LONGLEAF PINE SEEDLINGS ARE EXTREMELY RESILIENT TO THE COMBINED EFFECTS OF EXPERIMENTAL FIRE AND DROUGHT

by

LUKE WILSON

(Under the Direction of Daniel Johnson)

ABSTRACT

The longleaf pine ecosystem is marked by frequent fire, and climate change will change the conditions under which *P. Palustris* currently exists. This study aimed to understand the interaction of drought and known fire intensities within *P. palustris* for the first time. We used droughted and well-watered *P. palustris* seedlings and burned at three different fuel loads. While two treatments had similar levels of fire intensity per seedling, we were able to cause mortality. Needle fuel moisture of burned seedlings was not different between droughted and well-watered groups. Mortality and resprouting only occurred at over 1.5MJ $M^{-2}$ of fire intensity and drought that exceeded -1.7MPa of predawn water potential when burned. Future measures of growth on burned and droughted seedlings may elucidate the impacts on long-term growth and survival. Compared to Western US species, *P. palustris* is much more resistant to the combined effects of known fire energies and drought.

INDEX WORDS: grass stage, *Pinus palustris*, prescribed fire, recovery, transpiration, water potential

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# CHAPTER 1

# INTRODUCTION

## A Changing Planet Under Fire

The terrestrial biomes are expected to undergo major changes in the coming decades in response to climate change, largely caused by an increase in atmospheric [$CO_2$], air temperature, and land use changes (IPCC 2014). Some of the most visible impacts of climate change will be forest die off or lack of recruitment as a result of extreme events such as fire, drought and pests and their interaction (Hartmann et al. 2018). Throughout the southeast United States, climate change is expected to result in a change in precipitation regime with event intensity increasing and frequency decreasing, resulting in more frequent droughts and flooding (Easterling et al. 2017). Extreme fire conditions will become more common in the southeast United States under future climate as plants experience longer drought. While past plant ecophysiology research has focused on responses to changes in mean rainfall and temperature, more recently the field has shifted towards understanding the extremes such as heatwaves and extended drought (Smith 2011, Seneviratne et al. 2012).

## Longleaf Pine Ecosystem

The longleaf pine (*Pinus Palustris* Mill.) ecosystem was once dominant of the southeastern coastal plain of the United States (Frost 1993). The area is an economically, ecologically, and culturally significant ecosystem has been severely threatened by land use practices over the last two centuries. *P. palustris* occupies only 3% of its former range with its distribution currently dictated by land use practices and frequency of fire (Van Lear et al. 2005). Much of the *P. palustris* ecosystem has been replaced by loblolly (*P. taeda* L.) and slash (*P. elliottii* Engelm.) pine plantations, conversion to agricultural land, or due to a lack of fire has been converted to a mix of other hardwood species (Glitzenstein et al. 1995, Addington et al. 2012). Where fire is maintained, the interaction of resinous and quickly consumed needles and herbaceous understory creates a positive feedback response among fuels, fire and vegetation to allow *P. palustris* to persist under many soil and environmental conditions (Fonda 2001, O'Brien et al. 2008, Mitchell et al. 2009). Conservation of longleaf pine ecosystems is essential to provide habitat for endangered and threated wildlife species such as the gopher tortoise, red-cockaded woodpecker and over 100 common plant associates (Walker and Peet 1984).

*P. palustris* is fairly unique in its growth forms, where it begins its life underfoot as a "grass stage", a morphology usually found in other frequent fire landscapes (e.g., *Pinus merkusii* Jungh. & Vriese ex Vriese, *P. montezumae* Lamb, *P. michoacana* Lindl.; Keeley 2012). Grass stage seedlings only have a few centimeters of woody tissue above the soil with a dense tuft of needles insulating a singular apical bud from low intensity fire (Wahlenberg 1946, O'Brien et al. 2008). After five to ten years in the grass stage, seedlings allocate carbon to stem production to grow up to two meters in a single year to escape further fire. The exact conditions that promote this stem growth are currently unknown (Boyer 1990).

Seedling Physiology Under Drought and Fire

Understanding how seedlings respond to fire and drought has been of interest for decades in wildfire research (Ahlgren 1960). Past investigations have been focused on tracking seedling regeneration after wildfire based on fuel loading and fuel moisture (Gisborne et al. 1928, Brethauer et al. 2021). There have been recent advancements in understanding *P*. *palustris* response to fire. *P*. *palustris* seedlings with small root collar diameters, high litter accumulations, or burned during the growing season were more likely to be killed during fire, or rely on resprouts for survival (Jack et al. 2010, Knapp et al. 2018, Jin et al. 2019). High levels of *P*. *palustris* needle starch, which is reduced during drought, can promote growth after fire (Sayer et al. 2020). Belowground seedling carbon reserves are considered vital for *P*. *palustris* to emerge from the grass stage, however selecting for genetics to hasten growth may make the seedlings less resilient to fire and drought (Aubrey 2021). How plant carbon, hydraulics, weather, and fuel conditions interact likely dictate the survival of *P*. *palustris* under drought and fire.

There are few studies that have looked at the physiological consequences of drought and fire, and their interaction at known fire radiative energies doses (FRED), the energy released by fire per area (Smith et al. 2017, Partelli-Feltrin et al. 2020, 2021, Ruswick et al. 2021). The most heavily studied, *Pinus ponderosa* Doug. C, a western US species dependent upon frequent fire regimes can survive low to intermediate fire intensity at a well-watered state, but under drought it is likely to die (Steady et al. 2019, Partelli-Feltrin et al. 2020, 2021). We must better understand how fire radiative energy doses and drought will change flammability, plant mortality and survival under changing land use practices and climate change (Jolly and Johnson 2018).

Study Purpose

Seedling responses to fire energy doses are generally unknown, especially for trees of the southeast United States (Ruswick et al. 2021). *P. palustris* is an ideal species to investigate seedling response to fire as it not only tolerant of fire, but dependent upon it as well for regeneration and growth (Wahlenberg 1946). Within this study we aim to understand what energy dose it takes to top kill (kill all needles but cause resprouting from buds) or even completely kill *P. palustris* and how does hydraulic drought impact potential mortality and recovery. We hypothesize that our highest fuel loads will completely kill longleaf, but the combined effects of drought and fire will kill *P. palustris* at lower fuel loads. We hope to understand if higher burn energy doses impede a seedlings ability to regrow as quickly as unburned or less intensely burned seedlings. Lastly, we aim to understand the time scale that recovery from fire becomes apparent. This study aims to better understand fire energy doses and hydraulic soil drought on *P. palustris* seedling mortality.

