}$$

where c_a is ambient CO₂ concentrations and c_i is intercellular CO₂ concentration. Stomatal limitation of photosynthesis is the resistance to CO₂ uptake imposed by stomatal closure and is a proxy for plant stress (Geber and Dawson 1997, Johnson and Smith 2007).

Analysis approach and statistics

For leaf-level gas exchange measurements in the field experiment we used a single factor generalized linear model with watering treatment as the main effect. To assess the sensitivity of leaf-level gas exchange in the *freq* plots we use a two-factor generalized linear model, with pre- and post-watering as time effects and 1- and 3-week photosynthesis as the treatment effects. For individual trees where we had soil moisture data, we modeled the relationship between A_{max} and soil water potential at 5-cm soil depth using the curve fitting function in SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA.). We also modeled the relationship between average seasonal soil water potential and seasonal mortality using the same function. We chose between linear, exponential growth, and power functions and selected the model with the highest R² that passed the Shapiro-Wilk Normality test at $p>0.05$. Survival functions were developed for seedlings planted in the five water treatments using the survival analysis package in R (Therneau 2014).

Results

There was a microclimate effect created by the shelters. For the *freq* shelters, average maximum temperatures were decreased by 0.85 °C and minimum temperatures were increased by 0.14 °C. Average soil temperatures at -5 cm across all treatments were within 0.3 °C of each other and did not vary systematically. Photosynthetically active

Draft: Soil moisture controls at timberline

radiation was decreased under shelters. On August 9, 2012 between 12:00 and 15:00, hourly sampling showed that *freq* shelters reduced PAR from an average of 1065 W m⁻² outside the shelters to 927, a 13% decrease, with -30% shelters decreasing PAR to 918, and -70% decreasing PAR to 882. All these PAR values exceeded saturating PAR for *P. engelmannii* seedlings grown in a growth chamber (unpublished data).

As intended, soil water was changed due to our shelters (Figure 1). We found that during the growing season 2012, our control plots on average had 20% of the days when water potential was below -0.5 MPa, with all of the growing season above -1.0 MPa. The next wettest treatment was our 3-week frequency, where 40% of the days were below -0.5 MPa and 20% of the days were below -1 MPa. The one-week application produced 50% of the days below -0.5 MPa and 37% of the days below -1.0 MPa. The 30% reduction had 65% of the growing season below -0.5 MPa and 52% below -1.0 MPa. The 70% reduction shelters had 70% of the days below -0.5 MPa and 63% of the days below -1.0 MPa. For the 70% reduction treatment, 49% of the days had water potentials below -3.0 MPa.

Three-Year Survivorship

At the end of three growing seasons (2010-2012) the total surviving individuals ranged from 5% in our driest treatment to 50% in our treatment with the least variable water availability (1-week watering treatment)(Figure 2). When accounting for the censored individuals—those who survived the 28 months of the experiment—mean survivorship ranged from 134 days for those in the -70% treatment to 1220 days for the 1-week watering. Survivorship for individuals in the -70% treatment was significantly less than the control treatment ($p=0.0125$) while survivorship in the 1-week watering was significantly greater than the control ($p=0.048$).

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We found that average water potential of a plot was the best predictor of seasonal mortality. Seedling survival during the summer was linearly correlated with average water potential at 5-cm (Figure 3a, $R^2=0.50$, $p<0.005$). In plots where average water potential was above -1 MPa, mortality rates were relatively low. However, when our drought treatments created conditions where average water potentials were below -1 MPa, mortality rates climbed to over 25%. There was also a significant relationship between mortality rates and late season (mid-September to mid-October) water stress (Figure 3b, $R^2=0.54$, $p<0.02$). We saw much larger death rates during winter than in summer, with the linear regression indicating mortality rates $>50\%$ for plots where late season water potentials were below -1 MPa with much higher survival rates in plots that were wet prior to the start of winter.

Photosynthesis, Conductance, and Stomatal Limitation

Photosynthetic flux densities were sensitive to watering frequency and were responsive to changes in soil moisture (Figure 4b-c, 5). During the 2012 growing season maximum photosynthetic flux density (A_{max}) was higher for trees in the 3-week watering treatment, $2.45 \mu\text{mol m}^{-2} \text{sec}^{-1}$, than in the 1 week watering treatment, $1.37 \mu\text{mol m}^{-2} \text{sec}^{-1}$ ($p<0.0001$). We found that A_{max} increased 1 day after individual watering events by 42%, showing how tightly coupled A_{max} was to water status. Stomatal conductance followed a similar pattern, with conductance being significantly higher in the 3-week treatment, $0.027 \text{ mol m}^{-2} \text{sec}^{-1}$, than in the 1-week treatment, $0.016 \text{ mol m}^{-2} \text{sec}^{-1}$. Stomatal conductance was even more responsive than photosynthetic flux density to water addition, increasing on average 67% one day after watering ($p<0.001$) from $0.0147 \text{ mol m}^{-2} \text{sec}^{-1}$ to $0.0246 \text{ mol m}^{-2} \text{sec}^{-1}$.

