# WARMING AND INTENSIFIED SUMMER DROUGHT INFLUENCE LEAF DARK RESPIRATION AND RELATED PLANT TRAITS IN THREE DOMINANT SPECIES OF THE SOUTHERN OAK SAVANNA

A Thesis

by

KOURTNEE MARR LINDGREN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Molecular and Environmental Plant Sciences

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Approved by:

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# ABSTRACT

Warming and Intensified Summer Drought Influence Leaf Dark Respiration and Related Plant Traits in Three Dominant Species of the Southern Oak Savanna. (May 2009)

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The short-term temperature-response of dark respiration may be altered by climate warming through temperature acclimation; however the role of drought in influencing thermal acclimation is not known. We hypothesized that leaf dark respiration in three dominant species of the southern oak savanna in Central Texas, *Schizachyrium scoparium*, *Juniperus virginiana*, and *Quercus stellata*, would respond differently to the effects of warming and intensified summer drought owing to their contrasting photosynthetic pathways, leaf habits, and drought tolerances. Furthermore, changes in respiration were predicted to be linked to alterations in leaf chemistry and structure, including leaf nitrogen and non-structural carbohydrates in response to warming and drought. Monocultures planted in replicated rainfall exclusion shelters were warmed (+ 1.5 °C) and rainfall events were manipulated to intensify summer drought and augment cool season rainfall compared to the long-term mean.

Both warming and drought affected the short-term temperature-response functions of dark respiration and species differed in their responses. Evidence of temperature acclimation through adjustment in $Q_{10}$ (temperature sensitivity) and $R_{10}$ (base rate at 10 °C) was found in *S. scoparium* and *Q. stellata* but not *J. virginiana*. All three species showed evidence of reduced temperature acclimation of respiration with progressive summer drought. Redistributed rainfall in *J. virginiana* increased respiration in midsummer compared to plants receiving the long-term mean rainfall, but differences disappeared in late summer when drought intensified. In response to rainfall events

during summer drought, rates in *S. scoparium* increased, and the effect was greater in unwarmed compared to warmed plants. In both *S. scoparium* and *Q. stellata*, $Q_{10}$ was reduced post-watering. Regression analyses of respiration against leaf N, soluble carbohydrates, and SLA revealed that relationships differed between species and temperature treatments. Respiration rates were uncoupled from changes in soluble carbohydrates in response to drought and rainfall pulses, suggesting that thermal acclimation is diminished by increasing drought stress in drying soils in contrasting tree and grass species. These findings suggest that models of respiratory carbon flux that incorporate temporal changes in respiratory temperature responses with drought and warming and unique species responses will be critical in predicting species and ecosystem-scale responses to climate change.

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# I. INTRODUCTION

As atmospheric concentrations of greenhouse gases rise over the next century, mean surface temperatures of the earth are expected to rise as well, with a doubling of $CO_2$ concentrations leading to an increase of 1.1-6.4 °C (Solomon *et al.* 2007). Along with rising temperatures, climate warming could potentially modify seasonal precipitation distributions. For example, the mid-latitude continental grasslands of North American are expected to experience a shift from summer to spring precipitation, which, along with higher temperatures, might intensify summer drought (Wetherald *et al.* 1995; Easterling *et al.* 2000). Because plants play an important role in the global carbon cycle, the effects of climate change drivers on plant physiological processes, such as photosynthesis and respiration, are of particular interest to scientists studying global climate change. In fact, one of the greatest uncertainties in modeling the future terrestrial carbon sink is the respiratory release of $CO_2$ by plants (Bruhn *et al.* 2007). Therefore, understanding the response of leaf dark respiration to climate change drivers and their effects on the relationship between respiration and other associated plant traits will play an important role in predicting potential feedback effects of plants on climate and will aid researchers in the development of more accurate global change models that have the ability to predict future atmospheric $CO_2$ concentrations.

Respiration, the process that provides the energy and carbon skeletons needed for biosynthesis, cellular maintenance, and active transport (Atkin *et al.* 2003) in autotrophs and heterotrophs of terrestrial ecosystems, has a large influence on plant, ecosystem, and global carbon exchange, ultimately effecting atmospheric $CO_2$ concentrations (Cox *et al.* 2000; Ryan 1991; Valentini *et al.* 2000) and is known to be particularly sensitive to both short- and long-term changes in temperature (Atkin *et al.* 2005b) as well as drought (Flexas *et al.* 2005). It is estimated that plant respiration releases approximately 50-60 Gt of C year $^{-1}$ into the atmosphere globally (Gifford 2003), representing about 65% of

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This thesis follows the style of *Global Change Biology*.

total $CO_2$ released into the atmosphere from terrestrial ecosystems (Armstrong *et al.* 2006a). These pools and fluxes of carbon play a significant role in the global carbon cycle, especially when one considers the combustion of fossil fuels, cement production, and changing land use is estimated to release about 7.2 Gt of C $year^{-1}$ (Solomon *et al.* 2007) While the respiratory release of $CO_2$ into the atmosphere by plants is largely balanced by carbon fixed during photosynthesis, climate warming has the potential to alter this balance through direct effects of increasing temperature on respiration (Tjoelker *et al.* 2008). However, respiratory acclimation to temperature, defined as an adjustment in the rate of respiration in response to a change in temperature (Atkin *et al.* 2003), may constrain respiratory release of carbon in plants in response to climate warming (Tjoelker *et al.* 2008).

It may be possible to predict the degree of acclimation of leaf dark respiration to temperature from other plant traits such as leaf nitrogen (N), total non-structural carbohydrates (TNC), and specific leaf area (SLA). The 'worldwide leaf economics spectrum' suggests that certain trait correlations occur globally for species grouped by growth form, biome, and climate and that these trait correlations may be useful in predicting plant responses to changing climate (Wright *et al.* 2004). Across diverse taxa and environments, rates of leaf dark respiration frequently correlate with leaf N concentrations (Ryan 1995; Reich *et al.* 1998; Reich *et al.* 2006; Tjoelker *et al.* 2005; Wright *et al.* 2006) and SLA (Reich *et al.* 1998). The relationship with nitrogen is likely due to the role of respiration in protein content, protein repair, and turnover, while the relationship with SLA might be associated with the degree of allocation of carbon to structural rather than metabolic components of the cell (Lee *et al.* 2005). Furthermore, it has been suggested that carbohydrates may also play a role in predicting acclimation of leaf dark respiration to temperature, due to the limitations of the supply of carbohydrates to respiration from photosynthesis in different environments (Dewar *et al.* 1999; Atkin *et al.* 2003). Acclimation of leaf dark respiration to temperature was related to changes in both leaf N and carbohydrates in seedlings of five boreal tree species grown under contrasting temperatures (Tjoelker *et al.* 1999b), in *Pinus banksiana* across wide-ranging

sites and populations (Tjoelker *et al.* 2008), and in three deciduous tree species following 3-day shifts in ambient temperature (Lee *et al.* 2005). These results suggest that a joint enzyme and substrate-based model of respiratory acclimation to temperature may be appropriate (Tjoelker *et al.* 2009).

While temperature will no doubt play a role in mediating the effects of global change on plant-related process such as leaf dark respiration, the effects of changing rainfall distribution (such as increased summer drought) and its interaction with warming should also be considered. The effects of water stress on plant respiration are not well-understood due to the small number of studies and the contradictions among the studies that do exist, and results have included increased, unaffected, and decreased rates of leaf dark respiration in response to water stress (Flexas *et al.* 2005). It has been suggested that the temperature sensitivity of leaf dark respiration might be reduced in plants experiencing drought due to a reduction in photosynthesis and the subsequent synthesis of sugars (Atkin *et al.* 2003). Some studies suggest that the responses of leaf dark respiration to water stress among species might be predicted from a species' growth form (Galmés *et al.* 2007). For example, Galmés *et al*. (2007) observed that herbaceous species showed the largest decrease in leaf dark respiration in response to water stress and the greatest recovery after re-watering, while leaf dark respiration in semi-deciduous and evergreen shrubs only slightly declined under water stress. In order to better understand how climate change will affect plant, ecosystem, and global carbon exchange, more information is needed about the response of plant respiration not only to warming, but rainfall distribution as well.

The overarching goal of our study was to investigate the independent and interactive effects of warming and annual rainfall distribution on leaf dark respiration rates and the associated leaf traits of leaf N, leaf TNC, and SLA in three dominant species of the southern oak savanna: the $C_4$ grass *Schizachyrium scoparium* (Michx.) Nash, a native invasive evergreen *Juniperus virginiana* L., and *Quercus stellata* Wang., a deciduous tree. These three dominant plant species possess contrasting photosynthetic pathways and leaf habits, which may play a role in directing their responses to global

change drivers. It was hypothesized that leaf dark respiration rates of the three study species would respond differently to the independent and interactive effects of increased temperature and rainfall redistribution and that these changes could be linked to inherent differences and treatment alterations in leaf N, leaf TNC, and SLA. Furthermore, warming and rainfall redistribution treatment effects could interact to change the relationship between leaf dark respiration rates and these plant traits.

The specific objectives of our study were to: 1) determine how long-term warming and rainfall redistribution, which intensifies summer drought, affect temperature-response functions and leaf dark respiration rates in our three study species (*S. scoparium*, *J. virginiana*, and *Q. stellata*), and 2) examine if the relationships between leaf dark respiration and leaf N, leaf TNC, and SLA are affected by long-term warming and drought. First, we examine how temperature-response parameters of leaf dark respiration and leaf N, leaf TNC, and SLA of each species are affected by long-term warming and rainfall redistribution. Then, we determine how those same parameters respond to rainfall pulses and declining soil water content during prolonged summer drought. Lastly, we examine how the relationships between leaf dark respiration and leaf N, leaf TNC, and SLA are affected by long-term warming and rainfall redistribution.

# II. RELEVANT LITERATURE

## Southern post oak savanna

Savanna ecosystems, which occupy nearly a third of the world's land surface including more than 50 million hectares in North America (McPherson 1997), are characterized by a continuous grass layer and scattered trees or shrubs. The savanna ecosystem is important both culturally and commercially to humans, as it provides forage for livestock, wood products, recreational uses, preferred vegetation around dwellings, clean air and water, open space, and biodiversity (McPherson 1997). Furthermore, savannas are an important component of the global carbon cycle, as its vegetation may provide long-term storage compartments for carbon (McPherson 1997).

The Post Oak Savanna area in Texas, which consists of about 2.8 million hectares (Hatch *et al.* 1990), is an ecotone between the temperate deciduous forests to the east and the tall-grass prairies to the west (McPherson 1997). The average annual precipitation is 76-114 cm, with peak precipitation occurring in May and September (Hatch *et al.* 1990). In the southern post oak savanna, three plant species dominate: *Schizachyrium scoparium* (Michx.) Nash, a native, perennial, warm-season, $C_4$ grass, *Juniperus virginiana* L., a native invasive evergreen $C_3$ tree, and *Quercus stellata* Wang., a native, over-story $C_3$ tree (Hatch *et al.* 1990; McPherson 1997).

Factors that affect the relative abundance of woody plants and grasses in this ecosystem are livestock grazing, fire, and climatic variability. Livestock grazing can increase woody plant recruitment by selecting for long-lived, unpalatable plants rather than short-lived palatable grasses (McPherson 1997). Long-term absence of fire can also promote woody plant dominance at the expense of savanna grasses, which are generally well-adapted to and maintained by periodic fires (McPherson 1997). The combination of geographic fragmentation and efficient fire suppression by humans has virtually eliminated wildfires from this ecosystem, aiding in the establishment of woody plant species (McPherson 1997). Changes in climate can also affect the structure and function of this ecosystem because it contains a combination of life-forms particularly sensitive to

environmental change. The combination of temperature and precipitation are important in determining the extent of savanna ecosystems, possibly through their effect on soil water balance (McPherson 1997). The vegetation in this ecosystem experiences high plant water stress every summer due to temperature and precipitation patterns (McPherson 1997). Climate warming is predicted to result in changes in the amount and distribution of precipitation, with a shift from summer to spring rainfall, and when coupled with the direct effects of warming on evapotranspiration, is expected to increase the severity and frequency of droughts in North America (Wetherald *et al.* 1995; Easterling *et al.* 2000). Temperature and drought stress could have major effects on ecotones such as oak savanna, potentially causing shifts in the distribution and abundance of plants (McPherson 1997). It is hypothesized that increases in winter precipitation will favor woody plant establishment and growth at the expense of $C_4$ grasses, as the water is able to percolate beyond the roots of dormant herbaceous plants, becoming available in the deeper soil layers; increases in temperature and summer precipitation are hypothesized to favor $C_4$ grasses at the expense of $C_3$ woody plants, as they are able to exploit the precipitation occurring during the warm-growing season (McPherson 1997).

**Acclimation of leaf dark respiration in response to temperature**

It is well-known that rates of respiration are highly responsive to changes in temperature. Short-term changes in temperature result in immediate alterations of respiration rates, with the degree of change determined by the $Q_{10}$, the proportional increase in respiration for a 10 °C increase in temperature (Atkin *et al.* 2003). Long-term acclimation, defined as an adjustment in the rate of respiration in response to a change in temperature (Atkin *et al.* 2003), can affect the short-term temperature-response function of respiration in two ways: 1) it can result in an adjusted slope (or $Q_{10}$), or 2) it can result in a shift in the intercept/elevation (Atkin *et al.* 2003). Acclimation resulting in an adjusted slope of the temperature-response function is known as 'Type I acclimation', with changes in respiration rates occurring more so at

higher than lower temperatures (Atkin *et al.* 2003). Type I acclimation has been observed in *Plantago lanceolata*, *Eucalyptus pauciflora*, *Picea abies*, and *Pinus banksiana*, and is more common in plants that have been shifted from one growth temperature to another (Atkin *et al.* 2005a). Acclimation resulting in a shift in the intercept of the temperature-response function is known as 'Type II acclimation', with changes in rates of respiration at both high and low measurement temperatures (Atkin *et al.* 2003). Type II acclimation appears to be more common in leaves that have developed at different growth temperatures (Atkin *et al.* 2003). It should be noted that the two types of acclimation do not have to occur independently, but can occur together as plants acclimate to changing temperature (Atkin *et al.* 2005a). Perfect acclimation is termed as 'respiratory homeostasis', which occurs when plants grown at contrasting temperatures exhibit similar rates of respiration when compared at their respective growth temperatures (Atkin *et al.* 2003).

The degree of respiratory acclimation in leaves has been shown to vary both within and among species (Atkin *et al.* 2005a). Loveys *et al.* (2003) found that the degree of acclimation differed among species of herbs, grasses, shrubs, and trees that exhibited different inherent maximum relative growth rates. Larigauderie *et al*. (1995) found that, in several different alpine and lowland species (*Poa alpine, Leucanthemopsis alpina, Luzula alpino-pilosa, Carex foetida, Cirsium alpinum, Saxifraga biflora, Luzula campestris, Carex caryophyllea, and Cirsium acaule*) little or no acclimation of respiration occurred when grown at low temperatures, while other species (*Ranunculus acris, Anthoxanthum odoratum, Leucanthemum alpinum, Poa pratensis, Taraxacum alpinum, T. officinale*) did exhibit acclimation of respiration (Atkin *et al.* 2005a). Tjoelker *et al.* (1999b) observed that broad-leaved tree species and conifer species differed in their degree of acclimation. Broad-leaved tree species exhibited a lower degree of acclimation than the conifer species, suggesting that certain structural and/or functional traits of plants may be useful in predicting their degree of acclimation to changing temperature.

Zhou *et al*. (2007) found that warming resulted in a reduced $Q_{10}$ of the short-term temperature-response function for several different herbaceous species in a tall-grass prairie when compared to control plants. However, the acclimation response was only found in August and not in the other measurement months (May or September), indicating that there were seasonal differences in the degree of observed acclimation. Furthermore, they found that warming increased the average rate of dark respiration throughout the study period (beginning in autumn and throughout the following year), being constantly stimulated by the warming treatment until midsummer, then unchanged after that point. In a study of field-grown *Eucalyptus pauciflora*, Bruhn *et al.* (2007) observed acclimation in leaves to experimental night-time warming that was manifested through adjustments in the base respiration rates rather than the $Q_{10}$ when compared to the ambient-temperature controls. In comparison, Tjoelker *et al*. (2009) found, in northern and southern populations of *Pinus banksiana*, that acclimation occurred primarily through shifts in the base respiration rate instead of the $Q_{10}$.

Observed differences in Type I acclimation (changes in the temperature-sensitivity) may be attributed to changes in the availability of substrate for respiration or to adenylate restriction of respiration (Atkin *et al.* 2003). Interspecific variation in the degree of Type II acclimation (changes in respiration rates at both high and low temperatures) might be attributed to differences in phenotypic plasticity among species (Atkin *et al.* 2005b). For example, species that produce long-lived leaves and generate new tissues slowly will be relatively limited in their ability to acclimate to long-term changes in temperature, while more phenotypically plastic species will likely exhibit greater acclimation associated with changes in glycolytic and/or mitochondrial proteins in leaves that have developed under the new temperature regime (Atkin *et al.* 2005b) or changes in leaf cell ultrastructure (Armstrong *et al.* 2006b).

Understanding how plant respiration acclimates to changes in temperature will be imperative in predicting annual $CO_2$ release by terrestrial plants. Many global change models use a constant $Q_{10}$ of 2.0, assuming that plant respiration will response to both short- and long-term changes in temperature in a fixed, exponential manner (Atkin *et al.*

2005b). However, if coupled climate and carbon cycle models are to accurately predict future concentrations of $CO_2$, then they will need to incorporate the variability of respiration into their models, including temperature acclimation (Wythers *et al.* 2005; King *et al.* 2006).

**Responses of leaf dark respiration to water stress**

Water stress is an important factor that influences plant productivity worldwide, mainly through its effects on plant carbon balance, the difference between photosynthesis and respiration (Galmés *et al.* 2007). While the response of photosynthesis to drought has been well-studied, the response of respiration to drought is largely unknown, due to a small number of studies on the subject and because of contradictions among those studies (Flexas *et al.* 2005). Because respiration proceeds continuously throughout the day and night, its response to water stress, when photosynthesis is often suppressed (Flexas *et al.* 2005), will be an important factor controlling plant carbon balance.

Various studies have found conflicting results when dealing with the response of leaf respiration among different species to water stress: results include increased (Kaul 1966; Shearman *et al.* 1972; Collier *et al.* 1996), unaffected (Wample *et al.* 1984), and decreased rates (Brix 1962; Boyer 1970; Wilson *et al.* 1980; González-Meler *et al.* 1997; Ghashghaie *et al.* 2001; Haupt-Herting *et al.* 2001). The degree of water deficit is also an important factor in determining the response of leaf respiration to water stress. In a study with *Saxifraga cernua* (a perennial herb), Collier and Cummins (1996) found that when water stress developed slowly, leaf respiration progressively declined, but when water stress developed rapidly, leaf respiration initially increased and then declined steeply. In a study of six different Mediterranean species with varying leaf habits developing water stress under the same conditions in the field, one species (*Rhamnus ludovici-salvatoris*, an evergreen shrub) showed a progressive decline in leaf respiration, another (*Quercus humilis*, a winter deciduous tree) showed an initial increase followed by a large decrease, and the four other species (*R. alaternus*, an evergreen shrub, *Q. ilex*,

an evergreen tree, *Pistacia lentiscus*, an evergreen shrub, and *P. terebinthus*, a winter deciduous tree) were unaffected (Gulías *et al.* 2002).

