

Effects of starch-based superabsorbent polymer on survival and physiology of *Pinus ponderosa* seedlings exposed to severe drought stress

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Abstract

Planting nursery-grown trees has become necessary to reach reforestation goals to promote forest recovery; however, these young transplants are vulnerable to drought-related stress and mortality. Land managers may want to consider implementing silvicultural applications, such as amending the soil water holding capacity, to reduce drought stress in plants. We applied a superabsorbent polymer in three different ways as a soil amendment for ponderosa pine seedlings in a greenhouse drought trial. We watered the seedlings for 12 weeks and then initiated a severe drought by completely cutting off water. We measured the effect of the polymer treatment on seedling survival, height relative growth rate, stomatal conductance (g_s), and chlorophyll fluorescence (Φ_{PSII} and F_v/F_m), and compared a null treatment model to test models for each response, using time, treatment, and an interacting time-treatment effect as covariates. We found that the polymer did improve survival probability and median time to death in treatments that distributed the polymer throughout the soil, but was unable to influence the seedlings' drought-avoidant strategy of early stomatal closure and cessation of growth. However, the addition of the polymer may buffer the seedling against photoinhibition, which may increase drought recovery capacity and thus has the potential to increase seedling survival in a reforestation setting. This experiment serves as a proof of concept that water-retaining soil amendments may be beneficial for conifer seedlings in severe drought, but more research must be done to understand the efficacy of using these in a reforestation project.

1. Introduction

A key strategy in supporting forest recovery is planting nursery-grown seedlings shortly after major wildfires, thus reducing the reliance on proximate seed sources for natural regeneration (Keyser and Westerling 2017; White and Long 2018; North et al. 2019; Stevens-Rumann et al. 2022). Coordinating planting with site preparation can assist with forest recovery, but survival rates of these transplanted seedlings are limited by water availability and competition from non-conifer species that are adapted to drier conditions and a shorter growing season (Zhang et al. 2008; Zhang et al. 2013; Dobrowski et al. 2015).

Ponderosa pine (*Pinus ponderosa* Laws.) is the most ecologically and economically important pine species in the Western U.S (Oliver and Ryker 1990). Ponderosa ecosystems are adapted to frequent, low-mixed severity wildfire and have experienced extensive losses to high-severity wildfire (Woolman et al., 2022) following decades of historical fire suppression (Reynolds et al., 2013). The natural reforestation of large burned areas of ponderosa pine is commonly unsuccessful due to lack of seed sources and aggressive shrub and hardwood competition (Haffey et al., 2018). Further, Ponderosa pine is only moderately drought-tolerant relative to other regional pine species, which manifests in early stomatal closure in periods of mild moisture stress and reduces their ability for growth and establishment (Barton and Teeri, 1993; Martínez-Ferri et al. 2000). Research on this species has highlighted the critical role of soil moisture to reforestation project success (López et al. 2008; Ouzts et al., 2015). Research has focused on microsite selection and competing vegetation control as the primary means for improving

seedling moisture status (Hill and Ex 2020; McDonald and Fiddler 2010). In turn, there has been less focus on investigating methods to directly improve the plant-available soil water by altering the physical environment of the planted seedlings, which may offer a novel means to improve success of container-grown seedlings or increase the flexibility of planting operations, such as extending planting season (Butnor et al. 2012).

One solution to ameliorate moisture stress is to amend the soil water-holding capacity locally around the seedling through the addition of superabsorbent polymers (SAPs; Dubrovskii et al. 1990). While multiple variations of SAPs exist, typical composition consists of hydrophilic polymers that form chemical or physical cross linkages to hold water through Van der Waals forces and hydrogen bonds, which provide a slow release of water when those bonds are broken (Guilherme et al. 2015). Research into these soil amendments has historically focused on their use in increasing agricultural water productivity, while comparatively few studies quantify their effect on the seedling's resource allocation – a key metric of drought recovery potential – particularly in a reforestation setting (Garbowski et al. 2019; Rowland et al. 2023).

We conducted a greenhouse drought experiment to investigate the following questions:

Q1: Does the application of the superabsorbent polymer prolong seedling survival under moisture stress?

Q2: Does the application of the superabsorbent polymer ameliorate constraints imposed by moisture stress on the growth and physiology of the seedling?

2. Methods

2.1 Study species and experimental design

Seedling characteristics

Our ponderosa pine seedlings were sourced from the USDA 525 Seed Zone in the Western Slope Cascades-Sierra region in California at 1060–1220 meters (3500–4000 feet) elevation, a seed zone that is collected and used for Forest Service reforestation projects. They were grown in Styroblock containers (Stuewe & Sons, Inc.) for 8 months before they were lifted and placed in a 5°C refrigerator for four weeks. Before planting, we selected the 500 highest-quality seedlings from the 860 available. See Online Resource 1 for full criteria of seedling screening.

Experimental Design

Our experiment consisted of a Control group and three treatments (Fig. 1). We used the Koasis™ polymer, developed by Kannar Earth Sciences, Ltd, to examine the interactions between drought and water-retaining soil amendments. This is classified as a natural, biodegradable superabsorbent polymer (SAP), and comes in a dry granular form. We added 5 grams of dry polymer to seedlings using three

application strategies while planting them in 10.16 x 10.16 x 35.56 cm (4 x4 x14 in) plastic pots (Stuewe & Sons, Inc.) with standard potting soil. Five pots of the same treatment were placed in a box. Seedlings were arranged in a checkerboard pattern with 10.16 cm between seedlings.

Around Week 3 of the experiment, we noticed masses of hydrated polymer had fallen out of Deposit treatment pots on the greenhouse floor. We found that this occurred beneath 3 boxes, implying that up to 15% of the Deposit seedlings had less than 5 grams of polymer in them throughout the course of the experiment. It was impossible to determine exactly which seedlings were impacted.

The seedlings were randomly assigned treatments and blocks (which we denoted “cohorts”) within the greenhouse. We rotated the boxes within their cohort around the four tables every three weeks throughout both the watering and drought stages (see Online Resource 2 for specifics on randomization and rotation).

We top-watered the seedlings to saturation every other week for 12 weeks (from early March until early June) to simulate the wet season in which the polymer would hydrate in the field. Over the summer, the humidity in the greenhouse was between 15–20% and midday temperatures ranged from 35.0-39.5°C, comparable outside temperature stress. Forty percent shade cloths strung on the roof of the greenhouse protected seedlings from direct sunlight exposure.

