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# The Effects of Drought and Shade on Bottomland Hardwood Regeneration

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# THE EFFECTS OF DROUGHT AND SHADE ON BOTTOMLAND HARDWOOD REGENERATION

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University
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in partial fulfillment of the
requirements for the degree of
Doctor in Philosophy

in

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by
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# ABSTRACT

Floodplain forests support a high diversity of tree species adapted to regenerate under fluctuating water and light availability. Regeneration regulates species composition, and shade and flood tolerance influence the likelihood of regeneration. Regeneration failure of shade-intolerant and flood-tolerant tree species commonly occurs in southcentral and southeastern floodplain forests of the United States, also known as bottomland hardwoods (BLHs). In many BLHs reduced flooding has resulted in a dryer floodplain. These changes in flooding are linked to recent shifts in species composition. The mechanisms controlling regeneration in BLHs and these composition shifts are poorly understood.

In a controlled germination experiment, I osmotically induced water stress in seeds of five BLH tree species. I found that germination decreased with decreasing water availability in all species except for overcup oak. Desiccation sensitive acorns of oaks germinated across a wider and lower range of water potentials but reached maximum germination slower than desiccation resistance seeds of green ash and sugarberry.

Morphology of over 300 seedlings covering 11 tree species was examined in the field, and a subset of five species were grown in a greenhouse under a factorial combination of shade and reduced water availability. In both experiments, 21 plant traits were examined to determine the variation in biomass allocation among species and the impacts of shade and water availability on morphology. Differences in morphology and treatment response coincided with species life history. Shade-intolerant species possessed flood-tolerant and drought-sensitive hydraulic architecture and allocated less biomass towards vertical growth and more towards structural carbon in response to shade. In contrast, shade-tolerant, flood-intolerant species possessed root and stem architecture that was more efficient at water exploration, cavitation resistant, and in response to shade, allocated biomass towards efficient vertical growth.

Shade-tolerant, flood-intolerant species, such as sugarberry and green ash, possessed seed and seedling traits that aid in regenerating within shaded drier floodplains, while shade-intolerant, flood-tolerant species, such as overcup oak, possessed traits that hinder their regeneration. These opposing regeneration strategies rely on conflicting hydrology to be effective. Shade-intolerant, flood-tolerant species benefit from regular flood disturbance removing competition while shade-tolerant, flood-intolerant species benefit from a drier less-connected floodplain.

# CHAPTER 1. GENERAL INTRODUCTION

Regeneration is the series of processes by which a species replaces itself in kind. For tree species, this typically includes the production, dispersal, and germination of a seed, as well as establishment of the seedling and its survival to maturity (Price et al. 2001). Regeneration helps regulate forest population dynamics thereby influencing species composition (Grubb 1977). Grubb (1977) established the concept of the regeneration niche. The regeneration niche includes species-specific requirements that maximize the probability of regeneration and the collection of mechanisms that a plant uses to tolerate conditions outside that ideal up to a point. Natural regeneration of tree species in forested floodplains is complex due to its high diversity of tree species, each with species-specific regeneration dynamics, interacting with multiple gradients of stressors. In southcentral and southeastern floodplain forests in the United States, also known as bottomland hardwood (BLH) forests, regeneration failure of shade-intolerant and flood-tolerant tree species commonly occurs, and stunted seedlings and lack of sapling regeneration are common (Battaglia et al. 2000; Brinson 1990; Streng et al. 1989).

Flood and shade tolerance of tree species strongly affect their occurrence and distribution within the floodplain (Allen et al. 2001, Battaglia and Sharitz 2006, Wharton et al. 1982). Hydrogeomorphic features correlate with flooding regimes and allow for broadly predictable distributions of tree species across these features based upon their flood and drought tolerance (Allen et al. 2001; Junk et al. 1989; Streng et al. 1989; Wharton et al. 1982). Furthermore, localized variability in canopy cover and light availability, adds an important secondary filter on species composition controlled by shade tolerance (Battaglia and Sharitz 2006; Harcombe and Marks 1978; Streng et al. 1989). The temporal and spatial variability in hydrogeomorphic processes facilitates diverse species assemblages (Huffman 1980; Junk et al. 1989; Streng et al. 1989; Tonner and Keddy 1997).

The large diversity of tree species that thrive in BLH have adapted to their heterogenous environment. However, the construction of dams, levees, and other water control structures, have led to disconnects between floodplain forests and their rivers (Bejarano et al. 2011; King and Keim 2019; Mac Nally et al. 2011). In many BLHs altered flooding patterns have resulted in a dryer floodplain which has disrupted the associations among species and their hydrogeomorphic settings and caused cascading effects on regeneration (Gergel et al. 2002; King and Keim 2019). Numerous studies link these hydrologic changes to rapidly occurring shifts in historic species composition (Gee 2012; King and Keim 2019; Kroschel and King 2021; Streng et al. 1989). For example, Gee et al. (2014) found that a ring levee resulted in shifts from flood-tolerant and shade-intolerant *Quercus lyrata* (Walter) to flood-intolerant and shade-tolerant *Celtis laevigata* (Willd.). Similarly, a dam along the Apalachicola River in Florida created an overall drier floodplain resulting in previously wetter microsites showing the greatest decline in regeneration of flood-dependent species (Stallins et al. 2010). Similar shifts in floodplain and riparian species composition have been observed globally and these composition shifts have also been attributed to anthropogenic changes in river flows (Palmer and Ruhi 2019; Poff et al. 2007; Tonner and Keddy 1997). In the Murrary-Darling floodplain, lack of overbank flooding caused severe die backs of floodplain forest trees (Mac Nally et al. 2011). Similarly, in floodplain forests in Germany, removal of flood disturbance shifted multiple flood-adapted alluvial forest cover types to more flood-intolerant cover (Glaeser and Wulf 2009). The mechanisms behind these shifts in species composition are poorly understood. Recent work demonstrated that in some BLHs, altered flood dynamics increased dominance of less flood-tolerant species and further decreased light and water availability (D’Amato et al. 2013). This novel hydrogeomorphic setting has likely altered the regeneration environment to perpetuate flood-intolerant and shade-tolerant species and the regeneration niches of flood-tolerant and shade-intolerant species are no longer adequately supported.

Light and water availability affect a variety of plant regeneration processes (Grubb 1977; Kroschel et al. 2016; Price 2001; Streng et al. 1989; Taiz and Zeiger 2010), but our understanding of drought impacts on regeneration and how drought interacts with other stressors are lacking. For example, the effects of flooding on seed germination are known for a select few BLH species (Guo et al. 1998; Hawkins 2019; Pierce and King 2007), but less is known about the effects of reduced water availability (Bonner 1968; Bonner 1996; Krajicek 1968). A drier floodplain increases the risk of seed desiccation and germination failure and the moisture threshold required for adequate imbibition in BLH seeds is largely unknown. Some seeds demonstrate high tolerance to desiccation, but others lose viability quickly with reduced moisture (Baskin and Baskin 2014). Desiccation tolerance, however, may or may not correlate with an increased ability to germinate under reduced moisture conditions.

Following germination, the mechanisms that seedlings use to acclimate to changes in their environment strongly influences their ability to survive (Grime 1977; Grubb 1977). Changes in water and light availability are particularly detrimental to seedlings because of their overall low biomass and limited ability to capture light and water resources (Grime 1977; Grubb 1977; Taiz and Zeiger 2010). However, within limits, seedlings can alter plant traits to allocate proportionally more biomass towards structures to capture limiting resources (Bloom et al. 1985). As such, to tolerate shade, some plants maintain a positive carbon balance (Pierik and Testerink 2014; Kuehne et al. 2014) by altering the proportion of structural carbon invested into leaves, stems, and roots, and shift growth towards rapid vertical growth (Caldwell and Pearcy 1994; Pugnaire and Valladares 2007), while other plants slow growth and increase carbohydrate storage (King 1986; Markesteijn and Poorter 2009; Poorter and Garnier 1999; Pugnaire and Valladares 2007). To tolerate drought plants must maintain a continuous water supply and preserve their hydraulic architecture (Tyree and Ewers 1991). To achieve this, some plants increase the ratio of below to above ground biomass (Ledo et al. 2018; Nash and Graves 1993; Parolin et al. 2010; Tyree and Ewers 1991), increase water use efficiency by altering plant traits to decrease evapotranspiration (Landsberg and Gower 1997; Poorter and Garnier 1999; Tyree and Ewers 1991), or alter hydraulic architecture to balance hydraulic conductance (Bucci et al., 2004; Meinzer et al., 2009; Gonzalez-Benecke et al., 2010), capacitance (Kotowska et al. 2015; McCullough et al. 2014), and cavitation resistance (Ogasa et al. 2013).

Differences among BLH tree species in their ability to avoid or endure multiple cooccurring and sometimes atypical stressors are not well known. In other forest environments multiple stressors lead to distinct trade-offs where the presence of one stressor inhibits the tolerance of an added stressor (Delgado et al. 2018; Kotowski et al. 2010; Laanisto and Niinemets 2015; Lin et al. 2004; Mann et al. 2008; Niinemets and Valladares 2006). Alternatively, the effects of two or more stressors could be additive (Lucas et al. 2013; Niinemets and Valladares 2004; Sack and Grub 2002) or independent of one another (Lucas et al. 2013; Markesteijn and Poorter 2009). Lastly, in some cases the presence of one stress can improve the environment in such a way that the stress improves or facilitates the tolerance of another (Amissah et al. 2015; Holmgren 2000; Huang et al. 2008). Understanding the differences in and limitation of species and their responses to a changing environment can help provide a foundation for a process-based understanding of regeneration and compositional change in floodplain forests.

My research focuses on the early stages of regeneration including seed germination and seedling establishment. I aim to elucidate how changes in the environment impact the mechanisms that support regeneration. Specifically, I investigate how reduced water availability impacts seed germination timing and success, how morphology differs among naturally regenerating BLH tree species of different stress tolerances, and how shade and reduced water availability interact with each other to alter seedling morphology. Here, I present three experiments:

Chapter 2: Determining the effects of reduced water availability on seed germination of five bottomland hardwood tree species.

I conduct a controlled germination experiment to determine if the effects of reduced water availability on seed germination differs among BLH species.

Chapter 3: Biomass allocation patterns of co-occurring bottomland hardwood tree species with differing stress tolerances.

I evaluate the variation in biomass allocation among naturally regenerated seedlings of 11 BLH tree species to characterize their morphology and to determine if seedling flood, shade, and drought tolerances are associated with specific morphological characteristics.

Chapter 4: Determining the effects of reduced light and water availability on biomass allocation patterns of five bottomland hardwood tree species with differing stress tolerances.

Lastly, I conduct a controlled greenhouse experiment to investigate the main and interactive effects of shade and reduced water availability on seedlings of five BLH tree species to test whether trade-offs in responding plant traits exist.

# CHAPTER 2. DETERMINING THE EFFECTS OF REDUCED WATER AVAILABILITY ON SEED GERMINATION OF FIVE BOTTOMLAND HARDWOOD TREE SPECIES

## Introduction

Floodplain environments and their ecological processes are shaped by flood pulses and the lateral connection of the floodplain to the river (Junk et al. 1989). Dynamic disturbances and dramatic swings in water availability interact with microtopography to create a matrix of microsites capable of supporting a wide diversity of regeneration niches for tree species (Allen et al. 2001; Grubb 1977; Wharton 1982). Within a single growing season flood and drought cycles can impact regeneration by altering germination, emergence, and survival, (Kroschel et al. 2016; Price et al. 2001; Streng et al.1989) thereby shifting species composition of entire cohorts (Battaglia et al. 2000; Collins and Battaglia 2008; Kroschel and King 2021; Price et al. 2001; Toner and Keddy 1997). Many floodplain forests in the southcentral and southeastern United States, also known as bottomland hardwood (BLH) forests, have become drier due to stream alterations decreasing overbank flooding (Gee et al. 2014; King and Keim 2019; Stallins et al. 2010). This novel and drier hydrogeomorphic setting alters regeneration processes and contributes to shifts in BLH species composition (Gee et al. 2014; King and Antrobus 2005; King and Keim 2019; Oliver et al. 2005). In some scenarios, reduced flooding in floodplain forests increases the abundance of flood-intolerant and shade-tolerant tree species (Hanberry et al. 2012) which further exacerbates shade and drought stress on understory seedlings and saplings (D'Amato et al. 2013). The underlying mechanisms controlling regeneration in BLHs, however, are poorly understood. For example, only a general understanding of germination dynamics exists for a few BLH species. As such, while the effects of flooding on germination are known for a few species (Guo et al. 1998; Hawkins 2019; Pierce and King 2007), comparatively less is known about the effects of reduced water availability (Bonner 1968; Bonner 1996; Krajicek 1968).

Reduced water availability often leads to desiccation and germination failure in seeds. In BLHs, the seeds of some tree species are more tolerant to desiccation than others. For example, acorns of oaks (*Quercus* spp.) are recalcitrant, and thus considered sensitive to moisture loss. Sugarberry and green ash possess orthodox seeds that are tolerant to moisture loss (Baskin and Baskin 2014; Bonner and Karrfalt 2008). However, desiccation tolerant seeds still need adequate imbibition of water to germinate, and in BLH seeds it is uncertain if desiccation tolerance corresponds to the ability to germinate under reduced moisture conditions. In defining desiccation tolerance, recalcitrant seeds demonstrate sensitivity to desiccation and lose viability if seed moisture falls below 30-60% (Chin et al. 1989). After dispersal, recalcitrant seeds typically remain viable for a single growing season, and survival of the seed until germination depends on maintaining seed moisture via mechanisms such as leaf litter coverage or submergence (Farmer 1997), or by germinating and forming root structures quickly (Baskin and Baskin 2014). In contrast, orthodox seeds tolerate very low seed moisture (Haynes et al. 1988; Roberts 1973) and many orthodox seeds remain dormant and viable as a stable component in the soil seed bank for years (Baskin and Baskin 2014: Meadows et al. 2006; Kennedy 1990).

Regardless of species, imbibition of water initiates the process of germination. Imbibition is a passive process driven by the water potential gradient between the seed and the soil (Kigel and Galili 1995). As the water potential gradient between the soil and the seed decreases the rate of imbibition slows and stops when the seed's water potential reaches equilibrium with the soil's water potential. A viable, nondormant seed will germinate if this water potential has allowed for sufficient water to be imbibed, and all other species-specific requirements (e.g. temperature, light, oxygen) are met. The amount of water required and therefore the minimum water potential (also known as base water potential), and the time required for seed germination are species-specific (Bradford 1990; Gummerson 1986, Kigel and Galili 1995). This water potential threshold is largely unknown for many BLH seeds.

As germination proceeds and the radicle emerges, the rate of imbibition increases again as plant cells expand and increase turgor pressure. A reduction in water potential at this point in the germination process causes growth to cease and likely induces death of the seed (Kigel and Galili 1995). Timing and rate of germination relative to the timing and duration of flooding and water availability is an important factor controlling species composition in floodplain forests (Kroschel 2020; Toner and Keddy 1997). Kroschel (2020) found first-year seedling species composition was influenced by the interrelationship between flood timing relative to species' germination windows. Flooding prevented germination when it fully overlapped the germination window of early spring germinating species, but time since flooding also affected germination rates, presumably because of interactions between soil moisture and seed germination processes.

The construction of dams, levees, and other water control structures, as well as channel incision and decreases in channel bed elevation have led to widespread disconnects of floodplain forests from their rivers resulting in altered flood timing and an overall drying of many BLH forests (Gergel 2002, Gergel et al. 2002, King and Keim 2019). A better understanding of the water potential thresholds of seeds and the impacts of reduced water availability on germination are critical for better understanding of how regeneration from seed is impacted by altered hydrology.

The objective of this study was to determine if the effects of reduced water availability on seed germination differs among BLH species. I conducted a controlled germination experiment with both recalcitrant and orthodox seeds from tree species that commonly occur in southeastern BLHs, and particularly, those species whose abundance has been notably altered by this novel hydrogeomorphic landscape (Gee et al. 2014; King and Antrobus 2005; King and Keim 2019; Oliver et al. 2005). Specifically, I evaluated the effects of reduced water availability by reduced osmotic water potential on seed germination of three oak and two non-oak species. These species included one white oak subgenus *Lepidobalanus* overcup oak (*Quercus lyrata* Walter; QULY), two red oaks subgenus *Erythroblanus* willow oak (*Quercus phellos* L.; QUPH) and water oak (*Quercus nigra* L.; QUNI) which all have recalcitrant seeds, and green ash (*Fraxinus pennsylvanica* Marshall; FRPE), and sugarberry (*Celtis laevigata* Willd.; CELA) which both have orthodox seeds (Baskin and Baskin 2014; Farmer 1997; Roberts 1973). I hypothesized that reduced water availability impacts germination of recalcitrant seeds more negatively than orthodox seeds.

## Methods

### *Plant material and experimental design*

Seeds were purchased from Louisiana Forest Seed Company (Lecompte, LA). Wings of green ash samaras and the acorn cap of willow and water oak were removed by the supplier. Green ash and sugarberry seeds were collected manually from the tree after maturation, typically after leaf fall, and all oak acorns were collected from the ground after naturally dropping (Delaney, LAFSC, Personal Communication). After collection and prior to purchase, green ash and sugarberry seeds were dried to approximately 10% moisture and stored in fiber board boxes with plastic liners at approximately -17°C, and all oaks were stored in plastic weave bags at approximately 1-2°C (Delaney, LAFSC, Personal Communication). After purchase, all seeds of each species were cold stratified by storing the seeds, separated by species, in clear plastic storage containers affixed with a transparent lid on sand moistened with distilled water at 4°C for 90 days. After cold stratification, seeds were inspected and discarded if rot, mold, or insect damage was present, or if germination during cold stratification had occurred. In addition, after cold stratification, acorns of willow and water oak were float tested for approximately one hour in distilled water and any floating acorns were discarded.

To simulate reduced water availability, I created eight different solutions of distilled water and polyethylene glycol (PEG 8000) to target water potentials of: 0.0 (distilled water), -0.2, -0.4, -0.6, -0.8, -1.0, -1.2, and -1.4 MPa following the equation and methods from Michel (1983):

$$\Psi=1.30(PEG)^2T-137(PEG)^2$$

Where $\Psi$ is the water potential of the solution in bars (1 bar = 0.1 MPa), PEG is grams of polyethylene glycol 8000 per gram of distilled water, and T is the temperature of the solution (25°C for incubation). To confirm the water potential of solutions, samples from each were taken and measurements of water potential were made using a vapor pressure osmometer (Vapro 5600, EliTechGroup). Actual water potential values from osmometer measurements and target values did not differ ($P=0.863$; Table A.1). The eight original target water potential values were used to label the eight treatments.

In each water potential treatment 20 seeds were placed in either 100 mm petri dishes (sugarberry and green ash) or 22.5x16.0x6.5cm plastic food storage containers (oak species) on heavy weight germination paper (SD7630 Anchor paper Co.) saturated in one of the eight water potential solutions. Each container was affixed with a transparent lid and wrapped in cling film. Seeds were separated by species and each species/water potential treatment combination was replicated three times. Seeds were incubated in a climate-controlled germination chamber (Percival GR-36VL, Percival Scientific Inc.) for 28 days at a constant temperature of 25°C and a 12-hour light/dark cycle. Approximately every three days seeds were transferred to new containers and placed upon freshly saturated germination paper. Germination was defined as when the radicle emerged and measured approximately 1-2 mm. The number of seeds germinated were counted daily and germinated seeds were removed.

After 28 days of incubation, ungerminated seeds were removed from their treatment, rinsed with distilled water, and transferred into new containers with new germination paper saturated with distilled water. These ungerminated seed were incubated for an additional 7 days under the same temperature and light conditions. This 7-day period was referred to as the recovery period. Seeds were checked daily for germination. After 7 days, seeds that had not germinated during the recovery period were opened, their embryos were excised, and the cause for non-germination was identified when possible. All discolored, rotted, and damaged embryos were considered non-viable. All whole, firm, fully developed, and non-discolored embryos were tested for viability using the tetrazolium method (Elias et al. 2012). Embryos were soaked in a 1% solution of 2,3,5 triphenyl tetrazolium chloride (TZ) for approximately 12 hours at 25°C and viability was determined based on staining color and its uniformity with no stain coloration being considered non-viable and uniform red staining being viable (Elias et al. 2012).

### *Data analysis*

Cumulative germination was calculated as the proportion of germinated seeds to the number of viable seeds after 28 days. Germination recovery was calculated as the percentage of ungerminated seeds remaining after the initial water potential treatments that germinated after being transferred to distilled water during the seven-day recovery period. Maximum germination was defined as the highest mean cumulative germination reached. All analyses were performed in R (R Core Team, 2020). A two-way analysis of variance (ANOVA) was used to test the effects of species and water potential on cumulative germination, germination recovery, and days to first and maximum germination. Differences among treatment and species were evaluated using Tukey's HSD. All effects were considered significant at $\alpha \leq 0.05$

## Results

Cumulative germination was affected independently by water potential (P<0.001) and species (P<0.001). As water potential decreased, cumulative germination decreased in all species except overcup oak (QULY) (P=0.975) (Table 2.1). Averaged across all water potential treatments, water oak (QUNI) and willow oak (QUPH) had the highest cumulative germination while sugarberry (CELA) and green ash (FRPE) had the lowest (Table 2.1). Averaged across all species cumulative germination was highest in both the 0.0 MPa treatment and the -0.2 MPa treatment and was lowest from -0.8 MPa to -1.4 MPa (Table 2.2). Lastly, there was no single water potential treatment where cumulative germination did not differ among species (Table 2.2).

Table 2.1. Mean (±SE) cumulative germination by species averaged across all water potential treatments, and results (P>F) of ANOVAs for the effect of water potential on germination among and within species. Dissimilar lower case letters (Tukey's HSD) indicate differences in germination among species.

| Species | Cumulative germination (%) | P>F |
|---|---|---|
| CELA | 12.4 (3.3) c | <0.001 |
| FRPE | 22.6 (4.2) c | <0.001 |
| QULY | 40.3 (3.1) b | 0.975 |
| QUNI | 58.1 (3.9) a | <0.001 |
| QUPH | 47.0 (6.0) ab | 0.004 |
| P>F | | |
| Water potential | <0.001 | |

Table 2.2. Mean (±SE) cumulative germination by water potential treatment, and results (P>F) of ANOVAs for the effect of species on germination among and within treatments. Dissimilar lower case letters (Tukey's HSD) indicate differences in germination among water potential treatments.

| Water potential (MPa) | Cumulative germination (%) | P>F |
|---|---|---|
| 0.0 | 63.8 (6.2) a | 0.018 |
| -0.2 | 54.8 (6.1) ab | 0.007 |
| -0.4 | 46.6 (6.5) bc | 0.002 |
| -0.6 | 36.7 (5.7) cd | 0.005 |
| -0.8 | 23.3 (5.3) de | 0.012 |
| -1.0 | 25.0 (5.6) de | 0.001 |
| -1.2 | 19.5 (5.5) e | 0.017 |
| -1.4 | 18.1 (5.0) e | 0.005 |
| P>F | | |
| Species | <0.001 | |

The water potential at which cumulative germination significantly decreased from the 0.0 MPa treatment differed in each species, but among species differences in cumulative germination were not observed until the -0.4 MPa treatment (Figure 2.1). First, in CELA, cumulative germination decreased from 41.7 ± 3.0% in the 0.0 MPa to 13.2 ± 5.7% in -0.4 MPa. Next, cumulative germination in FRPE did not decrease until -0.8 MPa where cumulative germination decreased from 58.1 ± 12.7% in the 0.0 MPa to 7.1 ± 3.2% in -0.8 MPa. Both QUNI and QUPH maintained relatively high cumulative germination across water potential treatments compared to CELA and FRPE. In QUNI, cumulative germination did not decrease until -1.0 MPa where it decreased from 86.3 ± 2.4% in 0.0 MPa to 47.6 ± 4.2% in -1.0 MPa. Lastly, QUPH's cumulative germination was not significantly impacted by water potential until -1.2 MPa where it decreased from 81.3 ± 7.5% in 0.0 MPa to 16.9 ± 13.7% in -1.2 MPa.

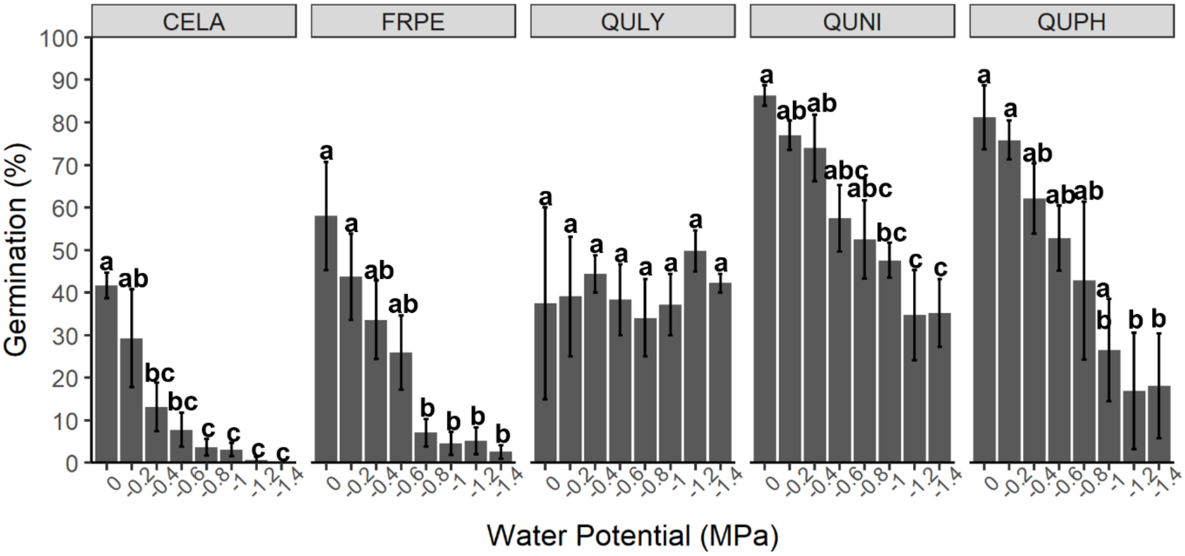


Figure 2.1. Mean (±SE) cumulative germination (%) in seeds by species in response to water potential. Within each species dissimilar lower case letters (Tukey's HSD) indicate differences among water potential treatments.

In all water potential treatments, including the 0.0 MPa treatment, differences in cumulative germination among species occurred (Figure 2.2). For example, QUNI and QUPH both maintained higher germination than CELA and FRPE where QUNI's cumulative germination was higher than CELA across all water potentials and QUPH's cumulative germination was higher than CELA's in the -0.2, -0.4, and -0.6 MPa treatments only. The 0.0 MPa treatment was the only treatment in which QULY, had lower cumulative germination than QUNI, in every other treatment cumulative germination among the three oaks was similar. As water potential decreased, QULY's germination was higher than CELA and FRPE's starting at -1.0 MPa. Additionally, in the 0.0 MPa treatment, all species reached or exceeded a cumulative germination of 30% while only FRPE, QUNI, and QUPH reached 50% cumulative germination (Figure 2.2). CELA only reached ≥30% cumulative germination in the 0.0 MPa treatment whereas FRPE maintained ≥30% cumulative germination to -0.4 MPa. Of the three oaks, QUPH maintained ≥30% cumulative germination to -0.8 MPa while QUNI and QULY both maintained ≥30% cumulative germination in all treatments.

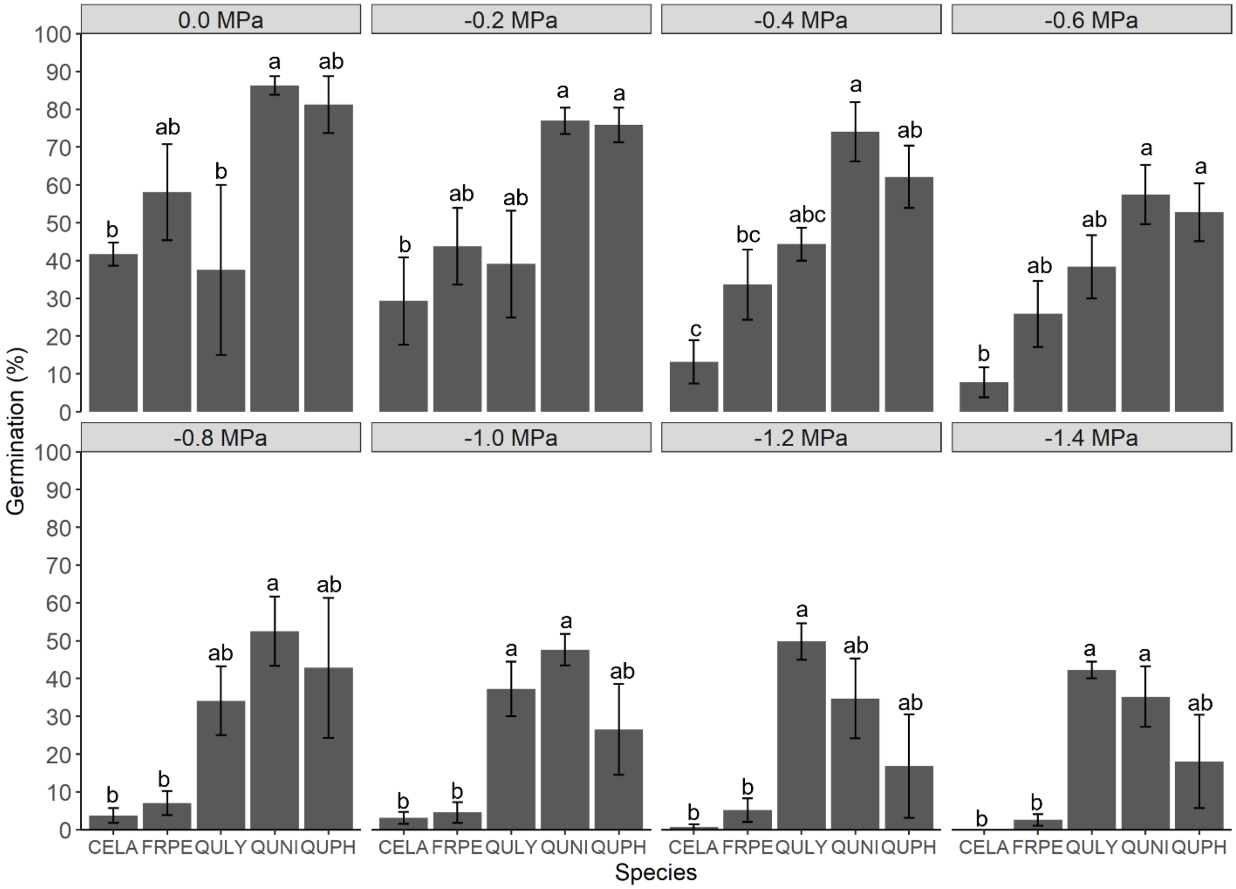


Figure 2.2. Mean (±SE) cumulative germination percentages by water potential treatment. Within each treatment, dissimilar lower case letters (Tukey's HSD) indicate differences among seed species.

The number of days to first germination differed by species (P=0.033; Table 2.3). QULY, QUNI, and FRPE germinated first after 2 days of incubation, whereas CELA was the slowest to begin germinating. In addition, the number of days to reach maximum germination also differed among species (P<0.001; Table 2.3). The three oak species, QUNI, QUPH, and QULY, took longer to reach maximum germination than the non-oak species, Days to reach maximum germination ranged from 25 days for QUNI seeds to 10 days for FRPE seeds.