Responses of leaf dark respiration to water stress among species might be predicted from a species' growth form. Galmés *et al*. (2007) investigated the effects of water deficit on leaf respiration in eleven different Mediterranean species including four evergreen sclerophyll shrubs, three summer semi-deciduous shrubs, and four herbs. Herbaceous species had the highest respiration rates, followed by evergreen shrubs and semi-deciduous shrubs, which were not statistically different from each other. These variations were possibly related to SLA, with evergreen shrubs exhibiting the lowest SLA and leaf respiration rates, and herbaceous species exhibiting the highest values of SLA and leaf respiration rates. In response to water stress, the herbs showed the largest decrease in leaf respiration and the most complete recovery after re-watering, while leaf respiration in the semi-deciduous shrubs and evergreen shrubs only slightly declined under water stress. Because herbaceous and semi-deciduous plants generally have leaves with shorter life-spans than evergreen plants, they tend to capitalize on their carbon balance over shorter periods of time, potentially explaining their higher respiration rates and higher photosynthesis to respiration ratios during drought (Galmés *et al.* 2007). However, the evergreen species are productive over long periods of time and have deeper roots systems which allow them to minimize water stress, potentially explaining the relatively unresponsiveness of leaf dark respiration to water stress in those plants (Galmés *et al.* 2007).

Combined data from several studies show a biphasic response of leaf dark respiration to relative water content, with an initial decrease in leaf respiration followed by an increase in leaf respiration with decreasing relative water content in drought-stressed plants in 14 different species including herbs, shrubs, and trees (Flexas *et al.* 2005). The initial decrease in respiration due to mild water stress could be attributed to a decrease in cell expansion, cell-wall synthesis, protein synthesis, reduced stomatal conductance, and reduced photosynthesis, which in turn results in a decrease in growth respiration (Flexas *et al.* 2005). An increase in respiration under severe water stress can

be attributed to increases in maintenance respiration due to the accumulation of proline and other compatible solutes or to water-stress induced senescence and its associated metabolic processes (Flexas *et al.* 2005).

In a study of three deciduous oak species growing on two sites with varying water availability, $Q_{10}$ values of leaf respiration were lower at the drier site than at the wetter site and differed among species (Turnbull *et al.* 2001). Furthermore, leaf respiration rates were higher and light-saturated rates of photosynthesis lower at the drier compared to the wetter site, indicating a reduced leaf-level carbon balance in species at the drier site (Turnbull *et al.* 2001). These findings suggest that under conditions of low soil water availability, long-term adjustments in leaf structure may increase leaf respiration but reduce its temperature-sensitivity (Atkin *et al.* 2005a). Reductions in the temperature sensitivity of leaf dark respiration might be the result of limitations on photosynthesis and the subsequent synthesis of sugars (Atkin *et al.* 2003).

**Linking leaf respiration and leaf respiratory acclimation to leaf N, leaf TNC, and SLA**

Many studies have found that the degree of acclimation of leaf dark respiration to temperature varies among plants species, but it is possible that such variation could be predicted from other plant traits such as leaf N, SLA, and TNC. It has been suggested that a 'worldwide leaf economics spectrum' exists that is largely independent of plant growth form, plant functional type, and biome (Wright *et al.* 2004). This spectrum reflects inherent tradeoffs that exist in plant function and structure, and it might allow us to estimate plant processes, such as leaf dark respiration, due to the predictability of its relationship with other leaf traits, such as leaf N, SLA, and TNC. However, a better understanding of how this proposed leaf economics spectrum is affected by climate is imperative in determining its usefulness in predicting plant responses to global change.

Specific rates of leaf dark respiration frequently correlate with tissue N concentration across diverse taxa and environments (Tjoelker *et al.* 2008). Mass-based leaf dark respiration rates were highly correlated with leaf N concentration for several

boreal and subalpine woody plants (Ryan 1995). Reich *et al.* (1998) found that, for 69 species from four functional groups (forbs, broad-leafed trees and shrubs, and needle-leafed conifers) in six different biomes, leaf dark respiration was highly related to leaf N on both mass and area bases. Furthermore, for any given level of leaf N, leaf dark respiration was highest in the species with a high SLA and short leaf life-span (forbs) and lowest in the species with low SLA and long leaf life-spans (needle-leafed conifers). Similar results were found in another study, where leaf N was correlated with leaf dark respiration among 39 grassland and savanna species (Tjoelker *et al.* 2005). In the same study, $C_3$ grasses had lower SLA, greater leaf N (area and mass based), and higher leaf respiration rates (area and mass based) than $C_4$ grasses. In a study including 208 woody species from 20 sites around the world, leaf dark respiration correlated with both SLA and mass-based N (Wright *et al.* 2006).

Rates of leaf dark respiration and its acclimation to temperature may also be related to leaf carbohydrate content, the substrate for respiration. In a review of the effects of light intensity and carbohydrate status on respiration, Noguchi (2005) reports many examples in which carbohydrate levels were positively related to leaf respiration (e.g. spinach, barley, wheat, *Arabidopsis thaliana*, and *Beta vulgaris*); however, there were also several studies where no correlations were found. In a study of five boreal tree species, it was concluded that respiration rates were positively correlated to both leaf N and carbohydrate concentrations and that the long-term acclimation of leaf dark respiration to temperature was associated with changes in both leaf N and carbohydrate concentrations (Tjoelker *et al.* 1999a). In three deciduous tree species, temperature acclimation of leaf dark respiration was related to changes in leaf N and carbohydrate content following a three-day shift in ambient temperature (Lee *et al.* 2005). More recently, it was found that acclimation in *Pinus banksiana* across wide-ranging sites and populations was associated with changes in both N and carbohydrate concentrations (Tjoelker *et al.* 2008).

# III. MATERIALS AND METHODS

## Experimental site and growth conditions

The study site is located at the Texas warming and rainfall manipulation (WaRM) project, located near Texas A&M University in College Station, Texas (N 30°34" W 96°21"). The facility (Appendix A), constructed in 2003, was designed to investigate the effects of rainfall distribution and climate warming on three dominant species of the southern oak savanna. Rainout shelters (Appendix A, numbers 1-4 and 6-9) are covered with polypropylene film and receive two different rainfall treatments: long-term mean Appendix A, white boxes) and summer redistributed (Appendix A, grey boxes). The sides below 1.5 m are open to help maintain ambient climatic conditions within the plots as near ambient as possible while excluding precipitation. Covering the two open ends of each shelter, a fine mesh shade cloth matching the radiation attenuation of the film, excludes wind-blown precipitation from entering the shelters. Sheet metal flashing (40 cm in width) inserted 30 cm into the soil penetrates the clay hard pan, isolating each shelter from surface and subsurface water flow.

Rainfall in each shelter is simulated using an overhead irrigation system (17 pressure-regulated spray nozzles per shelter) by supplying reverse osmosis water from four 11,500 L holding tanks. On average, in comparison to unsheltered controls (Appendix A, numbers 5 and 10), mean daily temperatures in the shelters are 0.3 °C higher, relative humidity values in the shelters are 2% lower, and ambient photosynthetically active radiation in the shelters is 30% lower. The two precipitation patterns vary in seasonal distribution and event size, but maintain the same total annual precipitation (1018 mm) and total number of events. The long-term (50 year) precipitation pattern characteristic of the region, including the frequency and intensity (amount) of events, is applied within four of the shelters (Appendix A, white boxes). The precipitation redistribution treatment is applied within the other four shelters (Appendix A, grey boxes). In the redistribution treatment, each summer precipitation event (May-September) is reduced by 40% compared to the long-term mean and evenly

redistributed to the two preceding spring (March and April) and two subsequent autumn (October and November) months. Shelters 5 and 10 (Appendix A, open boxes) are not covered and receive natural precipitation.

Located in each shelter are 10 2x2 m plots composed of *Schizachyrium scoparium* (Michx.) Nash, *Juniperus virginiana* L., and *Quercus stellata* Wang. grown in monoculture and two tree-grass combinations. The plots within each shelter were established in 2003 from local transplants of little bluestem, 1-yr-old bare-root post oak, and containerized juniper from regional seed sources. Five plots, one of each species combination, are heated 24 h per day with infrared heaters (Kalglo Electronics, Bethlehem, PA; model MRM-1208L) at 100 W $m^{-2}$ (Appendix A, hatched squares), and suspended at 1.5 m. The other five plots have dummy lamps (Appendix A, open squares). The warming treatment increase soil temperature (at 3 cm depth) by about 0.6 °C among the species plots and canopy leaf temperatures by about 1.0-1.5 °C, although temperatures vary with wind speed (Kimball 2005). Warmed plots have 1% lower volumetric soil water content as measured throughout the top 20 cm of soil. The unsheltered control plots (Appendix A, shelters 5 and 10) have no lamps. In the study described herein, only plants grown in monoculture were sampled. While each plot contains 25 plants, samples were taken from the center nine plants to minimize any edge effects (Appendix A, numbers 1-9 in Plot Layout).

**Long-term warming and rainfall redistribution**

Temperature-response functions of leaf dark respiration were determined during the summer months of 2007 throughout the summer rainfall redistribution phase. In order to examine the effects of the warming and rainfall redistribution treatments and potential warming x rainfall treatment interaction effects, samples were collected from both warmed and unwarmed plots in the shelters receiving each rainfall treatment in both June and August of 2007. Measurements were split among four replicate plots and blocked between two consecutive days (June 6, 7 and August 24, 25). In June, only little bluestem and juniper leaves were sampled (one plant per replicate plot, $n = 4$ plots). In

August, all three species were sampled (one plant per replicate plot, $n = 4$ plots, except for warmed oaks in the long-term mean rainfall treatment, where $n = 3$).

**Rainfall pulses during summer drought**

To determine whether leaf dark respiration responded to intermittent rainfall events during prolonged summer drought, warmed and unwarmed plants in the redistributed (summer drought) rainfall treatment were sampled before and after two individual rainfall events in August of 2007 (one plant per replicate plot, $n = 4$ plots). Only plants receiving the redistributed rainfall treatment were collected because it was expected that the effects of water stress would be greater than in plants receiving the long-term mean rainfall treatment. In the first precipitation event, plants received 11.68 mm of simulated rainfall and were measured 2 days prior to (August 9) and 3 days after (August 14) the rainfall event. In the second precipitation event, plants received 19.30 mm of simulated rainfall and were measured 1 day prior to (August 21) and 1 day after (August 24) the rainfall event.

**Progressive dry-down of the soil**

To assess whether or not leaf dark respiration responded to summer drought in concert with soil water content, measurements were conducted throughout the course of a dry-down period of the soil (over 24 days) in both warmed and unwarmed plants in the redistributed rainfall treatment (one plant per replicate plot, $n = 4$ plots). Respiration rates in all three species were measured on five dates in the summer of 2007 (July 17, 20, 26, August 6, and 9), with the exception that post oak was not measured on August 9.

**Temperature-response of leaf dark respiration**

Temperature-response curves of leaf dark respiration were generated in a controlled environment growth chamber (Environmental Growth Chambers, Chagrin Falls, Ohio) on detached leaves at the following measurements temperatures in a

randomized order: 10, 15, 20, 25, 30, and 35 °C. Rates of leaf dark respiration were measured using two Li-COR 6400 photosynthesis systems (LI-COR, Inc. Lincoln, NE, USA) equipped with temperature and $CO_2$ controls and conifer chambers to enable measurement of adequate amounts of plant tissue. By making measurements on detached leaves, the possibility of $CO_2$ leaks by diffusion were reduced because the chamber could be completely sealed (Amthor 2000). It has been shown for a number of species that measuring rates of leaf dark respiration does not differ between attached and detached leaves and that those rates may remain stable for longer than 12 h in the dark (Tjoelker *et al.* 2001; Mitchell *et al.* 1999). Checks ran in the field indicated that rates of leaf dark respiration did not differ between attached and detached leaves, and checks in the growth chamber revealed that leaf dark respiration rates may remain stable for at least 12 h in the dark (Appendix B). All measurements in the growth chamber were completed in approximately 12 h or less on the same day, depending on the number of species being measured. Leaf dark respiration rates are expressed on a dry mass basis (nmol $CO_2$ $g^{-1}$ $s^{-1}$).

To control for possible diurnal variations in leaf carbohydrate status (Tjoelker *et al.* 2001), leaf samples were collected at approximately the same time each morning (between 0600 and 0700 h) on each sample date. Because rates of respiratory $CO_2$ release from individual leaves can be low, it can be difficult to accurately measure small changes in $CO_2$ flux when using differential analysis of $CO_2$ concentrations (Tjoelker *et al.* 2001). Therefore, to ensure adequate amounts of tissue, multiple leaves were removed from each plant: in the juniper monoculture plots, several upper canopy leaves from the same branch were collected; from the grass monoculture plots, two or three blades of grass were collected; from the oak trees, one or two upper canopy leaves were collected. To ensure that leaves remain turgid and that respiratory carbon loss was minimized (Tjoelker *et al.* 2001), leaves were enclosed in a plastic bag with a wet paper towel and stored in a cooler on ice during transfer to the growth chamber. The leaves were placed inside the controlled-environment chamber, and allowed to equilibrate to

the first measurement temperature for approximately 30 min before measurements began.

**Leaf structure and chemistry**

After the dark respiration measurements were conducted, the leaf samples were digitally scanned for one-sided, projected leaf area (WinRhizo, Régent Instruments, Inc. Québec City, Québec, Canada). Leaves were oven-dried at 65 °C to determine dry mass and specific leaf area (SLA, leaf area $cm^2$ $g^{-1}$ leaf dry mass). Oven-dried and powdered samples were used to determine leaf N, using an elemental analyzer (Thermo Finnigan, FlashEA™ 1112, Milan, Italy), and leaf total nonstructural carbohydrate (TNC) content, using methods described by (Oleksyn *et al.* 2000). Soluble sugars were extracted from oven-dried, ground plant tissue using methanol:chloroform:water (12:5:3: by volume), and the residue was used for determination of starch content. Soluble sugar concentrations in the extract were determined spectrophotometrically at 625 nm using anthrone within 30 min. Starch content in the tissue residue was gelled by adding ethanol and sodium acetate-NaF buffer and boiling. Starch was then converted to glucose using amyloglucosidase (Sigma, St. Louis, MO, USA) by incubating at 50°C for 24h. Glucose concentrations were determined by assaying with glucose oxidase. To accomplish this, samples were mixed with peroxidase-glucose oxidase-*o*-dianisidine dihydrochloride and incubated at 25 °C for 30 min. Absorbance was then measured at 450 nm. Concentrations of glucose in the samples were then calculated by using linear regression equations based on glucose standards. Leaf carbohydrate content is expressed as soluble sugars (mg $g^{-1}$), starch (mg $g^{-1}$), and their sum, TNC (mg $g^{-1}$) throughout the paper. Rates of leaf dark respiration are also expressed on a leaf N-basis (μmol $CO_2$ $mol^{-1}$ $s^{-1}$) and a leaf soluble sugar-basis (nmol $CO_2$ $g^{-1}$ $s^{-1}$).

**Soil water availability**

To determine how progressive drought in the summer during a drying down period of the soil affects soil water availability, volumetric soil water content in the top

20 cm in each plot was determined using permanently installed time-domain reflectometry (TDR) probes (MiniTrase 6050X3, Soil Moisture Equipment Corporation, Goleta, CA). When possible, these measurements were taken on the same day that respiration measurements were conducted.

**Data analysis**

The short-term temperature-response function of leaf dark respiration was determined using nonlinear regression of leaf respiration rates for each sample at the six measurement temperatures (T) of 10, 15, 20, 25, 30, and 35 °C with the following formula (Atkin *et al.* 2005b):

$$\text{Predicted R} = R_{10} \times Q_{10}^{\left(\frac{T-10}{10}\right)}$$

where $R_{10}$ is the estimated specific respiration rate at the base temperature of 10 °C. $Q_{10}$ is the temperature sensitivity, defined as the ratio of respiration at one temperature to that at 10 °C lower (Atkin *et al.* 2005b), and represents the value for the entire measurement temperature range (10-35 °C).

The precipitation and warming treatments were arranged as a completely randomized split-plot factorial design. The precipitation treatments (long-term mean, redistributed) constituted the whole-plot factor (with four replications), while the temperature treatments (warmed, unwarmed) were assigned as a within-plot factor. For the data comparing all treatment combinations in June and August, 2007, treatment effects on temperature-response parameters ($Q_{10}$, $R_{10}$), mass-based respiration measured at 25 °C ($R_{25}$), and leaf chemistry and structure (leaf N, soluble sugars, starch, and SLA) were analyzed separately for each species using *F*-tests in ANOVA. Precipitation effects (1 df) were tested against the "between shelter" error (6 df), and temperature treatment effects (1 df) and the precipitation x temperature treatment interaction (1 df) were tested against the residual error (6 df).

The effects of rainfall pulses (pre-rainfall and post-rainfall, designated as pre/post) during prolonged summer drought on temperature-response parameters ($Q_{10}$, $R_{10}$), mass-based respiration measured at 25 °C ($R_{25}$), and leaf chemistry and structure

(leaf N, soluble sugars, starch, and SLA), were analyzed separately for each species using *F*-tests in repeated measures MANOVA, with shelters serving as blocks with respect to temperature treatments (plots in the shelters receiving the redistributed rainfall treatment were sampled). The effects of rainfall pulses (1 df), temperature treatments (1df), and the pre/post x temperature treatment interaction (1df) were tested against the "between shelter" error (3 df).

To examine the effects of declining soil water content on temperature-response parameters ($R_{10}$, $Q_{10}$), $R_{mass}$ at 25 °C ($R_{25}$), leaf N, soluble sugars, starch, and SLA, and the linear relationships among these variables, linear regression and analysis of covariance was used. First, we tested for separate slopes between the regression lines for each of the temperature treatments. If the slopes did not differ, then a homogeneity of intercepts test was used to determine whether the temperature treatments differed from each other in terms of elevation of the linear relationship. All statistical analyses were performed using JMP 7.0 (SAS Institute, Cary, NC, USA).

# IV. RESULTS

## Long-term warming and rainfall redistribution

In order to determine the effects of long-term warming and rainfall redistribution on leaf dark respiration and other leaf traits associated with leaf chemistry and structure, we examined temperature-response functions of respiration and determined leaf N, leaf TNC, and SLA in mid-growing season (June) and late-growing season (August) of 2007. In June and August, temperature-response functions of leaf dark respiration differed among the measured species and in some instances, between warming and rainfall treatments within species (Fig. 1, Fig. 2). Inspection of the fitted functions revealed lower $R_{mass}$ in the warmed plants compared to the unwarmed plants in both rainfall treatments, particularly at higher measurement temperatures (Fig. 1, Fig. 2)

In June, *S. scoparium* had higher rates of mass-based respiration ($R_{mass}$) than *J. virginiana* at a given measurement temperature. The two species exhibited similar temperature sensitivities ($Q_{10}$, 10 – 35 °C) of leaf dark respiration to short-term changes in temperature, which ranged from 1.89 to 2.01 across species and treatment combinations (Table 1). However, the two species differed in their response to the rainfall redistribution treatment. In *S. scoparium,* there were no statistically significant treatment effects on the intercept, $R_{10}$, or $Q_{10}$, of the temperature-response functions or on mean $R_{mass}$ at 25 °C ($R_{25}$) (Table 1). By comparison, mean $R_{10}$ values in *J. virginiana* were increased by 25% ($P = 0.07$, Table 1) and mean $R_{25}$ values by 30% ($P = 0.11$, Table 1) in trees receiving the redistributed rainfall treatment compared to the long-term mean. While warmed and unwarmed *J. virginiana* did not differ in $R_{10}$ or $R_{25}$, there was a significant treatment interaction effect ($P = 0.03$, Table 1) on $Q_{10}$ values. $Q_{10}$ was reduced by 5.5% in the warmed compared to the unwarmed treatment in the long-term mean rainfall treatment and unaffected in the redistributed treatment.