# CHAPTER 2

# MATERIALS AND METHODS

## Plant Material and Growth Conditions

*Pinus palustris* (Mill.) grass stage seedlings were acquired from a state nursery (Georgia Forestry Commission, Byromville, GA, USA) from open pollinated 1-0 seedstock in January of 2020 and stored in a cooler at 4 °C for approximately one week before being planted into 10 L round plastic pots. A 5 cm layer of playground sand followed by 30 cm commercially available sand was placed into each pot as a growing medium. Plastic mesh was placed at the bottom of each pot to prevent potting sand mixture from spilling through drainage holes while watering. Plants were fertilized with 20-10-20 liquid fertilizer (J.R. Peters, Inc., Allentown, PA, USA) at 75 ppm of nitrogen equivalent once to prevent transplant shock, and then at 200 ppm equivalent thereafter approximately every 12 weeks. Seedlings were grown in a glasshouse for the following year. In March 2021, seedlings were placed in two Conviron BDW40 (Controlled Environments LTD., Winnipeg, MB, CA) growth chambers on 14/10-hour day/night cycle with 800 μmol $m^{-2}$ $s^{-1}$ of photosynthetically active radiation. Temperatures were set to 25 °C during the day and 18 °C at night and relative humidity was set at 50% during the day and 85% at night. Chamber [$CO_2$] was not controlled but averaged 445 ppm ± 0.36 SE (Table 1).

Drought and Fire Treatments

We used an incomplete factorial design with eight treatment groups to test the interaction of fire intensity (3 levels of fuel loading representing a relative scale of low (L), medium (M), and high (H) fire intensities in addition to controls that did not receive a burn (C and DD) and drought (two levels; well-watered control (W) and drought (D)) on survival of *P. palustris* grass stage seedlings (Table 2). The drought treatment seedlings were not watered for 14 days prior to experimental burn, whereas well-watered controls were watered at least two times per week throughout the entire experiment. Fire intensity treatments consisted of three fuel amounts of 0.24 kg $m^{-2}$, 0.49 kg $m^{-2}$, and 0.99 kg $m^{-2}$ (Table 2). The lowest fuel load was based on average pine needle fuel loading for managed longleaf stands throughout the Southeast United States (Wiggers et al. 2013).The control group (three seedlings) was well watered throughout the experiment and not burned and used as comparison for greenness. A dry down curve was performed on five seedlings and was used in developing relationship between pre-dawn water potential and leaf level transpiration (described below). Preliminary data suggested (data not shown) and dry down data confirmed (described and analyzed below) that drought alone would not cause whole plant mortality, so drought only treatment groups were not implemented (resulting in an incomplete factorial design) as to increase sample size of fire and drought treatment groups.

Eight experimental burns took place across two days at the Athens Prescribed Fire Lab, part of the United States Forest Service Southern Research Station. The low and medium intensity burns were completed on May 13th, 2021 with the highest intensity burns taking place on July 29th, 2021.We built a 2.44 m by 2.44 m platform using plywood elevated on lumber supports with holes cut into the plywood to place ten seedlings at a time (Figure 1) (Ruswick et al. 2021). Seedling pot rims were cut to be flush with the soil surface and plywood platform. We dried bagged longleaf fuel (Vigoro, Atlanta, GA, UGA) at 105 °C for at least 24 hours prior to each of the burns and live fuel moisture averaged 5.8% across all burns. Longleaf fuel was also sorted manually to remove leaves, sticks and cones. We spread longleaf fuel by hand into a level bed across the platform prior to each burn.

We ignited a head fire with a propane torch which was exposed to a wind of 1.5 m $s^{-1}$ produced by fans to produce a uniform flame front. Each fire took approximately one to two minutes to move across the platform and consume the fuel, with the higher intensity burns taking longer than the less intense burns. A FLIR A655 camera (Teledyne FLIR LLC., Wilsonville, OR, USA) pointing down toward the burn platform was used to capture temperature, and radiative power of each fire on a 640x480 pixel grid at one frame per second.

Pre-dawn leaf water potential ($\Psi_{pd}$) was measured using a pressure chamber (Model 600D, PMS Instruments, Corvallis, OR, USA) on the day of the burn on all droughted seedlings and a subset of 3 seedlings per growth chamber per well-watered group (Scholander et al. 1965). We also collected approximately 1 gram of fresh needles from all droughted seedlings and a subset of well-watered seedlings minutes before burning to test potential differences in Live Fuel Moisture (LFM), a metric related to plant water status and flammability (Pivovaroff et al. 2019). LFM was calculated using Equation 1:

$$\% \, Live \, Fuel \, moisture = \frac{Fresh \, sample \, mass - Dry \, sample \, mass}{Dry \, sample \, mass} x100 \quad \text{Equation 1:}$$

Plant Imaging and Tracking of Recovery

We took RGB images using an iPhone 8 (Apple Inc. Cupertino, CA, USA) of all seedlings at various timepoints, representing pre-burn, day after burn, and then periodically after the burn to track regrowth of foliage. Seedlings that were not burned were imaged on the same days for comparison. All seedlings were placed in front of a white fabric background at 1.5 m from the tripod affixed camera within a BW40 growth chamber during daytime light conditions. The reflective walls within the chambers allowed seedlings to be illuminated from all sides to reduce shadows. Images taken more than two days after the second burn were done at the glasshouse on sunny days at the above distance and in front of the white background. Images taken with the growth chamber appeared very warm due to bulb temperature, so image color temperature was adjusted to 3000K to appear similar to those at the greenhouse.

Plant Conductance Measures

We calculated whole plant conductance by measuring $\Psi_{pd}$, mid-day water potential ($\Psi_{md}$), and changes in pot mass (i.e., water lost via transpiration) within growth chambers on all droughted seedlings and a subset of well-watered seedlings at the beginning of drought and the day or two days before burn. $\Psi_{pd}$ was measured before lights turned on within growth chambers. Between 9:00 and 10:00 seedlings on were placed on balances (Ohaus EX35001, Corp. Parsippany, NJ, USA) to measure initial mass ± 0.1g, placed back into the growth chamber, and then final mass measured again approximately two hours later. Stopwatches were used to calculate time between mass measurements for each seedling. $\Psi_{md}$ was measured at the time of the final mass measurement. During transpiration measurements, 6 mil thick plastic was placed on the top of the soil in seedling posts to prevent soil evaporation. Whole plant conductance was then calculated using Equation 2 (Tsuda and Tyree 2000):

$$K = \frac{Inital\ mass\ (g) - Final\ mass\ (g)}{\Psi pd - \Psi md * \text{elapsed time (s)}} \qquad \text{Equation 2:}$$

After experimental burns, eight seedlings that were never burned or droughted were used to construct a dry down transpiration curve within the glasshouse beginning on September 24th, 2021. Three seedlings were used as controls and watered regularly while five were droughted. All measurements were performed once before water was withheld and at least twice per week thereafter until plants reached a $\Psi_{pd}$ of -2.5 MPa at which leaf level transpiration had completely stopped. Seedlings were rewatered and re- measured one week and two weeks after cessation of drought. Leaf level gas exchange was measured using a LI-6800 infra-red gas analyzer (LICOR Biosciences, Lincoln, NE, USA) at ambient temperature and humidity but under a constant light level of 800 $\mu mol^{-1}$ $m^{-2}$ $s^{-1}$ and under 415ppm of [$CO_2$]. For each measurement two fascicles, approximately 5-6 needles, were placed into the cuvette and allowed to equilibrate for approximately 1-2 minutes before being logged. Needles within the chamber were then cut and corrected for leaf area using a portable scanner (Canon LiDE220, Ōta, Tokyo, Japan) and ImageJ software (Abramoff et al. 2004). The LI-6800 IRGAs were matched using the auto match function between every measurement. Pre-dawn (before 07:00, $\Psi_{pd}$) and mid-day (11:00-12:30, $\Psi_{md}$) leaf water potentials were measured on the same day as leaf level transpiration using a pressure chamber. $\Psi_{md}$ were taken on fascicles adjacent to those being measured for gas exchange.

