THE IMPACTS OF HYDRAULIC REDISTRIBUTION ON THE PHYSIOLOGY AND GROWTH OF UNDERSTORY PLANTS IN LONGLEAF PINE (*PINUS PALUSTRIS*) SANDHILLS

by

PHOEBE ALEXIS JUDGE

(Under the Direction of Ricardo Holdo)

ABSTRACT

Understory plants can take up water lifted by trees, but the effects of hydraulic lift on these plants remain unknown. The objective of this study was to determine how hydraulic lift affects the physiology and growth of understory plants in longleaf pine sandhills. We measured soil moisture, and compared water potentials, gas exchange, and growth across four understory species in root exclusion and control plots. We did not find evidence of hydraulic lift during an unusually wet year. Soil moisture, physiological measurements, and growth either did not differ between treatments, or were greater in root exclusion plots, against expectations. Competition for water may therefore dominate overstory - understory interactions during wetter periods. Physiological parameters were correlated with soil moisture, suggesting that water does indeed limit plant functioning and productivity in these systems. The four species differed in their water usage over the growing season.

INDEX WORDS: Hydraulic lift, water potential, gas exchange, plant physiology, *Pinus palustris, Aristida beyrichiana*

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PHOEBE ALEXIS JUDGE

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PHOEBE ALEXIS JUDGE

Major Professor: Ricardo Holdo

Committee: Steven Brantley
Nina Wurzburger

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
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CHAPTER 1

Introduction

Longleaf pine woodland is one of the most biodiverse ecosystems in the Western Hemisphere (Peet and Allard, 1993). This ecosystem supports an incredibly species-rich understory groundcover community that includes over 120 rare and endangered plants (Noss, 1989), including the federally endangered American chaffseed (*Schwalbea americana*) (Walker, 1993). and the globally imperiled crestless plume orchid (*Pteroglossaspis ecristata* Fern.). Longleaf pine groundcover is also home to many rare animal species such as the gopher tortoise (*Gopherus polyphemus*), and to culturally important game species such as bobwhite quail (*Colinus virginianus*) that depend on the dense and diverse groundcover for survival. Longleaf pine once dominated much of the southeastern Coastal Plain, but timber harvesting for turpentine and lumber converted much of the land to agriculture (Peet and Allard, 1993). Fire suppression and soil disturbance in the last half century have further reduced the extent of longleaf pine forest. Less than three percent of its former area remains today, and only a portion of this remaining area has intact groundcover vegetation (Cox et al., 2004; Simberloff, 1993).

In addition to a drastic decrease in land area, longleaf ecosystems could be under further threat from climate change. The southeastern US has already experienced a significant decline in precipitation during the summer months over the past century, while extreme precipitation events have become more common (Kunkel et al., 2013). Future climate is even more uncertain, with some models predicting insignificant changes in precipitation patterns across the region, while

others show localized increases or decreases in annual precipitation (McNulty et al., 2019). Where most models agree is that the Southeast is likely to experience more climatic extremes in the future: droughts will become more frequent and severe, and rainfall will be more intense (USGCRP, 2018). Some models also predict a multiple-degree temperature increase in the region by the middle of this century (Kunkel et al., 2013). This could increase rates of evapotranspiration, leading to soil moisture deficits and water supply shortages (McNulty et al. 2019; Zhao and Dai, 2015). Given the probability of more frequent, severe droughts, understanding the role that different water resources play in maintaining diversity and function in longleaf pine savannas will be crucial to our understanding of how to preserve these special ecosystems.

In ecosystems that experience soil drought, hydraulic redistribution may be an important water source for plants. Hydraulic redistribution is the process whereby plants rooted in wetter soil strata passively transfer water to drier strata (Burgess et al., 1998). Hydraulic redistribution usually occurs at night, when transpiration ceases and dry soils can accept water from the root system (Richards and Caldwell, 1987, but see Donovan et al., 2003). Water moves into plant roots from adjacent soils with water potentials that exceed those of the roots, and this water then exits the roots in soil layers with low water potential (Ryel, 2004). Water potential gradients can occur across soil depths and amongst heterogeneous soil patches, and plant roots can therefore redistribute water in many directions (Burgess et al., 1998).

Hydraulic lift is a form of hydraulic redistribution whereby roots take up water in deep soil layers and transport it to shallower soil layers (Richards and Caldwell, 1987). Hydraulically-lifted water (HLW) has been found to make up a sizeable proportion of the water used by plants in various ecosystems. Richards and Caldwell reported that sagebrush (*Artemisia tridentata*) was

capable of lifting about 1 l/m² of water per night during the summer drying cycle. Domec et al. (2010) found that the hydraulic lift during the driest part of the year (July to October, in this study) accounted for about 10% of stand-level water use over the entire year in a coastal plain loblolly (*Pinus taeda*) forest in North Carolina. Meinzer et al. (2004) found that hydraulic lift replaced about 80-90% of the topsoil water removed by plants daily in six different forest and savanna sites, and ultimately curtailed the seasonal decline in soil water potential. In longleaf pine woodlands, recent work in Southwest Georgia has shown that longleaf pines can redistribute as much as a third of the water they transpire on a daily basis (Belovitch et al., 2022).

In some cases, shallow-rooted understory plants can take up this HLW. In a study of trees and grasses in a South African savanna with seasonal rainfall patterns, Priyadarshini et al. (2015) found that grasses, whose roots were confined to shallow soil layers, took up water redistributed by deeply-rooted trees during the dry season when topsoil water was depleted. Domec et al. (2010) observed that grasses, shrubs, and small trees took up most of the water deposited in upper soil layers by loblolly pines in the early growing season (May – August), and surmised that this likely elongated the growing season for these understory plants. Yu and D'Odorico (2015) modeled the interaction between several tree and grass species from southern Africa and found that grass species were predicted to be very effective at taking up the water lifted by the trees. These authors hypothesized that the ability of grasses to exploit this water source could at least partially explain the coexistence of trees and grasses in savannas (Yu and D'Odorico, 2015).

The extra water deposited in the topsoil via hydraulic lift beyond precipitation inputs may help buffer understory plants against the effects of water stress. In a split-root study looking at the effect of hydraulic lift by deeper-rooted legumes, Pang et al. (2013) found that shallow-

rooted legume individuals had higher water potentials (or water potentials stayed higher for longer, in some cases) when planted with the deeply-rooted legumes than when planted without them. Domec et al. (2004) observed much higher soil water potentials in old-growth ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga menziesii*) forest sites where hydraulic lift was occurring compared to sites with younger trees that lifted less water. The authors found that that HLW greatly reduced rates of root embolism in old-growth trees during the dry season. In longleaf pine, a field study in Southwest Georgia found similar results: in plots that were trenched to prevent access to HLW, understory plants from four functional groups experienced significantly lower pre-dawn leaf water potentials during the latter half of the growing season compared with conspecifics in untrenched controls (Belovitch, 2018).

This water supplement that shallow-rooted sandhill plants receive via hydraulic lift may impact their physiology and growth. HLW could enable plants to maintain greater cell turgor, which is required for growth (Boyer, 1986). Moreover, the main defense plants have against water loss in the short term is to close their stomates, which reduces leaf-level carbon uptake (i.e. photosynthesis). In the long term, this could result in reduced plant growth, although the extent to which a plant will be affected by drought will depend on the species and growing conditions (Farooq et al., 2012). Conversely, increases in available soil water could augment stomatal conductance and therefore growth. Prieto and Ryel (2013) found that sagebrush shrubs irrigated with deep water had higher stomatal conductance than unwatered controls, and they observed up to an 80% increase in transpiration rates the following day. Bogie et al. (2018) found that millet plants grown near shrubs performing hydraulic lift produced nearly ten times the biomass of millet plants grown in monoculture. Pang et al., (2013) also observed a 56–67% increase in shoot biomass in shallow-rooted legumes planted with deeply-rooted legumes performing hydraulic

lift. Enhanced growth and survivorship in plants with access to HLW could even substantially impact carbon fluxes at the ecosystem scale. Modeling work on a loblolly pine (*Pinus taeda*) plantation in the southeastern coastal plain has shown that gross ecosystem productivity in the presence of HLW could be about 750 grams of C m^{-2} per year greater than in the absence of HLW (Domec et al., 2010).

Along with increased carbon assimilation, HLW may also benefit plants by reducing cavitation in shallow roots and preventing hydraulic failure (Domec et al., 2010) although this may depend on woody stem density (Meinzer et al., 2004). Results from a modeling study in which hydraulic lift was simulated showed that dry-season lift by blue oak (*Quercus douglasii*) maintained soil moisture at high enough levels to prevent hydraulic failure among plants (Gou & Miller, 2014). There is also evidence that plant use of HLW in times of drought can prevent desiccation of shallow fine roots (Bauerle et al., 2008) and their mycorrhizal partners (Querejeta et al., 2006), and can increase nutrient uptake in some cases (Armas et al., 2011). Priyadarshini et al (2015) hypothesized that HLW could be a crucial water source for African savanna trees during the dry season by keeping the shallow roots functioning properly. They noted that moisture content of grasses under tree canopies was greater than in grasses outside of the canopy, and they observed more basal grass meristems under the canopy. Thus, shallow-rooted understory species may benefit from HLW in a similar manner to trees.

HLW may be an important water source for shallow-rooted understory plants in many ecosystems, and yet relatively little is known about its effects on understory plant community structure and functioning. The sandy soils of southwestern Georgia are an ideal study system to answer this question because substantial hydraulic lift by pines has been documented there (Belovitch et al., 2022). Furthermore, we know that understory species in this system are taking

up redistributed water, and that plants which are able to take up this water during the growing season experience less water stress than plants that cannot access HLW (Ford, 2004; Belovitch, 2018). Understanding how HLW impacts plant growth and functioning may enable us to predict how this process will influence these longleaf groundcover communities under future climate scenarios.

The goal of this study was to determine the effect of hydraulic lift on the physiology and growth of the dominant plant functional types present in xeric longleaf pine woodlands. We compared soil moisture and the physiological responses of understory plants with access to HLW to those without access to HLW. We hypothesized that soil moisture would be higher in plots with access to HLW and that plants in these plots would have greater stomatal conductance and photosynthetic flux densities, and have lower water use efficiencies, when compared to plots where HLW was excluded. We also compared growth and biomass in plants with and without access to HLW. Lastly, we compared water use efficiency and plot-level differences between midday and predawn water potentials among the four plant functional types. We expected that plants with access to HLW would have greater growth rates and overall biomass than plants without access to HLW. We also expected to see significant differences in water usage and response to HLW among the four plant functional types.

Methods

Study site

The study took place at the Jones Center at Ichauway, an 11,400-ha preserve located in the Dougherty Plain physiographic zone of southwest Georgia. This region is characterized by long, hot summers and mild winters. Mean annual temperature for the region is 20°C, with average

daily highs reaching 34°C in summer. Mean annual rainfall is 1310 mm and is distributed fairly evenly throughout the year (Goebel et al., 2001).

The study was conducted in an upland sand ridge adjacent to the Ichawaynochaway Creek. This part of the property is characterized by deep, excessively-drained soils in the Lakeland and Lucy soil series underlain by a shallow, unconfined karst aquifer (Kirkman et al., 2004). The vegetation is typical of a sandhill community, with an overstory dominated by longleaf pine *(Pinus palustris)* and xeric oaks such as turkey oak (*Quercus laevis)* and sand post oak (*Quercus margaretta*). The understory is dominated by wiregrass (*Aristida beyrichiana*) and other warm-season grasses, but forbs and sub-shrub form xeric oaks are common as well. This area is burned on a two-year rotation. The last burn occurred in February 2021, just before the start of this study.