Table 2.3. Mean (±SE) number of days to reach first and maximum germination and results (P>F) of ANOVAs for the effects of species and water potential. Different lowercase letters and uppercase letters indicate a significant difference among species for days to first and days to max respectively (α≤0.05).

| Species | Days to first germination | Days to max germination |
|---|---|---|
| CELA | 6.9 (1.4) a | 15.4 (1.7) BC |
| FRPE | 2.2 (0.3) bc | 10.1 (1.4) C |
| QULY | 2.3 (0.6) bc | 20.0 (1.7) AB |
| QUNI | 2.2 (0.1) c | 25.2 (1.1) A |
| QUPH | 4.8 (0.9) ab | 21.7 (1.2) A |
| P>F | | |
| Species | 0.033 | <0.001 |
| Water potential | 0.526 | 0.291 |

Percent germination recovery differed by species (P<0.001) (Table 2.4). Percent recovery in QULY was lower than the other four species and averaged 3% across all water potential treatments. Percent germination recovery in seeds of the remaining four species ranged from 25% in QUPH to 14% QUNI. After the 7-day recovery period treatment, 86% of non-germinated seeds were non-viable based on visual inspection or TZ testing of excised embryos. Of the non-viable seeds, 46% of embryos were visibly rotted, discolored, or damaged in some manner, 51% were determined nonviable with TZ testing, and the remaining nonviable seeds were either empty or had insect damage most likely from acorn weevils (*Cuculio* spp.).

Table 2.4. Mean (±SE), cumulative germination recovery averaged across all water potential treatments, and results (P>F) of ANOVAs for the effects of species and water potential. Dissimilar lower case letters (Tukey's HSD) indicate differences among seed species

| Species | Germination Recovery (%) |
|---|---|
| QUPH | 24.9 (2.5) a |
| FRPE | 21.9 (5.4) a |
| CELA | 21.6 (1.6) a |
| QUNI | 14.4 (1.5) ab |
| QULY | 3.1 (0.7) b |
| P>F | |
| Species | <0.001 |
| Water potential | 0.058 |

## Discussion

The ability to tolerate low seed moisture content did not correlate with an ability to germinate under lower water potential. Instead, sugarberry and green ash seeds, which are orthodox and tolerant to desiccation, germinated only at higher water potentials but reached maximum germination quickly. This was opposed to oak seeds, which are recalcitrant and sensitive to desiccation, which maintained a relatively high cumulative germination (>30%) over a wider range of water potential and reached maximum germination more slowly. In floodplain forests the growth phenology, life history, and composition of tree species are strongly correlated with disturbance events (Streng et al. 1989; Junk et al. 1989), and these two opposing germination behaviors lend more insight to our understanding of the mechanisms behind these correlations. Rapid seed germination at higher water potentials corresponds with wetter conditions typical in early spring and the likelihood of early seedling emergence. In contrast, germinating slowly with a wider range of water potential increases the likelihood of seed germination later in the season. These species-specific differences in germination timing and the response to water availability help further our understanding of regeneration and the mechanisms behind floodplain forest species composition.

The water potential at which cumulative germination significantly declined differed among species in a manner that reflected differences in life history of these species. Specifically, cumulative germination in green ash and sugarberry declined at higher (less negative) water potentials than water oak or willow oak. These results indicate that the ability to tolerate low seed moisture content does not correlate with an ability to germinate under lower water potential. Sugarberry and green ash seeds are orthodox and can tolerate seed moisture content as low as 7% (Burns and Honkala 1990), and germination of orthodox seeds typically improves following a drying period. Once dry however, orthodox seeds still require adequate water imbibition to germinate. In contrast, acorns of the three oak species are recalcitrant and intolerant of desiccation. Willow oak acorns, for example, lose viability rapidly if seed moisture content drops below 40% (Burns and Honkala 1990). In sugarberry and green ash, seed germination only at higher water potential means their seeds are more likely to germinate earlier in the spring when soil water availability is higher. Furthermore, if water availability is not conducive for germination, sugarberry and green ash seeds may both remain dormant or reenter dormancy and survive for multiple years in the seed bank (Baskin and Baskin 2014). Meadows et al. (2006) found viability in sugarberry seeds remained as high as 79% after 5 years of burial while green ash seeds were still viable after 2 years of burial. This ability to tolerate desiccation and remain viable in the seed bank helps maintain germinative capacity and means that these species will remain a stable component in the regeneration layer for years (Meadows et al. 2006; Kennedy 1990). In contrast to this life history strategy, acorns of oaks have the capacity to germinate under a wider range of water potentials which increases the probability of germinating later in the spring as conditions become drier. This is beneficial because unlike sugarberry and green ash, seeds of oak species are recalcitrant and sensitive to desiccation, Acorns will typically not survive more than a single growing season after dispersal; therefore, the ability to germinate under a wide range of water potentials helps maximize the probability of germination. Germinating under lower water potential, would also support regeneration in canopy gaps, which are innately dryer than the closed canopy (De Jager et al. 2015; Price 2001), but is where regeneration success for oaks is highest (Burns and Honkala 1990; Collins and Battaglia 2008; Hodges et al. 2005; McNab 2021).

Species-specific differences in the number of days to reach maximum cumulative germination also reflected differences in life history among species. Contrary to my hypothesis and to other studies (Daws et al.2008; Li et al. 2006; Ludewig et al. 2014; Zhang et al. 2020), the number of days to reach maximum germination differed by species but was independent of water potential. This could be due to the use of a single constant temperature during incubation, which was needed to maintain constant osmotic potential of the PEG solutions (Michael 1983). Temperature is known to affect germination rate. Hawkins (2019a) found that alternating light/dark temperatures of 25/15°C and 30/20°C caused greater biomass accumulation in cherrybark oak (*Quercus pagoda* Raf.), as well as higher and faster cumulative germination in cherrybark oak, Nuttall's oak (*Quercus texana* Buckley), and willow oak seeds (Hawkins 2019b). In this study, sugarberry and green ash seeds reached maximum cumulative germination sooner than those of willow oak and water oak. Germination phenology and flood disturbance are naturally correlated with the spring growing season in BLHs (Streng et al. 1989). Therefore, differences in species' germination timing may affect differences in regeneration success and species composition. Kroschel (2020) found that following spring flooding, green ash and sugarberry emerged before willow oak, Nuttall oak and overcup oak which all emerged later in the spring and over a longer period. Reaching maximum cumulative germination quickly in the spring allows species to get an earlier start to growth and benefit from a longer effective growing season. (Streng et al. 1989, Jones et al. 1997). In contrast, reaching maximum germination later and spreading germination over a longer period allows species to avoid early-season disturbance such as prolonged or secondary flood events. However, this effectively shortens the growing season length experienced by the seedling (Streng et al. 1989).

My results are generally in line with other studies which have shown that germination decreases with reduced water potential (Daws et al.2008; Li et al. 2006; Ludewig et al. 2014; Zhang et al. 2020). However, in contrast to these studies, cumulative germination in overcup oak seeds was not affected by water potential. The reason for overcup oak's exception could be due to overcup oak possessing a non-dormant radicle and physiological epicotyl dormancy, while the other species in this study possess physiologically dormant radicles (Baskin and Baskin 2014). Overcup oak is a white oak, *Quercus* sect. *Lepidobalanus*, where epicotyl physiological dormancy has been well described (Baskin and Baskin 2014), while water and willow oak are red oaks, *Quercus* sect. *Erythroblanus* which instead require long periods of cold stratification to break radicle dormancy (Peterson 1983; Hopper et al. 1985; Hawkins 2019a; 2019b). In overcup oak, the non-dormant radicle emerges shortly after dispersal, allowing for earlier root development and access to soil water (Berjak and Pammenter 2013; Johnson et al. 2009). Germination in overcup oak could be less sensitive to water potential because seed moisture content at dispersal was sufficient to promote radicle emergence (Baskin and Baskin 2014; Berjak and Pammenter 2013). Similar results were found during a pilot study preceding this one, where cumulative germination in overcup oak seeds was similarly not affected by water potential (Table A.2). However, anecdotal observations from the pilot study indicated that radicle elongation was slower in lower water potential treatments, suggesting that secondary imbibition that promotes radicle growth may still be sensitive to water potential (Gummerson 1986, Kigel and Galili 1995).

Changes to floodplain hydrology in southeastern BLHs have created an often drier, ecosystem which has disrupted historic patterns of ecological processes such as regeneration and species composition (Gee et al. 2014; Hanberry et al. 2012; King and Antrobus 2005; King and Keim 2019; Oliver et al. 2005; Stallins et al. 2010). Regeneration is a series of processes, and germination is one of the earliest stages in this series that my results show is impacted by changes to floodplain hydrology. Reduced secondary flood events or early season drawdowns increases the emergence of early season species such as sugarberry and green ash (Kroschel 2020; Streng et al. 1989; Toner and Keddy 1997). This emergence is likely due in part to these species reaching maximum germination quickly, allowing them to respond to changes in water availability quickly, and germinating at higher water potentials. In addition, sugarberry seedlings are intolerant to flooding while green ash seedlings are moderately tolerant, but require extensive energy input into extra adventitious roots to manage this (Burns and Honkala 1990; McKnight et al. 1980). By only germinating at higher water potentials quickly, sugarberry and green ash are more likely to germinate prior to canopy leaf out allowing greater access to light, to increase vertical growth rapidly (Burns and Honkala 1990), and to reduce the chances of being overtopped should a second flood event occur. Historically, with longer periods of frequent over bank flooding, the number of seedlings of early emergent species would be reduced by flood mortality. Oak seedlings tend to emerge later in the season, and overcup oak seedlings are considered one of the most flood tolerant oak seedlings (Burns and Honkala 1990; McKnight et al. 1980). In addition to emerging later in the season than many other species, overcup oak also leafs out a month or more later than other species (Burns and Honkala 1990). This growth phenology helps contribute to its flood tolerance but further delays access to light. Historically these later emerging species would have emerged after flooding had reduced the numbers of earlier emerging species. However, in a drier disconnected floodplain, later germination, leaf out, and intolerance to shade (Burns and Honkala 1990; McKnight et al. 1980) poses a hinderance to regeneration. I hypothesized that reduced water availability impacts germination of recalcitrant seeds more negatively than orthodox seeds. In the broader context of regeneration and life history of the species I investigated, the results of my study indicate that my hypothesis was not clearly supported or refuted. Instead, my results suggests that the impact of water availability is not necessarily more negative, but that water availability is integrated into the life history and regeneration strategies of these species and therefore the response to water availability are simply different.

# CHAPTER 3. BIOMASS ALLOCATION PATTERNS OF CO-OCCURRING BOTTOMLAND HARDWOOD TREE SPECIES WITH DIFFERING STRESS TOLERANCES

## Introduction

Evolutionary processes and plant responses to the current environment interact to drive patterns of plant biomass allocation, thus affecting morphological, physiological, and phenological characteristics (Valladares et al. 2007). Within limits, plants can shift biomass allocation towards specific plant traits that aid in capturing limiting resources and facilitate avoiding or enduring stress. Seedlings are particularly sensitive to changes in environment, and growth patterns during early establishment determine the likelihood of the plant regenerating successfully (Grime 1977; Grubb 1977). These interactions among the plant, its environment, and regeneration means plant traits and biomass allocation are linked to species distribution and forest composition (Kattage et al. 2020).

In floodplain forest environments, many seedlings encounter multiple stressors including flooding and dense shade. Many rivers in the southeastern U.S. and their associated floodplain forests, or bottomland hardwoods (BLHs), have experienced altered flooding patterns which have created or amplifyed drought conditions (King and Keim 2019). Polytolerance, the ability to tolerate multiple stressors simultaneously, seldom occurs because the traits that convey tolerance to one stress are typically opposed to tolerating others (Niinemets and Valladares 2006). Biomass allocation is one mechanism plants use to regulate stress tolerance (Niinemets and Valladares 2006). However, biomass allocation may or may not differ among stressors or species (Laanisto and Niinemets 2015; Niinemets and Valladares 2004; 2006; Portsmuth and Niinemets 2007), because multiple morphological traits are involved in conveying stress tolerance, and any one plant trait may aid in multiple tolerance strategies. While flood and shade tolerance are generally understood in bottomland hardwood trees, little research has been conducted on drought tolerance. Furthermore, I am unaware of studies that have investigated tradeoffs in biomass allocation across multiple southeastern floodplain forest tree species. Most studies focusing on stress tolerance tradeoffs have investigated individual occurrence across environmental gradients in multiple species (Battaglia and Sharitz 2006) or in survival following disturbance (Mann et al. 2008). Globally, tradeoffs between flood and shade, shade and drought, and flood and drought tolerance, have been identified across phylogeny (Niinemets and Valladares 2006). However, due to the near infinite combination of stressors and species-specific stress responses, evidence both in support of (Battaglia and Sharitz 2006; Delgado et al. 2018; Huang et al. 2008; Kotowski et al. 2010; Mann et al. 2008) and against (Amissah et al. 2015; Holmgren 2000; Huang et al. 2008; Lucas et al. 2019; Markesteijn and Poorter 2009) these tradeoff hypotheses exist.

Optimal partitioning theory (Bloom et al. 1985) can be used to explain biomass allocation in response to stress and its tradeoffs. With optimal partitioning plants allocate proportionally more biomass towards traits that assist in acquiring the most limiting resource. In addition, species that can tolerate conditions where a resource is limiting are better able to compete for that resource in this manner. In flooded conditions the limiting resources are oxygen and light (Taiz and Zeiger 2010). Therefore, shifts in biomass allocation to avoid or tolerate flood stress may include traits to increase air movement, to avoid being over topped by flood waters, or to reduce metabolic expenditures (Kozlowski 1997; Sauter 2013; Whitlow and Harris 1979). Associated plant traits could include creating low density stems and roots and or producing adventitious roots, to better transport air through the stems and roots to tolerate low soil oxygen and endure these conditions in a low growth state until flooding subsides (Kozlowski 1997; Sauter 2013; Whitlow and Harris 1979). Alternatively, increased vertical growth can maintain leaf production above water to facilitate photosynthesis and gas exchange and in this manner escape or avoid being overtopped by flood water (McKnight et al. 1980; Whitlow and Harris 1979). In shaded conditions light is limiting (Taiz and Zeiger 2010). Two broad plant strategies to tolerate low light conditions include shade avoidance/escape, and shade tolerance/endurance (Gommers et al. 2013). To tolerate shade, plants may allocate biomass towards storage and structural carbon by creating longer-lived and denser (high biomass per surface area or length) organs (Kozlowski 1997, Eissenstat and Volder 2005). To avoid shade, plants may allocate biomass to vertical growth to maximize light access and minimize structural carbon investment to produce organs quickly and efficiently (Caldwell and Pearcy 1994; King 1986; Markesteijn and Poorter 2009; Poorter and Garnier 1999). Lastly, drought stress limits water availability and plants can maintain a positive water balance by increasing water capture while decreasing water loss typically through increasing the ratio of root biomass to stem and leaf biomass (Ledo et al. 2018; Nash and Graves 1993; Parolin et al. 2010; Tyree and Ewers 1991). Alternatively, or in conjunction with this, plants can alter the ratios of stem length, width, and density to balance hydraulic conductance (Bucci et al. 2004; Gonzalez-Benecke et al. 2010; Meinzer et al. 2009), capacitance (Kotowska et al. 2015; McCullough et al. 2014), and cavitation resistance (Ogasa et al. 2013).

In this study I evaluated the variation in biomass allocation among naturally regenerated seedlings of 11 commonly occurring bottomland hardwood tree species. Several plant traits were investigated based on their importance in physiological function and stress tolerance. My goal was to characterize the morphology of these seedlings and to determine if seedling flood, shade, and drought tolerances were associated with specific morphological characteristics. I hypothesized that seedlings possess morphology that enhances the acquisition of limited resources associated with the stress to which the species is most tolerant to. In addition, because of the connection between stress tolerance and morphology I hypothesized that tradeoffs in biomass allocation among stress tolerances exist.

## Methods

### *Site Descriptions*

I collected seedlings from four wildlife management areas (WMAs) in eastern Texas (Figure 3.1). These WMAs included: Richland Creek (31°54’03’’N 96°00’55’’W), Old Sabine Bottom (32°35’56.74’’N 95°20’13’’W), Alazan Bayou (31°29’14’’N 94°45’08’’W), and Gus Engeling (31° 54’ 28’’N 95° 54’ 11’’W). The 30-year average annual precipitation across all sites is 1175 mm, and the 30-year average daily maximum and minimum temperatures are 25.1°C and 12.1°C, respectively (NOAA National Centers for Environmental Information, Asheville, North Carolina, Annual Summaries http://www.ncdc.noaa.gov accessed February 2018). Vegetation monitoring plots were established at all four WMAs in conjunction with other research activities (see Lemon 2020; Nguyen 2021) and these plots were used to determine dominant canopy composition and regeneration availability for sampling as part of this study.

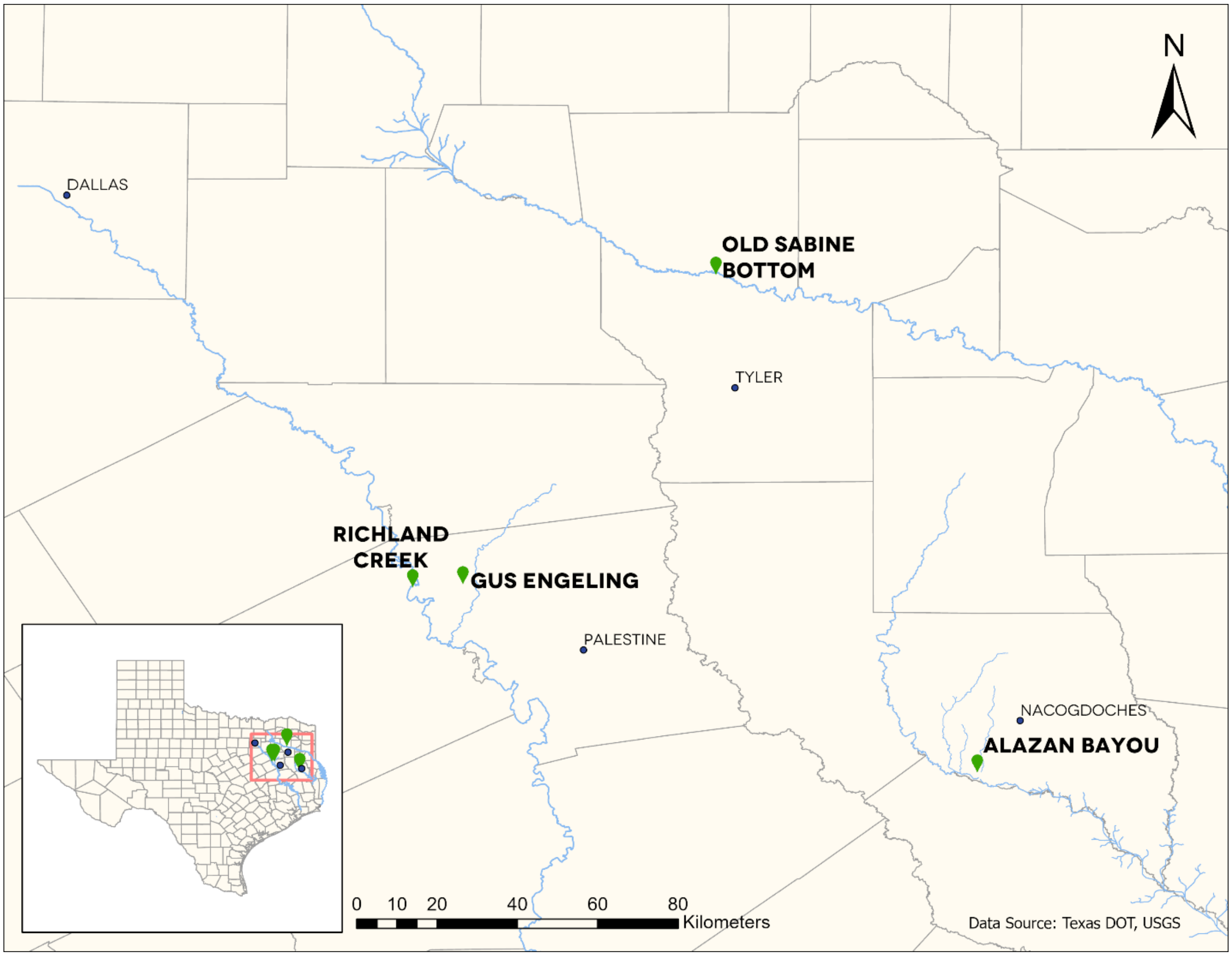


Figure 3.1. Location of four WMAs in Texas, United States, used as seedling collection sites: Old Sabine Bottom, Gus Engeling, Richland Creek, and Alazan Bayou.

Richland Creek WMA is within the Trinity River floodplain. The Trinity River is highly incised along the WMA's boundary, and the Richland Chambers Reservoir and a water-level controlling dam are located approximately 3 km to the west. The two predominant soil series are mapped as Kaufman and Trinity series (NRCS, accessed February 2018). Both soils are very deep, moderately well drained, and very slowly permeable clays. Both series are very fine, smectitic, thermic, Typic Hapluderts. Trinity series soils are also calcareous. Dominant overstory species include cedar elm (*Ulmus crassifolia* Nutt.*)*, sugarberry (*Celtis laevigata* Wild.), and green ash (*Fraxinus pennsylvanica* Marshall), with honey locust (*Glenditsia triacanthos* L.), boxelder (*Acer negundo* L.), black willow (*Salix nigra* Marshall), and overcup oak (*Quercus lyrata* Walter) also locally dominant.

Old Sabine Bottom WMA occurs within the Sabine River floodplain with the Sabine River to the north and the old Sabine River channel to the south. The predominant soil series is mapped as Gladewater (NRCS, accessed February 2018). Gladewater soils are very deep, somewhat poorly drained, very slowly permeable clays. These soils are very fine, smectitic, thermic Chromic Epiaquerts. Dominant overstory species include cedar elm, water oak (*Quercus nigra L.*), sweet gum (*Liquidambar styracaflua* L.), sugarberry, willow oak (*Quercus phellos* L.), overcup oak, American elm (*Ulmus Americana* L.), bitter pecan (*Carya aquatic* Nutt), and isolated patches of bottomland post oak (*Quercus similis* Ashe).

Alazan Bayou WMA lies within the Angelina River floodplain with the Angelina River to the south and Alazan Bayou to the east. The two predominant soil series are mapped as Mantachie and Tuscosso (NRCS, accessed February 2018). Both soils are very deep, somewhat poorly drained, moderately slowly permeable loams. Mantachie soils are fine-loamy, siliceous, active, acid, thermic Fluventic Endoaquepts, and Tuscosso soils are fine, mixed, active, thermic Dystric Fluventic Eutrudepts. Dominant overstory species include willow oak, water oak, sweetgum, bitter pecan, overcup oak, green ash, with locally dominant planer tree (*Planera aquatic* J.F.Gmel.) and laurel oak (*Quercus laurifolia* Michx.) throughout.

Gus Engling WMA is predominantly comprised of upland post oak savannah. However, bottomland hardwood forests surround and intersect these upland areas primarily along Catfish Creek to the east. Within the bottomland hardwood ecotype the predominant soil series are mapped as Nahatche and Pluck (NRCS, accessed February 2018). Both soils are very deep, poorly to somewhat poorly drained, moderately permeable fine loams. Nahatche is a fine-loamy, siliceous, active, nonacid, thermic Aeric Fluvaquent and Pluck is a fine-loamy, siliceous, active, acid, thermic Fluvaquentic Endoaquept. Within the bottomland hardwood ecotype, the dominant tree species include water oak, willow oak, overcup oak, and green ash with locally dominant planer tree and hornbeam (*Carpinus caroliniana* Walter).

### *Seedling Collection*

Sites were visited in April, May, June, October, and November 2018, April, August, and September 2019, and June 2020 as flood timing and accessibility allowed. Areas of high regeneration of commonly occurring tree species were located. Eleven species were chosen to examine species-specific tradeoffs in biomass allocation. These species were chosen based on their abundance of regeneration, prevalence in BLH forests with and without modified hydrology, and their range in drought, flood, and shade-tolerances (Table 3.1). When an area of abundant regeneration was identified, a maximum of 3 conspecific seedlings within a 10m radius were collected. Young, first- and second-year seedlings were preferentially collected. To reduce the probability of a seedling being older than one to two years, several selection criteria were used including height, the presence of seed remnants, cotyledons, and terminal bud scale scars from previous years' growth. Average height differed among species ($P<0.001$) and average height across species was 22.0±0.5 cm. In addition, I consulted with WMA staff to estimate emergence timing of species.

Seedlings were excavated by hand by carefully loosening the soil around the plant, following along lateral roots, and further removing as much of the bulk soil surrounding the rooting zone as possible to minimize the loss of fine roots. Seedlings were stored in plastic bags with moist paper towels and stored in a dark cooler at 4°C until biomass could be measured. Soil was carefully rinsed off from the plants and towel dried prior to biomass measurements.

Table 3.1. Species and common name, abbreviations (Abbr.), and assumed flood, drought, and shade-tolerance ranking of all collected species.

| Species (common name) | Abbr. | Flood Tolerance | Drought Tolerance | Shade Tolerance |
|---|---|---|---|---|
| *Acer negundo* (boxelder) | ACNE | Moderate | Tolerant | Moderate |
| *Acer rubrum* (red maple) | ACRU | Moderate | Moderate | Tolerant |
| *Celtis laevigata* (sugarberry) | CELA | Moderate | Moderate | Tolerant |
| *Fraxinus pennsylvanica* (green ash) | FRPE | Moderate | Moderate | Moderate |
| *Gleditsia triacanthos* (honey locust) | GLTR | Intolerant | Tolerant | Intolerant |
| *Planera aquatica* (water elm) | PLAQ | Tolerant | Intolerant | Tolerant |
| *Quercus lyrata* (overcup oak) | QULY | Tolerant | Intolerant | Moderate |
| *Quercus nigra* (water oak) | QUNI | Moderate | Intolerant | Intolerant |
| *Quercus texana* (Nuttall's oak) | QUTE | Moderate | Intolerant | Intolerant |
| *Quercus phellos* (willow oak) | QUPH | Moderate | Intolerant | Intolerant |
| *Ulmus crassifolia* (cedar elm) | ULCR | Intolerant | Moderate | Tolerant |

All species were assigned a tolerance ranking of tolerant, moderate, or intolerant for flood, drought, and shade stress (Table 3.1). These rankings were assigned based on the most consistent ranking across comparative studies as well as professional expertise (see acknowledgments). Whenever possible, rankings specific to the seedling age class were used, and for species with large native ranges and occurrences outside of BLH forests, rankings specific to either BLHs or within the Atlantic and Gulf Coastal Plain region were used (Baker 1949; Burns and Honkala 1990; McKnight et al. 1980, Niinemets and Valladares 2006; Putnam et al. 1960 USDA, NRCS 2021; Zon and Graves 1911). Burns and Honkala (1990) and McKnight et al. (1980) define a flood-tolerant seedling as one that can survive and grow in saturated or flooded soils for an extended time during the growing season and may possess morphological or physiological adaptations that allow their survival in these conditions. The US Department of Agriculture's Natural Resources Conservation Service (USDA, NRCS 2021) defines drought tolerance as the likelihood of a seedling being found naturally occurring and surviving in high lying, coarse textured, and/or low soil moisture soils. In addition, Burns and Honkala (1990) define a drought-tolerant seedling as one that can survive low soil moisture conditions during the growing season either by halting growth or actively adjusting root and shoot architecture, and resumes growth rapidly when soil moisture becomes favorable. Lastly, McKnight et al. (1980) and Putnam et al. (1960) describe shade-tolerance as a relative characterization of growth rate and survival of a seedling under limited light and/or closed canopy conditions.

*Biomass measurements*

To measure biomass, seedlings were separated into roots, stems, and leaves and the fresh mass of each component was measured. The number of leaves were counted, and to measure single-sided leaf area a flatbed scanner (HP Officejet 6500A Plus) and pixel counting software were used (Leaf Area Measurement, Askew 2003, Sheffield, UK). Stem volume was calculated as the volume of a truncated cone:

$$SV = (\pi L/12))(D^2_{top}+D_{top}D_{base}+D^2_{base})$$

where SV is stem volume ($cm^3$), L is the stem length (cm), $D_{top}$ is the diameter at the top of the stem (cm) just below the apical bud, and $D_{base}$ is the diameter at the base of the stem (cm) just above the root collar (Markesteijn and Poorter 2009). For seedlings that were branched, stem volume was calculated using the same formula, with each branch treated as an individual stem and $D_{base}$ as the diameter just above the branch node, and seedling stem volume being calculated as the total sum of the individual branches. Total root length was measured using the same flatbed scanner and Image J (Image J, National Institute of Health, Bethesda, MD) using methods described by Kimura et al. (1999) and Kimura and Yamasaki (2001). A subsample of roots covering the full range of root lengths and species was selected to also be measured by hand to compare methods. Measurements produced by these two methods did not differ ($P=0.077$; Table B.1). Rooting depth was measured in the field as the approximate depth of the deepest primary root. Following these measurements, all plant material was placed in a forced air oven to dry at 70°C and weighed periodically until dry weight was stable. Morphological traits (Table 3.2) were selected based on their importance in physiological functions including resource capture, resource use efficiency, mechanical support, and for their ability to quantify resource partitioning tradeoffs (Markesteijn and Poorter 2009).

Table 3.2. Morphological traits (abbreviation), description, grouping used in MANOVA, and units.

| Seedling Trait | Description | Grouping | Units |
|---|---|---|---|
| Biomass | Total dry mass | Seedling Growth | g |
| Leaf area | Total one-sided leaf area | Seedling Growth | $cm^2$ |
| Average leaf size (LS) | Average one-sided individual leaf area | Seedling Growth | $cm^2$ |
| Height | Vertical growth from root collar | Seedling Growth | cm |
| Stem area | Cross sectional area at root collar | Seedling Growth | $mm^2$ |
| Stem density (SD) | Stem mass per stem volume | Seedling Growth | $g\ cm^{-3}$ |
| Root length | Total root length | Seedling Growth | cm |
| Rooting depth | Depth of longest primary/tap root | Seedling Growth | cm |
| Specific leaf area (SLA) | Leaf area per leaf mass | Allocation Efficiency | $cm^2\ g^{-1}$ |
| Specific stem length (SSL) | Height per stem mass | Allocation Efficiency | $cm\ g^{-1}$ |
| Specific root length (SRL) | Root length per root mass | Allocation Efficiency | $cm\ g^{-1}$ |
| Secondary To primary root mass ratio (SecPrimRMR) | Secondary root mass per primary root mass | Allocation Efficiency | $g\ g^{-1}$ |
| Root water content (RWC) | Mass of water in root per dry root mass | Allocation Efficiency | $g\ g^{-1}$ |
| Leaf area ratio (LAR) | Leaf area per seedling biomass | Whole Plant Tradeoff | $cm^2\ g^{-1}$ |
| Leaf area per stem area (LASA) | Leaf area per cross sectional stem area | Whole Plant Tradeoff | $cm^2\ mm^2$ |
| Root length per biomass (RLBiom) | Root length per seedling biomass | Whole Plant Tradeoff | $cm\ g^{-1}$ |
| Stem length per biomass (SLBiom) | Height per seedling biomass | Whole Plant Tradeoff | $cm\ g^{-1}$ |
| Root to shoot ratio (RSR) | Root mass to combined stem and leaf mass | Whole Plant Tradeoff | $g\ g^{-1}$ |
| Leaf mass fraction (LMF) | Proportion of leaf mass to seedling biomass | Whole Plant Tradeoff | $g\ g^{-1}$ |
| Stem mass fraction (SMF) | Proportion of stem mass to seedling biomass | Whole Plant Tradeoff | $g\ g^{-1}$ |
| Root mass fraction (RMF) | Proportion of root mass to seedling biomass | Whole Plant Tradeoff | $g\ g^{-1}$ |

### *Data analyses*

All biomass data were combined across WMAs and sampling periods. All analyses were performed in R (R Core Team, 2020). Plant traits were grouped by their general physiological function and measurement scale to reduce multicollinearity (Table 3.2). Then, multivariate analysis of variance (MANOVA) was used to test for differences in grouped seedling traits among species and tolerance rankings. Pillai's trace was selected as the test statistic because of its robust general use in dealing with departures from the assumptions of MANOVA. Differences among species and within functional groups were considered significant at $\alpha \leq 0.05$. To further analyze differences ($\alpha \leq 0.05$) from MANOVA, univariate analysis of variance (ANOVA) was used. Family-wise error rate was controlled using the Holm-Bonferroni method. Differences among species and tolerance rankings, were assessed with Tukey's HSD. Variable normality and error variances were inspected visually. To improve normality and reduce heteroscedasticity of error variances all morphological traits were log transformed prior to analyses except for proportional traits which were arcsine transformed. All variables were transformed back to their original values for presentation and ease of interpretation. Lastly, linear discriminant analysis (LDA) was used to simultaneously compare multiple biomass traits for the purposes of visualizing overall differences in biomass allocation among species and tolerances rankings. Several plant traits were omitted from the LDA to reduce multicollinearity and the distortions in discriminant space caused by traits being represented multiple times (e.g. leaf area, SLA, LAR, LMF). Partial correlation analysis using Pearson's correlation coefficient was used and plant traits with high correlation or redundancy were omitted from the LDAs (Table B.2; Table B.3).

