Effects of post oak *Quercus Stellata* and Smooth Brome Bromus Inermis Competition on Water Uptake and Root Partitioning of Eastern Redcedar Juniperus Virginiana

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Research Article

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Abstract

Water availability may alter species competitive interactions, resulting in different outcomes as plants compete for available water. Eastern redcedar *Juniperus virginiana* (hereafter *ERC*) will likely continue to encroach into new habitats, which will affect soil and water budgets. We designed a greenhouse experiment to investigate changes in water uptake and rooting depths of two-year old ERC saplings in the presence of an invasive grass (*Bromus inermis*) and a native tree (*Quercus stellata*). We measured soil moisture content over two growing seasons. When grown together, ERC took up water from the deeper layers (21–40 cm) whereas *B. inermis* used water from the top layers of the soil (0–20 cm). Similarly, when ERC grew with *Q. stellata*, ERC took up water from the deeper layers and *Q. stellata* used water mostly from the top layers. This root partitioning can allow the co-existence of ERC, grasses, and other trees, which can facilitate ERC encroachment into grasslands and woodlands. However, when the three species grew together, we found root overlap between the ERC and *Q. stellata*. This overlap can affect ERC establishment and encroachment in habitats where grasses and trees co-occur. A major factor that affected ERC performance was higher mortality in treatments with *B. inermis* than in the ERC-alone or with *Q. stellata*. This indicates that competition with grasses is a major factor affecting ERC sapling establishment.

Introduction

Much competition between plants occurs belowground[1,2]. Studying the differentiation of root systems has been thought of as a mechanism that reduces competition and facilitates species coexistence, and can be used to understand the spatial distribution of plants, plant coexistence, and soil and water budgets[3,4,5]. Many studies show that niche differentiation can occur if plant species occupy different environmental niche axes, including soil moisture and root depth[6,7,8].

Competition for water is considered to occur by reduction in availability, favoring plants that can withstand the lowest water potential[9]. However, competition for water is less studied than other factors such as nutrients or light[9]. Water availability is a particularly important factor in the survival and establishment of very young plants[10]. The impact of soil-moisture depletion on plants is expressed in lower emergence rate, diminished plant size, and decreased biomass[11]. Moreover, plants growing in water-limited soils are more prone to hydraulic failure and drought-induced mortality. Drought leads to higher xylem tensions, as the tension of water within the xylem exceeds atmospheric pressure, leading to xylem embolism or cavitation. This embolism (gas bubble) blocks water flow through xylem conduits and reduce the delivery of water from the soil to other parts of the plant[12,13]. Water limitation can also affect a plant's nutrient uptake, alter plant-microbe interactions, and affect transpiration rates. This will result in a decline in available resources for the different parts of the plant, including the leaves and seeds[14]. The presence of trees in a system can alter soil water content, affecting all associated plants.

In one of the most prominent theories of niche separation between trees and grasses, Walter (1939) proposed the *two-layer hypothesis* as an equilibrium explanation for the coexistence of savanna trees and grasses. Trees and grasses have very distinct root morphologies and different water-use strategies. Root-morphology differences can affect the efficiency of trees and grasses to extract water, independently of rooting depth. Walter (1939) proposed that grasses predominate over trees in savannas because grasses have shallow roots, allowing them to use water efficiently and take advantage of the available rainfall. Grasses are considered superior competitors for water in the upper soil profile[15] due to their shallow rooting system with high root length and surface area. Contrastingly, trees have access to deeper water because of their extensive rooting systems[4],[16]. However, in grasslands, trees and grasses may use overlapping areas in the soil profile, especially while trees are growing their roots through the soil profile[6,17]. Daly *et al*. (2000)[18] studied tree-grass interactions in water-limited ecosystems and established that in the absence of fire, trees eventually become the dominant life form because they are more deeply rooted than grasses. Conversely, grass dominance is expected if trees and grasses occupy the same rooting region because of their greater water-use efficiency[4]. One aspect that Walter's (1939) two-layer hypothesis did not focus on is how the roots of two or more tree species interact to allow these trees to coexist[8]. A few studies comparing the depth of water uptake by co-occurring woody species reported some tree species use only deep soil water, while others take water from both shallow and deep layers[4,8,19].

Eastern redcedar (*Juniperus virginiana*; hereafter *ERC*) is the most widespread conifer in the eastern United States[20]. Due to drought and fire exclusion in the past century, ERC is encroaching into new habitats in eastern and Central US, including grasslands in the Great Plains (grassland located in the interior of North America)[21,22], and midwestern prairies as far west as Nebraska[23,24]. ERC are known to be drought-tolerant[25] and have the ability to photosynthesize in low soil-moisture conditions[26,27]. In addition, ERC has a relatively high leaf-level water-use efficiency under water limitation[27], giving this tree a competitive advantage over other species in water-limited environments[19,22].

An important factor that contributes to the improved growing conditions of ERC is reduced plant competition with neighboring plants[28], such as grasses (Ward, 2020). A major grass competitor is *Bromus inermis* (hereafter *smooth brome*). Smooth brome is an invasive perennial grass that dominates many grasslands and old fields in every state of the contiguous United States[30]. An additional competitor for ERC are *Quercus* spp. ERC is encroaching in areas where oak (*Quercus*) trees, such as *Quercus stellata* (hereafter *post oak*), are dominant[22,25]. For example, ERC encroached into the Cross Timbers (Western edge of the eastern deciduous forest of the U.S.), transforming post oak-dominated forests to ERC and post oak co-dominant woodlands[22].

In order to accurately model and forecast species dynamics and the impact of climate change on ecosystem processes, identifying the source of water uptake of species and their role in the water balance of an ecosystem is important[31]. In this study we sought to determine the rooting depth and water uptake across soil layers for ERC both growing alone and in competition with smooth brome and/or post oak.

We conducted a greenhouse experiment and used frequency domain reflectometry (FDR) to measure water availability at different depths. We also accounted for ERC root length and water status by examining midday leaf water potential and relative water content. We further tested Walter's (1939) two-layer hypothesis by focusing on how the roots of two or more species interact to allow trees to coexist.

We made three predictions based on Walter's (1939) two-layer hypothesis:

1) When ERC and smooth brome co-occur, smooth brome will take up water from the topsoil layers, because of their shallow roots, leaving less water for ERC. As a result, ERC, having deeper roots than smooth brome, will invest in belowground development and will further extend their roots to get to the deeper water in the soil profile.