At the beginning of the growing season in 2021, we randomly selected four trees from a pool of suitable candidate trees in the area. Suitability was based on size, distance from neighboring trees, and lack of groundcover disturbance. Tree sizes ranged between 40 and 55 cm diameter at breast height (dbh). We established two 4 x 4 m plots near each tree (Fig. 1). Plots were arranged in a blocked design, with two plots (one trenched and one control) randomly assigned to either the eastern or western side of each tree. Two of the trees had a greater density of woody stems on their eastern side than on their western side, so we randomly selected the eastern plot at one of these trees to be trenched, and assigned the plot on the eastern side of the other tree to be a control, to account for a possible confounding effect of woody plant cover with treatment. We randomly assigned the treatment and control plots to opposing sides for the remaining two trees. Plots were located 2.5 m from the base of each tree.

We trenched treatment plots with an automatic trencher (Ditch Witch, Perry, OK) to a depth of approximately 1 m. Trenched plots were 6 x 6 m to allow for a 1-m buffer zone between the trench and the 4 x 4 m experimental plot inside it (Fig. 1a). We lined trenches with two layers of 4-mm plastic sheeting with a layer of soil about 15cm thick between them, following the methods of Pecot et al. (2007).

Plant Diversity

We conducted diversity surveys in early May 2021 to determine the most abundant species from each plant functional group within each plot. We recorded vascular plant diversity and abundance within two randomly-chosen 1 x 1 m nested subplots located at the corners of each plot. We then surveyed the whole plot and recorded the presence of additional species not found within the nested subplots.

We selected study species (one from each of four PFTs) based on commonness (presence in all plots), abundance, and ease of measurement by the Li-6400. We selected species with leaves that would cover the surface area of the leaf cuvette, although some of the forb individuals did not have leaves large enough to do so, so we measured one forb basal leaf at a time, for which we then obtained the area to calculate physiological measurements on a per-area basis. We selected one species from four major functional groups in sandhill savanna understories: sand post oak (*Quercus margaretta,* subshrub), wiregrass (*Aristida beyrichiana,* graminoid), silkgrass (*Pityopsis aspera,* forb), and butterfly pea (*Clitoria mariana*, a leguminous herbaceous vine). To avoid confusion with common names, we refer to each species henceforth by functional type, with the exception of wiregrass (i.e., the oak, the legume, and the forb). We randomly selected three healthy study individuals from each species within each plot. In certain plots, only three individuals per species were present. We tagged each unique individual at the beginning of the

study, and sampled the same individuals throughout the study. The forb, the legume, and the oak are all clonal species, and in some cases, it was difficult to define distinct individuals (genets). For the purposes of this study, individuals that were located ≥ 1m apart were assumed to be hydraulically independent. One ramet (the 'main ramet,' hereafter) was tagged on each individual of these three species.

Environmental Conditions

We monitored soil moisture throughout the growing season using Teros10 (METER Group, Pullman, WA) probes. Data were logged every half hour with Em50 loggers (METER). We installed two probes horizontally in each of the eight plots at depths of 10 and 50 cm (Fig. 1). Cumulative rainfall data was also measured every half hour by a tipping bucket rain gauge (Onset, Bourne, MA).

Physiological Measurements

We measured pre-dawn leaf water potential on all study individuals every other week throughout the growing season using a Schölander – type pressure chamber (PMS Instrument Co., Corvallis, Oregon). Measurements began the last week of June and ended the second week of November. We measured pre-dawn leaf water potentials on all study individuals that still had leaves every week of the late growing season (starting the first week of October). We measured midday water potentials on two non-study individuals for each species within each plot. Non-study individuals were selected for the midday measurements to prevent over-harvesting of leaf material from the study individuals. We measured two leaves – rather than three - per species in each plot at midday due to time and resource constraints.

We measured photosynthesis (net carbon assimilation, or A_{net}) and stomatal conductance to water vapor (g_s) with a Li-COR 6400XT Portable Photosynthesis System (Li-COR, Lincoln,

Nebraska) on the same days we took water potential measurements. CO_2 flux was set to 410 µmol m^2 s^{-1} to reflect ambient conditions. Photosynthetic photon flux density (PPFD) was set at 1000 µmol m^2 s^{-1} for the C_3 species (the forb, the legume, and the oak) and 2000 µmol m^2 s^{-1} for wiregrass. Relative humidity in the chamber was kept between 50 and 80%, and temperatures were kept as close to ambient as possible. Since wiregrass leaves are very thin, we filled the chamber with one layer of leaves. Leaves were arranged side by side with minimal gaps and overlaps. During the first measurement period, we measured all three individuals of each species in each plot. On subsequent measurement periods, we had to reduce the number of individuals measured to two per species due to time constraints. Measurements occurred between 9 am and 2 pm over the course of two consecutive days with similar weather conditions. During the final measurement period (11/10/21), we took measurements on the newest sun leaves at the top of the plant on the oaks because the older leaves were dead or dying. These measurements may therefore not be comparable to oak measurements from earlier measurement periods.

In addition to the above measurements, we measured stomatal conductance on the oak and legume with an LI-600 porometer (Li-COR, Lincoln, Nebraska) during weeks when we were not measuring photosynthesis. This purpose of this was to increase the temporal coverage of our data without damaging the plants through repeated use of the LI-6400. We were unable to successfully measure wiregrass and the forb in the porometer without substantial leakage from the cuvette. We used the same type of oak and legume leaves that we measured with the LI-6400 (i.e., fully expanded, sun-exposed leaves large enough to fit in the $2cm^2$ LI-6400 chamber). We took two measurements per leaf using the auto-logging configuration and calculated their mean. We did not measure stomatal conductance on wiregrass or the forb because we were unable to

fill the porometer's open-system leaf chamber without substantial gas leakage occurring. Values with a leak percentage >10% were removed from the data.

Growth Rates

To assess changes in plant growth over the course of the experiment, we measured each individual at the beginning, middle, and end of the growing season. Initial measurements took place at the beginning of June, the second round took place at the end of August, and final measurements occurred at the end of November. Due to wide variations in growth forms among the study species, we used different growth metrics for different species.

For wiregrass, we measured the circumference at the base of each tussock at all three time points. For the oak and the forb, we measured the height of the main ramet up to the terminal bud. For the legume, we measured the length of the main ramet to the terminal node. We also measured the basal diameter of each oak main ramet by averaging two diameter measurements taken 90 degrees from one another at the base of the stem, just above the basal swelling.

For the forb, we counted the total number of ramets, leaves per main ramet, and total leaves on each individual during both the initial and final measurement periods. Some of the flowering stalks had died by the final measurement period, but there were basal leaves present for all individuals. Similarly, most of the aboveground legume biomass was dead by the time of the final measurement period. Additionally, we measured specific leaf area (SLA) on one representative leaf from every legume individual, and two representative leaves for each the forb individual (one stem leaf and one basal leaf). We scanned the leaves with a Plustek OpticPro scanner at 300 dpi and calculated leaf area using imageJ. We dried the leaves in a drying oven at 70°C for 72 hours and obtained specific leaf area by dividing leaf area by dry weight.

During the second measurement period (late August), we measured the number of ramets, the number of main ramet leaves, and height for both the legume and the forb. We also measured the length and width of leaves from each individual as a non-destructive measurement of leaf area. We harvested aboveground tissue of wiregrass, the oak, and the forb in early December 2021. For the wiregrass, we clipped the leaves at the base of the crown (current season's growth). For the forb and the oak, we clipped the entire shoot, as close to the ground as possible. Because the forb and the oak are both clonal, we also harvested secondary ramets originating from the same genet as the main ramet. Due to the patchier nature of the forb growth, it was more difficult to identify the genets. For this species, we placed a 0.1-m^2 quadrat around the main ramet, with the main ramet directly in the center of the square, and harvested all aboveground the forb biomass that fell within the square. We weighed the main ramets and secondary ramets for each individual plant separately, but we combined all aboveground biomass sampled from each individual when calculating final biomass. We dried all individuals in a drying oven at 70°C for 72 hours before weighing.

Statistical Analyses

We assessed differences in soil moisture between treatments using a linear mixed-effects model (function *lme* in the *nlme* package, Pinheiro et al., 2022) with treatment as the fixed effect and block as a random intercept with an autocorrelation structure of order 1. We first detrended the data from the effects of precipitation by subtracting the daily mean volumetric water content for all treatments and blocks (grand mean) from individual plot means, then finding the weekly average of these daily differences. We analyzed two probe depths separately.

We analyzed physiological responses using a repeated measures approach. We used linear mixed-effects models for these data as well (*lmer* function in package *lme4* **(**Bates et al.,

2015). We tested the fixed effect of treatment on detrended water potential, photosynthesis, stomatal conductance, and water use efficiency values for each species, with block and plant individual specified as random intercepts. We did not include plant individual as a term in the midday water potential models since different individuals were sampled each time. We then tested the effect of soil moisture at different depths on the raw photosynthesis, stomatal conductance, and water use efficiency values using the same model framework. Since soil moisture was collected at 30-minute intervals, each gas exchange measurement was linked to the volumetric water content from the previous 30 mins for a given depth.

We also tested the effect of the interaction between treatment and sampling date on the various growth metrics to determine whether or not species increased in size over the course of the growing season. We conducted post-hoc comparisons of means using the *emmeans()* function in the *emmeans* package **(**Lenth 2020), correcting for multiple comparisons with the Tukey method.

Finally, we compared differences in metrics of water use across species. First, we subtracted mean predawn water potentials from mean midday water potentials in each plot to estimate the mean daily water potential drop for each species. We used a one-way ANOVA (*aov()* function in package *stats*) to test the differences in this metric between species. To compare water use efficiency across species, we used a linear mixed-effects model with species as the fixed effect and block as a random intercept, with an autocorrelation structure of order 1.

For all models, we removed random effects if they explained no (or negligible) variance. We conducted t-tests using Satterthwaite's degrees of freedom method in the *lmerTest* package (Kuznetsova et al., 2017). If necessary, we transformed data with either the natural log or square

root function to meet test assumptions of normally-distributed residuals. All analyses were conducted in R (version 3.6.3, R Development Core Team, 2016), in the RStudio environment.

Results

Treatment effects

Cumulative rainfall for the study period was 565mm (Fig. 2), which was 53% higher than normal for the months of July – November, based on a 24-year mean (1996-2020) on Ichauway. Soil moisture at 10 cm depth did not differ between trenched and control plots ($p = 0.13$) (Fig. 3). Trenched plots were significantly wetter than control plots at 50 cm depth ($p = 0.0$), but this difference was small (1.1% VWC). The mean VWC for the period during which gas exchange measurements were taken (6/24/2021 – 11/10/2021) was 9.5% in the control plots and 10.6% in the trenched plots.

