

The private lives of C₄ grasses: Drought responses across biochemical subtypes

by

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Abstract

With changes in global climate, drought events have shifted towards more frequent and more severe regimes. Quantifying plant responses to changes in these drought conditions is important for understanding future ecosystem changes and forecasting the impacts on human populations. Droughts can elicit acute temporary environmental changes such as increased leaf thermal stress, high atmospheric moisture demand and reduced soil moisture. However, long-lasting consequences in ecosystem structure and function often coincide with prolonged intensity and duration of drought events. While we understand general trends of changes in drought events on plant function, we still require empirical evidence that translates species-level responses in an altered climate, particularly in C₄-dominated grasslands.

Grasslands dominate terrestrial biomes and provide a wide array of ecosystem services. C₄ grasses are the dominant species in many tropical grasslands worldwide, as well as in temperate regions including the tallgrass prairie of the Flint Hills ecoregion. C₄ grasses play essential roles in mediating carbon, nitrogen and hydrological cycles. Nearly half of all grass species worldwide use C₄ photosynthesis, a modified anatomical and biochemical form of C₃ photosynthesis that provides an ecological advantage during hot, dry conditions.

Over the past 24 – 35 million years, parallel evolution of C₄ photosynthesis has occurred over 24 times in the grass family, *Poaceae*, and over 66 times in all flowering plants. As a result, the evolutionary lineage and the biochemistry of C₄ grasses influence the photosynthetic and water use strategies we observe in modern C₄ lineages. Species are classified into three distinct subtypes of C₄ photosynthesis based on unique carbon-fixation biochemistry, and are each adapted to the climatic conditions in which they evolved (wet-adapted NADP-ME subtype, intermediate PCK subtype, and dry-adapted NAD-ME subtype).

All C₄ grasses are presumed to have roughly similar physiological responses to drought events, and yet these processes are rarely compared across species or distinct evolutionary lineages. While the physiological C₄ grass responses to soil moisture declines have been studied extensively, similar investigation into changes in atmospheric drought are lacking. Vapor pressure deficit (VPD), a proxy for atmospheric drought, is a dynamic, yet critical regulator of ecosystem processes. For most herbaceous species, VPD reduces photosynthetic capacity while accelerating terrestrial water loss. In my thesis, I measured physiological, structural, and anatomical traits of the three C₄ biochemical subtypes related to water use safety/efficiency and framed my hypotheses around the role of ancestry and biochemistry driving responses to changes in VPD. Using a suite of traits measured across multiple temporal scales, I assessed how structural and anatomical traits drive physiological responses of C₄ subtypes during drought.

In Chapter 2, I generated 87 twelve-hour response curves to quantify the gas exchange responses of 15 species of C₄ grass. I found that all C₄ subtypes showed relatively low stomatal sensitivity to changes in VPD, but no recovery in gas exchange rates were observed over the experimental time scale. The wet-adapted NADP-ME subtype showed the lowest tolerance to high VPD across the 12-hour period because these species allocated resources towards anatomical traits that maximize photosynthetic capacity during ideal conditions. The dry-adapted NAD-ME subtype maintained photosynthetic stability by prioritizing drought-safe anatomical traits, despite downregulating stomatal conductance under high VPD. The intermediate PCK subtype had the highest initial gas exchange rates but showed the steepest decline across the VPD response curve. Both the dry-adapted and intermediate subtypes exhibited anatomical traits that prioritize safety during droughts, but differences in leaf $\delta^{13}\text{C}$, stomatal traits and gas

exchange responses indicate the coordination between anatomical and physiological traits vary across PCK and NAD-ME groups.

In Chapter 3, I tested whole-plant responses of 6 species for 10 consecutive days in a modified drying oven that simulated high, temperature-driven VPD. I found that C₄ subtype gas exchange responses did not vary under isolated VPD driven by high temperatures, but subtype responses diverged under combined VPD and soil water deficits. Differences in microanatomical traits across C₄ subtypes were consistent with Chapter 2 results, and we further identified subtype-specific tradeoffs in specialized leaf water-holding cells. This short-term experiment was paired with a season-long study of the same species under ambient environmental conditions at Konza Prairie. Throughout the growing season, water loss strategies of C₄ subtypes varied, and showed the largest differences on the warmest sampling day. Physiological and structural traits indicated that the C₄ subtypes occupied different seasonal optima with distinct nitrogen use strategies and integrated stomatal behaviors.

In both chapters, coordination between anatomical traits and physiological function was evident, with distinction by photosynthetic subtype. Thus, a combination of physiological, anatomical, and structural traits was required to illustrate the covert differences in water loss strategies across C₄ subtypes. Together, these results demonstrate that atmospheric and soil droughts interact to shape plant community structure and function in grassland ecosystems, with subtype-specific responses linked to physiological and anatomical trait variation. As rising VPD continues to reshape the terrestrial plant water balance, understanding these responses will be critical for predicting grassland responses to droughts and informing ecosystem management under a shifting climate.

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Dedication

To all the wonderful and powerful women in my life, especially my mother, Mary Magdalen.

Introduction

Photosynthesis has evolved alongside our planet, shaping atmospheric conditions to foster the chemical, physical, and ecological processes that constitute our modern landscapes. The act of harnessing light energy from the sun provides the metabolic foundation for survival of all higher taxa, including the agricultural infrastructure to sustain over 8 billion human beings. As photosynthesis oxygenated the earth, selective pressures on plants drove acute levels of evolutionary convergence, particularly related to photosynthetic biochemistry. C₄ photosynthesis is a specialized strategy that evolved 24-35 million years ago in low atmospheric [CO₂] conditions in a variety of hot and dry conditions (Hatch & Slack, 1966). The C₄ strategy is a classic example of structural convergence, evolving at least 66 times across diverse plant lineages, including 24 separate times in the grass family, Poaceae (Sage *et al.*, 1999; Sage 2016; Grass Phylogeny Working Group II, 2012). The C₄ photosynthetic pathway is characterized by specialized, wreath-shaped anatomy (hence, Kranz anatomy) that concentrates CO₂ at the site of carboxylation and increases the enzymic affinity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) for CO₂ in the bundle sheath cells. This suite of biochemical and anatomical traits allows C₄ plants to reduce photorespiration and maintain higher photosynthetic efficiency than C₃ plants in hot, arid conditions (Hatch 1987).

C₄ grasses play a key role in global nutrient and hydrological cycles, occupying over 20% of terrestrial plant cover and accounting for approximately 23% of total gross primary productivity (35.3 Pg C year⁻¹) in terrestrial systems (Shantz, 1954; Still *et al.*, 2003). While C₄-dominated grasslands evolved in variable climate conditions, predicted shifts in precipitation patterns and seasonality will exacerbate and increase the frequency of drought events, hindering grass function at leaf- to landscape-scales (Morgan *et al.*, 2024; Post & Knapp, 2019; Knapp *et*

al., 2020). Drought, or any anomalous water deficit, can manifest in a variety of ways and elicits a multitude of impacts on plant and ecosystem function. Droughts are a result of reduced soil moisture availability, high atmospheric evaporative demand, or both (Lui *et al.*, 2023). Water deficits may be acute (i.e. ‘flash’ or ‘pulse’ droughts, Christian *et al.*, 2021) or chronic (e.g. North American Dust Bowl and The Millenium Drought in Australia, Knapp *et al.*, 2020; Van Dijk *et al.*, 2013). Drought negatively impacts plant function at the leaf level by hindering stomatal conductance and transpiration rates, reducing photosynthetic capacities (Hammond *et al.*, 2022; Knapp *et al.*, 2024). At the ecosystem level, drought often accelerates terrestrial water loss (Massmann *et al.*, 2019) and dampens aboveground net primary productivity, especially in C₄-dominated grasslands (Xu *et al.*, 2019; Carroll *et al.*, 2021). Water deficits subsequently alter ecosystem characteristics, including shifts in carbon sink-source dynamics, plant community composition, and plant adaptation and resilience to future droughts (Sellers *et al.*, 2018; Griffin-Nolan *et al.*, 2019; Müller & Bahn 2022). The impacts of soil moisture limitation on plant function are well established, however, plant responses to atmospheric drought, measured as changes in the vapor pressure deficit (VPD), remain underrepresented in literature and in models, particularly for C₄ grasses (Still *et al.*, 2019). Thus, there remains a critical gap in understanding the impacts of atmospheric drought on C₄ grass function in natural grassland systems.

Quantifying plant water use strategies during water deficits is important to our understanding of the global water budget, as plants transpire approximately 65% of terrestrial water into the atmosphere (Good *et al.*, 2015; Schlesinger & Jasechko, 2014). Historically, Land Surface Models and Earth System Models group plant ecological processes into plant functional types (PFTs) to encompass a large breadth of plant strategies across broad temporal and spatial scales. This expansive yet granular approach often fails to capture the inter- and intraspecific

ecological dynamics inside PFTs (Medlyn *et al.*, 2016). Recent efforts to account for the large variation within PFTs, particularly of C₄ grasses, have called for lineage-based functional types (LFTs), which reduce granularity and better represent C₄ functional traits than the typical C₄ grass PFT (Monson *et al.*, 2025; Donnelly *et al.*, 2023; Griffith *et al.*, 2020). Standard PFTs neglect the nuance of plant responses throughout phenological stages and during drought events (Anderegg *et al.*, 2015; Powell *et al.*, 2013).

Following the discovery of the C₄ syndrome in the 1970s, biochemists separated C₄ photosynthesis into three biochemical subgroups (hereby subtypes) closely linked with the evolutionary history of various C₄ taxa (Gutierrez *et al.*, 1974; Hatch *et al.*, 1975; Hattersley 1984). Named after their respective decarboxylating enzymes, there are three classical biochemical subtypes of C₄ photosynthesis: Nicotinamide adenine dinucleotide phosphate-dependent malic enzyme (NADP-ME) subtype, phosphoenolpyruvate-carboxykinase (PCK) subtype, and nicotinamide adenine dinucleotide-dependent malic enzyme (NAD-ME) subtype. As a consequence of C₄ lineages evolving across a variety of precipitation regimes (300 – 850 mm mean annual precipitation (MAP)), ecological evidence suggests that C₄ subtype distribution still varies across a climatic gradient (Ocheltree *et al.*, 2015; Taub, 2000).

With increasing MAP in North American, Southern African, and Australian grasslands, the abundance of the NADP-ME subtype increased while the abundance of the NAD-ME subtype simultaneously decreased (Ellis *et al.*, 1980; Taub, 2000; Wan & Sage, 2001). In response to drought events, instances of decreasing cover of the wet-adapted NADP-ME subtype, coinciding with increasing cover of the dry-adapted NAD-ME subtype have been reported (Wigley-Coetsee & Staver, 2020; Albertson & Tomanek, 1965). The PCK subtype does not follow ecological patterns as consistently relative to the other biochemical subtypes. In North

America, PCK abundance was negatively correlated with MAP, and positively correlated with MAP in Australia (Taub, 2000; Hattersley, 1992). Based on biochemical, phylogenetic, and ecological evidence illustrating different water use strategies of the C₄ subtypes, we designate the NADP-ME subtype as mesic-, or wet-adapted, the PCK subtype as intermediate, and the NAD-ME subtype as dry-adapted.

Species of the two C₄ subfamilies of Poaceae dominating North American grasslands, Andropogoneae and Chloridoideae, often coexist on the landscape and possess specialized physiological, structural, and anatomical traits to persist in mesic and/or hot, dry conditions, respectively (Lehmann *et al.*, 2019). The linkages between taxonomy and biochemistry of C₄ grasses are represented by the distribution of decarboxylating enzymes within taxa. Most C₄ Panicoideae species use NADP-ME, and nearly all C₄ Chloridoideae species use NAD-ME or PCK (Chapman, 1992; Sage & Monson, 1999). The observed ecological and physiological behavior of C₄ subtypes has been exclusively reported in relation to soil water deficits (Wigley-Coetsee & Staver, 2020; Ghannoum *et al.*, 2002; Taub, 2000; Wan & Sage, 2001; Hattersley, 1992; Ellis *et al.*, 1980; Albertson & Tomanek, 1965). Further, researchers speculate that structural and anatomical characteristics, such as stomatal regulation, may explain success under drought conditions when physiological data prove inconclusive (Ghannoum *et al.*, 2002). Establishing empirical evidence for C₄ subtype responses to atmospheric water deficits in wild grass species is critical to our understanding of plant-mediated interactions between atmospheric and terrestrial processes.

Heat tolerance and dissipation vary with plant age, plant type, and within a given plant depending on the age of leaves and their proximity to radiation and canopy fluxes (Vogel 2009; Medlyn *et al.*, 2002). For these reasons, to adequately understand water vapor dynamics at the

leaf-atmosphere surface, we must account for the temperature of leaves that we measure. Thus, we use VPD_{leaf} (Equation 2) rather than VPD_{air} (Equation 1) to assess plant function under atmospheric water deficits.

Equation 1:

$$VPD_{air} = e_s(T_{air}) \left(\frac{1-RH}{100} \right)$$

Equation 2:

$$VPD_{leaf} = e_s(T_{leaf}) - e_a$$

Where e_s is the saturation vapor pressure (kPa) and e_a is the actual vapor pressure (kPa), RH is air humidity relative to the saturation vapor pressure (%) of the atmosphere at the air temperature, and T_{air} and T_{leaf} are the temperatures (°C) of the atmosphere and leaf, respectively. Elevated VPD_{leaf} is a key driver of atmospheric drought stress on plants and is associated with reductions in transpiration, photosynthetic, and stomatal conductance rates across angiosperms (Hammond *et al.*, 2022; Farquhar 1978). Evidence for high VPD_{leaf} reducing carbon uptake and limiting crop yields is present in ecological and agricultural systems, respectively (Knapp *et al.*, 2024; López *et al.*, 2021). However, the assumption that all C_4 plants have more responsive stomata under atmospheric droughts than their C_3 counterparts lacks nuance, as current evidence assumes C_4 crop and wild C_4 grass physiology are functionally equivalent (Franks & Farquhar 2007). Naturally established C_4 grasses succeed in highly variable conditions, but C_4 crops have been bred well beyond their ancestral forms, so physiological processes are simply incomparable (Fay & Knapp, 1993). On the landscape, large water vapor deficits cause positive feedbacks that intensify terrestrial water loss, accelerating soil drying via evapotranspiration and negatively impacting hydrology at local to continental scales (Chandanpurkar *et al.*, 2025; Ficklin *et al.*, 2022; Zhou *et al.*, 2019).

My thesis explores the mechanistic underpinnings of atmospheric drought responses in native, field-collected C₄ prairie grasses across three temporal scales. In Chapter 2, I quantify grass species responses to elevated VPD with a 12-hour response curve. In Chapter 3, I assess responses to high VPD across 10 consecutive days and periodically throughout the growing season. In both projects, I measure physiological, structural, and anatomical traits related to water use safety/efficiency and frame my hypotheses around the role of ancestry and biochemistry in atmospheric drought tolerance.

I completed this research at Konza Prairie Biological Station, a mesic grassland site in northeastern Kansas, USA (39.095385, -96.574987). Konza Prairie hosts a broad diversity of C₄ grasses, allowing the collection of data to represent form and function within and across the three C₄ subtypes. In my second chapter, I generated 87 twelve-hour response curves to assess the physiological responses of the C₄ subtypes to leaf-level atmospheric drying. To investigate patterns in leaf architecture and success under high VPD, we measured the microanatomical and stomatal traits of 15 species of native C₄ grasses from Konza Prairie. The second chapter provides empirical evidence to strengthen our understanding of C₄ subtype responses to elevated VPD, improve C₄ representation in process-based ecosystem models, and support future grassland management under predicted shifts in precipitation regimes.

The third chapter of this thesis explores C₄ subtype responses to atmospheric drying across two temporal scales. I measured physiological responses of six species of C₄ grass to elevated atmospheric drying and declining soil moisture for ten consecutive days and periodically throughout the growing season. In collaboration with Kansas State University Veterinary Medicine Histopathology Lab, we assessed the microanatomical traits driving success under high VPD at diurnal and seasonal scales. In my third chapter, I aim to contextualize C₄

subtype responses to atmospheric drying at the plant-level and examine how atmospheric water and soil water deficits interact over time. Together, these chapters share a novel perspective on C₄ grass function across different drought regimes while providing a structural and anatomical basis for the physiological responses measured.

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C₄ grass biochemical subtypes have varied responses to atmospheric drought

Introduction

Grassy ecosystems dominate global terrestrial systems, covering ~23% of surfaces and hosting broad species and functional diversity (MacDougall *et al.*, 2026; Peterson *et al.*, 1998). Grasslands played an integral role in human evolution, and many grass species today are critical components of our agricultural and economic systems. In addition, warm-season, or C₄ grasses, mediate terrestrial and atmospheric processes and are essential to global nutrient and hydrological cycles (Still *et al.*, 2019). Despite contributing nearly 20% of terrestrial photosynthesis, many aspects of C₄ plant-atmosphere interactions, especially those related to atmospheric drought responses, require further study to understand shifting drought behaviors (Morgan *et al.*, 2024; Luo *et al.*, 2024; Knapp *et al.*, 2020).

Evolution of the C₄ photosynthetic pathway began 24 – 35 million years ago when the selective pressures of the atmosphere forced plants to respond to low atmospheric CO₂ concentrations, high temperatures, and/or water deficits (Hatch & Slack, 1966; Sage *et al.*, 2018). The complex evolutionary history of C₄ photosynthesis varies within and across taxa, though the most dramatic and rapid evolutionary radiation of the C₄ strategy has occurred in the order Poales (Elliott *et al.*, 2023; Edwards & Smith, 2010). A hallmark of C₄ photosynthesis is modified mesophyll structures, called bundle sheath cells. Spatially separating photochemistry in the mesophyll cells from biochemistry in the bundle sheath cells concentrates CO₂ at the site of RuBisCO activity, reducing photorespiration and increasing photosynthetic capacities in hot, arid environments (Hatch 1987). Throughout the wide range of plant lineages and environmental

gradients in which C₄ grasses evolved, many iterations of anatomical, biochemical, and physiological traits have arisen and varied over time. Upon distinguishing three biochemical variants of C₄ photosynthesis (coined the C₄ subtypes), biochemists, histologists, and biologists subsequently identified specific differences among the C₄ subtypes in structure and ecology.

The C₄ subtypes are named after their decarboxylating enzymes: NADP-malic enzyme subtype (NADP-ME), PEP-carboxykinase subtype (PCK), and NAD-malic enzyme subtype (NAD-ME). The 4-carbon acid product of mesophyll carboxylation and the site of decarboxylation both vary among the subtypes. NADP-ME decarboxylates malate in the bundle sheath chloroplasts and NAD-ME decarboxylates aspartate in the bundle sheath mitochondria. The PCK subtype hosts a more complex biochemical strategy, decarboxylating aspartate in the bundle sheath cytoplasm and/or malate in the bundle sheath mitochondria. The subtypes show unique bundle sheath chloroplast arrangements, where centrifugal arrangements in the NADP-ME and PCK subtypes contrast centripetal chloroplast arrangements in the NAD-ME bundle sheath cells, perhaps impacting enzyme-chloroplast interactions related to photoassimilation and/or leakiness of CO₂ from bundle sheath cells (Ghannoum *et al.*, 2025; Fravolini *et al.*, 2002; Farquhar, 1983).

In tandem with anatomical and biochemical differences, the C₄ subtypes exhibit ecological traits that suggest water use strategies vary. Evidence from three separate continents, North America, Australia, and South Africa, showed increases in NADP-ME abundance and simultaneous decreases in NAD-ME abundance along an increasing rainfall gradient (Ellis *et al.*, 1980; Taub, 2000; Wan & Sage, 2001). Plant community composition responses to drought events indicate the same relative pattern in response to water stress for the NADP- and NAD-ME subtypes (Wigley-Coetsee & Staver, 2020; Albertson & Tomanek, 1965). Reflecting the

complexity of its biochemistry, the PCK subtype is not associated with consistent ecological responses to water stress. Reports of PCK subtype positively correlating with increasing mean annual precipitation (MAP) in Australia conflict negative correlations with increasing MAP reported in North America (Taub, 2000; Hattersley, 1992).

I posit that differences in photosynthetic machinery and drought responses interact to yield distinct water use strategies in each of the C₄ subtypes. Despite biochemical and ecological nuances, PCK was measured as one subtype and called “intermediate.” Thus, I grouped NADP-ME-dependent species together as mesic- or wet-adapted, the PCK-dependent species as intermediate, and the NAD-ME-dependent species as dry-adapted. Here, I aimed to identify functional differences in the C₄ biochemical subtypes by quantifying physiological, structural, and anatomical traits of 15 species of C₄ grass representing different biochemical pathways. I generated a novel VPD response curve to assess leaf-level responses under atmospheric water deficits. I tested my hypotheses on field-collected grass specimens from unplowed, C₄-dominated prairie.

Methods

Field collection and growth conditions

I collected grasses from a long-term ecological research station, Konza Prairie Biological Station (KPBS), near Manhattan, Kansas, USA (latitude: 39.095385, longitude: -96.574987) in the Flint Hills ecoregion. KPBS hosts native tallgrass prairie and spans 3,487 hectares. The site maintains two long-term experimental prescribed burning and ungulate grazing treatments at the watershed level. The role of fire, fire return intervals, and grazing were excluded from this analysis, as I used species from ungrazed sites and simply measured responses each species existing in their unique ecological niches. While all three C₄ subtypes coexist at KPBS, the NADP-ME subtype dominates plant community composition because KPBS receives

approximately 835 mm MAP. Species from six C₄ grass tribes (Andropogoneae, Cynodonteae, Eragrostideae, Paniceae, Paspaleae, and Zoysieae) were collected. Fifteen species (Table 1) were chosen to represent the three C₄ subtypes: NADP-ME, PCK, and NAD-ME. Each subtype was represented by five species with five measured replicates per species (n = 75 total, n = 25 per subtype).

The intact shoots and roots of each grass replicate was sampled during the growing seasons of 2024 and 2025. Aboveground growth was cut to 10 cm at the time of transplant to ensure only new leaves were measured. I planted each replicate in 10 cm x 10 cm square containers for indoor laboratory measurements. To support the naturally established root system, I attempted to retain the native soil in which grasses were growing; however, I supplemented native soil with Pro-Mix Ultimate Vegetable & Herb Mix Soil ([phthorticulture.com]) as needed. Indoor laboratory air temperatures were maintained at 22 – 26 °C. Plants were grown under HortiBloom Mega Optic 500W horticultural grow lights ([hortibloom.com]) for 12 hours each day with photosynthetically active radiation (PAR) ranging between 1,600 – 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. To ensure soil moisture deficits were eliminated before measurement, each plant container was watered to field capacity approximately 2 – 3 days before gas exchange data collection.

Drying treatment and gas exchange

Gas exchange data was collected with LI-COR 6800 infrared gas analyzers (LI-COR Inc., Lincoln, NE, USA). The 3 cm x 3 cm cuvette provided a controlled chamber environment with CO₂ concentration set to 400 ppm and PAR to 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. I built a background program to manipulate VPD at the leaf-air interface (VPD_{leaf}) to generate a twelve-hour response curve, making stepwise changes of 1 kPa each hour across the time series. My focal gas exchange metrics were photosynthesis (A; $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), stomatal conductance (g_{sw}; $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$),

and transpiration (E ; $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). In this study, I used VPD_{leaf} , rather than the VPD of the atmosphere (VPD_{air}), to account for water vapor dynamics acting on leaves at a given leaf temperature. To reduce mechanical bias, gas analyzers were alternated with each species replicate to ensure balanced measurements (2 – 3 response curves per species) on a given machine. In addition, the same portion of leaf sampled in the cuvette was photographed and measured using ImageJ software, imputing the exact leaf area measurement into the instrument equations. I grouped the drying treatment into four phases: Increasing VPD_{leaf} (1 – 3 kPa, hours 1 – 3); high VPD_{leaf} (4 – 4.5 kPa, hours 4 – 6); decreasing VPD_{leaf} (3 – 1 kPa, hours 7 – 9), and steady-state VPD_{leaf} (1 kPa, hours 10 – 12). Using the LI-COR Autolog function, data points were logged every 120 seconds for 12 consecutive hours, totaling approximately 370 observations per response curve and approximately 39,000 total observations after data cleaning. The results of 87 response curves collected in 2024 and 2025 were used in these analyses (NADP-ME $n = 33$; PEP-CK $n = 26$; NAD-ME $n = 28$).

Microanatomical and stomatal traits

To ensure gas exchange data directly corresponded to microanatomical and stomatal measurements, I sampled leaf cross sections and stomatal peels from the same 3 cm portion of leaf that was measured inside the infrared gas analyzer. A Lumenera Infinity 2 camera ([teledyneimaging.com]) was attached to an Olympus BH-2 compound light microscope equipped for imaging. Images of leaf cross sections were collected and analyzed on Infinity CAPTURE software and ImageJ software, respectively. Leaf cross sections and stomatal peels were collected from fresh, unstained leaves within 3 days of gas exchange data collection.

The leaf blade was hand sliced into 3 – 5 μm cross sections and promptly plated the tissue onto slides for imaging. Cross sectional areas were imaged at 0.13x and 10x

magnification. From each grass replicate, one tissue region was selected on either side of the midvein to measure anatomical traits between two major vascular bundles (Bachle & Nippert, 2018). We sampled: vessel area (V_a), interveinal distance (IVD), leaf thickness (Lt; structural trait), xylem area (X_a), xylem diameter (X_d), bundle sheath cell area (BSC_a), mesophyll cell area (MSC_a), and the ratio of BSC_a to MSC_a ($BSC:MSC$). Interveinal distance was calculated by using the distance between the midpoint of adjacent vessels and averaged. Leaf thickness was measured at four points across the width of the leaf and averaged. Xylem area and xylem diameter were collected and averaged across two major veins. Vessel, bundle sheath cell, and mesophyll cell areas were measured on minor and major veins and averaged separately.

Stomatal impressions were prepared by coating a small 1 – 2 cm section of leaf with clear nail polish, gently pressing clear tape on top of the dried nail polish, and attaching the tape with the indentation to a slide. With a standardized field of view, images were collected at 10x magnification for stomatal density, and 20x magnification for stomatal length. I measured: stomatal density on the adaxial (SD_{ad}) and abaxial (SD_{ab}) sides of the leaf and stomatal length on the adaxial (SL_{ad}) and abaxial (SL_{ab}) sides of the leaf. To calculate the density of stomata, each image was divided into four quadrants. In two of the quadrants, all stomata in the quadrant were counted including those partially cut off from the field of view (i.e. stomata along the margins of the field of view). In the remaining quadrants, the stomata along the margins of the quadrants were excluded and only “complete” stomata present in the image were counted. The stomatal counts were summed and divided by the field of view for SD_{ad} and SD_{ab} . To measure the mean stomatal length, 10 stomata per adaxial and abaxial image across the field of view were selected, ensuring at least one stoma per vein (across leaf width) was measured and selection of stomata was randomized vertically (across leaf length) before averaging adaxial and abaxial

measurements. On the adaxial side of the blade, when <10 stomata were not present in the impression, I measured the length of all stomata in the field of view and averaged stomatal length for each leaf surface. Some replicates of certain species, such as *Andropogon gerardii*, *Spartina pectinata*, *Bouteloua gracilis*, and *Tridens flavus*, had zero stomata present in the field of view on the adaxial surface, and had to be excluded from analyses that use SD_{ad} and SL_{ad} .

Leaf tissue processing

Leaf tissue was clipped within 2 – 3 days of response curve measurements and dried at 60 °C. An amalgamator was used to grind leaf tissue into a homogenous mix. The ground samples were combusted and measured with an Elementar vario Pyro cube and a subsequent Elementar Vision mass spectrometer to measure leaf C:N and $\delta^{13}C$. The abundance of isotopic ratios was converted to standard notation with Equation 1, where R is the ratio of the heavier atom (^{13}C) in the sample to the lighter atom (^{12}C) in the standard reference. The laboratory standards were calibrated against Vienna Pee-Dee Belemnite, the internationally accepted standard for $\delta^{13}C$.

Equation 1:

$$\delta = \left[\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right] * 1000$$

Data analysis

To preprocess data, I removed the entire replicate of any response curve that did not generate adequate stepwise changes in VPD_{leaf} due to instrument constraints, thermocouple contact with leaves, and/or misaligned timing of stepwise changes of VPD_{leaf} . Any data points where $A < 0 \mu mol CO_2 m^{-2} s^{-1}$ and $> 50 \mu mol CO_2 m^{-2} s^{-1}$, $g_{sw} < 0 mol H_2O m^{-2} s^{-1}$, and $E < 0 mmol H_2O m^{-2} s^{-1}$ were filtered out. When $[CO_2]$ of air inside the chamber $< 350 ppm$ because of chemical and/or biological constraints, the entire data point was removed. To visualize response

patterns over the 12-hour drying treatment, the mean with ± 1 standard error (SE) of each subtype response was plotted using LOESS smoothing method.

The effects of C₄ subtype, VPD_{leaf} phase, and their interaction on A, g_{sw}, and E rates of response of each species were analyzed using linear mixed-effects models (LMMs) with species nested within subtype groups and sample included as random intercepts to account for experimental hierarchies and repeated measures across drying phases. In an unnested design, I tested the effects of species, VPD_{leaf} phase, and their interaction on A, g_{sw}, and E rates within each of the three subtypes using LMMs and with sample included as a random intercept to account for repeated measures across drying phases. Fixed effects and the interactions between them were estimated with Type III ANOVA using the Kenward-Rogers method in the *emmeans* package (Lenth & Piaskowski, 2025). Pairwise comparisons of C₄ subtypes were conducted using Tukey-adjusted marginal means when significant effects or interactions were found ($\alpha = 0.05$).

To calculate stomatal sensitivity (m) for each of the subtypes, minutes 45 – 60 were subsampled from each one-hour change in VPD_{leaf} for “steady state” median g_{sw} values (Equation 2).

Equation 2:

$$m = -\Delta g_{sw} / \Delta \ln(\text{VPD}_{\text{leaf}})$$

Stepwise changes with insufficient log-scale changes in VPD_{leaf} were removed from calculations to avoid skewed estimates from small denominators. To normalize m across plants (m/g_{ref}), I created g_{ref}, which was calculated from the g_{sw} rates from the first hour of the response curve at 1 kPa VPD_{leaf}. To test if normalized sensitivity was different across C₄ subtypes, LMMs were run on each VPD_{leaf} transition (i.e., 1 → 2 kPa or 3 → 4 kPa) with subtype as a fixed effect and

sample as a random intercept. Type I ANOVA and Wald chi-squared tests for fixed effects on LMMs were completed with pairwise subtype comparisons using model estimated marginal means. $\delta^{13}\text{C}$ and microanatomical, stomatal, and structural traits were analyzed separately with one-way ANOVA and Bonferroni-adjusted pairwise comparisons for significantly different effects when identified ($\alpha = 0.05$). All statistical analyses were completed in RStudio (v4.4.2; R Core Team).