Seedlings that died during the watering period were removed. The number of seedlings in each group during the Week 0 measurement period became the basis for 100% survival (Table 2). We measured seedling physiology – stomatal conductance (g_s) and chlorophyll fluorescence ($\Phi PSII$ and F_v/F_m) – on a random subset of 25 seedlings per treatment group, while growth and survival were censused over the entire population.

Table 1
Specifications for each group. This includes the name of the treatment, the associated box numbers for that treatment, the number of seedlings alive in each treatment at Week 0.

Treatment	Box #s	# alive at Week 0	# in Subset	% of Treatment in Subset
Control	0–40	196	25	12.8
Deposit	41–60	99	25	25.3
Rolling	61–80	99	25	25.3
Mixed	81–99	96	25	26.0
Total		490	100	

Table 2

Responses table including description and interpretation. The units, test population (census vs. subset), frequency of measurement, and the question the response is addressing is also provided.

Name	Question	Description	Interpretation	Units	Census vs. Subset	Frequency
Survival	Q1	Denoting whether a seedling is alive ("0") or dead ("1")	Higher proportions of 0 compared to 1 = higher survival	%	Census	Weekly
HRGR	Q2	Height relative growth rate	Higher values = higher growth rate, less indication of drought stress	$\text{cm cm}^{-1} \text{ week}^{-1}$	Census	Bi-weekly
ΦPSII	Q2	Operating efficiency of Photosystem II	Higher ratios = higher PSII functionality, less indication of drought stress	Unitless	Subset	Weekly
F_v/F_m	Q2	Maximal efficiency of Photosystem II	Higher ratios = higher PSII functionality, less indication of drought stress	Unitless	Subset	Weekly
g_s	Q2	Stomatal conductance	Higher values = higher rate of $\text{CO}_2\text{-H}_2\text{O}$ gas exchange	$\text{mol m}^{-2} \text{ s}^{-1}$	Subset	Weekly

2.2. Q1: Does the polymer prolong seedling survival under drought?

We censused the seedlings weekly for deaths. Once a seedling experienced death, we ceased the other growth and physiological measurements. We defined "dead" seedlings as those that did not have a green cambium and could not be bent gently without breaking. If there was some bend to the stem, that indicated that there was still living material. If it was brittle and felt like it was about to snap, we considered the seedling dead. The experiment ended once all the seedlings died.

2.3. Q2: Does the application of the polymer ameliorate constraints in growth and physiology during drought?

Measurements of growth

We took bi-weekly measurements of height (in cm) and basal diameter (in mm) from a standard marked point 2 cm above the starting soil line of each seedling. For diameter, we made two perpendicular measurements around this point and averaged them.

As a plant stem is unlikely to grow laterally during periods of severe resource limitation, we decided to only analyze height as our growth response (Lanner 1985). We excluded any measurements that showed negative growth, and then calculated the height relative growth rate (HRGR) using the following equation:

$$\frac{\ln(\text{height})_2 - \ln(\text{height})_1}{t_2 - t_1}$$

1

Where t represents the week the measurement was taken, and therefore t_1 is the week before t_2 .

Stomatal conductance

We measured weekly stomatal conductance (g_s) *in vivo* during the midday hours of 1100–1400 using a Li-Cor LI-600N steady-state Porometer/Fluorometer with an open gas-exchange chamber (Li-COR, Lincoln, NE, U.S.A). We marked a full-size, south-facing needle on the top whorl closest to the apical bud of the subset seedlings to measure conductivity in light-saturated conditions. We made a marker point on the chosen leaf, directly above the fascicle sheath, where it would not obstruct our Li-Cor measurements. When we rotated the boxes of seedlings around the greenhouse, we ensured that the selected needle always pointed towards the south. We used the same needle for all porometer and fluorometer measurements each week unless the leaf was no longer viable. If the selected needle was shed or browned significantly over the course of the drought, we took the measurement from the topmost green needle. All seedlings in the study dropped their selected needle at some point, though we did not note which week for individual seedlings.

Chlorophyll a fluorescence

Chlorophyll fluorescence provides an indirect way of measuring photosynthetic efficiency through quantifying the yield of photochemical reactions within Photosystem II (PSII), which in effect measures the integrity of the thylakoid membrane (Genty et al. 1989; López et al. 2008). We measured the quantum yield of fluorescence for seedlings in both light- and dark-adapted states, representing Φ_{PSII} and F_v/F_m , respectively. We took these measurements using the Pulse-Amplitude Modulated (PAM) fluorometer portion of the LI-600N (Li-COR, Lincoln, NE, U.S.A).

Φ_{PSII} measures the proportion of energy absorbed by PSII receptors in light-adapted needles that is converted into linear electron flow for biochemical reactions (Martínez-Ferri et al. 2000). Φ_{PSII} and g_s were measured simultaneously using the same selected needle. Using the PAR sensor on the Li-COR, we kept the Q_{amb} between 450 and 550 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. As pines have lower light saturation

thresholds, and the seedlings were adapted to these light conditions throughout the watering and drought period, we do not anticipate that the lack of direct sunlight influenced our physiological measurements (Leverenz 1987; Awada et al. 2003). We avoided measuring on days without full sun. If the position of the seedling did not have a Q_{amb} in the desired range, we moved the seedling and adapted it to the desired light range for 30 minutes before taking the measurement.

F_v/F_m describes maximum photochemical efficiency, which is measured by a light-saturating pulse on dark-adapted needles and is a proxy for overall PSII functionality (Genty et al. 1989; Wang et al. 2018). We measured this between 0600–0800 h, on the same day as the other porometer/fluorometer measurements. We placed the subset seedlings into three incubators with $0 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of light for at least 30 minutes prior to measurement. We strung a shade cloth over the incubator doors to block ambient light when measuring with the PAM fluorometer.

2.4: Statistical analysis

We used the hypotheses outlined in Table 3 to compare the null model to test models. For Q1, we incorporated two approaches to analyze whether the polymer prolonged survival in our seedlings: creating mixed-effects Cox proportional hazards models and visualizing survival probability through the Kaplan-Meier method. Our first approach used the *coxme* package in *R* (Ripatti and Palmgren, Biometrics 2002; Therneau 2024) to model survival using a null model with just random effects as covariates and an alternative model with the added treatment effect (Table 3). We nested boxes within cohorts for our random effects to account for heterogeneity in our greenhouse. We then used the concordance function in the performance package (Lüdecke et al. 2021) to compare the fit of the models using the concordance statistic (c-index; Hosmer et al. 2013).