Physiological measurements

Predawn water potentials did not differ between treatments for any of the species except for oak ($p = 0.002$). Water potentials were just 0.04 ($\pm$ 0.01) MPa higher in trenched plots compared to control plots for this PFT. Pre-dawn water potentials generally remained high over the course of the growing season for all species except for the forb (Fig. 4a). The forb experienced the lowest predawn water potential (< -1.5 MPa) at the beginning of the growing season, but water potentials remained high for the forb thereafter, until a small dip near the end of the growing season. No other species experienced water potentials below -0.7 MPa at any point during the study. Midday water potentials differed by treatment for the oak ($p = 0.02$) and the forb ($p = 0.01$), but not for wiregrass ($p = 0.76$) or the legume ($p = 0.67$) (Fig. 4b). Water potentials were 0.10 ($\pm$0.04) MPa higher in the trenched plots than controls for oak and 0.18 ($\pm$ 0.05) MPa

higher for the forb at midday. Most species experienced a noticeable dip in midday water potentials during the end of the growing season. This pattern was the same for both treatments.

Net carbon assimilation (A_{net}) differed significantly by treatment for wiregrass (p = 0.02) and the legume (p = 0.02), but not for the oak (p = 0.86) or the forb (p = 0.56) (Fig. 5). When averaged over the entire growing season, A_{net} was 1.6 μmol m^2 s^{-1} higher in trenched plots for wiregrass and 1.29 μmol m^2 s^{-1} for the legume. Stomatal conductance differed significantly by treatment for wiregrass (p = 0.001), and the forb (p = 0.048), but not for the legume (p = 0.11) or the oak (0.57) (Fig. 6). When averaged over the entire growing season, g_s was 37 μmol m^2 s^{-1} higher in trenched plots for wiregrass and 43 μmol m^2 s^{-1} higher for the forb. There was no difference in water use efficiency (WUE) between treatments for any of the species (Fig. 7).

Growth

In general, treatment had little effect on plant growth over the course of the growing season. For a few species, growth rates were slightly higher in trenched plots compared with control plots. Wiregrass circumference did not increase in size over time, nor did it differ significantly between treatments (p = 0.32) (Fig. 9). Oak diameters generally increased in size throughout the growing season (p < 0.001), but most of the growth occurred between the first and second sampling periods. During this period, oak DBH increased by about 2.88 mm on average (Fig. 9a). Oaks did not significantly increase in size between the second and final sampling periods in either treatment (1.13 cm, 95% CI [-2.58, 0.33]). Diameter growth did not differ between treatments over the course of the season (p = 0.93). Oaks also increased in height over time (p < 0.001), but there was no difference in growth between treatments (p = 0.65), nor was there an interaction between date and treatment (p = 0.65) (Fig. 9b). The legume increased in length (of the tallest ramet) between the beginning and end of the growing season in trenched plots (p = 0.04), but not

in controls (p = 0.82) (Fig. 10). Forb stem heights increased between the initial and second sampling periods (p < 0.001). Forb stems were higher on average in control plots than in trenched plots in the beginning of the season (p = 0.004), but plants in trenched plots grew faster than plants in control plots (Fig. 11). Stem heights increased in trenched plots by about 16.3cm ($\pm$ 2.72), while heights increased by 11.3 ($\pm$ 2.67) in control plots. There was no significant difference in final biomass (total plant weight harvested at the end of the growing season) between trenched and control plots for any of the species measured (wiregrass: p = 0.17 oak: p = 0.36, forb: p = 0.51) (Fig. 12).

Environmental conditions and plant performance

Photosynthesis was correlated with VWC at 10cm for wiregrass (p = 0.002), the oak (p = 0.022) and the legume (p < 0.001), but not for the forb (p = 0.71) (Fig. 13). Photosynthesis was correlated with VWC at 50cm for all species (wiregrass: p < 0.001, oak: p < 0.001, forb: p = 0.03, legume: p < 0.001). Stomatal conductance (g_s) was correlated with VWC at 10cm for the forb (p = 0.008) and the legume (p < 0.001), but not for wiregrass (p = 0.13) or the oak (p = 0.95) (Fig. 14). Stomatal conductance was significantly correlated with VWC at 50cm for all species (wiregrass: p = 0.04, oak: p = 0.041, forb: p < 0.001, legume: p = 0.001). Both gas exchange metrics increased with increasing soil moisture. Water use efficiency (WUE: A_{net}/E) also varied significantly with soil moisture for all plants at both 10cm and 50cm. As VWC increased, WUE decreased for all plants (Fig. 15).

There were large differences in both predawn and midday water potentials among the four species (p < 0.001) (Fig. 16). The daily differences between midday and predawn water potentials within each plot also varied significantly by species (p < 0.001). The seasonal average difference between predawn and midday water potentials was greatest for the oak (-2.33 $\pm$ 0.07

MPa) and wiregrass (-2.07 ± 0.09 MPa). This value was much lower for the forb (-1.14 ± 0.05 MPa) and the legume (-0.95 ± 0.05 MPa). The oak also displayed the maximum difference during growing season (-4.07 MPa), followed by wiregrass (-3.63 MPa), the forb (-2.32 MPa), and the legume (-2.07 MPa). WUE also differed significantly by plant species ($p < 0.001$) (Fig. 17). Species WUE varied from a seasonal average of 0.45 (± 0.01) μmole C/mmol H_2O for wiregrass, a C_4 species, to 0.25 (± 0.19) μmole C/mmol H_2O for the forb.

Discussion

We predicted that plants with access to HLW would have greater stomatal conductance and net carbon assimilation, and lower water use efficiencies, than plants without access to HLW. We also expected plants with access to HLW to have higher growth rates. These hypotheses were not supported. The unusually high rainfall during this study may have precluded hydraulic lift from occurring, or from having a measurable effect on plant physiology or growth. Shallow soil moisture did not differ by treatment, while soil moisture at 50cm depth was slightly higher in trenched plots. Consequently, we observed no negative physiological effect of tree root exclusion, and in some cases, there was greater leaf-level photosynthesis and stomatal conductance in our trenched plots. There were also no differences in water use efficiency between trenched and control plots for any species. Growth rates did not differ by treatment for wiregrass and oak, but forbs and legumes had higher growth rates in trenched plots. These results suggest that the pines may have been competing with the understory species for water in our study.

If hydraulic lift had occurred, we would have expected to observe nocturnal peaks in soil moisture in the shallow soil of control plots as water was deposited into this layer by longleaf

pine roots. We also would have expected to see a general pattern of higher mean daily soil moisture in control plots compared to trenched plots as soil dried. We did not observe this in any plot. The exceptionally wet growing-season conditions may have prevented shallow soils from drying down enough to create the water potential gradient between deep and shallow soil moisture necessary for hydraulic lift to occur.

We also would have expected to see greater leaf water potentials in plants in control plots compared with plants in trenched plots. Our hypothesis was not supported, however. Both predawn and midday water potentials were generally greater in trenched plots or did not differ between treatments. Furthermore, predawn water potentials for wiregrass, the oak, and the legume remained consistently high (the seasonal minimum for these three plant types was -0.7 MPa) throughout the growing season. The predawn values that we found for these three plant types appear on average higher than the predawn values averaged across four understory species from the same system in Belovitch (2018). The forb differed from the other species in that it experienced several dips in predawn water potentials during the growing season. These dips in some cases coincided with periods of lower soil moisture, and in other cases could have been the result of phenological changes (the leaves on this species senesced toward the middle of the season, then regrew). These results indicate that, with the exception of the forb, our study plants may not have experienced meaningful water stress at any point during the growing season.

We also hypothesized that stomatal conductance and photosynthesis would be greater in control plots compared with trenched plots. As with the water potential measurements, however, we found that both physiological parameters either did not differ between treatments, or were greater in trenched plots compared to controls. These results are consistent with the findings of Barron-Gafford et al. (2021). They conducted a trenching study similar to ours and found that

leaf-level gas exchange values in grasses and plot-level gross ecosystem productivity were greater in trenched plots during a year with high rainfall. During a subsequent dry period, however, they recorded greater gas exchange and gross ecosystem productivity in control plots than in trenched plots. They also observed hydraulic lift occurring during this dry period, and recorded higher soil moisture in control plots. These findings suggest that in wetter years, HL may not occur, and competition – rather than facilitation - may dominate overstory tree – understory plant interactions. Under drier conditions, therefore, we might have been able to see increased soil moisture and photosynthetic flux densities in our control plots relative to the trenched plots.

A similar trenching experiment in longleaf pine stands on Ichauway found contrasting results, however. Pecot et al., (2007) reported that, despite record drought conditions, trenched plots in these stands were on average wetter than untrenched plots in the first 30cm of soil. They attributed this to the exclusion of pine roots from the trenched plots. There could be several explanations for this. Even in a drier period, it is possible that competition for shallow soil water can overwhelm the effects of facilitation via hydraulic lift. The shallow roots of longleaf pines could have been taking up any water that they lifted during this study. Alternatively, it is possible that conditions were too dry for hydraulic lift to occur. If the water table receded past the depth at which the pines could reach it during the dry-down period, hydraulic lift might not have been possible.

The findings of Pecot et al. contrast those of Belovitch (2018), who found facilitative effects of HL on understory species of varying functional types in a nearby xeric site on Ichauway. Pecot et al. also found that herbaceous understory plants responded mainly to changes in light, while woody plants responded to changes in belowground resources. This contradicts

our finding that there were no differences in gas exchange and growth between treatments for the oak for the three herbaceous species. However, Pecot et al.'s study was conducted on a more mesic site, where many species, including pines, may concentrate roots in shallower soils where water is relatively abundant. This may not be the case in more xeric sites, although fine root demography may change with precipitation patterns. The xeric site at which our study took place is also less productive, and is therefore likely less light-limited than the more mesic parts of property.

We observed trends in plant growth that were analogous to those we observed in the physiological variables. We found greater growth rates in trenched plots than in control plots for both the legume (measured as ramet length) and the forb (stem height), suggesting that the differences in gas exchange values between treatment types, although small, could have been physiologically relevant for these two species. There was no difference, however, in wiregrass growth between the two treatments, despite greater stomatal conductance and photosynthesis in trenched plots than in control plots. Wiregrass did not grow in circumference during the study period. In some plots, circumferences decreased over the course of the growing season. However, we did observe that wiregrass individuals in trenched plots appeared to have more, longer, and healthier-looking leaves than individuals in control plots. There could be several reasons for this. First, at the beginning of the season, we may have measured dead material at the base of the tussocks that was removed over time. It is also possible that resources from the crown were redistributed to other parts of the plant (i.e. elongating leaves). Either way, it is likely that measuring basal circumference - while successful for other tussock grasses – was not the best way to capture wiregrass growth.

We also found no difference in oak growth between treatments. This finding contrasts that of Pecot et al. (2007), who reported that hardwood growth increased in trenched plots on Ichauway during their study. They attributed this to the removal of competition with adult pine trees for belowground resources. Other studies have also shown that excluding longleaf pine roots from gaps increases nutrient availability and root mass production of non-pine species (Jones et al., 2003). It is possible that the oaks in our study rooted deeply enough to access a steady source of deep soil water, and were thus largely unaffected by the trenching treatment. The short stature of the oaks in our study likely do not correlate with age because frequent fire may restrict aboveground growth while encouraging growth belowground.

Competition for shallow soil water between longleaf pines and understory species could explain why we saw higher soil moisture, water potentials, stomatal conductance, photosynthesis, and growth in some plants in trenched plots, compared with those in the controls. By trenching, we may have isolated the understory plants from competition with the longleaf pine roots, rather than isolating them the effects of hydraulic lift. Longleaf pines, like many trees, can alter patterns of root water uptake based on growing conditions (Belovitch et al., 2022). During drier periods, these trees likely rely on deeper soil water stores, while during wetter periods, they are known to favor water uptake in shallow soils (Belovitch et al., 2022). Even with higher-than-average moisture in the shallow soil layers during this study, the extensive lateral roots of the pines could have been more effective at taking up this water than the small-statured understory species, with the possible exception of the shrub form oaks. If conditions had been drier and shallow soil water potential were low, the pines would likely have switched to absorbing groundwater via their deeper roots (Belovitch et al., 2022).