## Results

A total of 319 seedlings were used in analyses (Table 3.3). Differences in the number of seedlings among species was due to differences in regeneration abundance for those species. Combined seedling growth traits, biomass allocation efficiency, and whole plant allocation, all differed by species and for all functional group classifications (all $P<0.001$).

Table 3.3. Number of seedlings used in analyses. Flood, drought, and shade-tolerances. Number of species in each tolerance ranking.

| Species | Number of seedlings investigated | Flood tolerance | Drought tolerance | Shade tolerance |
|---|---|---|---|---|
| ACNE *(Acer negundo)* | 12 | Moderate | Tolerant | Moderate |
| ACRU *(Acer rubrum)* | 27 | Moderate | Moderate | Tolerant |
| CELA *(Celtis laevigata)* | 24 | Moderate | Moderate | Tolerant |
| FRPE *(Fraxinus pennsylvanica)* | 14 | Moderate | Moderate | Moderate |
| GLTR *(Gleditsia triacanthos)* | 14 | Intolerant | Tolerant | Intolerant |
| PLAQ *(Planera aquatica)* | 17 | Tolerant | Intolerant | Tolerant |
| QULY *(Quercus lyrata)* | 83 | Tolerant | Intolerant | Moderate |
| QUNI *(Quercus nigra)* | 16 | Moderate | Intolerant | Intolerant |
| QUTE *(Quercus texana)* | 33 | Moderate | Intolerant | Intolerant |
| QUPH *(Quercus phellos)* | 55 | Moderate | Intolerant | Intolerant |
| ULCR *(Ulmus crassifolia)* | 24 | Intolerant | Moderate | Tolerant |
|  | Tolerance rank | Number of species in each ranking |  |  |
|  | Intolerant | 2 | 5 | 4 |
|  | Moderate | 7 | 4 | 3 |
|  | Tolerant | 2 | 2 | 4 |

### *Seedling growth traits*

All seedling growth traits differed by species (Table 3.4) and varied both within and among species (Table 3.4). The species with the lowest intraspecific variability was QULY while the highest was PLAQ (Table 3.5). High variability among and within species increased the variability within stress tolerance groupings (Figure 3.2). The highest variability among stress tolerance groupings was in drought-tolerant species. Drought-tolerant species had the greatest variation in multiple growth traits including biomass, leaf area, leaf size, and root length (Figure 3.2).

In seedlings of drought-tolerant species, roots were deeper and leaf area was higher compared to drought-intolerant species. However total root length did not differ with drought tolerance. Seedlings of shade-tolerant species were shorter, with lower leaf area and smaller average leaf size while shade-intolerant seedlings were taller, with deeper roots, and denser stems. Stems in flood-tolerant species were less dense with higher stem area than flood-intolerant species, and in addition, average leaf size was higher in flood-tolerant species (Figure 3.2).

Table 3.4. Results of ANOVAs (P values) showing the effects of species, and shade, drought, and flood tolerance category on seedling growth traits.

| | Biomass | Leaf area | Leaf size | Rooting depth | Root length | Stem area | Height | Stem density |
|---|---|---|---|---|---|---|---|---|
| Species | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Shade | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Drought | <0.001 | <0.001 | <0.001 | 0.036 | 1.000 | <0.001 | 0.010 | <0.001 |
| Flood | 0.662 | <0.001 | <0.001 | 0.704 | 0.472 | 0.001 | 1.000 | <0.001 |

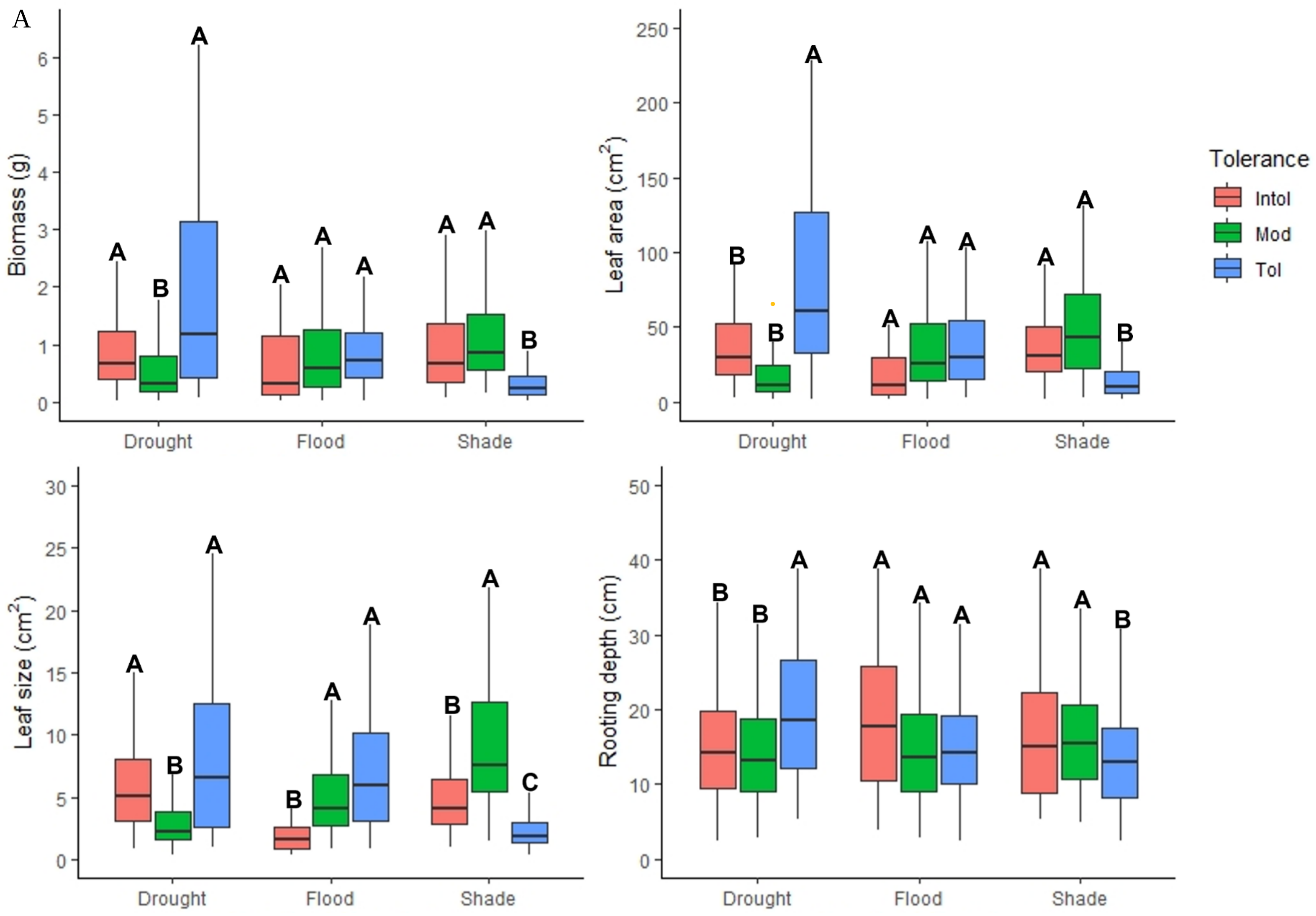


Figure 3.2. Mean (±SE) (A) seedling biomass, leaf area, leaf size, rooting depth, (B) root length, stem area, height, and stem density by drought, flood, and shade-tolerance rankings. Within each stress grouping, different letters (Tukey's HSD) indicate differences among rankings at α≤0.05.
(fig. cont'd.)

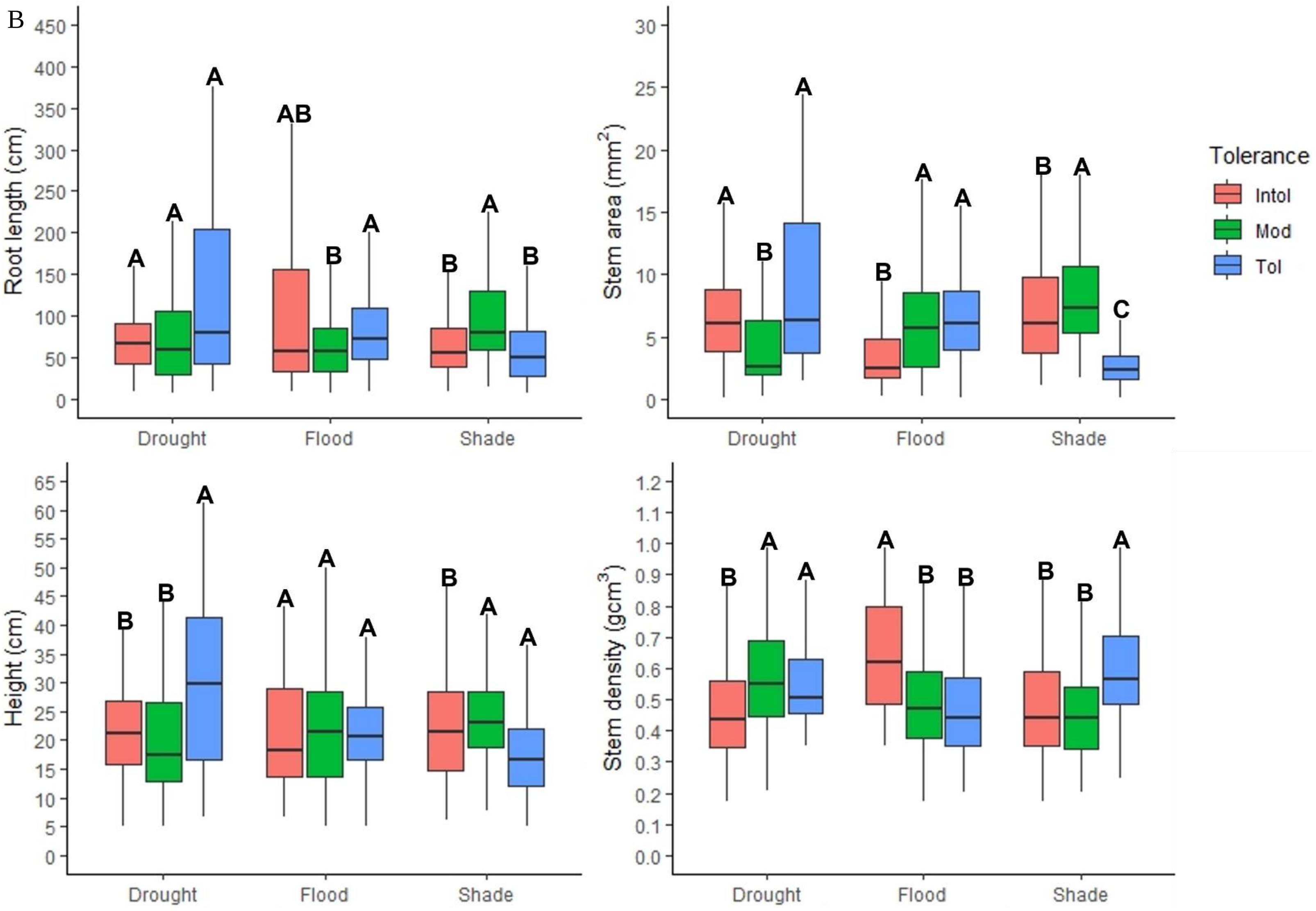

B
Root length (cm)
0
50
100
150
200
250
300
350
400
450
A
A
A
AB
B
A
B
A
B
Drought
Flood
Shade
Stem area (mm$^2$)
0
5
10
15
20
25
30
A
B
A
B
A
A
B
A
C
Drought
Flood
Shade
Tolerance
Intol
Mod
Tol
Height (cm)
0
5
10
15
20
25
30
35
40
45
50
55
60
65
B
B
A
A
A
A
B
A
A
Drought
Flood
Shade
Stem density (gcm$^3$)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
B
A
A
A
B
B
B
B
A
Drought
Flood
Shade

Table 3.5. Mean (±SE) seedling growth traits for all species. Different letters (Tukey's HSD) on mean trait values indicate difference ($\alpha \leq 0.05$) among species for that trait.

| Species | Biomass (g) | Leaf area (cm$^2$) | Leaf size (cm$^2$) | Rooting depth (cm) | Root length (cm) | Stem area (mm$^2$) | Height (cm) | Stem density (g cm$^{-3}$) |
|---|---|---|---|---|---|---|---|---|
| ACNE | 1.6 (0.5)ab | 107.8 (19.4)a | 13.6 (1.8)a | 16.2 (1.6)abc | 99.9 (29.4)bcd | 8.9 (2.3)ab | 30.8 (3.0)ab | 0.52 (0.02)abc |
| ACRU | 0.3 (0.0)c | 10.7 (1.2)f | 3.3 (0.3)cd | 9.1 (0.7)d | 32.4 (3.8)e | 2.8 (0.2)cd | 14.0 (0.7)d | 0.53 (0.02)abc |
| CELA | 1.2 (0.4)bc | 37.5 (11.9)def | 2.0 (0.2)de | 16.8 (1.4)abc | 179.4 (39.6)ab | 5.3 (1.3)cd | 22.9 (2.7)bcd | 0.65 (0.04)ab |
| FRPE | 2.9 (0.6)a | 96.6 (23.2)ab | 17.3 (3.4)a | 21.4 (2.9)ab | 255.0 (69.7)a | 16.5 (2.0)a | 32.4 (2.5)a | 0.40 (0.02)cd |
| GLTR | 2.5 (0.8)ab | 57.0 (13.7)abcd | 4.0 (0.8)cd | 23.2 (2.8)a | 171.5 (36.2)abc | 12.1 (2.9)ab | 29.8 (4.9)ab | 0.57 (0.05)abc |
| PLAQ | 0.6 (0.3)c | 19.3 (5.6)ef | 2.0 (0.3)de | 12.9 (1.5)bcd | 174.6 (106.5)bcd | 3.9 (1.2)cd | 20.7 (3.1)bcd | 0.53 (0.03)abc |
| QULY | 1.0 (0.1)b | 43.5 (3.5)bcd | 7.8 (0.4)b | 15.8 (0.8)abc | 101.7 (8.6)bc | 7.6 (0.4)b | 21.8 (0.7)bc | 0.45 (0.02)cd |
| QUNI | 0.7 (0.2)bc | 33.3 (8.8)cde | 3.7 (0.4)c | 12.4 (2.0)cd | 45.8 (6.7)de | 5.2 (1.2)bc | 16.6 (2.2)cd | 0.45 (0.03)cd |
| QUNU | 0.6 (0.1)bc | 45.6 (5.1)abc | 8.5 (0.8)ab | 13.9 (1.3)abcd | 81.2 (11.9)bcd | 7.2 (0.7)b | 22.3 (1.1)abc | 0.34 (0.01)d |
| QUPH | 1.3 (0.2)ab | 36.8 (4.06)cd | 4.2 (0.3)c | 17.3 (1.0)abc | 64.7 (4.5)cd | 7.3 (0.6)b | 22.5 (1.1)abc | 0.55 (0.03)bc |
| ULCR | 0.4 (0.1)c | 11.4 (1.9)f | 1.7 (0.5)e | 16.0 (2.0)abc | 64.9 (13.3)cde | 2.2 (0.4)d | 19.4 (1.7)bcd | 0.70 (0.05)a |

*Biomass Allocation Efficiency*

Several biomass allocation efficiency traits differed among tolerance rankings (Table 3.6) and every trait differed among species (all P<0.001; Table 3.6). In addition, there appeared to be a high degree of intraspecific variability across most variables (Table 3.7). One exception was SLA, which was the only biomass efficiency variable that was comparatively consistent both within and among species. Drought-tolerant and flood-tolerant species were the most variable however these group were composed of only two species each (Table 3.3)

Compared to drought-intolerant seedlings, drought-tolerant seedling's leaves were thinner with higher leaf area per leaf weight, seedling stems had higher structural carbon investment per stem height, and their roots possessed a greater proportion of fine roots (Figure 3.3). The water content of these roots was also relatively low. Seedlings of flood-intolerant species produced stems with lower structural carbon resulting in a greater height per carbon investment in comparison to flood-tolerant species, while roots had lower water storage capacity than flood-tolerant species (Figure 3.3). Lastly, leaves of shade-tolerant species were thinner with higher leaf area per leaf weight than those of shade-intolerant species, and stems were produced with lower structural carbon resulting in a greater height per carbon investment. The roots of shade-tolerant seedlings possessed a greater proportion of fine roots with less water storage than roots of shade-intolerant seedlings (Figure 3.3).

Table 3.6. Results of ANOVAs (P values) showing the effects of species, and shade, drought, and flood tolerance ranking on biomass allocation efficiency traits.

| | SLA | SRL | SecPrimRMR | SSL | RWC |
|---|---|---|---|---|---|
| Species | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Shade | <0.001 | <0.001 | 0.207 | <0.001 | <0.001 |
| Drought | <0.001 | 0.012 | 0.004 | <0.001 | <0.001 |
| Flood | 0.521 | 0.023 | 1.000 | 0.037 | <0.001 |

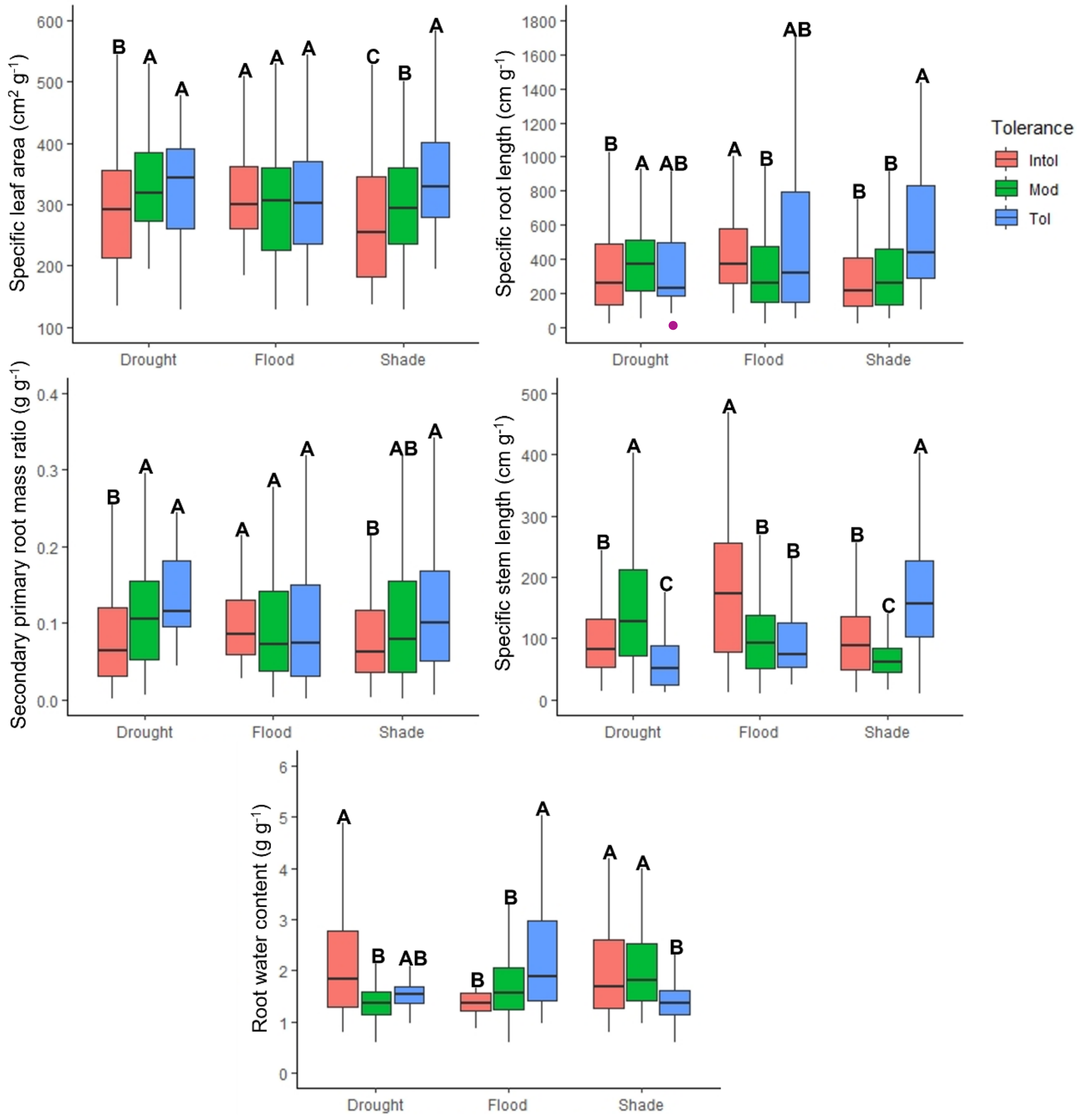


Figure 3.3. Mean (±SE) specific leaf area, specific root length, secondary to primary root mass ratio, specific stem length, and root water content by drought, flood, and shade-tolerance rankings. Within each stress grouping, different letters (Tukey's HSD) indicate differences among rankings at $\alpha \leq 0.05$.

Table 3.7. Mean (±SE) seedling biomass allocation efficiency traits for all species. Different letters (Tukey's HSD) on mean trait values indicate differences ($\alpha \leq 0.05$) among species for that trait.

| Species | SLA ($cm^2$ $g^{-1}$) | SRL (cm $g^{-1}$) | SecPrimRMR (g $g^{-1}$) | SSL (cm $g^{-1}$) | RWC (g $g^{-1}$) |
|---|---|---|---|---|---|
| ACNE | 357.2 (27.5)ab | 330.6 (55.3)bcde | 0.16 (0.04)ab | 61.9 (12.3)cd | 1.8 (0.2)bcd |
| ACRU | 314.3 (12.9)abc | 299.2 (26.9)cde | 0.09 (0.01)ab | 133.7 (8.9)a | 1.7 (0.2)cd |
| CELA | 377.1 (19.5)a | 1284.0 (346.06)ab | 0.17 (0.02)a | 205.5 (43.7)ab | 1.3 (0.1)d |
| FRPE | 310.2 (13.6)abc | 242.4 (50.0)de | 0.14 (0.03)ab | 40.4 (4.8)d | 1.5 (0.1)cd |
| GLTR | 346.5 (51.5)abc | 374.0 (80.4)cde | 0.17 (0.03)a | 102.1 (31.5)bcd | 2.0 (0.4)bcd |
| PLAQ | 394.4 (19.1)a | 1606.6 (547.3)a | 0.24 (0.07)a | 312.4 (93.2)a | 2.0 (0.3)bcd |
| QULY | 286.0 (8.1)bc | 419.4 (47.9)cde | 0.12 (0.02)ab | 81.4 (4.9)bc | 2.4 (0.1)ab |
| QUNI | 250.9 (24.3)cd | 279.3 (47.5)cde | 0.07 (0.01)ab | 149.8 (21.5)ab | 2.0 (0.2)abc |
| QUNU | 364.5 (10.6)a | 422.9 (45.6)bcd | 0.13 (0.02)ab | 114.8 (8.8)abc | 2.7 (0.1)a |
| QUPH | 219.3 (9.7)d | 228.2 (32.8)e | 0.06 (0.01)b | 93.4 (11.0)bcd | 1.6 (0.1)cd |
| ULCR | 341.8 (24.8)ab | 697.5 (182.7)abc | 0.12 (0.04)ab | 241.6 (30.8)a | 1.3 (0.1)cd |

*Whole plant biomass allocation*

Every whole plant allocation trait differed by species (all $P<0.001$; Table 3.8). In addition, several of the traits investigated showed one or more tolerance groupings with a high degree of variation. This variability appeared to be driven by high interspecific variability and low numbers of representative species in the respective functional groups.

Root to shoot ratio was lower in drought-tolerant species than in drought-intolerant species due to a greater allocation of biomass to stem mass and a lower allocation to root mass (Figure 3.4). Stems in seedlings of flood-intolerant species had more vertical height stem mass and less leaf mass compared to stems of flood-tolerant seedlings (Figure 3.4). Root length and stem length and mass were higher in shade-tolerant seedlings and leaf mass was lower when compared to shade-intolerant species (Figure 3.4).

Table 3.8. Results of ANOVAs (P values) showing the effects of species, and shade, drought, and flood tolerance ranking on whole plant allocation traits.

| | LAR | RLBiom | LASA | SLBiom | RSR | LMF | SMF | RMF |
|---|---|---|---|---|---|---|---|---|
| Species | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Shade | 1.000 | <0.001 | 1.000 | <0.001 | 1.000 | 0.047 | 0.011 | 1.000 |
| Drought | 0.190 | <0.001 | 0.048 | <0.001 | <0.001 | <0.001 | <0.001 | 0.005 |
| Flood | 0.767 | 0.016 | 1.000 | 0.061 | 1.000 | 0.101 | 1.000 | 1.000 |

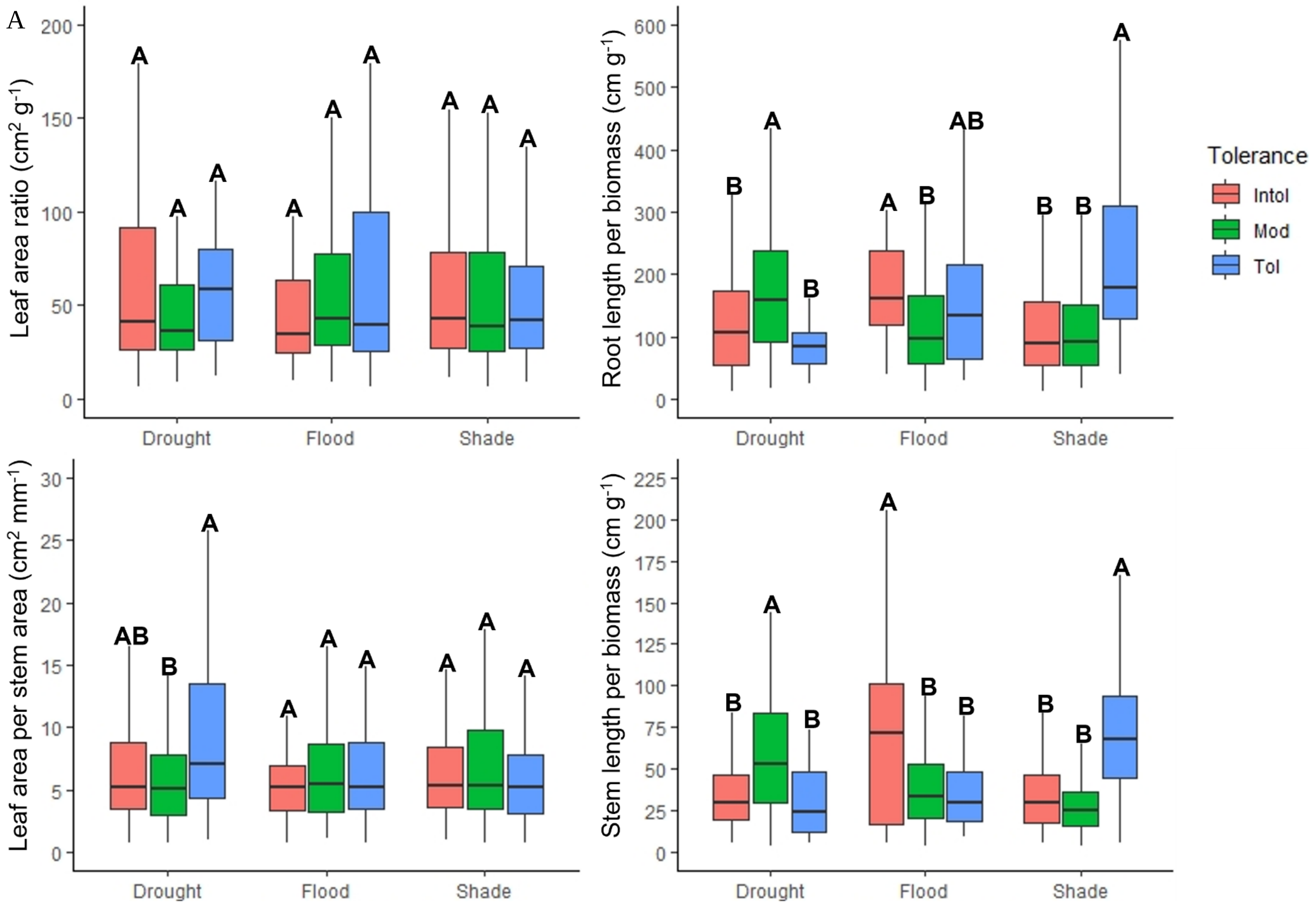


Figure 3.4. Mean (±SE) (A) leaf area ratio, root length per biomass, leaf area per stem area, stem length per biomass, (B) root to shoot ratio, leaf mass fraction, stem mass fraction, and root mass fraction by drought, flood, and shade-tolerance. Within each stress grouping, different letters (Tukey's HSD) indicate differences among ranking at α≤0.05.
(fig. cont'd.)