Results

Subtype-level responses to atmospheric drought

Overall, the wet-adapted NADP-ME subtype had the lowest gas exchange rates across the duration of the VPD response curve (Figure 2.1; Appendix Figure A.1). While the intermediate PCK subtype maintained the highest gas exchange rates of the three biochemical groups, the dry-adapted NAD-ME subtype followed strikingly similar temporal patterns, but with lower gas exchange rates (Figure 2.1; Appendix Figure A.1). Photosynthetic rates differed in the NADP-ME and PCK subtypes across the drying treatment and all C_4 subtypes declined in each of the four VPD_{leaf} phases, showing no post-drying exposure recovery in the 12-hour period ($p = 0.033$; Table 2.1; Figure 2.1; Appendix Figure A.1). Pairwise contrasts showed subtype differences were driven by PCK and NADP-ME groups. The PCK subtype maintained 31% and 35% higher mean photosynthetic rates than the NADP-ME subtype under increasing 1 – 3 kPa and high 4 – 4.5 kPa VPD_{leaf} , respectively ($p = 0.022$; $p = 0.040$; Appendix Table A.1). However, PCK subtype mean photosynthetic rates were only 12% and 7.5% higher, and not significantly different, than the NAD-ME subtype during the same drying periods ($p = 0.67$ and $p = 0.84$; Appendix Table A.1).

The dry-adapted NAD-ME subtype showed the smallest relative reductions in carbon capture over the 12-hour response curve (absolute decrease = $-6.0 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), with NADP-ME showing similar declines ($-7.0 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; Figure 2.1; Appendix Table A.1; Appendix Figure A.1). Due to higher initial rates of photosynthesis, the greatest magnitude of photosynthetic decline across the VPD_{leaf} regime was in the PCK subtype ($-9.1 \mu\text{mol m}^{-2} \text{ s}^{-1}$; Figure 2.1; Appendix Table A.1; Appendix Figure A.1). Despite differences in mean photosynthetic rates among the C_4 subtypes during the increasing and high VPD_{leaf} periods, no difference in species-specific photosynthetic responses to elevated VPD_{leaf} were found (Table 2.4).

Stomatal conductance rates did not vary across C_4 subtypes but decreased in all drying phases ($p = 0.15$; Figure 2.1; Table 2.3; Appendix Figure A.1). Over the 12-hour period, the NAD-ME subtype showed a 31% reduction in stomatal conductance rates across the VPD_{leaf} treatment, compared to a 34.9% decrease for intermediate PCK, and a 37.5% decrease for wet-adapted NADP-ME. The steepest negative downward trends in stomatal conductance for all C_4 subtypes were observed during the increasing 1 – 3 kPa (slope = -0.013 ± 0.00021) and high 4 – 4.5 kPa (slope = -0.012 ± 0.0015) phases of VPD_{leaf} (Appendix Figure A.1; Appendix Table A.3). No significant recovery in stomatal conductance was observed in the subtypes at this time scale, but rates of decline in all subtypes lessened during the decreasing 1 – 3 kPa and steady-state 1 kPa phases (Appendix Figure A.1; Figure 2.1).

I calculated sensitivity of stomatal conductance (m) to stepwise changes in VPD_{leaf} and report a normalized m/g_{ref} in Figure 2.5 (Oren 1999). Normalized stomatal sensitivity showed a clear trend that mirrored VPD_{leaf} increases and decreases across the response curve, with the highest sensitivities observed under 4 – 4.5 kPa VPD_{leaf} (Figure 2.5). Normalized stomatal

sensitivity was only different for subtypes within the first 1 → 2 kPa VPD_{leaf} timestep ($p = 0.027$; Table 2.5) and m/g_{ref} did not vary across C₄ subtypes in any other VPD_{leaf} timesteps. At the beginning portion of the response curve, m/g_{ref} of the PCK subtype varied from that of the NAD-ME subtype ($p = 0.042$; Appendix Table A.5) but did not significantly vary from the NADP-ME subtype ($p = 0.051$; Appendix Table A.5). The NADP-ME and NAD-ME subtypes showed remarkably similar stomatal sensitivities to high VPD_{leaf} ($p = 0.99$; Appendix Table A.5).

To contextualize these results with prior reported m values, I provided non-normalized m results in Appendix Table A.10. During increasing VPD_{leaf} ranging from 1 – 3 kPa, there were low to moderate stomatal sensitivities for all C₄ subtypes, with median m ranging between 13.1 – 60.5 mmol m⁻² s⁻¹ ln(kPa)⁻¹ (interquartile range (IQR) = -25.0 – 106.6; Figure 2.5; Appendix Table A.10). Stomatal sensitivity at the 3 → 4 kPa transition ranged between 92.4 – 117.1 mmol m⁻² s⁻¹ ln(kPa)⁻¹ across subtypes (IQR = 60.0 – 179.1; Figure 2.5; Appendix Table A.10). As VPD_{leaf} peaked at 4 → 4.5 kPa, median m across subtypes ranged between 123.8 – 201.9 mmol m⁻² s⁻¹ ln(kPa)⁻¹ (IQR = 100.6 – 239.9; Figure 2.5; Appendix Table A.10). Once the decline of VPD_{leaf} began at 4.5 → 4 kPa, there was a steep reduction in stomatal sensitivity with median m ranging from 10.9 – 89.04 mmol m⁻² s⁻¹ ln(kPa)⁻¹ (IQR = -18.4 – 133.6; Figure 2.5; Appendix Table A.10). During decreases in VPD_{leaf} from 3 → 2 kPa and 2 → 1 kPa negative median m values ranging from -39.9 to -14.9 mmol m⁻² s⁻¹ ln(kPa)⁻¹ were recorded (IQR = -40.8 – 6.9 Figure 2.5; Appendix Table A.10).

Transpiration rates were not different among C₄ subtypes ($p = 0.13$; Figure 2.1; Table 2.3). Subtype transpiration rates showed the greatest relative separation under high VPD_{leaf} at 4 – 4.5 kPa, but subtypes responses converged when VPD_{leaf} returned to 1 kPa (subtype × VPD_{leaf}

phase $p = 0.024$; Figure 2.1; Table 2.3). Transpiration increased alongside VPD_{leaf} during the increasing 1 – 3 kPa VPD_{leaf} phase and promptly decreased under high 4 – 4.5 kPa VPD_{leaf} , trending downward for the remainder of the response curve (Figure 2.1; Appendix Figure A.1; Appendix Table A.3). By the steady-state 1 kPa phase, transpiration rates of all C_4 subtypes stabilized, with the dry-adapted NAD-ME subtype and intermediate PCK subtype exhibiting nearly identical behavior during the last phase of the curve ($p = 0.998$; Appendix Table A.1).

Species-level responses to atmospheric drought

No species-level differences in photosynthetic rates were found within each of the C_4 subtypes, and no differences in stomatal conductance and transpiration were found in NADP-ME and PCK species responses (Table 2.4; Figures 2.2, 2.3, and 2.4). Stability in NAD-ME photosynthetic function under elevated VPD_{leaf} was supported by variation in species-specific water use within the NAD-ME subtype (Figure 2.4). Notably, *Schedonnardus paniculatus* nearly recovered to starting photosynthetic rates (beginning at $\sim 25 \mu\text{mol m}^{-2} \text{s}^{-1}$ and ending at $\sim 23 \mu\text{mol m}^{-2} \text{s}^{-1}$) when VPD_{leaf} returned to 1 kPa at hour 9 (Figure 2.4). Despite similarities in photosynthetic rates within the NAD-ME group ($p = 0.096$; Table 2.4), NAD-ME-dependent species showed intra-subtype differences in stomatal conductance and transpiration ($p = 0.047$ and $p = 0.024$, respectively; Table 2.4; Figure 2.4).

During the decreasing 3 – 1 kPa and steady-state 1 kPa phases of the curve, NAD-ME species *Eragrostis spectabilis* and *Muhlenbergia cuspidata* showed the largest differences in stomatal conductance rates ($p = 0.038$ at 3 – 1 kPa VPD_{leaf} and $p = 0.0056$ at 1 kPa VPD_{leaf}). Recovery of stomatal conductance rates post-drying was only observed in the NAD-ME, dry-adapted *Muhlenbergia cuspidata* at this time scale (Figure 2.4; Appendix Figure A.2). Under high 4 – 4.5 VPD_{leaf} , transpiration responses of *Muhlenbergia cuspidata* behaved differently

from *Bouteloua gracilis* ($p = 0.027$), *Eragrostis spectabilis* ($p = 0.00019$), and *Schedonnardus paniculatus* ($p = 0.022$), and almost differed from *Panicum virgatum* ($p = 0.052$; Appendix Table A.2). Species responses within each of the NADP-ME and PCK groups did not vary in g_{sw} or E responses to the VPD_{leaf} drought treatment ($p = 0.198$ and $p = 0.083$; Table 2.4; Figures 2.2 and 2.3).

Microanatomical, stomatal, and structural traits and stable isotope signatures

Large differences in subtype bundle sheath cell area were found in both major and minor veins ($p < 0.001$ and $p < 0.001$, respectively; Figure 2.6; Table 2.6). Differences in bundle sheath cell sizes drove differences in BSC:MSC for major and minor veins ($p < 0.001$ and $p < 0.005$, respectively; Figure 2.6; Table 2.6), but mesophyll cell area did not vary. Differences in bundle sheath cell areas across subtypes were driven by the NADP-ME group, which had significantly smaller bundle sheath cells than the PCK and NAD-ME groups ($p < 0.01$; Appendix Table A.6). Vessel area of minor and major veins and xylem area and diameter did not vary across C_4 subtypes, but the average distance between vessels was different ($p < 0.005$; Figure 2.6; Table 2.6) due to large differences in interveinal distance between the NADP-ME and NAD-ME subtypes ($p < 0.01$; Appendix Table A.6).

Stomatal size and density varied widely across subtypes, with the NADP-ME-dependent species having larger, sparsely distributed stomata relative to the other subtypes (SD_{ad} $p < 0.001$ and SD_{ab} $p < 0.05$; SL_{ad} and SL_{ab} $p < 0.01$; Table 2.7; Figure 2.7; Appendix Table A.7). The PCK and NAD-ME plants showed similar stomatal lengths on both sides of leaves, and similar stomatal density on the tops of leaves (Figure 2.7; Appendix Table A.7). The only significant difference observed in the abaxial density of stomata was between the intermediate PCK and wet-adapted NADP-ME subtypes.

Enzymatic discrimination and bundle sheath leakiness of CO₂ were assessed using $\delta^{13}\text{C}$ isotopic signatures and differed by C₄ subtype ($p = 0.0299$) and species ($p = 0.00551$; Table 2.10; Figure 2.8; Figure 2.9). The NADP-ME group had the highest $\delta^{13}\text{C}$ of the subtypes and the lowest variation (-15.9 ± 0.144), while $\delta^{13}\text{C}$ values for the PCK and NAD-ME subtypes were similar (PCK = -17.4 ± 0.402 and NAD-ME = -17.2 ± 0.574). Global variation in $\delta^{13}\text{C}$ shown in the subtype-level model were due largely to contrasts between the intermediate PCK and wet adapted NADP-ME groups ($p = 0.0421$; Figure 2.8; Appendix Table A.8). Species differences in $\delta^{13}\text{C}$ across subtypes were driven by *Bouteloua gracilis*, which varied from the wet-adapted *Andropogon gerardii* and *Sorghastrum nutans*, the intermediate *Spartina pectinata*, and the dry-adapted *Panicum virgatum* ($p < 0.05$; Figure 2.9; Appendix Table A.9). No differences in leaf C:N were found across subtypes or species ($p > 0.05$, Figures 2.10 and 2.11).

Discussion

The main objective of this research was to quantify the physiological and anatomical strategies driving C₄ biochemical subtype responses to atmospheric water deficits. To isolate the effects of high VPD_{leaf} treatment on subtype function, I eliminated soil water deficits and measured the gas exchange rates of 15 species for 12 consecutive hours. Post-drought, I included a return to initial low VPD_{leaf} and held chamber conditions steady for the last 3 hours of the response curve in an effort to measure the recovery of the C₄ subtypes. To complement these leaf-level physiological metrics, microanatomical and stomatal traits were measured on the same portion of each leaf we droughted. Together these data illustrate how the water use strategies of the C₄ subtypes vary.

Stomatal sensitivity and recovery of C₄ grasses to VPD_{leaf}

Stomatal sensitivity to water vapor deficits plays a critical role in leaf carbon and water regulation (Ocheltree *et al.*, 2014). Our current understanding of how C₄ plant stomata respond to elevated VPD_{leaf} is largely based on crop physiology, yet the history of domestication in C₄ crops like maize, sorghum, and millet limits application of that knowledge to natural grassland systems (Lobell *et al.*, 2014). Prior morphological and physiological evidence suggests that grasses should have highly responsive stomata due to their dumbbell-shaped guard cells (i.e., epidermal cells responsible for regulating stomatal opening and closure), acting quickly to reduce water loss via transpiration during water deficits, and recovering quickly once stress has ceased (Johnsson *et al.*, 1976; Franks & Farquhar 2007). Franks and Farquhar (2007) posited that anatomical coordination in guard cell turgor and adjacent subsidiary cell turgor yield an osmotic ‘see-saw’ that allows both rapid stomatal opening under high humidity and rapid stomatal closure under high VPD.

Here, I review non-normalized m results from this study alongside results of published stomatal sensitivities, calculated according to Equation 2 in “Methods” (Appendix Table A.10; Ho *et al.*, unpublished; Oren 1999). During the increasing phase, where sensitivity of stomatal conductance to water vapor deficits (m) was calculated for timesteps 1 → 2 kPa and 2 → 3 kPa, median m values for C₄ subtypes were comparable to stomatal sensitivities of non-monocots (including both herbaceous and woody species) ranging between ~ 0 – 100 mmol m⁻² s⁻¹ ln(kPa)⁻¹ (Ho *et al.*, unpublished; Appendix Table A.10). At the 3 → 4 kPa transition, m results ranged between 90 - 120 mmol m⁻² s⁻¹ ln(kPa)⁻¹ and were most comparable with those of Magnoliids and ferns (Ho *et al.*, unpublished; Appendix Table A.10). Only as VPD_{leaf} changed from 4 → 4.5 kPa did we see higher m values that may be comparable to C₄ crops, where median m ranged from 120 to 200 mmol m⁻² s⁻¹ ln(kPa)⁻¹ (Ho *et al.*, unpublished; Appendix Table A.10).

The lack of stomatal recovery following reductions in high VPD_{leaf} exposure suggests that dumbbell-shaped guard cells do not elicit rapid recovery post-drought (Figure 2.5; Figure 2.1; Appendix figure A.1). According to Equation 2, negative m values indicate stomata continued to downregulate conductance although VPD_{leaf} declined and eventually returned to 1 kPa in the treatment (Figure 2.5; Appendix Table A.10). The lag in recovery at this time scale may be explained by increases in foliar abscisic acid (ABA) inside guard cells under high VPD_{leaf} (McAdam & Brodribb, 2016; McAdam & Brodribb, 2015). ABA increases guard cell turgidity, allowing tighter stomatal regulation during water vapor deficits. In herbaceous angiosperms, hysteretic stomatal dynamics emerge, as slow declines in foliar ABA correspond with very slow recovery in stomatal conductance rates after exposure to elevated VPD_{leaf} (McAdam & Brodribb, 2015).

NADP-ME subtype prioritizes efficiency over safety

Throughout the response curve, the C_4 subtypes ultimately restricted water loss at similar rates but had varying rates of assimilation under elevated VPD_{leaf} at this time scale. In all gas exchange metrics, the NADP-ME type showed consistently lower tolerance to high VPD_{leaf} and displayed more wet-adapted physiological and anatomical traits overall. The lowest relative stomatal conductance and transpiration rates were observed in NADP-ME-dependent plants, indicating a strong downregulation of stomatal conductance and simultaneous reductions in photosynthetic rates (Figure 2.1). These wet-adapted species contain larger, sparsely packed stomata on both sides of their leaves (Figure 2.7). This permits high photosynthetic maxima during ideal conditions but during drought, forces the few available stomata to close and drastically reduce carbon assimilation (Figure 2.1; Figure 2.7). Notably, the lowest relative amount of intra-subtype variation in physiological and anatomical traits were seen in NADP-

ME-dependent species, including gas exchange responses to high VPD_{leaf} , $\delta^{13}C$, mesophyll and bundle sheath cell areas, and stomatal density.

PCK and NAD-ME subtypes prioritize safety

The PCK group maintained the highest relative gas exchange rates during the increasing, high, and decreasing portions of the VPD_{leaf} response curve (Figure 2.1). However, PCK and NAD-ME gas exchange responses, particularly transpiration rates, converge when VPD_{leaf} returns to 1 kPa for the last 4 hours of the treatment ($p = 0.998$; Appendix Table A.1). The parallel shape of the PCK and NAD-ME response curves, although exhibiting different relative rates, may be explained by similar selective pressures acting on Chloridoideae throughout the evolutionary history of the PCK and NAD-ME biochemical pathways. All 5 PCK species, and 4 of 5 NAD-ME species collected from KPBS (excluding *Panicum virgatum* from Panicoideae in the MPC clade) reside in Chloridoideae. In addition to gas exchange responses, I found similar bundle sheath cell areas, BSC:MSC, adaxial and stomatal length and density, and $\delta^{13}C$ isotopic signatures among the PCK and NAD-ME subtypes.

Coordination across physiological and anatomical traits

Variation in anatomical traits and the magnitude of difference between subtype gas exchange responses demonstrate the coordination in grass form and function under atmospheric drought. The differences in photosynthetic rates of the C_4 subtypes may be explained in part by variations seen in anatomical traits. These results show larger bundle sheath cells in PCK- and NAD-ME-dependent species in contrast with the smaller bundle sheath cells observed in the NADP-ME subtype. Recent evidence suggests that enlarged bulliform (not measured here) and bundle sheath cells act as water capacitors, storing subcellular water to maintain photosynthesis

during drought (Ho 2024; Bachle 2021). This interpretation is supported by measured anatomical traits and gas exchange responses (Figure 2.1; Figure 2.6).

We also observed variation in mesophyll chloroplast density across subtypes, which may interact with varying interveinal distance across C₄ groups. The NADP-ME anatomical type showed small bundle sheath cells with tightly packed chloroplasts in adjacent mesophyll cells. The lower interveinal distance in the NADP-ME group facilitates increased chloroplast density in the mesophyll, increasing CO₂ diffusion in the mesophyll and thus, photosynthetic capacity (Figure 2.6; Ghannoum *et al.*, 2025; Flexas *et al.*, 2012; Evans 2020). Conversely, the NAD-ME subtype anatomy showed larger relative bundle sheath cell areas and higher interveinal distance, but with sparsely packed mesophyll chloroplasts (Figure 2.6). With fewer chloroplasts residing in the mesophyll, the bundle sheath cell chloroplasts receive more light penetration and can thus increase adenosine triphosphate (ATP) production and regeneration of RuBisCO downstream (Bellasio & Lundgren 2016; Sage 2004). The PCK subtype, however, showed a combination of these patterns, hosting large bundle sheath cells and sparsely packed mesophyll chloroplasts like the NAD-ME type, but lower relative interveinal distance like the NADP-ME type (Figure 2.6).

Differences in isotopic composition across the C₄ subtypes indicated variation in bundle sheath leakiness, integrated stomatal behavior, and/or metabolic divergence (Hatch *et al.*, 1995; Farquhar 1983; Furbank 2011; Isreal *et al.*, 2026). The highest relative $\delta^{13}\text{C}$ of the subtypes in the NADP-ME group suggests NADP-ME-dependent species had the lowest rates of bundle sheath leakiness and corroborates the low relative stomatal conductance rates observed under elevated VPD_{leaf} (Farquhar 1983; Hatch *et al.*, 1995; Ouyang *et al.*, 2024). The similarities in $\delta^{13}\text{C}$ between PCK and NAD-ME subtypes aligns with published leakiness values (Ouyang *et*

al., 2024), and the parallel stomatal behaviors observed during the drought response curve (Figure 2.1).

Conclusion

Based on these results, I argue that all three C₄ subtypes use distinct water loss strategies that impact their photosynthetic capacity. This is supported by subtype patterns observed in i) bundle sheath cell area and photosynthetic rate, ii) stomatal length and density and stomatal conductance and sensitivity, and iii) $\delta^{13}\text{C}$ stable isotope signatures. Only with this combination of physiological and anatomical data was it possible to parse out the covert behaviors of water and carbon regulation in the C₄ biochemical subtypes. The nuanced biochemistry of these C₄ plants reflects their expansive evolutionary origins, particularly for the PCK and NAD-ME types (Furbank 2011; Chapman 1992). While significant overlap in PCK and NAD-ME responses to high VPD_{leaf} was observed, many traits, including photosynthetic rates, abaxial stomatal density, $\delta^{13}\text{C}$, and interveinal distance, were unique to each subtype. In addition, because plant taxa and biochemical subtypes do not follow the same geographic distributions, it is appropriate to infer that they do not have synonymous impacts on plant water use.

These results illustrate the inherent complexity of the biochemical variants in C₄ photosynthesis. As a tool for simplification, one can imagine a spectrum of plant water use, where the wet-adapted, NADP-ME group resides near the “efficient” end of the spectrum, and the NAD-ME group exists near the “safe” end of the spectrum. While I initially assumed the “intermediate” PCK group may reside in the middle of the spectrum because of the highly variable ecological and physiological responses to water availability, these results suggest that, at least in North American grasslands, the PCK subtype physiology is more aligned with the dry-adapted, NAD-ME subtype. Despite that, I argue that we must maintain the “intermediate”

designation for PCK-dependent species, because many geographical and biochemical caveats remain, and may only be addressed with measurements of enzyme activity and physiological metrics across a global representation of C₄ grasses.

Understanding the responses of C₄ grasses to atmospheric drought is a critical part of quantifying ecosystem processes, especially with a rapidly changing climate. As the frequency and magnitude of drought events is further exacerbated by increasing global temperatures and its downstream effects, preparation through knowledge of the ecological and physiological processes is essential. Navigating this uncertainty, I expect that efficacy of grouping the functional roles of C₄ grass together by subtype or by lineage (as LFTs) will vary by location, temporal and spatial scale, and by the ecological and/or the physiological responses researchers aim to measure.

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Tables and Figures

Table 0.1: List of the 15 species of C₄ grass measured.

Tribe	Biochemical Subtype	Species	Species Code
Andropogoneae	NADP-ME	<i>Andropogon gerardii</i>	ANGE
Andropogoneae	NADP-ME	<i>Schizachyrium scoparium</i>	SCSC
Andropogoneae	NADP-ME	<i>Sorghastrum nutans</i>	SONU
Cynodonteae	NAD-ME	<i>Bouteloua gracilis</i>	BOGR
Cynodonteae	PCK	<i>Bouteloua hirsuta</i>	BOHI
Cynodonteae	PCK	<i>Chloris verticallata</i>	CHVE
Cynodonteae	NAD-ME	<i>Muhlenbergia cuspidata</i>	MUCU
Cynodonteae	PCK	<i>Muhlenbergia racemosa</i>	MURA
Cynodonteae	NAD-ME	<i>Schedonnardus paniculatus</i>	SCPA
Cynodonteae	PCK	<i>Tridens flavus</i>	TRFL
Eragrostideae	NAD-ME	<i>Eragrostis spectabilis</i>	ERSP
Paspaleae	NADP-ME	<i>Paspalum pubiflorum</i>	PAPU
Paniceae	NADP-ME	<i>Digitaria cognata</i>	DICO
Paniceae	NAD-ME	<i>Panicum virgatum</i>	PAVI
Zoysieae	PCK	<i>Spartina pectinata</i>	SPPE

Table 0.2: List of all traits measured, abbreviations, and units.

Type	Trait	Abbreviations	Units
Anatomical	Vessel Area	V_a	μm^2
	Interveinal Distance	IVD	mm
	Xylem Area	X_a	μm^2
	Xylem Diameter	X_d	μm
	Bundle Sheath Cell Area	BSC_a	μm^2
	Mesophyll Cell Area	MSC_a	μm^2
	Bundle Sheath Cell:Mesophyll Cell	BSC:MSC	dimensionless ratio
	Adaxial Stomatal Density	SD_{ad}	stomata/ μm^2
	Abaxial Stomatal Density	SD_{ab}	stomata/ μm^2
	Adaxial Stomatal Length	SL_{ad}	μm
	Adaxial Stomatal Length	SL_{ab}	μm
Structural	Leaf Thickness	Lt	mm
	Leaf Carbon:Leaf Nitrogen	C:N	dimensionless ratio
Physiological	Vapor Pressure Deficit of air	VPD_{air}	kPa
	Vapor Pressure Deficit at leaf-air interface	VPD_{leaf}	kPa
	^{13}C stable isotopic signature	$\delta^{13}\text{C}$	(‰)
	Photosynthetic Rate	A	$\mu\text{mol m}^{-2} \text{s}^{-1}$
	Stomatal Conductance	g_{sw}	$\text{mol m}^{-2} \text{s}^{-1}$
	Leaf Transpiration Rate	E	$\text{mmol m}^{-2} \text{s}^{-1}$
	Stomatal Sensitivity	m	$\text{mmol m}^{-2} \text{s}^{-1} \ln(\text{kPa})^{-1}$
	Normalized Stomatal Sensitivity	m/g_{ref}	dimensionless log scale

Table 0.3: Type III ANOVA results of LMM testing the effects of biochemical subtype, VPD_{leaf} phase, and their interaction on mean A, g_{sw}, and E of 15 species of C₄ grass.

Response	Effect	NumDF	DenDF	F-value	p-value	Significance
A	Subtype	2	11.55192	4.63	0.0333	*
A	VPD _{leaf} phase	3	348.313	52.2	< 0.001	***
A	Subtype x VPD _{leaf} phase	6	348.305	0.45	0.842	ns
g _{sw}	Subtype	2	11.1618	2.26	0.149	ns
g _{sw}	VPD _{leaf} phase	3	348.3613	86.38	< 0.001	***
g _{sw}	Subtype x VPD _{leaf} phase	6	348.356	0.63	0.707	ns
E	Subtype	2	11.13777	2.5	0.126	ns
E	VPD _{leaf} phase	3	347.6519	187.16	< 0.001	***
E	Subtype x VPD _{leaf} phase	6	347.6441	2.47	0.0235	*

Table 0.4: Type III ANOVA results of LMM testing the effects of species, VPD_{leaf} phase, and their interaction on mean A, g_{sw}, and E of 15 species of C₄ grass.

Response	Subtype	Effect	NumDF	DenDF	F-value	p-value	sig
A	NADP-ME	Species	4	27.6	1.27	0.306	
A	NADP-ME	Species x VPD _{leaf} phase	12	120	0.29	0.99	
A	NADP-ME	VPD _{leaf} phase	3	120	16.37	5.55E-09	***
A	NAD-ME	Species	4	20.3	2.28	0.0958	.
A	NAD-ME	Species x VPD _{leaf} phase	12	106.5	0.16	0.999	
A	NAD-ME	VPD _{leaf} phase	3	106.6	14.18	7.61E-08	***
A	PCK	Species	4	21.7	1.21	0.333	
A	PCK	Species x VPD _{leaf} phase	12	86.2	0.3	0.989	
A	PCK	VPD _{leaf} phase	3	86.2	12.06	1.15E-06	***
E	NADP-ME	Species	4	27.7	1.38	0.266	
E	NADP-ME	Species x VPD _{leaf} phase	12	120.2	0.49	0.917	
E	NADP-ME	VPD _{leaf} phase	3	120.3	36.1	1.04E-16	***
E	NAD-ME	Species	4	19.8	3.58	0.0235	*
E	NAD-ME	Species x VPD _{leaf} phase	12	106.1	1.07	0.39	
E	NAD-ME	VPD _{leaf} phase	3	106.3	64.39	8.3E-24	***
E	PCK	Species	4	22.1	2.41	0.0796	.
E	PCK	Species x VPD _{leaf} phase	12	86.3	0.57	0.858	
E	PCK	VPD _{leaf} phase	3	86.3	75.82	4.12E-24	***
g _{sw}	NADP-ME	Species	4	27.8	1.62	0.198	
g _{sw}	NADP-ME	Species x VPD _{leaf} phase	12	120.2	0.32	0.985	
g _{sw}	NADP-ME	VPD _{leaf} phase	3	120.2	18.18	8.56E-10	***
g _{sw}	NAD-ME	Species	4	20.1	2.93	0.0467	*
g _{sw}	NAD-ME	Species x VPD _{leaf} phase	12	106.3	0.32	0.984	
g _{sw}	NAD-ME	VPD _{leaf} phase	3	106.4	22.09	3.39E-11	***
g _{sw}	PCK	Species	4	22.5	2.37	0.0826	.
g _{sw}	PCK	Species x VPD _{leaf} phase	12	86.6	0.7	0.751	
g _{sw}	PCK	VPD _{leaf} phase	3	86.6	40.52	1.85E-16	***

Table 0.5: Table of Type III ANOVA results using Satterthwaite's method to show subtype differences in normalized stomatal sensitivity (m/g_{ref}) at each VPD_{leaf} transition.

transition	term	Sum Sq	Mean Sq	NumDF	DenDF	F-value	P-value
1 $\rightarrow$ 2 kPa	subtype	2.246155	1.123077	2	99	3.740424	0.027165
2 $\rightarrow$ 3 kPa	subtype	0.003936	0.001968	2	108	0.023694	0.97659
3 $\rightarrow$ 4 kPa	subtype	0.181411	0.090706	2	104	0.621073	0.539349
4 $\rightarrow$ 4.5 kPa	subtype	0.773239	0.38662	2	14	0.730954	0.498947
4.5 $\rightarrow$ 4 kPa	subtype	0.000287	0.000143	2	11	0.302703	0.74478
4 $\rightarrow$ 3 kPa	subtype	0.02763	0.013815	2	53.34247	0.197799	0.821134
3 $\rightarrow$ 2 kPa	subtype	0.043462	0.021731	2	52.70759	0.363287	0.697115
2 $\rightarrow$ 1 kPa	subtype	0.179005	0.089503	2	77	1.127914	0.328997

Table 0.6: Type I ANOVA results showing differences in microanatomical traits across subtype.