Environmental conditions and plant performance

Gas exchange parameters were correlated with soil moisture in our study, indicating that water limitation is important in this system. Both stomatal conductance and photosynthesis increased with increasing volumetric water content for all relationships that were significant. This suggests the plants in our study were responding to differences in soil moisture and that if we had found greater soil moisture in control plots, we would likely have seen higher stomatal conductance and photosynthesis in these plots as a result. We also observed a greater correlation between both photosynthesis and stomatal conductance and volumetric water content at 50 cm depth than at 10 cm depth. Water content in the 50 cm layer therefore appears to be more physiologically relevant to these groundcover species than water content in the 10 cm layer. This could simply be because the plants in our study have higher root densities at 50 cm than in shallower soil layers.

Predictably, there were notable differences in water use among the four species studied. These differences could help explain different growth responses of these species among treatments. The diurnal difference between midday and predawn water potentials varied significantly by species. This difference is thought to reflect the degree to which a plant will regulate its water status under specific environmental conditions, primarily via changes in stomatal aperture (Ratzmann et al., 2019). Plants that allow midday (daily minimum) water potentials to drop in concert with predawn (soil) water potentials are said to behave more isohydrically, while plants that maintain high minimum water potentials are said to behave more anisohydrically (*sensu* Tardieu and Simonneau, 1998). The legume and the forb behaved isohydrically during this study, preventing midday water potentials from dropping much below predawn water potentials. The legume, in particular, maintained high water potentials throughout the growing season. Wiregrass and the oak, by contrast, behaved comparatively anisohydrically.

The maximum difference between predawn and midday water potentials was 3.63 MPa for wiregrass and 4.07 MPa for the oak. The fact that the species behaving the most anisohydrically in our study also had higher carbon assimilation is consistent with the traditional view of isohydricity, which posits that as soil dries, isohydric plants will forgo high rates of carbon assimilation in favor of halting water loss, while anisohydric plants will maintain high rates of assimilation at the risk of hydraulic failure (Garcia-Forner et al., 2017). More recent research has challenged this view, however. Several studies have reported lower rates of assimilation in so-called anisohyric species compared to isohydric species, calling into question both the usefulness of the concept and its implications for plant performance during drought (Garcia-Forner et al., 2017; Jiang et al., 2020). Certainly, collecting data on our species during drier conditions will provide insight into whether or not the differences in isohydricity we observed will both remain consistent under drought, and will have consequences for capacities for carbon assimilation and growth.

Water use efficiency (WUE) also differed significantly by species. Wiregrass, a C_4 grass, unsurprisingly had the highest WUE, a pattern which was largely driven by its high photosynthesis values, which were the highest of all four species (17.9 μmol C m^2 s^{-1} compared to 13.0 for oak, 11.0 for the forb, and 9.03 μmol for the legume). Transpiration rates for wiregrass were comparable to those of the other species (it had the second highest transpiration rate, behind the forb), despite the fact that it is a C_4 species. The forb had the lowest water use efficiency, despite behaving isohydrically throughout the study. The forb had low photosynthetic flux densities compared to the other species, while maintaining the highest transpiration rate. WUE increased with decreasing soil moisture for all species at both soil depths. This result is unsurprising: as water availability decreases and stomata close, transpiration generally decreases

more than photosynthesis because water vapor encounters less resistance on its path to the atmosphere than carbon. There is also evidence that plants can increase mesophyll conductance of CO_2 under drought conditions (Hommel et al., 2014). Some plants can also increase the tortuosity of the path of water through the mesophyll, thereby restricting water loss and increasing WUE (Hommel et al., 2014).

These findings could have implications for how future climate scenarios impact overstory-understory dynamics and understory plant functioning in longleaf pine sandhills. Competition between longleaf pine and understory species may be more important during wetter periods, while facilitation could be important during drier periods. There is evidence that facilitation of understory species by pines does indeed occur in this system: water potential data shows that plants in control plots experienced less water stress than plants in trenched plots (Belovitch, 2018). There is also evidence for midstory oaks facilitating the survival of longleaf pine seedlings in a longleaf pine sandhill, although the authors mainly attributed this effect to the reduced moisture stress associated with a lower vapor-pressure deficit under denser oak canopies (Loudermilk et al., 2016). There are not many midstory hardwoods at our site, so it is unclear how understory plants there would respond to the increased vapor-pressure deficit that often accompanies meteorological droughts, even if facilitation by hydraulic lift did occur.

HL may simply be more temporally variable in longleaf pine savannas than previously assumed and the conditions it requires simply did not occur, or did not occur consistently, during this study. We also cannot discount the possibility that HL is highly spatially variable, and that we simply did not capture it where it was occurring. When we re-excavated the trenches from our study down to a depth of 30cm, we found hundreds of severed pine roots smaller than 1cm in diameter, and at least two large pine roots greater than 5cm in diameter in each plot (Fig. 18).

These roots would have penetrated the trenched plots if they had not been severed by the trenching, and many of the roots would have terminated within the plots. This confirms that the trees in our study have many lateral pine roots exploring shallow soil in our plots and suggests that these trees are all capable of hydraulic lift under the right conditions. Nonetheless, it is possible that some other factor (i.e., soil conditions) would have precluded hydraulic lift from occurring in our plots.

It is unclear how precipitation patterns will change over the coming century in the Southeast. The region has been experiencing less frequent but more intense rainfall events over the past century (Kunkel et al., 2013). Summer months have become slightly drier, with longer periods of no rainfall. Some models predict that rainfall will stay the same, or even increase, in certain parts of the region. However, other simulations show rainfall becoming more variable in the region, with some very wet years and others very dry (Li et al., 2011; Wuebbels et al., 2014; McNulty et al., 2019). Hydraulic lift could be an important water source for understory plants in drier years but might not be happening at all during wetter years. An important next step in this research will be to investigate how frequently hydraulic lift actually occurs in these systems. If hydraulic lift only happens in very specific conditions that do not arise very often, any facilitative effects of this process on shallow-rooted species may not be crucial for their survival and performance. On the other hand, HLW water may keep shallow-rooted plants alive during occasional drought periods. Once we determine the frequency of hydraulic lift in this system, future research could focus on tracking changes in plant performance and community composition in relation to patterns of hydraulic lift across multiple years. This will provide further insight into the understudied effects of this process at the ecosystem scale.

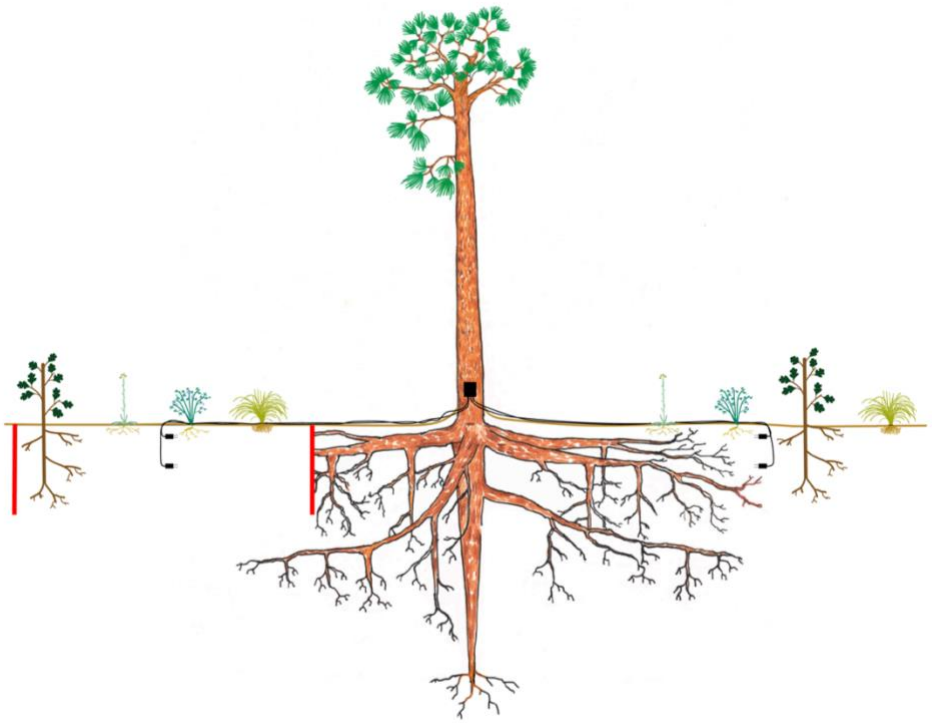

Figure 1. Example setup for an experimental block, showing trenched (left) and control (right) plots. Red bars represent trenches.

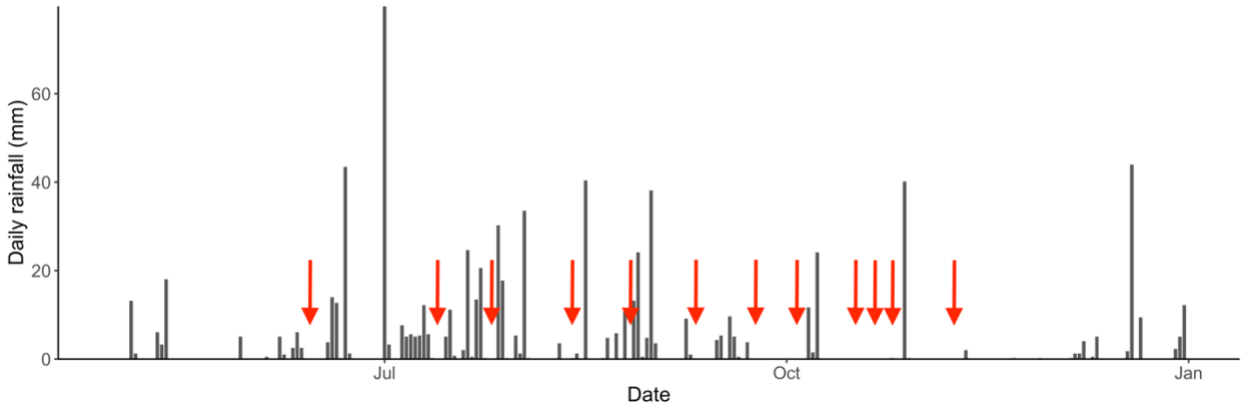

Figure 2**.** Total daily rainfall in mm over the course of the 2021 growing season. Red arrows indicate the timing of gas exchange measurements (2-day periods).