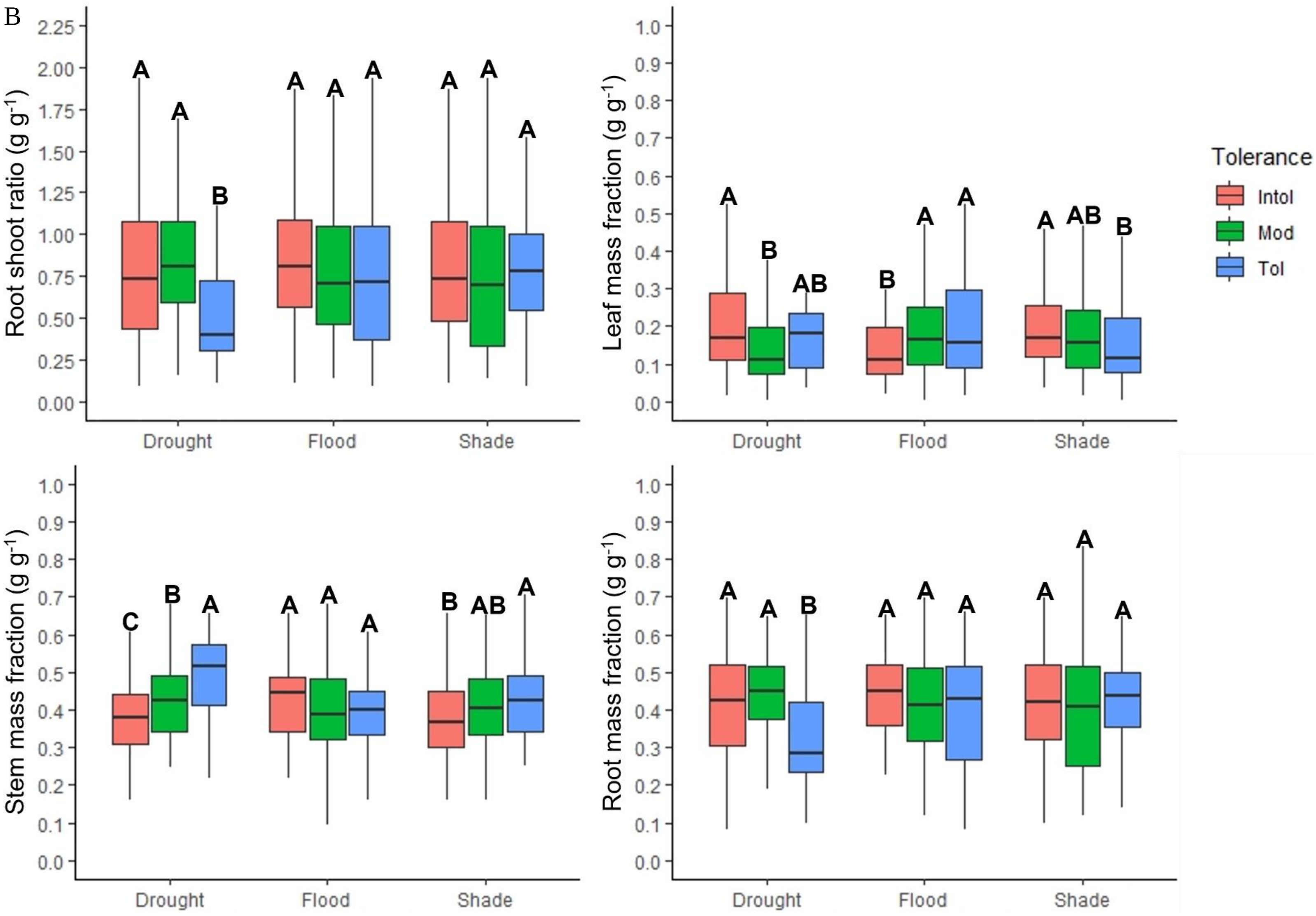

B
Root shoot ratio (g g$^{-1}$)
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
A A B
A A A
A A A
Drought
Flood
Shade
Leaf mass fraction (g g$^{-1}$)
A B AB
B A A
A AB B
Stem mass fraction (g g$^{-1}$)
C B A
A A A
B AB A
Root mass fraction (g g$^{-1}$)
A A B
A A A
A A A
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Tolerance
Intol
Mod
Tol

Table 3.9. Mean (±SE) whole seedling biomass allocation traits for all species. Different letters (Tukey's HSD) on mean trait values indicate differences (α≤0.05) among species.

| Species | LAR ($cm^2\ g^{-1}$) | RLBiom ($cm\ g^{-1}$) | LASA ($cm^2\ mm^{-2}$) | SLBiom ($cm\ g^{-1}$) | RSR ($g\ g^{-1}$) | LMF ($g\ g^{-1}$) | SMF ($g\ g^{-1}$) | RMF ($g\ g^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| ACNE | 88.6 (14.9)a | 68.8 (7.4)d | 14.1 (1.9)a | 31.2 (5.3)bcd | 0.31 (0.03)sb | 0.25 (0.03)ab | 0.52 (0.03)a | 0.23 (0.02)b |
| ACRU | 43.9 (5.1)b | 118.8 (10.6)cd | 4.4 (0.5)b | 58.5 (5.0)ab | 0.81 (0.07)a | 0.14 (0.02)b | 0.44 (0.02)ab | 0.42 (0.02)a |
| CELA | 56.0 (8.5)ab | 410.4 (78.9)a | 6.6 (0.7)ab | 76.2 (15.4)ab | 0.81 (0.07)a | 0.16 (0.03)b | 0.41 (0.02)ab | 0.43 (0.02)a |
| FRPE | 34.0 (3.9)b | 99.4 (16.8)cd | 5.3 (0.8)b | 167.0 (2.5)d | 1.12 (0.31)a | 0.11 (0.01)b | 0.42 (0.04)ab | 0.46 (0.04)a |
| GLTR | 46.2 (13.0)b | 127.6 (22.6)cd | 5.4 (0.9)b | 46.1(14.6)cd | 0.79 (0.12)a | 0.14 (0.04)b | 0.45 (0.04)ab | 0.41 (0.04)a |
| PLAQ | 87.2 (17.0)ab | 384.5 (65.1)a | 7.3 (1.4)ab | 112.7 (22.9)a | 0.60 (0.08)ab | 0.22 (0.04)ab | 0.44 (0.03)ab | 0.35 (0.03)ab |
| QULY | 58.2 (5.2)ab | 133.8 (11.7)cd | 6.4 (0.5)b | 31.0 (2.1)cd | 0.85 (0.06)a | 0.20 (0.01)ab | 0.38 (0.01)b | 0.42 (0.02)a |
| QUNI | 72.3 (11.7)ab | 105.9 (13.4)cd | 7.8 (1.1)ab | 38.5 (4.9)bc | 0.83 (0.13)a | 0.29 (0.03)ab | 0.29 (0.02)c | 0.42 (0.03)a |
| QUNU | 82.1 (5.9)a | 142.7 (17.2)bc | 7.0 (0.7)ab | 45.3 (3.2)b | 0.62 (0.06)ab | 0.23 (0.02)ab | 0.41 (0.02)ab | 0.36 (0.02)ab |
| QUPH | 43.7 (4.9)b | 90.4 (9.8)d | 6.0 (0.5)b | 30.5 (3.2)cd | 0.93 (0.06)a | 0.18 (0.01)ab | 0.36 (0.01)bc | 0.46 (0.01)a |
| ULCR | 47.7 (5.9)ab | 253.1 (39.9)ab | 7.4 (1.3)b | 102.7 (15.7)a | 1.01 (0.16)a | 0.14 (0.02)b | 0.40 (0.02)ab | 0.46 (0.03)a |

*Linear discriminant analysis*

When discriminating among species, the first (LD1) and second (LD2) discriminant functions accounted for 67% and 12% of the discriminative power respectively (Figure 3.5). The amount of discrimination described by the third and fourth discriminants accounted for 7% and 5% with the remaining 6 discriminants accounting for the remainder. LD1 was positively correlated with traits associated with higher biomass allocation efficiency, specifically towards vertical growth and root growth. These traits included higher SSL, SLBiom, RLBiom, and SRL. In addition, LD1, was negatively associated with traits associated with larger leaf area, higher RWC, and higher stem area which could be associated with flood tolerance. LD2 appeared to discriminate among species based on rooting architecture and the balance between above and below ground allocations. LD2 was positively associated with RMF and RSR and negatively associated with root length, SecPrimRMR, rooting depth, LASA and LAR.

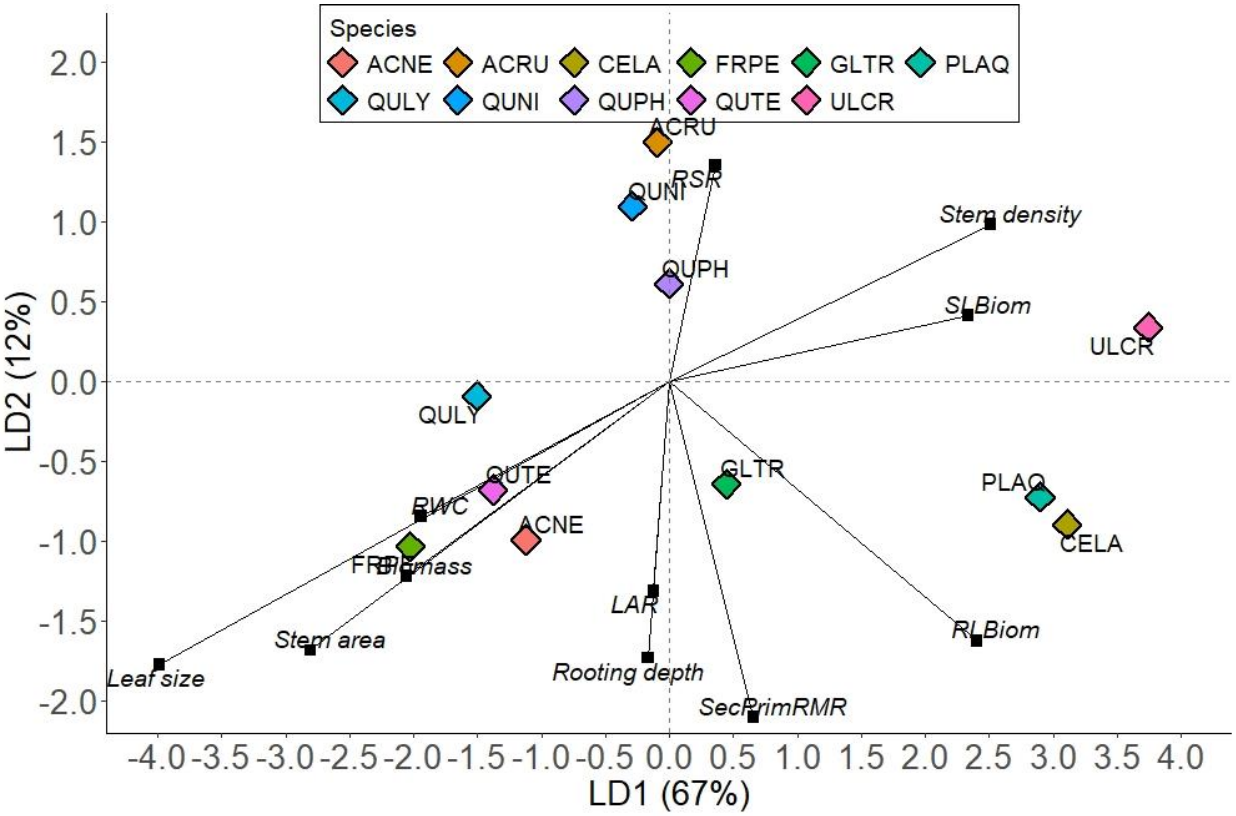


Figure 3.5. Linear discriminant analysis of plant traits (squares) for individual species (diamonds). Diamonds are the projected centroid for that species. Biomass traits are linearly scaled to fit projection space as an overlaid biplot.

When discriminating between flood-tolerance rankings, the first and second discriminant functions accounted for 78% and 22% of the discriminative power respectively (Figure 3.6). Flood-intolerant species were negatively associated with LD1 while flood-tolerant species were positively associated, and moderately flood-tolerant species fell between the two other groups. Of the flood-tolerant species investigated, PLAQ possessed plant traits more akin to flood-intolerant species. In addition, CELA, a moderately flood-tolerant species also appeared to possess traits more associated with flood-intolerant species. Difference between traits indicated that flood-intolerant species prioritized allocation to above ground vertical growth, while flood-tolerant species increased below ground biomass, altered root morphology, and increased leaf area.

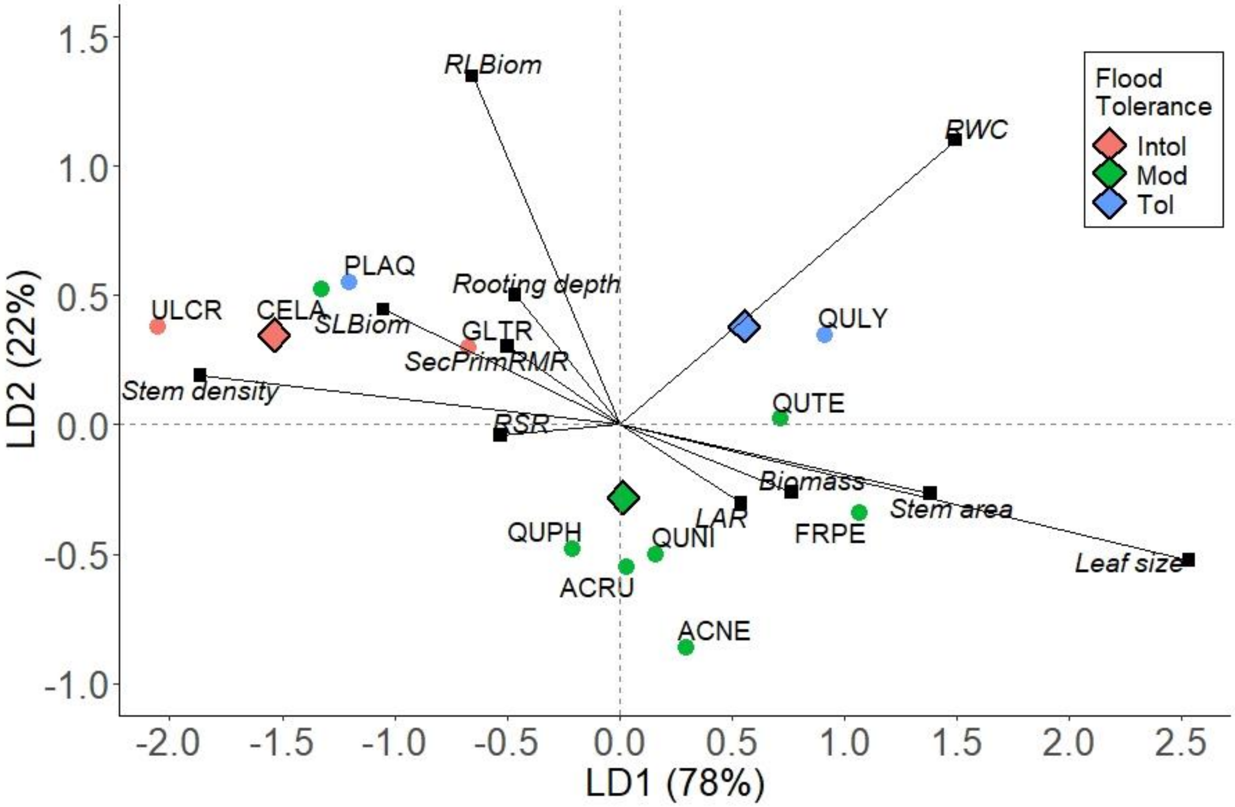


Figure 3.6. Linear discriminant analysis of plant traits (squares) for flood tolerance rankings (diamonds). Diamonds are the projected centroid for that tolerance ranking and circles are the projected centroid for that species. Color of circles denotes tolerance ranking membership. Biomass traits are linearly scaled to fit projection space as an overlaid biplot.

When discriminating between drought tolerance, the first and second discriminant functions accounted for 65% and 35% of the discriminative power respectively (Figure 3.7). Drought-tolerant species were weakly positively associated with LD1 while drought-intolerant species were weakly negatively associated. In addition, while only 33% of the discrimination among species was accounted for in LD2, the greatest difference between drought-tolerant and drought-intolerant species appeared to fall along this axis. Drought-intolerant and moderately-tolerant species appeared tightly grouped representing similarity in plant traits. FRPE, a moderately drought-tolerant species possessed traits more closely related to drought-tolerant species, and PLAQ, a drought-intolerant species appeared to possess traits more closely resembling moderately drought-tolerant species.

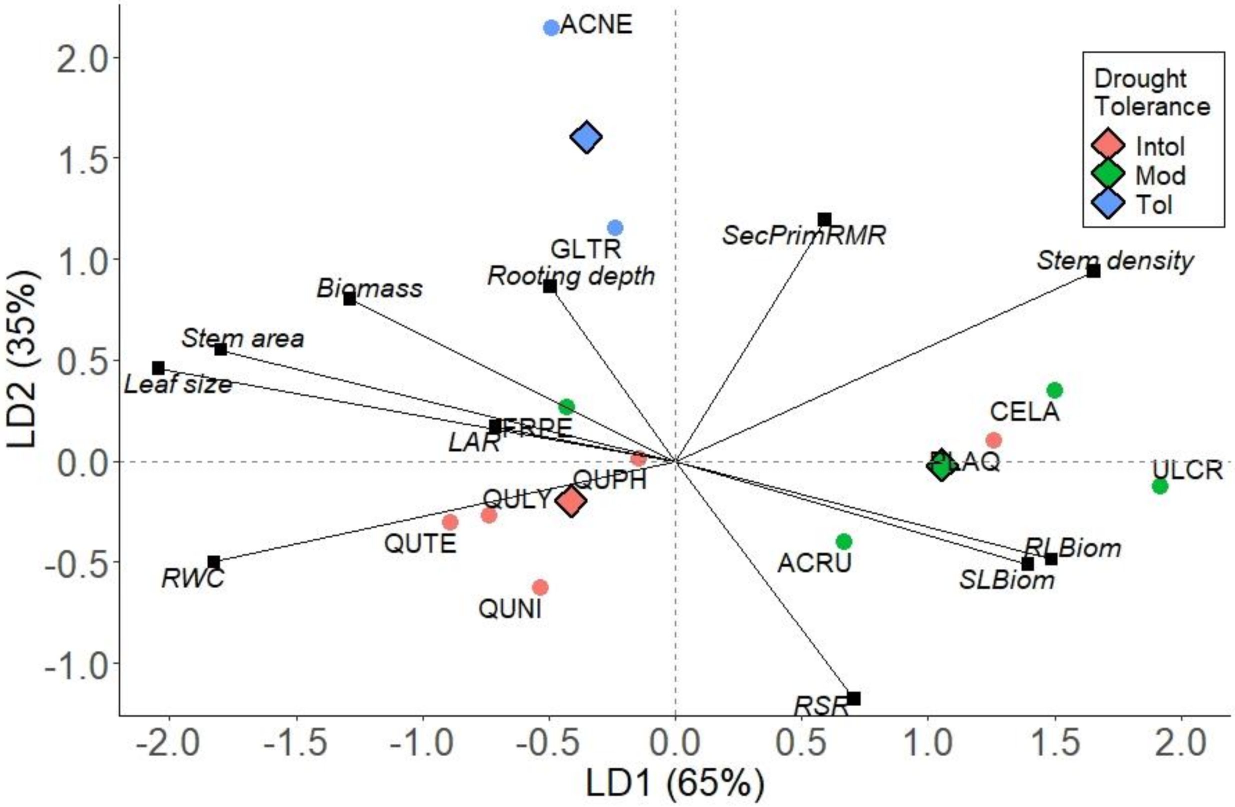


Figure 3.7. Linear discriminant analysis of plant traits (squares) for drought tolerance rankings (diamonds). Diamonds are the projected centroid for that tolerance ranking and circles are the projected centroid of that species. Color of circles denotes tolerance ranking membership. Biomass traits are linearly scaled to fit projection space as an overlaid biplot.

Lastly, when discriminating between shade tolerance, the first and second discriminant functions accounted for 89% and 11% of the discriminative power respectively (Figure 3.8). Shade tolerance rankings were almost entirely separated along LD1 with shade-tolerant species being positively associated and intolerant and moderately-tolerant species being negatively associated. Shade-tolerant species were associated with plant traits that prioritized vertical growth and higher efficiency of biomass allocation. In contrast, shade-intolerant species possessed traits that represented slower growth, longer lived organs, and greater carbon storage potential.

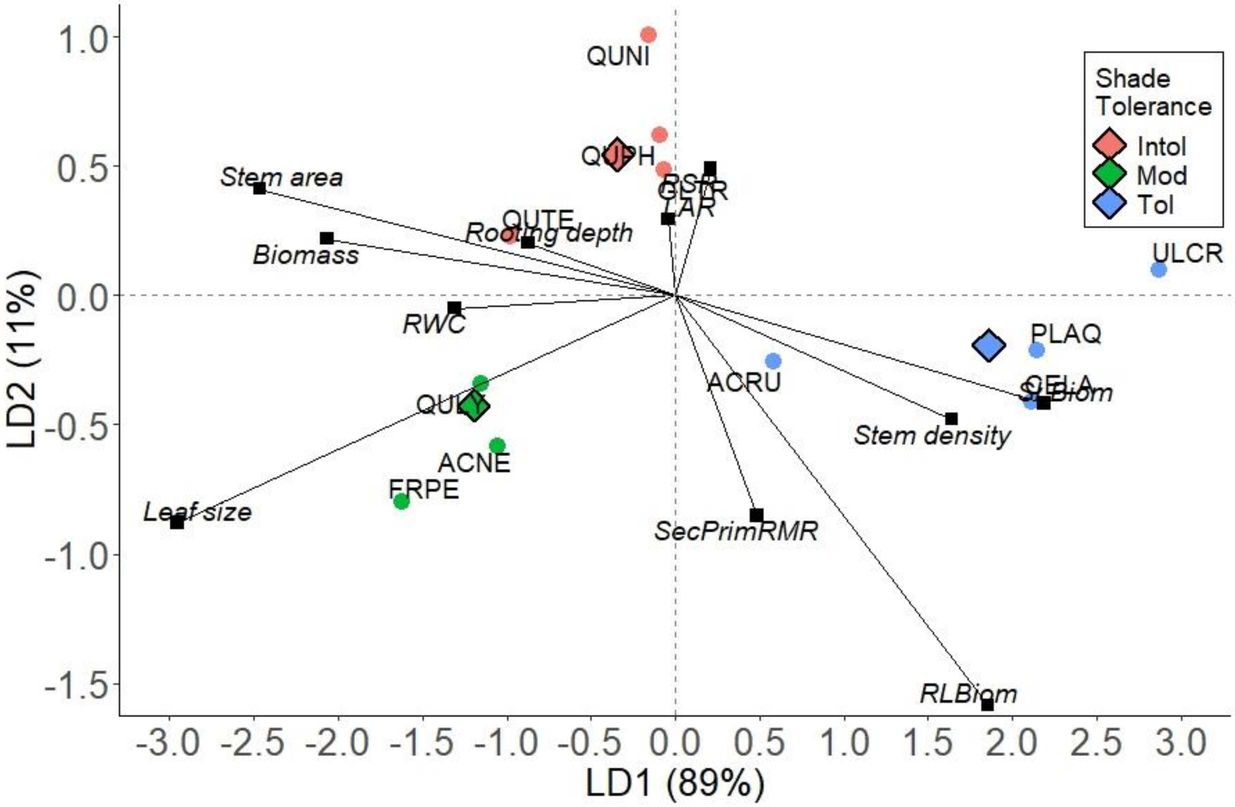


Figure 3.8. Linear discriminant analysis of plant traits (squares) for shade-tolerance rankings (diamonds). Each diamond represents the projected centroid for that tolerance ranking and circles are the projected centroid of that species. Color of circles denotes tolerance ranking membership. Biomass traits are linearly scaled to fit the projection space as an overlaid biplot.

## Discussion

Floodplain forest tree seedlings evaluated in this study possessed morphology associated with mitigating the effects of the most common stressor encountered by that species. Within a given tolerance ranking high intra- and interspecific variation indicated that no single morphology or tolerance strategy was universally favored. Furthermore, not all morphologies were compatible with one another and evidence of both polytolerance and polyintolerance were found.

Morphological differences between shade-tolerant and flood-tolerant species indicated that there was a tradeoff between these two tolerances. Shade/flood tolerance tradeoffs have been found globally and across phylogeny, however, outlier species and inconsistent responses to cooccurring shade and flood stress has spurred further investigation into the mechanisms behind this and similar tradeoffs (Niinemets and Valladares 2006). My results align with studies in other southeastern floodplain systems where shade/flood tradeoffs have been reported for the establishment and survival of multiple tree species (Battaglia and Sharitz 2006; Lin et al. 2004; Mann et al. 2008). Lin et al. (2004) found that shade-tolerant saplings grew faster in low light than shade-intolerant saplings, but they generally experience higher mortality during prolonged flooding events. Similarly, Mann et al. (2008) found in equally flood-tolerant species, flood mortality was highest in species that were also shade-tolerant and lowest in species that were shade-intolerant. Flood/shade tradeoffs, however, are found across floodplain systems globally. In agreement with my finding, it was reported that European wetland species of various moisture tolerances required a high degree of shade-tolerance to successfully establish under canopy (Kotowski et al. 2010). Furthermore, it was determined that more flood-tolerant species had lower regeneration under canopy due to slower germination and lower competitive ability for light compared to less flood-tolerant cooccurring species. In contrast, in Amazonian floodplains, Lucas et al. (2013) found no evidence for a flood/shade tradeoff among tree species, finding instead that cooccurring stressors such as mechanical damage, shade, and flood all acted independently. Lastly, counter to my results Delgado et al. (2018) found that while waterlogging decreased the survival of all temperate rainforest species they investigated, waterlogging had a greater negative impact on shade-intolerant species.

In my study, shade-intolerant species exhibited a shade endurance strategy which also coincided with more flood-tolerant plant traits. Flood-tolerance was associated with producing shorter, low-density stems with higher cross-sectional area and volume which are advantageous characteristics for flexibility under mechanical flood stress and supporting higher oxygen movement through the plant (Kozlowski 1997; Sauter 2013; Whitlow and Harris 1979). In contrast, shade-tolerant species exhibited a shade-avoidance strategy. The morphology behind this strategy included allocating a greater proportion of resources to vertical growth and producing thinner and denser stems to mechanically support branching, vertical growth, and compete for light access.

Tradeoffs between shade and drought tolerance have also been found globally, however, these tradeoffs are predominantly seen in species growing in deeply shaded environments (Niinemets and Valladares 2006). My results showed that while there were some morphological tradeoffs between shade and drought tolerance, primarily due to differences in stem density, there were also several overlaps among plant traits between the two stressors. It is possible that light or water availability at collection sites was not low enough to elicit a shade/drought tradeoff. This gradient of effects and dependence on shade intensity has been reported in other studies as well (Huang et al. 2008). While shade was not quantified across all collection sites, spot measurements of canopy intercepted photosynthetically active radiation (IPAR) were made at Alazan Bayou WMA concurrently with seedling collection, where IPAR averaged 72.9±10% (See Chapter 4 Methods). In BLHs light availability in the understory varies spatially and temporally (Battaglia and Sharitz 2006; Streng et al. 1986), and while similar light levels have been reported, lower values are equally as common (Battaglia et al. 2000; Battaglia and Sharitz 2006; Boerger et al. 2015; Gardiner and Hodges 1998). Similarly, Markesteijn and Poorter (2009) did not find a trade-off between shade and drought tolerance in moist and dry tropical forest tree seedlings. The authors concluded that the lack of tradeoff was because the two tolerances depended on different morphological adaptations. Holmgren (2000) also did not find support for a shade/drought tradeoff. Instead, their results indicated that shading led to a positive effect on plant growth under drought conditions. Improved performance or survival of seedlings in drought with shade versus drought without shade is an example of the facilitation hypothesis, where shade enhances plant performance in drought through improved microclimatic conditions. In contrast, the trade-off hypothesis postulates that shade and drought together have an additive negative impact because of shade and drought tolerance requiring opposing morphological adaptations (i.e., opposing leaf and root investments). In western China, Huang et al. (2008) found that drought led to increased root production and decreased above ground biomass in seedlings of the riparian species *Populus cathayana* (Rehder). When shade was added, low and moderate levels of shade acted to mitigate the negative effects of drought, but heavy shading exacerbated the effects.

While my results were similar to other southeastern U.S. floodplain studies finding tradeoffs between flood and shade tolerance, my results differed in that several plant traits were also correlated among multiple stressors indicating possible evidence for both polytolerance and polyintolerance. Less shade-tolerant and more flood-tolerant species (e.g., *Quercus* spp.) possessed morphology that could increase their vulnerability to drought stress. More shade-tolerant and less flood-tolerant species (e.g., sugarberry and cedar elm) possessed morphology that could increase drought resistance. More shade-tolerant and less flood-tolerant species possessed morphology capable of increasing water capture and decreasing water loss, while also increasing cavitation resistance in stems and roots. For example, seedlings of sugarberry and cedar elm had among the lowest leaf area and smallest average leaf size while also possessing among the deepest roots with a high proportion fine root mass. In addition, roots in sugarberry and cedar elm had low root water content per dry mass and high stem density, both of which have been associated with high cavitation resistance at the cost of having lower capacitance and hydraulic conductance (Bucci et al. 2004; Colangelo et al. 2018; Gonzalez-Benecke et al. 2010; Meinzer et al. 2009; Ogasa et al. 2013). In contrast, the morphology of less shade-tolerant and more flood-tolerant oak species appeared to be potentially vulnerable to sudden drought by having high evaporative surface area, low soil water exploration, and cavitation sensitive stems and roots. Oak species had large leaves, high seedling leaf area and among the highest leaf area per seedling biomass. In addition, oak seedlings had proportionally shorter and shallower roots with a low proportion of fine root mass. However, the water content of these roots was high and oak stems had low wood density. Together these two traits represent a high potential for water capacitance, which Colangelo et al. (2018) found to be one of the most important plant traits determining oak drought survival in floodplain forests with the risk that high water content roots and low-density stems tend to be more susceptible to cavitation (Colangelo et al. 2018; Ogasa et al. 2013).

In this study, I investigated carbon allocation and morphological tradeoffs across multiple species to elucidate the role plant traits have in controlling stress tolerance. However, biomass allocation is only one of the mechanisms that supports stress tolerance. For example, in floodplain forests growth phenology, life history, and stress tolerance are all strongly correlated with the timing and frequency of disturbance events (Streng et al. 1989; Junk et al. 1989). Early season emergent species are more likely to germinate prior to canopy leaf out. For species such as sugarberry or cedar elm (Burns and Honkala 1990; Kroschel 2020) early emergence allows for greater access to light, supports rapid vertical growth, and reduces the chances of being overtopped should a second or later flood event occur. In contrast, later emerging species historically would emerge after flooding had reduced competition from more flood-intolerant species, thus leaving less competition for light. Emerging later in the season for example in overcup oak, also corresponds to slower leaf production and bud break which further contributes to its flood tolerance (Burns and Honkala 1990; Kroschel 2020). Seed size has also been shown to contribute to stress tolerance of species (Adler et al. 2014; Grubb 1977; Streng et al. 1989). Larger seeded species tend to be more tolerant of drought and shade for a limited time after germination in part because larger seeded species more commonly exhibit hypogeal germination. This can help the seedling develop roots and shoots earlier by relying on cotyledon energy reserves and being less dependent on photosynthesis. Numerous other factors contribute to stress tolerance including but not limited to: tightly modulating water use efficiency through stomatal control and gas exchange (van der Sande 2019; Seiler and Johnson 1985; Zhang et al. 1996), increasing the density of chloroplasts and other cellular photosynthetic machinery within the leaf to improve photosynthetic capacity per leaf mass (Sterck 2006), or even the ability to upregulate expression of specific proteins and genes (Hacke et al. 2010).

Considering the multitude of stress tolerance mechanisms and strategies that are possible may help explain why in my study there was a high degree of interspecific variation even within similar tolerance rankings. For example, overcup oak and planer tree were the two most flood-tolerant species investigated, however they possessed near opposite patterns of biomass allocation. Plant traits and morphology in planer tree were more similar to sugarberry and cedar elm which are both significantly less flood-tolerant and more drought-tolerant. Planer tree's low leaf area, and high proportion of root length and fine root mass relative to its biomass was more aligned with the more drought-tolerant species. While these traits could be beneficial in drought conditions, these traits likely serve to increase flood-tolerance by increasing surface area in the soil to explore for aerobic areas and increase root to soil surface area contact for oxygen diffusion (Kozlowski 1997; Sauter 2013; Whitlow and Harris 1979). Similar conflicting morphology was found in green ash where seedlings of this moderately drought-tolerant species was more similar to the morphology of honey locust and boxelder which are both highly drought-tolerant. One possible explanation for the convoluting similarities between planer tree, sugarberry, and cedar elm, are due to phylogenetic similarities. All three species belong to the *Ulmaceae* family. However similar phylogenetic overlap was not apparent among the four *Quercus* spp. or the two *Acer* spp. investigated. In this study I showed that functional groups based on stress tolerance rankings were able to but limited in their ability to describe morphological differences among species. In general, patterns in biomass allocation followed functional group rankings and the expectations of the optimal partitioning theory (Bloom et al. 1985). While stress tolerance groupings such as the ones investigated here are useful for broad generalities, the outlier species and unique differences in biomass allocation among species help provide a foundation for a process-based understanding of how differences in stress tolerance contribute to shifts in species composition.