2) When ERC and post oak are competing, we predict that there will be niche separation between ERC and post oak roots[22]. ERC will avoid low water potentials by developing a deep root system that gives them access to deeper soil moisture.

3) When all three species (ERC, post oak, smooth brome) co-occur, soil moisture will be consistently low throughout the soil profile. Moreover, ERC will be more stressed (lower water potential and relative water content) when all three species co-occur compared to the other treatments (ERC-alone, ERC + post oak, and ERC + smooth brome). As a result, ERC sapling performance and encroachment will be negatively affected when the three species co-occur together due to lower water availability for ERC.

4) We expect that the water requirements for the ERC, post oak, and smooth brome will increase with time (higher in the second growing season) as the plants grow. As a result, there will be lower soil moisture content in second growing season for all ERC treatments.

Results

Soil moisture content changes in the four ERC treatments

Our observations showed that water from the drip irrigation system was percolating down the soil profile and was able to reach the bottom of the pots (70 cm). Pots with dead ERC were excluded from the analysis. The soil moisture content varied depending on ERC treatments ($F = 29.07$; $p < 0.001$; Fig. 1) and soil depths ($F = 270.62$; $p < 0.001$; Fig. 1). We did not find an overall shift in soil moisture content among the different measurement periods (Sep. 2018, Nov. 2018, Jan. 2019, May 2019, and Aug. 2019) ($F = 1.66$; $p = 0.14$; Fig. 1). Soil moisture content among the different measurement periods, however, was dependent on the ERC treatment combination (Table 1). In addition, we ran both linear regressions and cubic splines to examine possible seasonal shifts in soil moisture content. All regression lines were significant. Cubic splines had higher coefficients of determination than linear regressions (Table S1).

Table 1
Repeated measures analysis of variance results for the soil moisture content in the four ERC treatments among different measurement dates (Sep. 2018, Nov. 2018, Jan. 2019, May 2019, and Aug. 2019). Reported F values are equivalent F values based on Wilks' λ. Significant values indicated in bold (*).

Treatment	Wilks' λ	F	P
ERC (10 cm)	0.859	0.411	0.797
ERC (20 cm)	0.502	2.479	0.111
ERC (30 cm)	0.779	0.711	0.603
ERC (40 cm)	0.658	1.298	0.335
ERC + post oak (10 cm)	0.377	4,138	**0.031***
ERC + post oak (20 cm)	0.331	5.055	**0.017***
ERC + post oak (30 cm)	0.444	3.130	0.065
ERC + post oak (40 cm)	0.059	40.058	**< 0.001***
ERC + smooth brome (10 cm)	0.070	30.046	**< 0.001***
ERC + smooth brome (20 cm)	0.094	21.686	**< 0.001***
ERC + smooth brome (30 cm)	0.185	9.881	**0.002***
ERC + smooth brome (40 cm)	0.052	41.244	**0.005***
ERC + post oak + smooth brome (10 cm)	0.003	193.201	**0.001***
ERC + post oak + smooth brome (20 cm)	0.008	64.947	**0.015***
ERC + post oak + smooth brome (30 cm)	0.009	56.741	**0.017***
ERC + post oak + smooth brome (40 cm)	0.002	303.736	**0.003***

ERC

As predicted, ERC growing without competitors used more water from the deeper layers of the soil (30 and 40 cm) compared to the shallow depths (10 and 20 cm) (Figs. 1a and 1b) due to their extensive rooting system (Fig. 2). We found seasonal progression of soil moisture content between the two growing seasons (2018 and 2019 growing seasons; Fig. 1a). For example, there was significantly more moisture in the soil profile in the first growing season (2018) than in the second growing season (2019) ($F = 10.29$; $p = 0.002$) (Fig. 1a; Table S1), indicating that ERC used more water in the second growing season (as the saplings grew bigger). However, we did not find a significant change in water uptake among the different measurement periods at all depths for ERC-alone treatment ($F = 0.63$; $p = 0.82$; Table 1 and Fig. 1b).

ERC + post oak

When ERC grew with the post oak, we found lower soil moisture content at 10 cm and

20 cm depths compared to ERC-alone ($F = 4.47$; $p = 0.001$). We can attribute this decline in moisture content at the shallow depths to water uptake by the post oak because there was no significant change in water uptake at 30 cm and 40 cm compared to the ERC-alone treatment ($F = 0.007$; $p = 0.8$) (Figs. 1c and 1d). In addition, we found that the soil moisture content during the first growing season (2018) was greater than the 2019 growing season ($F = 64.94$; $p < 0.001$; Fig. 1c), indicating that the ERC and post oak used more water in the later growing season (Table S1). In addition, the repeated measures analysis indicated significant changes moisture content across the different measurement periods. Specifically, there was less soil moisture in May 2019 and Aug 2019 than on other measurement dates at 10, 20, and 40 cm depths ($F = 1.96$; $p = 0.029$). However, there was no significant difference in moisture content at 30 cm during the different measurement periods ($F = 1.63$; $p = 0.176$; Table 1, Fig. 1d).

ERC + smooth brome

When ERC occurred with smooth brome, there was a significant decline in soil moisture content at all depths compared to ERC-alone treatment (Figs. 1e and 1f). We can attribute this decline to the increase in water demand, mainly by the smooth brome. In direct dissension to the results of ERC-alone, the soil moisture content in the ERC + smooth brome treatment was significantly lower during the first growing season (2018) than the second growing season (2019) ($F = 83.89$; $p < 0.001$; Figs. 1e). This means that the ERC and smooth brome required less water later in the experiment (Table S1). In addition, we found significantly more moisture in the soil profile (the plants took up less water) later in the experiment (May 2019 and Aug 2019) at all depths ($F = 2.22$; $p = 0.011$; Table 1, Fig. 1f).

ERC + post oak + smooth brome

In the treatment with the most competitors, ERC + post oak + smooth brome, the soil moisture content varied across the experiment. The ERC + post oak + smooth brome treatment had significantly more moisture in the profile than ERC-alone throughout the experiment at all depths ($F = 20.69$; $p < 0.001$; Fig. 1g). Moreover, we found that the soil moisture content in 2018 growing season was lower than the 2019 growing season ($F = 412.88$; $p < 0.001$), indicating that the ERC, post oak, and smooth brome used less water in the 2019 growing season than 2018 growing season (Table S1). In addition, the repeated measures analysis indicated a significant change in soil moisture content throughout the experiment at all depths (Table 1).