In August, *S. scoparium* and *Q. stellata* exhibited higher rates of $R_{mass}$ than *J. virginiana* (Fig. 2). *J. virginiana* and *Q. stellata* exhibited similar $Q_{10}$ values ranging from 1.80 to 1.89 across treatment combinations (Table 1). $Q_{10}$ values in *S. scoparium*

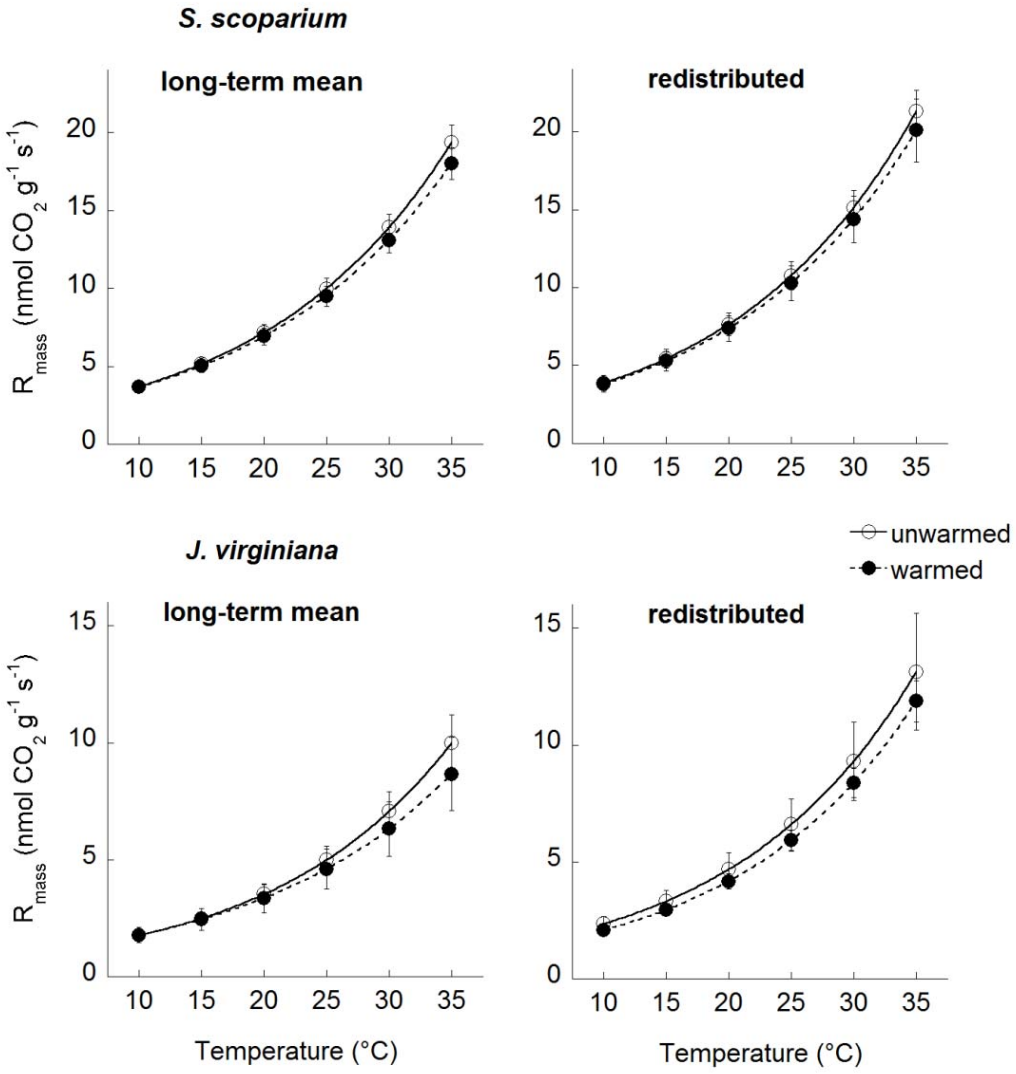


Fig. 1 Temperature-response functions of leaf dark respiration to short-term changes in temperature in *S. scoparium* and *J. virginiana* grown under different rainfall distribution treatments (long-term mean, redistributed) and temperature treatments (unwarmed (–○–), warmed (--●--)). Measurements were conducted in June of 2007 (mid-growing season). Shown are mean (± SE) values ($n$ = 4 plants for each treatment combination) and fitted regression models (see Table 1).

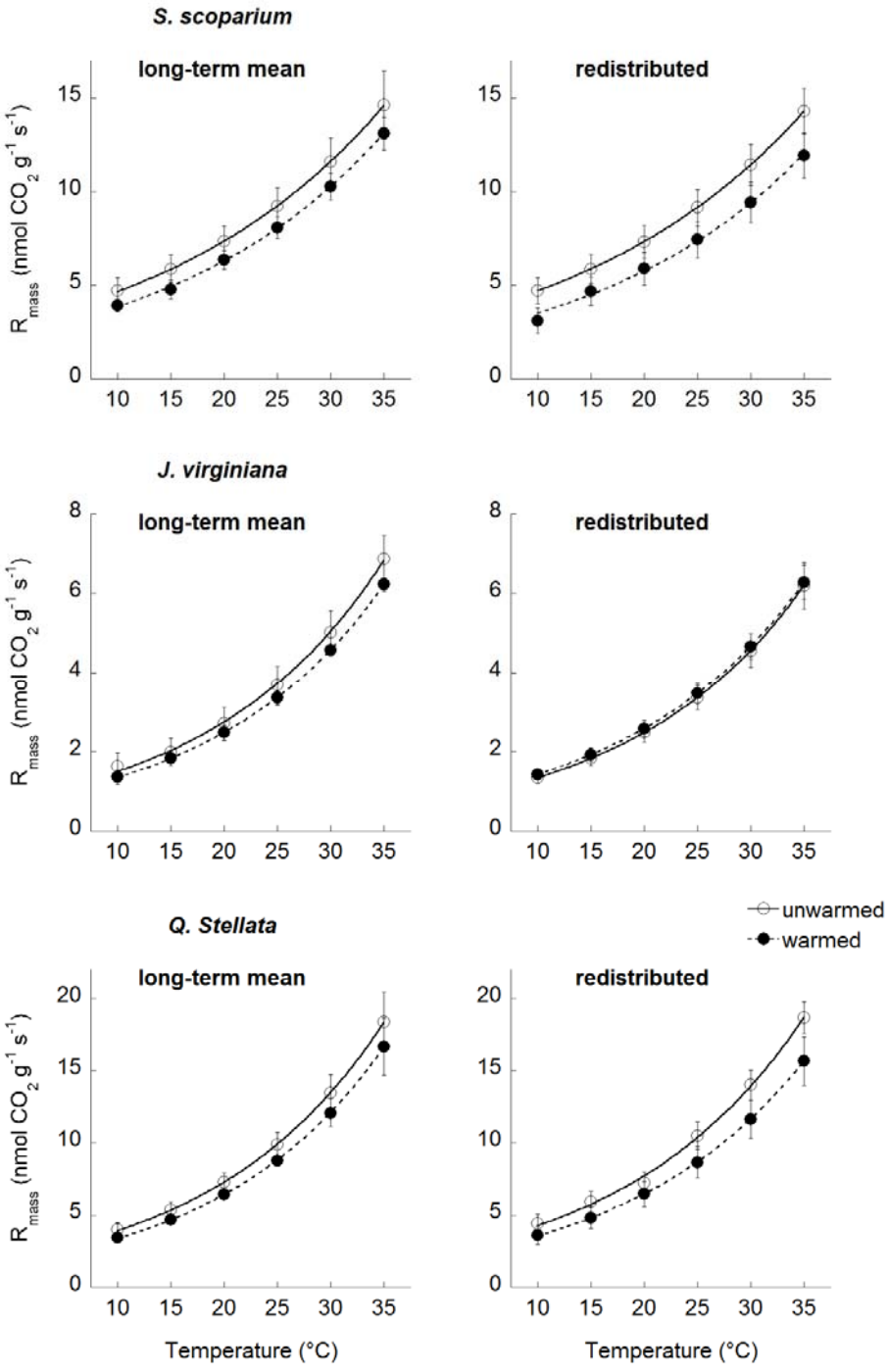


Fig. 2 Temperature-response functions of leaf dark respiration to short-term changes in temperature in *S. scoparium*, *J. virginiana*, and *Q. stellata* grown under different rainfall distribution treatments (long-term mean, redistributed) and temperature treatments (unwarmed (–○–), warmed (--●--)). Measurements were conducted in August of 2007 (late-growing season). Shown are mean (± SE) values ($n$ = 4 plants for each treatment combination, except where noted in methods) and fitted regression models (see Table 1).

were lower than the other two species and ranged from 1.58 to 1.69 across treatment combinations (Table 1). There were no statistically significant effects of temperature or precipitation treatment on $Q_{10}$, $R_{10}$, or $R_{25}$ in the three species in August, with the following exception. $R_{25}$ values in *Q. stellata* were increased by 3% in plants receiving the redistributed compared to the long-term mean rainfall treatment ($P = 0.02$). The values across measurement temperatures ranked lower in warmed compared to unwarmed plants in both rainfall treatments (Fig. 2).

Temperature-response functions of leaf dark respiration to short-term changes in temperature differed between June and August for *S. scoparium* and *J. virginiana*. Overall, values of $R_{mass}$ at higher measurement temperatures were lower in both *S. scoparium* and *J. virginiana* in August compared to June, largely reflecting lower temperature sensitivities ($Q_{10}$) in August. $Q_{10}$ values of *S. scoparium* and *J. virginiana* were reduced in August compared to June by 20% and 7% respectively (Table 1).

In June (mid-growing season), leaf chemistry and structure of *S. scoparium* differed among the rainfall and temperature treatments, while *J. virginiana* did not (Table 2). *S. scoparium* receiving the redistributed amount of rainfall had 24% higher leaf N than plants receiving the long-term mean amount of rainfall ($P = 0.07$). Overall, leaf soluble sugar and starch concentrations were lower in *S. scoparium* receiving the redistributed than the long-term mean rainfall treatment (soluble sugars, $P = 0.01$; starch, $P = 0.07$). Furthermore, starch concentrations were higher in the warmed compared to the unwarmed treatment ($P = 0.06$). However, a statistically significant rainfall x temperature treatment interaction effect on starch was observed ($P = 0.03$). Starch in unwarmed plants did not differ between precipitation treatments, while in warmed plants in the long-term mean treatments starch concentrations were six times that of the redistributed treatment. For *S. scoparium,* SLA exhibited a statistically significant rainfall x temperature treatment interaction effect ($P = 0.03$). In warmed plants, SLA increased by 38% in the redistributed rainfall treatment compared to the long-term mean, but in unwarmed plants, SLA did not differ between precipitation treatments (Table 2).

There were no treatment effects on leaf N, soluble sugars, starch, or SLA in *S.*

Table 1 Temperature-response parameters[i] of leaf dark respiration ($R_{mass}$, nmol $CO_2$ $g^{-1}$ $s^{-1}$) of three species of the post oak savanna grown under different rainfall (long-term mean, redistributed) and temperature treatments (unwarmed, warmed). Samples were collected during mid-growing season (June) and late-growing season (August).

| | | | Long-term mean | | Redistributed | | *P*-value[ii] | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | Species | | Unwarmed | Warmed | Unwarmed | Warmed | Rain | T | Rain x T |
| June (mid-growing season) | *S. scoparium* | $Q_{10}$ | 1.94 ± 0.03 | 1.90 ± 0.05 | 1.99 ± .06 | 1.95 ± 0.05 | 0.33 | 0.45 | 0.95 |
| | | $R_{10}$ | 3.70 ± 0.32 | 3.66 ± 0.35 | 3.88 ± .48 | 3.79 ± 0.48 | 0.64 | 0.85 | 0.94 |
| | | $R_{25}$ | 9.98 ± 0.68 | 9.52 ± 0.66 | 10.76 ± 0.91 | 10.29 ± 1.11 | 0.52 | 0.32 | 0.99 |
| | *J. virginiana* | $Q_{10}$ | 2.00 ± 0.01 | 1.89 ± 0.02 | 1.97 ± 0.04 | 2.01 ± 0.02 | 0.16 | 0.24 | **0.03** |
| | | $R_{10}$ | 1.76 ± 0.20 | 1.78 ± 0.34 | 2.35 ± 0.31 | 2.08 ± 0.17 | **0.07** | 0.58 | 0.52 |
| | | $R_{25}$ | 4.98 ± 0.59 | 4.60 ± 0.84 | 6.59 ± 1.10 | 5.91 ± 0.46 | 0.11 | 0.52 | 0.86 |
| August (late-growing season) | *S. scoparium* | $Q_{10}$ | 1.59 ± 0.09 | 1.62 ± 0.02 | 1.58 ± 0.05 | 1.62 ± 0.08 | 0.92 | 0.54 | 0.91 |
| | | $R_{10}$ | 4.71 ± 0.69 | 3.92 ± 0.34 | 4.71 ± 0.68 | 3.72 ± 0.64 | 0.88 | 0.17 | 0.86 |
| | | $R_{25}$ | 9.21 ± 1.00 | 8.08 ± 0.60 | 9.15 ± 0.97 | 7.44 ± 0.71 | 0.72 | 0.11 | 0.71 |
| | *J. virginiana* | $Q_{10}$ | 1.89 ± 0.08 | 1.87 ± 0.11 | 1.84 ± 0.04 | 1.82 ± 0.05 | 0.40 | 0.78 | 0.98 |
| | | $R_{10}$ | 1.47 ± 0.29 | 1.36 ± 0.18 | 1.35 ± 0.14 | 1.43 ± 0.14 | 0.86 | 0.96 | 0.72 |
| | | $R_{25}$ | 3.69 ± 0.48 | 3.36 ± 0.18 | 3.36 ± 0.32 | 3.47 ± 0.26 | 0.61 | 0.80 | 0.62 |
| | *Q. stellata* | $Q_{10}$ | 1.86 ± 0.11 | 1.89 ± 0.16 | 1.80 ± 0.06 | 1.84 ± 0.12 | 0.64 | 0.70 | 0.99 |
| | | $R_{10}$ | 3.99 ± 0.51 | 3.46 ± 0.34 | 4.46 ± 0.62 | 3.62 ± 0.61 | 0.46 | 0.33 | 0.85 |
| | | $R_{25}$ | 9.89 ± 0.85 | 8.79 ± 0.38 | 10.51 ± 0.96 | 8.67 ± 1.09 | **0.02** | 0.21 | 0.99 |

[i] Parameters (± SE) are derived from the short-term response of leaf dark respiration to measurement temperature (10-35 °C) using non-linear regression ($n$ = 4 plants for each treatment combination, except where noted in Methods).
[ii] *P*-values ≤ 0.10 are bolded.

Table 2 Leaf chemistry and structure of three different species of the post oak savanna grown in different rainfall (long-term mean, redistributed) and temperature (unwarmed, warmed) treatments collected during mid-growing season (June) and late-growing season (August). Means[i] of leaf N (mg $g^{-1}$), soluble sugars (abbreviated as sugars, mg $g^{-1}$), starch (mg $g^{-1}$), and SLA ($cm^2$ $g^{-1}$) are shown.

| | | | Long-term mean | | Redistributed | | *P*-value[ii] | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | Species | | Unwarmed | Warmed | Unwarmed | Warmed | Rain | T | Rain x T |
| June (mid-growing season) | *S. scoparium* | N | 11.2 ± 0.1 | 10.9 ± 1.0 | 13.2 ± 0.7 | 14.3 ± 1.6 | **0.07** | 0.60 | 0.36 |
| | | Sugars | 28 ± 5 | 44 ± 4 | 28 ± 3 | 24 ± 4 | **0.01** | 0.28 | 0.11 |
| | | Starch | 4 ± 2 | 35 ± 11 | 8 ± 4 | 4 ± 2 | **0.07** | **0.06** | **0.03** |
| | | SLA | 144 ± 7 | 130 ± 8 | 151 ± 3 | 179 ± 9 | **0.004** | 0.40 | **0.03** |
| | *J. virginiana* | N | 15.2 ± 1.0 | 14.7 ± 0.8 | 16.2 ± 1.1 | 16.0 ± 0.8 | 0.25 | 0.70 | 0.86 |
| | | Sugars | 34 ± 1 | 33 ± 2 | 32 ± 2 | 31 ± 2 | 0.43 | 0.69 | 0.98 |
| | | Starch | 9 ± 4 | 8 ± 5 | 6 ± 3 | 3 ± 1 | 0.26 | 0.61 | 0.82 |
| | | SLA | 34 ± 1 | 36 ± 1 | 35 ± 1 | 35 ± 1 | 0.77 | 0.55 | 0.47 |
| August (late-growing season) | *S. scoparium* | N | 10.3 ± 1.5 | 8.9 ± 0.5 | 9.2 ± 0.6 | 9.8 ± 0.8 | 0.93 | 0.66 | 0.34 |
| | | Sugars | 40 ± 6 | 54 ± 2 | 51 ± 9 | 49 ± 8 | 0.65 | 0.48 | 0.32 |
| | | Starch | 9 ± 3 | 15 ± 5 | 13 ± 7 | 10 ± 4 | 0.87 | 0.80 | 0.49 |
| | | SLA | 131 ± 14 | 115 ± 7 | 113 ± 6 | 114 ± 4 | 0.25 | 0.47 | 0.40 |
| | *J. virginiana* | N | 16.1 ± 1.8 | 14.2 ± 0.3 | 16.9 ± 1.4 | 16.3 ± 0.9 | 0.22 | 0.42 | 0.66 |
| | | Sugars | 38 ± 3 | 38 ± 5 | 43 ± 4 | 44 ± 3 | 0.25 | 0.78 | 0.87 |
| | | Starch | 6 ± 2 | 3 ± 1 | 3 ± 2 | 8 ± 2 | 0.56 | 0.40 | **0.009** |
| | | SLA | 31 ± 0.3 | 33 ± 1 | 29 ± 2 | 33 ± 2 | 0.52 | 0.19 | 0.59 |
| | *Q. stellata* | N | 19.9 ± 0.59 | 18.7 ± 1.12 | 21.5 ± 1.28 | 17.9 ± 1.16 | 0.69 | **0.08** | 0.36 |
| | | Sugars | 37 ± 4 | 33 ± 1 | 30 ± 2 | 45 ± 4 | 0.52 | 0.14 | **0.03** |
| | | Starch | 4 ± 1 | 4 ± 2 | 5 ± 1 | 4 ± 1 | 0.88 | 0.36 | 0.49 |
| | | SLA | 79 ± 3 | 75 ± 2 | 85 ± 8 | 79 ± 3 | 0.42 | 0.32 | 0.77 |

[i] ± SE of plants sampled within treatments; $n = 4$ plants for each treatment combination, except where noted in Methods.
[ii] *P*-values ≤ 0.10 are bolded.

*scoparium* or *J. virginiana* in August (late-growing season). However, treatment effects on leaf N and soluble sugars were evident in *Q. stellata* in August. Warmed trees had 12% lower leaf N than unwarmed trees ($P = 0.08$, Table 2). There was also a statistically significant rainfall x temperature treatment interaction effect on soluble sugar concentrations ($P = 0.03$). Warmed trees receiving the redistributed amount of rainfall had 51% greater soluble sugars than unwarmed trees, while soluble sugars did not differ between warming treatments in the long-term mean precipitation regime.

**Rainfall pulses during summer drought**

In order to examine the response of leaf respiration and leaf chemistry and structure to intermittent rainfall events during summer drought, we examined temperature-response functions of respiration and determined concentrations of leaf N, soluble sugars, starch, and SLA prior to and following two separate rainfall events in August. Leaves of each species were sampled in both warmed and unwarmed treatments in the redistributed rainfall treatment in which summer drought was accentuated. For the first precipitation event, volumetric soil water content (0 – 20 cm depth) across species and temperature treatments ranged from 5.0 to 9.3% pre-rainfall and from 5.2 to 9.1% post-rainfall (Table 3). For the second precipitation event, soil water content across species and temperature treatments ranged from 5.1 to 7.7% pre-rainfall and from 7.9 to 12.4% post-rainfall (Table 3).