# CHAPTER 4. DETERMINING THE EFFECTS OF REDUCED LIGHT AND WATER AVAILABILITY ON BIOMASS ALLOCATION PATTERNS OF FIVE BOTTOMLAND HARDWOOD TREE SPECIES WITH DIFFERING STRESS TOLERANCES

## Introduction

Differences in stress tolerance among tree species and the mechanisms they use to acclimate to changes in their environment influences their distribution as well as forest tree species composition (Grubb 1977). Water and light availability are critical for plant growth and changes in their availability are particularly detrimental to seedlings because of their overall low biomass and limited ability to capture light and water resources (Grime 1977; Grubb 1977; Taiz and Zeiger 2010). One way in which seedlings can respond to changes in resource availability is to alter plant traits to allocate proportionally more biomass towards structures to capture the limiting resource. Understanding how seedlings react to stressors and how they can acclimate to changes in their environment can help us achieve a mechanistic understanding of vegetation dynamics and better understand shifts in species composition.

Light availability affects a variety of plant regeneration processes (Grub 1977; Kroschel et al. 2016; Price 2001) and shade-exposed plants must maintain a positive carbon balance (Pierik and Testerink 2014; Kuehne et al. 2014). Two possible strategies to do so are shade escape/avoidance and shade tolerance/endurance. Shade escape strategies commonly occur in fast growing or pioneer species (Valladares et al. 2016). This strategy involves the rapid production of leaves, stems, and roots, typically with lower structural carbon investment cost, and rapid vertical growth by proportionally increasing above ground allocation more than below ground (Caldwell and Pearcy 1994; Pugnaire and Valladares 2007). In contrast, shade tolerance/endurance strategies commonly occur in slow growing species that regenerate entirely in the understory and in shade-intolerant gap species that exhibit temporary shade-tolerance and die back if not released (Oliver 1978). These species typically produce longer lived carbon dense leaves, stems, and roots that are resilient to damage, and often increase carbohydrate storage (King 1986; Markesteijn and Poorter 2009; Poorter and Garnier 1999; Pugnaire and Valladares 2007).

Reduced water availability also impacts a variety of plant regeneration processes (Grub 1977; Kroschel et al. 2016; Price 2001) and drought exposed plants must maintain a continuous water supply and preserve their hydraulic architecture (Tyree and Ewers 1991). Tolerating drought requires plants to increase the ratio of water capture relative to demand, which is commonly achieved by increasing the ratio of root biomass to stem and leaf biomass (Ledo et al. 2018; Nash and Graves 1993; Parolin et al. 2010; Tyree and Ewers 1991). This may include reducing leaf surface area and/or increasing leaf thickness, which reduces cell collapse (Krishnadas et al. 2020; Poorter and Garnier 2007) and can increase water use efficiency by decreasing evapotranspiration (Poorter and Garnier 1999; Tyree and Ewers 1991; Landsberg and Gower 1997). Prolonged drought often reduces stem diameter, xylem diameter, and increases stem density due to low turgor pressure reducing cell expansion. High stem and root density are associated with lower hydraulic conductance (Bucci et al., 2004; Meinzer et al., 2009; Gonzalez-Benecke et al., 2010) and capacitance (Kotowska et al. 2015; McCullough et al. 2014), but greater resistance to cavitation and hydraulic failure (Ogasa et al. 2013). Lastly, drought stress can stimulate (Maurel and Nacry 2020; Wang et al. 2015; Lucas et al. 2013) or inhibit root growth (Maurel and Nacry 2020; Hu et al. 2017) and/or alter the position and depth of roots within the soil (Wang et al. 2015) to maximize water capture.

Smith and Huston (1989) hypothesized that in response to simultaneous drought and shade stress, a trade-off exists such that plants cannot tolerate both stressors equally. This trade-off hypothesis evolved from evidence that under low soil moisture conditions plants most commonly allocate proportionally more biomass to roots than to above ground structure (Bloom et al. 1985; Ledo et al. 2018). This response increases the amount of respiring biomass to photosynthetic biomass and limits the plant's ability to maintain a positive carbon balance under shaded conditions (Smith and Huston 1989). Therefore, under dry conditions the plant becomes less shade tolerant. Likewise, in shade, the plant becomes less drought tolerant as it allocates proportionally more biomass to above ground structures. Due to these trade-offs in biomass allocation as well as other physiochemical constraints, polytolerance, or the ability to tolerate multiple stressors, rarely occurs. Niinemets and Valladares (2006) determined in a meta-analysis across 40% of northern hemisphere trees and shrubs that tradeoffs in shade and drought, shade and waterlogging, and waterlogging and drought, occur across phylogeny.

In contrast to a tradeoff between shade and drought tolerance, other studies suggest a non-linear relationship between shade and drought tolerance that is dependent on the intensity of shade and drought relative to each other and relative to the conditions normally experienced by the plant (Holmgren et al. 2012; Huang et al. 2008). Others investigating this relationship concluded that the effects are simply additive (Sack and Grub 2002). Lastly, some studies show that the two stress responses are uncoupled and unrelated because the tolerances do not solely require mutually exclusive alterations to biomass allocation (Sack and Grubb 2002; Sack et al. 2003). For example, there can be overlaps in trait functionality such as low specific leaf area increasing water use efficiency by decreasing respiration and water loss (Hoffman et al. 2005; Rad et al. 2011; Tyree and Ewers 1991), or dense stems and roots being more resistant to cavitation (Ogasa et al. 2013; Reich 2014). Lastly, the facilitation hypothesis is a counter argument to the trade-off hypothesis, where shade can alleviate the negative impacts of drought through improved microclimate conditions such as lowering vapor pressure deficit and oxidative cell damage (Canham et al. 1996; Holmgren 2000; Pearcy 2007).

In floodplain forests, trade-offs between shade-tolerant and shade-intolerant species demonstrates the tendency for shade-tolerant species to replace shade-intolerant species over time in canopy gaps following disturbance. (Battaglia et al. 1999; Lin et al. 2004; McNab et al. 2021). In addition, trade-offs between flood-tolerance and shade-tolerance limit this replacement in some cases (Hall & Harcombe 1998; Battaglia et al. 1999; 2000; 2004; 2006). Many floodplain forests in the southcentral and southeastern United States, also known as bottomland hardwood (BLH) forests, have become drier due to stream regulations decreasing overbank flooding (Gee et al. 2014; King and Keim 2019; Stallins et al. 2010). This drier hydrogeomorphic setting has altered regeneration processes and contributed to shifts in BLH species composition (Gee et al. 2014; King and Antrobus 2005; King and Keim 2019; Oliver et al. 2005). While the effects of flooding and shade on trees has received ample attention (Battaglia et al. 2000; Collins and Battaglia 2008; Lin et al. 2004; Streng et al. 1989), the effects of drought and shade has received comparatively little, particularly for BLH tree species.

Currently, the mechanisms that BLH trees use to tolerate multiple co-occurring stressors and how these species respond to drought are poorly understood. In addition, limited information exists on the possible tradeoffs associated with shade and drought tolerance in BLHs. In other forest environments, shade/drought trade-off studies demonstrate equivocal results. For example, Markesteijn and Poorter (2009) reported a lack of tradeoffs in moist and dry tropical forests, which they attributed to the two tolerances depending on different morphological changes. Furthermore, in eastern North American deciduous forests, shade improved drought tolerance in seedlings of *Liriodendron tulipifera* (L.), a common colonizer of forest canopy gaps (Holmgren 2000). Lastly, in western China, seedlings of the riparian species *Populus cathayana* (Rehder) when exposed to a gradient of shade and drought stress exhibited both tradeoff and facilitation responses depending on the intensity of shade (Huang et al. 2008).

In this study, I conducted a controlled greenhouse experiment to investigate the main and interactive effects of shade and reduced water availability on seedlings of five BLH tree species. I hypothesized that both shade and drought stress cause seedlings to allocate biomass towards structures that help tolerate those stressors, and that differences in stress tolerance among these species is due to differences in the degree to which species can adjust biomass allocation in response to these stressors. In addition, I hypothesized that because of tradeoffs in morphology, an interaction between shade and water availability would result in shaded plants with reduced water availability not adjusting plant traits to increase water capture and decrease water loss as much as their non-shaded counterparts.

## Methods

### *Plant material and experimental design*

This experiment was conducted in a climate-controlled greenhouse facility near Louisiana State University in Baton Rouge, Louisiana during the summers of 2019 and 2020. Greenhouse temperature averaged 27°C and relative humidity was allowed to fluctuate and averaged 45% over the course of the experiments.

Seedlings of five bottomland hardwood tree species were grown under a factorial combination of water availability and shade treatments. The species included: overcup oak (*Quercus lyrata* Walter; QULY), willow oak (*Quercus phellos* L.; QUPH), water oak (*Quercus nigra* L.; QUNI), green ash (*Fraxinus pennsylvanica* Marshall; FRPE) and sugarberry (*Celtis laevigata* Willd.; CELA). These species were chosen based on their prevalence in southeastern and southcentral bottomland hardwood forests both with and without altered hydrology, as well as their range in drought, flood, and shade tolerances at the seedling stage (Table 4.1) (Baker 1949; Burns and Honkala 1990; McKnight et al. 1980, Niinemets and Valladares 2006; Putnam et al. 1960 USDA, NRCS 2021; Zon and Graves 1911) (See Chapter 3 Methods for details on tolerance rankings).

Table 4.1 Five bottomland hardwood seedlings studied and their corresponding flood, drought, and shade tolerance rankings.

| Species (common name) | Flood tolerance | Drought tolerance | Shade tolerance |
|---|---|---|---|
| *Celtis laevigata* (sugarberry) | Moderate | Moderate | Tolerant |
| *Fraxinus pennsylvanica* (green ash) | Moderate | Moderate | Moderate |
| *Quercus lyrata* (overcup oak) | Tolerant | Intolerant | Moderate |
| *Quercus nigra* (water oak) | Moderate | Intolerant | Intolerant |
| *Quercus phellos* (willow oak) | Moderate | Intolerant | Intolerant |

Seeds were purchased from Louisiana Forest Seed Company (See Chapter 2 Methods for details on seed collection and handling). All seeds of each species were cold stratified by storing the seeds, separated by species, in clear plastic storage containers affixed with a lid to limit evaporation on sand moistened with distilled water at 4°C for 90 days (Baskin and Baskin 2014). After cold stratification, seeds were inspected and discarded if rot, mold, or insect damage was present, or if germination during stratification had occurred. In addition, acorns of willow and water oak were float tested for approximately one hour in distilled water and any floating acorns were discarded.

After cold stratification, seeds were sown in 7.6 L pots (22.9 cm x 22.9 cm) filled with heat treated montmorillonite clay (Turface MVP, Profile Product, Buffalo Grove, IL). A one-time application of slow-release fertilizer was evenly mixed into each pot (Osmocote 15-9-12, ICL-SF, Summerville, SC). Over the course of the experiment, pots were rotated and moved weekly to reduce placement bias. After sowing, each pot received ambient sunlight and was watered daily until water freely flowed from the pot. As seedlings emerged, the date of emergence was recorded, and each emerged seedling continued to receive daily watering and ambient sunlight for 30 days post-emergence. After 30 days post-emergence, seedlings were randomly assigned one of four treatments. Seedlings were grown under their randomly assigned treatment for an additional 30 days (60 days of total growth).

The experimental design was a randomized complete 2x2x5 factorial combination of water availability and shade treatments with five species. Water availability treatments consisted of a well-watered control treatment and a reduced-water treatment. In the well-watered treatment, seedlings were watered daily during the treatment period (30 days) until water freely flowed from the pot. In the reduced-water treatment seedlings were watered once during the treatment period 15 days after the treatment period started. Water was then withheld for the remaining 15 days of treatment.

The two shade treatments consisted of an ambient-sun control and a 25% of ambient treatment (i.e. 75% reduction of ambient light). The 25% of ambient light treatment was based on periodic spot measurements of intercepted photosynthetically active radiation (IPAR) taken at Alazan Bayou WMA; a BLH forest in east Texas (See Chapter 3 Methods). Spot measurements of below canopy transmitted radiation and above canopy ambient radiation were made using a quantum sensor (LI191, Li-COR Inc., Lincoln, NE). Mean (±S.E.) below canopy transmitted radiation was 27.1±10% of ambient. In BLHs light availability in the understory varies spatially and temporally (Battaglia and Sharitz 2006; Streng et al. 1986). However, similar light availability has been reported in BLH forests (Battaglia and Sharitz 2006; Boerger et al. 2015) and have been used in controlled studies (Battaglia et al. 2000; Gardiner and Hodges 1998).

The 25% of ambient treatment was achieved by constructing a 3 m long, 1 m wide, and 1 m tall PVC pipe scaffolding positioned around and above the seedlings. A knitted screen made from metalized HDPE rated for 80% reduction in light transmission (Aluminet, shade cloth store, Mundelin, IL) was draped over the frame to cover all sides and the top of the frame down to the ground. Light reduction was confirmed with periodic spot measurements of photosynthetically active radiation (PAR) and illuminance over the course of the experiment using a quantum sensor and lux meter (LX13380B, Dr. Meter, Union City, CA) respectively. Shade treatment reduced PAR by an average of 75±2% and reduced illuminance by 76±1%.

### *Biomass measurements*

After 30 days of treatment, seedlings were removed from their pots in their entirety, letting excess soil fall away. Seedlings were placed in plastic bags with wet paper towels and stored in a dark cooler at 4°C to minimize desiccation. Prior to measurements, seedlings were rinsed to remove any remaining soil and patted dry with paper towels to remove excess moisture. Each seedling was then separated into roots, stems, and leaves. No cotyledons were present on any seedling. The fresh mass of each component was measured. The number of leaves were counted, and seedling leaf area was measured using a flatbed scanner (HP Officejet 6500A Plus) and pixel counting software (Leaf Area Measurement, Askew 2003, Sheffield, UK). Stem volume was calculated as the volume of a truncated cone:

$$SV = (\pi L/12)(D^2_{top}+D_{top}D_{base}+D^2_{base})$$

where SV is stem volume (cm$^3$), L is the stem length (cm), $D_{top}$ is the diameter at the top of the stem (cm) just below the apical bud, and $D_{base}$ is the diameter at the base of the stem (cm) just above the root collar (Markesteijn and Poorter 2009). For seedlings that were branched, stem volume was calculated using the same formula, with each branch treated as an individual stem and $D_{base}$ as the diameter just above the branch collar, and seedling stem volume was calculated as the sum of the individual branches and main stem. Total root length was measured using the same flatbed scanner and Image J (Image J, National Institute of Health, Bethesda, MD) with methods modified from Kimura et al. (1999) and Kimura and Yamasaki (2001). A subsample of roots covering the range of total root lengths, species, and treatments was selected to also be measured by hand. Measurements produced by these two methods did not differ ($P=0.765$; Table B.1). Rooting depth was restricted by the depth of the pot to approximately 23 cm and many seedlings had roots that had begun to spiral at the pot's bottom. Because of this, rooting depth was estimated by uncurling and measuring the length of the longest live dominant primary root. Following these measurements, all plant material was placed in a forced air oven to dry at 70°C and weighed periodically until dry weight was stable. Total seedling water content was then calculated as the proportion of mass lost during drying, which was assumed to be only water, per total fresh mass of the seedling. All measured and calculated morphological plant traits (Table 4.2) were selected based on their importance in key physiological functions including resource capture, resource use efficiency, mechanical support, and for quantifying proportional resource partitioning (Markesteijn and Poorter 2009).

Table 4.2. Morphological traits (abbreviation), description, grouping used in MANOVA, and units.

| Seedling Trait | Description | Grouping | Units |
|---|---|---|---|
| Biomass | Total dry mass | Seedling Growth | g |
| Leaf area | Total one-sided leaf area | Seedling Growth | $cm^2$ |
| Average leaf size (LS) | Average one-sided individual leaf area | Seedling Growth | $cm^2$ |
| Height | Vertical growth from root collar | Seedling Growth | cm |
| Stem area | Cross sectional area at root collar | Seedling Growth | $mm^2$ |
| Stem density (SD) | Stem mass per stem volume | Seedling Growth | $g\ cm^{-3}$ |
| Root length | Total root length | Seedling Growth | cm |
| Rooting depth | Depth of longest primary/tap root | Seedling Growth | cm |
| Specific leaf area (SLA) | Leaf area per leaf mass | Allocation Efficiency | $cm^2\ g^{-1}$ |
| Specific stem length (SSL) | Height per stem mass | Allocation Efficiency | $cm\ g^{-1}$ |
| Specific root length (SRL) | Root length per root mass | Allocation Efficiency | $cm\ g^{-1}$ |
| Secondary To primary root mass ratio (SecPrimRMR) | Secondary root mass per primary root mass | Allocation Efficiency | $g\ g^{-1}$ |
| Root water content (RWC) | Mass of water in root per dry root mass | Allocation Efficiency | $g\ g^{-1}$ |
| Leaf area ratio (LAR) | Leaf area per seedling biomass | Whole Plant Tradeoff | $cm^2\ g^{-1}$ |
| Leaf area per stem area (LASA) | Leaf area per cross sectional stem area | Whole Plant Tradeoff | $cm^2\ mm^2$ |
| Root length per biomass (RLBiom) | Root length per seedling biomass | Whole Plant Tradeoff | $cm\ g^{-1}$ |
| Stem length per biomass (SLBiom) | Height per seedling biomass | Whole Plant Tradeoff | $cm\ g^{-1}$ |
| Root to shoot ratio (RSR) | Root mass to combined stem and leaf mass | Whole Plant Tradeoff | $g\ g^{-1}$ |
| Leaf mass fraction (LMF) | Proportion of leaf mass to seedling biomass | Whole Plant Tradeoff | $g\ g^{-1}$ |
| Stem mass fraction (SMF) | Proportion of stem mass to seedling biomass | Whole Plant Tradeoff | $g\ g^{-1}$ |
| Root mass fraction (RMF) | Proportion of root mass to seedling biomass | Whole Plant Tradeoff | $g\ g^{-1}$ |

*Data analyses*

All analyses were performed in R (R Core Team, 2020). Multivariate analysis of variance (MANOVA) was used to test the main and interactive effects of shade and water availability treatments and species on combined seedling traits (Table4.2) Seedling traits were combined based on the scale of their physiological function, interpretation in the context of plant function, and to reduce multicollinearity. Variable normality and error variances were inspected visually. To improve normality and reduce heteroscedasticity of error variances all morphological traits were log transformed prior to analyses except for proportional traits which were arcsine transformed prior to analyses. All variables were transformed back to their original values for presentation and ease of interpretation. Pillai's trace was selected as the test statistic because of its robust general use in dealing with departures from the assumptions of MANOVA. All differences were considered significant at $\alpha \leq 0.05$. To further analyze effects ($\alpha \leq 0.05$) from MANOVA, post hoc univariate and two-way analysis of variance (ANOVA) were used. Family-wise error rate was controlled using the Holm-Bonferroni method. Differences among species ($\alpha \leq 0.05$) were assessed with Tukey's HSD. Lastly, linear discriminant analysis (LDA) was used to visualizing differences in biomass allocation among species and tolerances rankings with respect to multiple plant traits simultaneously. Several plant traits were omitted from the LDA to reduce multicollinearity and the distortions in discriminant space caused by traits being represented multiple times (e.g., leaf area, SLA, LAR, LMF). Traits were selected using partial correlation analysis using Pearson's correlation coefficient. Plant traits with high correlation and/or redundancy were omitted (Table B.2; Table B.3).

## Results

No species demonstrated mortality due to treatment and all observed mortality occurred within 30 days of emergence and prior to treatment initiation. Across all species, 37% of QULY, 25% of CELA, and 15% of FRPE germinants died prior to treatment while receiving full sun and daily watering. No mortality occurred in QUNI or QUPH. A sixth species, cedar elm (*Ulmus crassifolia* Nutt.), was to be included in this study but was excluded due to germinants experiencing 74% pre-treatment mortality leading to inadequate replication. Anecdotally, pre-treatment mortality in all species appeared to be caused by desiccation and/or rotting of the hypocotyl in a manner similar to damping off disease (Grabowski 2018 accessed October 2021).

When examining whole seedling water content, a two-way interaction existed between water availability and shade treatments (P=0.009) and a two-way interaction between shade treatment and species (P=0.049) (Table C.1). The interaction between water availability and shade treatments indicated that across all species, reduced water availability reduced seedling water content only in the ambient sun treatment from 73.4±0.01% to 67.8±0.01%. Furthermore, the shade treatment and species interaction indicated that ambient sun reduced seedling water content in CELA, FRPE, and QUPH regardless of water availability treatment.

Combined seedling growth traits differed with water availability treatment independent of species, and an interaction between shade treatment and species indicated that the effects of shade on combined seedling growth traits differed among species (Table 4.3). For combined biomass allocation efficiency traits, two-way interactions between shade and water availability treatments, species and shade treatments, and species and water availability treatments indicated that the effects of both shade and water availability treatments differed among and averaged across species (Table 4.3). Lastly, both shade and water availability treatments independently affected combined whole plant biomass partitioning traits, but the effects of both treatments differed among species (Table 4.3).

Table 4.3. Results of MANOVA (P value) showing the effects of species, shade and water availability treatment, and their interactions on combined growth traits, biomass allocation efficiency (Efficiency), and whole-plant biomass partitioning (Whole).

| | Growth | Efficiency | Whole |
|---|---|---|---|
| Species | <0.001 | <0.001 | <0.001 |
| Shade | <0.001 | <0.001 | <0.001 |
| Water availability | <0.001 | <0.001 | <0.001 |
| Species x Shade | 0.014 | <0.001 | <0.001 |
| Species x Water availability | 0.055 | 0.024 | <0.001 |
| Shade x Water availability | 0.100 | 0.012 | 0.194 |
| Species x Shade x Water availability | 0.076 | 0.091 | 0.441 |

*Seedling growth*

A three-way interaction among shade and water availability treatments and species on average leaf-size and root length (Table 4.4) indicated that the effect of shade treatment on these traits differed among species and depended on water availability treatment. In reduced-water treatment average leaf size did not differ among species, regardless of shade treatment. However, within well-watered treatments FRPE had higher average leaf size than all other species but only within ambient-sun treatment (Figure 4.1). In FRPE seedlings, shade treatment decreased root length, only within well-watered treatments, while conversely shade treatment decreased root length in CELA only in reduced-water treatments (Figure 4.1).

Shade treatment decreased seedling biomass, leaf area (Figure 4.2a), stem area, rooting depth (Figure 4.2b), and height (Figure 4.2c) (Table 4.4). The only main effect of reduced water availability on seedling growth traits was on leaf area (Table 4.4) which was lower in reduced-water treatments compared to well-watered treatments (Figure 4.2a).

Table 4.4. Adjusted P values from ANOVA for the effects of species, shade and water availability treatment, and their interactions on seedling growth traits.

| | Biomass | Leaf area | Leaf size | Rooting depth | Root length | Stem area | Height | Stem density |
|---|---|---|---|---|---|---|---|---|
| Species | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Shade | <0.001 | <0.001 | 0.019 | 0.003 | <0.001 | <0.001 | <0.001 | 0.597 |
| Water availability | 1.000 | 0.045 | 0.392 | 1.00s0 | 1.000 | 0.725 | 0.407 | 1.000 |
| SpeciesxShade | 0.271 | 0.200 | 0.050 | 0.995 | 0.007 | 0.995 | 0.995 | 0.995 |
| SpeciesxWater availability | 1.000 | 0.833 | 0.468 | 0.557 | 1.000 | 1.000 | 1.000 | 0.530 |
| ShadexWater availability | 1.000 | 0.717 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| SpeciesxShadexWater availability | 0.240 | 0.074 | 0.005 | 0.249 | 0.001 | 0.076 | 0.594 | 0.594 |

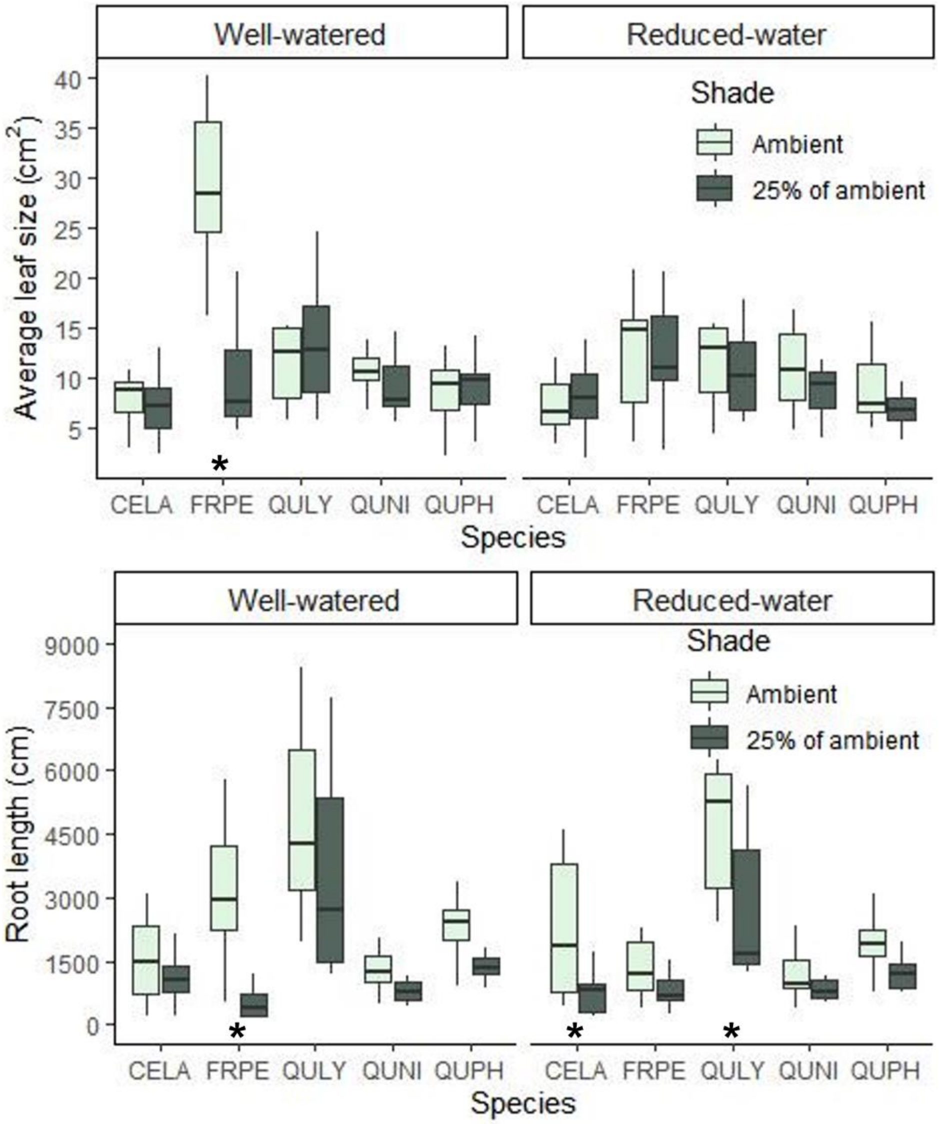


Figure 4.1 Mean (±SE) average leaf size and root length by species in response to shade treatment within each level of water availability treatment. Asterisk indicates an effect ($\alpha \leq 0.05$) of shade on a species, within that level of water availability treatment.