## Statistical Analyses

A combination of ResearchIR (Teledyne FLIR LLC., Wilsonville, OR, USA) and, Python were used to analyze infrared images using the Stefan-Boltzmann Equation (O'Brien et al. 2016, Ruswick et al. 2021). Total energy release was integrated for the duration of each burn and corrected for the size of the burn area. We also aggregated pixels around each individual pot ($646 cm^2$) to calculate FRED for each seedling. FRED, opposed to measures of fuel or maximum temperature, can be used to compare species of different fuel loads, moistures and burning environments as to better understand plant response to fire energy. All statistical analyses were performed in program R version 4.0.2 using the 'Tidyverse' and 'cowplot' suite of packages (Wilke 2019, Wickham et al. 2019, R Core Team 2020). To synthesize data from previous studies testing the interaction of FRED probability of mortality, we replotted data along the same axis (Smith et al. 2017, Steady et al. 2019, Partelli-Feltrin et al. 2020).

CHAPTER 3

RESULTS

Drought Treatments and Live Fuel Moisture

At time of the first burn, seedling diameter at soil level was 29.10 mm ± 0.26 (mean ± SE), plant height was 6.58 cm ± 0.77) for all seedlings. $\Psi_{pd}$ were statistically different between droughted and watered control groups on the day of burn (Students T test, n = 51, t= 16.23, P= >0.001). However, there were no differences (α = 0.05) in water potential between the different droughted groups or between the different well-watered groups on the day of burn (Figure 2). Live Fuel Moisture (LFM) of droughted and well-watered seedling needle foliage was not statistically different immediately prior to each burn (n = 49, t = 0.44, P= 0.65, Figure 3).

Fire Radiative Energy Doses and Mortality

FRED released by each fuel loading treatment ranged from 0.24 MJ $m^{-2}$ to 2.39 MJ $m^{-2}$ and are summarized in table within Table 2 and Figure 4. While fuel loading of the high fire treatment (H and HD at 0.99 kg $m^{-2}$) was double that of the medium fire treatment (M and MD at 0.49 kg $m^{-2}$), FRED was similar (Figure 4). A timeseries of images representing all seedlings outcomes (alive, resprout, and dead) and combination of fire intensities and drought conditions at time of burn are represented within Figure 5 and 6. Our fire intensity treatments only killed one seedling and caused two seedlings to resprout (Figure 5 - Row 1 and 2, Figure 7). Control seedling images were taken at the same points in time (Figure 5 - Row 3). Well-watered seedlings that received a low intensity burn still had green needles near the apical bud approximately 1 week after burn (Figure 6 - Row 3). Many seedlings that had moderate to severe drought (indicated by $\Psi_{pd}$ on day of burn) and high intensity fires appeared completely brown after fire but flushed new needles within a month (Figure 6 - Row 1 and 2). The seedlings that resprouted or died experienced fire intensity of at least 1.6 MJ $M^{-2}$ and a $\Psi_{pd}$ of at least -1.8 MPa the morning of the burn (Figure 7). The dry down curve of unburned seedlings showed that seedlings had halted mid-day leaf level transpiration and carbon assimilation by a $\Psi_{pd}$ of -0.7 MPa (Figure 8 A & C), while transpiration halted at a $\Psi_{md\ of}$ -1.2 MPa (Figure 8 B.). Two weeks following rewatering, physiological measures of plant status had recovered compared to controls on the same day, those measures being transpiration ($n = 7$, $t = 2.47$, $P= 0.143$), carbon assimilation ($n = 7$, $t = 1$, $P= 0.356$), and $\Psi_{pd}$ ($n = 7$, $t = 2.099$, $P= 0.198$, Figure 8 A. – C.). Whole plant transpiration leading up to experimental burns had also stopped by -0.7 MPa (Figure 8 D.)

Comparison of FRED

As a comparison of this study to others we visualized the interaction of FRED and probability of mortality for droughted and watered conifer seedlings (Figure 9). Other studies (Smith et al. 2017, Steady et al. 2019, Partelli-Feltrin et al. 2020) observed complete seedling mortality in droughted and well-watered seedlings by 1.5 MJ $m^{-2}$, while this study had no mortality for well-watered seedlings and one seedling death within the drought group at 1.5 MJ $m^{-2}$. All droughted seedlings were binned together at each FRED level for this studies data, although actual $\Psi_{pd}$ varied at the time of burn.

# CHAPTER 4

# DISCUSSION AND CONCLUSIONS

## Tracking Mortality and LFM

While fuel loading of the high fuel loading treatment was twice that of the medium treatment and flame height was visually larger, there was no statistical increase in FRED (Figure 1 B. & C, Figure 4). A lack of increase in FRED for the high group could be due to increased packing of needles on each other due to broken needles which could limit oxygen during the burns. Fuel combustion may have been lower in high fuel loading treatment, relative to the others treatment but was not measured.

The only seedling that died (Figure 5, Row 2) had the most extreme drought response of any seedling burned (Figure 8, Triangular point). One week after burn, foliage was brown, but one-month and two-months post burn foliage appeared red (Figure 5, Row 2). Other seedlings that recovered from fire initially appeared brown, but quickly recovered with new needle growth based around the apical bud (Figure 6, Row 3). These images indicate that the presence of brown scorched foliage alone cannot be used to predict mortality of *P. Palustris* seedlings, but red scorched foliage may indicate prior mortality.

## Hydraulic Stress at Time of Burn

The drought treatments prior to burns were effective at creating a range of $\Psi_{pd}$ at time of burn and not differing between groups (Figure 2). The dry down curve showed that this population of *P. palustris* halted gas exchange at -0.7 MPa $\Psi_{pd}$. Two weeks after rewatering of dry down seedlings, gas exchange had completely recovered (Figure 8). Three of the five dry down seedlings lost needles towards the bottom of the stem after rewatering, but otherwise recovered. Leaf transpiration at time of burn for droughted seedlings had likely stopped for almost all the droughted seedlings (Figure 7, red dashed vertical line). It is likely that there was some hydraulic stress at the time of burn for our droughted samples as seedlings within our dry down curve lost needles after rewatering. The hydraulic stress was likely limited to the rhizosphere and recovered relatively quickly as gas exchange values were not statistically different than controls two weeks after rewatering (Figure 8). Under field conditions, *P. palustris* that has had prior drought and dropped needles, may not only increase future fire severity due to additional dry fuel, but may also have less protection of the apical bud.

Grass stage *P. palustris* seedlings in sandy soil were able to adjust turgor loss point depending on season/precipitation. Turgor loss point at that site varied between -1.3 and -2.28 MPa over the course of two years (Harmon et al. 2020). Within a population of 15 California chapparal species changes in LFM were very small and unlikely to impact flammability until after the turgor loss point during drought (Pivovaroff et al. 2019). It is likely that our seedlings were near the turgor loss point at time of burn based on seedling data from the same species, providing further explanation as to why there was no difference in LFM at time of burn between droughted and well-watered seedlings.