Examining water uptake at the different depths, our results indicate that the smooth brome mostly took moisture from the shallow depths (10 cm and 20 cm) due to their shallow roots, whereas ERC and post oak from the deeper soil layers. There was an overlap in water uptake at the greater depths (30 cm and 40 cm) between ERC and post oak (Figs. 1g and 1h). We also found a decline in ERC rooting depths in the ERC + post oak + smooth brome combination compared to ERC-alone and an overlap between ERC and post-oak root lengths (Fig. 2).

Rooting depths and total biomass

ERC root depths and total biomass were dependent on the ERC treatment ($F = 35.80$; $p < 0.001$ and $F = 12.30$; $p < 0.001$ respectively; Figs. 2 and 3). The maximum ERC root length was longer than the pot depths (70 cm). However, our observations and data showed no sign that the ERC saplings were root-restricted or rootbound as they were exploring the full volume of the soil. The rooting depth of ERC saplings was not significantly affected by the post oak ($F = 0.45$; $p = 0.83$; Fig. 2). However, in treatments containing smooth brome (ERC + smooth brome and ERC + post oak + smooth brome), ERC saplings had significantly shorter roots compared to the ERC saplings growing alone or with the post oak ($F = 39.34$; $p < 0.001$; Fig. 2). Moreover, we found an overlap between ERC and post-oak root lengths in the ERC + post oak + smooth brome treatment (Fig. 2).

The presence of post oak did not have a significant effect on ERC total biomass ($F = 0.01$; $p = 0.93$; Fig. 3). However, ERC growing alone had higher mean total biomass than when ERC grew with smooth brome or post oak + smooth brome ($F = 25.20$; $p < 0.001$; Fig. 3). Overall, smooth brome negatively affected ERC's performance.

ERC water status

There was no significant difference in ERC leaf relative water content ($F = 1.64$; $p = 0.195$) between the four ERC treatment levels (Fig. 4). However, there was a significant difference in ERC water potential between the ERC + post oak and ERC + smooth brome treatments ($F = 4.18$; $p = 0.011$; Fig. 5). This indicates that water stress was not the main factor affecting ERC mortality rate.

ERC Mortality

ERC sapling mortality was affected by the ERC treatment ($\chi^2 = 10.49$, $df = 3$; $p = 0.01$; Fig. 6). We did not find a significant change in ERC mortality when grown alone, with the post oak ($\chi^2 = 0$; $p = 1$), or with the smooth brome ($\chi^2 = 0.8$; $p = 0.37$). There was, however, significantly higher ERC mortality when grown with both post oak + smooth brome in the same pots ($\chi^2 = 6.79$; $p = 0.009$; Fig. 6).

Discussion

Plants use various mechanisms to avoid and minimize competition with other plant species[37,38]. One important factor affecting plant distribution is water and nutrient availability. A major mechanism to avoid water stress and enhance the competitive advantage of a plant is its ability to change its root distribution to exploit deeper stores of soil water[39]. For example, plants with different root-distribution patterns (e.g., root length and lateral distribution) might be able to coexist in the same habitat if their roots can occupy different spaces and are able to exploit resources from distinct areas[40]. On the other hand, plant species with similar root distributions will have stronger competition between the individual plants, reducing plant growth and survival due to competition for the same limiting resources (e.g., water). Our experimental design allowed us to investigate changes in ERC water uptake and rooting depths to competition with an invasive grass (*B. inermis)* and a native tree (*Q. stellata*). We found a

separation of soil-moisture depletion zones by the different treatment combinations, allowing ERC to co-exist with grasses and other woody species.

ERC is known to expand its range and survive in different environments due to its ability to alter its root morphology, allowing for enhanced water uptake, especially during periods of low moisture[41]. Plant interspecific competition had a significant effect on ERC water uptake, root lengths, total biomass, and mortality (Figs. 1, 2, 3, and 6, respectively). As predicted, we found that ERC growing without competitors used water mostly from the deeper layers of the soil (30 and 40 cm) due to their extensive rooting system. It has been reported that ERC roots can reach up to 7 m deep for mature trees[19]. However, because we used saplings in this experiment, the ERC roots were not as extensive. Sapling establishment can be strongly suppressed by other plant species, including grasses, than larger trees[42], which makes the sapling stage critical in developing and understanding demographic and encroachment models[43].

One main mechanism in which ERC tolerates drought conditions and facilitates its encroachment is niche partitioning. For instance, differences in rooting depths reduce water competition between trees and grasses[4] and among different tree species[22]. Consistent with Walter's two-layer hypothesis (1939, 1979)[3,44], our results suggest root separation between ERC and smooth brome roots, in which smooth brome used up more water than ERC saplings at shallow depths (10 cm and 20 cm), due to their shallower rooting system, whereas ERC used the most water at greater depths (30 cm and 40 cm). Eggemeyer *et al*. (2009)[19] and Awada *et al*. (2013)[45] have shown that ERC competes for soil moisture with grasses in the top layers when water is available. However, when surface moisture decreases, ERC shift their water uptake to deeper layers[46].

We also found root partitioning between the ERC and post oak, where ERC had deeper roots than the post oak (Fig. 2) and took up the water from the deeper layers of the soil. On the other hand, the post oak used water from the shallower depths. Torquato *et al*. (2020)[22] found that ERC and post oak may be using water from different depths in the oak-dominated Cross Timbers reserve in Oklahoma. The differences in rooting depths can reduce competition for water and allow ERC encroachment into the oak-dominated woodlands[22,47]. In their experiment, Torquato *et al*. (2020)[22] found that ERC's roots occupied the shallower soil depths (45 cm), whereas the post oak roots accessed water from the deeper soil layers. These results are not consistent with what we found in our experiment. Based on our data on root lengths, root biomass, and growth rate, we believe that ERC extended their roots to the deeper soil layer in our pots (40 cm), whereas the post oak limited their roots and water uptake to the shallower depths (20 cm) to avoid competition. We suggest that the differences between our study and Torquato *et al*. (2020)[22] may be due to ontogenetic differences; Torquato *et al.* (2020)[22] used mature trees whereas we used saplings. The trees may reverse partitioning strategies as a result of ontogeny, but more research is needed to understand the relationship between tree age, size, and water uptake behavior.