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What was particularly interesting was the temporal dynamics of A_{max} . On our first measurement day on June 11, 2012, two weeks after snow melt, all our treatments had similar flux densities of A_{max} (Figure 4a) and did not respond to watering. Then, for the 1-week treatment, A_{max} was highly consistent and responsive to water beginning June 18 through July 9, with pre-watering values near $1 \mu\text{mol m}^{-2} \text{sec}^{-1}$, increasing to $1.7 \mu\text{mol m}^{-2} \text{sec}^{-1}$ one day after watering. However, the 3-week treatment had more than a three-fold increase in A_{max} following its much larger watering event on June 18, 2012, with photosynthetic flux densities increasing from $1.1 \mu\text{mol m}^{-2} \text{sec}^{-1}$ to $3.7 \mu\text{mol m}^{-2} \text{sec}^{-1}$. While A_{max} declined from that point until the next watering, they were consistently higher than the 1-week watering treatment. The initial, much larger pulse was sufficient to maintain higher A_{max} than the frequent, smaller events.

We found a consistent exponential relationship between water potential at 5-cm and A_{max} in our one-week frequency plots (Figure 4b, $R^2=0.47$, $p<0.001$) while no such relationship existed for our 3-week plots (Figure 4c). For the 1-week frequency plots, A_{max} was substantially reduced when water potential was below -1 MPa. There were no significant differences between A_{max} in the pre- and post-watering trees after accounting for soil water potential. However, A_{max} in the 3-week frequency plots was not correlated with water potential at 5-cm and there were no significant differences between pre- and post-watering conditions in the A_{max} -water potential relationship. Stomatal limitation ranged from 30-60% over the course of the experiment (Figure 4b). There was a significant linear increase in l_g from the beginning of the growing season through mid-July for both the 1-week and 3-week treatments (1-week: $R^2=0.58$, $p=0.01$; 3-week: $R^2=0.44$, $p=0.03$). There were no significant differences between the treatments.

Draft: Soil moisture controls at timberline

318 Discussion

319 As we hypothesized, soil moisture in our ecosystem exerted substantial control over
320 the persistence and physiology of seedlings. We saw that survivorship was much lower in
321 drought treatments and that the frequency of watering did very little to determine
322 survivorship. We observed that, in surviving seedlings, the 3-week frequency generated
323 significantly higher photosynthetic flux density that persisted for several weeks after the
324 individual watering event compared to frequent, small rainfall events. With higher
325 photosynthetic rates, we would expect that the 3-week treatment seedlings would capture
326 more C and be able to increase rooting depth and canopy size.

327 We observed that stomatal limitation to photosynthesis increased from the
328 saturated conditions after snowmelt to late in the growing season. This mirrors the midday
329 pattern that was observed by Johnson and Smith (2007) on *Abies lasiocarpa* seedlings
330 exposed to drought at treeline. Our measured relative stomatal limitations were
331 significantly higher than those measured by Johnson and Smith (2007), with our mid-July
332 measurements showing that over 50% of the limits to photosynthesis were caused by
333 stomatal resistance. For most weeks, the small, one-week watering events were insufficient
334 to substantially alter stomatal limitation. This contrasts with the larger, less frequent
335 events where the watering decreases the stomatal limitation by 12-15% (Figure 4).

336 The most robust result that we found was the influence of water availability on
337 seedling survival and photosynthesis. Others have reported that summer soil moisture
338 influenced photosynthetic flux densities and survival of seedlings of treeline species
339 (Brodersen et al. 2006, Moyes et al. 2013). After three growing seasons, there was only a
340 5% survival rate in our 70% reduction treatment, compared to 50% survival in the 1-week

Draft: Soil moisture controls at timberline

frequency plots. When we looked closely at the influence of water on A_{max} in the frequency plots we found that trees in both the 1-week and 3-week plots had similar A_{max} soon after snowmelt. However, as the soils began to dry down there was a divergence in the treatments. The small, frequent rain events elevated A_{max} in the 1-week plots, but only marginally. By the following week, A_{max} had returned to the pre-watered levels. However, the single, large event in mid June in the 3-week plots elevated A_{max} and those increased levels were maintained for the subsequent two weeks. One potential explanation for these different responses is patterns of water loss from these fine textured soils. During the period of analysis in 2012, this site had extremely low relative humidity and maximum daily temperatures over 15°C, which can cause substantial evaporation from the upper soil profile. The watering events in the 1-week treatment likely replenished this layer, with the typical wetting front not moving substantially below the 5-cm layer at which we made our soil moisture measurements. This is likely why A_{max} was closely correlated with water potential in this soil layer. However, the large, infrequent event would have had a much deeper wetting front, moving water below the layers subject to evaporation. This additional water would then be available to tree seedlings with roots below the soil layer sensitive to evaporation. We saw that the seedlings in the 3-week treatment were able to maintain high photosynthetic flux densities even when water potentials at 5-cm dropped below -1.5 MPa, something that the 1-week treatment trees were unable to do, perhaps due to greater penetration of individual watering events and higher water potentials lower in the soil profile. This result likely underestimated the influence of water on newly emerged seedling survival (Bansal et al. 2011). Our seedlings were much larger than new