Resprouting and Regrowth Following Fire

Two seedlings utilized buds at the root collar to sprout approximately one month following fire (Figure 5 row 2). One seedling was planted high with the root collar above the soil level, while the other's root collar was just below the soil level. In a study of another southern pine species, *P. echinata*, seedlings resprouted following fire when dormant buds are below soil due to a strong morphological basal crook, but not when planted high and buds are exposed. The same study found that *P. echinata* x *taeda* hybrids do not resprout following fire even with a weak basal crook (Bradley et al. 2016). Within *P. palustris*, exposed root collars and smaller seedlings had higher rates of mortality, while small seedlings depending on resprouting for survival (Jin et al. 2019). Our sand based potting soil may have further increased fire intensity as sand content increases soil thermal conductivity, while water saturation decreases soil thermal conductivity (Nikoosokhan et al. 2016). We expect that longleaf found in less sandy soil textures would receive less sensible heat through the soil indicating less damage at the root collar for a given fire intensity. *P. palustris'* ability to recover from fire, without resprouting is dependent on apical bud protection, which may be unlikely at high levels of drought and fire intensity (Knapp et al. 2018, Brethauer et al. 2021).

Longleaf are Resistant to Fire Related Mortality

While this experiment showed that extreme drought and fire can kill longleaf pine seedlings (see dead and resprout points in Figure 7), we were unable to determine the threshold of fire intensity and level of drought at which this occurs. Future investigation must use a greater number of plants, drought trees even further and implement even more intense fire treatments to properly quantify the conditions that push *P. palustris* to mortality. However, our results are encouraging for restoration efforts for *P. palustris*. The severity of our experimental drought is likely beyond the point that which safe prescribed fire would occur (Addington et al. 2015). Even our highest fuel load was only able to kill one seedling and cause two to resprout, further showing that fuel loads on managed stands are unlikely to die from fire even under extreme drought without the presence of coarse woody debris to further increase intensity.

Comparing Species Response to Fire and Drought

Understanding how species respond to varying levels of fire intensity are of utmost importance under climate change because fire intensity is likely to increase worldwide (Flannigan et al. 2000). Synthesizing previous studies results here, we can show that *P. palustris* seedlings are resilient to increased levels of experimental fire when compared to several western United States conifer species (Figure 9). Related studies of how FRED and drought influences mortality show that seedling populations of P. *ponderosa*, *P. contorta* and *L. occidentalis* have complete mortality at 1.5MJ $m^{-2}$ regardless of plant water status (Smith et al. 2017, Steady et al. 2019, Partelli-Feltrin et al. 2020). However, in the current study, mortality was only observed at FRED >1.5 MJ $m^{-2}$ indicating that *P. palustris* seedlings have different anatomical, morphological, or physiological differences, compared to the reported western species, that convey strong protection from fires.

Conclusions

While this study was unable to determine the FRED that is probable to kill *P. palustris,* morality may begin occurring around 1.5 MJ $m^{-2}$ when under hydraulic drought. The threshold for mortality in *P. palustris* is very high compared to western United States conifer species. Drought did not limit seedlings' ability to respond and regrow in the two months following fire. Long-term measurements of plant size following this experiment may elucidate the interaction of fire and drought intensity in long term seedling growth. Further measurements will also determine if resprouting trees are able to grow as quickly those that were just scorched. After *P. palustris* is burned it may be difficult to determine mortality until up to one month after a burn. This study adds to the growing knowledge of plant response to fire intensity, in addition to better understanding *P. palustris'* response to fire and drought.

**Table 1.** Table of environmental conditions while plants were in growth chambers. Each row corresponds to one of the chambers at either day or night. Temperature (Temp) was measured in °C, Relative Humidity (RH) was measured in percent, Carbon dioxide concentration [$CO_2$] was measured in ppm, photosynthetically active radiation (PAR) was measured in µmol $m^{-2}$ $s^{-1}$. Each environmental condition within the table has an average ± the Standard Error.

| Chamber | Time | Temp °C | RH (%) | [CO2] (ppm) | PAR (µmol $m^{-2}$ $s^{-1}$) |
|---|---|---|---|---|---|
| 2 | Day | 24.7 ± 0.001 | 52.9 ± 0.01 | 422.8 ± 0.06 | 774.7 ± 0.169 |
| 2 | Night | 17.05 ± 8.2 | 84.5 ± 0.002 | 441.5 ± 0.054 | 13.35 ± 0.004 |
| 3 | Day | 24.8 ± 0.001 | 51.0 ± 0.01 | 451.5 ± 0.06 | 773.6 ± 0.17 |
| 3 | Night | 16.9 ± 4.57 | 83.7 ± 0.007 | 471.4 ± 0.056 | 12.09 ± 0.010 |

**Table 2.** Pre-dawn water potential at time of burn, fuel load, and average seedling Fire Radiative Energy Dosage (FRED).

| Burn Treatment | Sample size | Water potential at time of burn (MPa) | Fuel loading (kg $m^{-2}$) | Average seedling FRED (MJ $m^{-2}$) |
|---|---|---|---|---|
| Control (C) | 3 | No burn | No burn | No burn |
| Dry down curve (DD) | 5 | No burn | No burn | No burn |
| Low fuel load (L) | 9 | -0.23 | 0.24 | 0.33 |
| Low fuel load + drought (LD) | 11 | -1.25 | 0.24 | 0.46 |
| Medium fuel load (M) | 19 | -0.18 | 0.49 | 1.45 |
| Medium fuel load + drought (MD) | 10 | -1.66 | 0.49 | 1.44 |
| High fuel load (H) | 10 | -0.21 | 0.99 | 1.51 |
| High fuel load + drought (HD) | 14 | -1.58 | 0.99 | 1.57 |