A

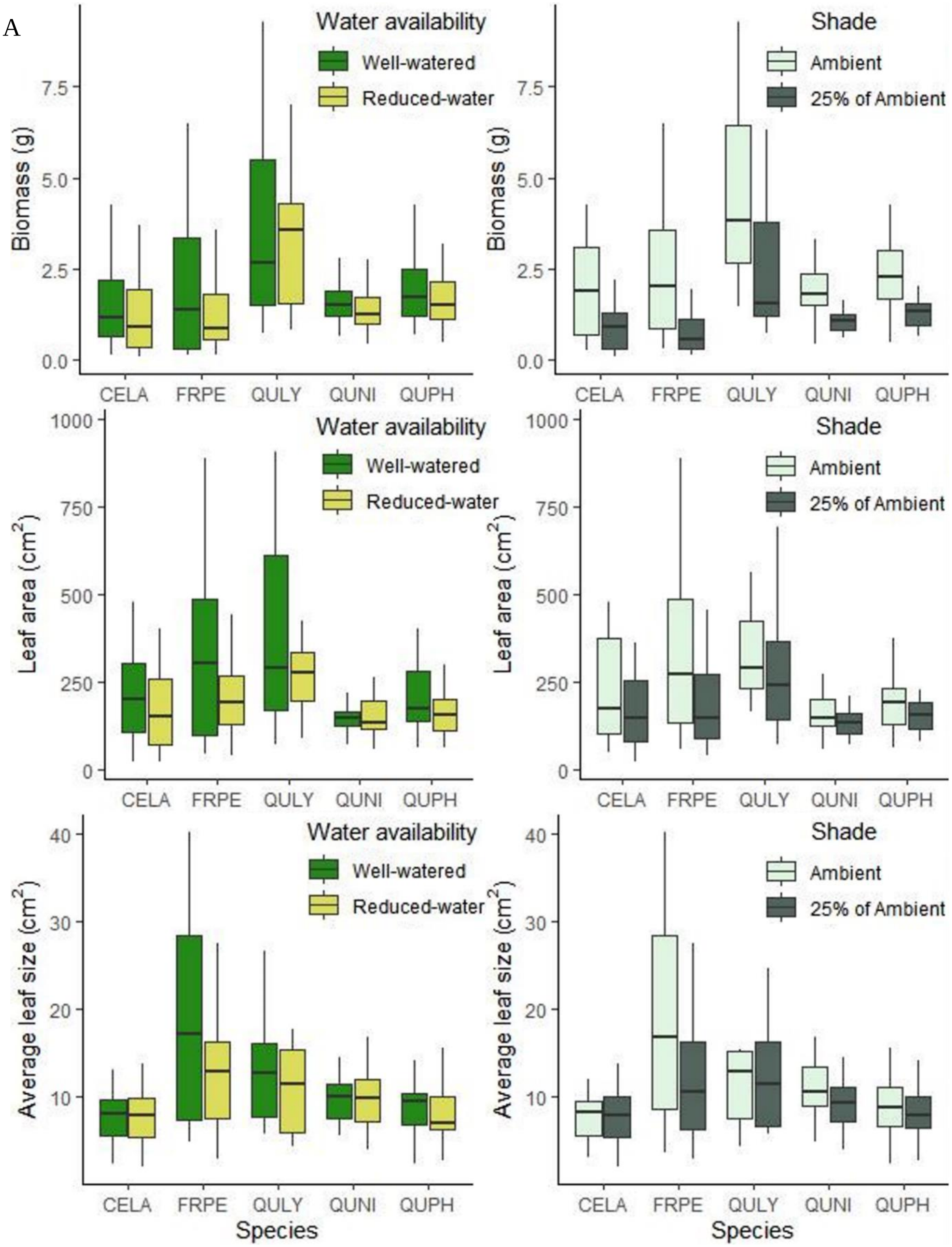
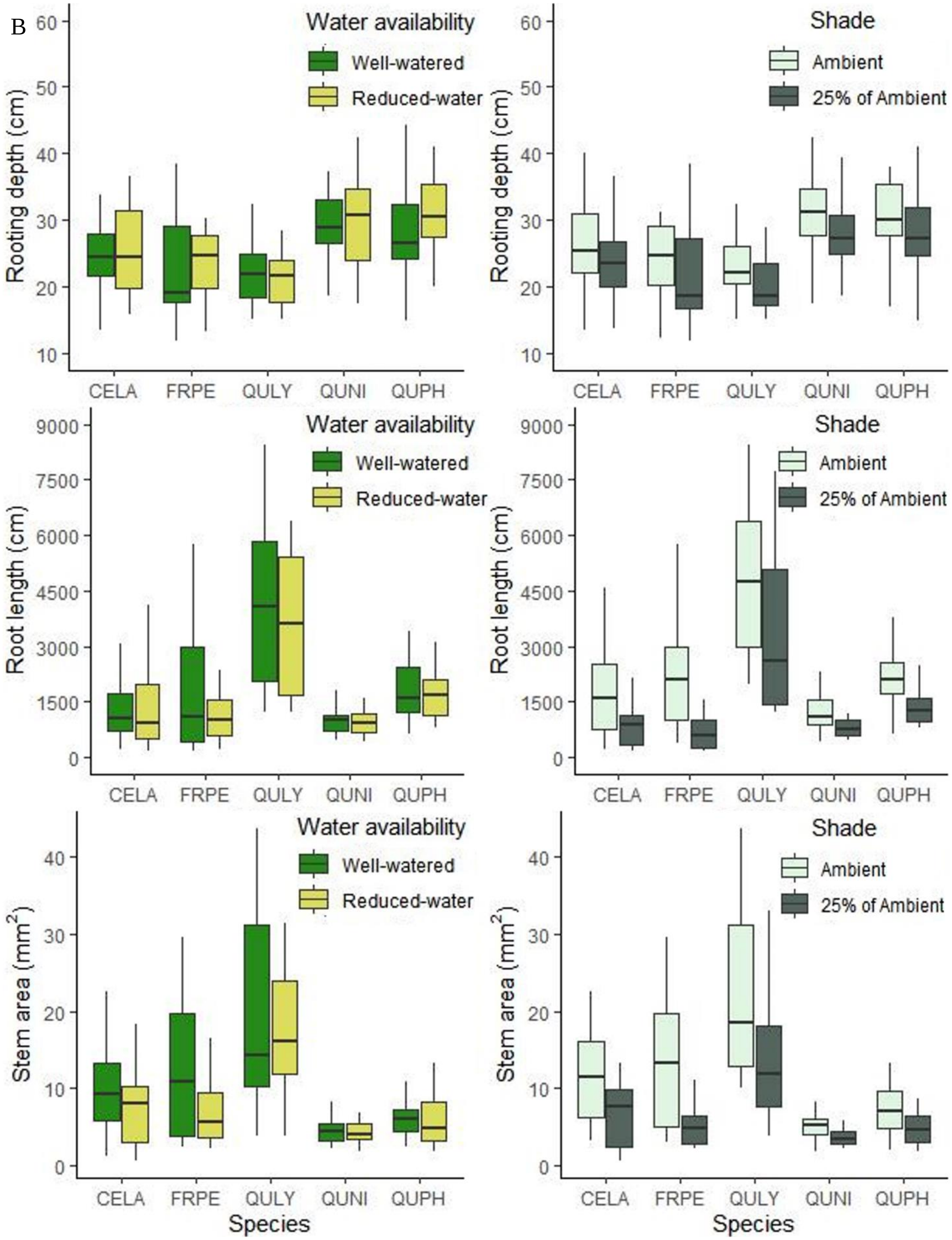
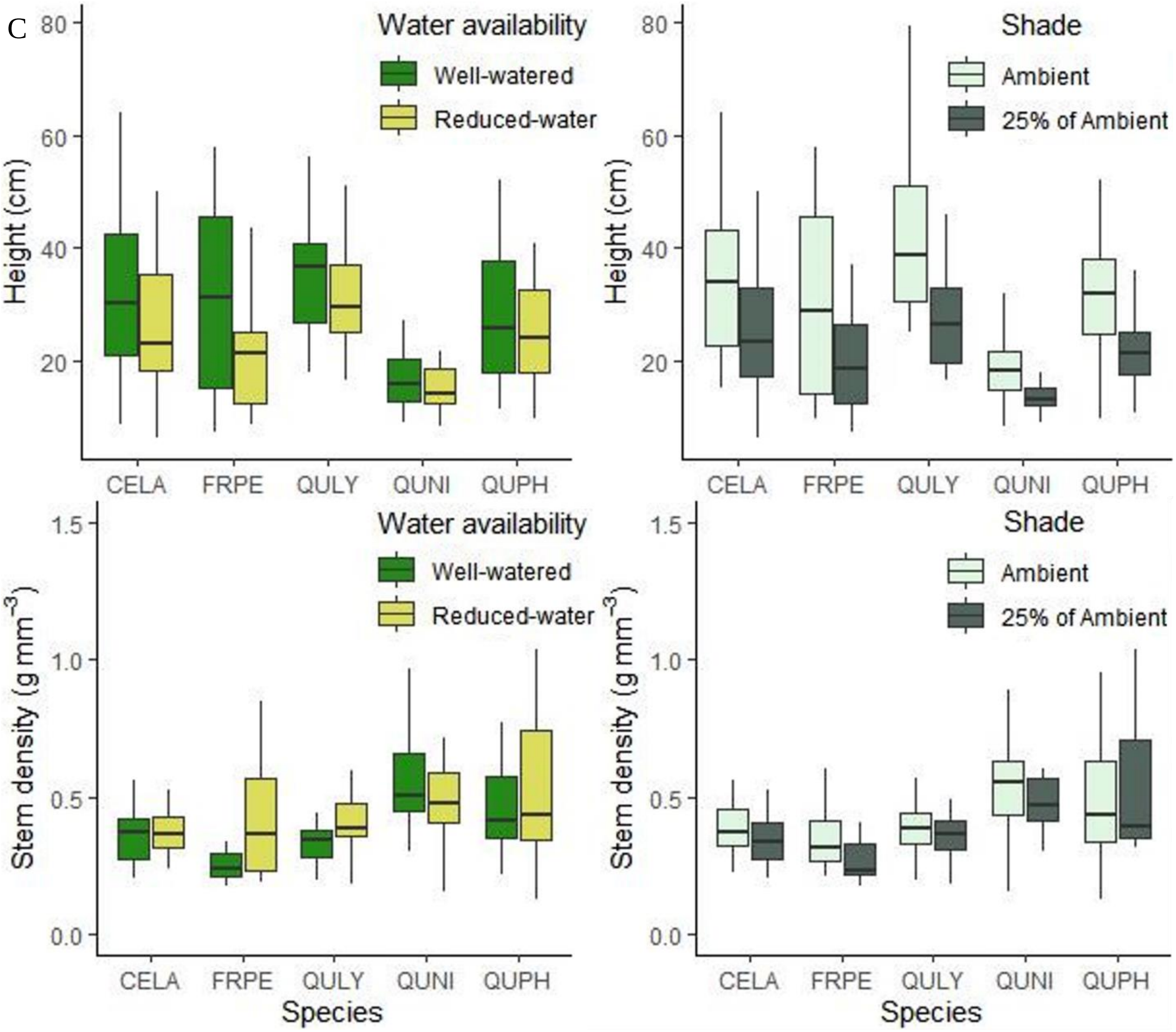


Figure 4.2 Mean (±SE) (A) biomass, leaf area, average leaf size, (B) rooting depth, root length, stem area, (C) height, and stem density by species in response to water availability and shade treatments
(fig. cont'd.)


B
Water availability
Well-watered
Reduced-water
Shade
Ambient
25% of Ambient
Rooting depth (cm)
10
20
30
40
50
60
Root length (cm)
0
1500
3000
4500
6000
7500
9000
Stem area ($mm^2$)
0
10
20
30
40
CELA
FRPE
QULY
QUNI
QUPH
Species

(fig. cont'd.)


C
Water availability
Well-watered
Reduced-water
Height (cm)
80
60
40
20
CELA
FRPE
QULY
QUNI
QUPH
Shade
Ambient
25% of Ambient
Height (cm)
Water availability
Well-watered
Reduced-water
Stem density (g mm$^{-3}$)
1.5
1.0
0.5
0.0
Species
Shade
Ambient
25% of Ambient
Stem density (g mm$^{-3}$)
Species

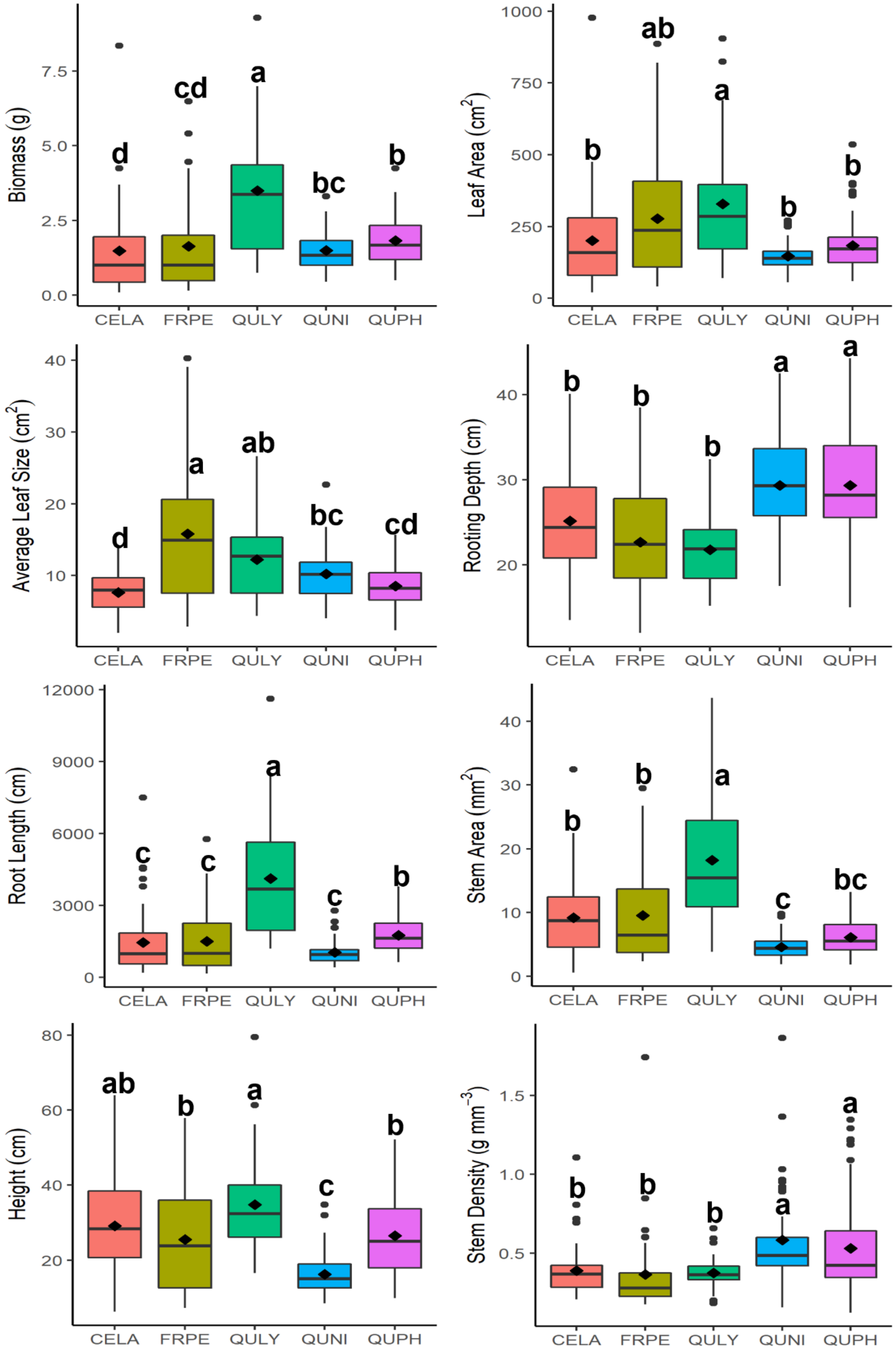


Figure 4.3. Mean (±SE) (diamond) seedling growth traits for five BLH seedlings averaged across all treatments. For each growth trait, dissimilar letters indicate difference among species ($\alpha \leq 0.05$).

*Biomass allocation efficiency*

A two-way interaction existed between shade treatment and species on SLA and SSL, as well as a two-way interaction between water availability treatment and species on SRL and SecPrimRMR (Table 4.5). Shade treatment increased SLA across all species instead and ranged in adjustment from 317.0 (7.9) cm$^2$ g$^{-1}$ to 525.7 (12.1) cm$^2$ g$^{-1}$ in FRPE to 216.2 (9.5) cm$^2$ g$^{-1}$ to 246.6 (7.8) cm$^2$ g$^{-1}$ in QULY (Figure 4.4a). Shade treatment also increased SSL in all species except for QULY, which also had the lowest SSL (Figure 4.4a). Decreased water availability decreased SRL only in QULY (Figure 4.4a), however, QULY's SRL was still one of the highest among species. In addition, decreased water availability increased SecPrimRMR only in CELA which had one of the lowest SecPrimRMRs among species (Figure 4.4b).

Shade treatment increased SRL (Figure 4.4a) and RWC (Figure 4.4b) across all species independent of water availability treatment (Table 4.5). Lastly, reduced water availability decreased RWC (Figure 4.4b) across all species independent of shade treatment (Table 4.5).

Table 4.5. Adjusted P values from ANOVA for the effect of species, shade and water availability treatment, and their interactions on seedling biomass allocation efficiency traits.

| | SLA | SSL | SRL | SecPrimRMR | RWC |
|---|---|---|---|---|---|
| Species | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Shade | <0.001 | <0.001 | <0.001 | 0.639 | <0.001 |
| Water availability | 0.713 | 1.000 | 1.000 | 1.000 | <0.001 |
| Species x Shade | <0.001 | 0.015 | 0.306 | 0.169 | 0.169 |
| Species x Water availability | 0.118 | 0.599 | 0.034 | 0.010 | 0.599 |
| Shade x Water availability | 0.099 | 1.000 | 1.000 | 0.075 | 0.056 |
| Species x Shade x Water availability | 0.088 | 0.068 | 1.000 | 1.000 | 0.650 |

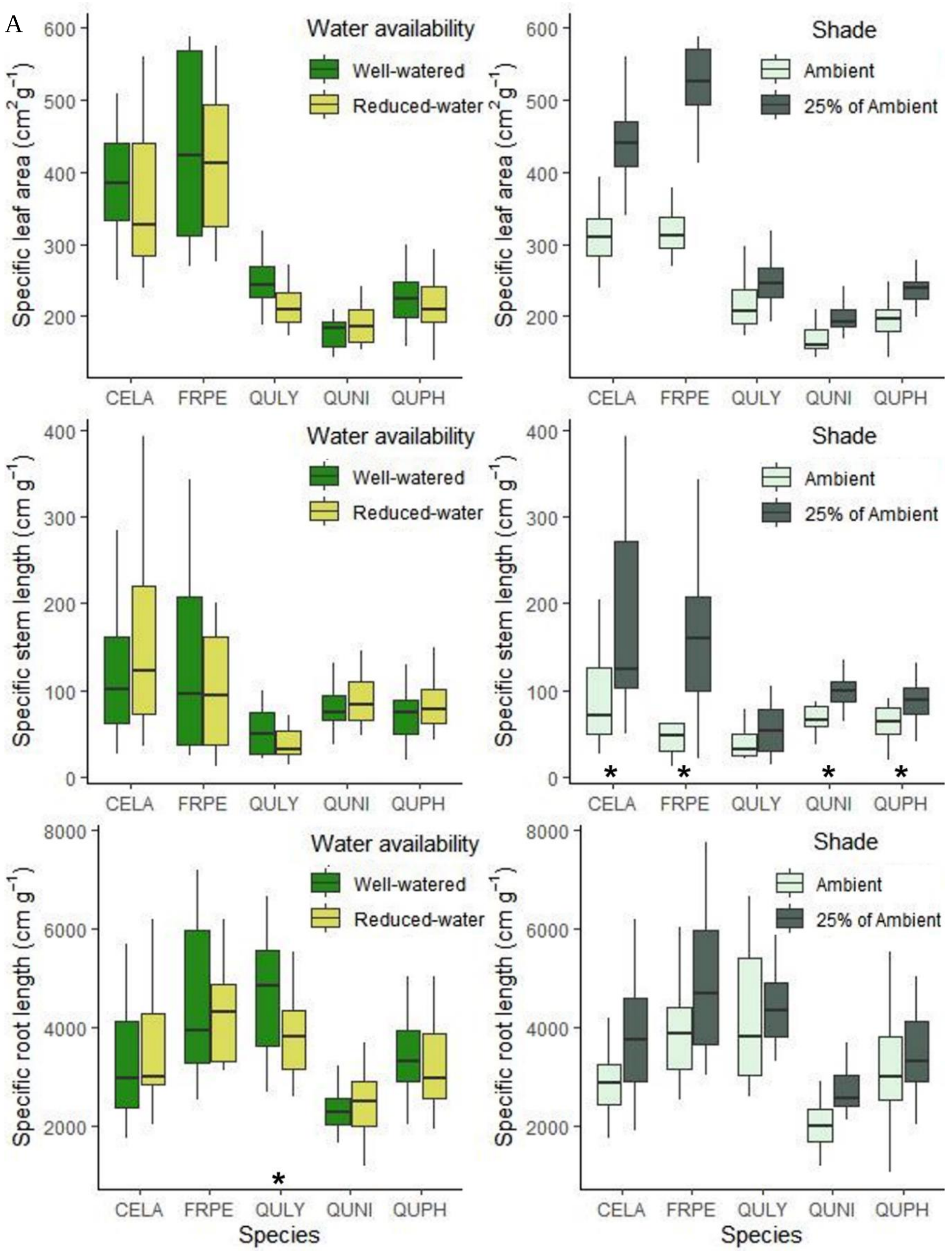
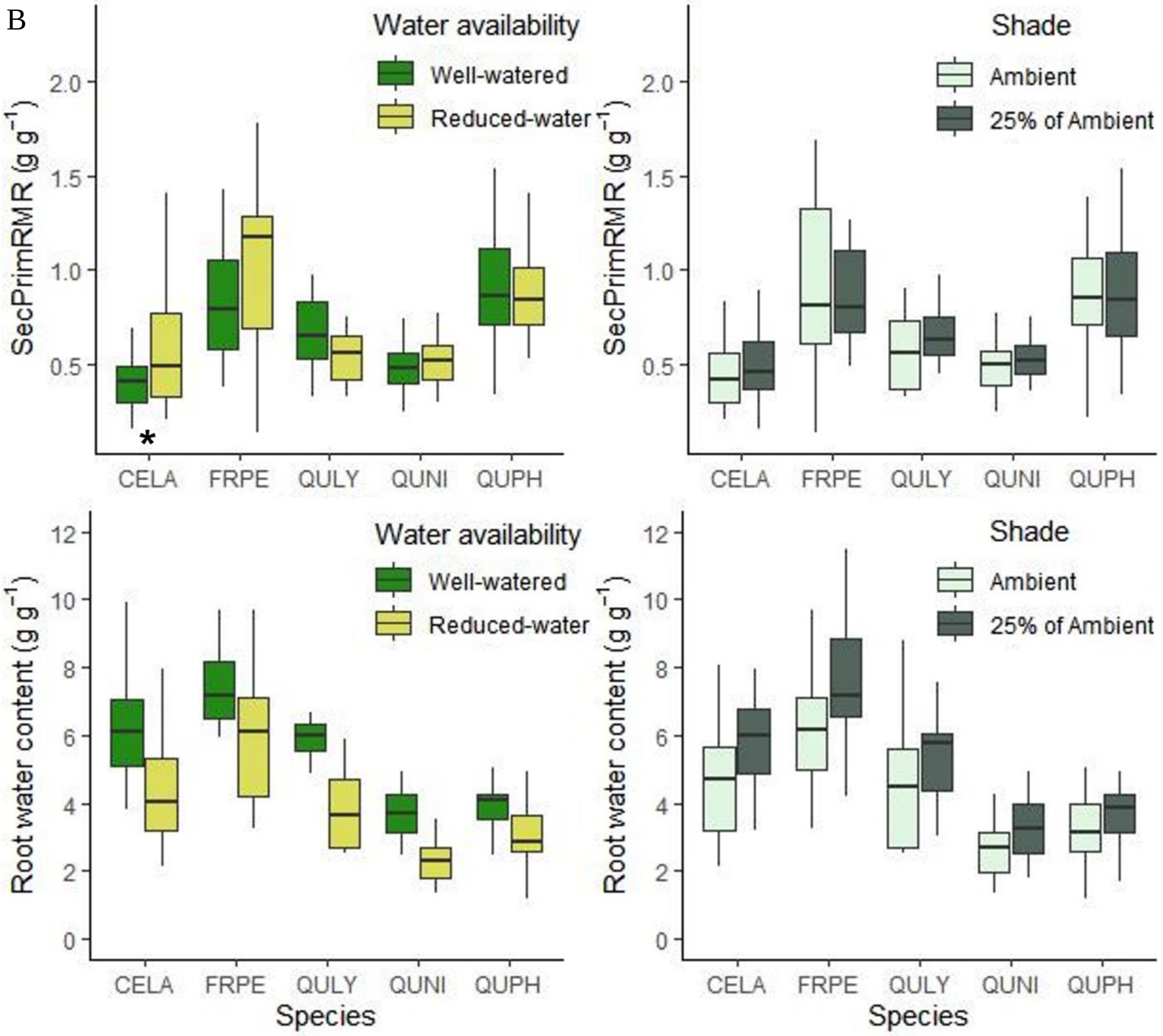


Figure 4.4. Mean (±SE) (A) specific leaf area, specific stem length, specific root length, (B) secondary to primary root mass ratio, and root water content by species in response to water availability and shade treatments. Asterisk indicates a species treatment effect (α≤0.05).
(fig. cont'd.)


B
Water availability
Well-watered
Reduced-water
SecPrimRMR (g g$^{-1}$)
2.0
1.5
1.0
0.5
*
CELA
FRPE
QULY
QUNI
QUPH
Shade
Ambient
25% of Ambient
Root water content (g g$^{-1}$)
12
10
8
6
4
2
0
Species

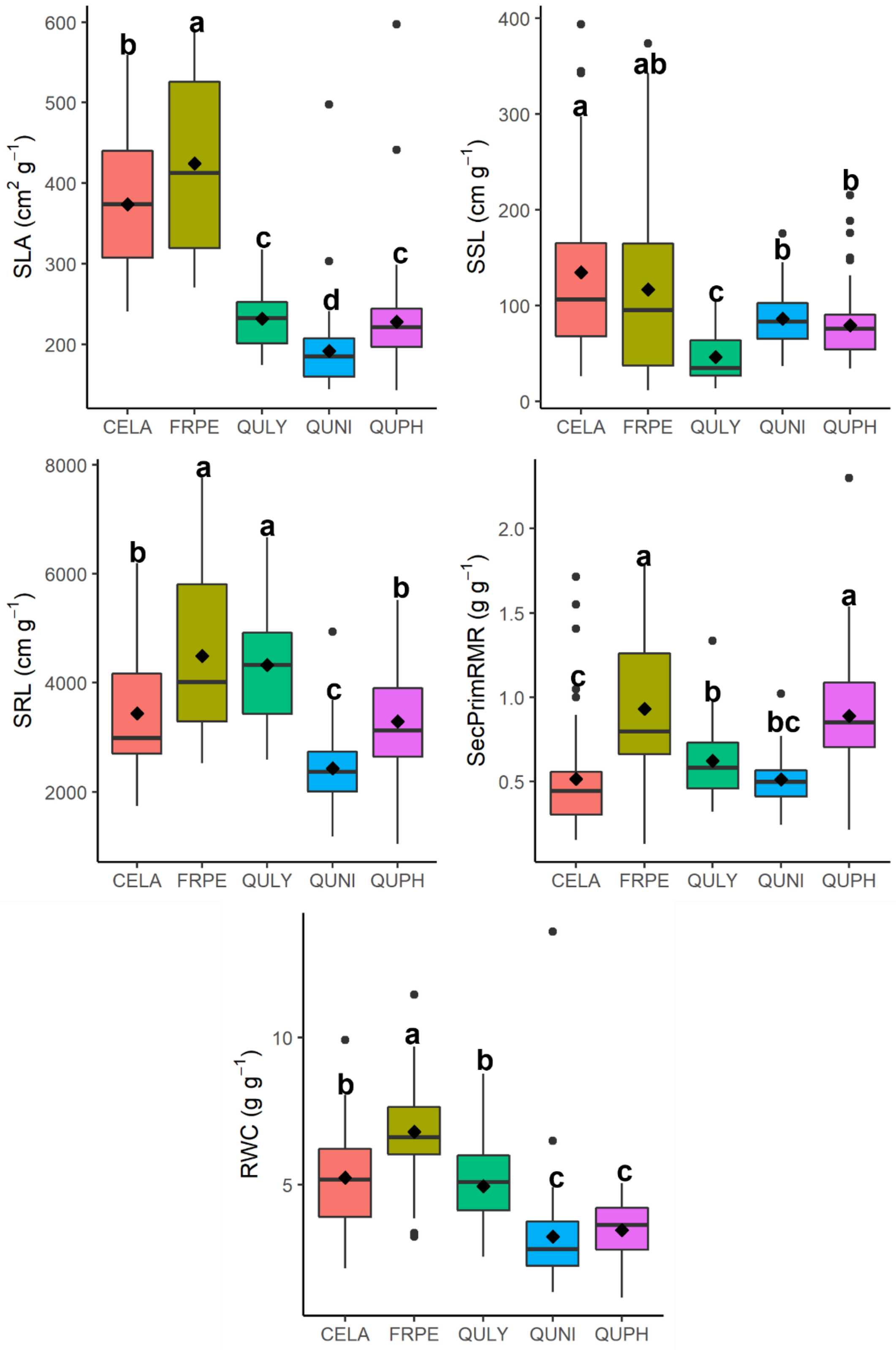


Figure 4.5. Mean (±SE) (diamond) seedling allocation efficiency traits for five BLH seedlings averaged across all treatments. For each growth trait, dissimilar letters indicate difference among species($\alpha \le 0.05$).

### *Whole plant biomass allocation*

A two-way interaction existed between shade treatment and species and between water availability treatment and species on LAR. In addition, a three-way interaction existed among shade and water availability treatments and species for SLBiom (Table 4.6). Shade treatment increased LAR across all species instead and ranged in adjustment from 151.4 (6.0) cm$^2$ g$^{-1}$ to 272.1 (10.7) cm$^2$ g$^{-1}$ in FRPE to 87.3 (5.9) cm$^2$ g$^{-1}$ to 109.3 (6.2) cm$^2$ g$^{-1}$ in QULY (Figure 4.7a). In addition, only QULY decreased LAR in response to decreased water availability. In response to shade treatment all species except for QULY increased SLBiom, however, in the other four species, the effect of shade treatment on SLBiom depended on water availability. In CELA and QUNI, shade treatment increased SLBiom regardless of water availability, whereas in FRPE and QUPH, shade treatment only increased SLBiom within well-watered treatments (Figure 4.6).

Independent of water availability treatment, shade treatment increased RLBiom (Figure 4.7a) and LMF (Figure 4.7b) and decreased SMF (Figure 4.7c) in all species (Table 4.6). Independent of shade treatment, decreased water availability increased RLBiom, RSR (Figure 4.7b), and RMF (Figure 4.7c), and decreased LMF in all species (Table 4.6).

Table 4.6. Adjusted P values from ANOVA for the effect of species, shade and water availability, and their interactions on whole plant biomass allocation traits.

| | LAR | LASA | RLBiom | SLBiom | RSR | LMF | SMF | RMF |
|---|---|---|---|---|---|---|---|---|
| Species | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Shade | <0.001 | 0.089 | 0.004 | <0.001 | 0.195 | <0.001 | <0.001 | 0.195 |
| Water availability | 0.001 | 1.000 | 0.038 | 1.000 | 0.002 | 0.033 | 0.819 | 0.002 |
| SpeciesxShade | <0.001 | 0.965 | 0.341 | <0.001 | 0.803 | 0.803 | 0.965 | 0.803 |
| SpeciesxWater availability | <0.001 | 0.426 | 0.212 | 0.338 | 0.338 | 0.267 | 0.267 | 0.338 |
| ShadexWater availability | 0.052 | 1.000 | 1.000 | 1.000 | 0.865 | 1.000 | 1.000 | 0.865 |
| SpeciesxShadexWater availability | 0.306 | 1.000 | 0.427 | 0.046 | 1.000 | 1.000 | 1.000 | 1.000 |

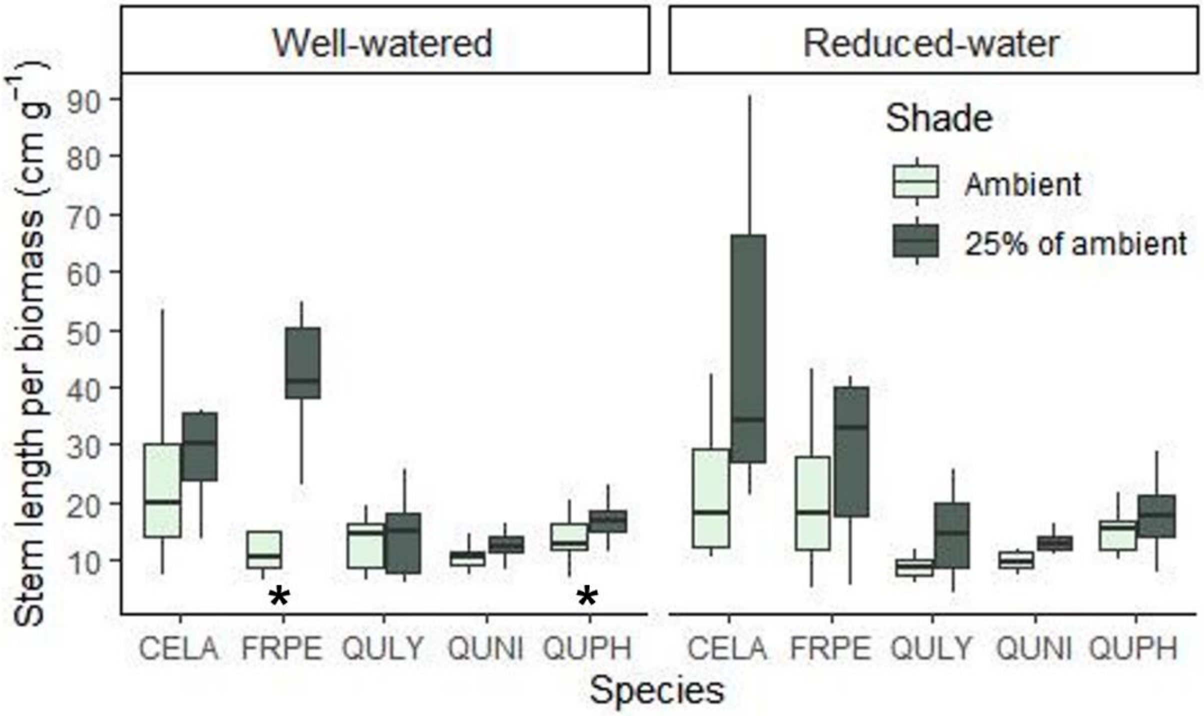


Figure 4.6. Mean (±SE) stem length per biomass by species in response to shade treatment within each level of water availability treatment. Asterisk indicates an effect (α≤0.05) of shade on a species, within that level of water availability treatment.