In our second approach, we visualized the survival probabilities of each treatment using a Kaplan-Meier curve (Kaplan and Meier 1958). This method projects the probability of surviving at time t , based on the time to death for the seedlings in each of the treatments. Because our data was non-censored (i.e., none of the seedlings survived beyond the scope of the study), the survival probability within the scope of our study is definitively known. To calculate if the difference between treatment groups is significant, we used a pairwise t -test with a Bonferroni adjustment. We visualized our survival curve using *ggsurvplot* in the survival package in *R* (Therneau and Grambsch 2000) and indicated median time to death for each treatment group.

Table 3

Model description table containing the combination of fixed and random effects and the associated response(s) being investigated for each question.

Question	Response(s)	Model	Varies as a function of:
Q1	Survival	M0	Null model ($\sim$ block + cohort)
Q1	Survival	M1	Treatment + block + cohort
Q2	Height RGR, g_s , Φ PSII, F_v/F_m	M0	Null model ($\sim$ block + cohort)
Q2	Height RGR, g_s , Φ PSII, F_v/F_m	M1	Treatment + block + cohort
Q2	Height RGR, g_s , Φ PSII, F_v/F_m	M2	Time + block + cohort
Q2	Height RGR, g_s , Φ PSII, F_v/F_m	M3	Treatment + time + block + cohort
Q2	Height RGR, g_s , Φ PSII, F_v/F_m	M4	Treatment + time + (treatment*time) + block + cohort

To analyze Q2, we created Generalized Linear Mixed Effects Models for all hypotheses of growth and physiological indicators with time, treatment, and random effects as covariates (Table 3). We utilized the glmmTMB package (Brooks et al. 2017) in R to build these models and run residual diagnostics, and then used the model.sel function in the MuMIn package (Bartoń 2025) to compare the models based on Akaike Information Criterion (AIC). The model that had an $\Delta AICc \geq 6$ from the next-best model was considered the best-supported model.

For the height relative growth rate (HRGR), we used a Tweedie error distribution to accommodate for the abundant zero values recorded in later weeks of the experiment. Zero values were common in our stomatal conductance (g_s) measurements towards the later weeks of the experiment as well, as gas exchange ceases during severe drought stress to conserve water (Faria et al. 1998). To remedy this issue, we truncated our data at Week 5, when more than 50% of our seedlings had a g_s measurement of $0 \text{ mol m}^{-2} \text{ s}^{-1}$. Unlike for g_s , we used all 13 weeks of data and a Gaussian error distribution in our analysis of Φ PSII and F_v/F_m .

After running residual diagnostics, we transformed our responses and fixed effects to meet model assumptions for linearity and homogeneity of variances. We did not have to transform HRGR, but we applied a scalar transformation on the time variable (week). We applied a square-root transformation to g_s and Φ PSII, and then applied a polynomial transformation to the time (linear for Φ PSII and quadratic

for g_s). We transformed F_v/F_m exponentially and then applied a scaling transformation to the time variable.

3. Results

Table 4
Model comparison table of tree performance metrics.

Response	Hypothesis	Terms	LogLik	$\Delta AICc$	C-index/ R^2
Survival	M1	~Treatment + block + cohort	-17290.5	0	0.56
Survival	M0	Null model (~ block + cohort)	-17214.9	42.84	0.50
HRGR	M2	~Time + block + cohort	823.176	0	0.90
HRGR	M0	Null model (~ block + cohort)	442.547	756.24	0.00
g_s	M2	~Time + block + cohort	769.212	0	0.75
g_s	M0	Null model (~ block + cohort)	183.398	1167.52	0.00
$\Phi PSII$	M3	~Treatment + time + block + cohort	581.328	0	0.62
$\Phi PSII$	M0	Null model (~ block + cohort)	155.247	844.05	0.00
F_v/F_m	M4	~Treatment + time + (treatment*time) + block + cohort	945.993	0	0.71
F_v/F_m	M0	Null model (~ block + cohort)	456.64	958.06	0.00

Notes: abbreviations are as follows: *logLik* = log-likelihood, $\Delta AICc$ = change in Akaike’s corrected information criterion, and *c-index/ R^2* provides the c-index of marginal R^2 (fixed effects only) depending on response. Where not part of the model set, the null model is provided for each response to quantify fit relative to plausible models.

3.1. Q1: Seedling Survival

Overall, we found that the polymer was able to extend the survival rate of the seedlings in the Mixed and Rolling treatment, but not in the Deposit treatment, when compared to the Control (Table 4). Our model that included the polymer treatment as a covariate (M1, c-index = 0.56) was better at correctly predicting survival outcomes than the null survival model (M0, $\Delta AICc$ = 42.8, c-index = 0.50). The c-index represents how well the model can predict the outcome of a binary event, with a value of 0.50 indicating that the probability of classifying the outcome correctly is no better than random chance. Thus, a c-index of 0.56 indicates that the treatment-inclusive model is slightly better than random at predicting the outcome of survival.

Through a Pairwise t -test between each of the treatment groups, we found that the Mixed treatment performed significantly better than the Control ($p = 3.3 \times 10^{-11}$, $\alpha = 0.05$), and had a significantly noticeable improvement on survival of all the treatments. Rolling treatment also made a significant improvement, though to a lesser degree than the Mixed treatment, on survival compared to the Control ($p = 0.0292$, $\alpha = 0.05$). Based on this t -test, the Deposit treatment did not differ from the Control treatment ($p = 1.000$), and the survival plot shows that the Deposit treatment may have performed worse than the Control (Fig. 2).

We found that the Mixed treatment prolonged the median survival time (time to 50% death) by approximately 1.5 weeks compared to the Control. Median survival time for the Mixed treatment occurred at 12 weeks of drought, while the median survival time for the Control and Deposit treatments occurred at 10.5 weeks. The median survival time for the Rolling treatment was 11 weeks after the initiation of drought.