In *S. scoparium,* comparisons of temperature-response functions before and after the first precipitation event (the smaller of the two events) revealed no statistically significant differences in the intercept, $R_{10}$, $Q_{10}$, or $R_{25}$ (Table 4, Appendix D). However, following the second, larger rainfall event, $R_{10}$ and $R_{25}$ values increased by 51% ($P = 0.04$) and 24% ($P = 0.05$), respectively (Table 4, Appendix D). In addition, $Q_{10}$ values were reduced by 11% post-rainfall compared to pre-rainfall values ($P = 0.09$). Temperature treatment effects were observed on $R_{10}$ in the first precipitation event, where values were 12% lower in warmed compared to unwarmed *S. scoparium* ($P = 0.05$). A comparable trend was observed one week later in the second precipitation event. In the second rainfall event, $Q_{10}$ values were 9% higher in warmed compared to

Table 3 Volumetric soil water content (%, 0-20 cm depth) of species monoculture plots measured before and after two summer precipitation events ($n$ = 4 plots). Data were obtained from warmed and unwarmed plots in the redistributed rainfall (summer drought) treatment.

| Date | Species | Temp | Pre-rainfall | Post-rainfall |
|---|---|---|---|---|
| Aug 9 & 14 | *S. scoparium* | unwarmed | 6.4 ± 1.0 | 6.7 ± 1.1 |
| | | warmed | 6.3 ± 0.8 | 6.6 ± 0.7 |
| | *J. virginiana* | unwarmed | 5.0 ± 0.7 | 5.2 ± 0.7 |
| | | warmed | 6.5 ± 0.4 | 6.7 ± 0.6 |
| | *Q. stellata* | unwarmed | 9.3 ± 1.6 | 9.1 ± 1.3 |
| | | warmed | 8.4 ± 0.6 | 8.2 ± 0.6 |
| Aug 21 & 24 | *S. scoparium* | unwarmed | 6.1 ± 0.9 | 9.0 ± 1.6 |
| | | warmed | 6.1 ± 0.8 | 8.2 ± 0.6 |
| | *J. virginiana* | unwarmed | 5.1 ± 0.5 | 7.9 ± 0.3 |
| | | warmed | 6.6 ± 0.6 | 8.4 ± 0.4 |
| | *Q. stellata* | unwarmed | 7.7 ± 1.2 | 12.4 ± 1.5 |
| | | warmed | 6.9 ± 1.0 | 11.3 ± 1.3 |

Table 4 Temperature-response parameters[i] of leaf dark respiration ($R_{mass}$, nmol $CO_2$ $g^{-1}$ $s^{-1}$) of three species of the post oak savanna grown under different temperature treatments (unwarmed, warmed) and measured before and after precipitation events in August 2007.

| | | | Pre-rainfall | | Post-rainfall | | *P*-value[ii] | | |
|---|---|---|---|---|---|---|---|---|---|
| Date | Species | | Unwarmed | Warmed | Unwarmed | Warmed | Pre/Post | T | Pre/Post x T |
| Aug 9 & 14 | *S. scoparium* | $Q_{10}$ | 1.74 ± 0.02 | 1.74 ± 0.03 | 1.73 ± 0.03 | 1.83 ± 0.03 | 0.31 | 0.16 | 0.20 |
| | | $R_{10}$ | 3.54 ± 0.34 | 3.03 ± 0.17 | 3.19 ± 0.12 | 2.91 ± 0.25 | 0.21 | **0.05** | 0.49 |
| | | $R_{25}$ | 8.08 ± 0.64 | 6.93 ± 0.33 | 7.26 ± 0.43 | 7.21 ± 0.72 | 0.40 | 0.27 | 0.14 |
| | *J. virginiana* | $Q_{10}$ | 1.75 ± 0.06 | 1.74 ± 0.07 | 1.88 ± 0.07 | 1.67 ± 0.07 | 0.57 | 0.34 | 0.16 |
| | | $R_{10}$ | 1.60 ± 0.06 | 1.47 ± 0.12 | 1.20 ± 0.21 | 1.44 ± 0.13 | 0.14 | 0.39 | 0.19 |
| | | $R_{25}$ | 3.71 ± 0.29 | 3.38 ± 0.35 | 3.04 ± 0.45 | 3.15 ± 0.44 | 0.15 | 0.73 | 0.41 |
| | *Q. stellata* | $Q_{10}$ | 2.04 ± 0.08 | 1.97 ± 0.06 | 1.76 ± 0.08 | 1.94 ± 0.05 | **0.03** | 0.67 | **0.05** |
| | | $R_{10}$ | 3.08 ± 0.49 | 3.02 ± 0.54 | 4.04 ± 0.71 | 2.55 ± 0.27 | 0.40 | 0.18 | 0.33 |
| | | $R_{25}$ | 8.74 ± 0.89 | 8.16 ± 1.19 | 9.15 ± 0.96 | 6.82 ± 0.49 | 0.84 | 0.52 | 0.27 |
| Aug 21 & 24 | *S. scoparium* | $Q_{10}$ | 1.68 ± 0.02 | 1.92 ± 0.09 | 1.58 ± 0.05 | 1.62 ± 0.08 | **0.09** | **0.02** | 0.34 |
| | | $R_{10}$ | 3.10 ± 0.16 | 2.50 ± 0.20 | 4.71 ± 0.68 | 3.72 ± 0.64 | **0.04** | 0.13 | 0.66 |
| | | $R_{25}$ | 6.79 ± 0.39 | 6.60 ± 0.29 | 9.15 ± 0.97 | 7.44 ± 0.71 | **0.05** | 0.29 | 0.19 |
| | *J. virginiana* | $Q_{10}$ | 1.96 ± 0.05 | 1.95 ± 0.12 | 1.84 ± 0.04 | 1.82 ± 0.05 | 0.17 | 0.85 | 0.89 |
| | | $R_{10}$ | 1.29 ± 0.21 | 1.32 ± 0.25 | 1.35 ± 0.14 | 1.43 ± 0.14 | 0.65 | 0.86 | 0.88 |
| | | $R_{25}$ | 3.49 ± 0.42 | 3.44 ± 0.30 | 3.36 ± 0.32 | 3.47 ± 0.26 | 0.88 | 0.96 | 0.80 |
| | *Q. stellata* | $Q_{10}$ | 1.84 ± 0.08 | 1.83 ± 0.11 | 1.80 ± 0.06 | 1.84 ± 0.12 | 0.89 | 0.84 | 0.84 |
| | | $R_{10}$ | 3.87 ± 0.18 | 3.56 ± 0.20 | 4.46 ± 0.62 | 3.62 ± 0.61 | 0.69 | 0.34 | 0.74 |
| | | $R_{25}$ | 9.71 ± 0.57 | 8.78 ± 0.69 | 10.51 ± 0.96 | 8.67 ± 1.09 | 0.70 | 0.34 | 0.62 |

[i] Parameters (± SE) are derived from the short-term response of leaf dark respiration to measurement temperature (10-35 °C) using non-linear regression ($n$ = 4 plants for each treatment combination).
[ii] *P*-values ≤ 0.10 are bolded.

unwarmed plants ($P = 0.02$), particularly in the pre-rainfall measurement.

Examination of temperature-response functions of *Q. stellata* and *J. virginiana* before and after the two precipitation events revealed no statistically significant effects of short-term rainfall pulses on the intercept, $R_{10}$, or on $R_{25}$. While there was no warming treatment effect on $Q_{10}$ in *Q. stellata* for either event, $Q_{10}$ values following the first precipitation event were reduced by 8% compared to the pre-event values ($P = 0.03$, Table 4). Furthermore, the $Q_{10}$ values of warmed and unwarmed plants appeared to respond differently to the rainfall event, declining 14% in the unwarmed plants while remaining unchanged in the warmed plants ($P = 0.05$, Table 4). By comparison, $Q_{10}$ in *J. virginiana* did not differ in response to the two rainfall events. Inspection of the fitted temperature-response functions for *S. scoparium* and *Q. stellata* revealed that $R_{mass}$ values in warmed plants were lower than unwarmed plants both before and after each of the two rainfall events (Appendix D).

The short-term effects of rainfall during summer drought and warming treatment effects on leaf N and SLA in the three species following both precipitation event were minimal (Appendix D). In contrast, TNC increased following precipitation events in all three species, particularly following the second rainfall event (Appendix D).

**Soil drying during summer drought**

We investigated the effects of progressive summer drought on temperature-response parameters ($Q_{10}$, $R_{10}$, $R_{25}$), and other leaf traits including leaf N, soluble sugars, starch, and SLA in both warmed and unwarmed plants grown in the redistributed rainfall treatment. A time course was determined through measurements conducted on five dates throughout a 24-day period in which soils dried following a summer rainfall event (Fig. 3). We determined how warming treatment and soil drying affected the relationships between $R_{25}$ and the other associated leaf traits.

*Progressive drought effects on temperature-response parameters of dark respiration*

For *Q. stellata*, both the intercepts, $R_{10}$, and the slopes, $Q_{10}$, of the temperature-

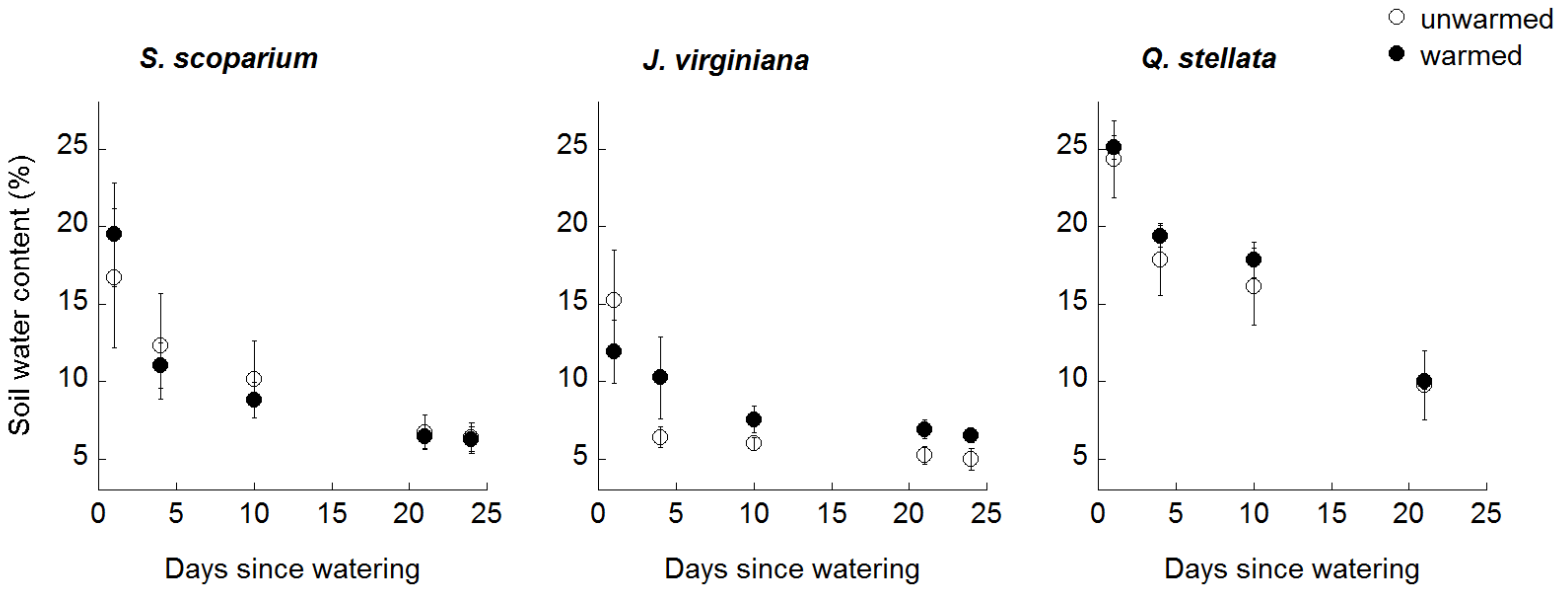


Fig. 3 Volumetric soil water content (%, 0-20 cm depth) of species monoculture plots measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007 ($n$ = 4 plots). Data were obtained from warmed (●) and unwarmed (○) plots in the redistributed (summer drought) rainfall treatment.

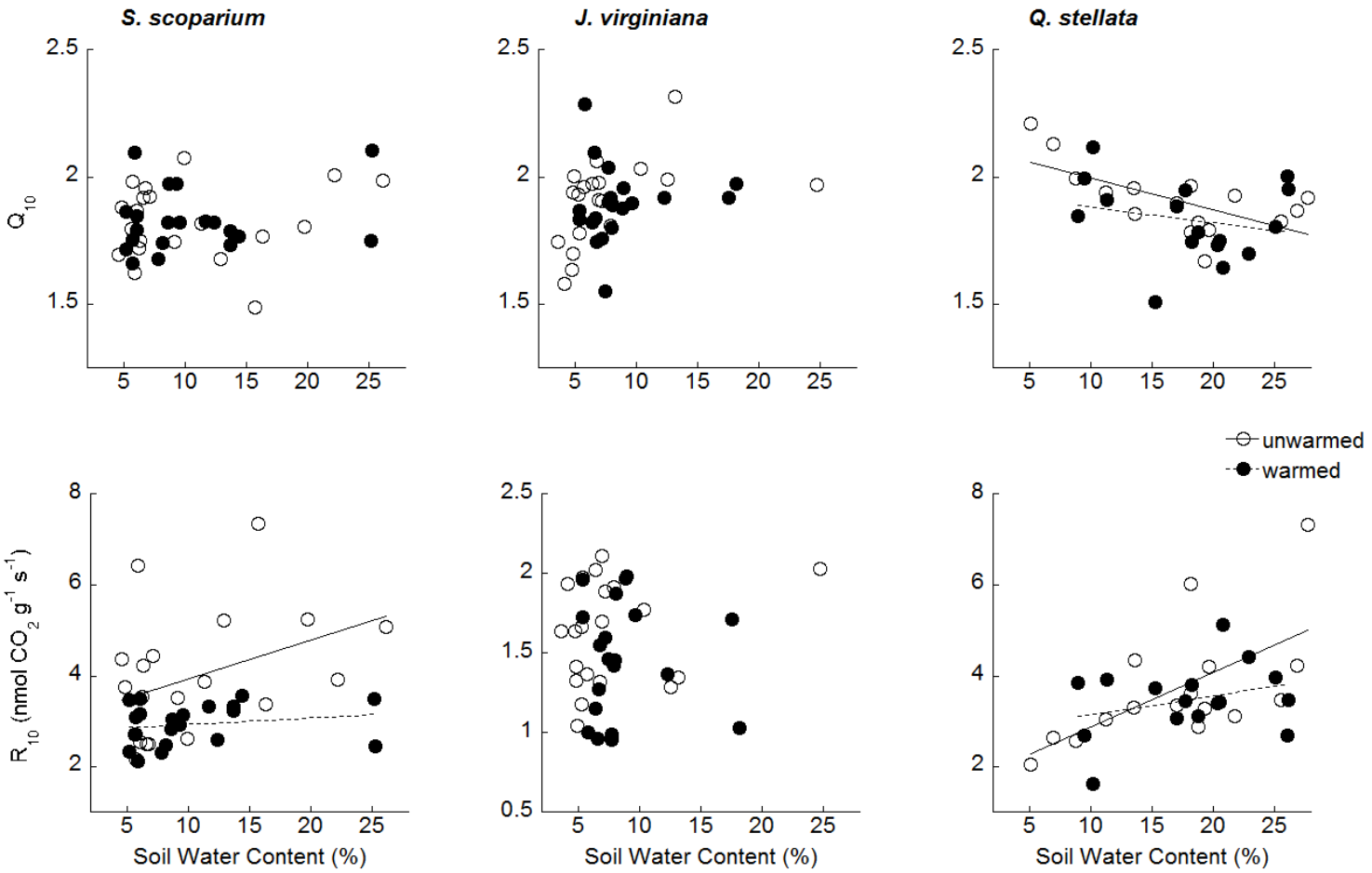


Fig. 4 Temperature-response parameters ($Q_{10}$, $R_{10}$) of leaf dark respiration in relationship to volumetric soil water content of three species of the post oak savanna grown under different temperature treatments (unwarmed (–○–), warmed (--●--) and measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007.

response functions were related to declining soil water content (Fig. 4, Appendix E). In comparison, neither $Q_{10}$ nor $R_{10}$ of leaf dark respiration in *S. scoparium* and *J. virginiana* showed a relationship with soil water content (Fig. 4). In *Q. stellata*, $Q_{10}$ exhibited a linear relationship with soil water content across warming treatments, increasing as soils dried ($r^2 = 0.19$, $n = 32$, $P = 0.01$). Conversely, $R_{10}$ values declined across warmed and unwarmed plants as soils dried ($r^2 = 0.24$, $n = 32$, $P = 0.004$). Warmed and unwarmed *Q. stellata* did not differ in either the slope or intercept of the relationships between either $Q_{10}$ (slopes, $P = 0.41$; intercept, $P = 0.17$) or $R_{10}$ (slopes, $P = 0.17$; intercept, $P = 0.35$) and soil water content. Warming treatment effects were evident in *S. scoparium*, where warmed plants exhibited reduced $R_{10}$ values compared to unwarmed plants ($P = 0.002$).

*Progressive drought effects on respiration rates measured at 25 °C*

In each species, $R_{25}$ tended to decrease with declining soil water content, but differed between temperature treatments (Fig. 5). In unwarmed *S. scoparium*, $R_{25}$ declined with decreasing soil water content (Fig. 5; $r^2 = 0.33$, $n = 20$, $P = 0.008$). Conversely, $R_{25}$ was unrelated to soil water content in warmed *S. scoparium* ($r^2 = 0.03$, $n = 20$, $P = 0.43$). $R_{25}$ in warmed and unwarmed treatments converged to comparable values through time with soil drying. The slope of the relationship between $R_{25}$ and soil water content differed between temperature treatments ($P = 0.02$), indicating the response of $R_{25}$ to soil drying differed between warmed and unwarmed plants.

In *J. virginiana*, $R_{25}$ also tended to decrease through time as the soils dried. In the unwarmed plants, $R_{25}$ declined with decreasing soil water content ($r^2 = 0.28$, $n = 20$, $P = 0.02$). However, in warmed plants no relationship was observed between $R_{25}$ and volumetric soil moisture content ($r^2 = 0.02$, $n = 20$, $P = 0.56$). Slopes of the relationship between $R_{25}$ and soil water content did not differ between temperature treatments ($P = 0.30$); however, the intercepts of the regression relationships did differ ($P = 0.02$), reflecting the fact that $R_{25}$ was lower in leaves of warmed compared to unwarmed plants.

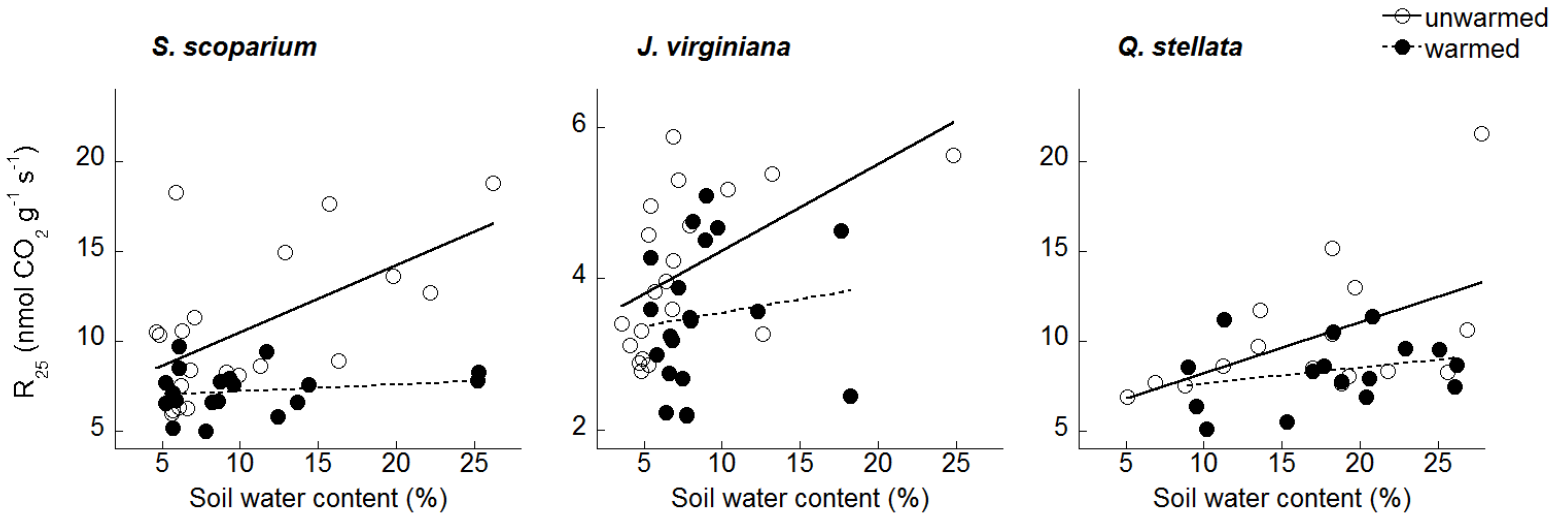


Fig. 5 Values of leaf dark respiration measured at 25°C, expressed on the basis of dry mass ($R_{25}$, nmol $CO_2$ $g^{-1}$ $s^{-1}$) in relationship to volumetric soil water content of three species of the post oak savanna grown under different temperature treatments (unwarmed (–○–), warmed (--●--)) and measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007.