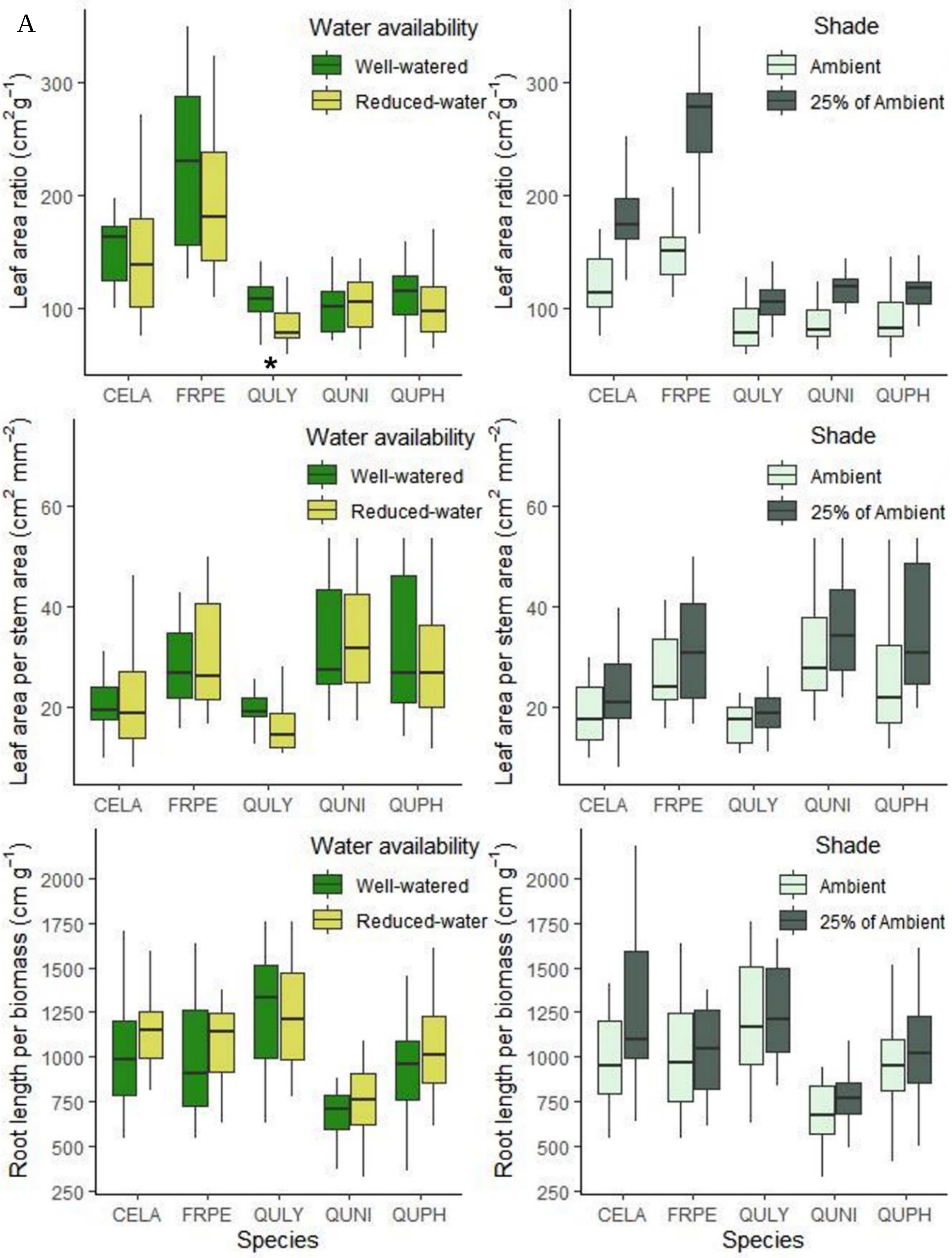
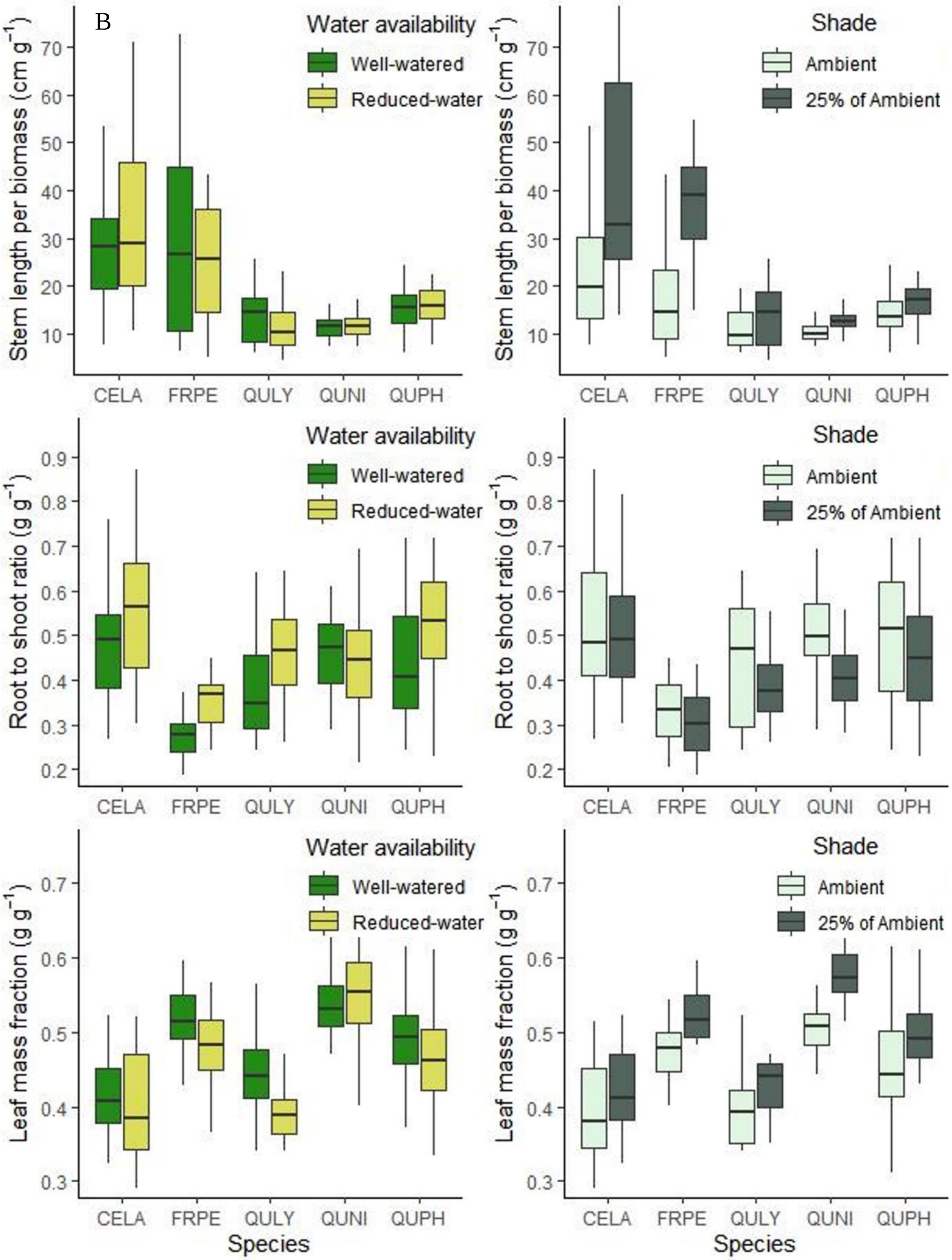
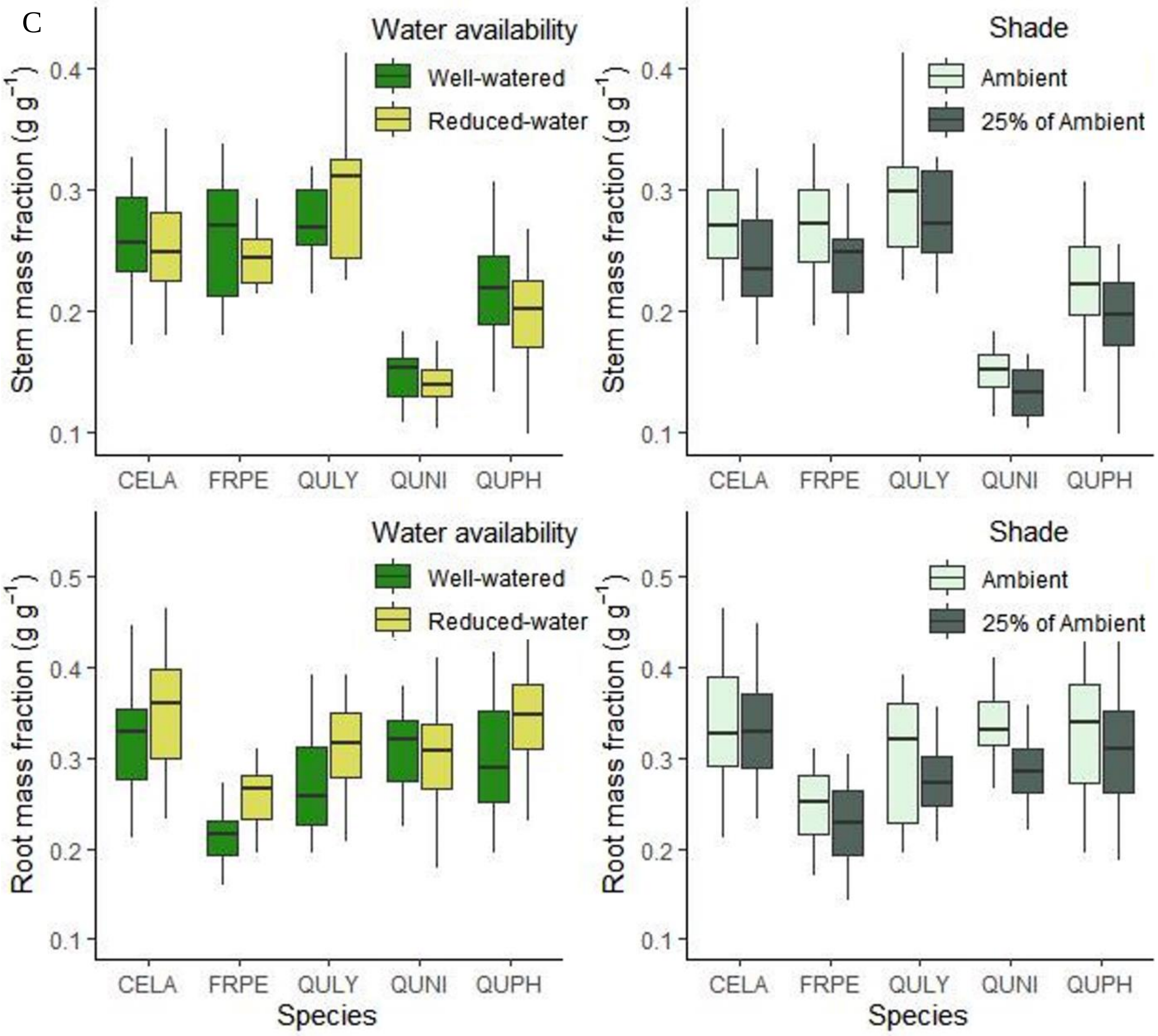


Figure 4.7 Mean (±SE) (A) leaf area ratio, leaf area per stem area, root length per biomass, (B) stem length per biomass, root to shoot ratio, leaf mass fraction, (C) stem mass fraction, and root mass fraction by species in response to water availability and shade treatments. Asterisk indicates a species treatment effect (α≤0.05).
(fig. cont'd.)


B
Water availability
Well-watered
Reduced-water
Shade
Ambient
25% of Ambient
Stem length per biomass (cm g$^{-1}$)
10
20
30
40
50
60
70
Root to shoot ratio (g g$^{-1}$)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Leaf mass fraction (g g$^{-1}$)
0.3
0.4
0.5
0.6
0.7
CELA
FRPE
QULY
QUNI
QUPH
Species

(fig. cont'd.)


C
Water availability
Well-watered
Reduced-water
Stem mass fraction (g g$^{-1}$)
0.1
0.2
0.3
0.4
CELA
FRPE
QULY
QUNI
QUPH
Shade
Ambient
25% of Ambient
Stem mass fraction (g g$^{-1}$)
0.1
0.2
0.3
0.4
CELA
FRPE
QULY
QUNI
QUPH
Water availability
Well-watered
Reduced-water
Root mass fraction (g g$^{-1}$)
0.1
0.2
0.3
0.4
0.5
CELA
FRPE
QULY
QUNI
QUPH
Species
Shade
Ambient
25% of Ambient
Root mass fraction (g g$^{-1}$)
0.1
0.2
0.3
0.4
0.5
CELA
FRPE
QULY
QUNI
QUPH
Species

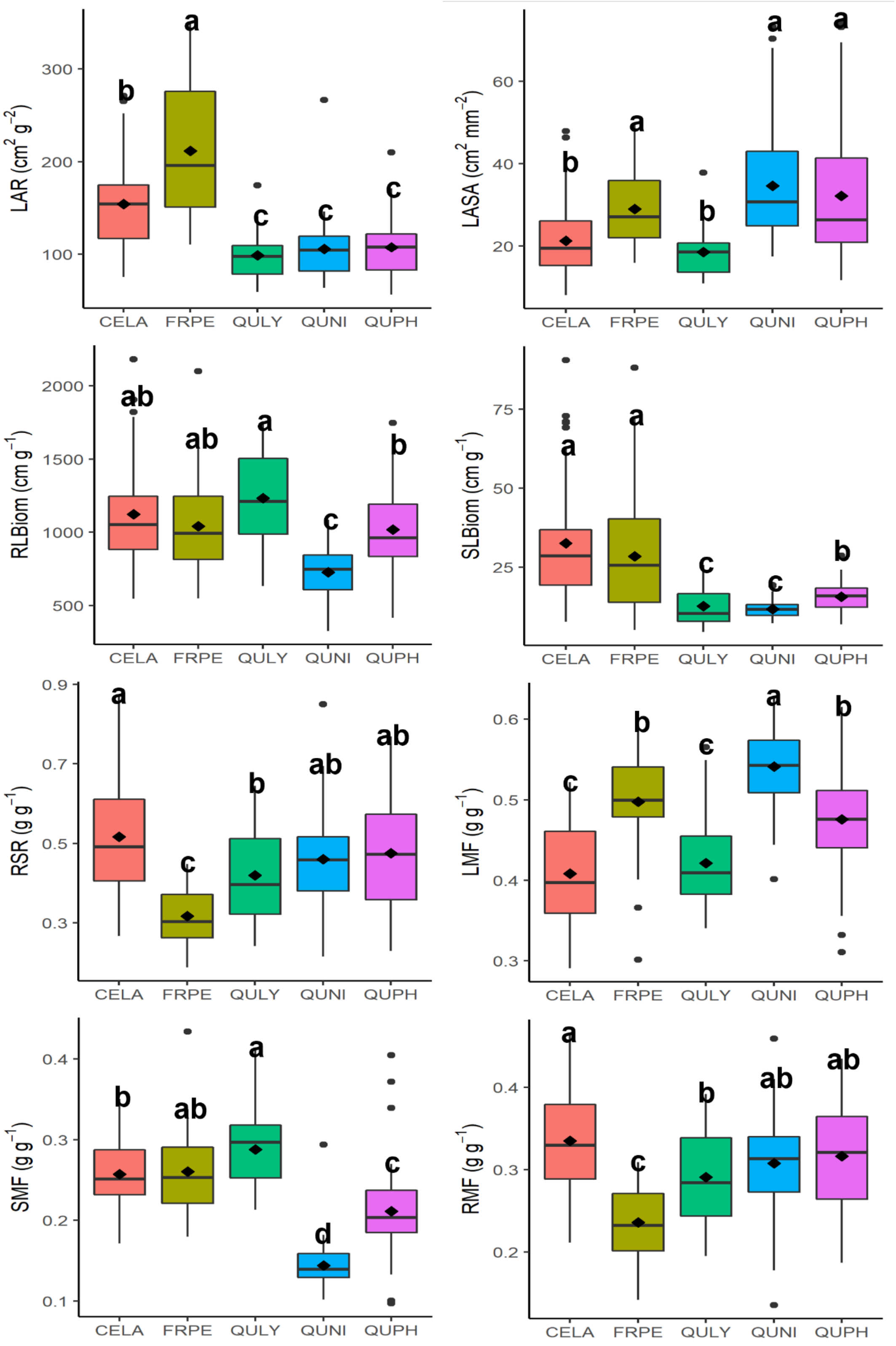


Figure 4.8. Mean (±SE) (diamond) whole plant allocation traits for five BLH seedlings averaged across all treatments. For each growth trait, boxplots with different letters indicate difference among species (α≤0.05).

*Linear discriminant analysis*

Combined across all treatments, linear discriminant analysis (LDA) discriminated between the five study species (Figure 4.9). The first and second discriminants accounted for 65% and 16% of the discriminative power with the third and fourth discriminants accounting for the remaining 11% and 8% respectively. The spatial distribution of species along the first two linear discriminants appeared to follow shade and drought tolerance rankings (Baker 1949; Burns and Honkala 1990; McKnight et al. 1980, Niinemets and Valladares 2006; Putnam et al. 1960 USDA, NRCS 2021; Zon and Graves 1911), with species separated by shade tolerance along LD1 and separated by drought tolerance along LD2. The clearest examples were the separation of more shade-tolerant species CELA and FRPE from shade-intolerant QUNI and QUPH along LD1 and separation of more drought-tolerant CELA from drought-intolerant QULY along LD2. Lastly, appearance of overlap between QULY and FRPE suggests these species had similar trait expression when investigated across all treatments. The first linear discriminant was strongly positively corelated with rooting depth and stem density while negatively corelated with RWC, SLBiom, RLBiom and LAR. The second discriminant appeared to balance above and below ground biomass and was positively associated with RSR and negatively correlated with SecPrimRMR and average leaf size.

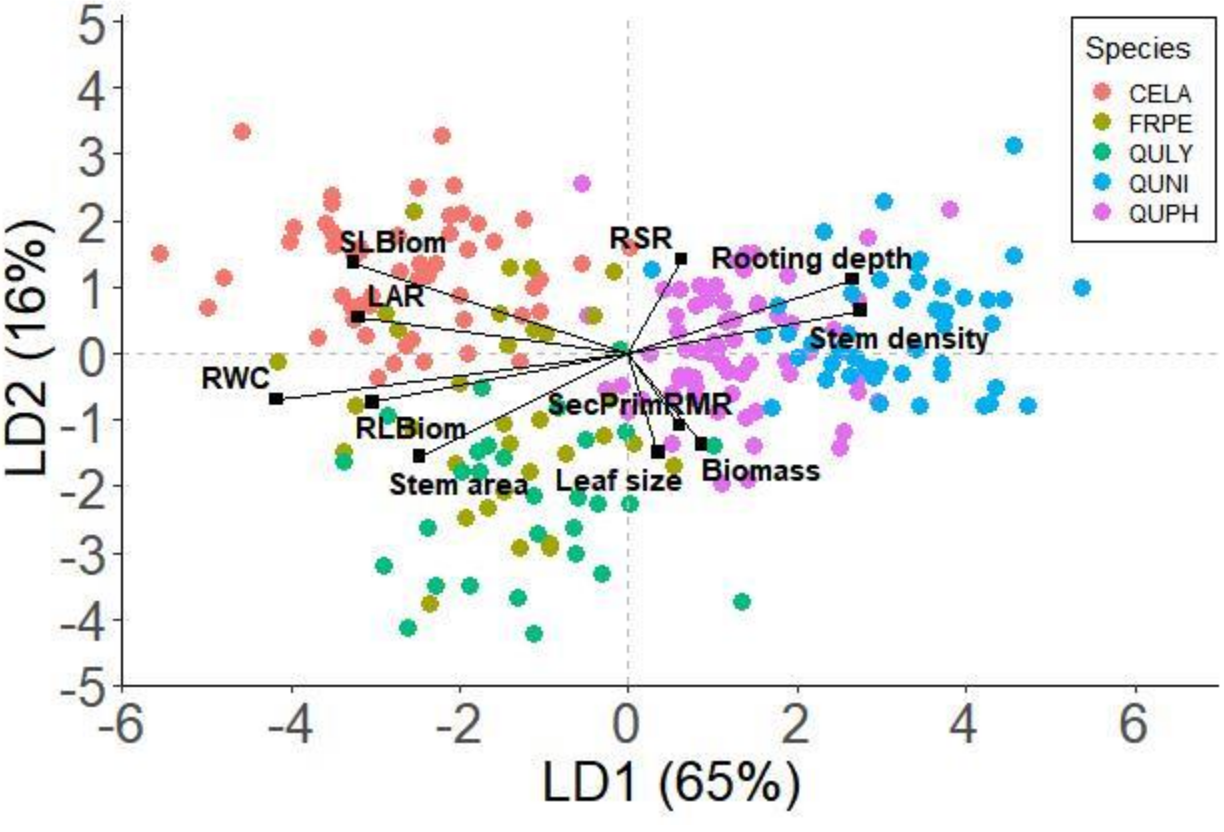


Figure 4.9. Linear discriminant analysis of plant traits (squares) for all species investigated (circles) across all treatments. Each point represents the projected value for an individual seedling. Biomass traits are linearly scaled to fit the projection space represented as an overlaid biplot.

In the LDA of species and shade treatment combinations (Figure 4.10), the first and second discriminant functions accounted for 58% and 20% of the discriminative power respectively. The first linear discriminant separated the five species from each other while the second linear discriminant primarily separated the ambient-sun and 25% of ambient treatments from each other within each species. Strong separation among species occurred across treatments but the degree of discrimination between shade treatments within each species varied. The first linear discriminant separating species was positively associated with traits related to efficient production of leaves, stems, and roots per total seedling biomass and negatively associated with the production of deeper roots, denser stems, and high root to shoot mass. The separation of shade treatment was explained by a greater adjustment of LAR, SLBiom, and RSR. CELA and FRPE showed the greatest adjustment in morphology in response to shade while QULY was the least responsive.

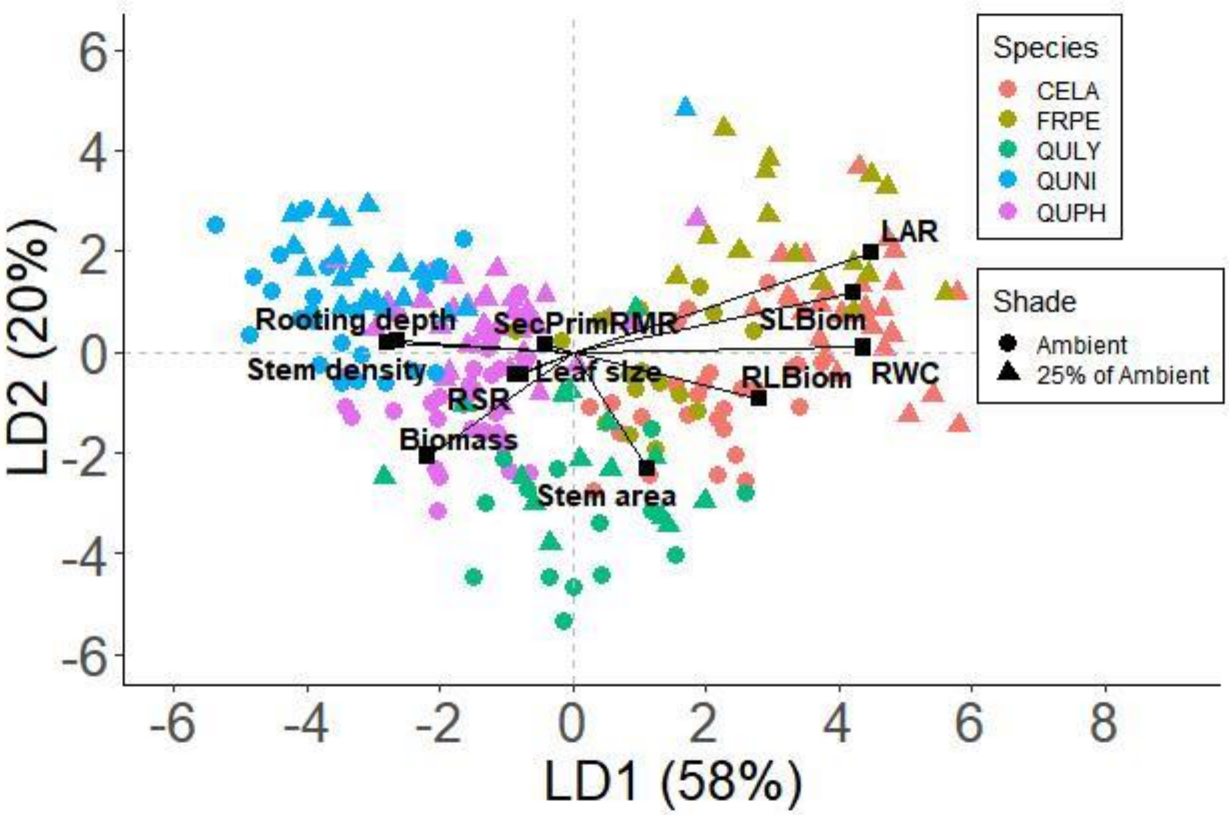


Figure 4.10. Linear discriminant analysis of plant traits (squares) for all species investigated by shade treatment. Each point represents the projection value for an individual seedling. Traits are linearly scaled to fit the projection space and represented as an overlaid biplot.

Lastly, the LDA of species and water availability treatment combinations (Figure 4.11), the first and second discriminant functions accounted for 60% and 17% of the discriminative power respectively. A high degree of overlap existed among species and treatments. The first linear discriminant separated both the five species from each other as well as water availability treatments within each species. The first linear discriminant positively associated with deep roots, dense stems, and high root to shoot mass and negatively associated with high production of leaves, stems, and roots per seedling biomass, high root water content and high stem area. QUNI and QUPH separated clearly from CELA FRPE and QULY along the first discriminant. A high degree of overlap between QULY and FRPE was present. CELA remained separated from FRPE and QULY primarily along the second linear discriminant with greater allocation to stem length and root length per total seedling biomass. In addition, the degree of discrimination between water availability treatments among species was greatest in CELA.

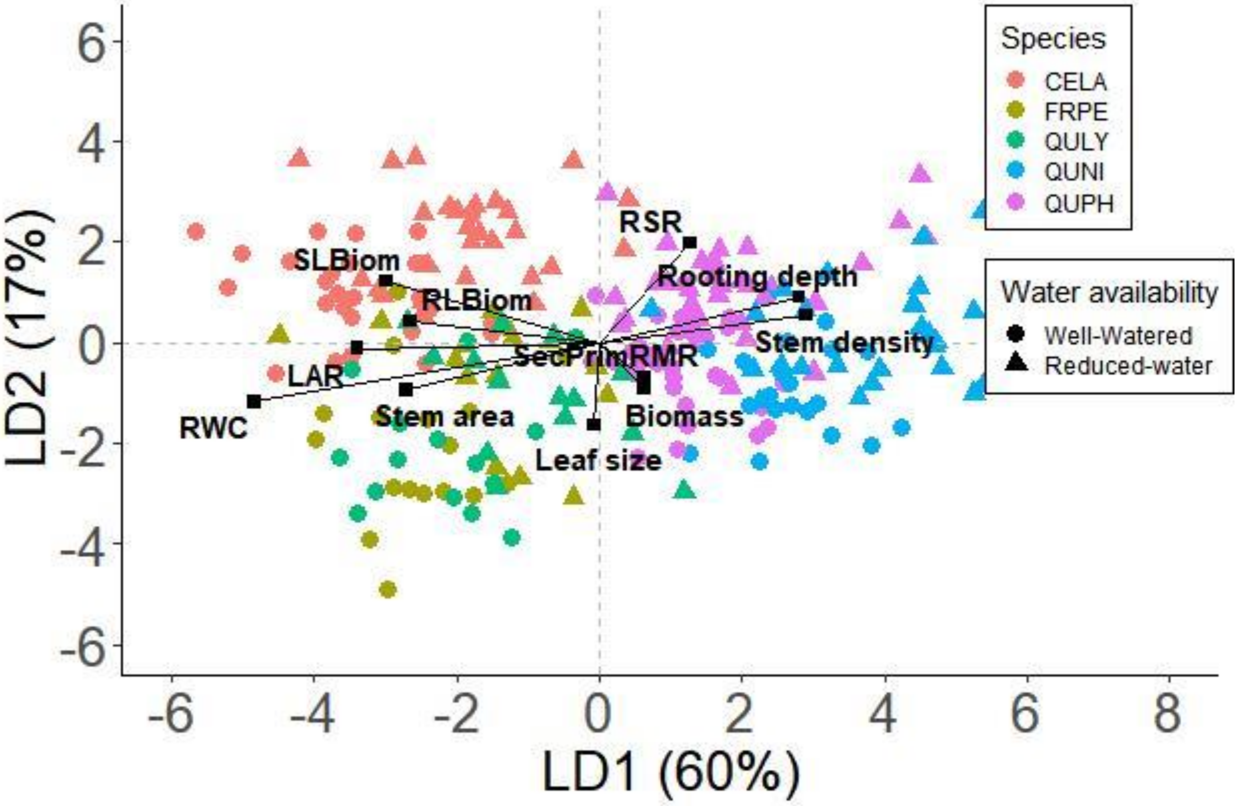


Figure 4.11. Linear discriminant analysis of plant traits (squares) for all species investigated by water availability treatment. Each point represents the projection value for an individual seedling. Traits are linearly scaled to fit the projection space and represented as an overlaid biplot.

## Discussion

Floodplain forest tree seedlings evaluated in this study adjusted morphology to mitigate the effects of shade and reduced water availability. Relatively few interactions between shade and water availability treatment on biomass allocation indicated that the two stressors primarily acted independently of one another, thus failing to support my facilitation hypothesis and the alternative tradeoff hypothesis. However, adjustments in morphology made in response to shade and water availability indicated the potential for future tradeoffs if longer exposure to or more intensive stressors occurred. Overall, species' responses to stressors highlighted broad differences in tolerance strategies, indicating that no single morphology or tolerance strategy was universally favored.

Polytolerance is rare, and trade-offs between tolerating shade and drought stress have been found across phylogeny (Laanisto and Niinemets 2015; Niinemets and Valladares 2006; Smith and Huston 1989). The results of my study do not corroborate those findings. However, I did not include direct measurements of drought status of seedlings (e.g. water potential), and therefore it is possible that in my study the difference between water availability treatments may not have been intense enough to elicit a tradeoff response. Instead, the results of my study align with the growing amount of experimental evidence suggesting that drought- and shade-tolerance tradeoffs may not be as universal as previously thought (Holmgren 2000; Markesteijn and Poorter 2009; Sack 2004, Sack and Grubb 2002). In my study, the effects of shade and water availability on seedling biomass allocation were primarily independent of one another. In the few interactions that were found, the effects were highly species-specific and not uniformly indicative of either a facilitation or tradeoff interaction.