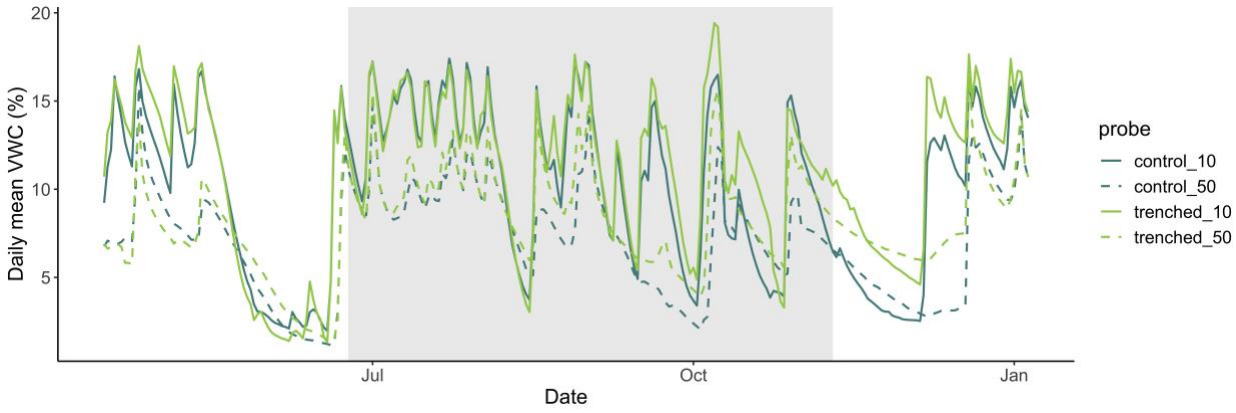

Figure 3. Daily volumetric water content in control (in dark green) and trenched plots (in light green), averaged across all blocks. Solid lines represent 10cm depth data, while dashed lines represent 50cm depth data.

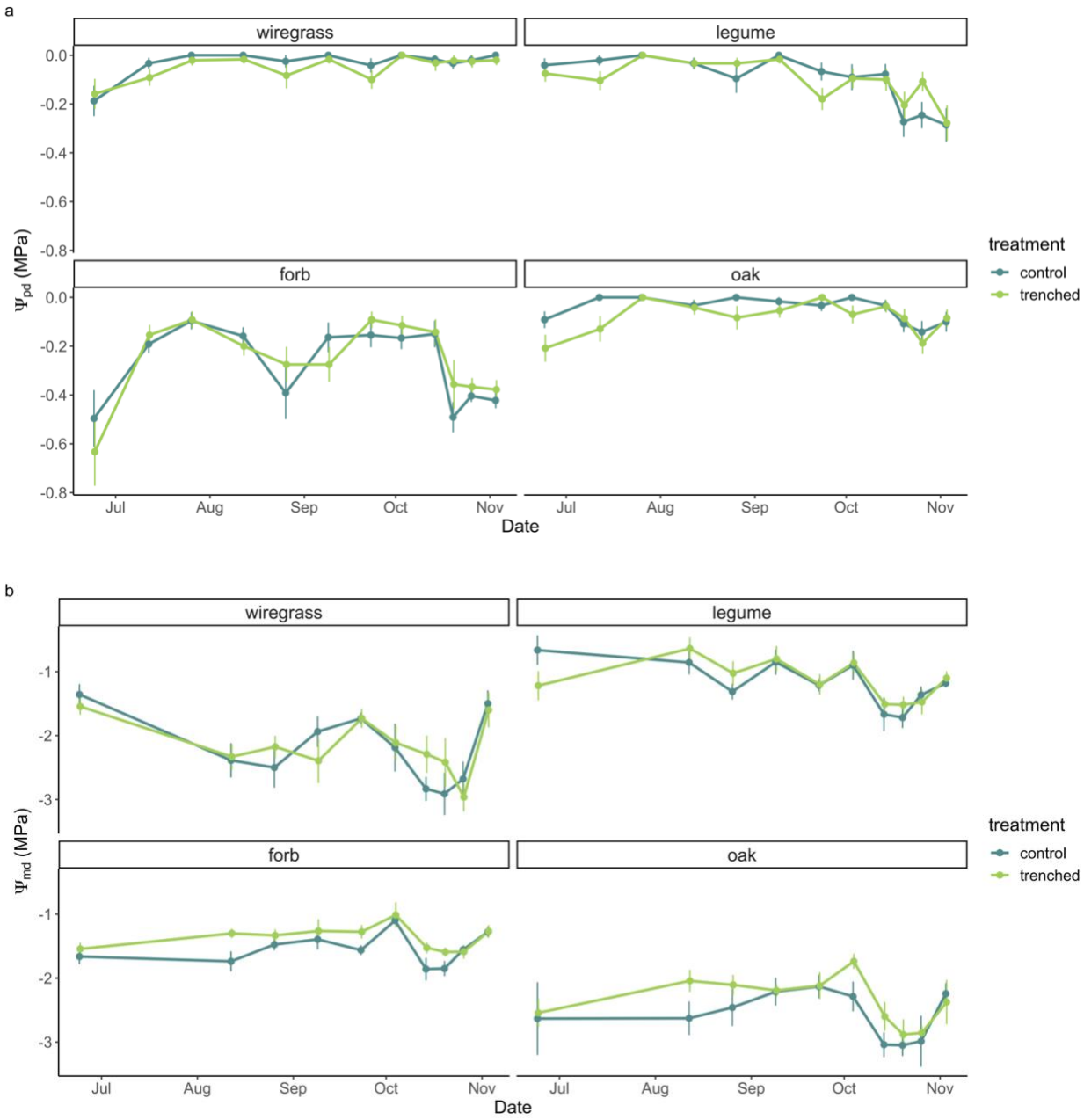

Figure 4. Predawn (a) and midday (b) water potentials (mean ± SE) for each species over the course of the growing season. Plants in trenched plots are represented by light green points, while plants in control plots are in dark green.

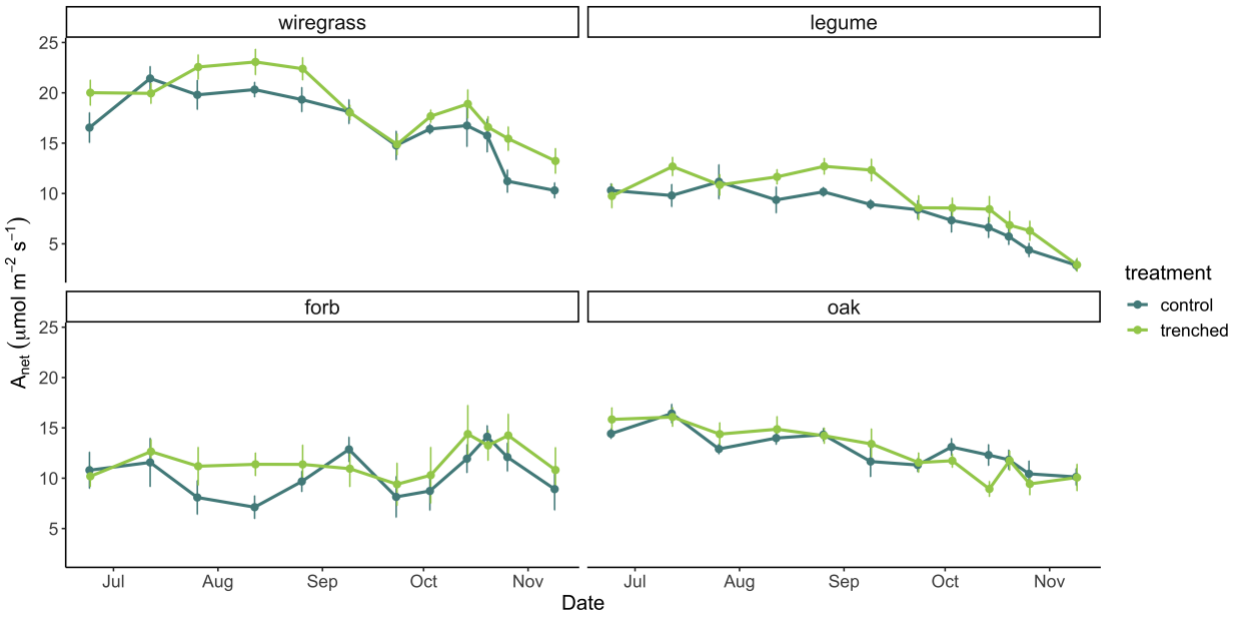

Figure 5. Photosynthesis (µmol m^2 s^{-1}) measurements (mean ± SE) at each sampling period for the four species. Light green points represent plants in trenched plots, while darker green data represents plants in control plots.

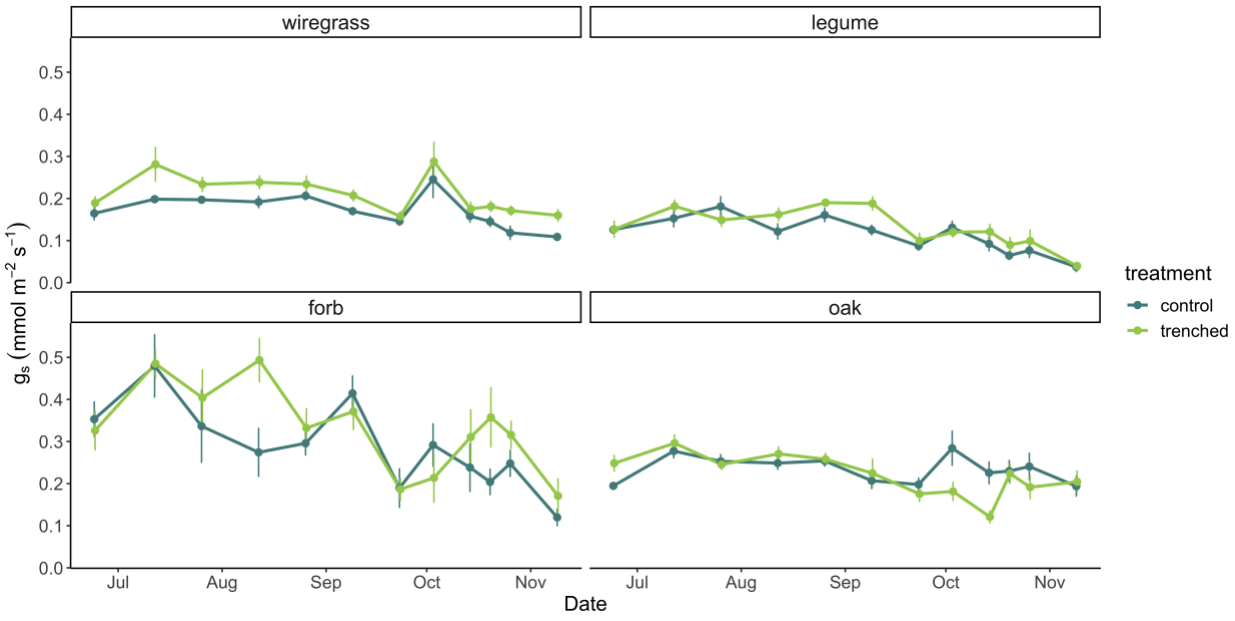

Figure 6. Stomatal conductance (mmol H2O m^2 s^{-1}) measurements (mean ± SE) at each sampling period for the four species. Light green points represent plants in trenched plots, while darker green data represents plants in control plots.