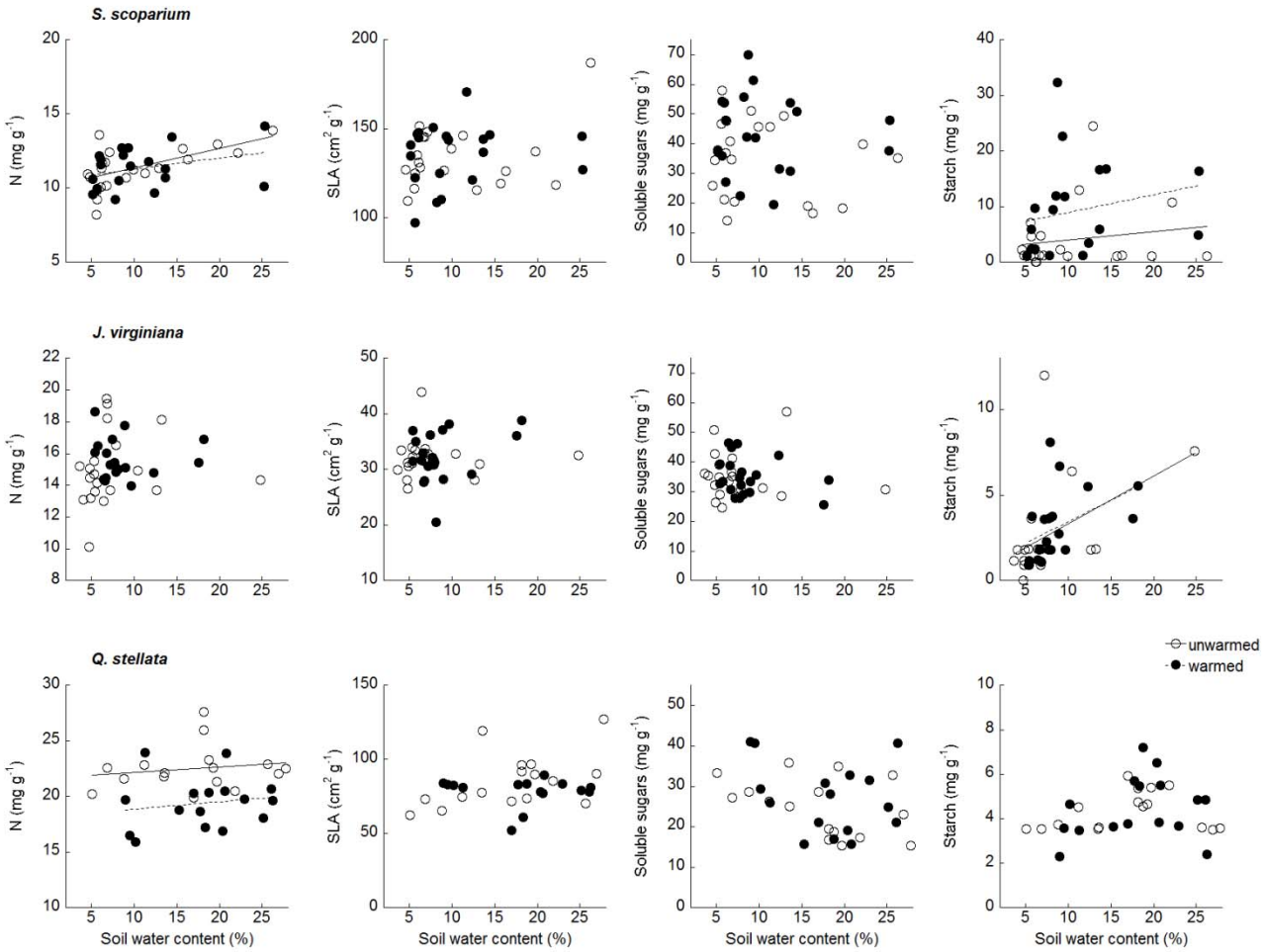


Fig. 6 Values of leaf N (mg $g^{-1}$), SLA ($cm^2$ $g^{-1}$), soluble sugars (mg $g^{-1}$), and starch content (mg $g^{-1}$) in relationship to volumetric soil water content of three species of the post oak savanna grown under different temperature treatments (unwarmed (–○–), warmed (--●--)) and measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007.

Similar to the other two species, $R_{25}$ in unwarmed *Q. stellata* declined with decreasing soil water content during summer drought ($r^2 = 0.26$, $n = 16$, $P = 0.04$), and there was no relationship between $R_{25}$ and soil water content in warmed plants. Slopes of the relationship between $R_{25}$ and soil water content did not differ between temperature treatments ($P = 0.23$), but the intercepts did differ ($P = 0.04$), indicating that $R_{25}$ was lower in warmed than unwarmed plants across the range of soil water contents.

*Progressive drought effects on leaf N, SLA, soluble sugars, and starch*

Effects of declining soil water content on leaf N, SLA, soluble sugars, and starch content varied among species and between temperature treatments (Fig. 6). Leaf N content decreased with declining soil water content in *S. scoparium* (Fig. 6, $r^2 = 0.21$, $n = 40$, $P = 0.002$). The slopes and intercepts of the relationship did not differ between temperature treatments ($P = 0.39$ and $P = 0.78$, respectively), indicating that warmed and unwarmed *S. scoparium* exhibited a comparable linear relationship of declining soil water content with leaf N. There was no relationship between SLA, concentrations of soluble sugars or starch and soil water content in *S. scoparium* in either warming treatment (Fig. 6). Overall, starch concentrations were higher in warmed compared to unwarmed *S. scoparium* (intercepts test, $P = 0.04$).

In *J. virginiana*, starch content in both warmed and unwarmed plants declined with decreasing soil water content during summer drought (Fig. 6). Similar slopes ($P = 0.87$) and intercepts ($P = 0.79$) indicated that warming treatments exhibited a comparable linear relationship. By comparison, there was no relationship between leaf N, SLA, or soluble sugars and soil water content for *J. virginiana* and temperature treatments did not differ in these traits (Fig. 6).

Leaf N was unrelated to soil water content in both temperature treatments of *Q. stellata* (Fig. 6). However, warmed plants had lower concentrations of leaf N than unwarmed plants, as reflected in a lower intercept ($P = 0.0003$). Similarly, SLA, soluble sugars, and starch were unrelated to soil water content in *Q. stellata* and temperature treatments did not differ in either slope or intercept of the relationships (Fig. 6).

*Relationships between respiration and other leaf traits*

In both temperature treatments, $R_{25}$ of *S. scoparium* declined with decreasing leaf N in drying soils (Fig. 7). However, the relationship was steeper and stronger in unwarmed ($r^2 = 0.64$, $n = 20$, $P < 0.0001$) compared to warmed plants ($r^2 = 0.26$, $n = 20$, $P = 0.02$). Furthermore, the slopes differed ($P < 0.0001$) between temperature treatments and values of $R_{25}$ converged to similar values at lower concentrations of leaf N. In comparison, there was no relationship between $R_{25}$ and SLA in *S. scoparium*. Contrary to expectations, $R_{25}$ declined with increasing soluble sugars in unwarmed *S. scoparium* ($r^2 = 0.22$, $n = 20$, $P = 0.04$), but was unrelated to soluble sugars in warmed *S. scoparium* ($r^2 = < 0.001$, $n = 20$, $P = 0.99$). The slopes of the relationships differed between temperature treatments ($P = 0.04$). Furthermore, there was no relationship between N-based respiration ($R_N$, μmol $CO_2$ mol $N^{-1}$ $s^{-1}$) at 25 °C and soluble sugars in 0.001), but was unrelated to SLA in warmed *Q. stellata*, as slopes differed between S. *scoparium* as soils dried (Fig. 8). However, for a given concentration of soluble sugars, warmed plants had lower $R_N$ than unwarmed plants ($P = 0.001$). On the other hand, as soils became drier, soluble sugar-based respiration ($R_{soluble\ sugar}$, $CO_2$ g soluble $sugars^{-1}$ $s^{-1}$) leaf N in unwarmed *S. scoparium* ($r^2 = 0.53$, $n = 20$, $P < 0.001$) but was unrelated to leaf N in warmed *S. scoparium* ($r^2 = <0.001$, $n = 20$, $P = 0.91$). Separate slopes indicated that temperature treatments of *S. scoparium* differed in the nature of the relationship between $R_{soluble\ sugar}$ and N ($P < 0.001$).

We found no relationship between $R_{25}$ and leaf N in *J. virginiana* (Fig. 7). However, warmed plants had lower rates of $R_{25}$ for the same N content than unwarmed plants ($P = 0.03$). Furthermore, $R_{25}$ was not related to either SLA or soluble sugars. While $R_N$ was unrelated to soluble sugars in *J. virginiana* warmed plants had lower values of $R_N$ for a given soluble sugar content than unwarmed controls, as intercepts differed between temperature treatments (Fig. 8, $P = 0.01$). Likewise, there was no relationship between soluble $R_{soluble\ sugar}$ and N in either temperature treatment of *J. virginiana*.

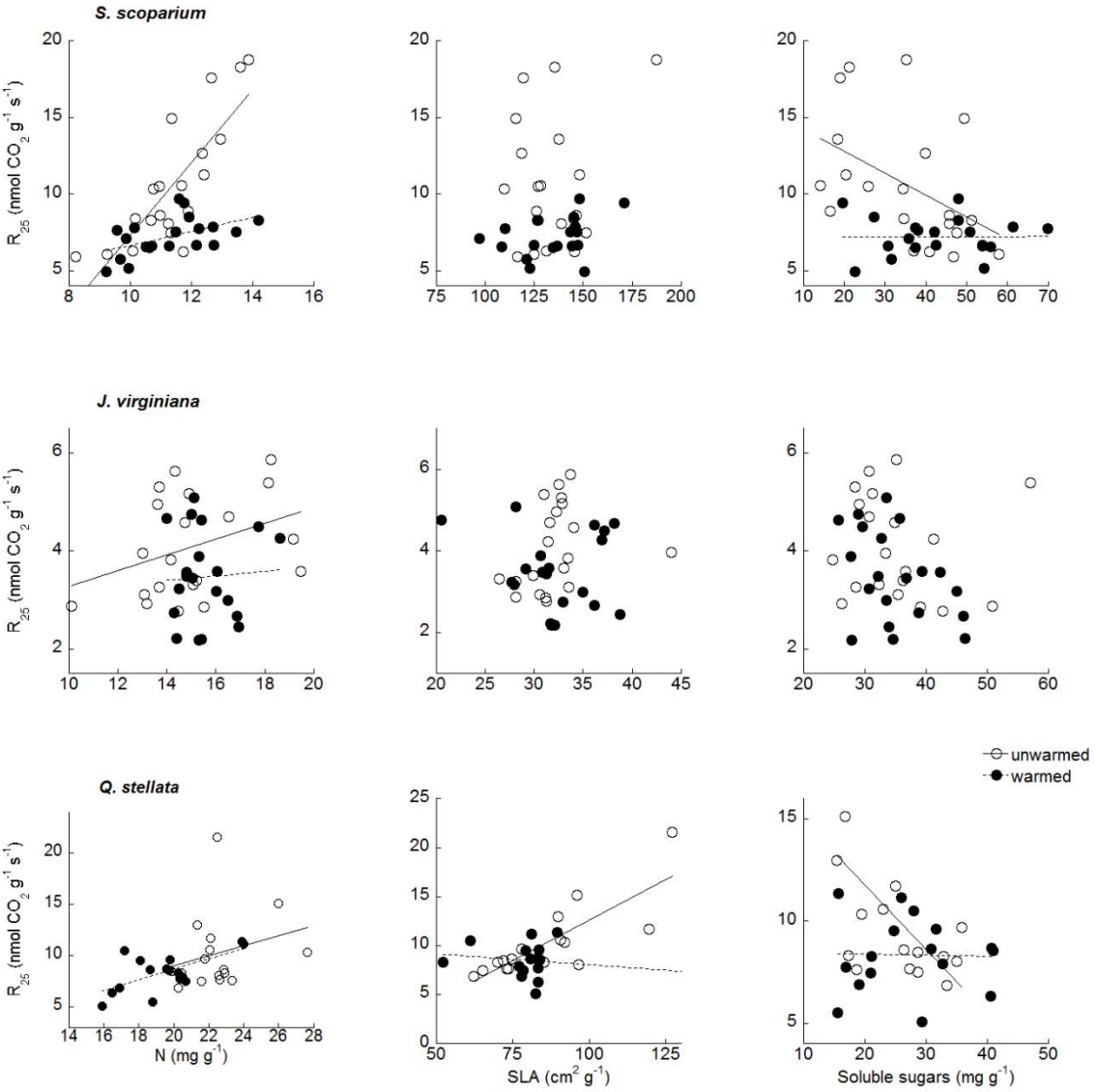


Fig. 7 Values of leaf dark respiration measured at 25°C in relationship to leaf N (mg $g^{-1}$), SLA ($cm^2$ $g^{-1}$), and soluble sugars (mg $g^{-1}$) of three species of the post oak savanna grown under different temperature treatments (unwarmed (–○–), warmed (--●--)) and measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007.

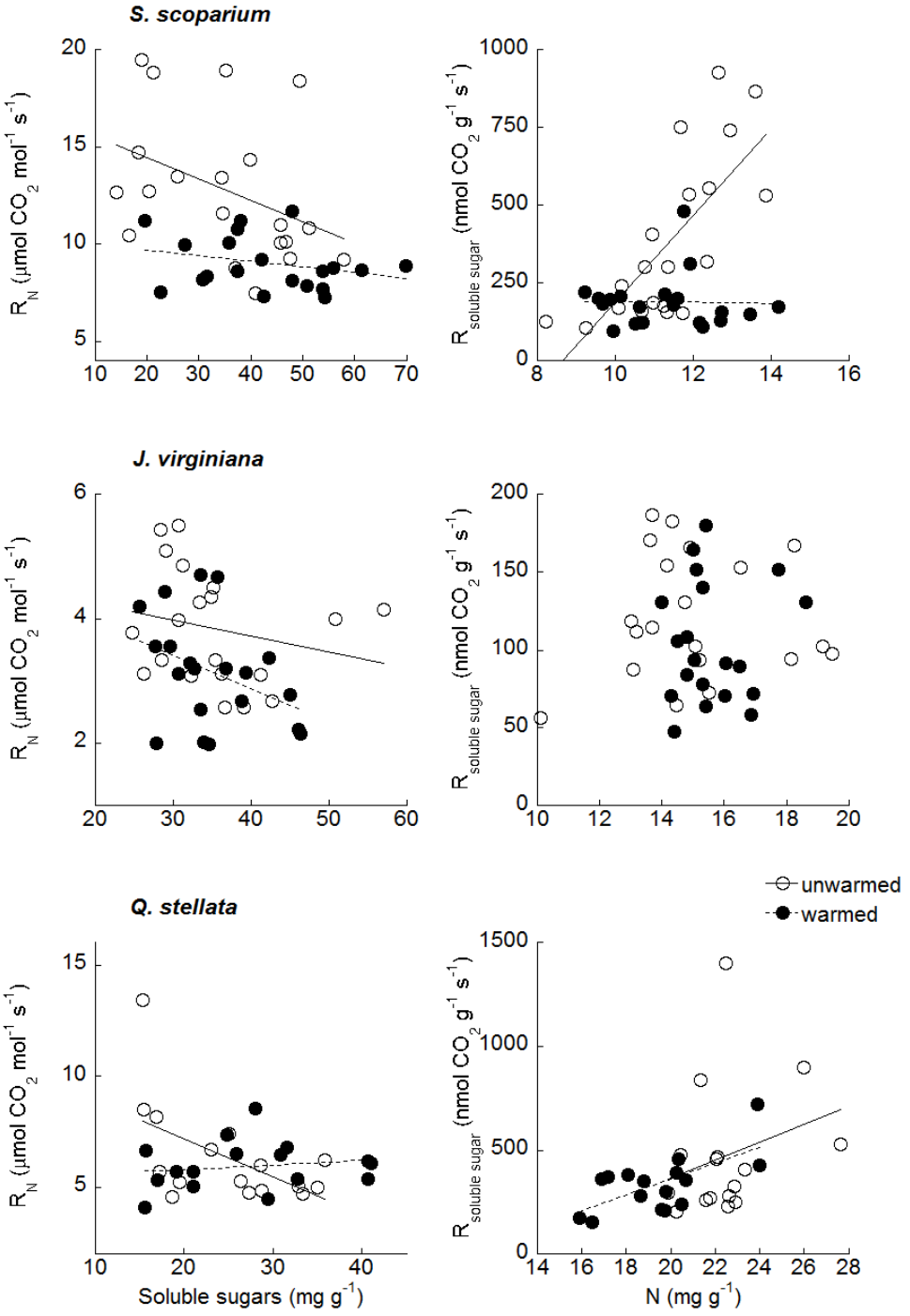


Fig. 8 Leaf dark respiration measured at 25°C, expressed on a nitrogen-basis ($R_N$, μmol $CO_2$ mol $N^{-1}$ $s^{-1}$) and a soluble carbohydrate-basis ($R_{soluble\ sugar}$, nmol $CO_2$ $g^{-1}$ $s^{-1}$) in relationship to leaf N (mg $g^{-1}$) and soluble sugars (mg $g^{-1}$) for three species of the post oak savanna grown under different temperature treatments (unwarmed (–○–), warmed (--●--)) and measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007.

Across temperature treatments, $R_{25}$ of *Q. stellata* declined with decreasing leaf N as soils dried during summer drought (Fig. 7, $r^2 = 0.21$, $n = 32$, $P = 0.008$). Likewise, $R_{25}$ declined with decreasing SLA in unwarmed *Q. stellata* ($r^2 = 0.65$, $n = 16$, $P < 0.001$), but was unrelated to SLA in warmed *Q. stellata*, as slopes differed between temperature treatments ($P = 0.01$). $R_{25}$ declined with increasing concentrations of soluble sugars in unwarmed *Q stellata* ($r^2 = 0.36$, $n = 16$, $P = 0.01$). However, no relationship was observed in warmed *Q. stellata,* and slopes differed between the two temperature treatments ($P = 0.02$). $R_N$ declined with increasing concentrations of soluble sugars in unwarmed *Q. stellata* (Fig. 8, $r^2 = 0.30$, $P = 0.03$) but was unrelated to soluble sugars in warmed *Q. stellata*. Temperature treatments differed in the slopes of the relationship between $R_N$ and soluble sugars ($P = 0.02$). In comparison, $R_{\text{soluble sugar}}$ across temperature treatments declined with decreasing concentrations of leaf N as soil water content declined ($r^2 = 0.19$, $n = 32$, $P = 0.01$). Warmed and unwarmed *Q. stellata* did not differ in the slope of the relationship ($P = 0.92$).