Previous studies indicate that the response of combined shade with low water availability may be quadratic instead of the assumed linear response (Holmgren 2012). In a quadratic response, the effects of low water availability are amplified at low and high levels of shade, but these effects are ameliorated at intermediate levels due to improved microclimate conditions such as reduced vapor pressure deficit (Holmgren 2012; Huang et al. 2008). In this study, reduced water availability decreased whole seedling water content, but only within ambient sun treatments, meaning that seedlings had higher water content in shaded reduced-water treatments than in ambient sun reduced-water treatments; however, this did not result in shade facilitating a reduced drought response in the plant traits investigated. In addition, water stress was likely not the same for each species as water utilization was likely different among species (Kassahun and Renninger 2021). This limits the potential to discuss the relations among treatments. This study also assumed that seedlings were developed enough to elicit differing responses to treatment. Different stress response traits take differing amounts of time to develop and change with age. For example, in oaks, larger-diameter vessels with high hydraulic conductance are produced in spring when soil moisture is typically high. As soil water declines later in the season these vessels may cavitate and lose function or lose conductance with the production of tyloses (Cochard and Tyree 1990). Smaller vessels can be produced during periods of low water availability which have lower hydraulic conductivity but higher resistance to cavitation (Cochard and Tyree 1990; Taiz and Zieger 2010; Tyree and Sperry 1988). In contrast, cell expansion is driven by turgor pressure and is sensitive to even small changes in water content. As such, traits such as leaf area and root length can be impacted at all developmental stages (Landsberg and Gower 1997).

Although no consistent interactions among treatments were found, shade altered morphology in such a way that increased sensitivity to decreased water availability is possible. To tolerate shade, plants must maintain a positive carbon balance and, to tolerate reductions in water availability, plants must maintain a continuous water supply. In response to shade, seedlings increased the efficiency of biomass allocation to leaves stems and roots and increased the proportion of total seedling biomass allocated towards leaves. Foliar traits commonly show the greatest response to shade, and in low light environments plants can increase light capture while minimizing carbon cost by increasing specific leaf area (Infante-mata et al. 2019; Veneklas and Poorter 1998). In contrast, shade decreased rooting depth and increased root water content. High root water content is beneficial for tolerating periods of low water availability due to increased capacitance (Colangelo et al. 2018), however these roots also typically have larger cortex area and can be more sensitive to cavitation which lowers hydraulic conductivity as soil dries (Tyree 2018). A higher proportion of leaf area and leaf mass per total seedling mass represents a proportionally higher amount evaporative surface area (Tyree 2018; Tyree and Ewers 1991) thereby increasing evapotranspiration. Lastly, while higher specific leaf area is an important shade acclimation response (Landsberg and Gower 1997), it is also negatively correlated with water use efficiency due to thinner leaves and lower boundary layer resistance increasing water loss (Choong et al. 1992; Poorter and Garnier 1999; Roden et al. 1900).

Water availability treatment impacted fewer plant traits than shade; and while drought status was not measured, the reduction in water availability still elicited morphological changes that could negatively impact future plant growth in shade. Reduced water availability increased seedling root to shoot ratio. This is consistent with drought studies on floodplain species globally (Ledo et al. 2018; Parolin et al. 2010) and among similar BLH species (Nash and Graves 1993). Adjustments to RSR in response to reduction in water availability are typically more apparent in seedlings than in mature individuals (Ledo et al. 2018) which is important for seedling survival and successful establishment (Mašková and Herben 2018). Higher RSR however can increase shade susceptibility by altering whole plant carbon balance by disproportionately increasing respiring tissue (i.e. roots) while decreasing photosynthetic tissue (i.e. leaves) (Ledo et al. 2018). Proportionally lower light capture area to metabolically active tissue is unsustainable in low light conditions and could potentially decrease the ability for seedlings to respond to changes in light availability such as with gap creation (McNab et al. 2021; Oliver 1978).

The distribution of biomass in sugarberry and green ash and their response to shade treatment are characteristic of rapid growing and colonizing species and a shade escape strategy (Poorter and Garnier 1999). High SLA and rapid vertical dominance are associated with successful regeneration in early emerging species with higher SLA species typically replacing lower SLA species (Dwyer et al. 2014; Schieving and Poorter 1999; Veneklaas and Poorter 1998). In contrast, the three oak species possessed a distribution of biomass indicative of shade endurance/tolerance with carbon dense stems and leaves and minimal adjustment to vertical growth in response to shade.

Stress tolerance is conveyed by multiple traits and adjusting biomass allocation is only one strategy plants use. Drought tolerance can be characterized by a plants ability to maintain gas exchange as water availability declines (Berger-Landefelt 1936; Tardieu and Davies 1993). The oak species in this study are anisohydric (Robert et al. 2017). Anisohydric species maintain high rates of gas exchange as water availability declines, allowing plant water potential to also decline (Tardieu and Simonneau 1998). Anisohydric species typically require either high hydraulic conductance and or cavitation resistant hydraulic architecture (McDowell 2011; Tyree and Sperry 1988). In contrast, sugarberry and green ash are isohydric (Caron and Kjelgren 2016; Montague et al. 2004). Isohydric species begin to reduce stomatal conductance early during periods of decreasing water availability to maintain a relatively high (less negative) water potential which typically also results in lower net carbon gain (Tardieu and Simonneau 1998). Isohydric species are sometimes considered more drought sensitive due to the risks of carbon starvation, while anisohydric species are more drought tolerant from a carbon balance perspective but risk catastrophic hydraulic failure (Berger-Landefelt 1936; Tardieu and Davies 1993). These classifications are not a perfect dichotomy, and only represent two ends of a gradient of water management strategies (Hochberg et al. 2018). Some species can change regulation strategies depending on water availability (Franks et al. 2007), and there is open debate on the carbon and hydraulic sensitivity of these two strategies (Garcia-Forner et al. 2016; Quero et al. 2011). In floodplain forests, with a well-connected floodplain, anisohydric species that maintain production between flood pulses may outcompete cooccurring isohydric species that reduce gas exchange to tolerate inter-flood periods. However, in a drier and disconnected floodplain high water demand of anisohydric species may exceed water supply, leading to hydraulic failure (Colengelo et al. 2018), while the more conservative water management strategy of isohydric species may be more productive. This idea was partially supported by Colangelo et al. (2018) where floodplain oak mortality during severe drought was associated with inefficient water use, low hydraulic conductance, and lower water capacitance. Further research into the ability of floodplain forest species to adjust water use efficiency at a physiological and morphological over different temporal and spatial scales could help further explain changes in BLH species composition (see Kassahun and Renninger 2021).

Many BLH forests are shifting from communities dominated by more flood-tolerant, shade-intolerant species such as overcup oak to less flood-tolerant, shade-tolerant species such as sugarberry and green ash (Gee et al. 2014; Hanberry et al. 2012; King and Antrobus 2005; King and Keim 2019; Oliver et al. 2005; Stallins et al. 2010). Research such as what is presented here helps integrate a mechanistic understanding of these changes. Sugarberry and green ash emerge early in the season (Burns and Honkala 1990; Kroschel 2020; Streng et al. 1989), and benefit from high light availability. The results of my study indicate that this early emergence is also accompanied by a morphology that facilitates fast rapid growth and maintaining vertical dominance. Furthermore, in response to low water availability, sugarberry and green ash showed minimal loss of traits that would support this vertical dominance. The shade and drought tolerance of these species as well as their life history creates an advantage to regeneration when compared to oak species (Grubb 1977). BLH oak species emerge later in the season (Burns and Honkala 1990; Kroschel 2020; Streng et al. 1989) and thus do not benefit from an open canopy and must also compete with earlier emerging species that exacerbate low light conditions. Furthermore, I found that the morphology and response to both shade and reduced water availability of oak species diminished their ability to compete for light. Oliver et al. (2005) determined shade-intolerant red oaks (e.g. water oak and willow oak) grow slower than many other species in shade, whereas Burns and Honkala (1990) cited that sugarberry commonly out competes most other species for early vertical dominance both in shade and when released. Silvicultural operations to promote regeneration of shade-intolerant species such as artificial gap creation could potentially become less effective if implemented in drier forests (Hodges et al. 2005; McNab et al. 2021), and this could be for several reasons. First, in this study, oaks were already at a disadvantage due to poor vertical dominance and lower ability to acclimate to shade and drought. Second, a canopy opening can temporarily further decrease soil moisture and vapor pressure deficit (De Jager et al. 2015; Price 2001). If the surrounding floodplain is already experiencing low water conditions, this may further hinder oak’s regeneration due to their negative response to drought whereas species such as sugarberry and green ash, which were shown to better tolerate reduced water conditions, are instead allowed to maintain their dominance. In contrast, in a well-connected floodplain with regular seasonal overbank flooding, late-season or a secondary flood would be advantageous to later emerging oak species by reducing competition from early emergent species (Junk et al. 1989; Streng et al. 1989; Tonner and Keddy 1997). Further evaluation of these shade/drought/flooding interactions are needed to more fully understand how floodplain modifications interact with stress tolerance mechanisms of BLH trees to drive species compositional changes.

# CHAPTER 5. OVERALL CONCLUSIONS

The goal of my research was to investigate how changes in the environment impact seed germination and biomass allocation of bottomland hardwood tree species. My results contextualize how plant traits interact with plant life history and changes in the environment and provide a mechanistic foundation to further understand regeneration and compositional change in floodplain forests. I found that more shade-tolerant, flood-intolerant species possessed multiple plant traits and seed phenology that likely provide these species with an advantage against shade as well as drought stress, thereby improving the likelihood of regeneration in drier floodplain settings. In contrast, the plant traits observed across less shade-tolerant more flood-tolerant species indicated that these species were either sensitive to shaded conditions and low water availability or responded to these stressors in a manner that could predispose them to being more susceptible to these stressors in future scenarios. Overall, my study suggests that differences in morphologies and stress response among species also coincided with species life history and phenology. For example, the shade endurance/tolerance strategy typified by the oak species I investigated relies on a well-connected floodplain with regular flood disturbance to have the best chance of being successful. These species germinated slower which could correspond to later season emergence (Kroschel 2020), possessed flood-tolerant and drought-sensitive hydraulic architecture, and responded poorly to both shade and reduced water availability. In contrast, more shade-tolerant, flood-intolerant species germinated sooner which could correspond to earlier emergence in the season (Kroschel 2020) and possessed a shade escape/avoidance mechanism as well, allowing them access to light sooner and rapidly securing vertical dominance. The traits that allow shade-tolerant, flood-intolerant species to tolerate and regenerate under shaded low soil moisture conditions thereby also act to hinder the regeneration of shade-intolerant, flood-tolerant species.

In my germination experiment, greater desiccation tolerance in seeds did not correspond with an enhanced ability to germinate under low water availability. Orthodox seeds, which are tolerant to desiccation and included seeds of sugarberry and green ash, germinated at higher water potentials than recalcitrant seeds which are sensitive to desiccation. Germinating with a higher water potential increases the likelihood of germinating sooner and earlier in the season when water and light availability are high. In contrast, desiccation sensitive recalcitrant seeds (e.g. seeds of *Quercus* spp.) maintained germination at lower water potentials. Recalcitrant seeds typically only survive one growing season, so the ability to germinate under a wide range of water potentials increases the probability of germination. In addition, the ability to germinate at lower water potential increases the probability of germinating later in the season or in stochastically created canopy gaps which are commonly drier than the surrounding area under closed canopy. In a well-connected floodplain with regular flooding, germinating later in the season usually reduces flood exposure and occurs after a flood-induced reduction of earlier season germinants, possibly reducing competition for light.

When characterizing the morphology of naturally regenerated seedlings I determined that more shade-tolerant species also tended to possess plant traits associated with drought-tolerance and flood-intolerance. In contrast, more shade-intolerant species possessed plant traits associated with flood-tolerance and drought-intolerance. Shade-tolerant species included sugarberry and green ash, and their morphology supported shade escape/avoidance mechanisms. This mechanism also supports submergence avoidance (Bloom et al. 1990; Voesenek et al. 2004; Whitlow and Harris 1979), assuming vertical growth can exceed flood depth (Kozlowski 1997; Sauter 2013; Whitlow and Harris 1979). In floodplain forests protected from flooding, or with reduced flood depths, rapid vertical growth can secure these species' dominance in the understory. However, drought-tolerant morphology increases in importance on floodplains with reduced flooding. More shade-tolerant and less flood-tolerant species possessed morphology capable of increasing water capture and decreasing water loss, while also increasing cavitation resistance in stems and roots. In contrast, shade-intolerant, and flood-tolerant species, primarily exhibited traits that allowed them to tolerate floods by increasing air movement through the stem and into the rhizosphere and allocating more structural carbon to longer lived leaves and stems and less towards vertical growth. This allocation pattern supports both a shade and flood endurance mechanism, however, this lowers these species' competitive ability for light from faster vertical growing and early emergent species which in turn further reduce light availability. Additionally, the traits of these less shade-tolerant and more flood-tolerant oak species can increase drought vulnerability through high evaporative surface area, low soil water exploration, and cavitation sensitive stems and roots. In an overall drier floodplain this morphology could potentially pose an additional challenge for regeneration.

Investigating the main and interactive effects of shade and reduced water availability on biomass allocation, showed that there was no interaction between the two treatments. However, both treatments independently led to morphological changes where one stressor could potentially hinder the ability for a seedling to tolerate the other perhaps under more intense stress. In addition, in response to shade, the more shade-tolerant species, sugarberry and green ash, responded with a greater adjustment in biomass allocation than the shade-intolerant oak species. In an already shaded understory, this response imposes a disadvantage to the less responsive and slower growing shade-intolerant species. Furthermore, reduced water availability stimulated a lower response among all plant traits and among all species. However, I used whole seedling water content as an indicator for the efficacy of reduced water treatment, which may not fully reflect the biological water status of the plant, thus interpret these results cautiously (Tyree and Ewers 1991). It is possible that the difference between water availability treatments may not have been intense enough to elicit response. As is, my results mirror results of other studies indicating that drought- and shade-tolerance tradeoffs may not be as universal as previously thought (Holmgren 2000; Markesteijn and Poorter 2009; Sack 2004, Sack and Grubb 2002). Other interaction responses could have been possible, as is evident from the variety of trade-off responses seen globally. In tropical forests in Ghana, shade facilitated drought tolerance and survival of tree seedlings by improving the microenvironment and reducing vapor pressure deficit (Amissah et al. 2015). Riparian species in China responded similarly, however, the response of shade ameliorating drought stress occurred only at intermediate levels of shade (Huang et al. 2008). In contrast, Lucas et al. (2013) determined that the factorial combination of stem damage, flooding, and shade on seedlings of tropical floodplain forests trees was additive with no interaction.

In conclusion, multiple plant traits convey stress tolerance and species use multiple strategies to adjust to their environment and maximize their likelihood of regeneration. Future research should continue to examine multiple plant traits across multiple species, but should also include responses over time, over a dynamic range of conditions, and across multiple stages of regeneration (Pearcy 2007). For example, Kotowski et al. (2010) investigating several floodplain species of various moisture tolerances from temperate floodplains across Europe reached similar findings to mine. Their study determined that seedling establishment under canopy required a high degree of shade-tolerance, and that more flood-tolerant species had lower regeneration under canopy due to both their slower germination later in the season, as well as their lower competitive ability for light compared to faster germinating, less flood-tolerant species. Understanding how the mechanisms species use to support regeneration differ among species, respond to the environment, and relate to life history as a whole, provides a foundation for a process-based understanding of forest species composition not only in BLH but in other floodplain forest systems as well.

# APPENDIX A. SUPPLEMENTAL DATA FOR CHAPTER 2

Table A.1 Mean (±SE) measured water potential (ψ) versus target water potential treatment values and the results of paired t-test on individual treatment levels and across all treatment levels.

| Target ψ | Measured ψ | P value |
|---|---|---|
| 0.00 | -0.01 | - |
| -0.20 | -0.18 (0.01) | 0.185 |
| -0.40 | -0.38 (0.01) | 0.136 |
| -0.60 | -0.56 (0.02) | 0.202 |
| -0.80 | -0.75 (0.03) | 0.212 |
| -1.00 | -0.94 (0.02) | 0.090 |
| -1.20 | -1.11 (0.02) | 0.069 |
| -1.40 | -1.34 (0.04) | 0.240 |
| P<F | | 0.863 |

Table A.2 The effect of water potential on mean (±SE) final percent germination for overcup oak (QULY) and results of ANOVA during a pilot study prior to Chapter 2.

| Ψ (MPa) | QULY |
|---|---|
| 0.0 | 60.0 (6.7) |
| -0.2 | 60.8 (2.5) |
| -0.4 | 75.4 (2.1) |
| -0.6 | 65.6 (1.2) |
| -0.8 | 50.0 (10.0) |
| -1.0 | 67.5 (7.5) |
| -1.2 | 57.5 (7.5) |
| -1.4 | 62.3 (4.2) |
| P>F | 0.274 |

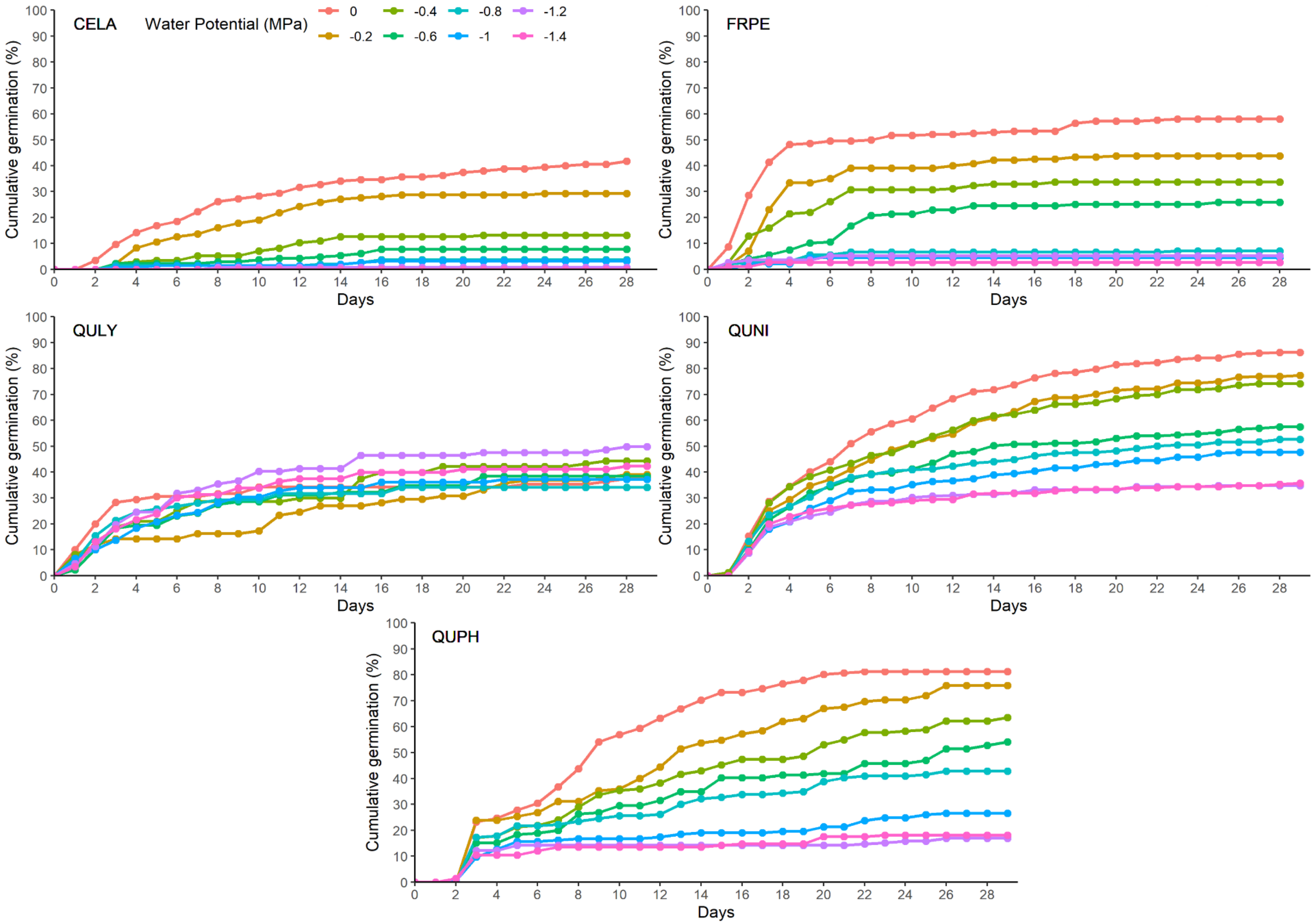


Figure A.1. Cumulative percent germination in seeds by species in response to water potential treatment. Points represents an average of replicates for species and water potential treatment. For illustrative purposes.

# APPENDIX B. SUPPLEMENTAL DATA FOR CHAPTER 3

Table B.1 Paired t-test results comparing manual measurements and automated measurements of subsamples from combined greenhouse and field seedlings.

| Measurement method | n | Mean (±SE) | t | df | P>F |
|---|---|---|---|---|---|
| Manual | 50 | 411.40 (51.13) | 0.300 | 49 | 0.765 |
| Automatic | 50 | 405.16 (50.39) | | | |

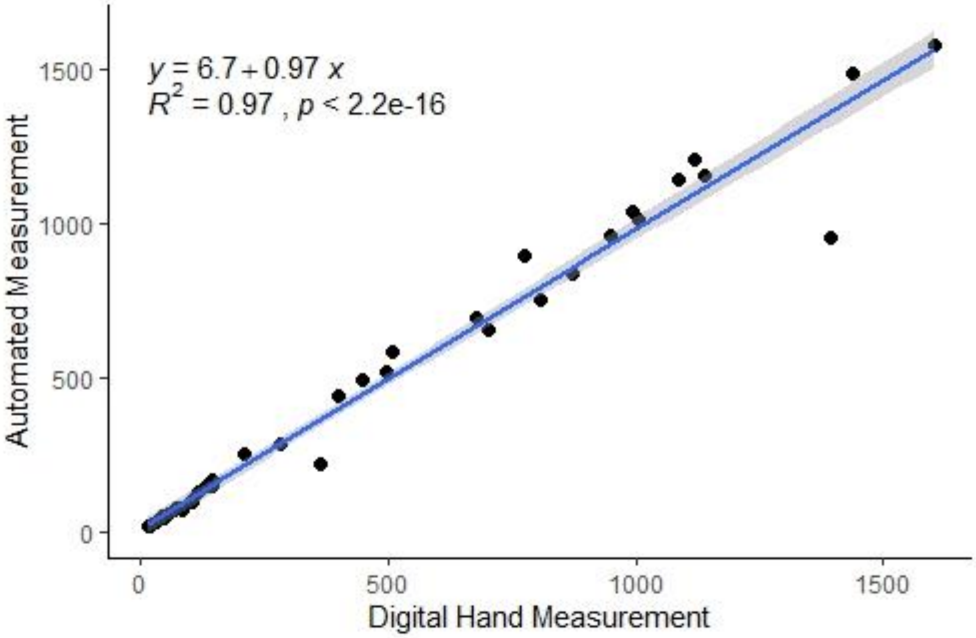


Figure B.1 Relationship between hand measurement and automated measurements of root length (cm) on subsamples of greenhouse (Chapter 4) and field seedling (Chapter 3) scanned root images. Each point represents a paired value of a scanned root image.

Table B.2. Partial correlations among all plant traits of all seedlings. Pearson’s correlation coefficients are shown. P<0.05 (†); P<0.01 (*); P<0.001(**).

| | Rooting depth | Height | Leaf area | Root length | Stem area | Biomass | LS | SLA | LAR | SD | SRL | RLBiom | SecPrim RMR | LASA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rooting depth | 1 | | | | | | | | | | | | | |
| Height | -0.09 | 1 | | | | | | | | | | | | |
| Leaf area | $-0.12^{\dagger}$ | 0.03 | 1 | | | | | | | | | | | |
| Root length | -0.04 | 0.01 | -0.02 | 1 | | | | | | | | | | |
| Stem area | -0.01 | $0.49^{**}$ | 0.00 | $0.65^{**}$ | 1 | | | | | | | | | |
| Biomass | 0.17* | $0.77^{**}$ | -0.02 | 0.01 | -0.02 | 1 | | | | | | | | |
| LS | -0.10 | -0.03 | 0.02 | 0.01 | -0.05 | 0.09 | 1 | | | | | | | |
| SLA | 0.09 | -0.01 | $0.77^{**}$ | 0.00 | 0.00 | 0.01 | 0.00 | 1 | | | | | | |
| LAR | 0.01 | $-0.49^{**}$ | 0 | $-0.65^{**}$ | $1.00^{**}$ | 0.02 | 0.05 | 0.00 | 1 | | | | | |
| SD | 0.02 | 0.02 | -0.08 | $-0.12^{\dagger}$ | 0.05 | 0.01 | $-0.37^{**}$ | 0.04 | -0.06 | 1 | | | | |
| SRL | 0.05 | -0.06 | $0.14^{\dagger}$ | 0.00 | 0.02 | 0.06 | $0.13^{\dagger}$ | $-0.18^{*}$ | -0.02 | 0.04 | 1 | | | |
| RLBiom | 0.04 | -0.01 | 0.02 | $1.00^{**}$ | $-0.65^{**}$ | -0.01 | -0.01 | 0.01 | $0.65^{**}$ | $0.12^{\dagger}$ | 0.03 | 1 | | |
| SecPrim RMR | $-0.27^{**}$ | $0.13^{\dagger}$ | $-0.12^{\dagger}$ | -0.04 | -0.08 | -0.06 | -0.02 | $0.13^{\dagger}$ | 0.08 | -0.1 | 0.04 | 0.04 | 1 | |
| LASA | -0.01 | $0.49^{**}$ | 0.00 | $0.65^{**}$ | $-1.00^{**}$ | -0.02 | -0.05 | 0.00 | $1.00^{**}$ | 0.06 | 0.02 | $-0.65^{**}$ | -0.08 | 1 |
| SSL | -0.08 | 0.03 | $0.27^{**}$ | 0.07 | -0.06 | -0.03 | $-0.17^{*}$ | $-0.24^{**}$ | 0.064 | $-0.18^{*}$ | $0.25^{**}$ | -0.07 | 0.00 | -0.06 |
| SLBiom | 0.20 | $1.00^{**}$ | -0.04 | -0.01 | $-0.49^{**}$ | $-0.77^{**}$ | 0.03 | 0.02 | $0.49^{**}$ | -0.02 | 0.06 | 0.013 | $-0.13^{*}$ | $-0.49^{**}$ |
| RWC | 0.06 | $0.30^{**}$ | -0.06 | -0.03 | -0.02 | $-0.34^{**}$ | 0.10 | $0.17^{*}$ | 0.02 | $-0.24^{**}$ | 0.07 | 0.03 | $-0.16^{**}$ | -0.02 |
| RSR | -0.01 | -0.06 | $-0.13^{\dagger}$ | 0.00 | 0.08 | 0.00 | -0.03 | 0.09 | -0.08 | -0.08 | $-0.31^{**}$ | 0.01 | 0.00 | 0.08 |
| LMF | 0.07 | 0.00 | $0.78^{**}$ | 0.00 | -0.02 | 0.00 | 0.03 | $-0.87^{**}$ | 0.017 | 0.06 | $-0.38^{**}$ | 0.00 | 0.07 | -0.02 |
| SMF | 0.02 | 0.05 | $0.68^{**}$ | 0.04 | -0.05 | -0.04 | 0.03 | $-0.72^{**}$ | 0.05 | 0.09 | $-0.30^{**}$ | -0.03 | 0.05 | -0.05 |
| RMF | 0.08 | 0.05 | $0.54^{**}$ | 0.00 | -0.05 | -0.02 | 0.11 | $-0.59^{**}$ | 0.05 | 0.11 | $-0.43^{**}$ | 0.01 | 0.03 | -0.05 |

(Table cont’d).

|  | SSL | SLBiom | RWC | RSR | LMF | SMF | RMF |
|---|---|---|---|---|---|---|---|
| SSL | 1 |  |  |  |  |  |  |
| SLBiom | -0.02 | 1 |  |  |  |  |  |
| RWC | 0.01 | -0.30$^{**}$ | 1 |  |  |  |  |
| RSR | 0.29$^{**}$ | 0.06 | -0.01 | 1 |  |  |  |
| LMF | -0.27$^{**}$ | 0.00 | 0.04 | 0.18$^{*}$ | 1 |  |  |
| SMF | -0.57$^{**}$ | -0.04 | 0.00 | 0.28$^{**}$ | -0.91$^{**}$ | 1 |  |
| RMF | -0.22$^{**}$ | -0.04 | 0.00 | 0.57$^{**}$ | -0.83$^{**}$ | -0.83$^{**}$ | 1 |

Table B.3. Partial correlations among plant traits selected for use in linear discriminant analyses in Chapters 3 and 4. Pearson's correlation coefficients are shown. P<0.05 (†); P<0.01 (*); P<0.001(**).

|  | Rooting depth | Stem area | Biomass | LS | LAR | SD | RLBiom | SecPrim RMR | SLBiom | RWC | RSR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rooting depth | 1 |  |  |  |  |  |  |  |  |  |  |
| Stem area | -0.08 | 1 |  |  |  |  |  |  |  |  |  |
| Biomass | 0.45$^{**}$ | 0.62$^{**}$ | 1 |  |  |  |  |  |  |  |  |
| LS | -0.07 | -0.03 | 0.14$^{\dagger}$ | 1 |  |  |  |  |  |  |  |
| LAR | 0.09 | -0.37$^{**}$ | -0.04 | 0.44$^{**}$ | 1 |  |  |  |  |  |  |
| SD | 0.07 | -0.68$^{**}$ | 0.33$^{**}$ | -0.24$^{**}$ | -0.30$^{**}$ | 1 |  |  |  |  |  |
| RLBiom | 0.30$^{**}$ | 0.12$^{\dagger}$ | -0.25$^{**}$ | -0.05 | 0.13† | 0.10 | 1 |  |  |  |  |
| SecPrimRMR | -0.27$^{**}$ | -0.10 | 0.24$^{**}$ | -0.01 | -0.03 | -0.08 | 0.67$^{**}$ | 1 |  |  |  |
| SLBiom | 0.14$^{\dagger}$ | -0.14$^{\dagger}$ | -0.52$^{**}$ | -0.24$^{**}$ | -0.20$^{*}$ | -0.24$^{**}$ | 0.16$^{*}$ | -0.05 | 1 |  |  |
| RWC | 0.04 | -0.03 | -0.02 | 0.10 | -0.01 | -0.36$^{**}$ | 0.15$^{*}$ | -0.09 | -0.01 | 1 |  |
| RSR | 0.24$^{**}$ | -0.38$^{**}$ | -0.08 | -0.02 | -0.55$^{**}$ | -0.43$^{**}$ | 0.27$^{**}$ | -0.25$^{**}$ | -0.50$^{**}$ | -0.33$^{**}$ | 1 |

# APPENDIX C. SUPPLEMENTAL DATA FOR CHAPTER 4

Table C.1. Results of ANOVA (P value) showing the effect of species, shade and water availability, and their interactions on seedling water content.

| | Seedling water content |
|---|---|
| Species | <0.001 |
| Shade | <0.001 |
| Water availability | <0.001 |
| Species x Shade | 0.049 |
| Species x Drought | 0.326 |
| Shade x Water availability | 0.009 |
| Species x Shade x Water availability | 0.421 |

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# VITA

Charles (C.J.) Pell was born in, California but has lived from coast to coast. C.J. completed his bachelors at Louisiana State University in 2013 where he received a B.S. in Forest Management with a concentration in ecological restoration and a second B.S. in Natural Resource Ecology and Management with a concentration in conservation biology. He attended Auburn University and graduated in 2015 with a M.S. in Forestry. While at Auburn his research focused on the effects of drought and fertilization on productivity and leaf-level physiology of loblolly pine. After Auburn, C.J. moved back to California where he worked for the US Forest Service stationed at the Lake Tahoe Basin Management Unit. He worked as a plant science technician and later as a regional field crew lead for the Southern Sierra Nevada Ecology Program partnered with the University of California Davis. In 2018 C.J. started his PhD program at LSU, where his academic journey began. Upon completion, C.J. hopes to continue his research into ecophysiology either in academia or with a government agency where he can conduct research to help improve policy and management of our natural systems.