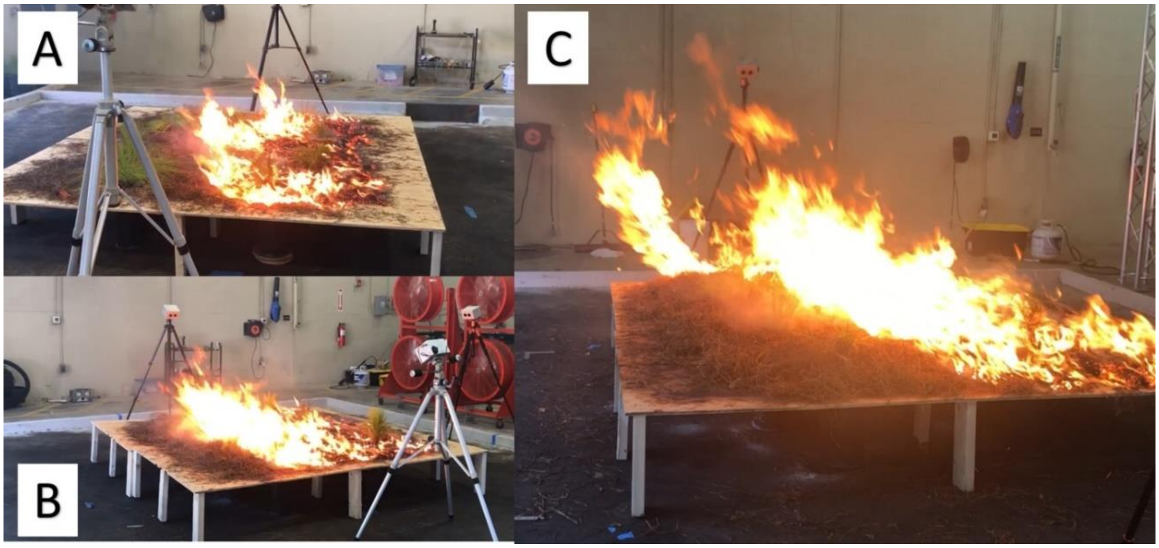


**Figure 1.** Low Medium and High fuel loads while being burned **A.** Burn platform with 0.24 $kg^{-1}$ $m^{-2}$ of fuel, low fuel load (L), mid burn. **B.** Burn Platform with 0.49 $kg^{-1}$ $m^{-2}$ of fuel, medium fuel load (M), mid burn. **C.** Burn platform with 0.99 $kg^{-1}$ $m^{-2}$ of fuel, high fuel load (H), mid burn.

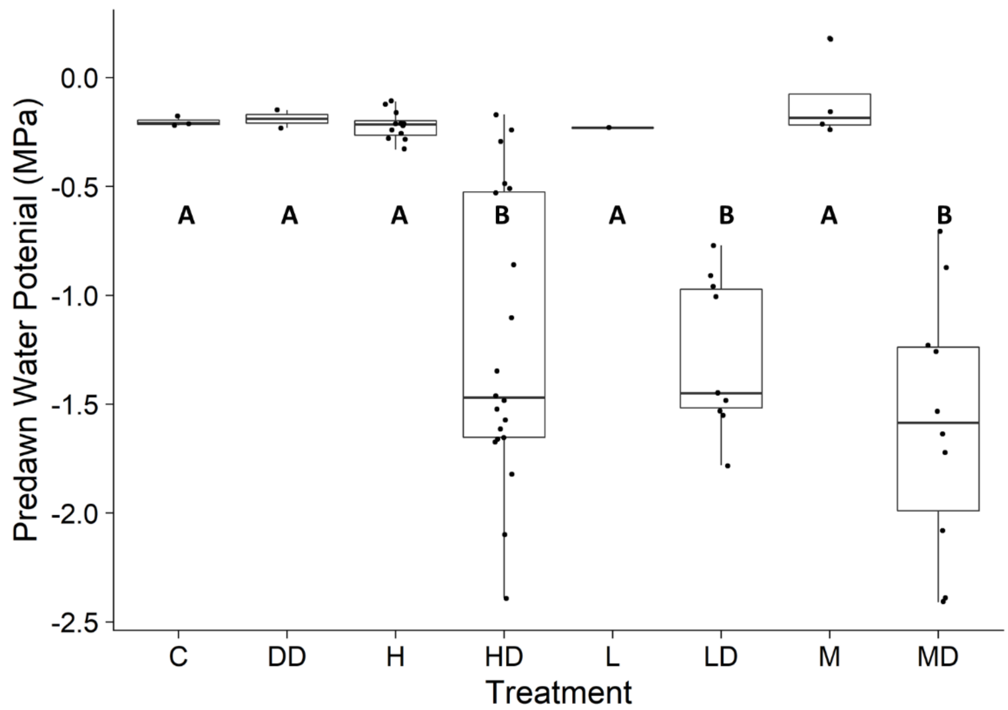


**Figure 2.** Pre-dawn water potentials at time of burn. Letters indicate significance between groups at α <0.05 using TukeyHSD *post hoc* test. Treatment acronyms are control (C), dry down curve (DD), low fuel loading (L), low fuel loading + drought (LD), medium fuel loading (M), medium fuel loading + drought (MD), high fuel loading (H), high fuel loading + drought (HD).

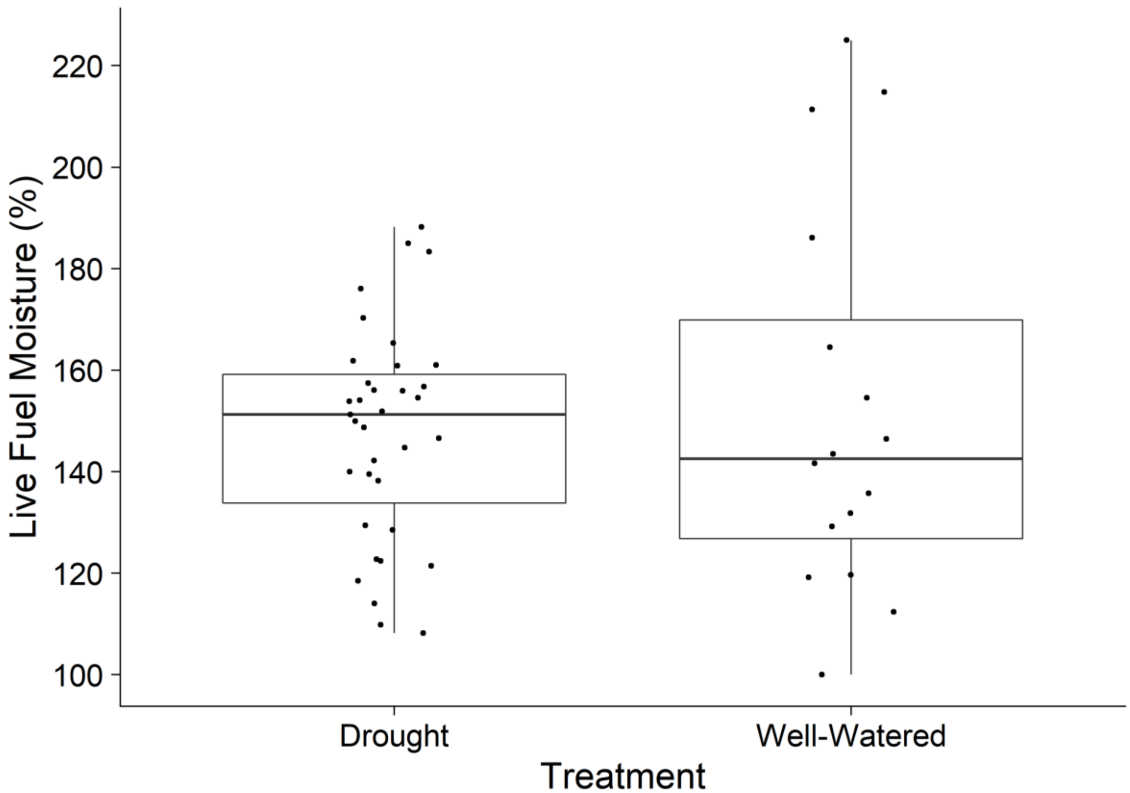


**Figure 3.** Comparison of seedling live fuel moisture of seedlings at time of burn between drought and well-watered treatment groups. Live Fuel Moisture (%) between drought and well-watered seedlings was not statistically different (n = 49, t = 0.44, P= 0.65). The bottom and top of each box represents the 1st and 3rd quartile ranges, with lines extending up and down to 1.5*IQR. Horizontal lines within each box are the median. Horizontal scatter of points within a treatment are jittered as to not appear on top of each other.