3.2. Q2: Seedling Growth and Physiology

We found that the patterns of height relative growth rate (HRGR) observed in this study were primarily determined by time. Time was the sole main effect in the only plausible models for HRGR (M2, Table 4). This model showed robust goodness-of-fit for HRGR patterns, with an R^2 value of 0.90 and strong support relative to M0 ($\Delta AICc = 756.2$). HRGR showed a pattern of logarithmic decline over the course of the drought experiment, with the growth rate dropping 65.5% from the pre-drought measurement (Week 0) to the first measurement period (Week 2; Fig. 3). This decline tapered off after Week 4, until there was no growth by approximately Week 6 based on this model with time as a fixed effect.

We also found that time was the main factor contributing to the stomatal conductance (g_s) patterns we observed (M2), which improved fit compared to the null model M0 ($\Delta AICc = 1167.52$). The trend in g_s also showed a logarithmic decline. Figure 3 shows the first 5 weeks of data after the initiation of the drought treatment to emphasize differences between time points while responses remained non-zero. Stomatal conductance decreased by 92.8% between Week 0 and Week 3, showing functionally full stomatal closure by this time period.

Both the effects of time and the polymer treatment influenced the patterns we observed in the operating quantum efficiency of PSII ($\Phi PSII$), but we found that the only plausible model (M3, $R^2 = 0.63$) did not include an interaction between these covariates. This model represented a strong improvement over the null model M0 ($\Delta AICc = 844.1$). Figure 4 shows $\Phi PSII$ for the time when the metric had positive values. Often, the trees had a reading of 0.0 for $\Phi PSII$ before we noted that they were dead. Zero values were not commonplace in our $\Phi PSII$ data in the early weeks of the drought, which led to it decreasing more linearly than our other two metrics that were primarily affected by time alone ($\Delta \Phi PSII$ (Week 1 – Week 0) = 19.6% decrease, $\Delta \Phi PSII$ (Week 2 – Week 1) = 21.7% decrease). A similar percentage decrease was sustained until Week 4, and then the values started to level out. $\Phi PSII$ dropped to 0.0 around Week 9, when the standard deviation of the values surpassed the readings.

The average Φ PSII over the course of the experiment was higher in the Mixed treatment relative to the Control group while other treatments were comparable to the Control. The Mixed treatment had an average Φ PSII value of 0.38, which indicates 38% of light energy was converted into usable excitation energy for photochemical reactions (Fig. 4). Comparatively, the Control and Deposit treatment had an average Φ PSII of 0.28, or a 10% lower conversion efficiency. Although the Rolling treatment had a positive effect on the conversion efficiency with an average Φ PSII of 0.33, it was considered comparable to the Control and Deposit treatments.

We found that the only plausible model for the optimal efficiency of Photosystem II (F_v/F_m), or the maximum quantum yield of Photosystem II, had the effects of time and the polymer treatment interacting to influence the functionality of Photosystem II. This model (M4) represented a strong improvement over the null model ($\Delta AICc = 958.1$). The Mixed treatment differed significantly from the Control group, while both the Mixed and Rolling treatments differed significantly from the Deposit treatment towards Week 8 of drought (Fig. 5). The plot shows the non-parametric decline patterns of F_v/F_m . The Control and Deposit groups began to decrease at a faster rate than the other two groups around Week 2, while the Mixed treatment did not begin decreasing noticeably until Week 5.

Overall, the seedlings started at the lower bound of the healthy range of F_v/F_m values (Table 5). We found that the Mixed treatment was the least responsive to the drought, with a decline of 14.1% between Weeks 4 and 9. Similarly, the Rolling treatment showed evidence of buffering F_v/F_m against significant decrease, with a 19.5% decline in efficiency between Weeks 4 and 9, compared to 26.7% and 32.0% for the Control and Deposit treatments, respectively.

Table 5
Responses table for the average F_v/F_m values per treatment for 0 (pre-drought), 4, and 9 weeks after the initiation of the drought treatment.

Weeks after initiation of drought treatment				
Parameters	Treatment	Week 0	Week 4	Week 9
F_v/F_m	Control	0.79 ± 0.02	0.75 ± 0.01	0.55 ± 0.02
	Deposit	0.80 ± 0.02	0.75 ± 0.01	0.51 ± 0.02
	Rolling	0.79 ± 0.02	0.77 ± 0.01	0.62 ± 0.01
	Mixed	0.79 ± 0.02	0.78 ± 0.01	0.67 ± 0.01

4. Discussion

4.1. Seedling Survival

As established through multiple reforestation studies, seedling mortality is a valuable metric to evaluate the efficacy of adaptation strategies to soil moisture stress (López et al. 1998; Stevens-Rumann et al. 2022; Rowland et al. 2023; Wu et al. 2023). Our results showed that overall, the treatment-inclusive model (M1) did not substantially improve over the null model (M0). The c-index of 0.56 for the M1 model falls below the threshold of what is considered a robust predictor of a binary-event model (c-index ≥ 0.70 ; Hosmer et al. 2013), and thus we can conclude that the presence of the polymer was not a strong predictor of survival. Nevertheless, the Mixed polymer treatment extended mean survival under drought by 1.5 weeks, which may provide managers with added flexibility in planting windows and thereby reduce time bottlenecks on reforestation capacity (Dobrowski et al., 2024). It is difficult to extrapolate the impact of any of the polymer applications on seedling field performance based on these results, so future studies may want to compare the addition of the polymer to other well-studied silvicultural treatments, such as competing vegetation control (McDonald and Fiddler 2010) or microsite selection (Hill and Ex 2020).

The lack of improved performance of the Deposit treatment may be due to the spatial distribution of roots in relation to the moisture distribution. Zhu et al. (2023) noted that the root placement within the soil column plays a significant role in water uptake pattern, and young seedlings are particularly sensitive to an inadequate allocation of water to support fine root mass. As the Deposit treatment was the least-homogeneous application of the SAP, we postulate that the water accumulated below the plug was not enough to nourish the fine root mass further up the soil column, leading to a decrease in survival (Hendrick and Pregitzer 1996; López et al. 1998; Zhu et al. 2023). This also may be why the Mixed and Rolling treatments were more effective: the thoroughness of moisture throughout the soil column prevented the fine root mass from desiccating, which would have stimulated more water uptake and increased seedling survival (Zhu et al. 2023).