We also examined water uptake at the different depths when ERC grew with post oak and smooth brome. Our results indicate that there was an overlap in water uptake at the greater depths between ERC and post

oak. Smooth brome has an extensive rooting system that mostly took moisture from the shallow depths, leaving less water for ERC and post oak at the 10 cm and 20 cm depths. As a result, the two woody species, ERC and post oak, invested in belowground development and shared water uptake from the 30 cm and 40 cm layers (Figs. 1g and 1h). We also found a decline in ERC rooting depths in the ERC + post oak + smooth brome combination compared to ERC-alone (Fig. 2) and an overlap between ERC and post-oak roots. This suggests that the post oak can be considered a major competitor affecting ERC performance only in the presence of a shallow-rooted species such as smooth brome.

When comparing water uptake between the two growing seasons for all treatments combinations, we found that ERC-alone used significantly more water in the second growing season (2019) than the first growing season (2018). The same amount of water was supplemented in both seasons but there was more moisture in the soil profile in 2018. This can be attributed to the increase in size of ERC as they grew older. Similarly, we found that the ERC + post oak treatment also used significantly more water in 2019 compared to 2018 growing season (there was a decline in water uptake by the ERC and post oak in 2019). However, in the treatments with smooth brome (ERC + smooth brome and ERC + post oak + smooth brome), the water uptake by the plants declined in 2019 compared to the 2018 growing season (there was more soil moisture in 2019 than 2018). This could be related to the plants, mainly the smooth brome, requiring more water to establish their roots and growing at the beginning of the experiment[4], which resulted in lower soil moisture content in the profile. In 2019, the ERC and smooth brome were already established, and their growth rate declined, which ultimately resulted in reduced water requirements and uptake. Another possible cause for the lower water uptakes in the second growing season is the negative effect of smooth brome on ERC and post oak survival, growth rate, and total biomass (aboveground and belowground biomass).

Grasses can regulate woody plant establishment directly, through competition for water, light, or nutrients, or indirectly as fuel for fires[48,49]. Sapling emergence, survival, and growth of woody plants are reported to be reduced by grasses[17,50]. Our results suggest that smooth brome had a significant negative impact on ERC performance. ERC total biomass significantly decreased when in competition with smooth brome or with smooth brome and post oak. Similar results have been obtained in other studies that determined the effects of competition by grasses on trees[48–51]. Ward (2020, 2021)[29,54] showed that shade is the most important factor affecting the growth rate and biomass of ERC. On the other hand, we found no evidence of the negative effect of post oak competition on ERC performance, likely due to the niche differentiation between the ERC and post oak. Torquato *et al*. (2020)[22] found that the soil moisture in pure ERC strands is more negative than in ERC-post oak stands, which may help ERC encroachment into oak woodlands. We need to recognize that grasses have the opposite effect and control ERC encroachment. Thus, the relative importance and effects of grasses and woody species competition needs to be better understood. Understanding plant interactions, competition, and co-existence will help in modeling and controlling the woody encroachment problem and its consequences for the biodiversity and economic productivity in grasslands and prairies.

Methods

Ethics statement

The plant materials used in this experiment were purchased from commercial nurseries. ERC saplings were obtained from Pinelands Nursery & Supply, New Jersey, the post oak saplings were obtained from Mossy Oak Nativ Nurseries, Mississippi, and the smooth brome seeds were obtained from Great Basin Seed company, Utah. The experiment did not involve any endangered or protected species, so no specific permissions were required for the collection and use of plant materials. The experimental research on plants, including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation. All methods were performed in accordance with the relevant guidelines and regulations.

Experimental design

We conducted an experiment to analyze changes in soil moisture content and rooting depths for ERC growing alone and in competition with smooth brome, and/or post oak. We used 95 L containers (55 cm diameter x 70 cm height) (hereafter *pots*) filled with PRO-MIX® All-Purpose Professional Grower's Potting Mix (BFG Supply). We used a completely randomized design in which we grew ERC in four treatments (no competition, post oak competition, smooth brome competition, and smooth brome and post oak competition). We used 16 pots for each treatment. ERC saplings were planted first, followed by the post oak and the smooth brome. We planted ERC-alone (one sapling; mean initial height ± S.E.= 226 ± 6.6 mm), ERC + post oak (one sapling each; mean initial height ± S.E.= 319 ± 8.3 mm), ERC + smooth brome, and ERC + post oak + smooth brome, for a total of 64 pots. In the treatments with smooth brome, we planted 200 grass seeds/pot with a germination rate of 97%.

We grew the plants in a controlled environment (average temperature = 25°C and four hours of supplemental light in winter). All pots were irrigated for approximately 10 min every other day through a drip-irrigation system at a rate of 40 ml/min. The same amount of water was supplemented in both growing seasons and for all treatment combinations. We monitored soil moisture throughout the experiment to ensure proper drainage to the deeper soil layers. Prior to planting the saplings, we installed a 1 m-long 50 mm-diameter polycarbonate access tube in every pot to measure moisture content in the soil profile.

Plant overall performance

Throughout the experiment, we recorded ERC height, trunk diameter (at root collar), and ERC tree mortality in the four treatments every week as an estimate of sapling quality and performance[32,33]. At the end of the experiment (24 months), all plant material was harvested. ERC and post oak roots were washed with water to remove soil, extended to their full length, and measured with a ruler to record the maximum root length. To account for the effect of competition on the performance of ERC, plants were dried in the oven at 60 ºC for 48 h, then weighed to measure total plant biomass, including root biomass, for ERC, post oak, and smooth brome (Figure S1).

Soil moisture content

We used a Diviner 2000→ soil-moisture probe (Sentek Pty Ltd, Stepney, Australia) to obtain moisture content readings every 10 cm in the soil profile[6]. The Sentek system uses frequency-domain reflectometry (FDR). Factory settings and calibration were used to derive percentage water (content θ%) at each depth. The access tubes, installed before planting, were used to measure moisture content in the soil profile. Soil moisture content were recorded every other week.