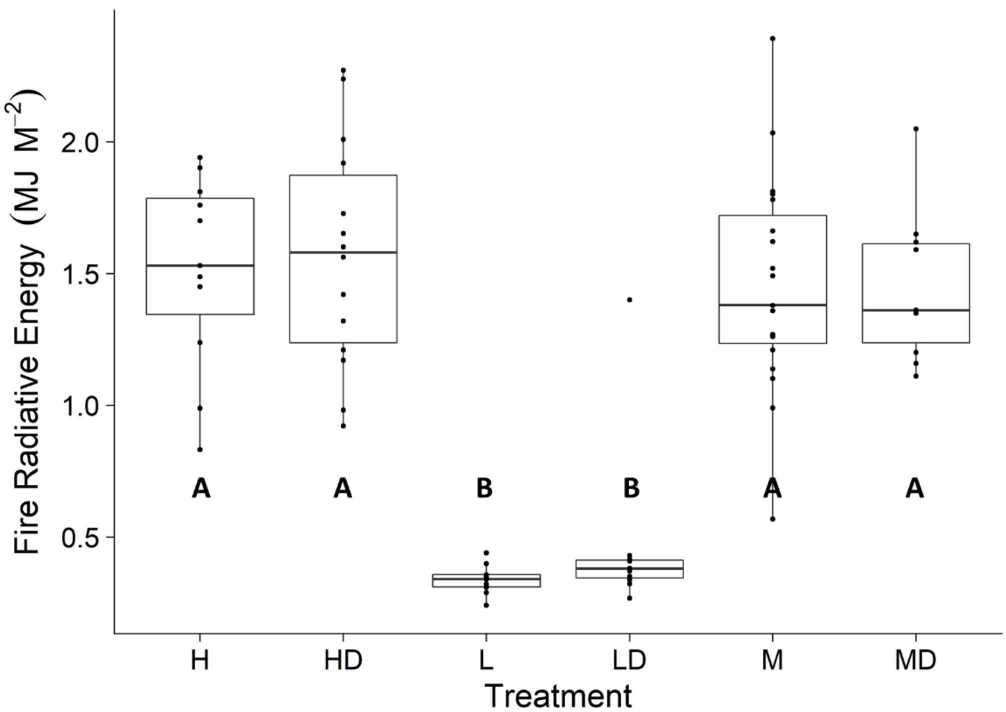


**Figure 4.** Fire radiative energy dose of seedlings by treatment group. The bottom and top of each box represents the 1st and 3rd quartile ranges, with lines extending up and down to 1.5*IQR. Horizontal lines within each box are the median. Treatment acronyms are control (C), dry down curve (DD), low fuel loading (L), low fuel loading + drought (LD), medium fuel loading (M), medium fuel loading + drought (MD), high fuel loading (H), high fuel loading + drought (HD). Letters above treatment (A or B) indicate significant differences ($\alpha$ <0.05) using TukeyHSD *post hoc* test.

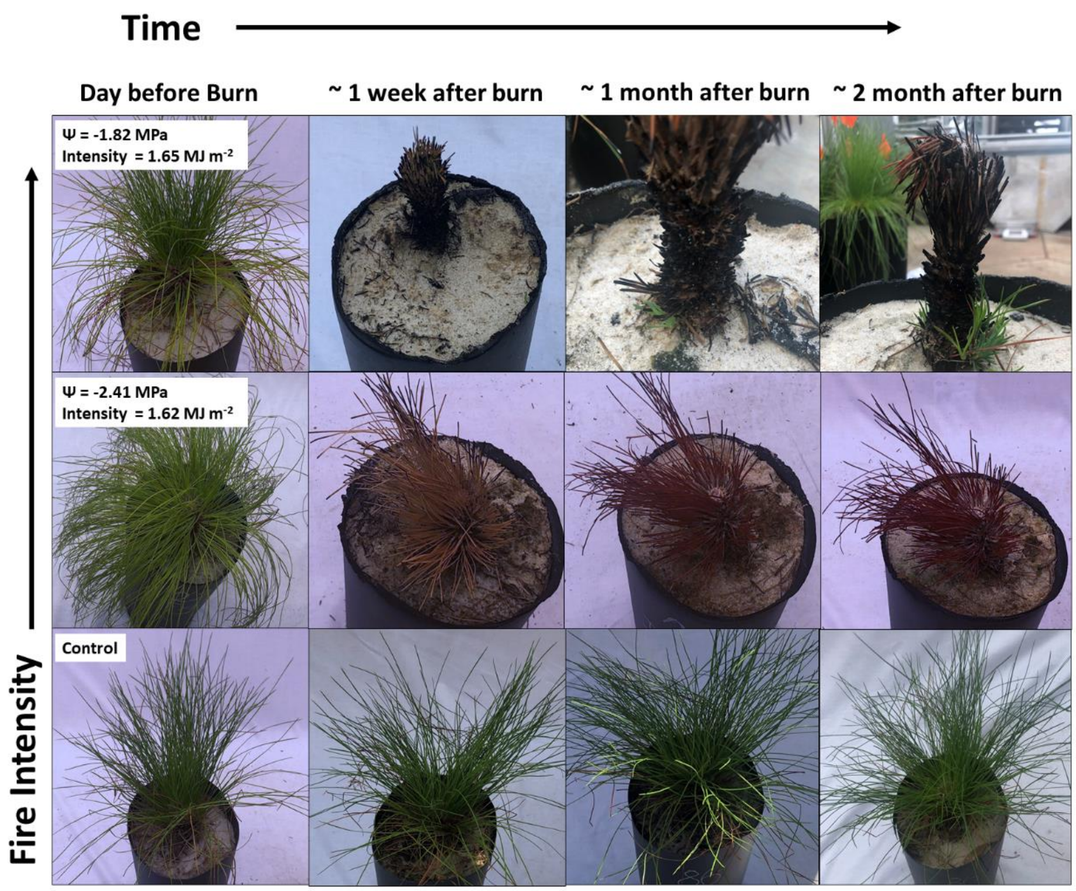


**Figure 5.** Burned and unburned longleaf seedlings over time. Each column represents a point in time relative to when burned with time since burn increasing to the right. Each row is an individual seedling with the pre-dawn water potential on day of burn (Ψ), and individual seedling burn intensity, with intensity between seedlings increasing bottom to top. The first row is one of the two seedlings that resprouted, while the second row is the only seedling that died. The third row is a control seedling with images at the same points in time.