One issue that we encountered was that polymer fell out of the bottom of the pot under three Deposit-treatment boxes. We do not expect that this had a major impacts on the results, as this accident affected at most 15% of the Deposit seedlings, but it is important to note that the polymer may have absorbed a significant amount of the water and then dropped out, leaving those Deposit seedlings affected with less available water than all other seedlings. While in the field SAP would logically be introduced into planting holes at the time of planting, our experience suggests that preparing container seedling with SAP deposits may be tricky to do reliably.

4.2. Seedling Growth and Physiology

Ponderosa pine is a characteristically drought-avoidant species and thus displays high stomatal sensitivity to a lack of available moisture (Martínez-Ferri et al. 2000; López et al. 2008). We hypothesized that the SAP might be able to increase seedling water availability, enhancing transpiration and CO₂ assimilation while reducing desiccation (Hoshika et al. 2018; Maseyk et al. 2019). In turn, this would increase photosynthetic productivity and promote growth, despite the tendency of pines to reduce indices of growth during periods of severe environmental stress (Martínez-Ferri et al. 2000; López et al. 2008; Rowland et al. 2023). In other words, we expected the presence of the polymer to reduce the pine

seedlings' sensitivity to environmental conditions, which could potentially promote their competitive capacity in disturbed environments, especially after fire (Buckley 2017; Maseyk et al. 2019). However, due to treatments impacting neither height relative growth rate (HRGR) nor stomatal conductance (g_s) we concluded that the addition of the polymer is unable to increase carbon assimilation and transpiration rates.

As noted above, pines have high dehydration sensitivity, which may result in stomatal closure even under mild moisture stress, which the polymer is unlikely to be able to overcome (Faria et al. 1998; Martínez-Ferri et al. 2000). This tendency to close stomates under mild stress represents a drought survival strategy prioritizing the preservation of the hydraulic conductivity needed to recover from drought over the potential exhaustion of carbohydrate reserves (Bucholz et al., 2020). The polymer may be potentially more effective at improving both survival outcomes and physiological metrics in species exhibiting the opposite drought survival strategy (Bigelow et al., 2014), such as white fir (*Abies concolor* Gordon & Glend.). Stomatal closure can also occur during high midday temperatures, even in the absence of any moisture stress treatment. We suspect that the high temperatures of the greenhouse, which resembled ambient field conditions, likely played a role in the early closure of the stomata and the cessation of growth, even if the polymer increased water availability to the seedling. Therefore, additional research should focus on less-severe temperature extremes to understand the effect of the polymer on stomatal conductance and how that translates into productivity and growth.

We found that the presence of the polymer positively impacted $\Phi PSII$ and made a significant improvement on F_v/F_m , particularly for the Mixed treatment. These fluorescence metrics measure the health of Photosystem II (PSII) and the electron transport chain which convert energy from photons to increase the electron transport rate to fuel either photosynthesis or photorespiration (Medrano et al. 2002; Murchie and Lawson 2013). Energy allocation will shift towards the latter process during periods of environmental stress, but the quantum efficiency measured through chlorophyll fluorescence may remain high (Krause and Weis 1991; Murchie and Lawson 2013). As $\Phi PSII$ measures the conversion efficiency in ambient light (Martínez-Ferri et al. 2000), it provides insight into how the polymer may be affecting the functionality of PSII and allows us to compare the extent of the polymer's effect between treatments. We found that compared to the average $\Phi PSII$ of the Control, the Mixed and Rolling treatments improved the conversion efficiency by 10% and 5%, respectively ($\Phi PSII_{Control} = 0.28$, $\Phi PSII_{Mixed} = 0.38$, $\Phi PSII_{Rolling} = 0.33$), indicating that a polymer throughout the soil column improved the *in situ* functionality of PSII during the drought. The Deposit treatment ($\Phi PSII = 0.28$) did not show any increase in $\Phi PSII$ compared to the Control.

A lower ratio of $\Phi PSII$ and F_v/F_m indicates an increase of absorbed light energy dissipated as heat instead of consumed by photosynthesis, resulting in a deregulation of excitation energy in a process called photoinhibition (Faria et al. 1998; Medrano et al. 2002; Murchie and Lawson 2013; Wang et al. 2018). Photoinhibition is a reduction in electron transfer that serves as a protective mechanism when the plant's photosynthetic capacity is limited by soil water deficits (Long et al. 1994; Zuo 2025). This process

results in a decrease in net photosynthesis and energy conversion to repair light-induced damage to the photosynthetic reaction center (Maseyk et al. 2019, Zuo 2025).

Studies have shown that the F_v/F_m ratio, one of the most common indicators of photoinhibition and PSII stress, typically stays within a range of 0.79–0.83 if non-inhibited (Murchie and Lawson 2013; Sharma et al. 2014; Zuo 2025), within which all our seedlings started (see Table 5). The quantum efficiency of pines may drop as low as 0.75 in mildly stressed conditions without severely impacting productivity (Sharma et al. 2014; Bęś et al. 2019). We found that the Control group reached an F_v/F_m of 0.75 around Week 4, while the Mixed treatment did not reach this threshold until around Week 8, implying that the water released from the polymer may buffer against photoinhibition. This may be due to the polymer providing enough water to be consumed by photosynthesis without impeding other biochemical processes, which results in an overall reduction in moisture stress on the seedling (Wang et al, 2018).

Our results showed that the SAP may be able to buffer some of the damage to PSII caused by drought, and we postulate that the slow release of moisture from the polymer was sufficient to support PSII function and prevent deregulation, even if it did not lead to more photosynthetic productivity. Plants that are photoinhibited can recover from values that fall below 0.75 to a certain point, below which the rate of photochemical reactions cannot upkeep biochemical processes and the chloroplasts begin to denature (Medrano et al. 2002; Murchie and Lawson 2013). Although there is some debate as to the threshold below which PSII cannot recover, Rico-Cambron et al. (2023) found that an F_v/F_m value below 0.55–0.60 yielded a higher chance of seedling death during a drought treatment, while seedlings with F_v/F_m readings above this number were more likely to survive and recover. While we did not test for recovery in this experiment, we found that both the Mixed and Rolling treatments had an average F_v/F_m value above 0.60 during Week 9 of the drought treatment, while the Control dropped to 0.55. In fact, the average F_v/F_m of the Mixed treatment, 0.67 (indicating 67% quantum yield efficiency), at Week 9 had high potential to recover from drought stress if there is another rain event (Medrano et al. 2002; Rico-Cambron et al. 2013).