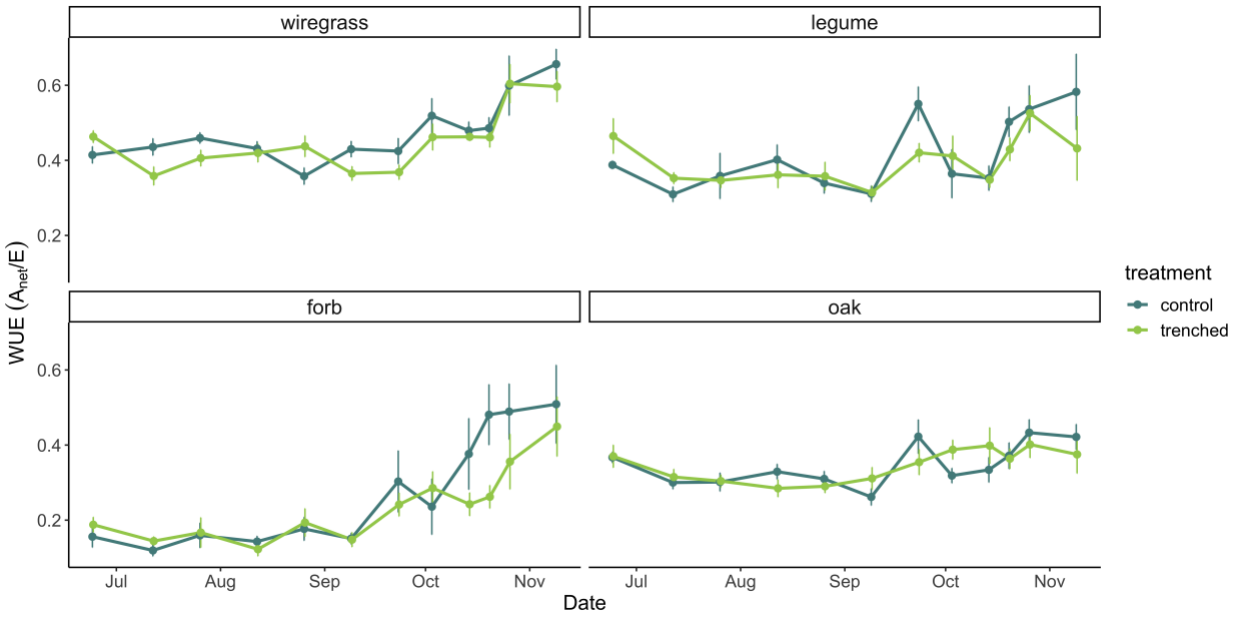

Figure 7. Water use efficiency (µmol C/mmol H_2O) measurements (mean ± SE) at each sampling period for the four species. Light green points represent plants in trenched plots, while darker green data represents plants in control plots.

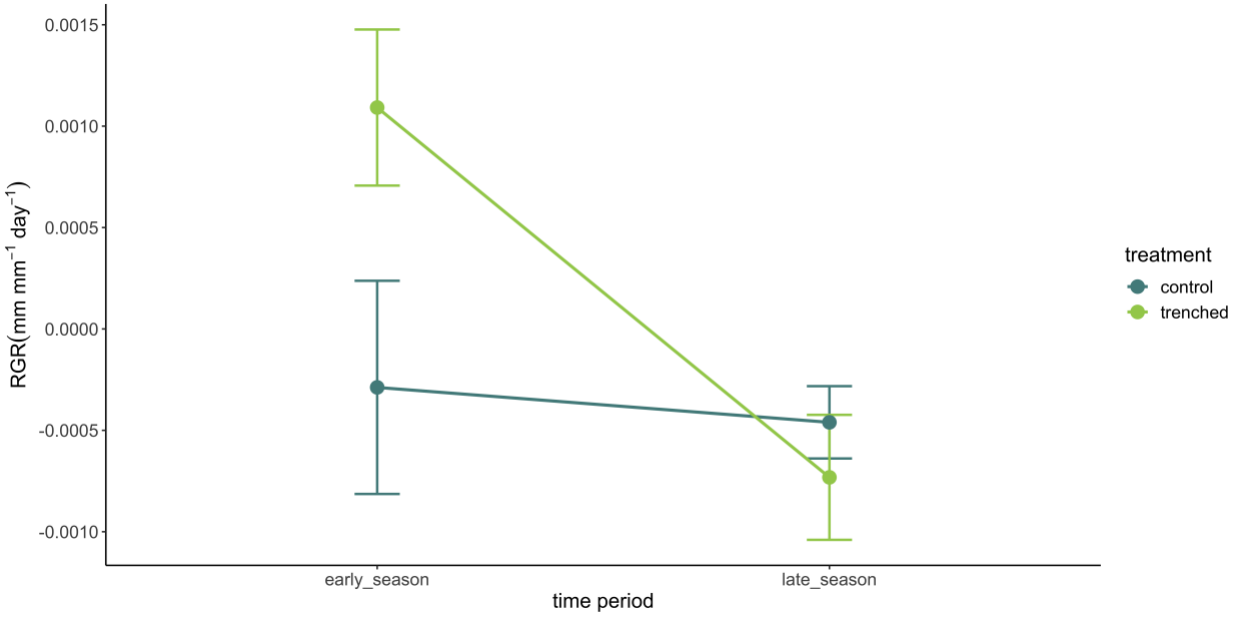

Figure 8. Change in tussock circumference (mean ± SE) of wiregrass plants over the course of the growing season. Light green lines represent plants in trenched plots, while the darker green lines represent plants in controls.

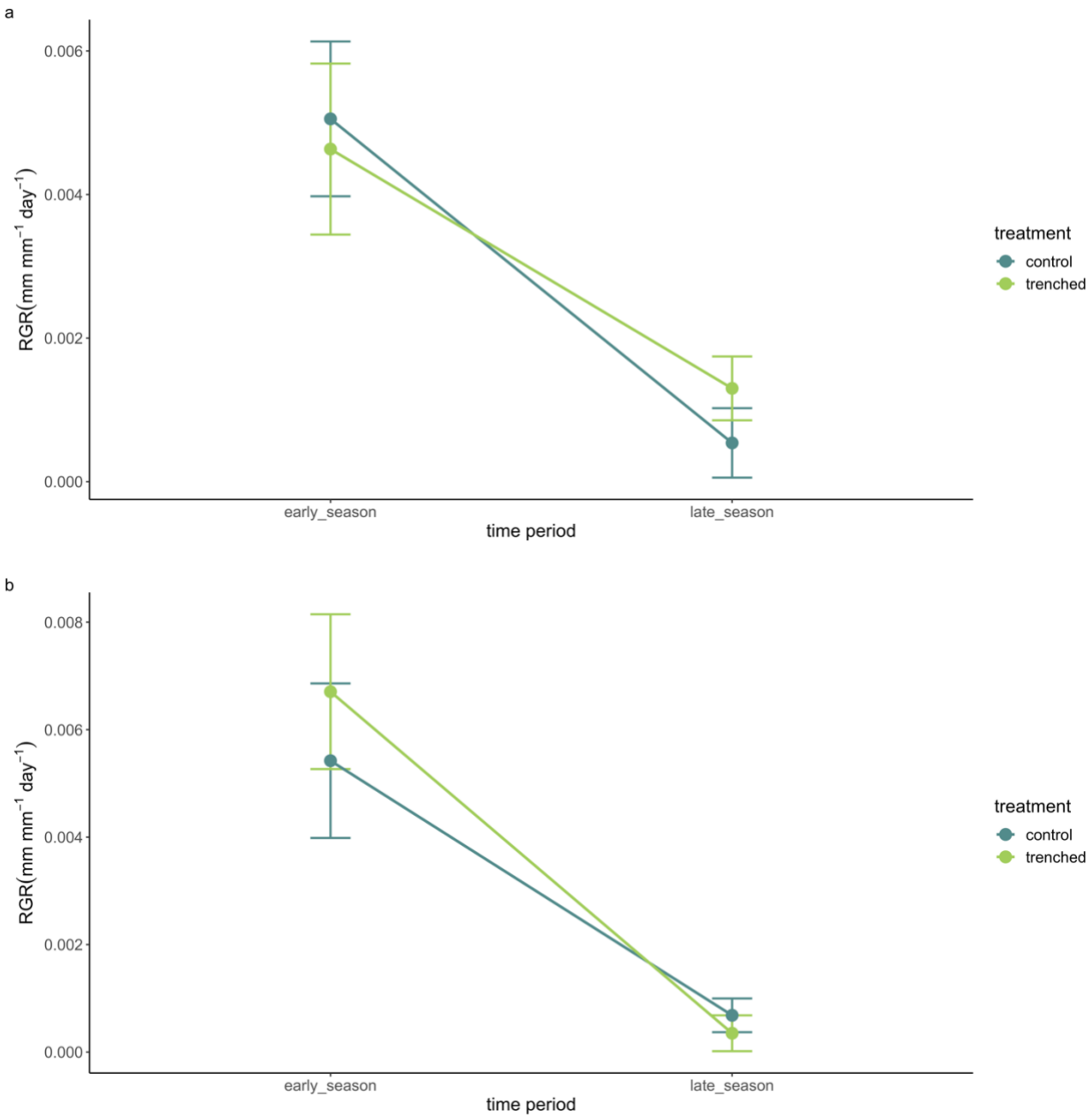

Figure 9. Change in diameter at breast height (a) and the change in height (b) (mean ± SE) of the main ramet of each oak individual over the course of the growing season. Light green lines represent plants in trenched plots, while the darker green lines represent plants in controls.

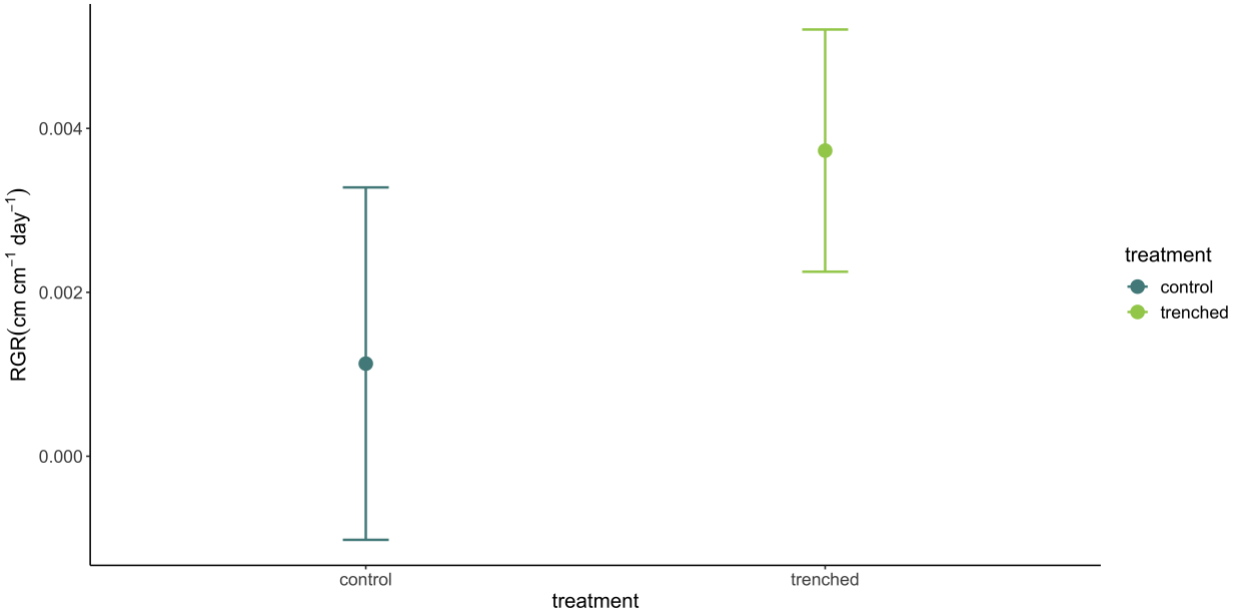

Figure 10. Change in the height of the main ramet (cm) of each legume individual over the course of the growing season. Light green lines represent plants in trenched plots, while the darker green lines represent plants in controls.