Response	df	statistic	p-value
Major xylem area	2	1.16	0.32
Major xylem diameter	2	1.38	0.259
Major vessel area	2	0.67	0.515
Minor vessel area	2	2.33	0.105
Major bundle sheath cell area	2	10.99	6.96E-05
Minor bundle sheath cell area	2	11.17	6.04E-05
Major mesophyll cell area	2	0.86	0.427
Minor mesophyll cell area	2	1.83	0.168
Major BSC:MSC	2	10.45	0.000107
Minor BSC:MSC	2	6.65	0.00227
Leaf thickness	2	0.04	0.956
Interveinal distance	2	6.64	0.00218

Table 0.7: Type I ANOVA results of stomatal traits across C₄ subtypes.

Trait	Df	Sum Sq	Mean Sq	F-value	p-value
Abaxial stomatal density	2	66914.15	33457.08	4.543634	0.013905
Adaxial stomatal density	2	229190.9	114595.5	15.92497	2.27E-06
Abaxial stomatal length	2	0.000699	0.00035	7.552285	0.001062
Adaxial stomatal length	2	0.00062	0.00031	9.802031	0.000182

Table 0.8: Type I ANOVA results table showing differences in leaf $\delta^{13}\text{C}$ and leaf C:N across the C_4 subtypes and across the 15 C_4 grass species measured.

Response	Effect	Df	Sum Sq	Mean Sq	F-value	p-value
$\delta^{13}\text{C}$	subtype	3	31.1594	10.3865	2.599	0.0584
C:N	subtype	3	490.7598	163.5866	2.304	0.0837
$\delta^{13}\text{C}$	species	14	125.8741	8.991	2.595	0.00551
C:N	species	14	1562.97	111.6407	1.718	0.0762

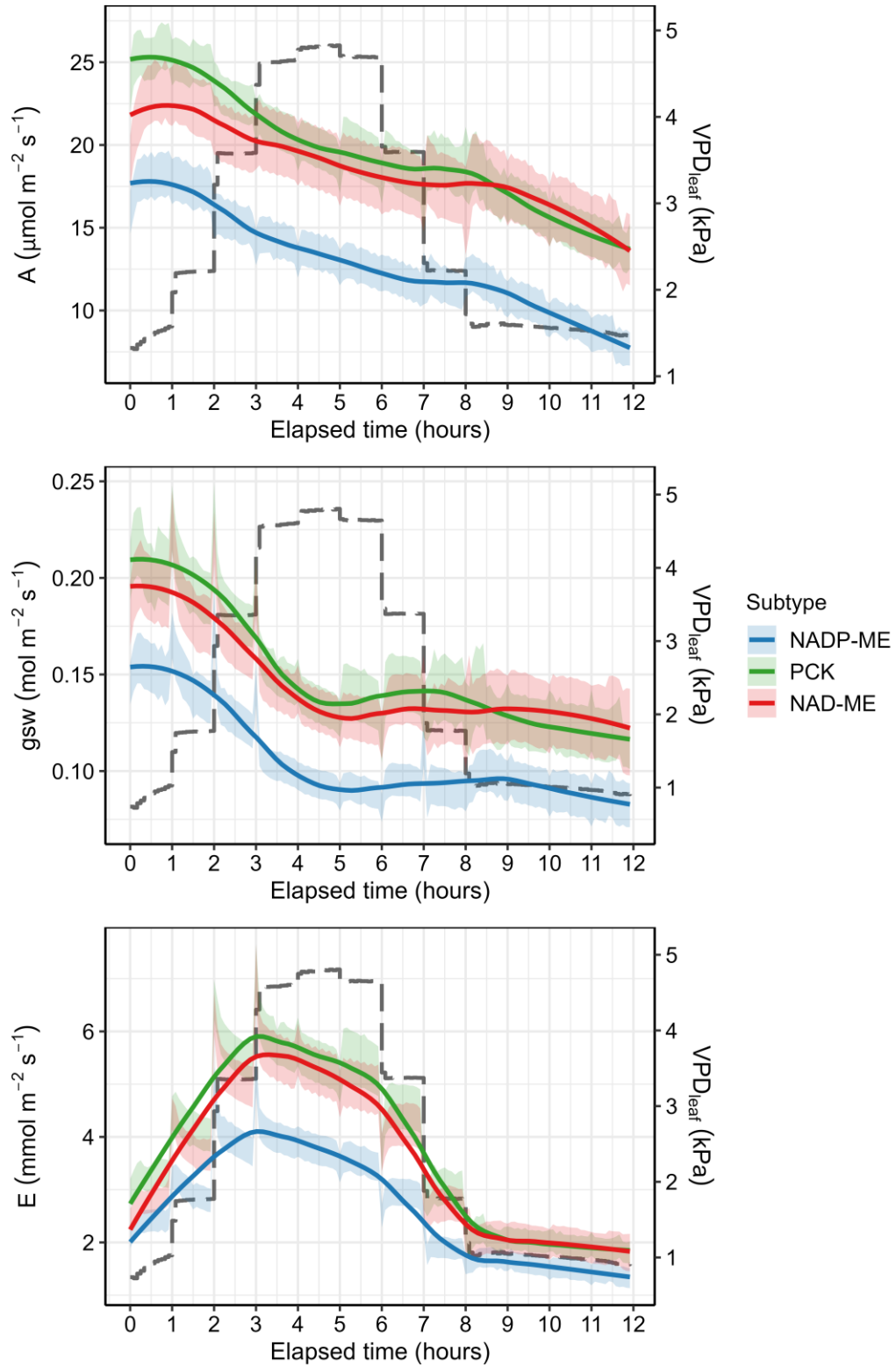


Figure 0.1: Mean responses of A , g_{sw} , and E to 12-hour VPD_{leaf} treatment across C_4 subtypes. Solid lines show LOESS-smoothed means with ± 1 SE. Dashed lines show mean VPD_{leaf} changes over time.

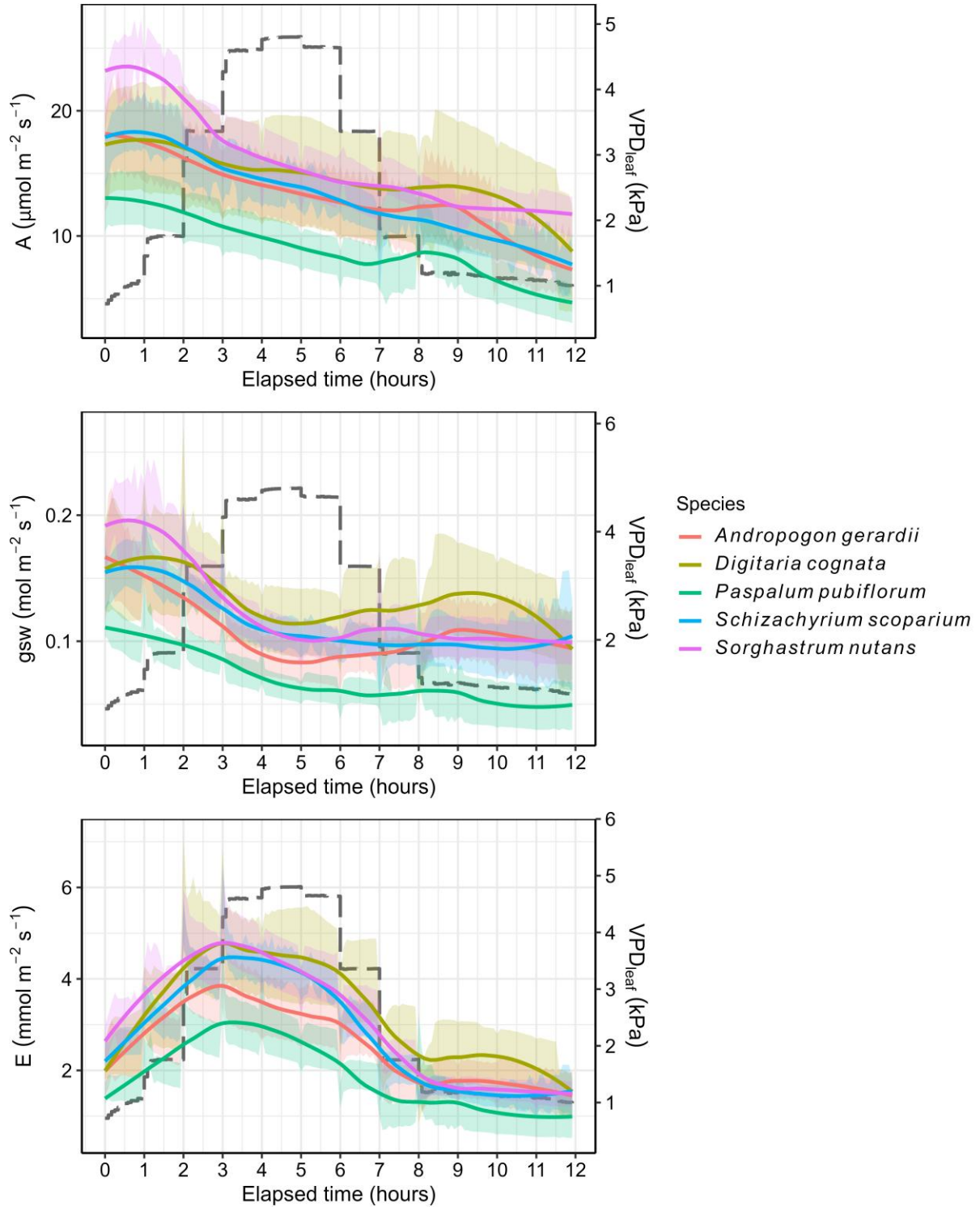


Figure 0.2: Mean responses of A , g_{sw} , and E to 12-hour VPD_{leaf} treatment within NADP-ME C₄ subtype. Solid lines show LOESS-smoothed means with ± 1 SE. Dashed lines show mean VPD_{leaf} changes over time.

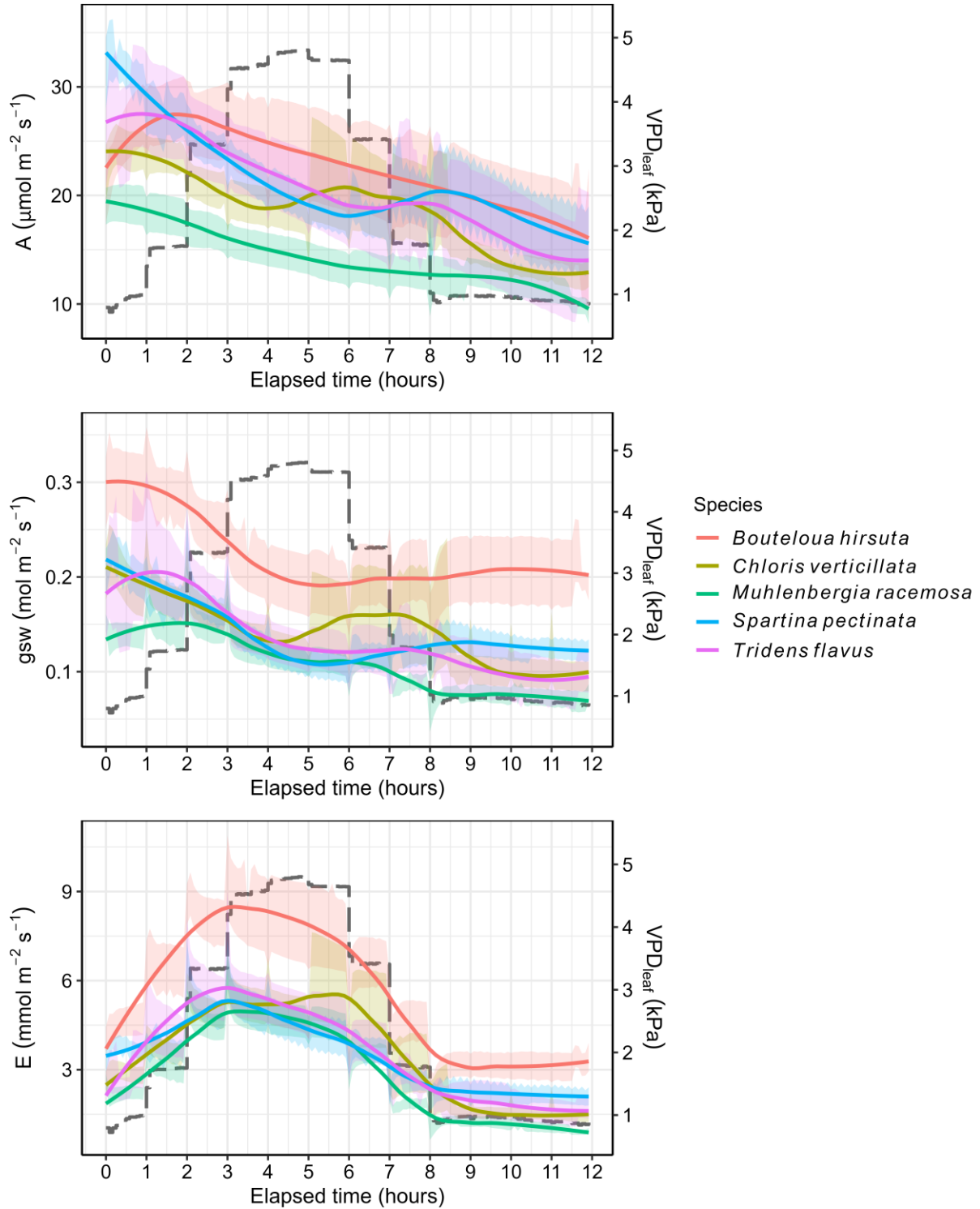


Figure 0.3: Mean responses of A , g_{sw} , and E to 12-hour VPD_{leaf} treatment within PCK C₄ subtype. Solid lines show LOESS-smoothed means with ± 1 SE. Dashed lines show mean VPD_{leaf} changes over time.

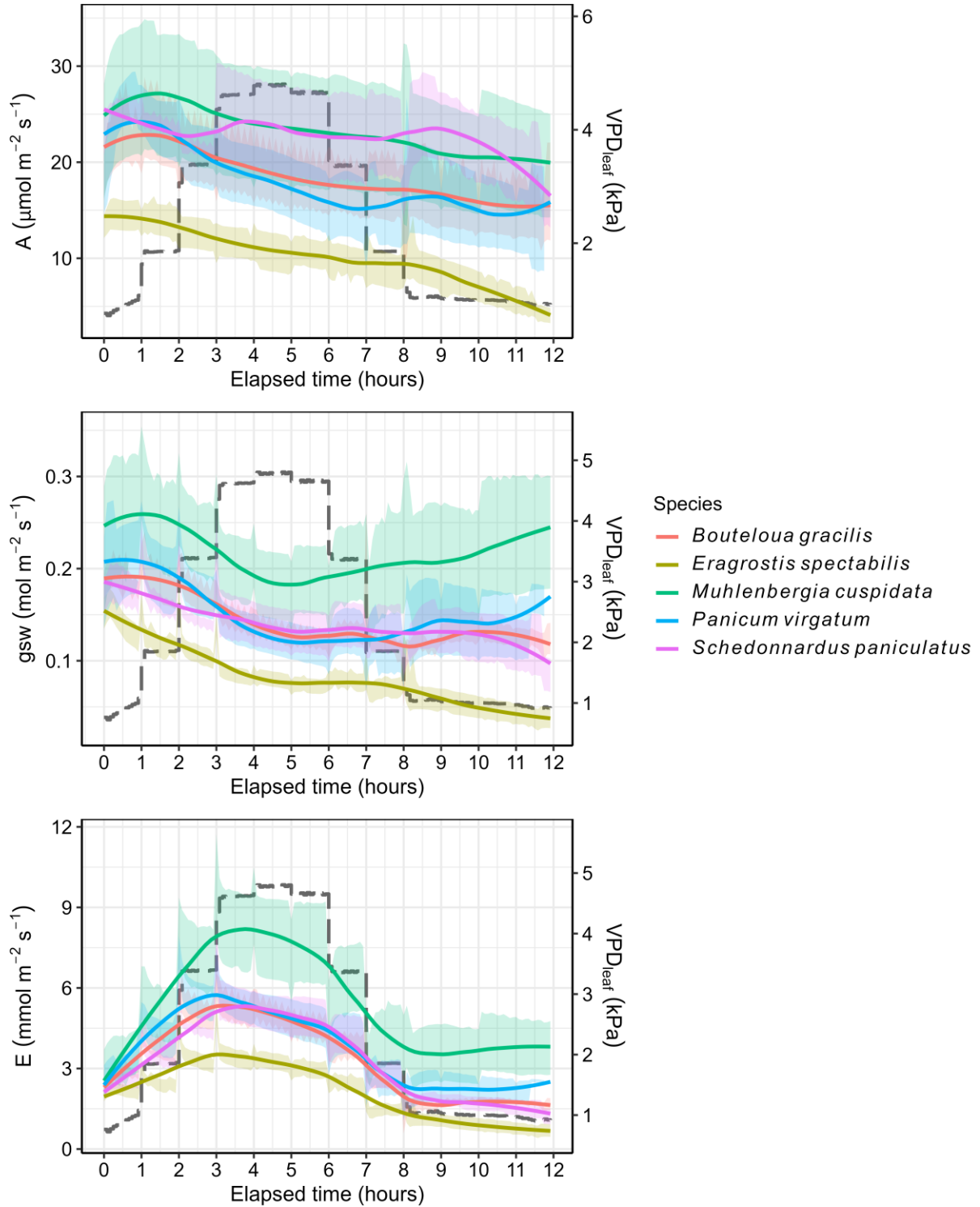


Figure 0.4: Mean responses of A , g_{sw} , and E to 12-hour VPD_{leaf} treatment within NAD-ME C₄ subtype. Solid lines show LOESS-smoothed means with ± 1 SE. Dashed lines show mean VPD_{leaf} changes over time.

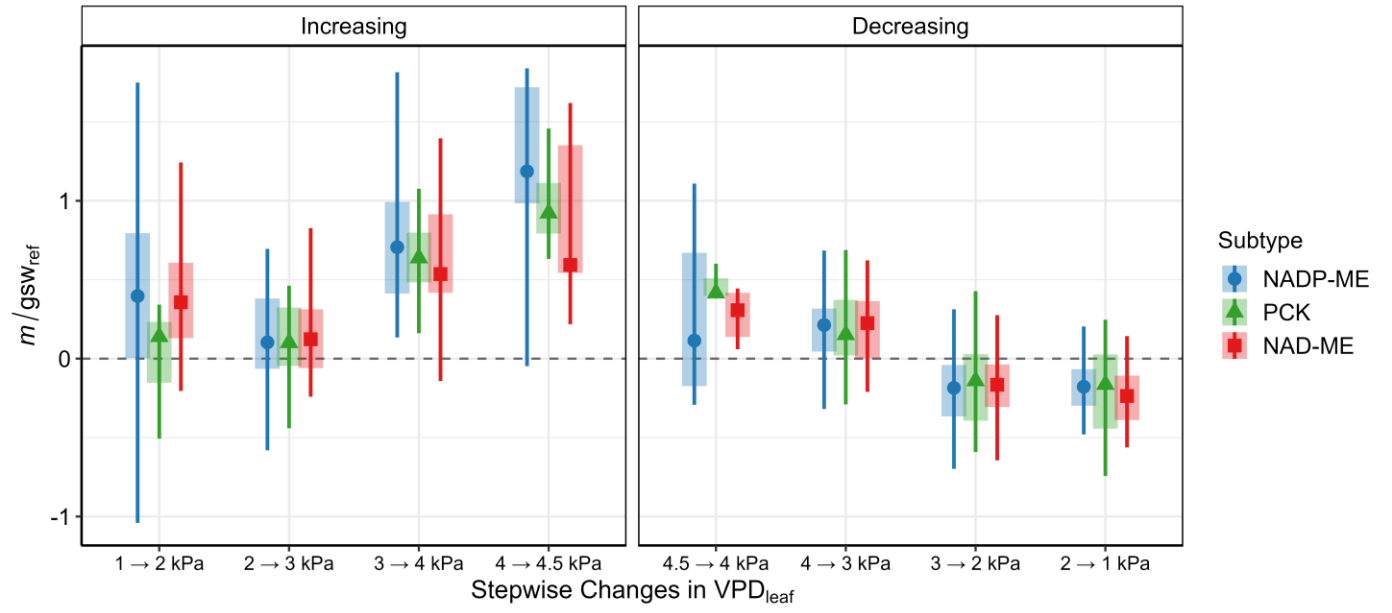


Figure 0.5: Normalized stomatal sensitivity (m/g_{ref}) of each C4 subtype calculated for each stepwise change in VPD_{leaf} .

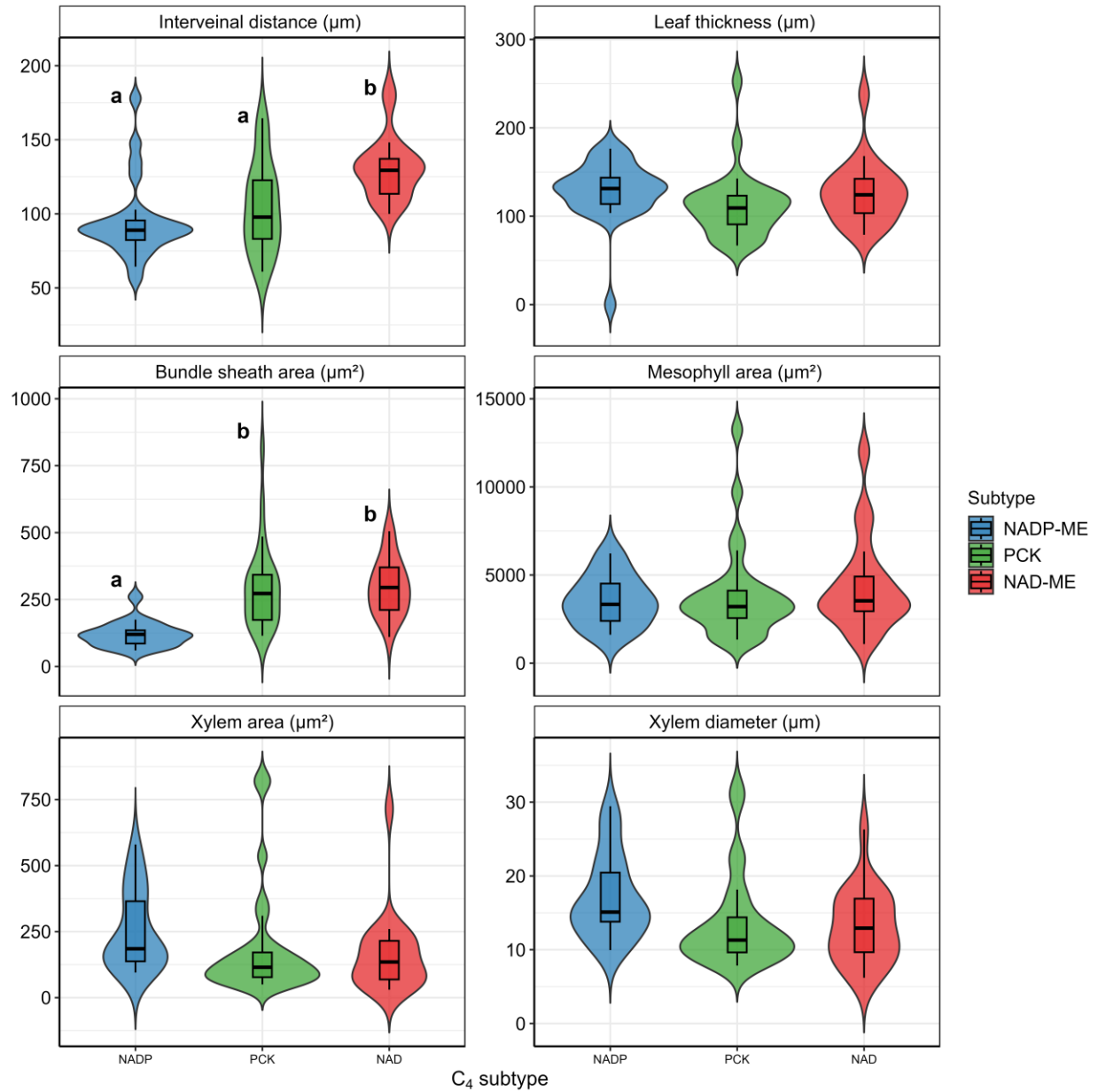


Figure 0.6: Violin plots showing microanatomical traits grouped by C₄ subtype. Letters indicate significant differences calculated with Bonferroni-adjusted pairwise comparisons.

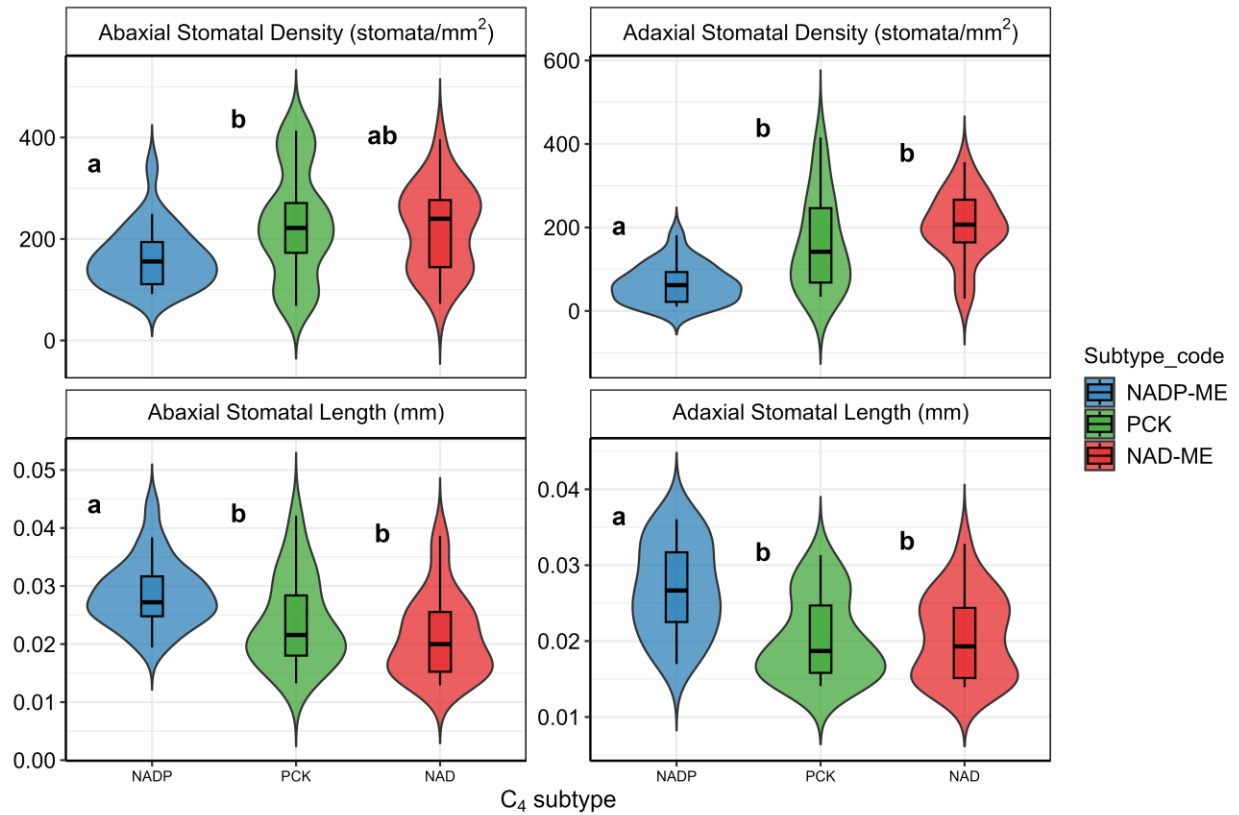


Figure 0.7: Violin plots show stomatal traits grouped by C_4 subtype. Letters indicate significant differences calculated with Bonferroni-adjusted pairwise comparisons.

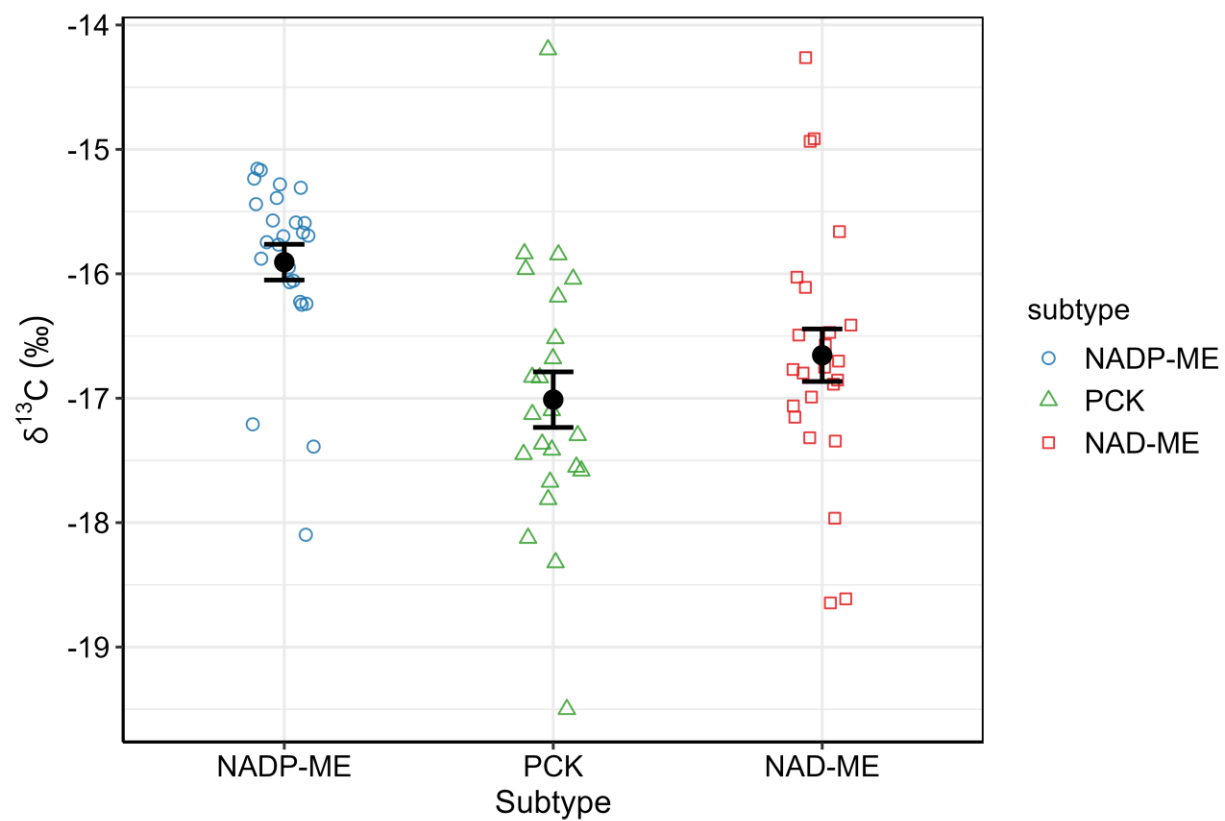


Figure 0.8: Mean ± 1 SE of $\delta^{13}\text{C}$ grouped by C₄ biochemical subtype.