ERC water status

ERC leaf relative water content (RWC) was determined for the four treatments at the end of the experiment (August 2019). ERC leaf samples were weighed immediately after harvesting (fresh weight), then placed in a beaker with distilled water for 18 h at room temperature and then weighed to determine the saturated weight. The samples were then dried in an oven at 60°C for 24 h to obtain their dry weights. The RWC was calculated using the following formula[34,35]:

$$RWC=\frac{\text{f}\text{r}\text{e}\text{s}\text{h} \text{w}\text{e}\text{i}\text{g}\text{h}\text{t} – \text{d}\text{r}\text{y} \text{w}\text{e}\text{i}\text{g}\text{h}\text{t}}{\text{s}\text{a}\text{t}\text{u}\text{r}\text{a}\text{t}\text{e}\text{d} \text{w}\text{e}\text{i}\text{g}\text{h}\text{t} – \text{d}\text{r}\text{y} \text{w}\text{e}\text{i}\text{g}\text{h}\text{t}}\times 100$$

1

We also measured ERC leaf-water potential, at the end of the experiment, between 11:00 am and 1:00 pm using a Scholander pressure chamber, Model 1505D (PMS Instrument Company, New York, USA). Midday water potential represents the time period when the plants are the most active, most water stressed, and have the greatest Vapor Pressure Deficit (VPD)[36].

Statistical analysis

A univariate repeated measures analysis of variance (ANOVA) was used to analyze differences in soil moisture content in the four ERC treatments among different soil depths and different measurement dates (Sep. 2018, Nov. 2018, Jan. 2019, May 2019, and Aug. 2019). To evaluate seasonal progression of soil moisture content for 2018 and 2019 growing seasons, we also performed an ANCOVA for each ERC treatment, with depth as the covariate and month and ERC treatments (ERC-alone, ERC + post oak, ERC + smooth brome, ERC + post oak + smooth brome) as the independent variables. Seasons were defined as the first growing season for soil moisture data collected in September 2018, and second growing season for soil moisture data collected in August 2019. To examine possible seasonal shifts in soil moisture content, we used a linear and a cubic regression spline. We also used a LOESS regression to visualize possible trends in soil moisture content between the first (2018) and second growing season (2019) in the four ERC treatments.

We ran a univariate analysis of variance (ANOVA) followed by Scheffé *post hoc* tests to analyze differences in total biomass, root-length, ERC RWC, and midday leaf-water potential in the four ERC treatments. Pots with dead ERC were excluded from the analysis. ERC sapling mortality in the different ERC treatments was analyzed using a Chi-square (χ^2) test. All statistical analyses were run in SPSS 26.0 statistical package (IBM SPSS, 2019).

Declarations

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Author contributions statement

D.W. designed and supervised the experiment. S.H. performed the experiment and data collection. S.H and D.W analyzed the results. S.H. wrote the manuscript. J.M and D.W. critically revised the manuscript. All authors have read and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

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Figures

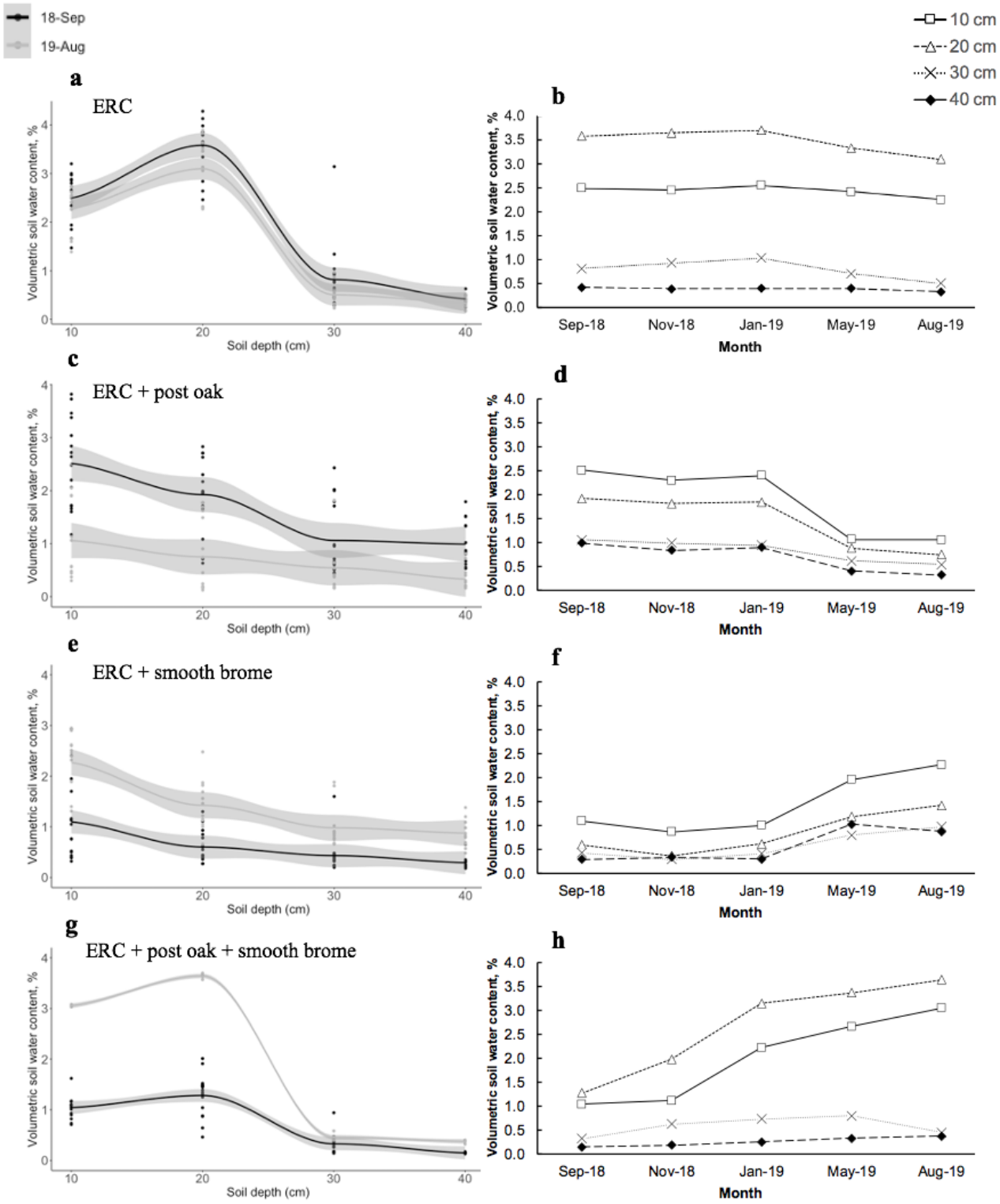

Figure 1

Relationship between volumetric soil water content (%) and soil depth (cm) in two growing seasons (September 2018 and August 2019) and mean soil moisture content at different depths over the experiment (two years). (a) volumetric soil water content at different depths for ERC-alone in 2018 (18-Sep) and 2019 (19-Aug) growing season. (b) mean volumetric soil water content at different depths for ERC-alone over different months. (c) volumetric soil water content at different depths for ERC + post oak