# V. DISCUSSION

**How do species differ in their temperature-response functions of leaf dark respiration in response to long-term warming and rainfall redistribution?**

The three species measured exhibited unique temperature-responses of leaf dark respiration to temperature, and we observed shifts in both the $Q_{10}$ and $R_{10}$ in response to warming and rainfall redistribution. Not surprisingly, *S. scoparium* and *Q. stellata* had higher rates of leaf dark respiration than *J. virginiana* across the range of measurement temperatures on all dates measured. Because *Q. stellata* and *S. scoparium* have relatively short-lived leaves in comparison to *J. virginiana*, it is perhaps expected that their rates of leaf dark respiration would be higher as well. In concert with the shorter life-span of the leaves in *S. scoparium* and *Q. stellata*, increased metabolic rates correspond with a suite of traits that maximize carbon gain and return on construction costs over shorter time periods (Mooney *et al.* 1997; Wright *et al.* 2004). In general, it is more difficult to predict how $Q_{10}$ will vary among species. We observed $Q_{10}$ values ranging from 1.58 to 2.08 across species and measurement dates, which are within the range of values observed for leaves in other studies (1.36-4.2; (Atkin *et al.* 2005b)). Although our three study species frequently overlapped in their $Q_{10}$, values within a species were dynamic through time, varying in response to rainfall pulses and declining soil water content during summer drought.

While the degree of acclimation to temperature varied across measurement dates, it appeared to be more pronounced in *S. scoparium* and *Q. stellata* compared to *J. virginiana*, and it generally manifested itself as a downshift in the intercept of the temperature-response function, $R_{10}$, resulting in lower values of leaf dark respiration in warmed compared to unwarmed plants across the range of measurement temperatures. In general, $Q_{10}$ across species and dates was insensitive to warming treatment. While several studies have reported effects of temperature on $Q_{10}$ values, these were usually seen in previously existing leaves that had been shifted to a new growth temperature (Atkin *et al.* 2005b). In contrast, no effects were observed in several studies when plants

were grown at a different temperature (rather than being shifted)(Atkin *et al.* 2005b), such as the plants in our current study.

The effects of rainfall redistribution on temperature-response functions also differed among species and measurement dates. Rainfall redistribution in June in *J. virginiana* increased base respiration rates compared to the long-term mean, resulting in increased rates of respiration across the range of measurement temperatures. Furthermore, the rainfall x temperature interaction effect on $Q_{10}$ translated into overall higher rates of respiration across the measurement temperature range in plants receiving the redistributed, or "intensified summer drought", rainfall treatment. These results indicate that future changes in rainfall patterns could potentially alter leaf acclimation responses to warming in *J. virginiana*. Intensified summer drought along with warming could increase the respiratory release of $CO_2$ from leaves of *J. virginiana*, possibly affecting not only the carbon balance of this particular tree species, but also the carbon balance of the entire ecosystem. Because *J. virginiana* is a dominant and often invasive evergreen tree, changes in its carbon balance in addition to potential increases in its abundance (due to overgrazing and fire suppression) could have a large influence on carbon fluxes from the southern oak savanna, especially if increases in canopy $CO_2$ release are not offset by increases in photosynthesis or decreases in root and/or soil respiration.

In comparison, we generally did not observe any effects of rainfall redistribution on temperature-response parameters in *S. scoparium* or *Q. stellata*; however, other data concerning rainfall pulses and declining soil water content during summer drought suggests that soil water availability does in fact affect leaf dark respiration rates in these two species on comparatively short time scales (days to weeks).

**How does leaf dark respiration respond to rainfall pulses as well as declining soil water content during summer drought?**

Rainfall pulses along with declining soil water content during summer drought affected the temperature-responses of leaf dark respiration in *S. scoparium* and *Q.*

*stellata* but less so in *J. virginiana*. While leaf dark respiration in *S. scoparium* was unresponsive to the first rainfall event, we found evidence of reduced temperature sensitivity but increased rates of respiration across the range of measurement temperatures following the second, larger rainfall event. Likewise, we found that, over a period of 24 days of soil drying, $R_{25}$ in *S. scoparium* was higher when soils were wetter. However, $Q_{10}$ was unresponsive to soil drying. These results are unlike those found when examining the effects of long-term rainfall distribution in *S. scoparium*, where no differences were observed in either $Q_{10}$ or $R_{10}$ in response to the rainfall redistribution treatment compared to the long-term mean rainfall treatment. In *Q. stellata*, on the other hand, we found a decrease in $Q_{10}$ after the first rainfall event, but only in unwarmed plants. $Q_{10}$ values in warmed *Q. stellata* remained the same, indicating that warmed plants had a greater temperature sensitivity of leaf respiration than unwarmed *Q. stellata* when soils were wetter. We found similar results in response to declining soil water content in *Q. stellata*, where $Q_{10}$ values were lower in wetter soils; however, temperature treatments did not differ, as they did in response to the rainfall pulse. In addition, $R_{10}$ values declined as soils became drier, possibly offsetting the increased sensitivity to temperature that was observed in drier soils. In general, *J. virginiana* was unresponsive to rainfall pulses and declining soil water content during summer drought. This evergreen's potentially deep rooting, overall low rates of respiration and photosynthesis, and ability to photosynthesize under low xylem pressure potentials might explain why its rates dark respiration appear to be unaffected by rainfall pulses during summer drought conditions (Lassoie *et al.* 1983).

Even though we found some evidence that leaf dark respiration in *S. scoparium* and less so *Q. stellata* were affected by rainfall pulses, the lack of consistency between rainfall events may be related to the degree to which the different events ameliorated plant water stress. This complicates drawing broader conclusions from these data, but suggests that responses may be coupled to short-term changes in plant water status. While we observed some effects of rainfall pulses and more so effects of declining soil water content during summer drought on leaf dark respiration in our study species, these

generally did not follow the long-term rainfall distribution patterns in early or late summer, where rainfall treatment effects were minimal. Long-term rainfall redistribution perhaps also affects leaf development independent of shorter-term drought effects, as evident in compensatory changes in leaf N and SLA *between rainfall treatments* in *S scoparium* in June.

In general, $R_{25}$ in all three species, but especially *S. scoparium*, declined with decreasing soil water content in unwarmed plants but was unrelated to soil water content in warmed plants. These data suggest that the magnitude of temperature acclimation of leaf respiration declines with progressive drought. In addition these findings suggest that climate warming reduces the sensitivity of respiration to declining soil water content. These findings are potentially important in that they suggest that drought and temperature effects interact in an important way in shaping leaf respiratory carbon fluxes. Plants exposed to summer drought in a warmer world might be less affected by declining soil water availability and less likely to acclimate to increases in temperature, resulting in higher rates of respiratory $CO_2$ release by leaves.

Turnbull *et al*. (2001) also observed that leaf dark respiration in three tree species growing on sites with varying soil water content was sensitive to water availability. Their results indicated that trees growing at a drier site had *increased* rates of leaf dark respiration compared to trees at a wetter site, which ultimately led to a reduced carbon balance in trees at the site with reduced water availability. However, trees at the wetter (lower) site tended to have higher $Q_{10}$. The results of Turnbull *et al* (2001) and those found in this study indicated that, in general, the relationships between soil water content and respiration may be useful in informing models of plant respiratory fluxes. The degree to which declining soil water content reduces thermal acclimation of respiration could have implications for respiratory losses at a range of temporal and spatial scales. However, more work is needed to elucidate how species differ in this regard.

**Is Type I or Type II acclimation more prevalent?**

Although the degree of acclimation to warming in *S. scoparium* and *Q. stellata* differed across the measurement dates, temperature and drought acclimation often manifested itself as a downward-shift in $R_{10}$, which resulted in decreased rates of respiration across the range of measurement temperatures. This type of response has been termed 'Type II' acclimation, and it has been suggested that temperature-mediated changes in respiratory capacity (differences in either the capacity per mitochondrion or the number of mitochondria) or enzymatic changes (changes in relative amounts of enzymes or in the proteins associated with the respiratory chain) might be responsible for this type of acclimation response (Atkin *et al.* 2003; Atkin *et al.* 2005a). In agreement with the mechanism responsible for the'Type II' response, we did observe some reductions in leaf N content along with the reductions in $R_{10}$, suggesting that enzymatic changes may play a role in acclimation of respiration to warming in *S. scoparium* and *Q. stellata*. Several studies have found contrasting results when examining the type of acclimation response to warming. In a study of four different herbaceous species in a tall-grass prairie, including one $C_4$ grass, it was found that warming resulted in a reduced $Q_{10}$ rather than $R_{10}$, and acclimation responses varied depending on the month in which measurements were made (Zhou *et al.* 2007). On the other hand, several other studies have observed acclimation responses that were attributed to adjustments in base respiration rate and not $Q_{10}$ (Bruhn *et al.* (2007) in the evergreen *E. paucliflora*; Ow *et al.* (2008) in *Populus deltoids* x *nigra*, Tjoelker *et al.* (1999b) in five boreal tree species, and Tjoelker *et al.* (2009) in the evergreen *P. banksiana*), which generally corresponds to the results found in the current study.

We also observed changes in $Q_{10}$ in response to long-term rainfall redistribution (*J. virginiana*), declines in $Q_{10}$ following rainfall pulses (*S. scoparium, Q. stellata*) and at higher than lower soil water contents during summer drought (*Q. stellata*). This type of response, termed 'Type I' acclimation, primarily occurs as a result of changes in the availability of respiratory substrate and/or adenylate restriction of respiration (Atkin *et al.* 2003). Changes in $Q_{10}$ in response to declining soil water content may reflect the

adverse affects of drought on plant growth, as the demand for respiratory substrates and adenylates decline as growth is restricted. Water deficits have been found to inhibit starch synthesis, causing a shift towards the production of soluble sugars (Correia *et al.* 2006; Freeden *et al.* 1991; Zrenner *et al.* 1991; Quick *et al.* 1992). In our study, concentrations of soluble sugars in all three species appeared to be relatively unaffected by declining soil water content; therefore, changes in $Q_{10}$ were more likely a result of adenylate restriction of respiration. These results highlight that warming along with other environmental variables, such as soil water availability, might affect the type and degree of acclimation of respiration to a changing climate.

**To what extent are responses in respiration to warming and drought associated with changes in leaf chemistry and structure?**

Effects of warming and intensified summer drought on leaf structure and chemistry did not always mirror that of the effects on leaf dark respiration. Furthermore, species differed in the degree to which they exhibited changes in leaf N, SLA, and soluble sugars; in general, leaf chemistry and structure in *S. scoparium* and *Q. stellata* was much more plastic than *J. virginiana* during the course of our measurements.

In *S. scoparium*, changes in leaf N, soluble sugars, starch, and SLA did not appear to coincide with any alterations in leaf dark respiration in response to long-term warming and drought. Furthermore, in August, when there was indication of acclimation to warming, no significant differences were observed in any of the other associated leaf characteristics. We did, however, find evidence of decreases in leaf N with decreasing soil water content in *S. scoparium*, indicating a progressive drought effect. While leaf respiration declined with decreased leaf N in unwarmed *S. scoparium* in relation to soil drying, leaf respiration in warmed *S. scoparium* was constrained across the range of observed leaf N concentrations. Thus, changes in N content alone could not account for observed differences in respiration between warming treatments. In addition, the $R_{25}$ – leaf N relationship differed between warming treatments and this difference appeared unrelated to soluble sugar content, suggesting that substrate

limitations were likely not important in explaining differences in respiration between warming treatments.

Long-term warming and rainfall redistribution appeared to have little effects on leaf chemistry and structure in *J. virginiana*, which coincides with the relative insensitivity of leaf dark respiration in this species throughout the course of our measurements. Although there was some indication that leaf dark respiration may increase with rainfall redistribution and in drier soils (as indicated by our rainfall pulse data), there were no associated changes in leaf N, TNC, or SLA. Some evidence of acclimation to warming was evident during declining soil water content during summer drought, where warmed plants had lower rates of respiration per leaf N content than unwarmed plants. Furthermore, starch concentrations in *J. virginiana* appeared to be sensitive to soil water content, declining with drier soils during drought, which was not observed in the other two species. However, all three species exhibited rapid increases in total non-structural carbohydrates following rainfall pulses, perhaps reflecting short-term shifts in source-sink balance with re-watering. Presumably, increased carbohydrate storage, even if transient, would be advantageous during drought, when photosynthesis is impaired by water stress.

In *Q. stellata*, we observed decreases in leaf N content in warmed compared to unwarmed plants which coincided with reduced rates of respiration in the warmed plants across the dates on which we sampled, suggesting that acclimation to temperature in this species may be manifest predominately through adjustments in leaf N. On the other hand, leaf N, SLA, and TNC appeared to be insensitive to summer drought. In *Q. stellata*, $R_{25}$ increased with increasing leaf N in both warmed and unwarmed plants and was driven mainly by warming treatment effects on leaf N.

Other studies have found that acclimation to temperature was associated with changes in both leaf N and soluble carbohydrate concentrations, suggesting a joint enzyme- and substrate-based model of acclimation of respiration to temperature (Tjoelker *et al.* 1999b; Tjoelker *et al.* 2008). Furthermore, while it has frequently been observed that leaf dark respiration increases with increasing concentrations of soluble

sugars (Tjoelker *et al.* 2008), we observed that respiration decreased with increasing soluble carbohydrates, especially in unwarmed *S. scoparium* and *Q. stellata*. Our results suggest that leaf respiration rates were uncoupled from changes in soluble carbohydrates, and that thermal acclimation of respiration was reduced in as soils dried during summer drought. Reduced adenylate demand and its feedback effects on respiration (Atkin *et al.* 2003) could account for the lack of correlation between leaf carbohydrates and respiration, particularly as growth is suppressed with increasing drought stress. Reduced energy demand in response to drought by ATP-demanding processes such as sucrose synthesis and phloem loading might effectively reduce the turnover of ATP to ADP (Atkin *et al.* 2008), causing respiration to be adenylate restricted even in the presence of adequate carbohydrate supply.

**Implications for the southern oak savanna**

Species differences in their ability to acclimate to changing temperatures and drought play an important role in plant and ecosystem-scale carbon cycling. In our study, *S. scoparium*, a $C_4$ grass, and *Q. stellata*, a $C_3$ deciduous tree, appeared to have a greater ability to acclimate to temperature than did the $C_3$ evergreen tree, *J. virginiana*. *S. scoparium* and *Q. stellata* also appeared to be more sensitive to soil water availability than did *J. virginiana*. Furthermore, *J. virginiana* exhibited the lowest respiration rates among the three species. Consequently, changes in species composition have the potential to alter ecosystem-scale carbon fluxes in leaf and canopy respiration and its sensitivity to climate warming. Our finding that temperature acclimation declines with increasing drought has important implications for carbon cycling modeling, suggesting that warming and soil water availability will jointly influence respiratory carbon fluxes from canopies. These findings are important for ecosystems such as the southern oak savanna, where changing land-use (grazing, fire frequency) and climate change (increasing temperature, changes in rainfall distribution) has and will have in the future the potential to affect the distribution and abundances of these dominant plant species. This study suggests that predicted increases in woody plant dominance, particularly the

invasive evergreen *J. virginiana*, will increase respiratory $CO_2$ flux into the atmosphere due to the tree's evergreen nature, its lack of suppression of leaf respiration by water stress, and its minimal acclimation of leaf respiration to warming, possibly influencing the carbon-sink strength of this ecosystem. While this study focused on leaf dark respiration, it will be important in the future to consider effects on photosynthesis as well as whole plant respiration and soil respiration to gain a better understanding of how increases in temperature and changing rainfall distribution will affect the carbon balance of the entire ecosystem.

## VI. SUMMARY

Our three study species, *S. scoparium*, *J. virginiana*, and *Q. stellata*, exhibited unique temperature-responses of leaf dark respiration to temperature. Shifts in both $Q_{10}$ and $R_{10}$ were observed in response to long-term warming and rainfall redistribution. *S. scoparium* and *Q. stellata* appeared to have a greater ability to acclimate to warming than did *J. virginiana*, and acclimation generally manifested itself as a downshift in the intercept of the temperature-response function, also known as 'Type II' acclimation. $Q_{10}$ was generally insensitive to warming across the three study species. The effects of long-term rainfall redistribution on temperature-response functions also differed among species and measurement dates. Rainfall redistribution in *J. virginiana* resulted in overall higher rates of respiration in plants receiving the "intensified summer drought" rainfall treatment, indicating that future changes in rainfall patterns might affect leaf acclimation responses to warming in *J. virginiana*. Rainfall pulses during summer drought tended to affect temperature-responses of leaf dark respiration in *S. scoparium* and *Q. stellata* more so than *J. virginiana*. We found evidence that respiration declined with decreasing soil water content more so in warmed compared to warmed plants, suggesting that temperature acclimation during summer drought may be limited. While we found evidence that decreases in respiration in response to drying soils were related to leaf N concentrations, the difference appeared to be unrelated to soluble sugar content, suggesting that thermal acclimation was diminished in drying soils. These findings have important implications for carbon cycle modeling, suggesting that species differences in response to both warming and soil water availability will be important in predicting plant respiratory $CO_2$ release.

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## APPENDIX A. LAYOUT OF THE TEXAS WARMING AND RAINFALL MANIPULATION PROJECT

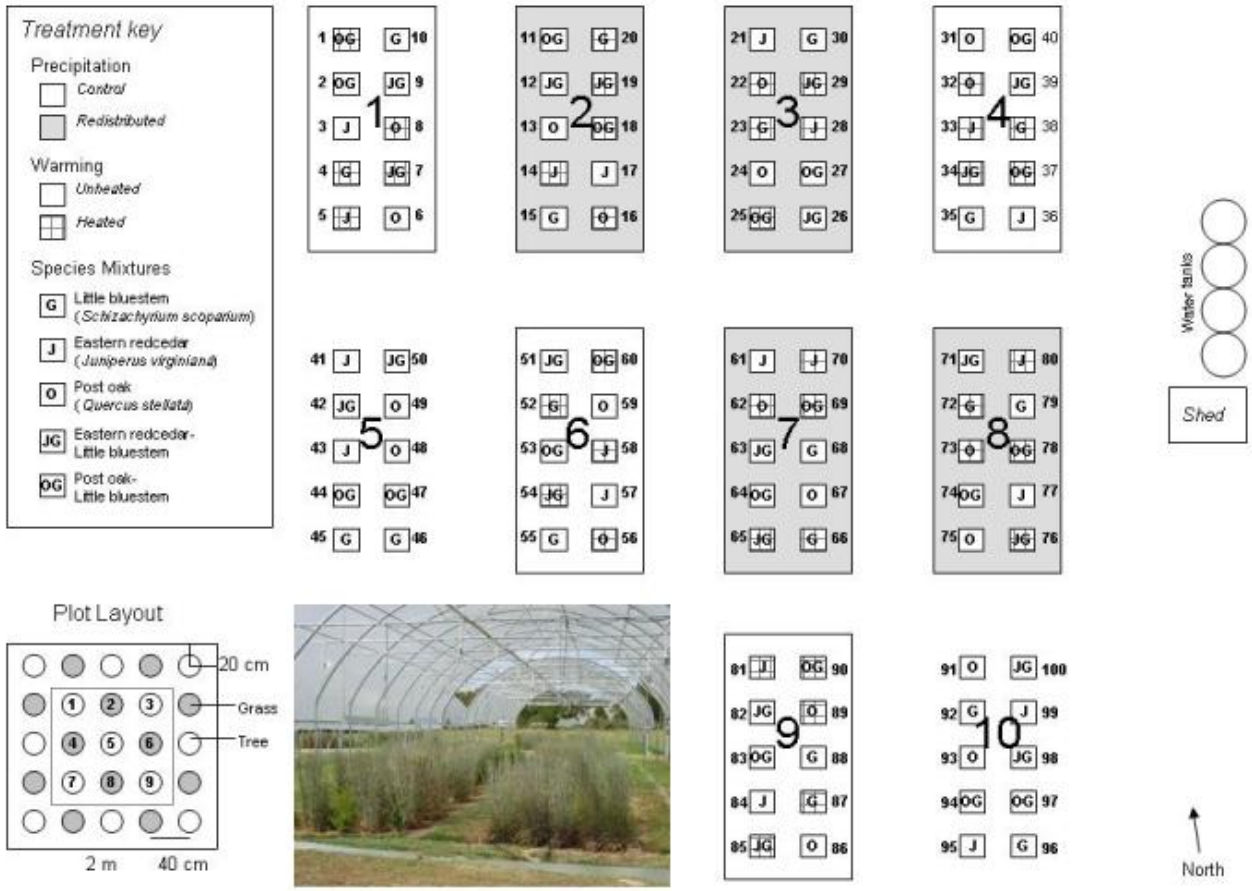


Fig. 9 Layout of the Texas Warming and Rainfall Manipulation project (Warm).