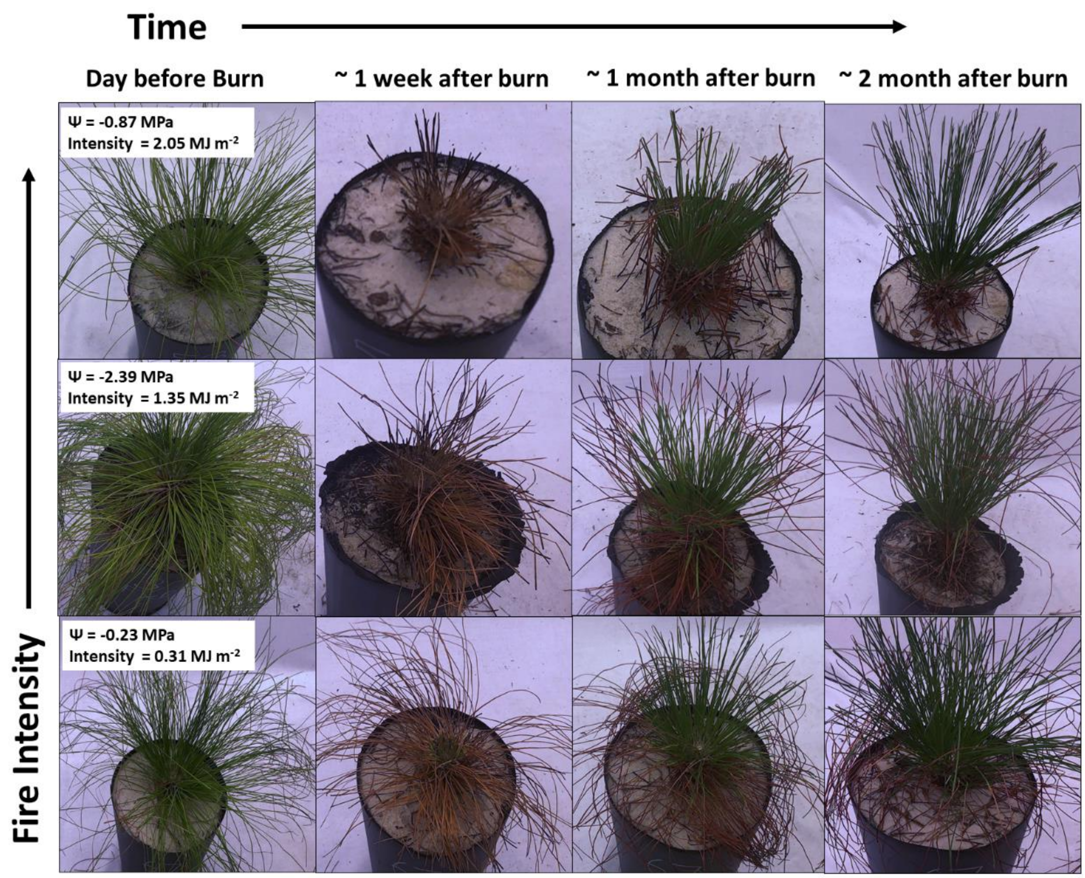


**Figure 6.** Burned and unburned longleaf seedlings over time. Each column represents a point in time relative to when burned with time since burn increasing to the right. Each row is an individual seedling with the pre-dawn water potential on day of burn (Ψ), and individual seedling burn intensity, with intensity between seedlings increasing top to bottom. The first row is of a seedling with moderate drought but very intense fire. The second row is of a very droughted seedling with moderate to severe intensity burn. The last row is a seedling that was well watered and the lowest fire intensity group.

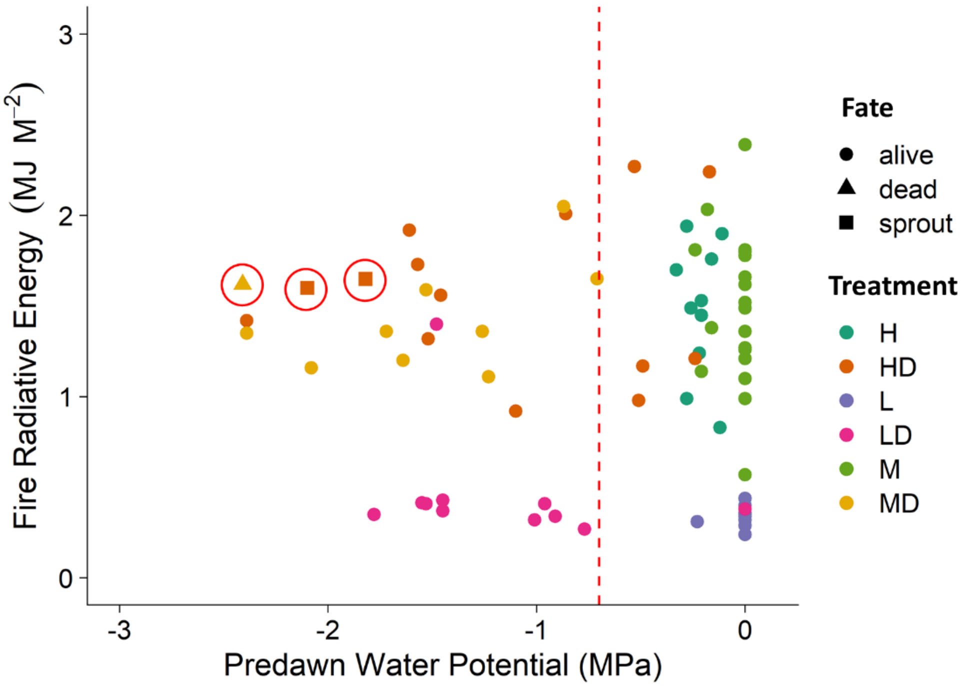


**Figure 7.** Scatter plot of pre-dawn water potential (MPa) and Fire Radiative Energy Dose (FRED, $MJ^{-1}$ $M^{-2}$) at time of burn. Different colors represent each treatment group, and shape represents seedling fate. Points circled in red are those that either sprouted or died. Points with a water potential of 0 were not measured but plotted to show the distribution of FRED for the whole population of burned plants. Treatment acronyms are control (C), dry down curve (DD), low fuel loading (L), low fuel loading + drought (LD), medium fuel loading (M), medium fuel loading + drought (MD), high fuel loading (H), high fuel loading + drought (HD). Vertical dashed red line is the point at which mid-day leaf level transpiration had stopped according to our dry down curve (see Figure 8 A).