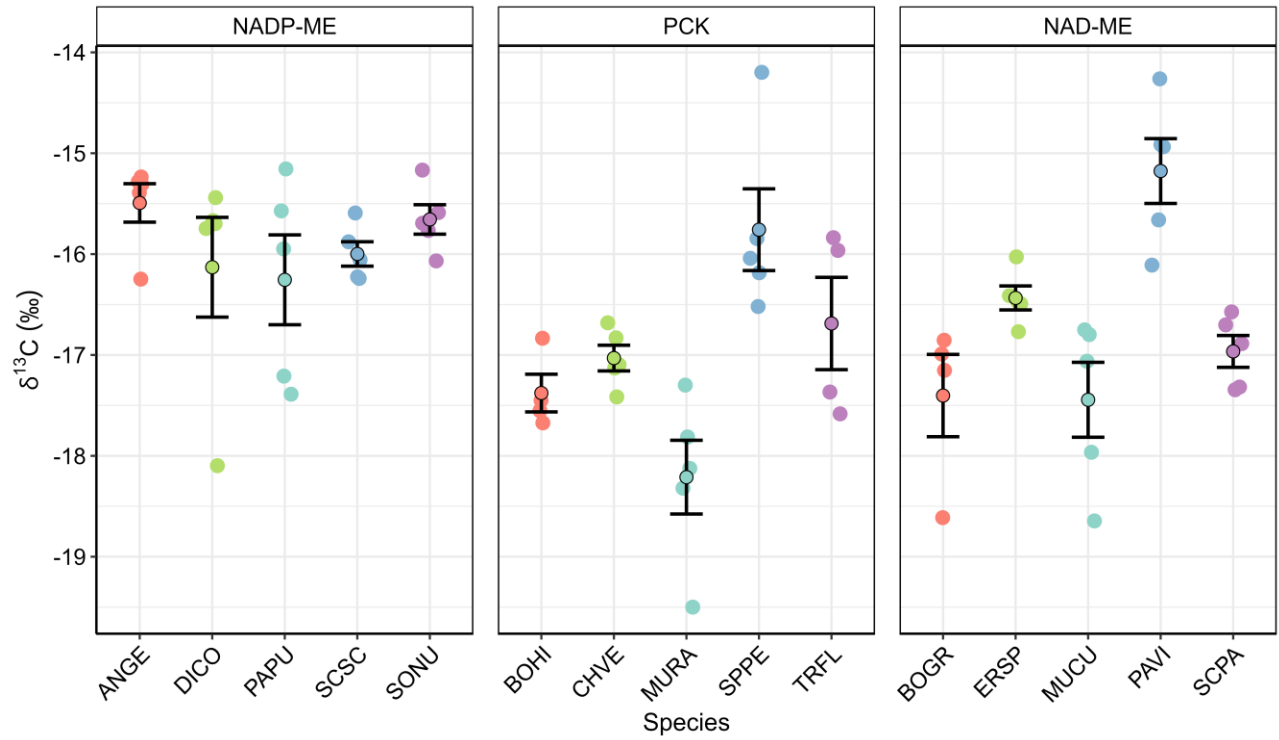


Figure 0.9: Mean ± 1 SE of $\delta^{13}\text{C}$ signature of each of the 15 species of C₄ grass measured. Full species names are listed as species codes seen in Table 2.1.

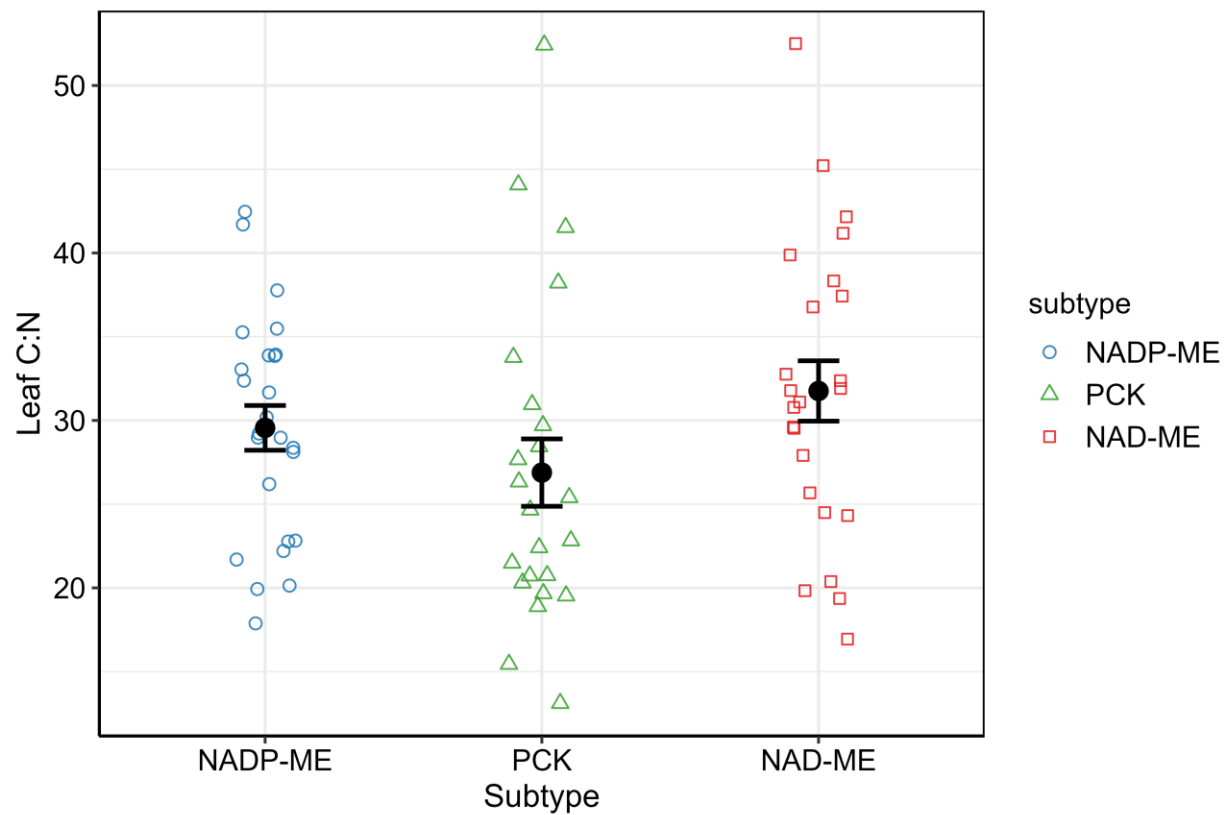


Figure 0.10: Mean $\pm$ 1 SE of leaf carbon to nitrogen ratios in each of the C₄ subtypes.

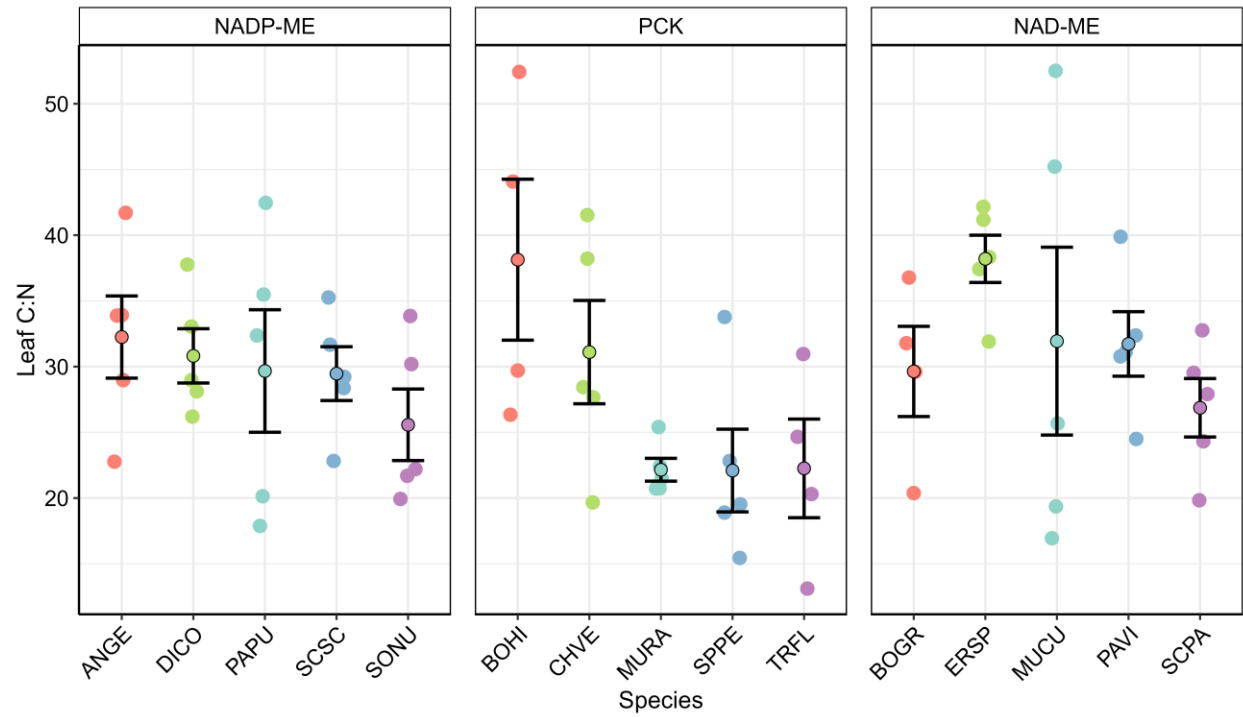


Figure 0.11: Mean $\pm$ 1 SE leaf carbon to nitrogen ratios in each of the C₄ subtypes. Full species names are listed as species codes seen in Table 2.1.

Biochemical subtypes of C₄ grass show divergent responses under high VPD and reduced soil moisture

Plants are key regulators of global carbon, nitrogen, and hydrological cycles (Still *et al.*, 2003; Chapman *et al.*, 2006; Schlesinger & Jasechko 2014). Grasslands are expansive and diverse globally and are responsible for nearly a quarter of aboveground net primary productivity (Still *et al.*, 2019; Knapp & Smith, 2001). As drought events continue shifting towards more frequent and more extreme regimes, quantifying plant responses during drought events has become increasingly critical (Morgan *et al.*, 2025; Anderegg *et al.*, 2015; Dai 2013). Beyond disruptions to terrestrial carbon and hydrological cycles (Frank *et al.*, 2015), drought events have the potential to shape the future structure and function of plant communities (Müller & Bahn 2022). In some instances, depending on the intensity and duration of droughts, changes in ecosystem characteristics such as plant adaptive strategies to drought, plant community composition, and/or soil properties can occur and push ecosystems towards new equilibria (Ratajczak *et al.*, 2017; Kannenberg *et al.*, 2020).

With meristems protected belowground, grasses are generally well-adapted to disturbances like fire, grazing, and rapid atmospheric changes (Post & Knapp 2020; Linder *et al.*, 2018; Hobbs & Huenneke, 1992). In the past 30 million years, parallel evolution of C₄ photosynthesis has occurred over 24 times in Poaceae, and over 66 times in all of flowering plants (Elliot *et al.*, 2024; Sage 2016; Grass Phylogeny Working Group II, 2012). The complex and widespread evolution of C₄ grasses has selected these unassuming plants to persist in a wide variety of dynamic atmospheric conditions (mean annual precipitation (MAP) 300 – 850 mm and mean annual temperature (MAT) 20 – 40 °C) and dominate open-canopy ecosystems like grasslands and savannas (Elliot *et al.*, 2024; Sage & Monson 1999; Chapman 1992). Specialized

leaf mesophyll cells associated with Kranz anatomy provide C₄ plants with an ecological advantage over the ancestral C₃ form during hot, dry conditions, because bundle sheath cells concentrate CO₂ at the site of RuBisCO activity, increasing carboxylation efficiency and reducing risk of photorespiration (Hatch 1987).

The evolutionary history of C₄ photosynthesis has been a source of academic contention for several decades. The C₄ strategy is thought to have evolved predominantly because of low atmospheric [CO₂], but evidence suggests that high temperatures and water availability also drove evolutionary selection for C₄ traits (Osborne *et al.*, 2014). It is safe to infer, considering the rapid global expansion of C₄ photosynthesis, that a given “type” of C₄ photosynthesis likely experienced different climate drivers in different environments. Looking at the evolution of the C₄ strategy through a phylogenetic lens, Chloridoid grasses, which dominate hot, arid environments today, evolved in hot, dry conditions over 20 million years ago. And at the opposite end of the spectrum, Panicoid grasses, which dominate mesic, cooler environments around the world, evolved under mild temperatures with higher water availability during similar times (Lehmann *et al.*, 2019). Despite the high functional diversity of C₄ grasses observed today, we still require empirical evidence that relates their complex phylogeny to their function in a modern climate, particularly during drought events.

Since its discovery over 50 years ago, our understanding of the biochemical, ecological, and anatomical traits associated with C₄ photosynthesis has drastically increased. In the 1970s, biochemists identified three variants of C₄ photosynthesis: NADP-dependent malic enzyme (NADP-ME) subtype, PEP-carboxykinase (PCK) subtype, and NAD-dependent malic enzyme (NAD-ME) subtype (Kanai & Edwards 1999; Gutierrez *et al.*, 1974). The C₄ subtypes are named

after the enzymes responsible for decarboxylating the 4-carbon acids, malate and/or aspartate, formed from the initial fixation of CO₂ in the mesophyll cells.

Frequently, the taxonomic distributions and biochemical pathways of C₄ grasses overlap, where Chloridoideae (mostly NAD-ME-dependent and some PCK-dependent in North America) and Andropogoneae (largely NADP-ME-dependent) operate over geographic gradients that reflect their evolutionary origins (Lehmann *et al.*, 2019). While the C₄ subtypes coexist in natural grassland systems, their relative abundance is strongly correlated with shifts in MAP. For instance, NADP-ME-dependent species tend to dominate mesic and wetter grassland sites, while NAD-ME-dependent species abundance increases in habitats receiving lower MAP (Ellis *et al.*, 1980; Wan & Sage, 2001; Taub 2000). Similar ecological behavior of C₄ biochemical subtypes following drought events has been reported, as the NAD-ME subtype increased in percent cover and abundance in plant community composition while the NADP-ME subtype decreased in response to drought (Albertson & Tomanek, 1965; Wigley-Coetsee & Staver, 2020).

The NADP-ME and NAD-ME groups have shown relatively consistent differences across Australian, South African, and North American grasslands, but the ecological role of the PCK subtype varies across continents. Taub (2000) found that PCK-dependent species decreased in abundance relative to increasing MAP in North America, while Hattersley (1992) observed the opposite trend in the PCK subtype in Australia. Debate surrounding the metabolic flexibility of the PCK subtype (Wang 2014; Furbank 2011), whether working alone, or in tandem with NAD-ME or NADP-ME, has prompted many to study only the drought responses of the latter two biochemical variants. In this project, we group and measure the three photosynthetic subtypes as described by enzymatic assays of C₄ plants by Kanai & Edwards in 1999. We aim to assess the responses of the C₄ subtypes to atmospheric drought by classifying the NADP-ME subtype as

wet-adapted, PCK as intermediate, and NAD-ME as dry-adapted. Despite strong evidence that C₄ subtype responses vary during soil water deficits, responses of C₄ subtypes to atmospheric drought are limited (Gan & Sage, 2024).

Atmospheric drought is measured as changes in the vapor pressure deficit (VPD). Alongside global temperatures, VPD is increasing at decadal scales and negatively impacting plant function (Novick *et al.*, 2019; Hammond *et al.*, 2022). Water vapor deficits vary widely across space and time, depending on the saturation of vapor pressure in the atmosphere and its temperature. VPD is inherently dynamic, fluctuating with air flow and humidity, and directly interacting with plant transpiration (Massmann *et al.*, 2019; Medlyn *et al.*, 2017). Plants absorb and dissipate heat differently across species, leaf age, and vertically across canopies, so leaf temperatures can vary significantly (Vogel 2009). Because of that, it's essential to differentiate between VPD of the air (VPD_{air}; Equation 1) and VPD at the leaf-air interface (VPD_{leaf}; Equation 2) when we assess the responses to atmospheric drought on plant function.

Equation 1:

$$VPD_{air} = e_s(T_{air}) \left(\frac{1-RH}{100} \right)$$

Equation 2:

$$VPD_{leaf} = e_s(T_{leaf}) - e_a$$

Where e_s is the saturation vapor pressure (kPa) and e_a is the actual vapor pressure (kPa), RH is humidity relative to the saturation vapor pressure (%) of the atmosphere at the air temperature, and T_{air} and T_{leaf} are the temperatures (°C) of the atmosphere and leaf, respectively. As VPD_{air} has increased globally, ecologists have quantified a clear feedback mechanism linking the evaporative demand of the atmosphere with plant water loss, or transpiration, which is regulated through stomatal opening and closure. Currently, we measure ecosystem processes,

including how transpiration and VPD_{leaf} interact, by assigning plant functional types (PFTs) to different forms of plant growth and photosynthetic pathways, such that C_4 plants are grouped into one PFT (Griffith *et al.*, 2020). Considering the long history of artificial selection in C_4 crops, which has bred plants like maize and sorghum (both NADP-ME-dependent species) for centuries, the photosynthetic capacities of the crops are maximized, and thus, are not comparable to naturally established C_4 grasses, nor those using NAD-ME or PCK decarboxylation pathways.

This research aims to establish an empirical basis for understanding whole-plant physiological responses of the C_4 subtypes to water vapor pressure deficits at diurnal and seasonal scales. I measured the same 6 species for each temporal scale, selecting 2 species to represent each of the C_4 subtypes. In the first experiment, I modified a large drying oven to simulate high VPD_{leaf} that persisted for 10 consecutive days and compared droughted plant responses to ambient plant responses, incorporating declining soil moisture into the last 4 days of the treatment. For the second experiment, I measured individuals of the same species periodically across the growing season. I examined how responses to atmospheric drought interact with cooccurring soil water deficits, and how the microanatomical traits of each species influenced physiological responses. To provide a basis for understanding resource allocation in the C_4 subtypes, isotopic signatures and structural traits were assessed across the growing season. Here, I focused on native prairie grasses and applied a theoretical framework of safety versus efficiency to the C_4 biochemical subtypes in a natural ecological context.

Methods

Field sampling and growth conditions

To test these questions on naturally established C_4 grasses, 6 species were sampled from Konza Prairie Biological Station (KPBS), an unplowed, native tallgrass prairie site in the Flint

Hills ecoregion of Northeastern, Kansas, USA (latitude: 39.095385, longitude: -96.574987). Covering nearly 3,500 hectares, KPBS maintains experimental grazing and prescribed burning regimes at the watershed-level, and hosts 100 species of grass, 57 of them being C₄ (Taylor *et al.*, unpublished). All replicates were collected for laboratory measurements or sampled in the field from ungrazed, frequently burned watersheds (fire return intervals of 1 – 4 years). I selected 6 species such that each of the biochemical subtypes was represented by 2 species of C₄ grass (Table 3.1; n = 18 – 24 total, n = 3 – 4 per subtype). Sampling was completed across two temporal scales.

Diurnal drought treatment

In the lab, two treatment conditions were established to compare controlled versus droughted responses to ambient or high VPD conditions, respectively, for 10 consecutive days. At KPBS, the intact roots and shoots of 24 plants were collected and transplanted in 5 L round plastic containers. Rather than further disturbing the root systems at transplant, I retained as much native soil as possible and supplemented containers with Pro-Mix Ultimate Vegetable & Herb Mix Soil ([porthorticulture.com]). Aboveground growth was trimmed to 10 cm upon collection and new leaf growth was subsequently measured. I allowed 15 days for plants to establish new leaves in laboratory (control) conditions. HOBO data loggers ([onsetcomp.com]) were placed in each environment to measure air temperature (°C), relative humidity (%), and dewpoint (°C), which was used to calculate VPD_{air} (kPa). Control conditions inside the laboratory kept air temperatures at approximately 24 - 27 °C. During acclimation and sampling periods, all plants were grown under HortiBloom Mega Optic 500W horticultural grow lights ([hortibloom.com]) for 12 hours every day with photosynthetically active radiation (PAR)

ranging between 1,600 – 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Once established, the 24 plants were split into two treatment groups: drought ($n = 12$, $n = 4$ per subtype) and control ($n = 12$, $n = 4$ per subtype).

To measure droughted plant responses, I modified a 2-meter $\times$ 1.5-meter drying oven, equipped with fans and electric heat typically used for drying plant biomass. Inside, the plant containers were positioned on the center shelf and grow lights were hung from the top of the oven, ensuring space for airflow and high radiation. Oven temperatures ranged from 30 – 40 °C and drove VPD_{air} to peak at 3.5 – 4.5 kPa each day (Appendix Figure B.1). The relative humidity of both treatments ranged between 40% – 55%. Soil moisture in each container was measured daily between 4:30pm and 5:30pm with the HydraGO Advanced Portable Soil Sensor System (Stevens Water Monitoring Systems, Portland, Oregon, USA).

Field sampling

To contextualize plant responses to VPD in a natural setting, individuals of the same set of species existing in their natural habitats were repeatedly measured throughout the growing season during midday (10am – 2pm). I selected 3 replicate plants of each species, with $n = 6$ per subtype, and $n = 18$ total. Individuals of all species measured occurred in well-drained upland soils, except for the biochemical “intermediate” *Spartina pectinata* (PCK), a wetland C_4 species that was restricted to lowland areas. Soil moisture was measured with the HydraGO sensor near the base of each plant at every sampling interval.

Physiological measurements

Gas exchange data were collected with a LI-COR 6800 infrared gas analyzer (LI-COR Inc., Lincoln, Nebraska, USA). Inside the cuvette (3 cm $\times$ 3 cm), we maintained CO_2 concentration at 400 ppm and PAR at 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. For gas exchange measurements across the diurnal time scale, RH was set to 50% and allowed VPD_{leaf} to reflect ambient conditions.

Seasonal gas exchange responses were measured with VPD_{leaf} set to 4.5 kPa inside the cuvette. I measured photosynthetic, stomatal conductance, and transpiration rates of the C_4 subtypes. Midday leaf water potential was collected using a Scholander pressure bomb (PMS Instruments, Albany, Oregon, USA). Meteorological data were retrieved from KPBS main weather station for daily precipitation during the growing season (Nippert, 2026).

Diurnal drought treatment

To capture diel patterns, daily gas exchange measurements were taken at the midpoint of the blade 4 times per day (morning, 7:30 am; midday, 11:30 am; afternoon, 3:30 pm; and evening, 6:30 pm). Measured leaves were alternated within and across days by marking 4 leaves on rhizomatous plants and sectioning bunching plants into 4 quadrants, subsampling each individual and randomizing the order of leaf selection across days. Due to a reduced number of adequately sized leaves available for destructive sampling, midday leaf water potential was measured every other day between 12:30 pm and 1:30 pm.

Field sampling

In-situ gas exchange, midday leaf water potential, and soil moisture data were measured approximately every 3 weeks between June and September 2025. The 18 individuals were measured at 5 intervals: Day of year = 162; 179; 210; 226; 247. All field measurements were restricted to midday, between 10 am and 2 pm, when plants are acclimated to warm, sunny conditions, but function has not yet declined from prolonged high radiation and temperatures. Gas exchange measurements were taken on the second newest leaf at the midpoint, and midday leaf water potential was measured on adjacent, mature leaves.

Anatomical, structural, and isotopic trait data collection

Before sampling the 24 individuals across the 10-day period, I collected 1 – 2 leaves per sample for structural and microanatomical traits. Leaves for microanatomical trait measurements were immediately placed in FAA solution (10% formalin/5% glacial acetic acid/50% ethanol), and tissues were vacuum infiltrated with FAA solution within 3 weeks of collection. The fixed leaves were sent to the Kansas State University Veterinary Medicine Histopathology lab for cross sectioning, mounting, staining, and imaging. Leaf tissue was sliced to 4 μm with a Leica RM2135 microtome (Leica Biosystems, Newcastle, UK) and mounted in paraffin. Samples were stained with Fast Green and Safranin-O (Ruzin 2000), then imaged with a confocal microscope equipped with high-speed imaging software. All images were analyzed on ImageJ software using methods described by Bachle & Nippert (2018).

Between two major vessels, I measured vessel area (V_a), xylem area (X_a) and xylem diameter (X_d), bulliform cell area (B_a), interveinal distance (IVD), and leaf thickness (L_t ; structural trait). Bundle sheath cell area (BSC_a) and mesophyll cell area (MSC_a) were recorded and used to calculate the ratio of BSC_a to MSC_a ($BSC:MSC$). See Figure 1 for example images of the 6 species sampled. Xylem area and xylem diameter were collected and averaged across the two major veins. The vessel, bulliform cell, bundle sheath cell, and mesophyll cell areas were collected on minor and major veins and averaged separately. I measured bulliform cells touching the adaxial and/or abaxial epidermis in images of leaf cross sections and distinguished these from air- and water-holding cells not touching the leaf epidermis. Interveinal distance was calculated by using the distance between the midpoint of adjacent vessels and averaged, while leaf thickness was collected and averaged across four points along the leaf width. The total leaf area per cross section was collected to calculate the percent cover of each cell type using the sums of

each cell area collected from every plant replicate. One *Bouteloua gracilis* species replicate was removed from the anatomy dataset (n = 23 total; NADP-ME n = 8; PCK n = 8; NAD-ME n = 7).

At each sampling interval across the season, leaf tissues were collected for structural (leaf carbon:leaf nitrogen; C:N) and isotopic ($\delta^{13}\text{C}$) signatures to assess how resource allocation changes across the 4-month period. Plant biomass was dried at 60 °C for 2 – 3 days and ground to a homogenous powder, then sent to Kansas State University in the Stable Isotope and Mass Spectroscopy Laboratory. Leaf C and N percent content and stable ^{13}C isotopic signatures were measured upon combustion of the tissue in an Elementar vario Pyro cube paired to an Elementar Mass Spectrometer. The isotopic abundance ratios were converted using Equation 3, where R is the ratio of heavier atom (^{13}C) in the sample to light atom (^{12}C) in the standard reference. The laboratory standards are calibrated against the Vienna Pee-Dee Belemnite international standard for $\delta^{13}\text{C}$.

Equation 3:

$$\delta = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] * 1000$$

Statistical Analysis

For data preprocessing, any gas exchange data points where $A < 0 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $> 50 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, $g_{\text{sw}} < 0$ and $> 0.5 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, and where $E < 0 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and $> 10 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ were removed. When biological or chemical behaviors inside the cuvette drove $[\text{CO}_2]$ inside the leaf and inside the chamber $< 350 \text{ ppm}$, the data points were removed to prevent skewed calculations of A and g_{sw} .

Drying oven

Due to low-light conditions in the control group for the first 2 days of the sampling campaign, all gas exchange data from the control group measurements were removed from

figures and statistics for this period. In the first 6 days of treatment, I droughted the plants with high VPD_{air} , and in the last 4 days, I used a combination of high VPD_{air} and declining soil moisture. All plant responses were measured, and statistical analyses were conducted relative to VPD_{leaf} . Because of the unbalanced temporal representations of each treatment group combined with the two iterations of drought treatments, we run all statistical tests for isolated high VPD_{leaf} treatment in days 2 – 6 and for combined VPD_{leaf} and low soil moisture treatment in days 7 – 10.

The daily mean gas exchange responses of each day were visualized and analyzed to capture the entirety of diel responses in our statistical models. Type III ANOVA tests on two linear mixed-effects models (LMMs) were used to evaluate the effects of treatment (control or drought), time across drought period (day), and C_4 subtype on A , g_{sw} , and E rates under each drought regime (VPD_{leaf} and $VPD_{leaf} \times$ declining soil moisture). To account for experimental hierarchies and repeated measures across each day, a nested design (subtype:species) and sample were included as random intercepts. The same LMM structure was used to assess midday leaf water potential across subtypes, treatment, and day of drought period. Differences in leaf microanatomical traits were analyzed with one-way ANOVAs on linear models with subtype as a fixed effect.

Seasonal measurements

A general LMM structure was created to test A , g_{sw} , E , and water potential responses of C_4 subtypes across the season (day of year), where subtype and day of year were fixed effects, and sample was included as a random effect for repeated measures. Subtype differences in $\delta^{13}C$ and leaf C:N across the growing season were assessed with type III ANOVA on LMMs using the same model structure for gas exchange and water potential. Model pairwise comparisons were made with estimated marginal means. All statistical analyses were completed in RStudio v.4.4.2.

Results

Drying oven results

In the first portion of the drought treatment under isolated VPD_{leaf} , there were no subtype differences in mean photosynthetic rates, stomatal conductance, or transpiration rates for control ($VPD_{air} = 1.5 - 2.5$ kPa) and droughted groups ($VPD_{air} = 3.5 - 4.5$ kPa, $p > 0.05$; Figure 3.2; Table 3.3). Stomatal conductance rates in the droughted plants were significantly lower than in the control ($p < 0.01$; Figure 3.2; Table 3.3) but did not increase or decrease over time ($p > 0.05$; Table 3.3). As the drought progressed, photosynthetic and transpiration responses of the droughted plants gradually increased throughout days 2 – 6 under isolated VPD_{leaf} stress ($p < 0.001$ and $p < 0.01$, respectively; Figure 3.2; Table 3.3).