## APPENDIX B. CHECKS ON ATTACHED/DETACHED FOLIAGE

In order to determine whether rates of leaf dark respiration differ between attached and detached foliage, rates of leaf dark respiration in *J. virginiana* were measured on both attached and detached leaves in the field in May and June of 2007. Rates were first measured on attached foliage, and then leaves were cut from the tree (while still in cuvette) with scissors. Rates were allowed to stabilize and then measurements were taken once again. In May, unwarmed *J. virginiana* receiving both rainfall treatments were examined ($n$ = 16 across rainfall treatments). In June, warmed and unwarmed *J. virginiana* receiving the long-term mean rainfall treatment were examined ($n$ = 16 across temperature treatments). Results indicate that attached and detached *J. virginiana* did not differ in their rates of leaf dark respiration (Table 5).

In order to examine whether rates of leaf dark respiration remain stable during the time period (for up to 12 h) in which detached leaves of *S. scoparium*, *J. virginiana*, and *Q. stellata* were measured repeatedly in the determination of temperature-response functions in the growth chamber, rates were first measured at 25 °C. After completing measurements at the remaining temperatures (10, 15, 20, 30, 35 °C in randomized order), the leaves were again measured at 25 °C. Results indicate that, with only one exception, rates of leaf dark respiration remained stable during the time period in which temperature-response functions were measured in the growth chamber (Table 6).

Table 5 Mean rates of leaf dark respiration (± SE) expressed on a mass basis (nmol $CO_2$ $g^{-1}$ $s^{-1}$), measured in the field in May (at 25 °C) and in June (at 30 °C) on attached and detached leaves of *J. virginiana*. In May, $n = 16$ plants (combinations of unwarmed plants receiving both rainfall treatments). In June, $n = 16$ plants (combinations of warmed and unwarmed plants receiving the long-term mean rainfall treatment).

| Species | Month | Attached | Detached | *P*-value |
|---|---|---|---|---|
| *J. virginiana* | May (25 °C) | 8.04 ± 0.60 | 9.11 ± 0.77 | 0.38 |
| | June (30 °C) | 7.63 ± 0.57 | 8.40 ± 0.66 | 0.29 |

Table 6 Rates of leaf dark respiration at 25 °C measured at the beginning and end of the day on the indicated dates on which temperature-response functions were measured in the growth chamber. Shown are mean values (± SE) across both warmed and unwarmed plants receiving the redistributed rainfall treatment ($n = 8$ plants). *P*-values ≤ 0.05 are bolded.

| | | $R_{25}$ (nmol $CO_2$ $g^{-1}$ $s^{-1}$) | | *P*-value |
|---|---|---|---|---|
| Date | Species | Beginning | End | Beginning/End |
| Jul 17 | *S. scoparium* | 10.08 ± 1.45 | 9.58 ± 0.77 | 0.77 |
| Jul 20 | | 8.41 ± 0.96 | 9.05 ± 0.79 | 0.62 |
| Jul 26 | | 11.34 ± 1.72 | 9.28 ± 0.72 | 0.29 |
| Aug 6 | | 7.54 ± 0.51 | 8.23 ± 0.48 | 0.34 |
| Aug 9 | | 7.35 ± 0.57 | 8.64 ± 0.40 | 0.09 |
| Jul 17 | *J. virginiana* | 4.65 ± 0.30 | 4.01 ± 0.25 | 0.12 |
| Jul 20 | | 3.59 ± 0.38 | 3.52 ± 0.23 | 0.87 |
| Jul 26 | | 4.38 ± 0.33 | 4.55 ± 0.37 | 0.74 |
| Aug 6 | | 3.19 ± 0.22 | 3.64 ± 0.27 | 0.22 |
| Aug 9 | | 3.12 ± 0.22 | 4.00 ± 0.29 | **0.03** |
| Jul 17 | *Q. stellata* | 10.52 ± 1.61 | 10.72 ± 0.75 | 0.91 |
| Jul 20 | | 8.59 ± 0.66 | 9.25 ± 0.44 | 0.41 |
| Jul 26 | | 9.58 ± 1.04 | 9.28 ± 0.87 | 0.83 |
| Aug 6 | | 8.38 ± 0.83 | 9.10 ± 0.75 | 0.53 |

## APPENDIX C. DIURNAL VARIATIONS IN LEAF DARK RESPIRATION AND NON-STRUCTURAL CARBOHYDRATE CONCENTRATIONS

To examine diurnal variation in leaf dark respiration rates, we measured rates in both temperature treatments of *S. scoparium* and *J. virginiana* receiving the long-term mean rainfall treatment during a 24-h time interval in July 2007. Conditions during the daylight hours of the 24-h measurement period varied from overcast to scattered clouds. Measures were conducted every 2 h during the day and every 3 h at night beginning and ending at 1700 h with sample times at 1700, 2000, 2300, 0200, 0500, 0700, 0900, 1100, 1300, 1500 h. Leaves were detached from plants, placed in a plastic bag with a dampened paper towel, and transferred to the laboratory where rates of dark respiration were measured at 25 °C using two Li-COR 6400 photosynthesis systems (LI-COR, Inc. Lincoln, NE, USA) equipped with temperature and $CO_2$ controls and conifer chambers. Immediately after measurements were made at each time interval, leaves were placed in a freezer to enable determinations of leaf non-structural carbohydrate contents (as described in Methods). Rates of leaf dark respiration measured at 25 °C were calculated on a mass-basis ($R_{25}$, nmol $CO_2$ $g^{-1}$ $s^{-1}$).

In *S. scoparium* we found that, while $R_{25}$ did not differ between warming treatments or across the 24-h measurement period, concentrations of soluble sugars and starch did. In comparison, $R_{25}$ in *J. virginiana* did differ between warming treatments and with measurement time. Likewise, diurnal variations in soluble sugar content in *J. virginiana* were observed. Overall, starch content was much lower in *J. virginiana* than in *S. scoparium* and unlike *S. scoparium* did not show diurnal variation.

While $R_{25}$ in *S. scoparium* (Fig. 10) did not vary significantly with temperature treatment ($P = 0.32$) or measurement time ($P = 0.24$), concentrations of soluble sugars and starch did (Fig. 11). Soluble sugar and starch content across measurement times were 18% ($P = 0.005$) and 34% greater ($P = 0.04$), respectively, in warmed compared to unwarmed *S. scoparium*. Mean soluble sugar concentrations varied with measurement time ($P = 0.0001$), ranging from 22 mg $g^{-1}$ to 43 mg $g^{-1}$, decreasing during the nighttime

hours of 1700 to 0700 and then increasing again to values comparable to the previous day. Mean concentrations of starch also varied with measurement time ($P < 0.0001$), ranging from 7.9 mg $g^{-1}$ to 39 mg $g^{-1}$ and showed the same general pattern as soluble sugars.

In *J. virginiana*, $R_{25}$ (Fig. 10) was reduced by 10% in warmed compared to unwarmed plants across the diurnal sampling period ($P = 0.05$). Likewise, $R_{25}$ varied significantly by time of day ($P = 0.006$), declining during the nighttime hours (from 1700 to 0700) by 37% across temperature treatments. While soluble sugar content (Fig. 11) did not differ significantly between temperature treatments ($P = 0.25$), it did differ across measurement times ($P = 0.0004$). Mean soluble sugar concentrations across warmed and unwarmed *J. virginiana* declined from 31 mg $g^{-1}$ at 1700 on the first day to 25 mg $g^{-1}$ at 0900 the following morning, then increased again to 35 mg $g^{-1}$ at 1700 on the second day. In contrast, starch content in *J. virginiana* (Fig. 11) did not differ significantly across temperature treatments ($P = 0.59$) or measurement times ($P = 0.72$). Mean starch concentrations in *J. virginiana* ranged from 1.1 mg $g^{-1}$ to 2.2 mg $g^{-1}$.

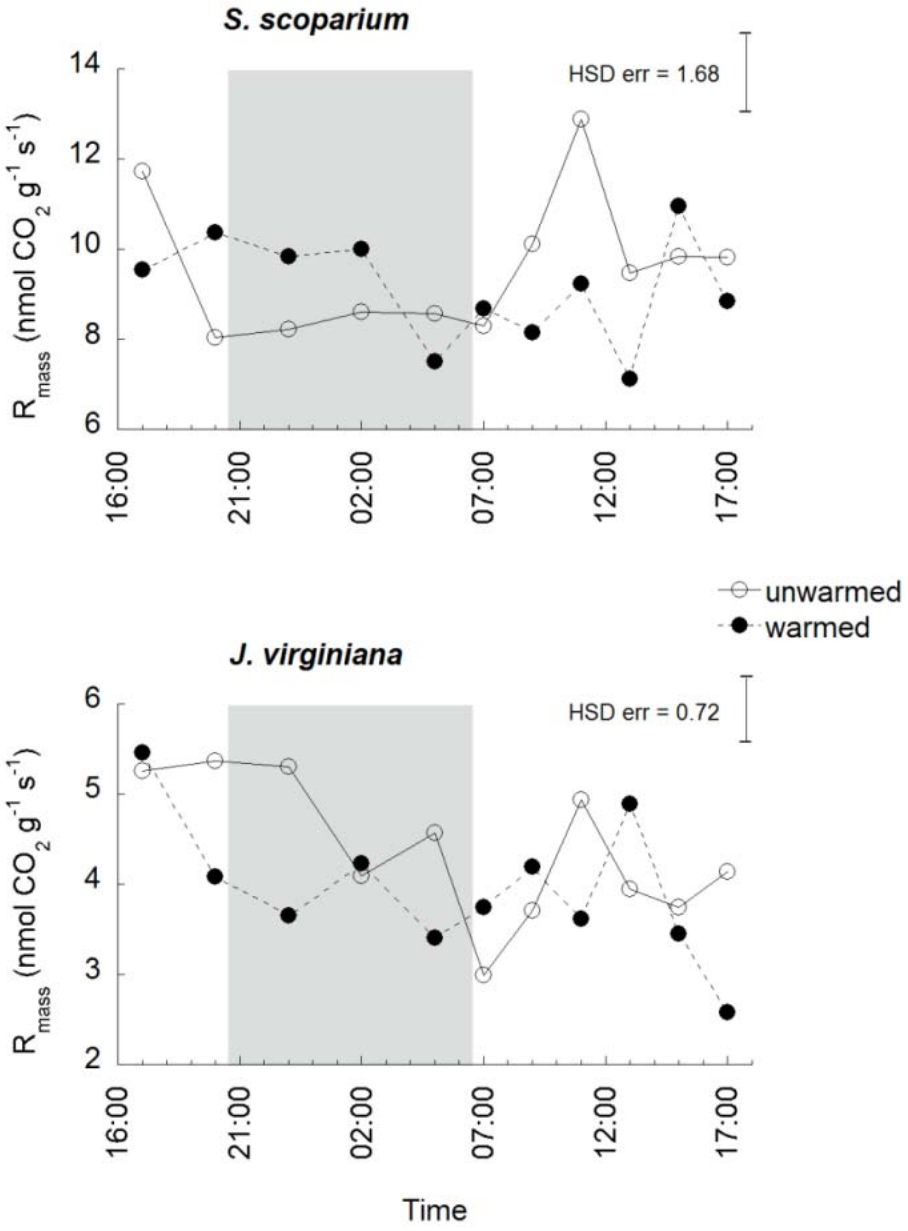


Fig. 10 Leaf dark respiration rates measured at 25 °C ($R_{25}$, nmol $CO_2$ $g^{-1}$ $s^{-1}$) throughout a 24-h period in July 2007 of *S. scoparium* and *J. virginiana* grown under different temperature treatments (unwarmed (–○–), warmed (--●--)) and receiving the long-term mean amount of rainfall. Shaded areas indicate leaves sampled at night (between 2030 and 0630 h CDT). Shown are mean values ($n$ = 4). Statistical differences were determined using Tukey's HSD. HSD errors are indicated.

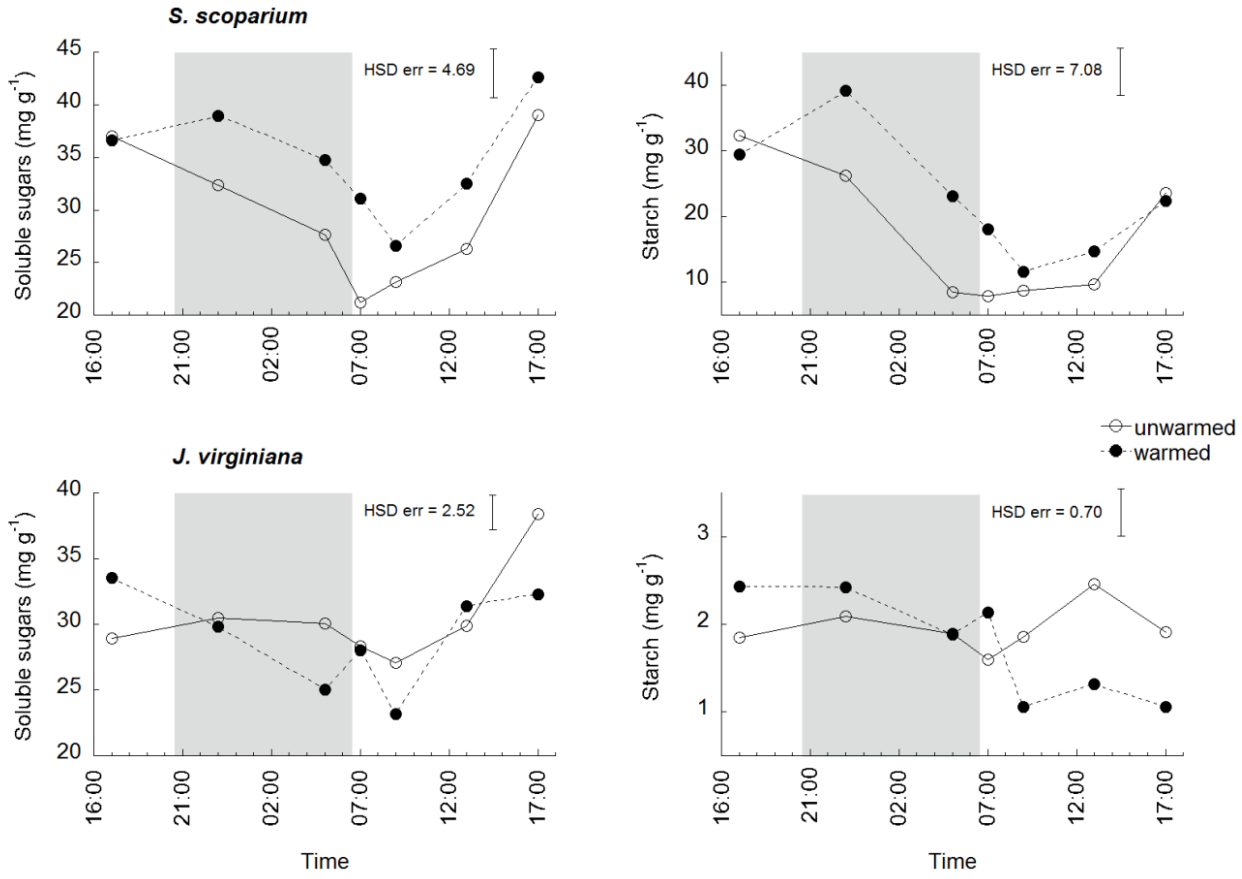


Fig. 11 Concentrations of soluble sugars and starch measured throughout a 24-h period in July 2007 of *S. scoparium* and *J. virginiana* grown under different temperature treatments (unwarmed (–○–), warmed (--●--)) and receiving the long-term mean amount of rainfall. Shaded areas indicate leaves sampled at night (between 2030 and 0630 h CDT). Shown are mean values ($n$ = 4). Statistical differences were determined using Tukey's HSD. HSD errors are indicated.

# APPENDIX D. SUPPLEMENTAL INFORMATION FOR 'RAINFALL PULSES DURING SUMMER DROUGHT'

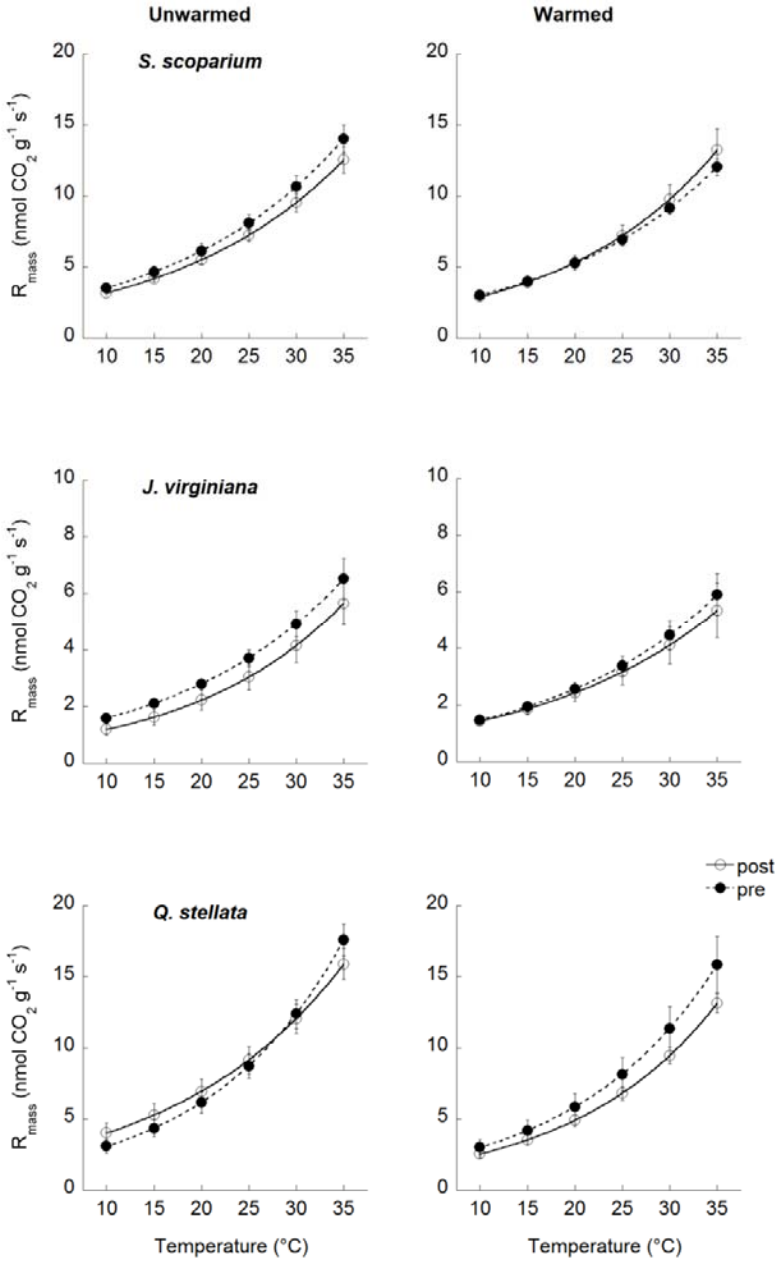


Fig. 12 Temperature-response functions of leaf dark respiration of three species of the post oak savanna grown under different temperature treatments (warmed, unwarmed) and measured before (Aug. 9, --●--)

and after (Aug. 14, –○–) a precipitation event in August 2007. Shown are mean (± SE) values and fitted regression models ($n$ = 4 plants for each treatment combination).