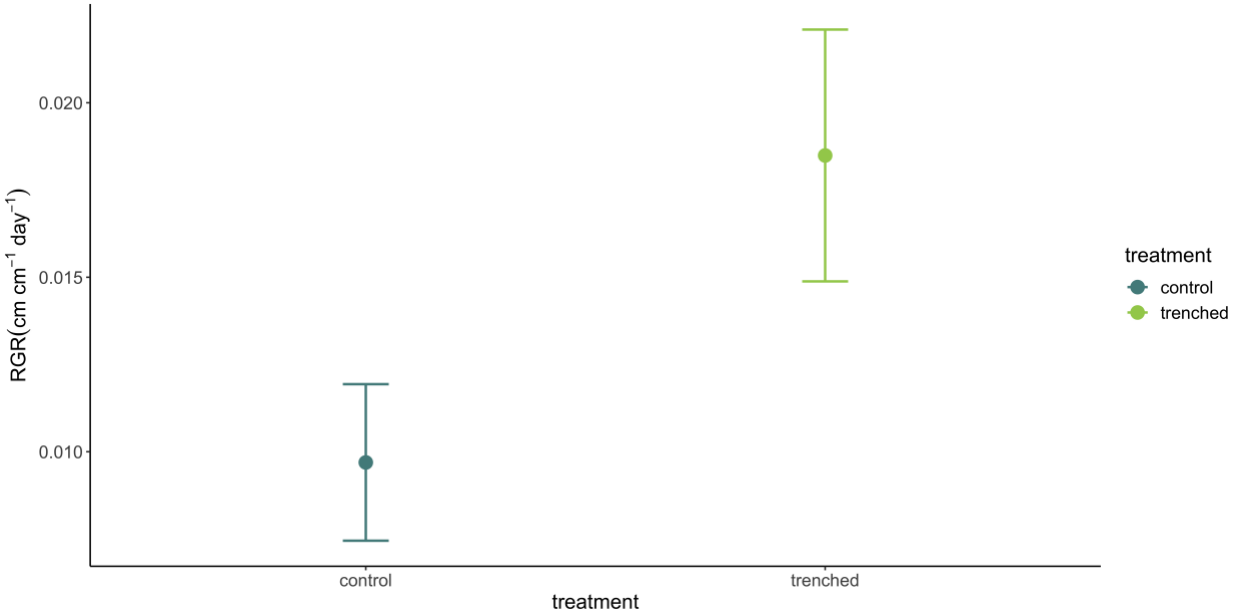

Figure 11. The change in the height (mean ± SE) of the main ramet (cm) of each forb individual over the first half of the growing season. Light green lines represent plants in trenched plots, while the darker green lines represent plants in controls.

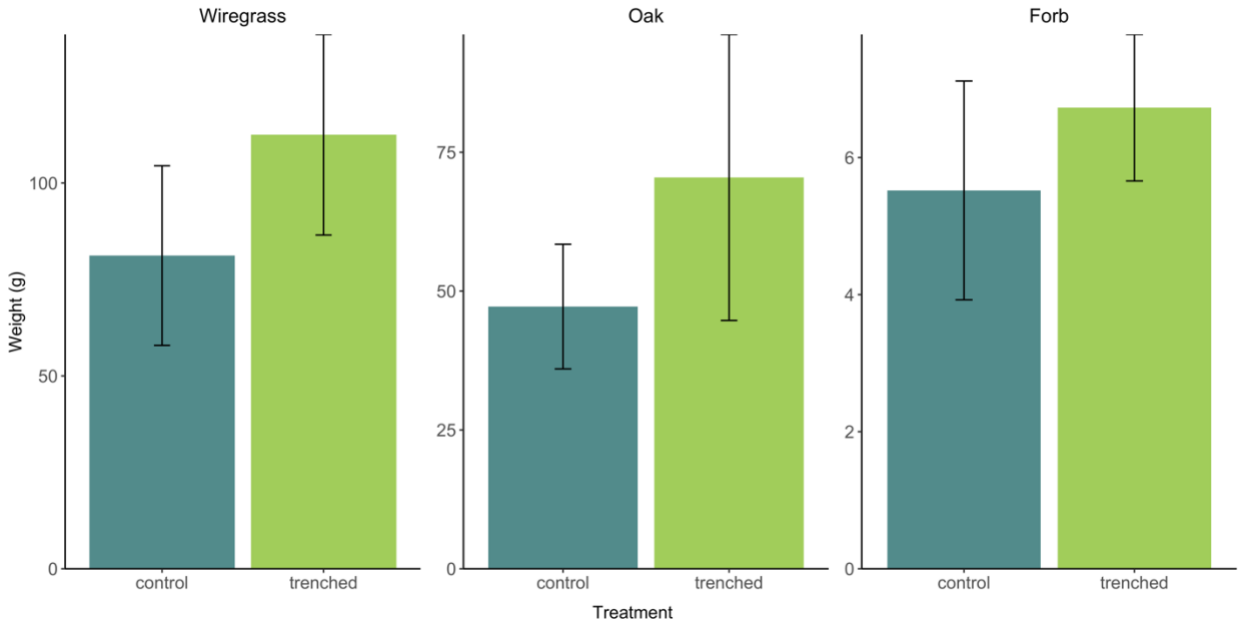

Figure 12. Final biomass (mean ± SE) in g dry weight of wiregrass, the oak, and the forb in trenched vs. control plots, collected at the end of the growing season. Y- axes scales differ by species.

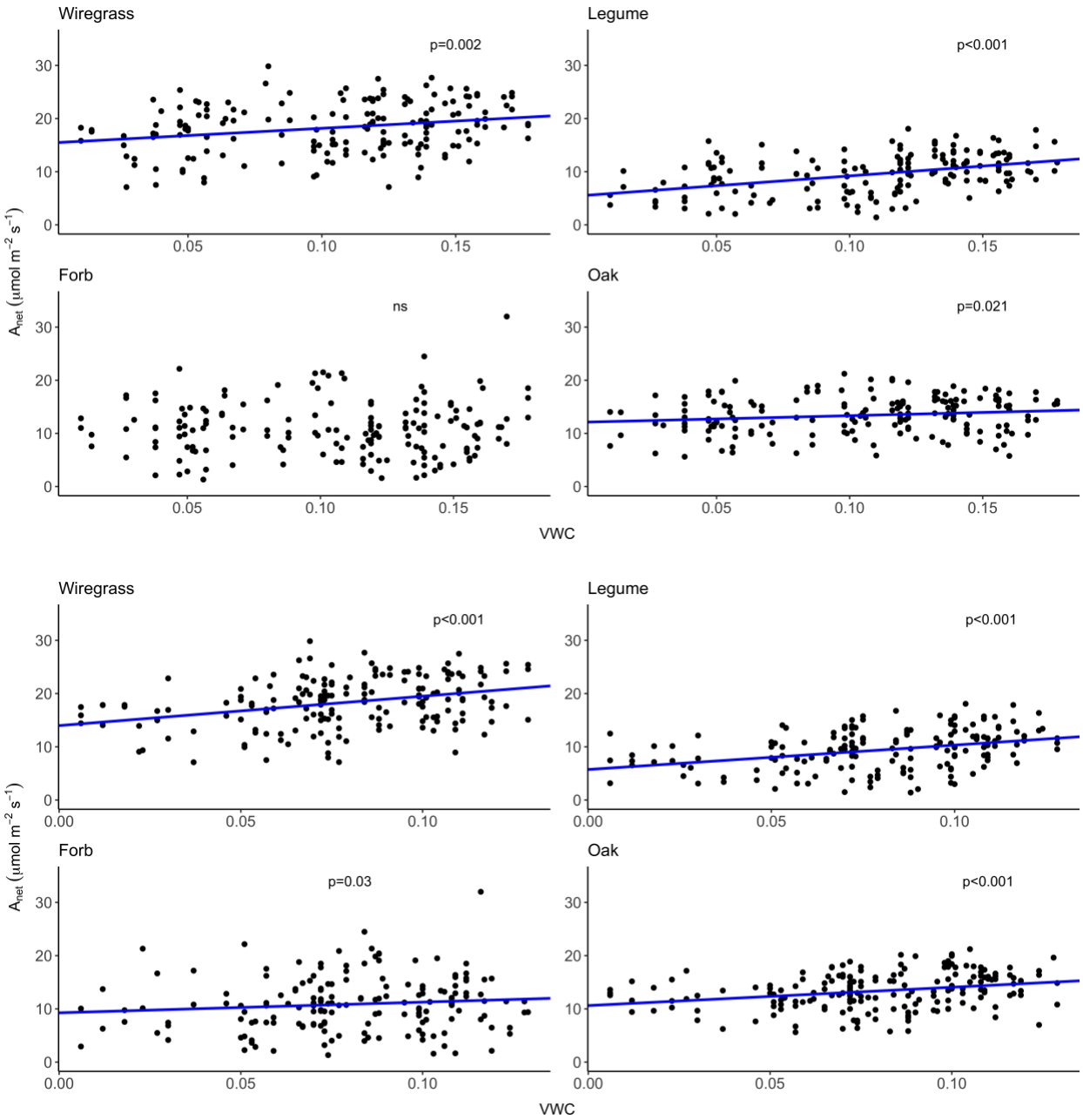

Figure 13. Photosynthesis (µmol C m^{-2} s^{-1}) as a function of volumetric water content at 10cm (top panel) and 50cm (bottom panel) depths for each species.