Based on these results, we suggest that the slow-release of water provided by the polymer may be able to alleviate some of the damage the PSII accumulates from severe moisture stress. To reiterate, the presence of the polymer, based on the results of this study, did not lead to an increase in productivity or growth of the seedling, which would have potentially given the seedling an advantage over competing vegetation while accelerating forest recovery. However, the polymer may be able to prevent permanent photoinhibition (and subsequent death) for the seedling, thus providing a greater chance that its photosynthetic infrastructure will survive until the next rain event, which will lead to seedling recovery and may increase the chance of establishment (Garbowski et al. 2019; North et al. 2019; Wu et al. 2023).

Conclusions

In this study, we report on the efficacy of three different applications of a biodegradable superabsorbent polymer (SAP) in decreasing mortality and physiological indicators of drought in container-grown *Pinus*

ponderosa seedlings subjected to severe drought stress in a greenhouse setting. Based on the results of our study, we conclude that the polymer may be effective in prolonging survival by about 1.5 weeks compared to seedlings that do not have the polymer if distributed evenly throughout the soil column, which may help the seedling reach the next rain event and recover before desiccation. We found that the polymer did not increase growth and productivity of the seedlings, due to a lack of available water to prevent the early-onset stomatal closure that is characteristic of pines. However, our chlorophyll fluorescence measurements revealed that the Mixed and Rolling treatments could keep the quantum efficiency of Photosystem II above the recovery threshold, which may be able to maintain the seedlings' photosynthetic infrastructure until it can recover during the next rain event. It is crucial that this polymer is rigorously tested in a field trial before it can become a tool for reforestation managers, especially as the Mixed treatment is not as implementable in the field as the Deposit and Rolling treatments.

Declarations

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Competing Interests

Kannar Earth Sciences, Ltd. supplied us with the starch-based biopolymer with no financial incentive, and had no influence on the writing and results of this paper due to limited correspondence. Our researchers have no personal, professional, or financial incentive to skew the results of the experiment for any particular result.

Author Contribution

K.S. created the research methodology and performed the data collection. C.C. assisted K.S. in experimental setup and data collection. K.S. produced the first draft of the manuscript along with all tables and figures, and performed data analysis and modeling under the supervision of C.L. and J.W. All authors contributed to writing, editing, and reviewing the final manuscript.

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Data Availability

The data generated from this experiment can be found in the Supplementary Information section under Online Resource 3. The R analysis conducted for the construction and testing of data models can be found under Online Resource 4.

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Figures

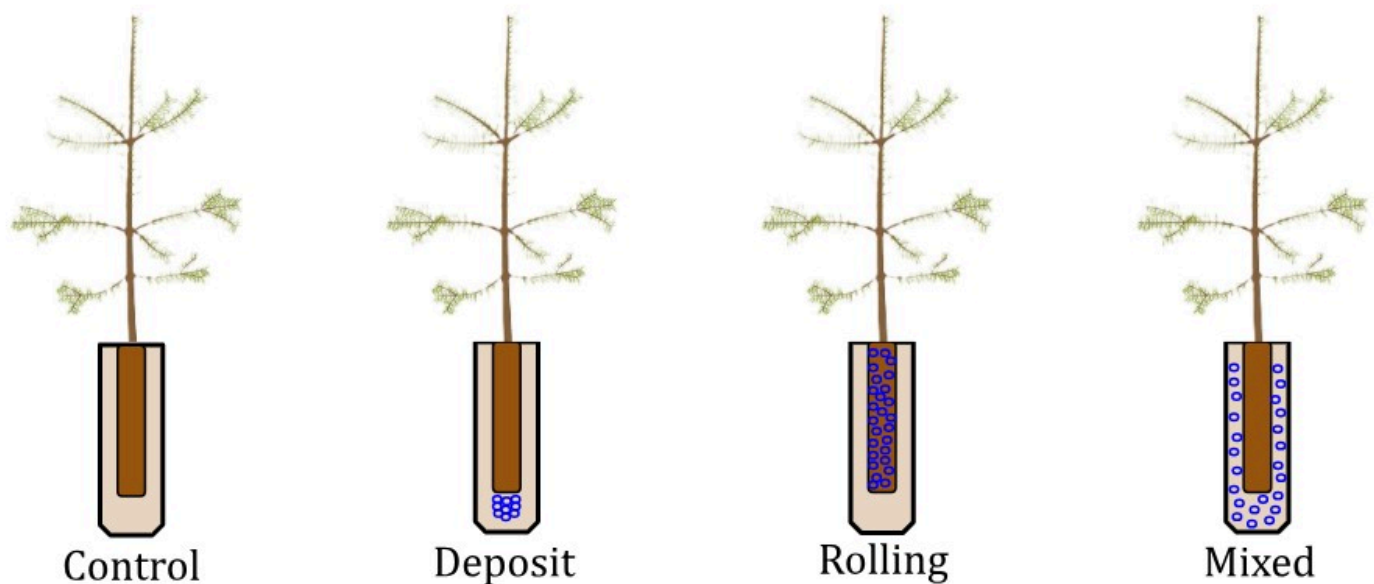


Figure 1

*Diagram of the Control group and three applications of the polymer (blue circles) in relation to the seedling plug. **Control:** contains no polymer. **Deposit:** we placed the polymer at the bottom of a planting hole and put the seedling on top. **Rolling:** we rolled the seedling plug in the polymer and then planted it. **Mixed:** after measuring how much soil we needed for a pot, we thoroughly mixed the polymer throughout the soil in a separate box, then used the mixture to step up the seedling.*