in 2018 and 2019 growing season. (d) mean volumetric soil water content at different depths for ERC + post oak for different months. (e) volumetric soil water content at different depths for ERC + smooth brome in 2018 and 2019 growing season. (f) mean volumetric soil water content at different depths for ERC + smooth brome for different months. (g) volumetric soil water content at different depths for ERC + post oak + smooth brome in 2018 and 2019 growing season. (h) mean volumetric soil water content at different depths for ERC + post oak + smooth brome for different months. The black circles and black lines (a, c, e, g) represent moisture content datapoints and LOESS regressions for 2018 growing season and the grey circles and grey lines represent moisture content datapoints and LOESS regressions for 2019 growing season. For the changes over time (b, d, f, h) the square symbols with the dashed line (–) represent water moisture data at 10 cm, the triangles and black line represent water moisture data at 20 cm, the circles and dotted line (..) represent water moisture content at 30 cm, and the and diamond long-dashed line (– –) represent water moisture data at 40 cm. Note that more soil water was available in the second measurement in cases where smooth brome was present (e, f, g, h) whereas the reverse was the case for combinations not including smooth brome (a, b, c, d).

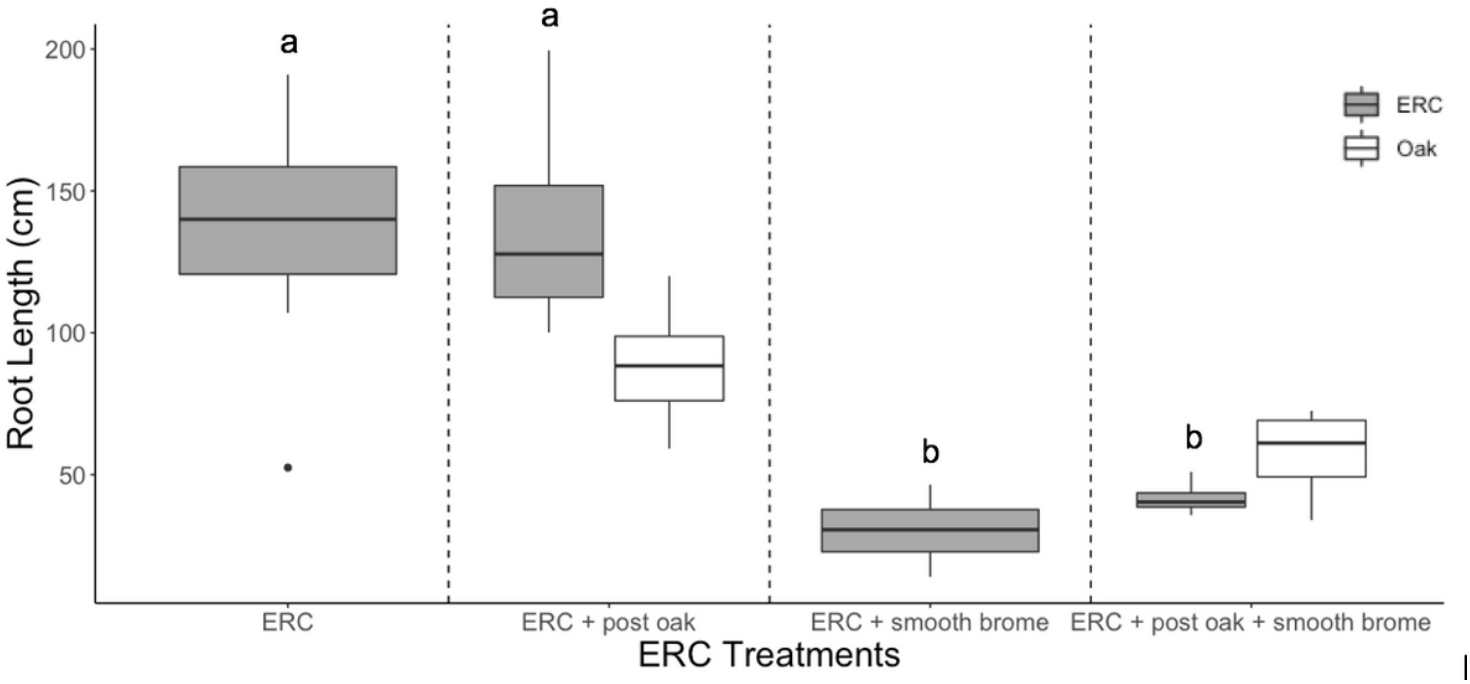

Figure 2

ERC and post oak root length (cm) in the four treatments. Smooth brome negatively affected ERC root length but there was not an effect of post oak competition. Different symbols represent significant differences (Scheffe´ post hoc test).