Draft: Soil moisture controls at timberline

germinants and had a well developed root system. They were outplanted at the typical time for recruitment when soils were saturated but many failed to survive the first year.

Persistence of seedlings will be required for treeline to migrate upslope or infill below current treeline with warming temperatures. Several studies have shown the sensitivity of newly emerged seedlings to microenvironmental effects (Germino et al. 2002, Johnson et al. 2004, Moyes et al. 2013). We found that survival of healthy, three year old seedlings with well developed root and shoot systems is likewise sensitive to water availability. The frequency of watering did not play a strong role in determining survivorship but did influence A_{max} . In addition, reductions in incoming water had a strong and consistent influence on seedling survival. The water stress present at the end of the growing season was a significant predictor of mortality rates, with over-winter mortality much larger than growing season mortality. This indicates that late season conditions were perhaps more important for determining long-term survival of these seedlings than even growing season drought. While snowmelt timing and conditions immediately after snowmelt are consistently drivers germination and establishment our results suggest that persistence may be controlled significantly by end-of-season water status.

During the past 33 years at our research site, the average longest duration between rain events during the June-September growing season was 30 days, with only 25% of years having precipitation events on average less than 3-weeks apart or less (NRCS, 2014). In 2011, when we observed low mortality and high soil water potentials, we had the second highest rainfall frequency in the historical record with the longest period between events being 15 days. The driest quartile of years had at least one period longer than 36 days without rain, with one year having no measurable rainfall in 81 days. During the 1/3 of

Draft: Soil moisture controls at timberline

century for which we have good daily precipitation measurements, there were never more than two years in a row that had dry periods shorter than 21 days. These historical data indicate that it is rare to have hydrological conditions where germination, establishment, and persistence are likely, potentially explaining the absence of seedlings and saplings in the herbaceous vegetation above timberline and below treeline on the Wasatch Plateau.

There is abundant evidence that the establishment phase of treeline species life history is critical to understand treeline migration and infilling (Germino et al. 2002, Smith et al. 2003, Bansal and Germino 2008). This work adds to that understanding by showing that a treeline below the forecast thermal treeline may be currently controlled by summertime drought, where warming temperatures would likely decrease the likelihood of upslope migration of trees unless there was an increase in summertime precipitation.

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Figure Legends

Figure 1: Weather at the summit of the Great Basin Experimental Range, Ephraim, UT measured at the Seeley Creek SNOTEL installation (a-c). Average soil water potential at 5-cm, calculated using moisture release curves and measured volumetric soil moisture, from our experimental plots for three years (d-f).

Figure 2: Survival curves for field-grown seedlings after three growing seasons. All treatments had at least one individual survive through the experiment, but there was 95% mortality in the 70% drought treatment and only 50% mortality in the 1-week ambient watering treatment. There were statistically significant differences between the -70 treatment and control, 1-week, and 3-week treatments and significant differences between the 1-week treatment and control, 30% reduction, and 70% reduction.

Figure 3: Survival- and photosynthesis-water relationships for field grown seedlings. (a) Survivorship percentage as a function of seasonal average water potential (summer, filled circles) or end of season water potential (Aug 15-Sept 15, open circles). (b) A_{max} as a function of soil water potential at 5-cm for seedlings grown in the 1-week frequency plots. There is a significant exponential increase in A_{max} with increasing water potentials. (c) A_{max} as a function of soil water potential at 5-cm for seedlings grown in the 3-week frequency plots. There is a no relationship between A_{max} and water potentials at 5-cm.

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530 Figure 4: (a) Seasonal measurements of A_{max} for seedlings grown in the 1-week (circles)
531 and 3-week (triangles) watering treatment. Filled circles indicate A_{max} prior to watering
532 and the open circles are A_{max} the day after watering. Filled triangles are A_{max} for the 3-week
533 treatment seedlings on the same date as the pre-watering measurements in the 1-week
534 treatments. The open triangles are the following date. The 3-week treatments were
535 watered on 6/18/12 and 7/9/12. (b) Seasonal changes in stomatal limitation (l_g). For both
536 the 1-week and 3-week treatments we observed a significant increase in stomatal
537 limitation as the growing season progressed and as soils dried after snowmelt. There were
538 no significant differences between treatments.

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