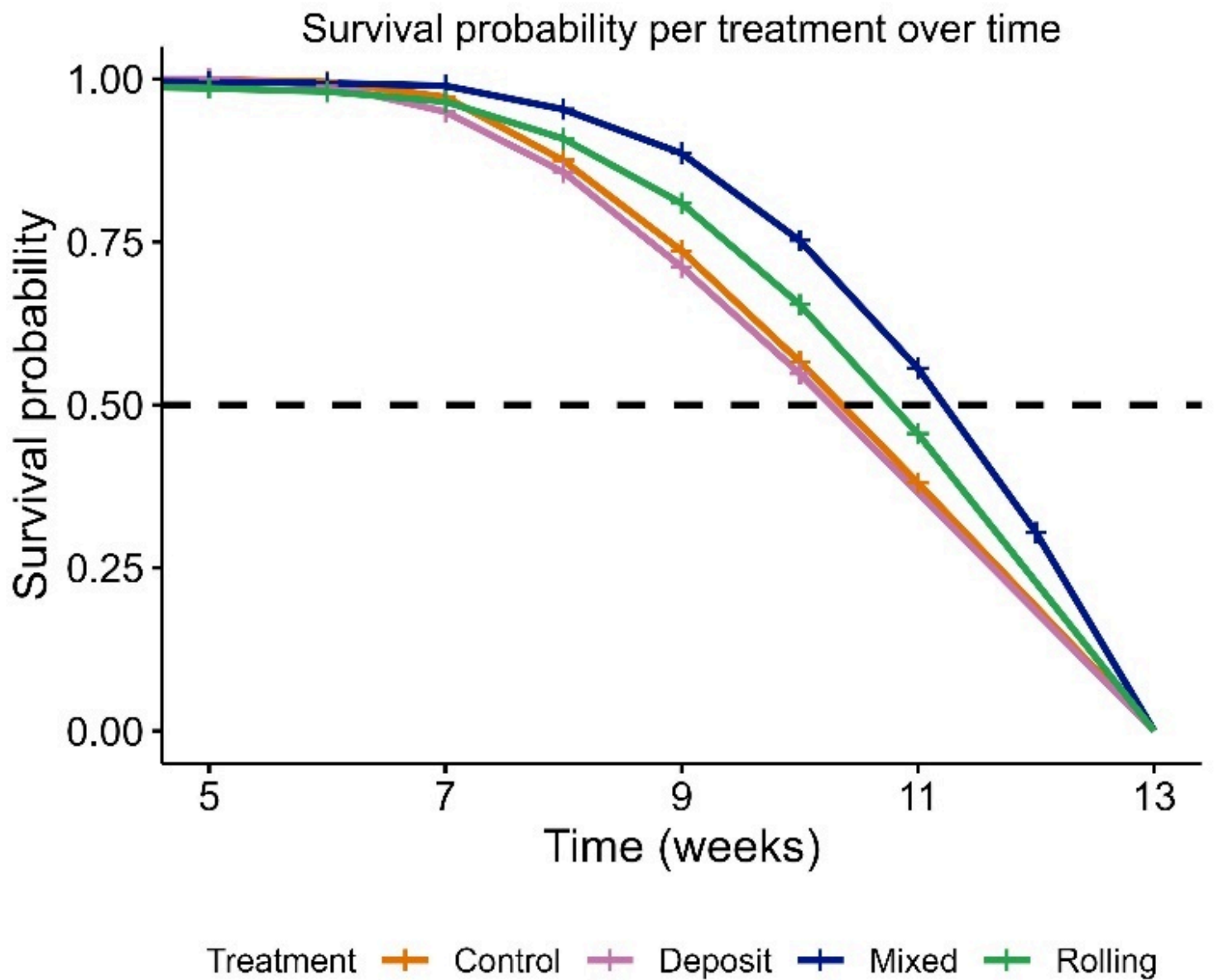


Figure 2

Survival probability for each treatment between Week 5 and Week 13 (latter weeks are shown to emphasize differences). The black dashed line represents the median time to death for each treatment.

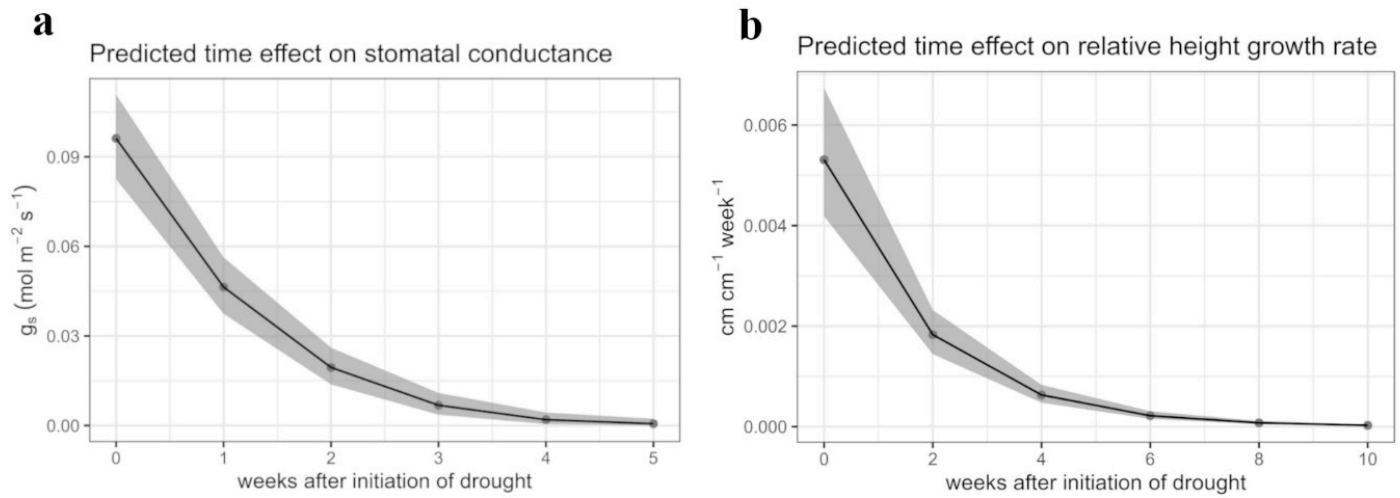


Figure 3

a) Predicted effect of time on stomatal conductance (g_s , in $\text{mol m}^{-2} \text{s}^{-1}$) patterns, truncated at Week 5; b) Predicted effect of time on height relative growth rate (HRGR, in $\text{cm cm}^{-1} \text{week}^{-1}$), shown over the 10 weeks of growth observed from the experiment. The shaded area represents 95% confidence intervals.