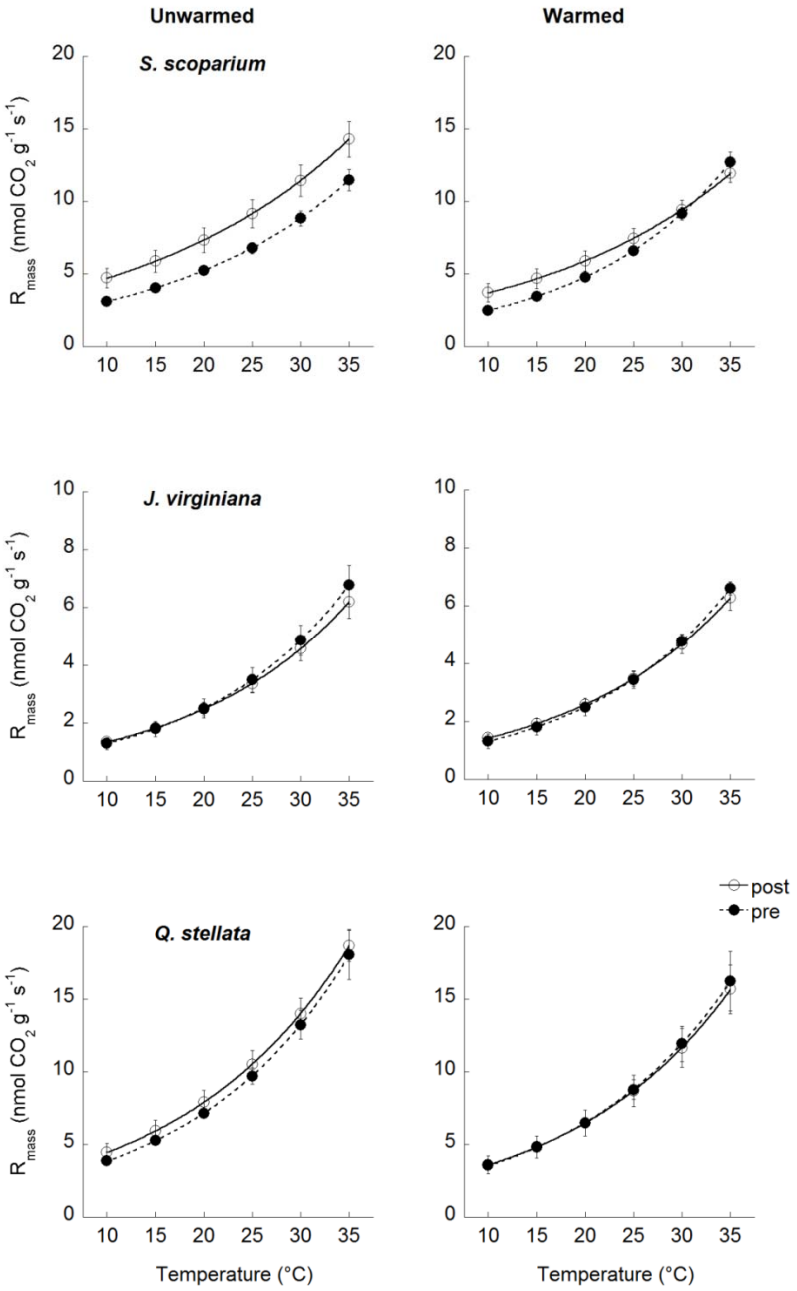


Fig. 13 Temperature-response functions of leaf dark respiration of three species of the post oak savanna grown under different temperature treatments (warmed, unwarmed) and measured before (Aug. 21, --●--) and after (Aug. 24, –○–) a precipitation event in August 2007. Shown are mean (± SE) values and fitted regression models ($n$ = 4 plants for each treatment combination).

Short-term effects of rainfall during summer drought and warming treatment effects observed on leaf N and SLA were minimal in the three study species following both precipitation events (Table 7). However, we did observe some short-term rainfall and warming effects on TNC concentrations in all three species. For the first rainfall event, warmed *S. scoparium* had 14% more TNC than unwarmed *S. scoparium* ($P$ = 0.04). However, this effect was not observed for the second rainfall event. While there were no short-term rainfall effects observed on TNC in *J. virginiana* for the first rainfall event, following the second rainfall event, TNC increased by 8% compared to pre-rainfall values ($P$ = 0.05). In *Q. stellata*, TNC increased by 15% ($P$ = 0.03) and 18% ($P$ = 0.008), respectively, following the first and second rainfall events. Warmed and unwarmed trees prior to the second rainfall event had similar concentrations of TNC; however, after the rainfall pulse, TNC in the warmed trees increased by 40% compared to pre-rainfall values ($P$ = 0.004).

After the second rainfall event, starch concentrations in *S. scoparium* increased by 57% compared to pre-rainfall values ($P$ = 0.008, Table 7). However, this trend was not observed for the first rainfall event. Starch concentration in *J. virginiana* increased by 175% following the second rainfall event ($P$ = 0.03). Furthermore, for the second rainfall event, warmed *J.virginiana* had significantly greater starch concentrations compared to unwarmed *J. virginiana* ($P$ = 0.003). Short-term rainfall effects were evident on soluble sugars in *Q. stellata*. Concentrations increased by 20% ($P$ = 0.02) and 15% ($P$ = 0.02) following the first and second rainfall events, respectively. Warmed and unwarmed trees prior to the second rainfall event had similar concentrations of soluble sugars; however, after the rainfall pulse, soluble sugars in the warmed trees increased by 41% compared to pre-rainfall values ($P$ = 0.006).

Table 7 Leaf chemistry and structure of three species of the post oak savanna grown under different temperature (unwarmed, warmed) treatments and measured before and after precipitation events in August 2007. Means[i] of leaf N (mg $g^{-1}$), TNC (mg $g^{-1}$), and SLA ($cm^2$ $g^{-1}$) are shown.

| | | | Pre-rainfall | | Post-rainfall | | *P*-value[ii] | | |
|---|---|---|---|---|---|---|---|---|---|
| Date | Species | | Unwarmed | Warmed | Unwarmed | Warmed | Pre/Post | T | Pre/Post x T |
| Aug 9 & 14 | *S. scoparium* | N | 10.3 ± 0.71 | 10.5 ± 0.74 | 11.1 ± 0.56 | 12.4 ± 1.12 | **0.07** | 0.44 | 0.35 |
| | | TNC | 46 ± 6 | 48 ± 6 | 44 ± 5 | 55 ± 6 | 0.63 | **0.04** | 0.44 |
| | | SLA | 131 ± 7 | 121 ± 9 | 130 ± 3 | 137 ± 9 | 0.24 | 0.93 | 0.23 |
| | *J. virginiana* | N | 14.7 ± 1.85 | 15.8 ± 0.52 | 16.3 ± 1.20 | 16.5 ± 0.87 | 0.28 | 0.66 | 0.62 |
| | | TNC | 42 ± 2 | 44 ± 1 | 43 ± 3 | 47 ± 4 | 0.48 | 0.40 | 0.68 |
| | | SLA | 30 ± 8 | 32 ± 2 | 30 ± 1 | 33 ± 2 | 0.97 | **0.08** | 0.66 |
| | *Q. stellata* | N | 21.7 ± 0.51 | 19.0 ± 1.85 | 21.6 ± 0.63 | 18.5 ± 0.53 | 0.69 | 0.17 | 0.73 |
| | | TNC | 34 ± 3 | 38 ± 4 | 41 ± 3 | 42 ± 5 | **0.03** | 0.46 | 0.46 |
| | | SLA | 83 ± 13 | 83 ± 1 | 84 ± 6 | 76 ± 1 | 0.67 | 0.58 | 0.56 |
| Aug 21 & 24 | *S. scoparium* | N | 8.48 ± 0.82 | 8.76 ± 0.48 | 9.23 ± 0.61 | 9.80 ± 0.80 | 0.14 | 0.39 | 0.77 |
| | | TNC | 48 ± 6 | 61 ± 11 | 71 ± 13 | 73 ± 17 | **0.08** | 0.61 | 0.46 |
| | | SLA | 103 ± 6 | 116 ± 8 | 113 ± 6 | 114 ± 4 | 0.35 | 0.42 | 0.23 |
| | *J. virginiana* | N | 16.1 ± 0.75 | 15.2 ± 1.34 | 14.5 ± 2.07 | 16.3 ± 0.92 | 0.86 | 0.66 | 0.33 |
| | | TNC | 44 ± 3 | 48 ± 4 | 47 ± 4 | 52 ± 3 | **0.05** | 0.26 | 0.41 |
| | | SLA | 30 ± 1 | 32 ± 2 | 29 ± 2 | 33 ± 2 | 0.97 | 0.26 | 0.68 |
| | *Q. stellata* | N | 19.7 ± 1.51 | 18.5 ± 0.26 | 21.5 ± 1.28 | 17.9 ± 1.16 | 0.61 | 0.17 | 0.32 |
| | | TNC | 36 ± 3 | 35 ± 1 | 35 ± 1 | 49 ± 4 | **0.008** | 0.19 | **0.004** |
| | | SLA | 77 ± 6 | 76 ± 6 | 85 ± 8 | 79 ± 3 | 0.36 | 0.51 | 0.68 |

[i] ± SE of plants sampled within treatments; $n = 4$ plants for each treatment combination.
[ii] *P*-values ≤ 0.10 are bolded.

# APPENDIX E. SUPPLEMENTAL INFORMATION FOR 'SOIL DRYING DURING SUMMER DROUGHT'

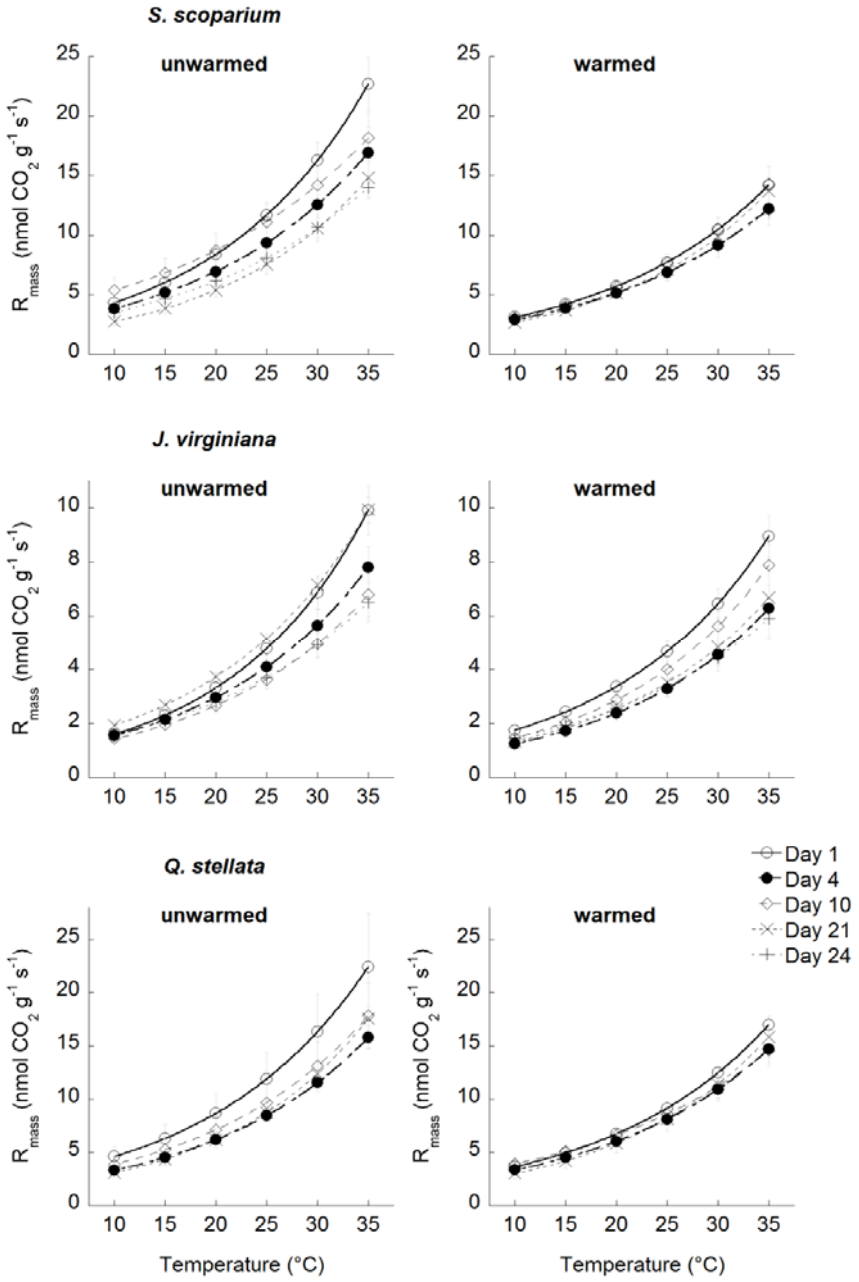


Fig. 14 Temperature-response functions of leaf dark respiration to short-term changes in temperate in three species of the post oak savanna grown under different temperature treatments (unwarmed, warmed) and measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007. Shown are mean (± SE) values ($n$ = 4) and fitted regression models.

Table 8 Regression relationships of temperature-response functions of leaf dark respiration ($R_{10}$, $Q_{10}$) and leaf dark respiration measured at 25°C, expressed on the basis of dry mass ($R_{25}$, nmol $CO_2$ $g^{-1}$ $s^{-1}$), against volumetric soil water content of three species of the post oak savanna grown under different temperature treatments (unwarmed, warmed) and measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007. In the case that there was no evidence for different slopes or intercepts between temperature treatments, only a single fit including both temperature treatments is shown. *P*-values ≤ 0.05 are bolded.

| Regression relationship | $r^2$ | *n* | *P*-value |
|---|---|---|---|
| $R_{10}$ – soil water content | | | |
| *S. scoparium* | | | |
| unwarmed | 0.16 | 20 | 0.09 |
| warmed | 0.03 | 20 | 0.45 |
| *J. virginiana* | | | |
| single fit | 0.004 | 40 | 0.69 |
| *Q. stellata* | | | |
| single fit | 0.24 | 32 | **0.004** |
| $Q_{10}$– soil water content | | | |
| *S. scoparium* | | | |
| single fit | 0.02 | 40 | 0.33 |
| *J. virginiana* | | | |
| single fit | 0.09 | 40 | 0.07 |
| *Q. stellata* | | | |
| single fit | 0.19 | 32 | **0.01** |
| $R_{25}$ – soil water content | | | |
| *S. scoparium* | | | |
| unwarmed | 0.33 | 20 | **0.008** |
| warmed | 0.03 | 20 | 0.43 |
| *J. virginiana* | | | |
| unwarmed | 0.28 | 20 | **0.02** |
| warmed | 0.02 | 20 | 0.56 |
| *Q. stellata* | | | |
| unwarmed | 0.26 | 16 | **0.04** |
| warmed | 0.08 | 16 | 0.30 |

Table 9 Regression relationships of leaf N (mg $g^{-1}$), SLA ($cm^2$ $g^{-1}$), soluble sugars (mg $g^{-1}$), and starch (mg $g^{-1}$) against volumetric soil water content of three species of the post oak savanna grown under different temperature treatments (unwarmed, warmed) and measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007. In the case that there was no evidence for different slopes or intercepts between temperature treatments, only the single fit including both temperature treatments is shown. *P*-values ≤ 0.05 are bolded.

| Regression relationship | $r^2$ | *n* | *P*-value |
|---|---|---|---|
| N – soil water content | | | |
| *S. scoparium* | | | |
| single fit | 0.21 | 40 | **0.002** |
| *J. virginiana* | | | |
| single fit | 0.01 | 40 | 0.53 |
| *Q. stellata* | | | |
| unwarmed | 0.03 | 16 | 0.54 |
| warmed | 0.03 | 16 | 0.53 |
| SLA – soil water content | | | |
| *S. scoparium* | | | |
| single fit | 0.05 | 40 | 0.16 |
| *J. virginiana* | | | |
| single fit | 0.02 | 40 | 0.36 |
| *Q. stellata* | | | |
| single fit | 0.08 | 31 | 0.11 |
| Soluble sugars – soil water content | | | |
| *S. scoparium* | | | |
| single fit | 0.009 | 40 | 0.56 |
| *J. virginiana* | | | |
| single fit | 0.01 | 40 | 0.53 |
| *Q. stellata* | | | |
| single fit | 0.10 | 32 | 0.07 |
| Starch – soil water content | | | |
| *S. scoparium* | | | |
| unwarmed | 0.02 | 20 | 0.49 |
| warmed | 0.05 | 20 | 0.34 |
| *J. virginiana* | | | |
| single fit | 0.21 | 40 | **0.003** |
| *Q. stellata* | | | |
| single fit | 0.04 | 32 | 0.26 |

Table 10 Leaf dark respiration measured at 25°C, expressed on a mass basis ($R_{25}$, nmol $CO_2$ $g^{-1}$ $s^{-1}$), against leaf N (mg $g^{-1}$), SLA ($cm^2$ $g^{-1}$), and soluble sugars (mg $g^{-1}$) for three species of the post oak savanna grown under different temperature treatments (unwarmed, warmed) and measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007. In the case that there was no evidence for different slopes or intercepts between temperature treatments, only a single fit including both temperature treatments is shown. *P*-values ≤ 0.05 are bolded.

| Regression relationship | $r^2$ | *n* | *P*-value |
|---|---|---|---|
| $R_{25}$ – N | | | |
| *S. scoparium* | | | |
| unwarmed | 0.64 | 20 | **<0.001** |
| warmed | 0.26 | 20 | **0.02** |
| *J. virginiana* | | | |
| unwarmed | 0.13 | 20 | 0.12 |
| warmed | 0.003 | 20 | 0.81 |
| *Q. stellata* | | | |
| single fit | 0.21 | 32 | **0.008** |
| $R_{25}$ – SLA | | | |
| *S. scoparium* | | | |
| single fit | 0.02 | 40 | 0.35 |
| *J. virginiana* | | | |
| single fit | .002 | 40 | 0.76 |
| *Q. stellata* | | | |
| unwarmed | 0.65 | 16 | **<0.001** |
| warmed | 0.001 | 15 | 0.90 |
| $R_{25}$ – Soluble sugars | | | |
| *S. scoparium* | | | |
| unwarmed | 0.22 | 20 | **0.04** |
| warmed | <0.001 | 20 | 0.99 |
| *J. virginiana* | | | |
| unwarmed | 0.006 | 20 | 0.74 |
| warmed | 0.17 | 20 | 0.07 |
| *Q. stellata* | | | |
| unwarmed | 0.36 | 16 | **0.01** |
| warmed | <0.001 | 16 | 0.93 |

Table 11 Leaf dark respiration measured at 25°C, expressed on a nitrogen basis ($R_N$, μmol $CO_2$ mol $N^{-1}$ $s^{-1}$) and a soluble carbohydrate basis ($R_{soluble\ sugar}$, nmol $CO_2$ $g^{-1}$ $s^{-1}$) against leaf N (mg $g^{-1}$) and soluble sugars (mg $g^{-1}$) of three species of the post oak savanna grown under different temperature treatments (unwarmed, warmed) and measured on five different dates throughout a period of soil drying during summer drought in July and August of 2007. In the case that there was no evidence for different slopes or intercepts between temperature treatments, only a single fit including both temperature treatments is shown. *P*-values ≤ 0.05 are bolded.

| Regression relationship | $r^2$ | *n* | *P*-value |
|---|---|---|---|
| $R_N$ – Soluble sugars | | | |
| *S. scoparium* | | | |
| unwarmed | 0.16 | 20 | 0.08 |
| warmed | 0.08 | 20 | 0.24 |
| *J. virginiana* | | | |
| unwarmed | 0.05 | 20 | 0.36 |
| warmed | 0.14 | 20 | 0.10 |
| *Q. stellata* | | | |
| unwarmed | 0.30 | 16 | **0.03** |
| warmed | 0.03 | 16 | 0.56 |
| $R_{Soluble\ sugars}$ – N | | | |
| *S. scoparium* | | | |
| unwarmed | 0.53 | 20 | **<0.001** |
| warmed | <0.001 | 20 | 0.91 |
| *J. virginiana* | | | |
| single fit | <0.001 | 40 | 0.88 |
| *Q. stellata* | | | |
| single fit | 0.19 | 32 | **0.01** |

# VITA


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