Soil water was withheld during days 7 – 10 to assess C_4 subtype responses under combined elevated VPD_{leaf} and declining soil moisture. No subtype-level differences were observed in photosynthetic, stomatal conductance, and transpiration rates under combined VPD_{leaf} and soil moisture drought, and these metrics did not change over time ($p > 0.05$; Figure 3.2; Table 3.3). Across treatment groups, stomatal conductance varied ($p < 0.001$; Figure 3.2; Table 3.3) and continued to separate in the dry-adapted NAD-ME and intermediate PCK subtypes as time progressed, but not NADP-ME (treatment $\times$ day interaction $p < 0.01$; Figure 3.2; Table 3.3). In addition, transpiration rates were more varied by subtype with each passing day under co-occurring high VPD_{leaf} and soil water deficits (subtype $\times$ day interaction $p < 0.05$; Figure 3.2; Table 3.3). During this 10-day sampling period, no differences were seen in midday leaf water potential among C_4 subtypes and between treatment groups ($p > 0.05$; Figure 3.3; Table 3.4), although midday leaf water potential in both treatment groups changed over time ($p < 0.01$; Figure 3.3; Table 3.4).

Seasonal drought results

Across the growing season, stomatal conductance and transpiration rates varied among C₄ subtypes ($p = 0.0141$ and $p = 0.0182$, respectively; Figure 3.4; Table 3.5), but subtype photosynthetic rates were not statistically different at this temporal scale ($p > 0.05$; Figure 3.4; Table 3.5). Differences in stomatal conductance and transpiration were most pronounced at day 210, when air temperatures were the highest recorded in our dataset (36.6 ± 0.439 °C; Appendix Table B.5). Pairwise contrasts for day 210 showed gas exchange rates were higher in NADP-ME than NAD-ME groups with corresponding differences in stomatal conductance ($p < 0.001$; Appendix Table B.5; Figure 3.4) and transpiration ($p < 0.001$; Appendix Table B.5; Figure 3.4). On day 226, the same pattern between the NADP-ME and NAD-ME subtypes emerged, though differences in stomatal conductance and transpiration were more pronounced on day 210 than on day 226 ($p < 0.05$ and $p < 0.05$, respectively; Appendix Table B.5; Figure 3.4).

The steepest decline in the NADP-ME subtype gas exchange rates was seen at the end of the growing season, though photosynthetic capacity decreased earlier (between days 210 and 226) than stomatal conductance and transpiration rates (days 226 and 247). In the period between days 210 and 226, NADP-ME photosynthesis declined by 45.03% and PCK declined by 22.38%, while the dry-adapted NAD-ME group increased by 4.17% (Figure 3.4). Between the last two sampling periods (days 226 and 247), stomatal conductance rates for NADP-ME decreased by 59.4%, by 25.5% for PCK, and 19.9% for NAD-ME groups (Figure 3.4). Declines in transpiration rates during the same time reflect similar patterns, where the wet-adapted NADP-ME decreased by 62.4%, PCK decreased by 32.1%, and the dry-adapted NAD-ME decreased 26.8% (Figure 3.4).

Midday leaf water potential varied among C₄ subtypes and throughout the season ($p < 0.001$ and $p < 0.005$, respectively; Table 3.5; Figure 3.5). On day 210, the dry-adapted NAD-ME subtype maintained the highest Ψ (-1.67 ± 0.08 MPa) of the three groups (NADP-ME, $p < 0.05$; PCK, $p < 0.05$; Appendix Table B.5; Figure 3.5). By day 226, midday Ψ in the intermediate PCK declined to -2.76 ± 0.15 MPa and was significantly lower than NADP- and NAD-ME subtype midday Ψ ($p < 0.001$ and $p < 0.001$, respectively; Appendix Table B.5; Figure 3.5).

$\delta^{13}\text{C}$ and C:N across the season

$\delta^{13}\text{C}$ differed among C₄ subtypes, and over time during the 2025 growing season ($p < 0.05$ and $p < 0.001$, respectively; Table 3.6; Figure 3.6). A pronounced decline in all C₄ subtype $\delta^{13}\text{C}$ values was observed on leaves sampled on day 210 (Figure 3.6). For the duration of the growing season, the NADP-ME-dependent species showed the lowest variation in $\delta^{13}\text{C}$ of the C₄ subtypes (Figure 3.6). PCK subtype $\delta^{13}\text{C}$ was significantly lower than the wet-adapted NADP-ME subtype $\delta^{13}\text{C}$ for days 162, 179, and 247 ($p < 0.05$; Appendix Table B.6). By the end of the season on day 247, the NADP-ME subtype again showed higher $\delta^{13}\text{C}$ than PCK group ($p < 0.05$; Appendix Table B.6).

Leaf C:N varied across C₄ subtypes and across the season ($p < 0.01$ and $p < 0.001$, respectively; Figure 3.7; Table 3.6). By day 179, leaf C:N was significantly higher in NAD-ME-dependent species than in PCK-dependent species ($p < 0.05$; Appendix Table B.6). NAD-ME and PCK differences persisted for the rest of the season ($p < 0.05$; Appendix Table B.6), showing the most separation on day 226 ($p < 0.01$; Appendix Table B.6).

Microanatomical trait results

Microanatomical traits related to photosynthesis and water use varied across C₄ subtypes. Xylem area was different among subtypes ($p = 0.015$; Table 3.7), with the highest percent area

observed in the wet-adapted NADP-ME subtype (2.15 ± 0.12 %) and the lowest percent xylem area in the intermediate PCK subtype (1.32 ± 0.37 %, Figure 3.8; Figure 3.9; Appendix Table B.7). Total bulliform cell area varied between subtypes ($p = 0.008$; Table 3.7) due to significantly higher relative abaxial bulliform cell cover in the NADP-ME group. The wet-adapted NADP-ME species showed 21.6 ± 1.19 % abaxial bulliform cell area, compared to 10.3 ± 0.62 % area in PCK and 15 ± 0.72 % in NAD-ME species ($p < 0.05$; Figure 3.8; Figure 3.9; Appendix Table B.7). The opposite pattern emerged for bundle sheath cell cover, with PCK bundle sheath occupying 25.1 ± 1.6 % of the cross-sectional area, NAD-ME bundle sheath occupying 21.2 ± 1.6 %, and NADP-ME bundle sheath cells covering only 10.8 ± 0.53 % ($p < 0.0001$; Table 3.7; Figure 3.8; Figure 3.9).

Discussion

The objective of this study was to measure the physiological responses of the C_4 subtypes to high VPD_{leaf} and soil water deficits across two temporal scales: 1) an intense short-period simulating a heat wave; and 2) responses to high VPD across a growing season. In my first project, I tested drought responses of C_4 subtypes in 24 plants for ten consecutive days in a modified drying oven that maintained VPD_{leaf} at 3.5 – 4.5 kPa during the day. In days 7 – 10, I stopped watering plant containers to assess subtype responses to combined high VPD_{leaf} and declining soil moisture. From these 24 individuals, leaf tissue was collected to examine microanatomical traits driving C_4 subtype responses under droughted conditions. In my second project, I measured the responses of 18 in situ grasses approximately every 3 weeks for 5 sampling periods, with VPD_{leaf} inside the chamber set to 4.5 kPa for the duration of the 2025 growing season. In the Flint Hills ecoregion, KPBS typically receives 835 mm MAP. Most rainfall occurs at the beginning of the growing season and is followed by an annual drought in

the latter half of the year. Here, I used these seasonal responses to my advantage with the expectation that cumulative drought impacts on plant function would become more prominent towards the end of the 2025 growing season. These data show distinct diurnal and seasonal responses by C₄ biochemical subtypes to high VPD_{leaf} and reduced soil moisture availability.

C₄ subtypes responses diverge under combined high VPD_{leaf} and low soil moisture

During the 10-day drought treatment, elevated VPD_{leaf} was driven exclusively by high temperatures maintained inside the oven (Appendix Figure B.1). The influences of temperature and VPD_{leaf} on plant function were difficult to disentangle because photosynthetic enzyme kinetics vary across photosynthetic types, across plant species adaptations, and with plant acclimation in the short term (Yamori *et al.*, 2014; Sage & Kubien 2007). In naturally established grasses, photosynthetic and biomass production rates across C₃ and C₄ species vary under different temperatures. Kemp & Williams (1980) found the maximum photosynthetic rates of C₄ *Bouteloua gracilis* were observed at 35 °C, while its C₃ counterpart, *Agropyron smithii*, maximized photosynthesis at 20 °C. Thermal optima of enzymatic processes may drive increases or decreases in photosynthetic rates, but empirical evidence for C₄ plant responses beyond 40 °C require further investigation (Sage & Kubien 2007).

High VPD_{leaf} dampens transpiration and stomatal conductance at leaf- to landscape-scales (Medlyn *et al.*, 2002; Oren *et al.*, 1999; Monteith 1995). I found that despite high temperatures (35 – 40 °C) driving VPD_{air} to peak at 4.5 kPa most days, only stomatal conductance consistently varied across treatment groups, with lower rates in the droughted group due to high VPD_{leaf} (Figure 3.2). Under isolated high VPD_{leaf}, gradual increases in photosynthetic and transpiration rates occurred over time ($p < 0.001$ and $p < 0.05$, respectively; Table 3.3), likely responding more to temperature than to VPD_{leaf} during the first 6 days of sampling. Differences in gas

exchange rates across treatment groups were more pronounced across treatment groups than across C₄ subtypes (Appendix Table B.1).

In days 7 – 10, when soil moisture declined but VPD_{leaf} remained high, similar results in C₄ subtype gas exchange responses were observed. Reduced stomatal conductance rates in the droughted plants continued to separate with time from the control group (treatment x day $p < 0.001$; Table 3.3). However, under the combined effects of reduced soil moisture and high VPD_{leaf}, transpiration diverged with time in the NAD-ME- and PCK-dependent species (subtype $\times$ day $p < 0.01$; Figure 3.2; Table 3.3; Appendix Table B.1). Only under cooccurring high VPD_{leaf} and limited soil water did the dry-adapted NAD-ME subtype show significantly lower transpiration rates than the wet-adapted NADP-ME subtype. Plant responses to atmospheric and soil water deficits frequently overlap, but differences in response depend on spatial scale, species composition, and water regulation traits (Grossiord *et al.*, 2020; Lopez *et al.*, 2021). Prior peer-reviewed evidence showed the combined effects of reduced soil moisture and atmospheric water vapor interact nonlinearly (i.e., effects were not additive; Grossiord *et al.*, 2020; Fu *et al.*, 2022). Rather, some reports showed that interactions of different drought drivers were synergistic, and VPD and soil drought amplified each other as time progressed (Cai *et al.*, 2023; Wang *et al.*, 2025).

C₄ subtypes operate at different seasonal optima

The wet-adapted NADP-ME showed higher rates of water loss than the dry-adapted NAD-ME subtypes during the 2025 growing season, but similar photosynthetic rates (days 210 and 226; Figure 3.4; Appendix Table B.5). Similarly, Ghannoum *et al.* in 2005 reported that photosynthetic capacities in NAD- and NADP-ME species did not vary under limited soil water availability. These results may be most influenced by decreasing water availability in surface

soils throughout the season, as C₄ prairie grasses (Nippert *et al.*, 2012), and C₄ crops (maize; Yu *et al.*, 2007) produce fine adventitious root systems that maximize soil water acquisition when soil moisture is available, but also permit maintenance of leaf gas exchange between periods of soil water availability.

Alongside subtype separation in gas exchange rates due to hot, dry field conditions on day 210, I found phenological differences across C₄ subtypes in the last two sampling periods (days 226 and 247). By day 226, the wet-adapted NADP-ME subtype showed a steep decline in photosynthesis relative to the other subtypes. By day 247, stomatal conductance and transpiration rates of the NADP-ME-dependent species mirrored this pattern, decreasing to the lowest relative gas exchange rates of the three biochemical subtypes (Figure 3.4). Midday leaf water potential (Ψ) varied across subtypes, with the dry-adapted NAD-ME group showing the highest relative midday Ψ during the warmest sampling day (Figure 3.5). Notably, the intermediate PCK subtype decreased to the lowest midday Ψ of the C₄ subtypes by day 226 and maintained the lowest relative midday Ψ for the remainder of the growing season (Figure 3.5).

Integrated stomatal behavior and nutrient dilution across the season

$\delta^{13}\text{C}$ varied over the season and showed an obvious decline during the warmest sampling day (day 210), which coincided with downregulating stomatal conductance during the same time (Figure 3.2; Figure 3.6). Lower relative $\delta^{13}\text{C}$ values observed in the intermediate PCK and dry-adapted NAD-ME subtypes reflect subtype-specific patterns in stomatal conductance to water vapor (Appendix Table B.5; Appendix Table B.6). These results support prior studies that suggests $\delta^{13}\text{C}$ is an effective proxy for integrated stomatal behavior in C₄ grasses (Isreal *et al.*, 2026; Ellsworth *et al.*, 2017).

During the 2025 season, leaf C:N varied across C₄ subtypes and across time (Table 3.6; Figure 3.7). The general upward trend in C:N in all subtypes over time is driven by nutrient dilution, as aboveground biomass increases and nutrient concentration per unit leaf area simultaneously decreases (Sternier & Elser 2003). Higher leaf C:N in NAD-ME than in PCK subtypes drove differences in leaf C:N for all sampling periods except day 162 (early season, when nutrient dilution is lowest). These results suggest the NAD-ME group has a lower nitrogen use efficiency across the season than its PCK and NADP-ME counterparts, which is consistent with previous reports showing that the NADP-ME subtype yields high nitrogen use efficiency due to rapid RuBisCO activity (Togawa-Urakoshi & Ueno, 2022; Ghannoum *et al.*, 2005; Taub & Lerdau 2000). Other studies found PCK (alongside NADP-ME) species had higher leaf nitrogen content, and thus, higher photosynthetic nitrogen use efficiency than NAD-ME species (Pinto *et al.*, 2015), perhaps due to higher bundle sheath [CO₂] and bundle sheath RuBisCO activity (Ghannoum *et al.*, 2005; Hatch 1987).

Tradeoffs in bulliform and bundle sheath cell cover

The differences in microanatomical traits across biochemical subtypes were largely driven by the efficiency-tailored anatomy seen in the wet-adapted NADP-ME species. Lower relative bundle sheath cell area and large xylem conduits provided the NADP-ME subtype with the architecture to maximize water uptake and photosynthetic rates during ideal conditions (Figure 3.4; Figure 3.8). In the dry-adapted NAD-ME and the intermediate PCK groups, bundle sheath cells covered 21 – 25% of the leaf cross-sectional area, compared to approximately 11% cover in the NADP-ME group (Figures 3.8 and 3.9). However, patterns in bundle sheath cell cover showed an inverse relationship with bulliform cell cover. While abaxial bulliform cell area covered 10 – 15% of the cross-sectional areas of NAD-ME and PCK subtypes, abaxial bulliform

cell covered over 20% of the measured cross section in the NADP-ME group (Figure 3.9). The ratio of bundle sheath to bulliform cell areas across the C₄ subtypes suggests that each group may utilize the spatial orientation of these cells to their advantage. Where interior bundle sheath cells might be more protected from changes in atmospheric demand experienced at the epidermis (Sage 2001), bulliform cells might provide protection against disruptions in stomatal function during water deficits. Bulliform cells are most well documented for their role in leaf rolling under stress (Matschi *et al.*, 2020), but recent evidence shows that both bulliform cells and bundle sheath cells may act as water storage capacitors for C₄ grasses (Ho 2024; Bachle 2021). At the cellular and subcellular level, bulliform cells may contract and expand, adjusting the flow of water in and out of the capacitors and subsequently adjusting cellular turgidity, and/or, depending on cellular proximity, may provide mesophyll structures with water to carboxylate CO₂ during periods of drought.

Convergence between anatomical and physiological data suggests bundle sheath cells and bulliform cells likely occupy unique functional roles across the growing season. Higher seasonal gas exchange responses and lower $\delta^{13}\text{C}$ values suggest higher abaxial bulliform cell cover in the NADP-ME subtype may facilitate water storage for ‘optimal’ stomatal behavior during hot, dry conditions (Figure 3.2; Figure 3.6; Figure 3.8). Simultaneously, lower gas exchange responses and $\delta^{13}\text{C}$ values observed in the PCK and NAD-ME groups suggest high bundle sheath cell cover may yield photosynthetic stability despite downregulation of stomatal conductance under the same environmental conditions (Figure 3.2; Figure 3.6; Figure 3.8). While the functional roles of bundle sheath cells are contested and vary across C₄ lineages, enlarged bundle sheath cells may protect PCK and NAD-ME photosynthetic machinery via water and ion capacitance (Sage 2001;

Williams *et al.* 1993), which allows adjacent mesophyll cells to be replenished quickly during periods of high atmospheric demand.

Conclusion

With this combination of metrics, I argue that the C₄ biochemical subtypes host unique physiological, structural, and anatomical traits that yield different water loss strategies during drought. Across the growing season, seasonal optima of the C₄ subtypes varied. This is supported by i) gas exchange and midday leaf water potential responses, ii) $\delta^{13}\text{C}$ stable isotope signatures and leaf C:N, and iii) tradeoffs between bulliform/bundle sheath cell cover and xylem area across subtypes. The wet-adapted NADP-ME group favored traits for optimizing carbon capture, and the intermediate PCK and dry-adapted NAD-ME groups allocated resources to protect photosynthetic function while suppressing water loss. VPD_{leaf} inside the infrared gas analyzer was driven by reductions in relative humidity and maintained at 4.5 kPa over the growing season. As the 2025 growing season progressed and precipitation largely waned, separation in C₄ subtype gas exchange and hydraulic responses became obvious.

Across the 10-day period, VPD_{leaf} driven by high temperatures did not produce subtype specific gas exchange responses to drought. Rather, C₄ subtype responses across treatment groups only began to diverge under combined temperature-driven VPD_{leaf} and reduced soil water availability at the diurnal scale. Data from the 10-day drought illustrates both the complex and dynamic nature of vapor pressure deficits and the growing urgency to standardize how VPD impacts on plant function are measured.

The functional complexity of C₄ photosynthesis cannot be generalized even to the three biochemical subtypes, as many caveats surrounding the PCK and NAD-ME types remain. Further, the functional role of different cell types, particularly bulliform and bundle sheath cells,

requires detailed investigation and provides an opportunity to explore how drought interacts with subcellular processes. Examining the nuance in “dry-adapted” or “wet-adapted” categorizations of C₄ plants strengthens our understanding of plant-mediated processes and will eventually, with enough evidence, dispel the myth that all C₄ plants are “drought-tolerant.” Quantifying plant responses during drought events of all kinds remains a critical mission in increasing our resilience to a changing climate.

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Tables and Figures

Table 0.1: List of six C₄ species measured.

Tribe	Subtype	Species	Species Code
Andropogoneae	NADP-ME	<i>Andropogon gerardii</i>	ANGE
Andropogoneae	NADP-ME	<i>Sorghastrum nutans</i>	SONU
Cynodonteae	PCK	<i>Bouteloua hirsuta</i>	BOHI
Cynodonteae	NAD-ME	<i>Bouteloua gracilis</i>	BOGR
Zoyiseae	PCK	<i>Spartina pectinata</i>	SPPE
Paniceae	NAD-ME	<i>Panicum virgatum</i>	PAVI

Table 0.2: Table showing all traits measured.

Type	Trait	Abbreviations	Units
Anatomical	Vessel Area	V_a	μm^2
	Xylem Area	X_a	μm^2
	Bundle Sheath Cell Area	BSC_a	μm^2
	Mesophyll Cell Area	MSC_a	μm^2
	Bundle Sheath Cell:Mesophyll Cell	BSC:MSC	dimensionless ratio
	Bulliform Cell Area	B_a	μm^2
	Adaxial Stomatal Density	SD_{ad}	stomata/ μm^2
	Abaxial Stomatal Density	SD_{ab}	stomata/ μm^2
	Adaxial Stomatal Length	SL_{ad}	μm
	Adaxial Stomatal Length	SL_{ab}	μm
Structural	Leaf Carbon:Leaf Nitrogen	C:N	dimensionless ratio
	Vapor Pressure Deficit at leaf-air interface	VPD_{leaf}	kPa
Physiological	Leaf Temperature	T_{leaf}	$^{\circ}\text{C}$
	^{13}C stable isotopic signature	$\delta^{13}\text{C}$	(‰)
	Photosynthetic Rate	A	$\mu\text{mol m}^{-2} \text{s}^{-1}$
	Stomatal Conductance	g_{sw}	$\text{mol m}^{-2} \text{s}^{-1}$
	Leaf Transpiration Rate	E	$\text{mmol m}^{-2} \text{s}^{-1}$
	Vapor Pressure Deficit of air	VPD_{air}	kPa
Environmental	Air temperature	T_{air}	$^{\circ}\text{C}$
	Relative Humidity	RH	%
	Soil Moisture	SM	%

Table 0.3: Type III ANOVA results from two LMMs testing effects of subtype, treatment, and day on A, g_{sw} , and E across the VPD and VPD_{leaf} and SM (soil moisture) treatments.

Drought	Response	Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
VPD _{leaf}	A	subtype	42.78357	21.39179	2	2.964652	0.66281	0.57819
VPD _{leaf}	A	treatment	1.144175	1.144175	1	14.12446	0.035451	0.853331
VPD _{leaf}	A	day	418.097	418.097	1	318.1146	12.95444	0.00037
VPD _{leaf}	A	subtype:treatment	28.79967	14.39984	2	14.0875	0.446169	0.648815
VPD _{leaf}	A	subtype:day	35.57066	17.78533	2	318.0382	0.551066	0.576884
VPD _{leaf}	A	treatment:day	0.100855	0.100855	1	318.9366	0.003125	0.955456
VPD _{leaf}	A	subtype:treatment:day	16.28558	8.14279	2	318.806	0.252299	0.777168
VPD _{leaf}	g_{sw}	subtype	0.008168	0.004084	2	3.008333	1.874273	0.296078
VPD _{leaf}	g_{sw}	treatment	0.019848	0.019848	1	13.83229	9.108384	0.009321
VPD _{leaf}	g_{sw}	day	0.000923	0.000923	1	318.2651	0.423631	0.5156
VPD _{leaf}	g_{sw}	subtype:treatment	0.007474	0.003737	2	13.81355	1.715029	0.216132
VPD _{leaf}	g_{sw}	subtype:day	0.001464	0.000732	2	318.1986	0.335897	0.71495
VPD _{leaf}	g_{sw}	treatment:day	0.001105	0.001105	1	318.7138	0.507016	0.476954
VPD _{leaf}	g_{sw}	subtype:treatment:day	0.000673	0.000336	2	318.617	0.154359	0.857028
VPD _{leaf}	E	subtype	1.34E-05	6.71E-06	2	3.008737	4.1821	0.135283
VPD _{leaf}	E	treatment	2.01E-07	2.01E-07	1	14.96057	0.125191	0.728408
VPD _{leaf}	E	day	1.67E-05	1.67E-05	1	319.6553	10.41292	0.001381
VPD _{leaf}	E	subtype:treatment	7.32E-06	3.66E-06	2	14.93526	2.279842	0.13675
VPD _{leaf}	E	subtype:day	1.98E-06	9.89E-07	2	319.5694	0.616207	0.540629
VPD _{leaf}	E	treatment:day	3.87E-07	3.87E-07	1	319.9797	0.241408	0.623528
VPD _{leaf}	E	subtype:treatment:day	2.09E-06	1.05E-06	2	319.8722	0.652364	0.521505
VPD _{leaf} × SM	A	subtype	64.55926	32.27963	2	2.929482	1.147659	0.428491
VPD _{leaf} × SM	A	treatment	26.71512	26.71512	1	14.06119	0.94982	0.346234
VPD _{leaf} × SM	A	day	6.596576	6.596576	1	316.9851	0.234532	0.628518
VPD _{leaf} × SM	A	subtype:treatment	11.23775	5.618876	2	13.99884	0.199772	0.821212
VPD _{leaf} × SM	A	subtype:day	139.5288	69.7644	2	316.4478	2.480379	0.085338
VPD _{leaf} × SM	A	treatment:day	83.0663	83.0663	1	316.6297	2.95331	0.086679
VPD _{leaf} × SM	A	subtype:treatment:day	20.05526	10.02763	2	316.1328	0.356519	0.70039
VPD _{leaf} × SM	g_{sw}	subtype	0.009471	0.004735	2	2.972467	2.216754	0.25749
VPD _{leaf} × SM	g_{sw}	treatment	0.040142	0.040142	1	16.05226	18.79162	0.000508
VPD _{leaf} × SM	g_{sw}	day	2.28E-05	2.28E-05	1	319.8292	0.010695	0.917698
VPD _{leaf} × SM	g_{sw}	subtype:treatment	0.006403	0.003202	2	15.954	1.498725	0.253243
VPD _{leaf} × SM	g_{sw}	subtype:day	0.004369	0.002184	2	319.3702	1.022576	0.360842
VPD _{leaf} × SM	g_{sw}	treatment:day	0.023417	0.023417	1	319.5453	10.96224	0.001036
VPD _{leaf} × SM	g_{sw}	subtype:treatment:day	0.001774	0.000887	2	319.1188	0.415337	0.660474
VPD _{leaf} × SM	E	subtype	2.02E-05	1.01E-05	2	2.980961	6.06825	0.088926
VPD _{leaf} × SM	E	treatment	4.38E-08	4.38E-08	1	16.041	0.026319	0.87315
VPD _{leaf} × SM	E	day	4.46E-07	4.46E-07	1	319.5809	0.267985	0.605045

$VPD_{leaf} \times SM$	E	subtype:treatment	1.3E-05	6.51E-06	2	15.95355	3.911868	0.041456
$VPD_{leaf} \times SM$	E	subtype:day	1.52E-05	7.61E-06	2	319.1019	4.57636	0.010977
$VPD_{leaf} \times SM$	E	treatment:day	3.17E-06	3.17E-06	1	319.5745	1.90853	0.168092
$VPD_{leaf} \times SM$	E	subtype:treatment:day	6.93E-06	3.46E-06	2	319.0967	2.083254	0.126215

Table 0.4: Type III ANOVA results table from LMMs testing the effects of C₄ subtype, treatment, day (time), and interactions on leaf water potential (Ψ).

Response	Effect	Sum Sq	Mean Sq	num_df	den_df	F_value	p_value
Ψ	Subtype	18.07802	9.039012	2	84	0.412	0.664
Ψ	Treatment	14.45018	14.45018	1	84	0.658	0.42
Ψ	Day	164.176	164.176	1	96	7.476	0.00745
Ψ	Subtype:Treatment	118.9641	59.48205	2	84	2.709	0.0724
Ψ	Subtype:Day	8.902083	4.451042	2	96	0.203	0.817
Ψ	Treatment:Day	59.50103	59.50103	1	96	2.709	0.103
Ψ	Subtype:Treatment:Day	56.45208	28.22604	2	96	1.285	0.281

Table 0.5: Type III ANOVA table showing LMM results testing subtype differences in A, g_{sw} , E, and midday leaf water potential (Ψ) as individual fixed effects, and their interactions across the growing season.

Response	term	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
A	subtype	55.0918	27.5459	2	15.06853	1.76524	0.204751
A	day of year	1990.597	497.6494	4	59.1659	31.89115	3.63E-14
A	subtype:day of year	423.9382	52.99228	8	59.16072	3.395935	0.002843
g_{sw}	subtype	0.036615	0.018307	2	15.24912	5.713634	0.014065
g_{sw}	day of year	0.115595	0.028899	4	59.58014	9.019169	9.17E-06
g_{sw}	subtype:day of year	0.064906	0.008113	8	59.5664	2.532095	0.019213
E	subtype	6.94E-05	3.47E-05	2	15.23698	5.272718	0.018186
E	day of year	0.000313	7.82E-05	4	59.55765	11.89718	3.62E-07
E	subtype:day of year	0.000131	1.64E-05	8	59.54418	2.496401	0.020801
Ψ	subtype	296.101	148.0505	2	14.7601	14.53929	0.000324
Ψ	day of year	198.9426	49.73564	4	59.12811	4.884283	0.001802
Ψ	subtype:day of year	354.7419	44.34274	8	59.11331	4.354674	0.000357

Table 0.6: Type III ANOVA results testing C₄ subtype, day of year (time across season), and their interaction on $\delta^{13}\text{C}$ and leaf C:N.

Response	term	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
$\delta^{13}\text{C}$	.subtype	3.302596	1.651298	2	15	4.582117	0.027983
$\delta^{13}\text{C}$	day of year	8.12443	2.031107	4	60	5.636033	0.000642
$\delta^{13}\text{C}$	subtype:day of year	5.094318	0.63679	8	60	1.767001	0.101667
C:N	.subtype	838.5826	419.2913	2	15	9.840733	0.001862
C:N	day of year	1667.358	416.8394	4	60	9.783187	3.69E-06
C:N	subtype:day of year	147.7515	18.46894	8	60	0.433464	0.896319

Table 0.7: One-way ANOVA testing mean percent cover of cell types among C₄ subtypes.