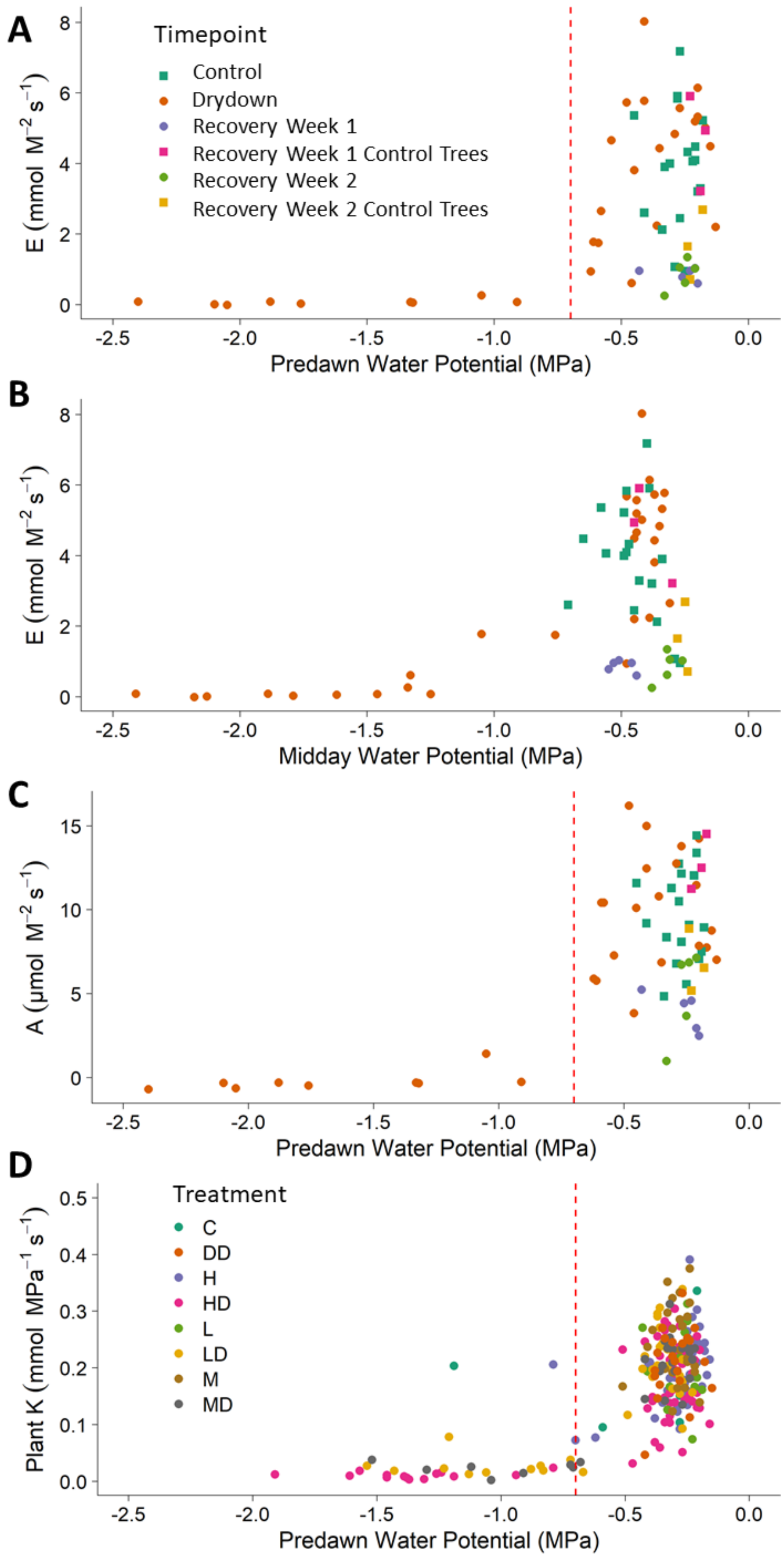
A
Timepoint
Control
Drydown
Recovery Week 1
Recovery Week 1 Control Trees
Recovery Week 2
Recovery Week 2 Control Trees
E (mmol M-2 s-1)
Predawn Water Potential (MPa)
B
E (mmol M-2 s-1)
Midday Water Potential (MPa)
C
A (µmol M-2 s-1)
Predawn Water Potential (MPa)
D
Treatment
C
DD
H
HD
L
LD
M
MD
Plant K (mmol MPa-1 s-1)
Predawn Water Potential (MPa)

**Figure 8. A.** Interaction of predawn water potential (MPa) and leaf level transpiration (E, mmol $M^{-2}$ $s^{-1}$). **B.** Interaction of midday water potential (MPa) and leaf level transpiration (E, mmol $M^{-2}$ $s^{-1}$). **C.** Interaction of predawn water potential (MPa) and leaf level carbon assimilation (A, µmol $M^{-2}$ $s^{-1}$). **D**. Interaction of predawn water potential (MPa) and whole plant conductance (K, mmol $MPa^{-1}$ $s^{-1}$) of trees prior to burn. For panels A-C, Control trees are squares, while dry down and dry drown recovery trees are circles. For panel D, each point color is a different burn treatment, acronyms are control (C), dry down curve (DD), low fuel loading (L), low fuel loading + drought (LD), medium fuel loading (M), medium fuel loading + drought (MD), high fuel loading (H), high fuel loading + drought (HD). Vertical dashed red line is the point at which mid-day leaf level transpiration had stopped during the post burn dry down curve (-0.7MPa).

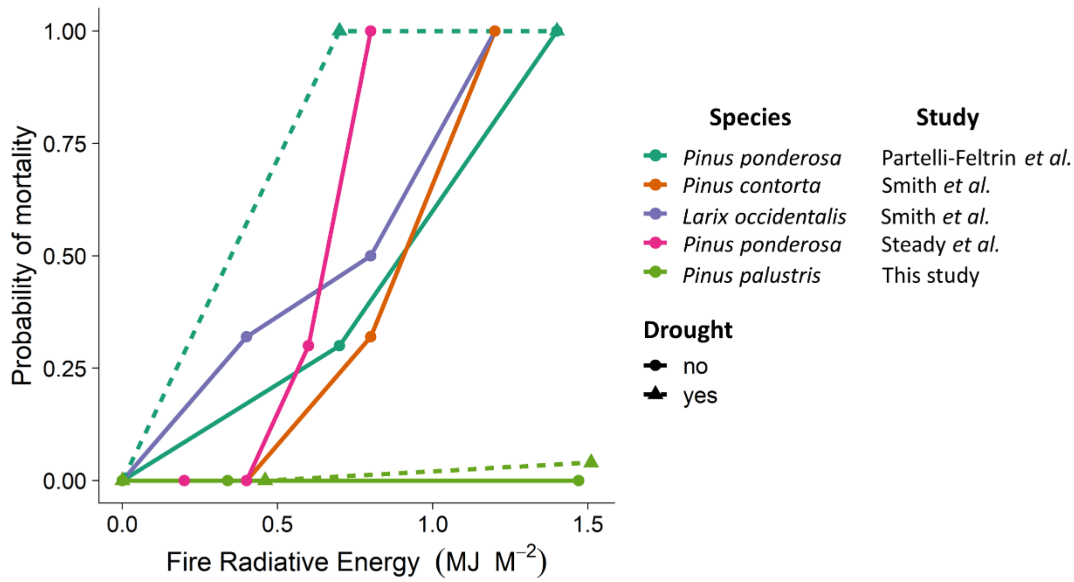


**Figure 9.** Interaction of fire radiative energy dose (FRED) (MJ M$^{-2}$) and probability of mortality for this study and others. Droughted samples have circular points and solid lines while droughted samples have triangular points and dotted lines. Points  from other studies are redrawn from (Smith et al. 2017, Steady et al. 2019, Partelli-Feltrin et al. 2020). Seedlings that resprouted are considered alive for the *P. palustris* data from this study. Medium and high fuel loads points from this study were also combined as FRED was similar. Individual points are aggregated from all droughted or well-watered samples at a given FRED.

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