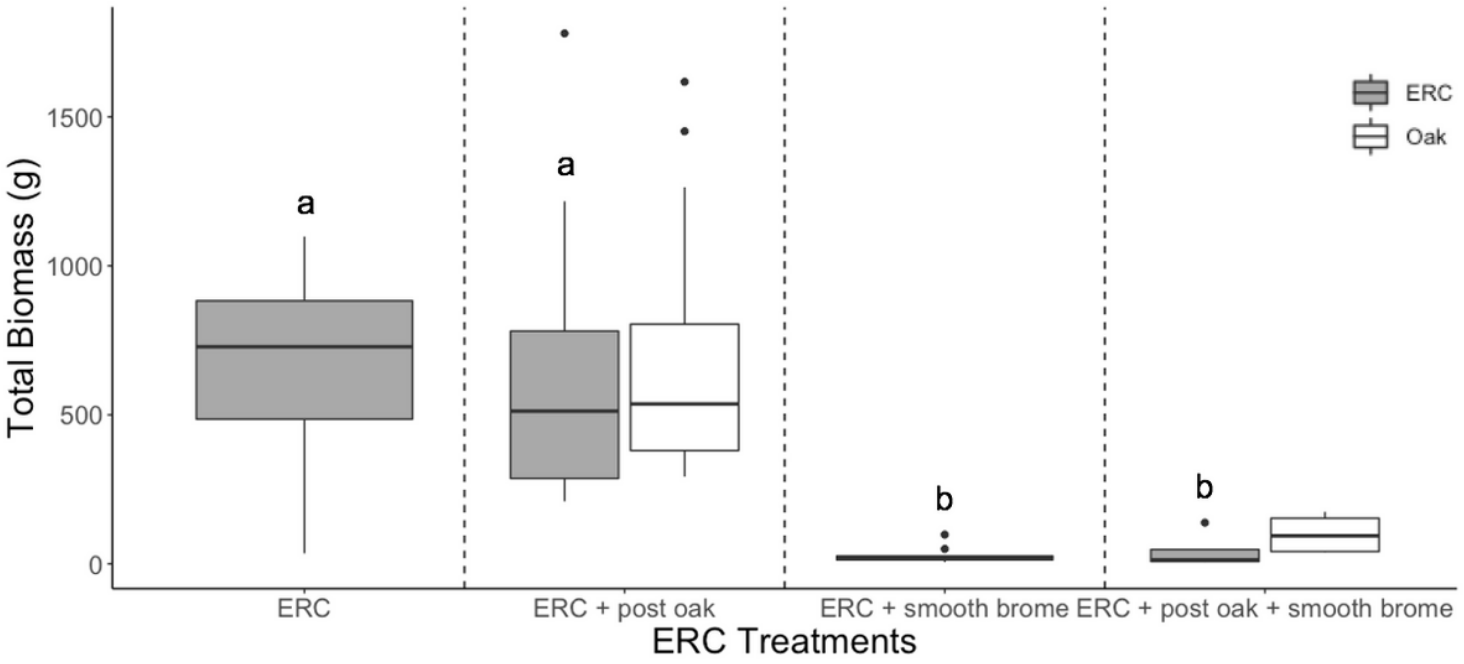

Figure 3

ERC and post oak total biomass (g) (above and below-ground) in the in four treatments. Smooth brome negatively affected ERC total biomass. Different symbols represent significant differences (Scheffe´ post hoc test).

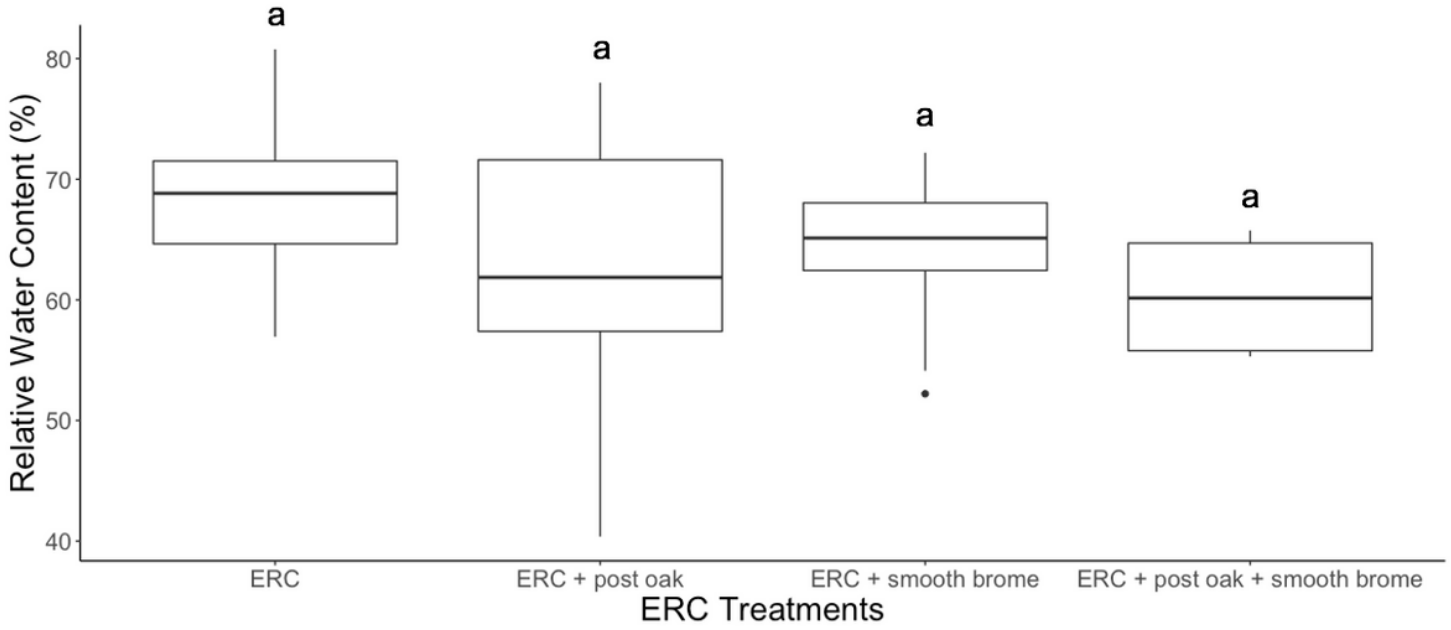

Figure 4

Relative water content (RWC) for ERC grown in four treatments. There was no significant difference in RWC across treatments, suggesting that it was not water stress that negatively affected the plants in the

smooth brome treatments. Different symbols represent significant differences (Scheffe´ post hoc test).