Trait	term	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Xylem area (%)	subtype	2	3.0513	1.5257	5.176	0.01543
Vessel area (%)	subtype	2	34.747	17.3735	1.6436	0.2183
Bundle sheath area (%)	subtype	2	865.3472	432.6736	32.9822	<0.0001
Mesophyll area (%)	subtype	2	0.8288	0.4144	0.0306	0.9699
Abaxial bulliform area (%)	subtype	2	513.4533	256.7267	40.634	<0.0001
Adaxial bulliform area (%)	subtype	2	2.1833	1.0916	0.2979	0.7456
Extra bulliform area (%)	subtype	2	4.105	2.0525	0.4571	0.6403
Total bulliform area (%)	subtype	2	604.5794	302.2897	6.2022	0.008022

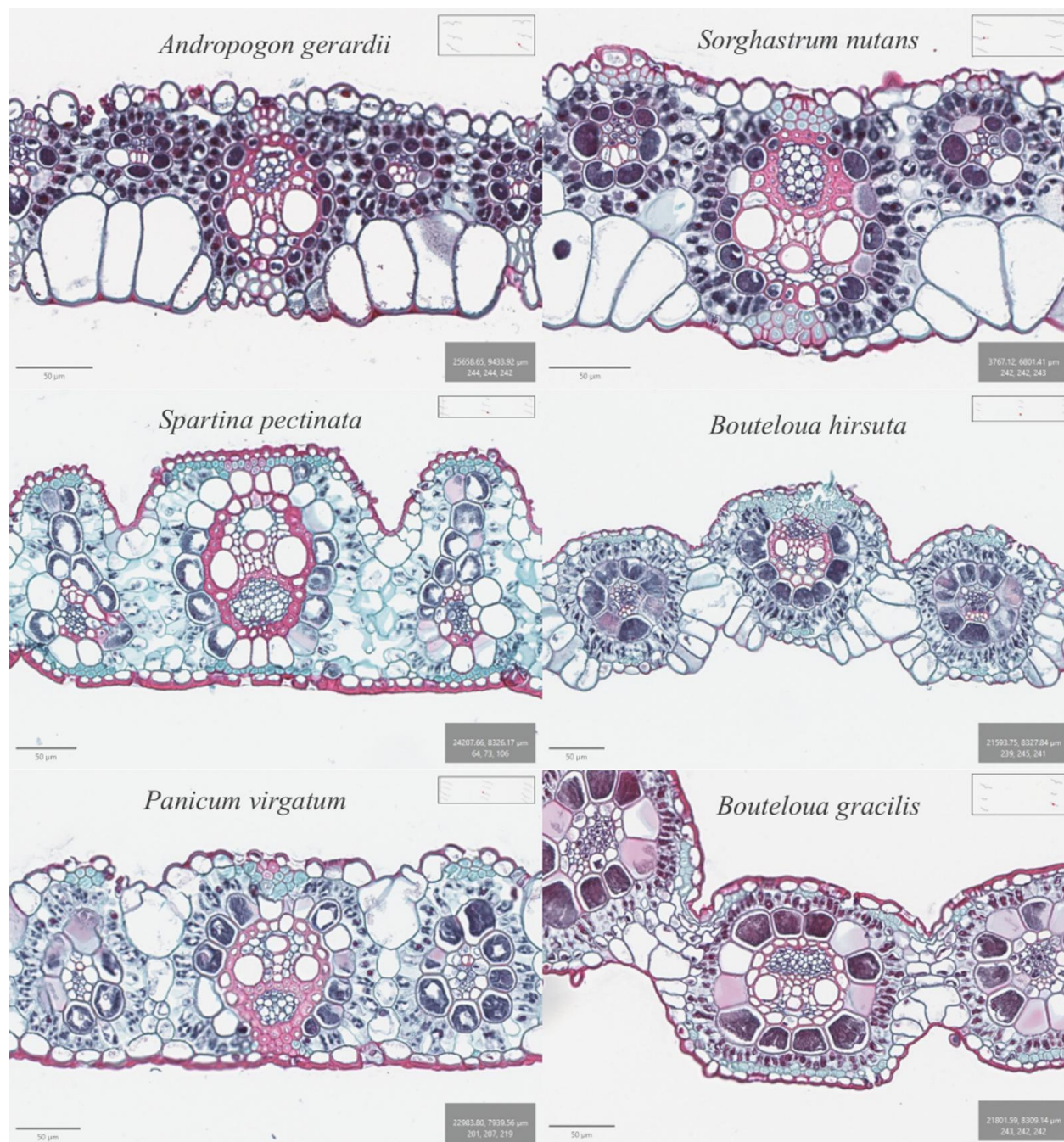


Figure 0.1: Example images of the 6 species of C₄ grass measured, with NADP-ME species in the top row, PCK species in the middle, and NAD-ME species in the bottom row. Species names are shown directly above each cross-section.

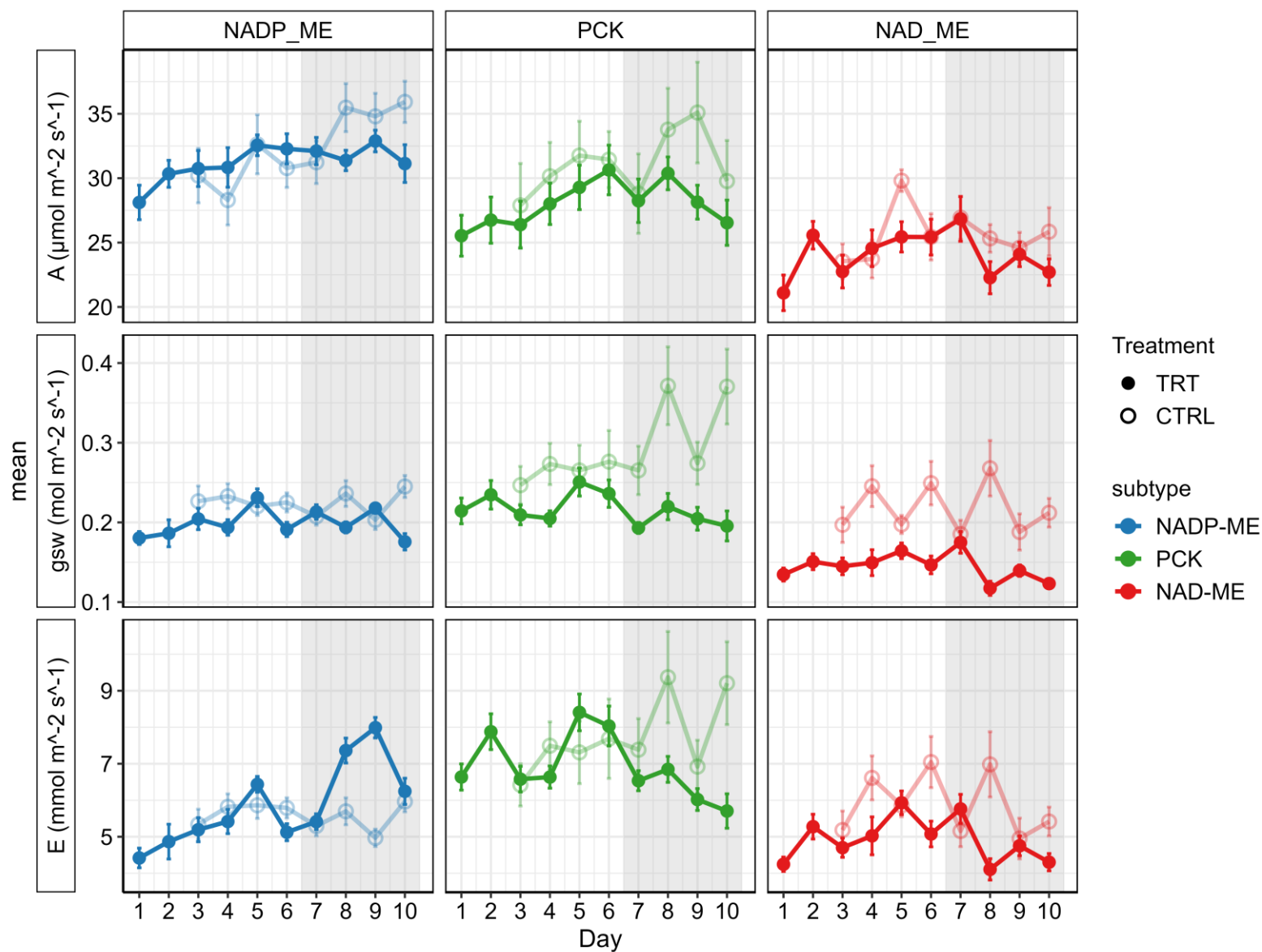


Figure 0.2: Means $\pm$ 1 SE of A, g_{sw} , and E responses across 10-day period grouped by C_4 subtype. Dark lines with filled points show drought group, and light lines with empty points show control group. Transparent grey overlay shows when watering ceased.

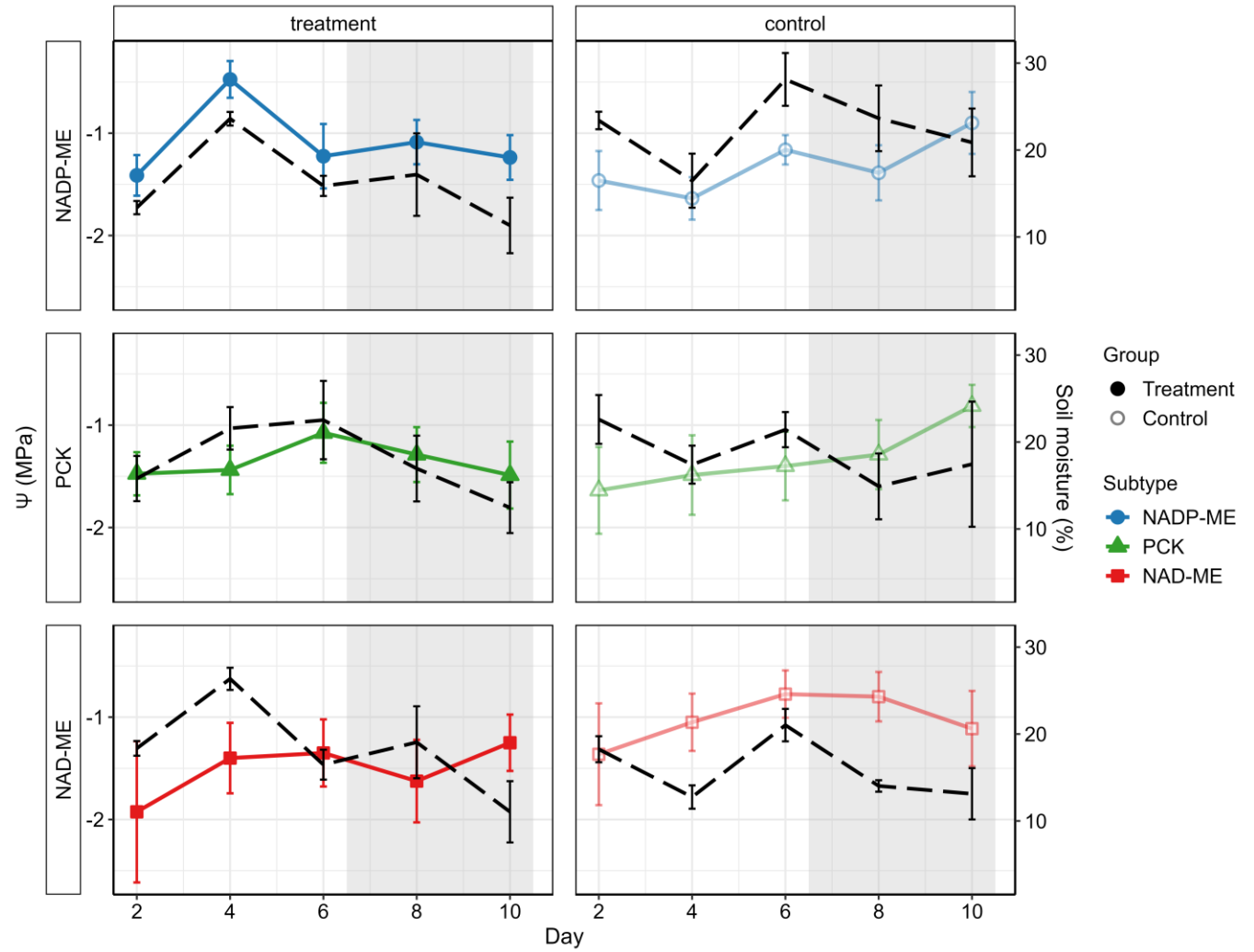


Figure 0.3: Mean ± 1 SE midday leaf water potential of each C₄ subtype across the 10-day drought treatment. Dark lines with filled points show the drought group, and light lines with empty points show the control group. Dashed, black lines show mean ± 1 SE soil moisture. Transparent grey overlay shows when watering ceased.

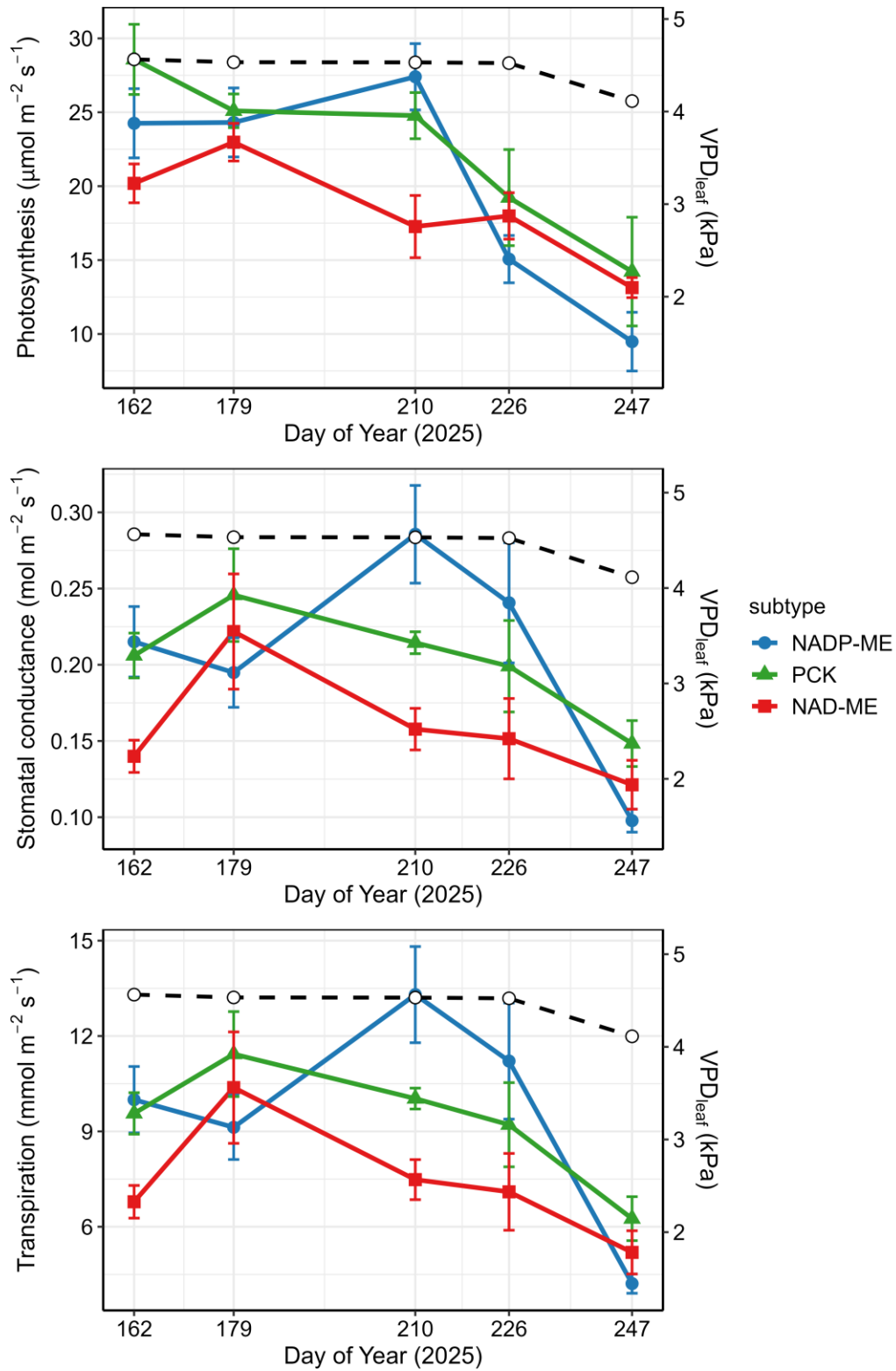


Figure 0.4: Mean ± 1 SE responses of A, g_{sw} , and E across growing season grouped by C₄ subtype. Dashed black line shows mean VPD_{leaf}.

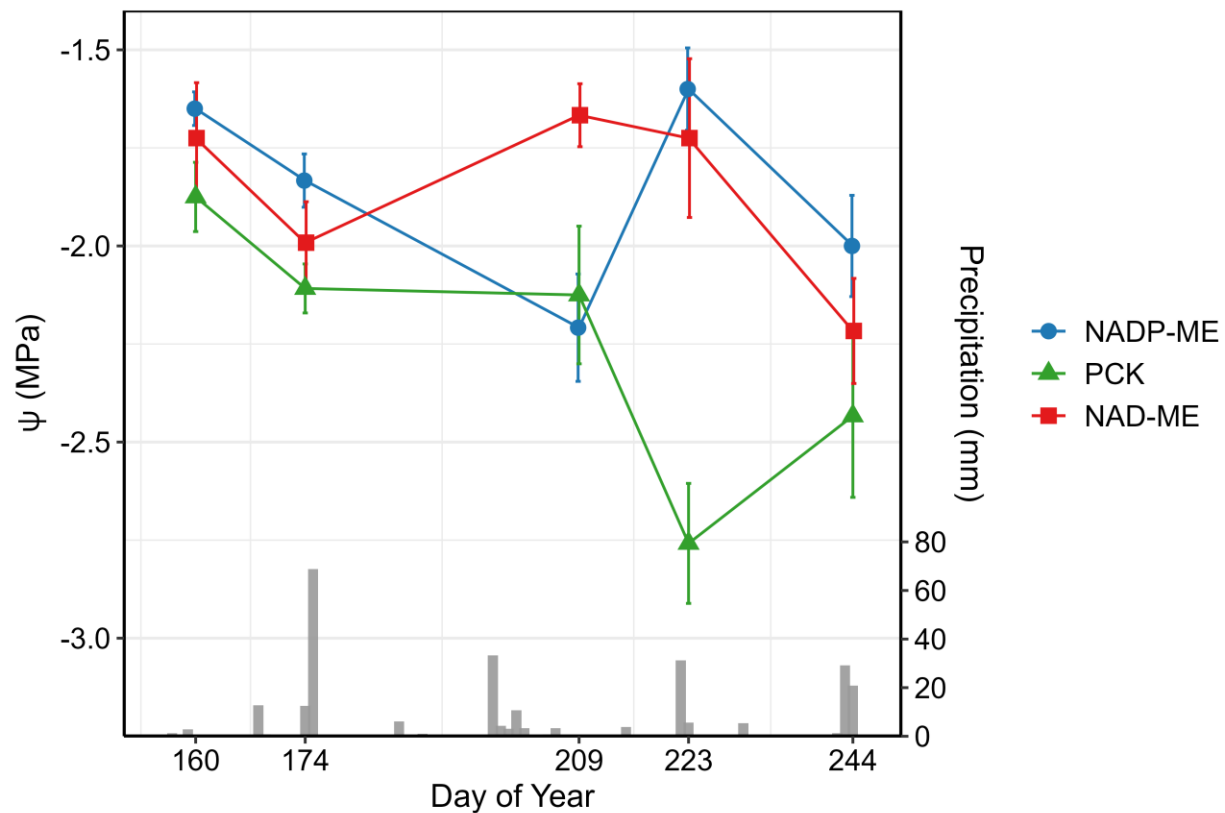


Figure 0.5: Mean ± 1 SE midday leaf water potential (Ψ) of C_4 subtypes across the growing season. Grey bars show daily precipitation at KPBS.

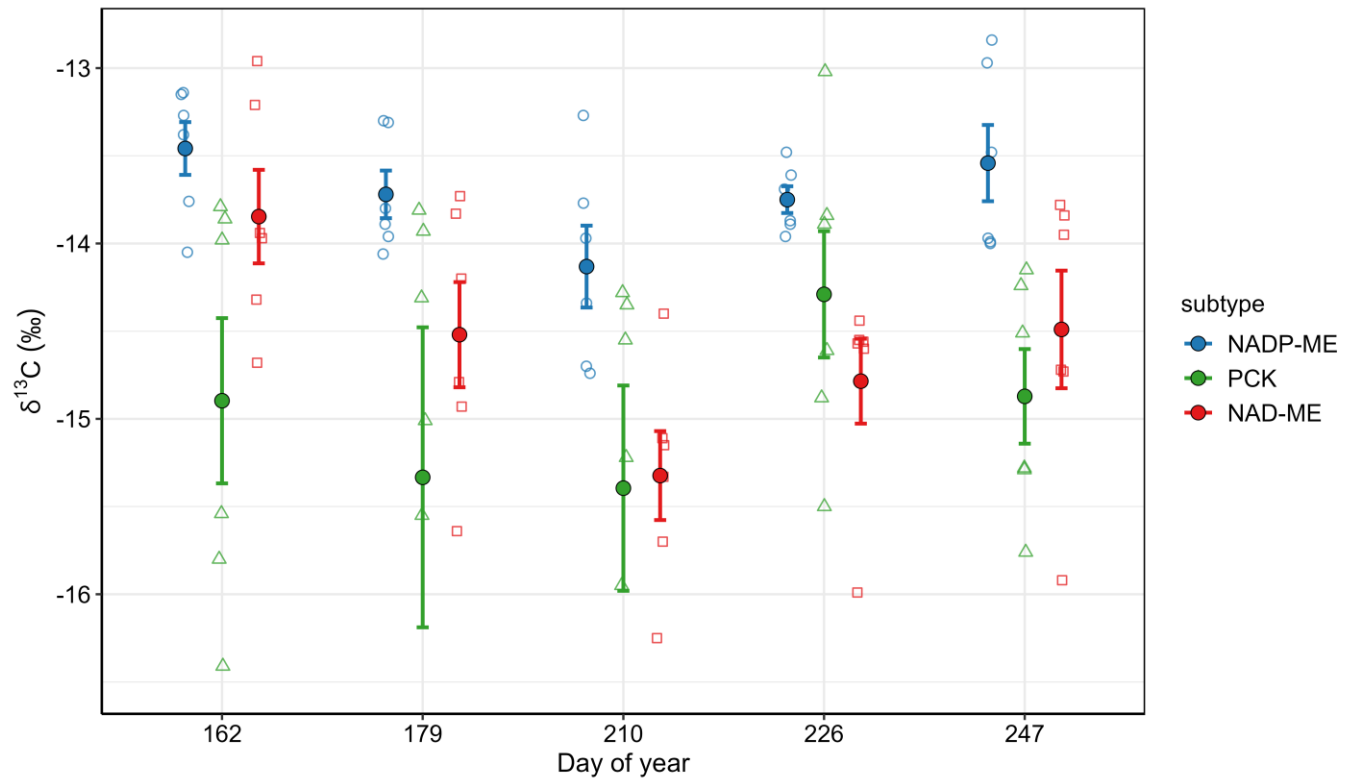


Figure 0.6: Mean ± 1 SE of $\delta^{13}\text{C}$ in C_4 biochemical subtypes across the growing season.

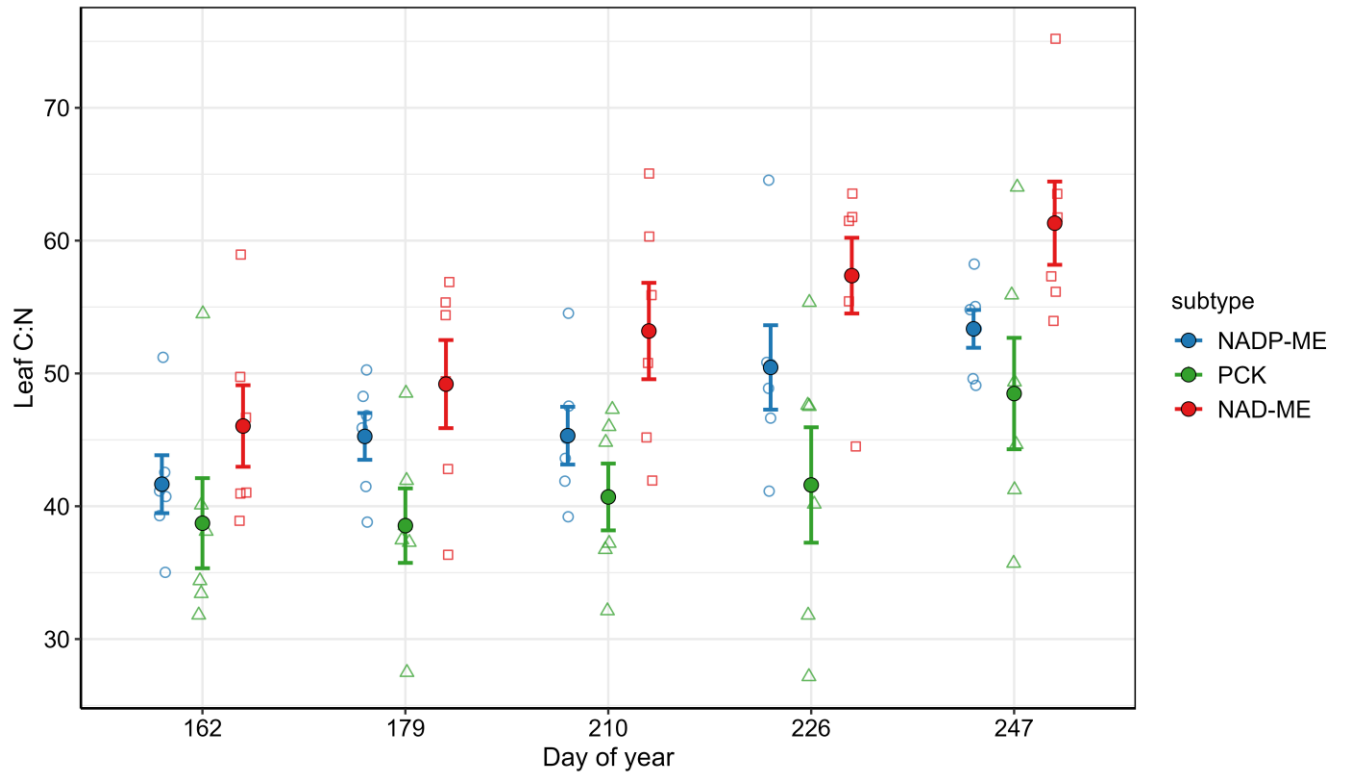


Figure 0.7 Mean $\pm$ 1 SE of leaf C:N in C₄ biochemical subtypes across the growing season.

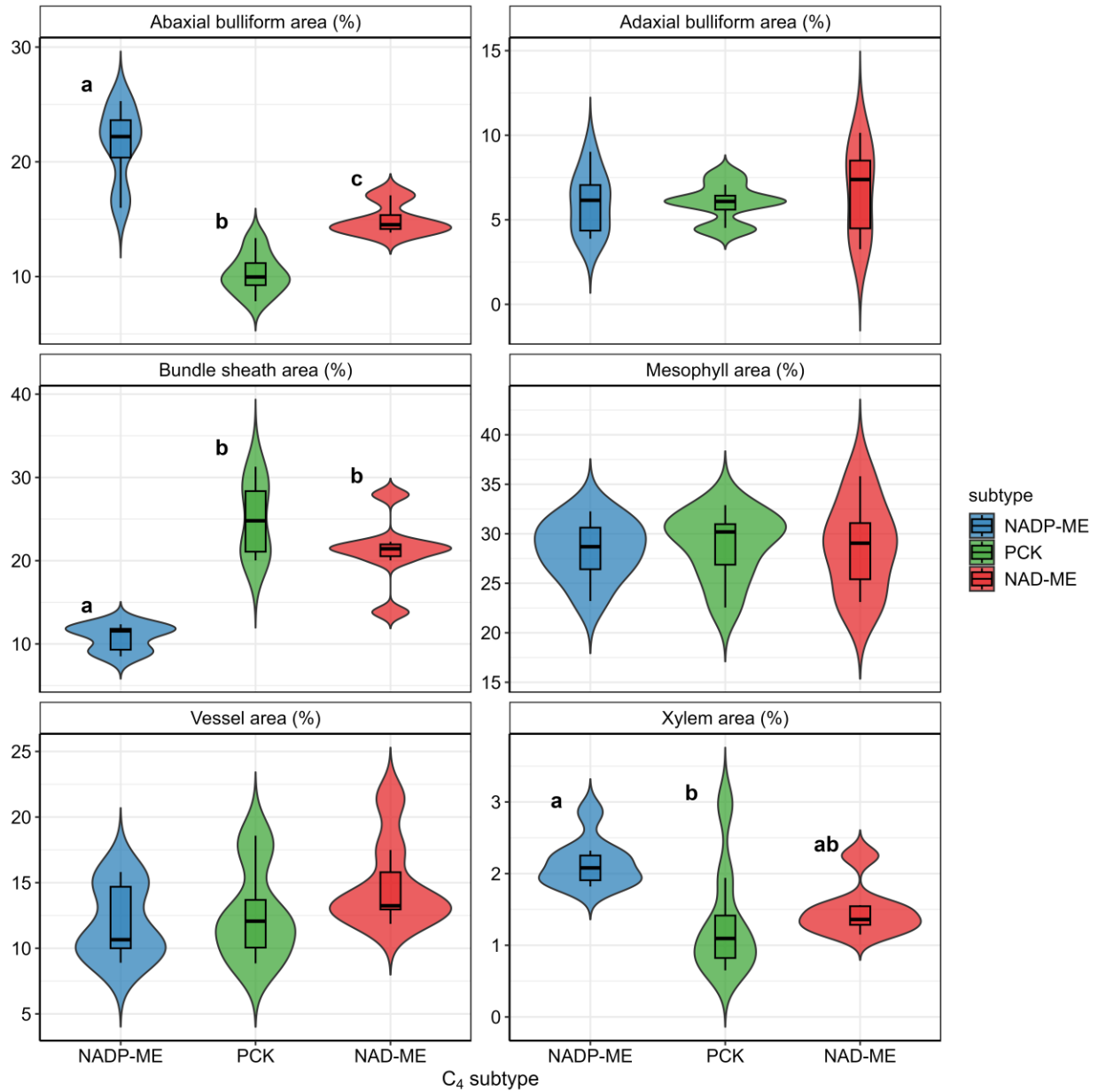


Figure 0.8: Mean $\pm$ 1 SE microanatomical traits grouped by C₄ subtype. Letters indicate significant differences between subtypes.