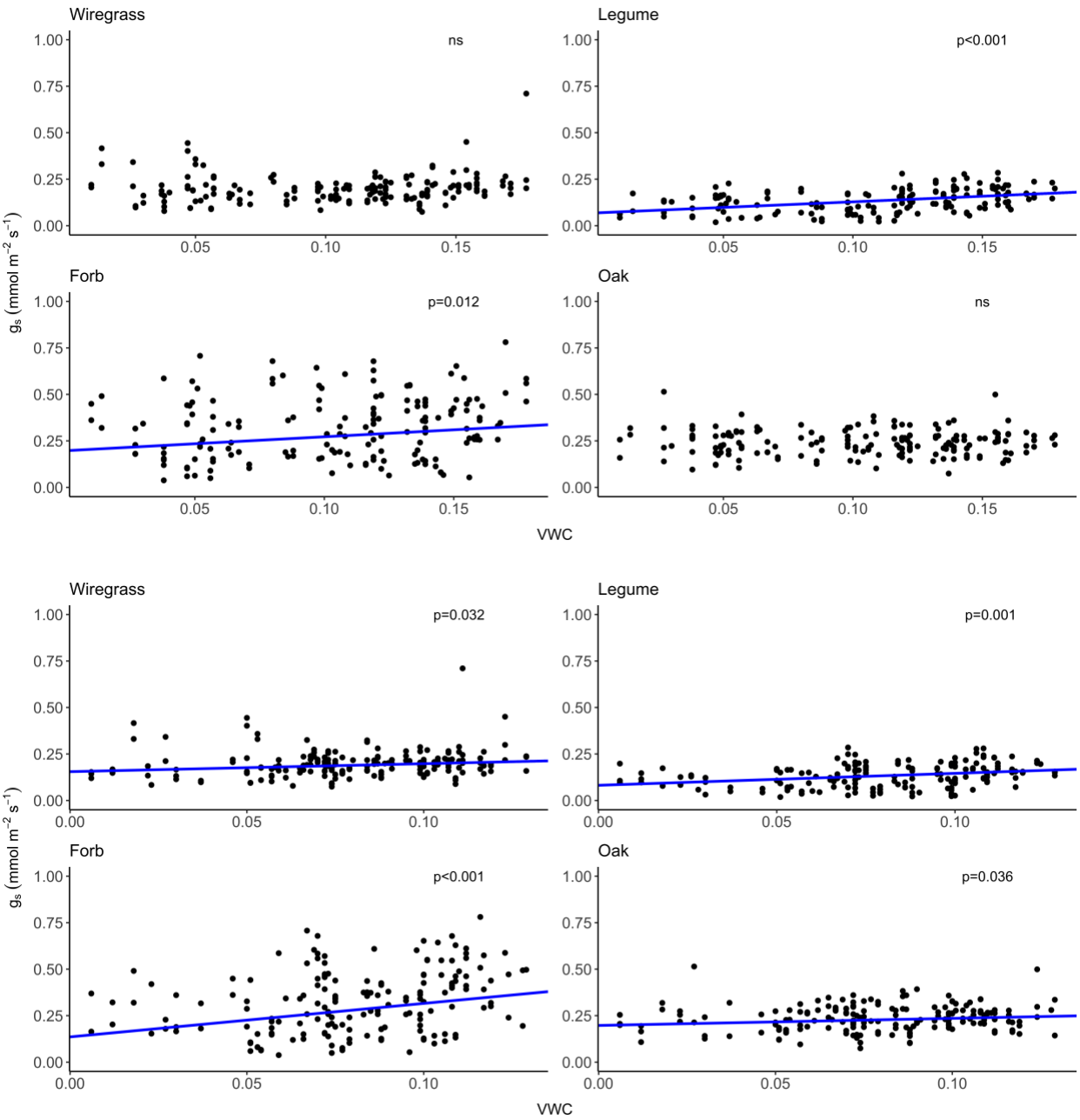

Figure 14. Stomatal conductance (mmol H_2O m^{-2} s^{-1}) as a function of volumetric water content at 10cm (top panel) and 50cm (bottom panel) depths for each species.

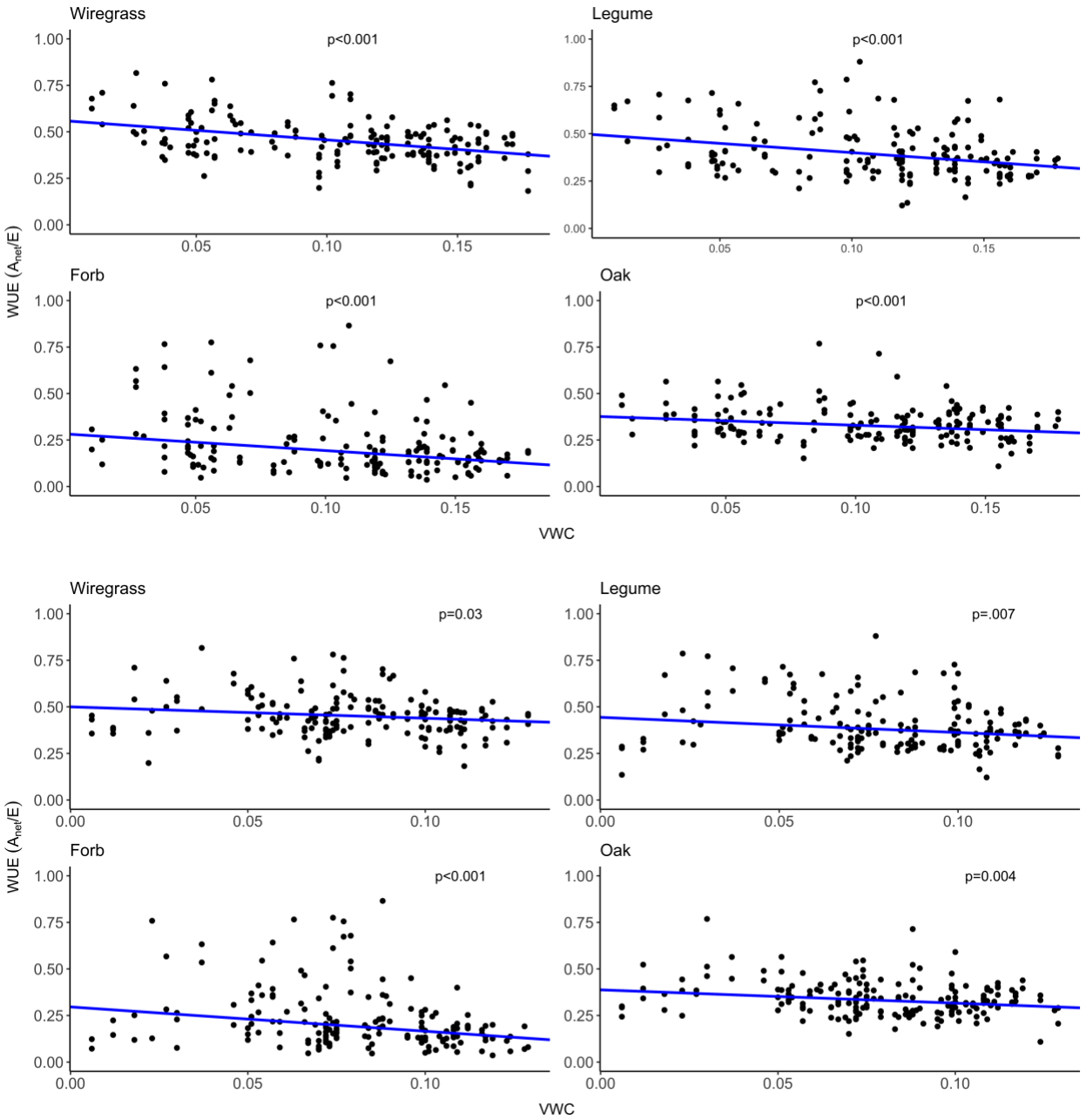

Figure 15. Water use efficiency (µmol C/mmol H_2O) as a function of volumetric water content at 10cm (top panel) and 50cm (bottom panel) depths for each species.

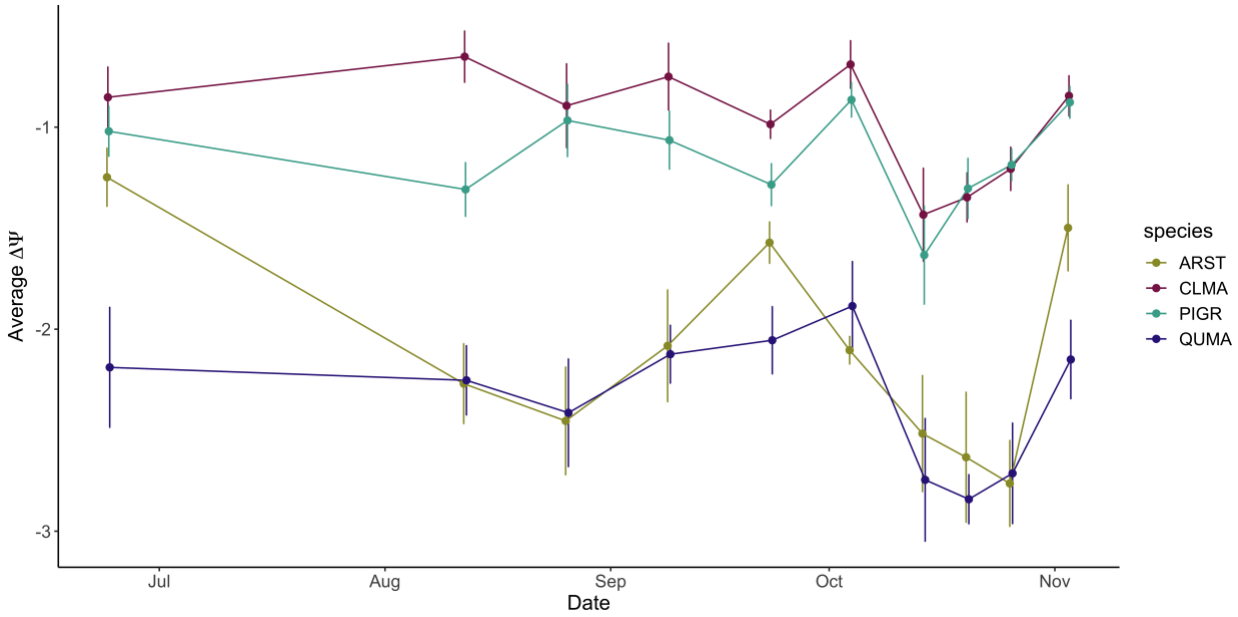

Figure 16. Average differences in daily plot-level predawn and midday water potentials for each species. Error bars show one standard error. Lower numbers (more negative) indicate larger changes between predawn and midday water potentials.

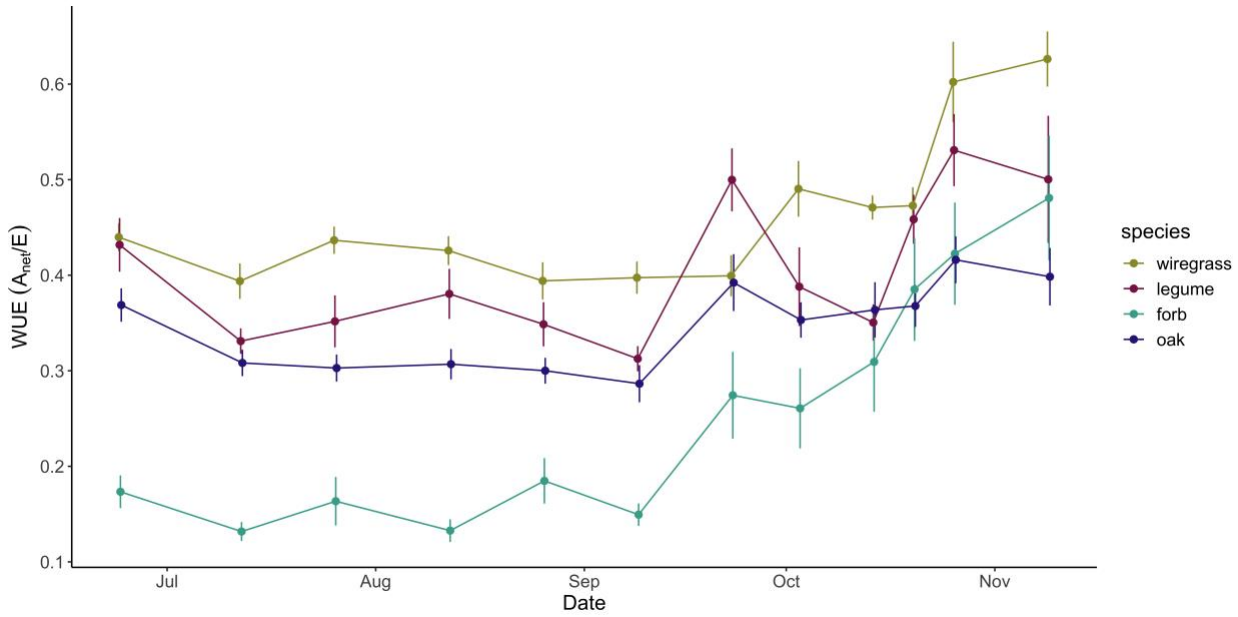

Figure 17. Water use efficiency values for each species over the course of the growing season, calculated from gas exchange data (photosynthesis/transpiration).

Figure 18. Longleaf pine roots (marked with pink flagging) between the tree and a trenched plot that were severed during the trenching process.

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