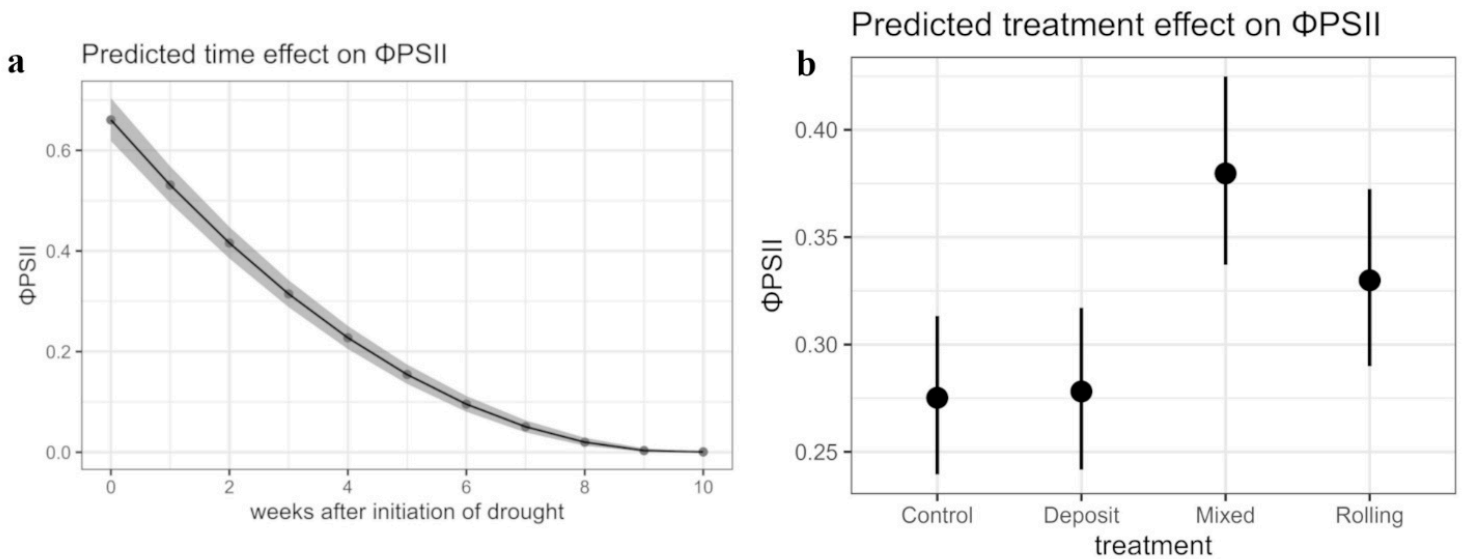


Figure 4

a) Predicted time effect on ΦPSII , shaded area represents 95% confidence interval; b) Point range plot of the predicted effect of the polymer on ΦPSII , showing the average ΦPSII values for each treatment group.

Predicted interacting effect of treatment and time on F_v/F_m

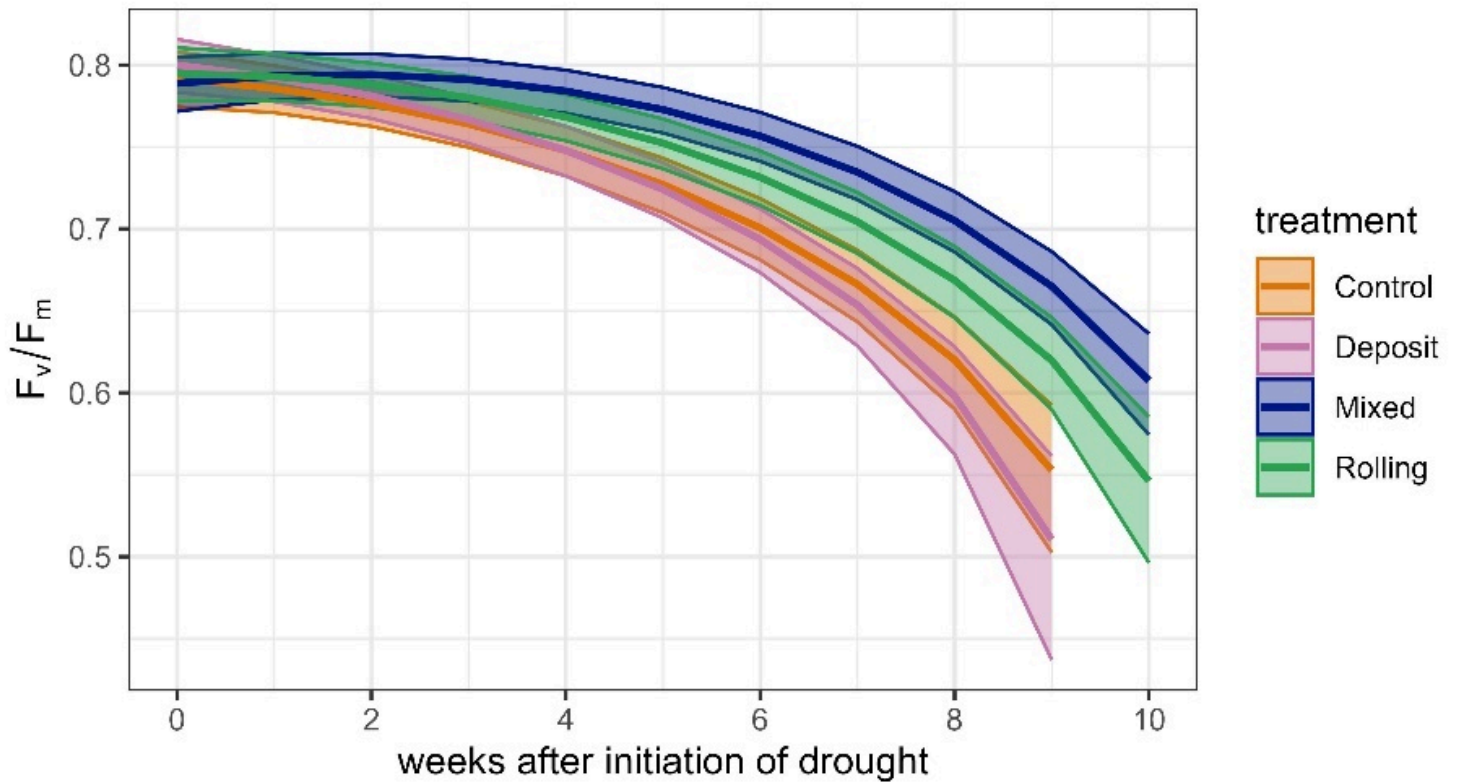


Figure 5

The predicted F_v/F_m with the interacting effects of treatment and time. Shaded areas represent 95% confidence intervals.

Supplementary Files

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