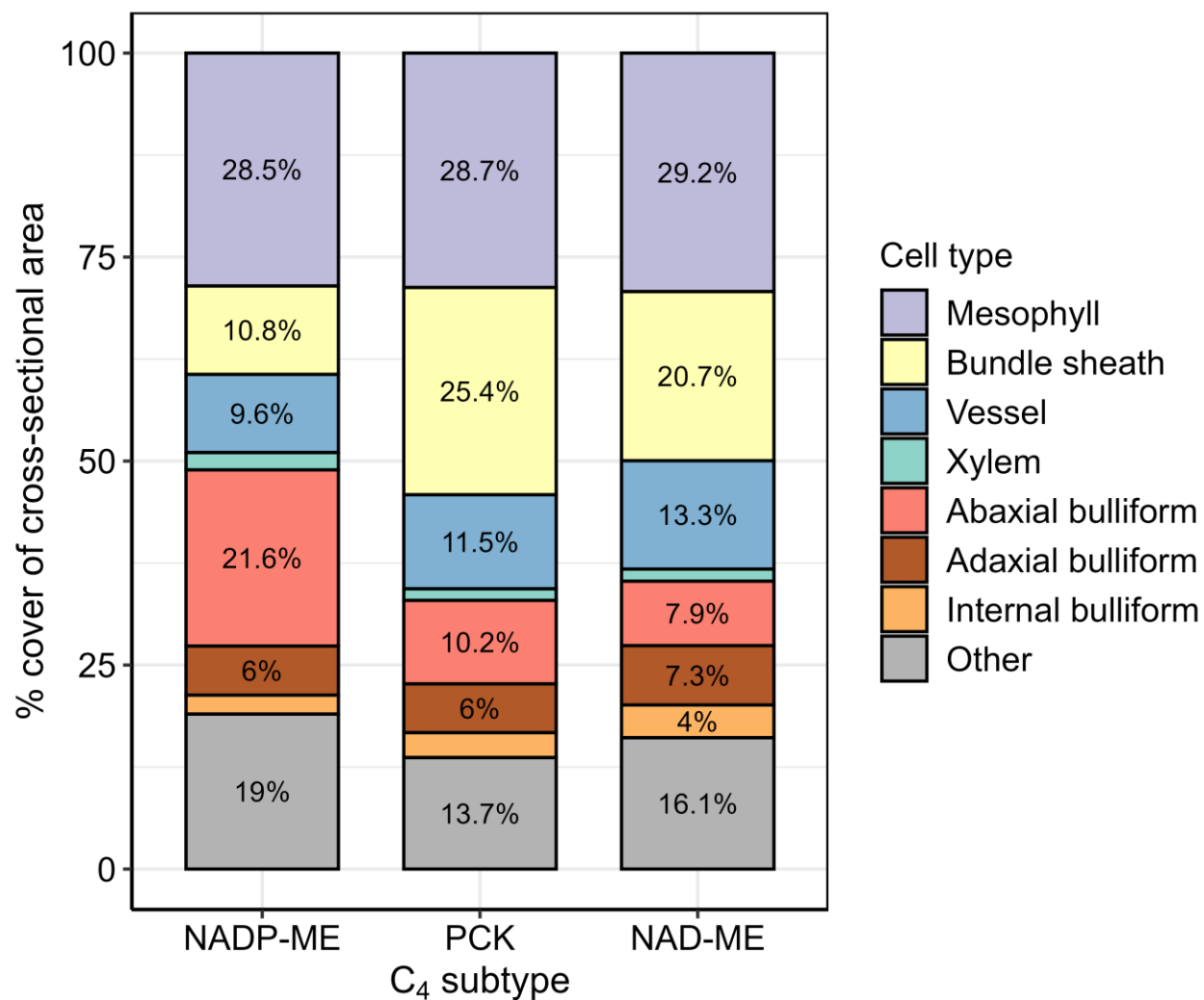


Figure 0.9: Mean percent (%) cover of different cell types in leaf cross-sectional area across C₄ subtypes. “Other” category includes structural cells and connective tissue not measured.

Conclusion

Grasses dominate terrestrial biomes and provide a wide array of ecosystem services (MacDougall *et al.*, 2026; Zhao *et al.*, 2020; Havstad *et al.*, 2007). Nearly half of all grass species use C₄ photosynthesis, providing them with an ecological advantage during hot, dry conditions (Hatch 1987; Christenhusz *et al.*, 2016). Despite comprising only 1% of plant species diversity, C₄ grasses are responsible for 20% of global photosynthesis and are essential to nutrient and hydrological cycles (Luo *et al.*, 2024; Osborne *et al.*, 2024). However, capturing the diversity of C₄ grass function during drought events, especially during atmospheric droughts, proves difficult because of the complex evolutionary history of C₄ photosynthesis. The frequency of atmospheric droughts continues to increase and negatively impact plant function at leaf- to landscape-scales (Dai 2013; Novick *et al.*, 2019). Vapor pressure deficit (VPD), a proxy for atmospheric drought, is a function of temperature and air humidity and is an inherently dynamic environmental characteristic that regulates ecosystem processes (Novick *et al.*, 2024; López *et al.*, 2021; Grossiord *et al.*, 2020). In the atmosphere and at the leaf surface, VPD can be heat-driven (VPD increases with high air and/or leaf temperatures) or driven by humidity deficits (VPD increases with reductions in water vapor concentration). Heat-driven VPD and humidity-driven VPD do not elicit synonymous impacts on plant function (Grossiord *et al.*, 2020; Eze *et al.*, 2024). As our knowledge of woody and herbaceous C₃ plant responses to VPD becomes more established, the responses of C₄ plants to elevated VPD, particularly wild C₄ grasses, remain underrepresented in literature and process-based models (Still *et al.*, 2019).

Quantifying C₄ grass responses to increased VPD facilitates a more holistic understanding of grassland drought responses (Post & Knapp 2020). In my thesis, I aimed to quantify the physiological responses of three photosynthetic groups of C₄ grass to high VPD.

Each group represents the three biochemical variants of C₄ photosynthesis, called subtypes (Gutierrez *et al.*, 1974). The C₄ biochemical subtypes encompass unique anatomical and biochemical traits that impact their photosynthetic and water regulation strategies. The C₄ subtypes are distributed along precipitation gradients and respond differently to soil drought events (Wigley-Coetsee & Staver, 2020; Ghannoum *et al.*, 2005; Ghannoum *et al.*, 2002; Taub, 2000; Wan & Sage, 2001; Hattersley, 1992; Ellis *et al.*, 1980; Albertson & Tomanek, 1965). Because of that, in my projects, I designate NADP-ME as wet-adapted, PCK as intermediate, and NAD-ME as dry-adapted. The goal of this research was to establish a functional and anatomical basis for understanding C₄ biochemical subtype responses to water deficits.

In my second chapter, I quantified the physiological responses of 15 species of C₄ grass over a 12-hour VPD response curve and examined patterns in microanatomical traits related to water use safety and efficiency. VPD at the leaf level was largely driven by humidity deficits. I found that the dry-adapted NAD-ME subtype maintained the most physiological stability under high VPD because of its large bundle sheath cells and high stomatal densities. Like the dry-adapted group, the intermediate PCK has large bundle sheath cells and anatomical traits associated with hydraulic safety. However, the PCK subtype also maintained the highest relative gas exchange rates and showed the steepest decline under elevated VPD. I found that the wet-adapted NADP-ME subtype is the most sensitive to atmospheric water deficits and prioritizes traits that maximize efficiency during ideal conditions like small bundle sheath cells and low stomatal density but operates at low relative gas exchange rates during high VPD.

In my third chapter, I modified a large drying oven to simulate temperature-driven VPD and assessed physiological responses of 6 species of C₄ grass to cooccurring high VPD and reduced soil moisture for 10 days. To contextualize these results at a standardized VPD stress on

the leaf surface, I sampled the same 6 species at five intervals across the 2025 growing season. During the 10-day drought, I found that C₄ subtype responses to temperature-driven VPD did not vary. Only under high temperature-driven VPD and when soil moisture was unavailable, did subtype responses diverge.

Over the growing season, the C₄ subtypes regulated water loss differently. The NADP-ME-dependent species maintained the highest relative gas exchange rates during the sampling day with the highest air temperatures but showed the greatest magnitude of decline at the end of the growing season. The intermediate PCK and dry-adapted NAD-ME subtypes maintained physiological stability across the growing season, with NAD-ME-dependent species also retaining hydraulic function more than their biochemical counterparts even during the warmest sampling day.

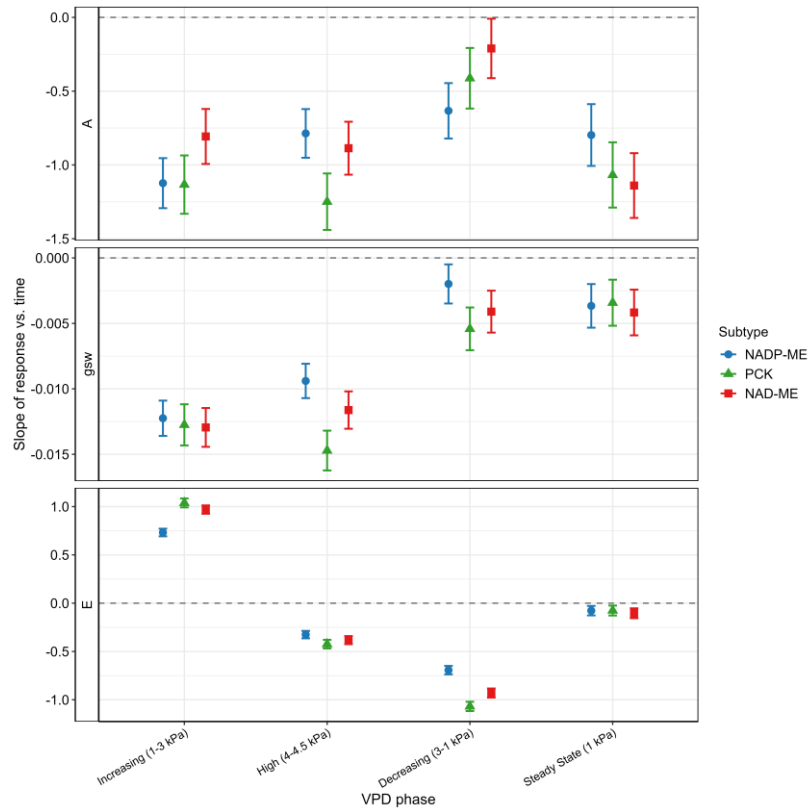
In my projects, the physiological differences of the C₄ subtypes were more obvious under humidity-driven VPD than temperature-driven VPD. While the intermediate PCK frequently behaved similarly to the dry-adapted NAD-ME, inconclusive patterns in geographic distributions and anatomical traits render this subtype difficult to categorize as more dry- or wet-adapted. Measuring water regulation strategies in the C₄ subtypes required a combination of physiological, anatomical, and structural data collected across several temporal scales. This thesis has added to our knowledge of the underlying mechanisms driving C₄ grass responses to atmospheric drought and provides valuable evidence to support developing hypotheses in plant physiological ecology. Together, the results of these studies help us understand during what times and under what conditions is it most appropriate to group functional processes together by C₄ subtype.

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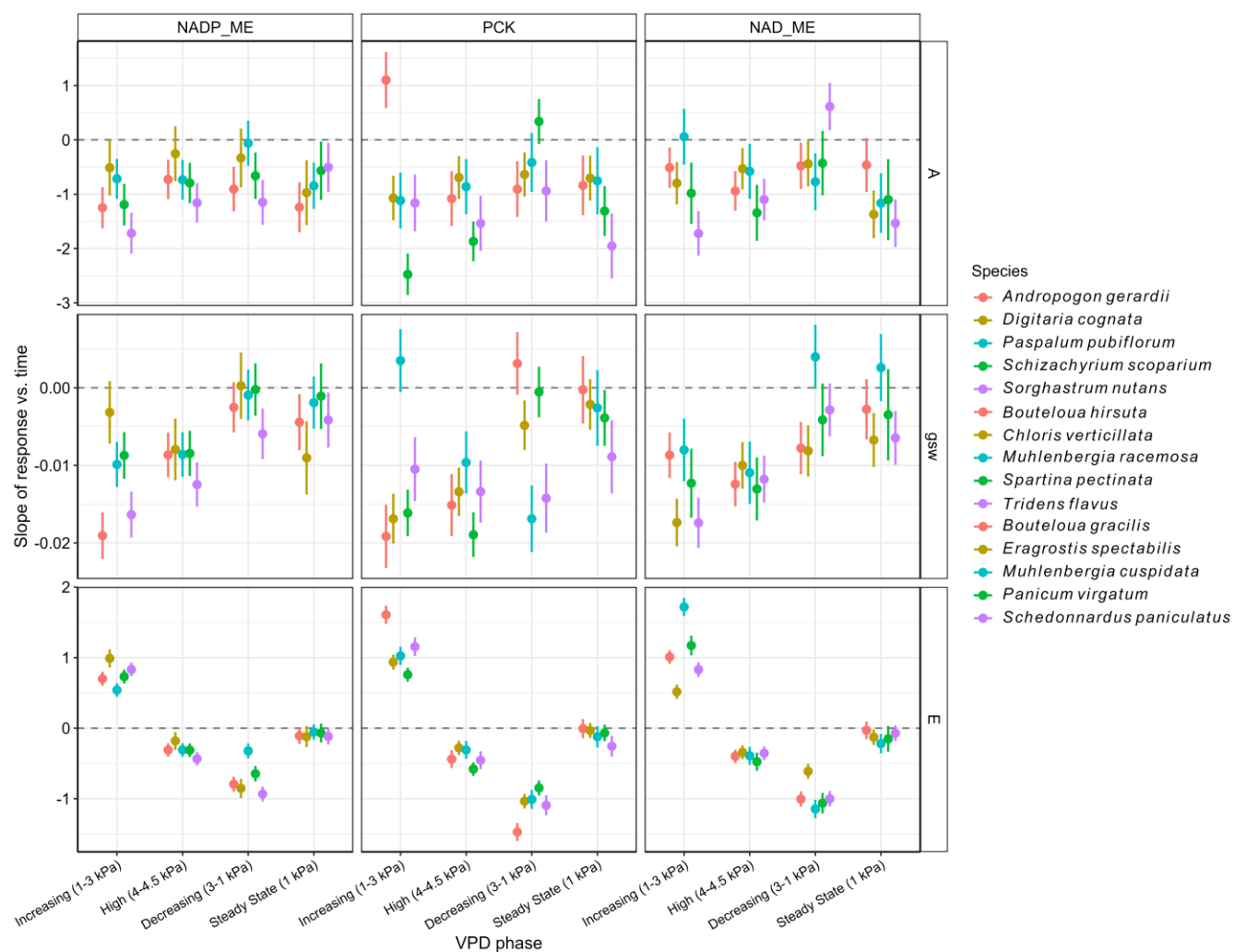
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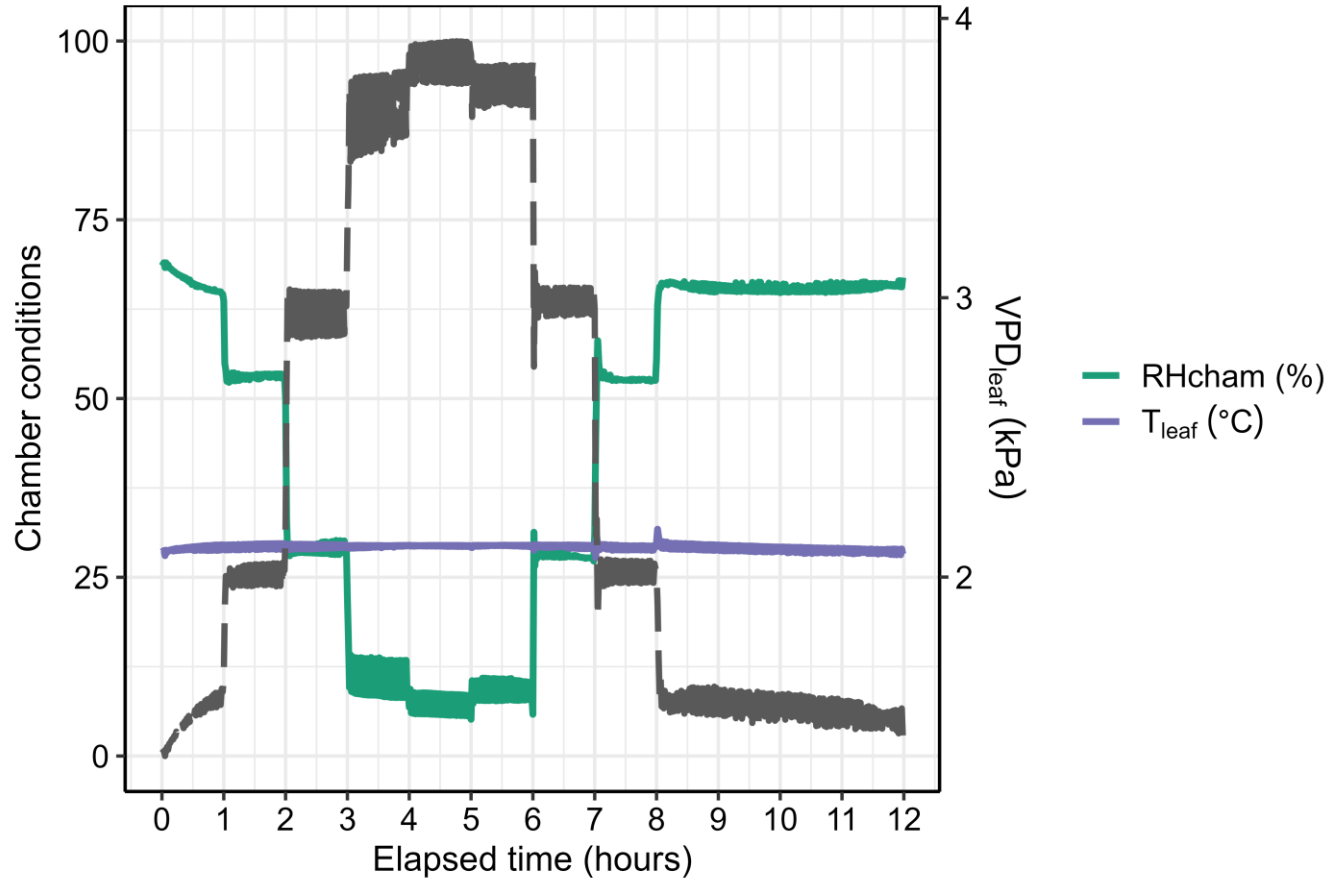
Appendix A - Chapter 2 Supplemental



Appendix Figure A.1: Estimated marginal slopes ($\pm 95\%$ CI) showing change in mean responses of A, g_{sw} , and E over time grouped by C₄ subtype within each VPD phase. Slopes were derived from LMMs with subtype and VPD_{leaf} phase as fixed effects.



Appendix Figure A.2: Estimated marginal slopes ($\pm$ 95% CI) showing change in mean responses of A, g_{sw}, and E over time grouped by species within each C₄ subtype for each VPD phase. Slopes were derived from LMMs.



Appendix Figure A.3: Chamber conditions inside the infrared gas analyzer. VPD_{leaf} is shown as grey, dashed lines, relative humidity in the chamber (RHcham) in green, and leaf temperature (T_{leaf}) in purple.

Appendix Table A.1: Table showing pairwise comparison of the effects of VPD_{leaf} phase and C₄ subtype on mean A, g_{sw}, and E across the VPD_{leaf} response curve.

Response	VPD _{leaf} phase	Contrast	Estimate	SE	df	p-value	sig
A	Increasing VPD _{leaf} (1-3 kPa)	NADP-ME - PCK	-7.82868	2.61	15.9	0.0219	*
A	Increasing VPD _{leaf} (1-3 kPa)	NADP-ME – NAD-ME	-5.52678	2.61	16	0.117	
A	Increasing VPD _{leaf} (1-3 kPa)	PCK – NAD-ME	2.301906	2.69	18.1	0.674	
A	High VPD _{leaf} (4-4.5 kPa)	NADP-ME - PCK	-7.02291	2.6	15.7	0.0401	*
A	High VPD _{leaf} (4-4.5 kPa)	NADP-ME – NAD-ME	-5.49336	2.6	15.8	0.119	
A	High VPD _{leaf} (4-4.5 kPa)	PCK – NAD-ME	1.529552	2.68	17.8	0.837	
A	Decreasing VPD _{leaf} (3-1 kPa)	NADP-ME - PCK	-6.40961	2.6	15.7	0.0629	.
A	Decreasing VPD _{leaf} (3-1 kPa)	NADP-ME – NAD-ME	-5.88365	2.6	15.8	0.0912	.
A	Decreasing VPD _{leaf} (3-1 kPa)	PCK – NAD-ME	0.525964	2.68	17.8	0.979	
A	Steady State VPD _{leaf} (1 kPa)	NADP-ME - PCK	-5.62191	2.68	17.7	0.119	
A	Steady State VPD _{leaf} (1 kPa)	NADP-ME – NAD-ME	-5.97192	2.68	17.8	0.0937	.
A	Steady State VPD _{leaf} (1 kPa)	PCK – NAD-ME	-0.35001	2.75	19.6	0.991	
g _{sw}	Increasing VPD _{leaf} (1-3 kPa)	NADP-ME - PCK	-0.0592	0.03	13.7	0.0806	.
g _{sw}	Increasing VPD _{leaf} (1-3 kPa)	NADP-ME – NAD-ME	-0.04369	0.03	13.8	0.226	
g _{sw}	Increasing VPD _{leaf} (1-3 kPa)	PCK – NAD-ME	0.015512	0.03	15.1	0.82	
g _{sw}	High VPD _{leaf} (4-4.5 kPa)	NADP-ME - PCK	-0.05031	0.03	13.6	0.148	
g _{sw}	High VPD _{leaf} (4-4.5 kPa)	NADP-ME – NAD-ME	-0.03917	0.03	13.8	0.295	
g _{sw}	High VPD _{leaf} (4-4.5 kPa)	PCK – NAD-ME	0.011143	0.03	14.9	0.902	
g _{sw}	Decreasing VPD _{leaf} (3-1 kPa)0029	NADP-ME - PCK	-0.0449	0.03	13.6	0.209	
g _{sw}	Decreasing VPD _{leaf} (3-1 kPa)	NADP-ME – NAD-ME	-0.03973	0.03	13.8	0.286	
g _{sw}	Decreasing VPD _{leaf} (3-1 kPa)	PCK – NAD-ME	0.005163	0.03	14.9	0.978	
g _{sw}	Steady State VPD _{leaf} (1 kPa)	NADP-ME - PCK	-0.03603	0.03	14.5	0.359	
g _{sw}	Steady State VPD _{leaf} (1 kPa)	NADP-ME – NAD-ME	-0.03531	0.03	14.7	0.374	
g _{sw}	Steady State VPD _{leaf} (1 kPa)	PCK – NAD-ME	0.000722	0.03	15.7	1	
E	Increasing VPD _{leaf} (1-3 kPa)	NADP-ME - PCK	-0.00141	0	14.7	0.0865	.

E	Increasing VPDleaf (1-3 kPa)	NADP-ME – NAD-ME	-0.00098	0	14.7	0.276	
E	Increasing VPDleaf (1-3 kPa)	PCK – NAD-ME	0.000429	0	16.1	0.773	
E	High VPDleaf (4-4.5 kPa)	NADP-ME - PCK	-0.00186	0	14.6	0.0209	*
E	High VPDleaf (4-4.5 kPa)	NADP-ME – NAD-ME	-0.00129	0	14.6	0.119	
E	High VPDleaf (4-4.5 kPa)	PCK – NAD-ME	0.000569	0	15.9	0.638	
E	Decreasing VPDleaf (3-1 kPa)	NADP-ME - PCK	-0.00108	0	14.6	0.215	
E	Decreasing VPDleaf (3-1 kPa)	NADP-ME – NAD-ME	-0.00091	0	14.6	0.319	
E	Decreasing VPDleaf (3-1 kPa)	PCK – NAD-ME	0.000163	0	15.9	0.963	
E	Steady State VPDleaf (1 kPa)	NADP-ME - PCK	-0.00055	0	16	0.662	
E	Steady State VPDleaf (1 kPa)	NADP-ME – NAD-ME	-0.00058	0	16	0.626	
E	Steady State VPDleaf (1 kPa)	PCK – NAD-ME	-3.7E-05	0	17.1	0.998	

Appendix Table A.2: Table showing pairwise contrasts of the effects of species and VPD_{leaf} phase on mean A, g_{sw}, and E of 15 C4 grasses. Significance levels were computed by 3-way ANOVA.

Response	Subtype	VPD _{leaf} phase	Contrast	Estimate	SE	df	p-value	sig
A	NADP-ME	Increasing VPD _{leaf} (1-3 kPa)	ANGE - DICO	-0.06561	4.181848	57.7	1	
A	NADP-ME	Increasing VPD _{leaf} (1-3 kPa)	ANGE - PAPU	4.965356	3.590507	52	0.641144	
A	NADP-ME	Increasing VPD _{leaf} (1-3 kPa)	ANGE - SCSC	0.141095	3.757379	47.3	1	
A	NADP-ME	Increasing VPD _{leaf} (1-3 kPa)	ANGE - SONU	-4.00674	3.664598	49.9	0.809102	
A	NADP-ME	Increasing VPD _{leaf} (1-3 kPa)	DICO - PAPU	5.030969	4.116639	59.9	0.738619	
A	NADP-ME	Increasing VPD _{leaf} (1-3 kPa)	DICO - SCSC	0.206708	4.262965	54.9	0.999999	
A	NADP-ME	Increasing VPD _{leaf} (1-3 kPa)	DICO - SONU	-3.94112	4.181417	57.7	0.87901	
A	NADP-ME	Increasing VPD _{leaf} (1-3 kPa)	PAPU - SCSC	-4.82426	3.684665	49.1	0.686798	
A	NADP-ME	Increasing VPD _{leaf} (1-3 kPa)	PAPU - SONU	-8.97209	3.590005	52	0.106471	
A	NADP-ME	Increasing VPD _{leaf} (1-3 kPa)	SCSC - SONU	-4.14783	3.756899	47.3	0.803546	
A	NADP-ME	High VPD _{leaf} (4-4.5 kPa)	ANGE - DICO	-1.24924	4.181848	57.7	0.998214	
A	NADP-ME	High VPD _{leaf} (4-4.5 kPa)	ANGE - PAPU	4.409937	3.590507	52	0.735093	
A	NADP-ME	High VPD _{leaf} (4-4.5 kPa)	ANGE - SCSC	-0.17264	3.757379	47.3	0.999999	
A	NADP-ME	High VPD _{leaf} (4-4.5 kPa)	ANGE - SONU	-1.87401	3.664598	49.9	0.985851	
A	NADP-ME	High VPD _{leaf} (4-4.5 kPa)	DICO - PAPU	5.659182	4.116639	59.9	0.645977	
A	NADP-ME	High VPD _{leaf} (4-4.5 kPa)	DICO - SCSC	1.076607	4.262965	54.9	0.999075	
A	NADP-ME	High VPD _{leaf} (4-4.5 kPa)	DICO - SONU	-0.62476	4.181417	57.7	0.999884	
A	NADP-ME	High VPD _{leaf} (4-4.5 kPa)	PAPU - SCSC	-4.58257	3.684665	49.1	0.726073	
A	NADP-ME	High VPD _{leaf} (4-4.5 kPa)	PAPU - SONU	-6.28394	3.590005	52	0.413073	
A	NADP-ME	High VPD _{leaf} (4-4.5 kPa)	SCSC - SONU	-1.70137	3.756899	47.3	0.991037	
A	NADP-ME	Decreasing VPD _{leaf} (3-1 kPa)	ANGE - DICO	-2.374	4.181848	57.7	0.979218	
A	NADP-ME	Decreasing VPD _{leaf} (3-1 kPa)	ANGE - PAPU	4.033231	3.590507	52	0.793539	
A	NADP-ME	Decreasing VPD _{leaf} (3-1 kPa)	ANGE - SCSC	-0.48488	3.757379	47.3	0.999935	
A	NADP-ME	Decreasing VPD _{leaf} (3-1 kPa)	ANGE - SONU	-1.53718	3.664598	49.9	0.993319	
A	NADP-ME	Decreasing VPD _{leaf} (3-1 kPa)	DICO - PAPU	6.407235	4.116639	59.9	0.530715	
A	NADP-ME	Decreasing VPD _{leaf} (3-1 kPa)	DICO - SCSC	1.889119	4.262965	54.9	0.991785	
A	NADP-ME	Decreasing VPD _{leaf} (3-1 kPa)	DICO - SONU	0.836826	4.181417	57.7	0.999631	
A	NADP-ME	Decreasing VPD _{leaf} (3-1 kPa)	PAPU - SCSC	-4.51812	3.684665	49.1	0.736285	
A	NADP-ME	Decreasing VPD _{leaf} (3-1 kPa)	PAPU - SONU	-5.57041	3.590005	52	0.534477	

A	NADP-ME	Decreasing VPDleaf (3-1 kPa)	SCSC - SONU	-1.05229	3.756899	47.3	0.998603	
A	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - DICO	-2.87416	4.520681	72.4	0.96871	
A	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - PAPU	2.671208	3.851798	65.1	0.957208	
A	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - SCSC	-0.86166	4.132843	64.9	0.999568	
A	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - SONU	-1.46297	3.946279	63	0.995874	
A	NADP-ME	Steady State VPDleaf (1 kPa)	DICO - PAPU	5.545367	4.366288	71.4	0.710222	
A	NADP-ME	Steady State VPDleaf (1 kPa)	DICO - SCSC	2.012496	4.616115	70.5	0.99233	
A	NADP-ME	Steady State VPDleaf (1 kPa)	DICO - SONU	1.411186	4.449859	69.4	0.997756	
A	NADP-ME	Steady State VPDleaf (1 kPa)	PAPU - SCSC	-3.53287	3.963371	63.2	0.899057	
A	NADP-ME	Steady State VPDleaf (1 kPa)	PAPU - SONU	-4.13418	3.768426	61.1	0.807373	
A	NADP-ME	Steady State VPDleaf (1 kPa)	SCSC - SONU	-0.60131	4.055254	61.4	0.999888	
E	NADP-ME	Increasing VPDleaf (1-3 kPa)	ANGE - DICO	-0.00043	0.00087	65.6	0.987505	
E	NADP-ME	Increasing VPDleaf (1-3 kPa)	ANGE - PAPU	0.000932	0.000743	58.5	0.719717	
E	NADP-ME	Increasing VPDleaf (1-3 kPa)	ANGE - SCSC	-7.8E-05	0.000774	52.5	0.999976	
E	NADP-ME	Increasing VPDleaf (1-3 kPa)	ANGE - SONU	-0.00061	0.000757	55.8	0.929578	
E	NADP-ME	Increasing VPDleaf (1-3 kPa)	DICO - PAPU	0.001363	0.000858	68.3	0.509235	
E	NADP-ME	Increasing VPDleaf (1-3 kPa)	DICO - SCSC	0.000354	0.000885	62.2	0.994483	
E	NADP-ME	Increasing VPDleaf (1-3 kPa)	DICO - SONU	-0.00017	0.00087	65.6	0.999631	
E	NADP-ME	Increasing VPDleaf (1-3 kPa)	PAPU - SCSC	-0.00101	0.00076	54.8	0.675358	
E	NADP-ME	Increasing VPDleaf (1-3 kPa)	PAPU - SONU	-0.00154	0.000743	58.5	0.247217	
E	NADP-ME	Increasing VPDleaf (1-3 kPa)	SCSC - SONU	-0.00053	0.000774	52.5	0.95949	
E	NADP-ME	High VPDleaf (4-4.5 kPa)	ANGE - DICO	-0.00108	0.00087	65.6	0.727628	
E	NADP-ME	High VPDleaf (4-4.5 kPa)	ANGE - PAPU	0.000637	0.000743	58.5	0.911059	
E	NADP-ME	High VPDleaf (4-4.5 kPa)	ANGE - SCSC	-0.00073	0.000774	52.5	0.880581	
E	NADP-ME	High VPDleaf (4-4.5 kPa)	ANGE - SONU	-0.00102	0.000757	55.8	0.661673	
E	NADP-ME	High VPDleaf (4-4.5 kPa)	DICO - PAPU	0.001716	0.000858	68.3	0.276442	
E	NADP-ME	High VPDleaf (4-4.5 kPa)	DICO - SCSC	0.000353	0.000885	62.2	0.994522	
E	NADP-ME	High VPDleaf (4-4.5 kPa)	DICO - SONU	5.76E-05	0.00087	65.6	0.999996	
E	NADP-ME	High VPDleaf (4-4.5 kPa)	PAPU - SCSC	-0.00136	0.00076	54.8	0.387951	
E	NADP-ME	High VPDleaf (4-4.5 kPa)	PAPU - SONU	-0.00166	0.000743	58.5	0.18231	
E	NADP-ME	High VPDleaf (4-4.5 kPa)	SCSC - SONU	-0.0003	0.000774	52.5	0.995361	
E	NADP-ME	Decreasing VPDleaf (3-1 kPa)	ANGE - DICO	-0.00086	0.00087	65.6	0.858922	