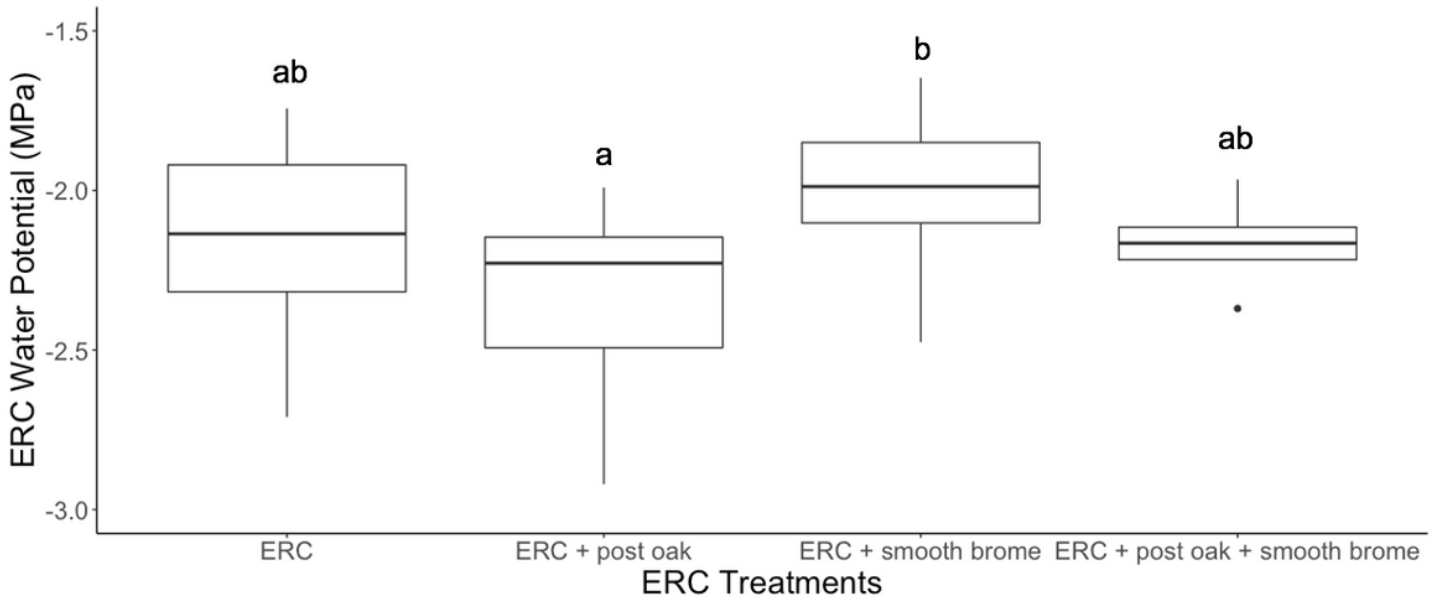

Figure 5

Midday water potential (MPa) at the end of the experiment for ERC grown in four treatments. There was a significant difference in water potential only between the ERC + post oak and ERC + smooth brome treatments, suggesting that it was not water stress that killed the ERC in the smooth brome treatments. Different symbols represent significant differences (Scheffe´ post hoc test).

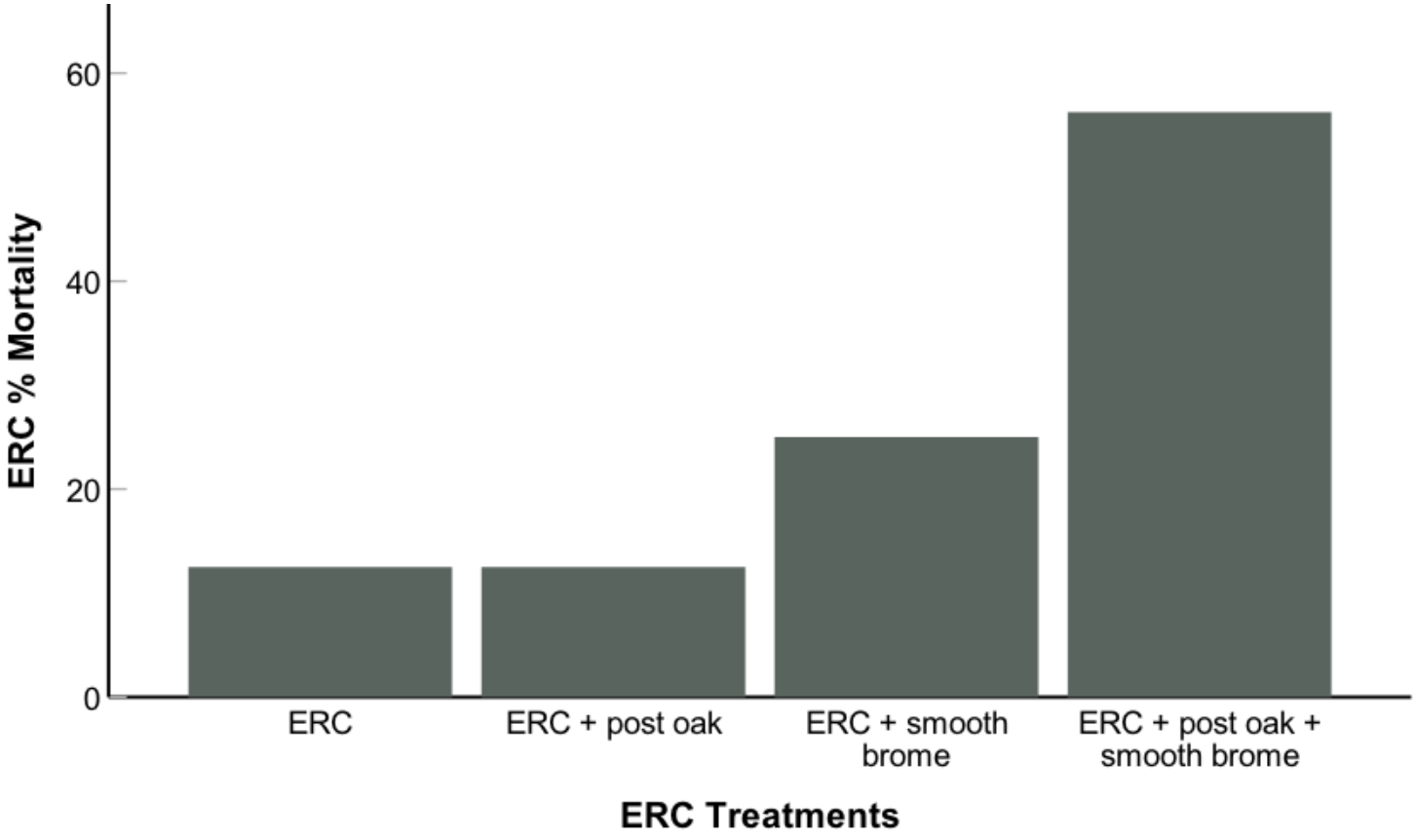

Figure 6

ERC mortality (%) at the end of the experiment in the four treatments. Smooth brome negatively affected ERC survival. The only significant difference was in the ERC + post oak + smooth brome.

Supplementary Files

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