E	NADP-ME	Decreasing VPDleaf (3-1 kPa)	ANGE - PAPU	0.000687	0.000743	58.5	0.886314	
E	NADP-ME	Decreasing VPDleaf (3-1 kPa)	ANGE - SCSC	-0.00034	0.000774	52.5	0.992337	
E	NADP-ME	Decreasing VPDleaf (3-1 kPa)	ANGE - SONU	-0.00037	0.000757	55.8	0.988298	
E	NADP-ME	Decreasing VPDleaf (3-1 kPa)	DICO - PAPU	0.001548	0.000858	68.3	0.379283	
E	NADP-ME	Decreasing VPDleaf (3-1 kPa)	DICO - SCSC	0.000524	0.000885	62.2	0.975684	
E	NADP-ME	Decreasing VPDleaf (3-1 kPa)	DICO - SONU	0.000493	0.00087	65.6	0.979428	
E	NADP-ME	Decreasing VPDleaf (3-1 kPa)	PAPU - SCSC	-0.00102	0.00076	54.8	0.663973	
E	NADP-ME	Decreasing VPDleaf (3-1 kPa)	PAPU - SONU	-0.00106	0.000743	58.5	0.617336	
E	NADP-ME	Decreasing VPDleaf (3-1 kPa)	SCSC - SONU	-3.2E-05	0.000774	52.5	0.999999	
E	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - DICO	-0.00071	0.00095	82.6	0.945844	
E	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - PAPU	0.000156	0.000806	74.2	0.999681	
E	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - SCSC	-0.00025	0.000864	74	0.998354	
E	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - SONU	-4.1E-05	0.000824	71.5	0.999999	
E	NADP-ME	Steady State VPDleaf (1 kPa)	DICO - PAPU	0.000861	0.000917	81.9	0.880897	
E	NADP-ME	Steady State VPDleaf (1 kPa)	DICO - SCSC	0.000452	0.000969	80.8	0.990114	
E	NADP-ME	Steady State VPDleaf (1 kPa)	DICO - SONU	0.000664	0.000933	79.2	0.953379	
E	NADP-ME	Steady State VPDleaf (1 kPa)	PAPU - SCSC	-0.00041	0.000828	72.5	0.987682	
E	NADP-ME	Steady State VPDleaf (1 kPa)	PAPU - SONU	-0.0002	0.000786	69.6	0.999105	
E	NADP-ME	Steady State VPDleaf (1 kPa)	SCSC - SONU	0.000212	0.000846	70	0.999111	
g_{sw}	NADP-ME	Increasing VPDleaf (1-3 kPa)	ANGE - DICO	-0.01508	0.034544	56.4	0.992251	
g_{sw}	NADP-ME	Increasing VPDleaf (1-3 kPa)	ANGE - PAPU	0.044752	0.029685	51	0.562456	
g_{sw}	NADP-ME	Increasing VPDleaf (1-3 kPa)	ANGE - SCSC	0.002468	0.031091	46.4	0.999991	
g_{sw}	NADP-ME	Increasing VPDleaf (1-3 kPa)	ANGE - SONU	-0.02521	0.030309	48.9	0.919399	
g_{sw}	NADP-ME	Increasing VPDleaf (1-3 kPa)	DICO - PAPU	0.05983	0.033995	58.5	0.406402	
g_{sw}	NADP-ME	Increasing VPDleaf (1-3 kPa)	DICO - SCSC	0.017546	0.035229	53.7	0.987211	
g_{sw}	NADP-ME	Increasing VPDleaf (1-3 kPa)	DICO - SONU	-0.01014	0.034541	56.3	0.998334	
g_{sw}	NADP-ME	Increasing VPDleaf (1-3 kPa)	PAPU - SCSC	-0.04228	0.030479	48.2	0.638595	
g_{sw}	NADP-ME	Increasing VPDleaf (1-3 kPa)	PAPU - SONU	-0.06997	0.029681	51	0.14403	
g_{sw}	NADP-ME	Increasing VPDleaf (1-3 kPa)	SCSC - SONU	-0.02768	0.031087	46.4	0.898995	
g_{sw}	NADP-ME	High VPDleaf (4-4.5 kPa)	ANGE - DICO	-0.03289	0.034544	56.4	0.875069	
g_{sw}	NADP-ME	High VPDleaf (4-4.5 kPa)	ANGE - PAPU	0.02043	0.029685	51	0.95815	
g_{sw}	NADP-ME	High VPDleaf (4-4.5 kPa)	ANGE - SCSC	-0.02105	0.031091	46.4	0.960409	

g_{sw}	NADP-ME	High VPDleaf (4-4.5 kPa)	ANGE - SONU	-0.02269	0.030309	48.9	0.943704	
g_{sw}	NADP-ME	High VPDleaf (4-4.5 kPa)	DICO - PAPU	0.053316	0.033995	58.5	0.523288	
g_{sw}	NADP-ME	High VPDleaf (4-4.5 kPa)	DICO - SCSC	0.011834	0.035229	53.7	0.997172	
g_{sw}	NADP-ME	High VPDleaf (4-4.5 kPa)	DICO - SONU	0.010192	0.034541	56.3	0.998297	
g_{sw}	NADP-ME	High VPDleaf (4-4.5 kPa)	PAPU - SCSC	-0.04148	0.030479	48.2	0.654991	
g_{sw}	NADP-ME	High VPDleaf (4-4.5 kPa)	PAPU - SONU	-0.04312	0.029681	51	0.597072	
g_{sw}	NADP-ME	High VPDleaf (4-4.5 kPa)	SCSC - SONU	-0.00164	0.031087	46.4	0.999998	
g_{sw}	NADP-ME	Decreasing VPDleaf (3-1 kPa)	ANGE - DICO	-0.03973	0.034544	56.4	0.779104	
g_{sw}	NADP-ME	Decreasing VPDleaf (3-1 kPa)	ANGE - PAPU	0.034518	0.029685	51	0.772175	
g_{sw}	NADP-ME	Decreasing VPDleaf (3-1 kPa)	ANGE - SCSC	-0.0087	0.031091	46.4	0.998606	
g_{sw}	NADP-ME	Decreasing VPDleaf (3-1 kPa)	ANGE - SONU	-0.01381	0.030309	48.9	0.990825	
g_{sw}	NADP-ME	Decreasing VPDleaf (3-1 kPa)	DICO - PAPU	0.074252	0.033995	58.5	0.200106	
g_{sw}	NADP-ME	Decreasing VPDleaf (3-1 kPa)	DICO - SCSC	0.031032	0.035229	53.7	0.902712	
g_{sw}	NADP-ME	Decreasing VPDleaf (3-1 kPa)	DICO - SONU	0.025919	0.034541	56.3	0.943431	
g_{sw}	NADP-ME	Decreasing VPDleaf (3-1 kPa)	PAPU - SCSC	-0.04322	0.030479	48.2	0.619307	
g_{sw}	NADP-ME	Decreasing VPDleaf (3-1 kPa)	PAPU - SONU	-0.04833	0.029681	51	0.486662	
g_{sw}	NADP-ME	Decreasing VPDleaf (3-1 kPa)	SCSC - SONU	-0.00511	0.031087	46.4	0.999829	
g_{sw}	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - DICO	-0.02995	0.03727	70.6	0.928657	
g_{sw}	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - PAPU	0.035492	0.031784	63.6	0.797186	
g_{sw}	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - SCSC	-0.00752	0.034105	63.3	0.999461	
g_{sw}	NADP-ME	Steady State VPDleaf (1 kPa)	ANGE - SONU	-0.00227	0.032572	61.6	0.999994	
g_{sw}	NADP-ME	Steady State VPDleaf (1 kPa)	DICO - PAPU	0.065444	0.036002	69.6	0.371805	
g_{sw}	NADP-ME	Steady State VPDleaf (1 kPa)	DICO - SCSC	0.022434	0.038066	68.7	0.976241	
g_{sw}	NADP-ME	Steady State VPDleaf (1 kPa)	DICO - SONU	0.027678	0.0367	67.7	0.94261	
g_{sw}	NADP-ME	Steady State VPDleaf (1 kPa)	PAPU - SCSC	-0.04301	0.032714	61.7	0.683188	
g_{sw}	NADP-ME	Steady State VPDleaf (1 kPa)	PAPU - SONU	-0.03777	0.031113	59.7	0.743411	
g_{sw}	NADP-ME	Steady State VPDleaf (1 kPa)	SCSC - SONU	0.005244	0.03348	60	0.999861	
A	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - CHVE	2.044464	4.874929	61.5	0.993364	
A	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - MURA	8.373431	5.309388	63.6	0.517346	
A	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - SPPE	-0.57124	4.820233	54.4	0.999954	
A	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - TRFL	-0.42977	5.309388	63.6	0.99999	
A	PCK	Increasing VPDleaf (1-3 kPa)	CHVE - MURA	6.328967	4.874929	61.5	0.693249	

A	PCK	Increasing VPDleaf (1-3 kPa)	CHVE - SPPE	-2.6157	4.337046	50.1	0.973963	
A	PCK	Increasing VPDleaf (1-3 kPa)	CHVE - TRFL	-2.47424	4.874929	61.5	0.986321	
A	PCK	Increasing VPDleaf (1-3 kPa)	MURA - SPPE	-8.94467	4.820233	54.4	0.353297	
A	PCK	Increasing VPDleaf (1-3 kPa)	MURA - TRFL	-8.8032	5.309388	63.6	0.466861	
A	PCK	Increasing VPDleaf (1-3 kPa)	SPPE - TRFL	0.141462	4.820233	54.4	1	
A	PCK	High VPDleaf (4-4.5 kPa)	BOHI - CHVE	2.375408	4.792535	59.1	0.987475	
A	PCK	High VPDleaf (4-4.5 kPa)	BOHI - MURA	9.796445	5.309388	63.6	0.35741	
A	PCK	High VPDleaf (4-4.5 kPa)	BOHI - SPPE	4.075354	4.820233	54.4	0.915062	
A	PCK	High VPDleaf (4-4.5 kPa)	BOHI - TRFL	2.915866	5.309388	63.6	0.981657	
A	PCK	High VPDleaf (4-4.5 kPa)	CHVE - MURA	7.421037	4.792535	59.1	0.535819	
A	PCK	High VPDleaf (4-4.5 kPa)	CHVE - SPPE	1.699946	4.244223	47.3	0.994393	
A	PCK	High VPDleaf (4-4.5 kPa)	CHVE - TRFL	0.540458	4.792535	59.1	0.999962	
A	PCK	High VPDleaf (4-4.5 kPa)	MURA - SPPE	-5.72109	4.820233	54.4	0.758767	
A	PCK	High VPDleaf (4-4.5 kPa)	MURA - TRFL	-6.88058	5.309388	63.6	0.694643	
A	PCK	High VPDleaf (4-4.5 kPa)	SPPE - TRFL	-1.15949	4.820233	54.4	0.999236	
A	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - CHVE	2.314147	4.792535	59.1	0.988649	
A	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - MURA	8.479278	5.309388	63.6	0.504797	
A	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - SPPE	3.857657	4.820233	54.4	0.929391	
A	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - TRFL	3.685234	5.309388	63.6	0.957059	
A	PCK	Decreasing VPDleaf (3-1 kPa)	CHVE - MURA	6.165131	4.792535	59.1	0.700485	
A	PCK	Decreasing VPDleaf (3-1 kPa)	CHVE - SPPE	1.54351	4.244223	47.3	0.996135	
A	PCK	Decreasing VPDleaf (3-1 kPa)	CHVE - TRFL	1.371086	4.792535	59.1	0.998493	
A	PCK	Decreasing VPDleaf (3-1 kPa)	MURA - SPPE	-4.62162	4.820233	54.4	0.872185	
A	PCK	Decreasing VPDleaf (3-1 kPa)	MURA - TRFL	-4.79404	5.309388	63.6	0.894734	
A	PCK	Decreasing VPDleaf (3-1 kPa)	SPPE - TRFL	-0.17242	4.820233	54.4	1	
A	PCK	Steady State VPDleaf (1 kPa)	BOHI - CHVE	4.468785	4.792535	59.1	0.883129	
A	PCK	Steady State VPDleaf (1 kPa)	BOHI - MURA	7.173437	5.544138	69.9	0.695812	
A	PCK	Steady State VPDleaf (1 kPa)	BOHI - SPPE	2.07024	5.067673	61.3	0.993999	
A	PCK	Steady State VPDleaf (1 kPa)	BOHI - TRFL	4.549818	5.544138	69.9	0.923401	
A	PCK	Steady State VPDleaf (1 kPa)	CHVE - MURA	2.704651	5.051361	66.8	0.983326	
A	PCK	Steady State VPDleaf (1 kPa)	CHVE - SPPE	-2.39855	4.523283	55.9	0.983849	
A	PCK	Steady State VPDleaf (1 kPa)	CHVE - TRFL	0.081033	5.051361	66.8	1	

A	PCK	Steady State VPDleaf (1 kPa)	MURA - SPPE	-5.1032	5.313113	68.2	0.871732	
A	PCK	Steady State VPDleaf (1 kPa)	MURA - TRFL	-2.62362	5.769344	75.5	0.991007	
A	PCK	Steady State VPDleaf (1 kPa)	SPPE - TRFL	2.479578	5.313113	68.2	0.990055	
E	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - CHVE	0.001809	0.001041	39.5	0.423104	
E	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - MURA	0.003124	0.001129	40.2	0.061339	.
E	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - SPPE	0.002302	0.001049	36.1	0.204708	
E	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - TRFL	0.002	0.001129	40.2	0.403525	
E	PCK	Increasing VPDleaf (1-3 kPa)	CHVE - MURA	0.001315	0.001041	39.5	0.714314	
E	PCK	Increasing VPDleaf (1-3 kPa)	CHVE - SPPE	0.000493	0.000954	34.6	0.985031	
E	PCK	Increasing VPDleaf (1-3 kPa)	CHVE - TRFL	0.000191	0.001041	39.5	0.999734	
E	PCK	Increasing VPDleaf (1-3 kPa)	MURA - SPPE	-0.00082	0.001049	36.1	0.933735	
E	PCK	Increasing VPDleaf (1-3 kPa)	MURA - TRFL	-0.00112	0.001129	40.2	0.855642	
E	PCK	Increasing VPDleaf (1-3 kPa)	SPPE - TRFL	-0.0003	0.001049	36.1	0.998422	
E	PCK	High VPDleaf (4-4.5 kPa)	BOHI - CHVE	0.002062	0.00103	38.2	0.284502	
E	PCK	High VPDleaf (4-4.5 kPa)	BOHI - MURA	0.003359	0.001129	40.2	0.037459	*
E	PCK	High VPDleaf (4-4.5 kPa)	BOHI - SPPE	0.003296	0.001049	36.1	0.025999	*
E	PCK	High VPDleaf (4-4.5 kPa)	BOHI - TRFL	0.00293	0.001129	40.2	0.090311	.
E	PCK	High VPDleaf (4-4.5 kPa)	CHVE - MURA	0.001296	0.00103	38.2	0.717637	
E	PCK	High VPDleaf (4-4.5 kPa)	CHVE - SPPE	0.001234	0.000942	33.2	0.687307	
E	PCK	High VPDleaf (4-4.5 kPa)	CHVE - TRFL	0.000867	0.00103	38.2	0.915688	
E	PCK	High VPDleaf (4-4.5 kPa)	MURA - SPPE	-6.3E-05	0.001049	36.1	0.999997	
E	PCK	High VPDleaf (4-4.5 kPa)	MURA - TRFL	-0.00043	0.001129	40.2	0.995397	
E	PCK	High VPDleaf (4-4.5 kPa)	SPPE - TRFL	-0.00037	0.001049	36.1	0.996665	
E	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - CHVE	0.001183	0.00103	38.2	0.779803	
E	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - MURA	0.002485	0.001129	40.2	0.20004	
E	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - SPPE	0.002056	0.001049	36.1	0.305508	
E	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - TRFL	0.001887	0.001129	40.2	0.46223	
E	PCK	Decreasing VPDleaf (3-1 kPa)	CHVE - MURA	0.001302	0.00103	38.2	0.71455	
E	PCK	Decreasing VPDleaf (3-1 kPa)	CHVE - SPPE	0.000873	0.000942	33.2	0.884475	
E	PCK	Decreasing VPDleaf (3-1 kPa)	CHVE - TRFL	0.000704	0.00103	38.2	0.958874	
E	PCK	Decreasing VPDleaf (3-1 kPa)	MURA - SPPE	-0.00043	0.001049	36.1	0.993885	
E	PCK	Decreasing VPDleaf (3-1 kPa)	MURA - TRFL	-0.0006	0.001129	40.2	0.983784	

E	PCK	Decreasing VPDleaf (3-1 kPa)	SPPE - TRFL	-0.00017	0.001049	36.1	0.999841	
E	PCK	Steady State VPDleaf (1 kPa)	BOHI - CHVE	0.001123	0.00103	38.2	0.810399	
E	PCK	Steady State VPDleaf (1 kPa)	BOHI - MURA	0.002035	0.001159	43.9	0.411803	
E	PCK	Steady State VPDleaf (1 kPa)	BOHI - SPPE	0.001445	0.00108	39.9	0.670004	
E	PCK	Steady State VPDleaf (1 kPa)	BOHI - TRFL	0.001731	0.001159	43.9	0.572046	
E	PCK	Steady State VPDleaf (1 kPa)	CHVE - MURA	0.000912	0.001063	42.4	0.910601	
E	PCK	Steady State VPDleaf (1 kPa)	CHVE - SPPE	0.000322	0.000977	37.6	0.997347	
E	PCK	Steady State VPDleaf (1 kPa)	CHVE - TRFL	0.000608	0.001063	42.4	0.978478	
E	PCK	Steady State VPDleaf (1 kPa)	MURA - SPPE	-0.00059	0.001112	43.8	0.983678	
E	PCK	Steady State VPDleaf (1 kPa)	MURA - TRFL	-0.0003	0.001188	47.5	0.999024	
E	PCK	Steady State VPDleaf (1 kPa)	SPPE - TRFL	0.000286	0.001112	43.8	0.998993	
g_{sw}	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - CHVE	0.066391	0.042111	31.3	0.522763	
g_{sw}	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - MURA	0.135632	0.045593	31.7	0.041255	*
g_{sw}	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - SPPE	0.100384	0.042898	29.6	0.160753	
g_{sw}	PCK	Increasing VPDleaf (1-3 kPa)	BOHI - TRFL	0.084627	0.045593	31.7	0.360918	
g_{sw}	PCK	Increasing VPDleaf (1-3 kPa)	CHVE - MURA	0.069241	0.042111	31.3	0.481614	
g_{sw}	PCK	Increasing VPDleaf (1-3 kPa)	CHVE - SPPE	0.033993	0.039178	28.9	0.906343	
g_{sw}	PCK	Increasing VPDleaf (1-3 kPa)	CHVE - TRFL	0.018236	0.042111	31.3	0.992312	
g_{sw}	PCK	Increasing VPDleaf (1-3 kPa)	MURA - SPPE	-0.03525	0.042898	29.6	0.921815	
g_{sw}	PCK	Increasing VPDleaf (1-3 kPa)	MURA - TRFL	-0.05101	0.045593	31.7	0.795512	
g_{sw}	PCK	Increasing VPDleaf (1-3 kPa)	SPPE - TRFL	-0.01576	0.042898	29.6	0.995899	
g_{sw}	PCK	High VPDleaf (4-4.5 kPa)	BOHI - CHVE	0.038075	0.041855	30.7	0.890979	
g_{sw}	PCK	High VPDleaf (4-4.5 kPa)	BOHI - MURA	0.085212	0.045593	31.7	0.354153	
g_{sw}	PCK	High VPDleaf (4-4.5 kPa)	BOHI - SPPE	0.081453	0.042898	29.6	0.340062	
g_{sw}	PCK	High VPDleaf (4-4.5 kPa)	BOHI - TRFL	0.070247	0.045593	31.7	0.544754	
g_{sw}	PCK	High VPDleaf (4-4.5 kPa)	CHVE - MURA	0.047136	0.041855	30.7	0.791572	
g_{sw}	PCK	High VPDleaf (4-4.5 kPa)	CHVE - SPPE	0.043378	0.038903	28.2	0.797305	
g_{sw}	PCK	High VPDleaf (4-4.5 kPa)	CHVE - TRFL	0.032171	0.041855	30.7	0.937664	
g_{sw}	PCK	High VPDleaf (4-4.5 kPa)	MURA - SPPE	-0.00376	0.042898	29.6	0.999986	
g_{sw}	PCK	High VPDleaf (4-4.5 kPa)	MURA - TRFL	-0.01496	0.045593	31.7	0.997357	
g_{sw}	PCK	High VPDleaf (4-4.5 kPa)	SPPE - TRFL	-0.01121	0.042898	29.6	0.998913	
g_{sw}	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - CHVE	0.044325	0.041855	30.7	0.825664	

g_{sw}	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - MURA	0.107144	0.045593	31.7	0.155998	
g_{sw}	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - SPPE	0.09038	0.042898	29.6	0.243915	
g_{sw}	PCK	Decreasing VPDleaf (3-1 kPa)	BOHI - TRFL	0.088808	0.045593	31.7	0.314129	
g_{sw}	PCK	Decreasing VPDleaf (3-1 kPa)	CHVE - MURA	0.06282	0.041855	30.7	0.569732	
g_{sw}	PCK	Decreasing VPDleaf (3-1 kPa)	CHVE - SPPE	0.046056	0.038903	28.2	0.760321	
g_{sw}	PCK	Decreasing VPDleaf (3-1 kPa)	CHVE - TRFL	0.044483	0.041855	30.7	0.823814	
g_{sw}	PCK	Decreasing VPDleaf (3-1 kPa)	MURA - SPPE	-0.01676	0.042898	29.6	0.994794	
g_{sw}	PCK	Decreasing VPDleaf (3-1 kPa)	MURA - TRFL	-0.01834	0.045593	31.7	0.994207	
g_{sw}	PCK	Decreasing VPDleaf (3-1 kPa)	SPPE - TRFL	-0.00157	0.042898	29.6	1	
g_{sw}	PCK	Steady State VPDleaf (1 kPa)	BOHI - CHVE	0.076553	0.041855	30.7	0.376052	
g_{sw}	PCK	Steady State VPDleaf (1 kPa)	BOHI - MURA	0.133298	0.04633	33.6	0.050512	.
g_{sw}	PCK	Steady State VPDleaf (1 kPa)	BOHI - SPPE	0.102874	0.043655	31.6	0.154115	
g_{sw}	PCK	Steady State VPDleaf (1 kPa)	BOHI - TRFL	0.127275	0.04633	33.6	0.067732	.
g_{sw}	PCK	Steady State VPDleaf (1 kPa)	CHVE - MURA	0.056745	0.042658	32.9	0.674791	
g_{sw}	PCK	Steady State VPDleaf (1 kPa)	CHVE - SPPE	0.026321	0.039736	30.5	0.96291	
g_{sw}	PCK	Steady State VPDleaf (1 kPa)	CHVE - TRFL	0.050722	0.042658	32.9	0.757463	
g_{sw}	PCK	Steady State VPDleaf (1 kPa)	MURA - SPPE	-0.03042	0.044424	33.7	0.958403	
g_{sw}	PCK	Steady State VPDleaf (1 kPa)	MURA - TRFL	-0.00602	0.047056	35.5	0.999936	
g_{sw}	PCK	Steady State VPDleaf (1 kPa)	SPPE - TRFL	0.024401	0.044424	33.7	0.981287	
A	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - ERSP	8.677212	5.547965	25.6	0.53256	
A	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - MUCU	-4.02486	5.763679	29.6	0.955253	
A	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - PAVI	-0.17457	5.763679	29.6	1	
A	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - SCPA	-5.24487	5.433855	27.9	0.868291	
A	NAD-ME	Increasing VPDleaf (1-3 kPa)	ERSP - MUCU	-12.7021	5.763679	29.6	0.206177	
A	NAD-ME	Increasing VPDleaf (1-3 kPa)	ERSP - PAVI	-8.85179	5.763679	29.6	0.548394	
A	NAD-ME	Increasing VPDleaf (1-3 kPa)	ERSP - SCPA	-13.9221	5.433855	27.9	0.105572	
A	NAD-ME	Increasing VPDleaf (1-3 kPa)	MUCU - PAVI	3.850287	5.971605	33.9	0.966451	
A	NAD-ME	Increasing VPDleaf (1-3 kPa)	MUCU - SCPA	-1.22001	5.653924	32.4	0.999491	
A	NAD-ME	Increasing VPDleaf (1-3 kPa)	PAVI - SCPA	-5.07029	5.653924	32.4	0.896002	
A	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - ERSP	7.682242	5.547965	25.6	0.642525	
A	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - MUCU	-4.94144	5.763679	29.6	0.909998	
A	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - PAVI	0.925551	5.763679	29.6	0.999841	

A	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - SCPA	-4.49736	5.383248	26.9	0.917154	
A	NAD-ME	High VPDleaf (4-4.5 kPa)	ERSP - MUCU	-12.6237	5.763679	29.6	0.211193	
A	NAD-ME	High VPDleaf (4-4.5 kPa)	ERSP - PAVI	-6.75669	5.763679	29.6	0.766722	
A	NAD-ME	High VPDleaf (4-4.5 kPa)	ERSP - SCPA	-12.1796	5.383248	26.9	0.188233	
A	NAD-ME	High VPDleaf (4-4.5 kPa)	MUCU - PAVI	5.866995	5.971605	33.9	0.861267	
A	NAD-ME	High VPDleaf (4-4.5 kPa)	MUCU - SCPA	0.444081	5.605304	31.4	0.999991	
A	NAD-ME	High VPDleaf (4-4.5 kPa)	PAVI - SCPA	-5.42291	5.605304	31.4	0.867604	
A	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - ERSP	7.691857	5.547965	25.6	0.641468	
A	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - MUCU	-5.34687	5.763679	29.6	0.883868	
A	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - PAVI	2.103532	5.763679	29.6	0.995999	
A	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - SCPA	-5.29129	5.383248	26.9	0.860588	
A	NAD-ME	Decreasing VPDleaf (3-1 kPa)	ERSP - MUCU	-13.0387	5.763679	29.6	0.185631	
A	NAD-ME	Decreasing VPDleaf (3-1 kPa)	ERSP - PAVI	-5.58832	5.763679	29.6	0.866574	
A	NAD-ME	Decreasing VPDleaf (3-1 kPa)	ERSP - SCPA	-12.9831	5.383248	26.9	0.142865	
A	NAD-ME	Decreasing VPDleaf (3-1 kPa)	MUCU - PAVI	7.4504	5.971605	33.9	0.723983	
A	NAD-ME	Decreasing VPDleaf (3-1 kPa)	MUCU - SCPA	0.055577	5.605304	31.4	1	
A	NAD-ME	Decreasing VPDleaf (3-1 kPa)	PAVI - SCPA	-7.39482	5.605304	31.4	0.681554	
A	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - ERSP	8.814759	5.743993	29.2	0.549254	
A	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - MUCU	-4.77647	5.927042	32.9	0.926951	
A	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - PAVI	2.206371	6.274702	40.2	0.996586	
A	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - SCPA	-6.36402	5.557804	30.4	0.781522	
A	NAD-ME	Steady State VPDleaf (1 kPa)	ERSP - MUCU	-13.5912	5.789966	30.2	0.158053	
A	NAD-ME	Steady State VPDleaf (1 kPa)	ERSP - PAVI	-6.60839	6.145386	37.3	0.817892	
A	NAD-ME	Steady State VPDleaf (1 kPa)	ERSP - SCPA	-15.1788	5.411383	27.5	0.063879	.
A	NAD-ME	Steady State VPDleaf (1 kPa)	MUCU - PAVI	6.982837	6.316814	41.2	0.802702	
A	NAD-ME	Steady State VPDleaf (1 kPa)	MUCU - SCPA	-1.58756	5.605304	31.4	0.998513	
A	NAD-ME	Steady State VPDleaf (1 kPa)	PAVI - SCPA	-8.57039	5.971725	39.3	0.609305	
E	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - ERSP	0.001098	0.000904	28.7	0.742768	
E	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - MUCU	-0.00128	0.000958	35.7	0.67346	
E	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - PAVI	-0.00055	0.000958	35.7	0.978098	
E	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - SCPA	8.81E-05	0.000896	32.5	0.999978	
E	NAD-ME	Increasing VPDleaf (1-3 kPa)	ERSP - MUCU	-0.00237	0.000958	35.7	0.118288	

E	NAD-ME	Increasing VPDleaf (1-3 kPa)	ERSP - PAVI	-0.00165	0.000958	35.7	0.434697	
E	NAD-ME	Increasing VPDleaf (1-3 kPa)	ERSP - SCPA	-0.00101	0.000896	32.5	0.790619	
E	NAD-ME	Increasing VPDleaf (1-3 kPa)	MUCU - PAVI	0.000726	0.001008	43	0.950668	
E	NAD-ME	Increasing VPDleaf (1-3 kPa)	MUCU - SCPA	0.001364	0.00095	40.3	0.608771	
E	NAD-ME	Increasing VPDleaf (1-3 kPa)	PAVI - SCPA	0.000637	0.00095	40.3	0.961511	
E	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - ERSP	0.001695	0.000904	28.7	0.352586	
E	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - MUCU	-0.003	0.000958	35.7	0.026856	*
E	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - PAVI	-0.00015	0.000958	35.7	0.99987	
E	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - SCPA	4.42E-06	0.000883	31	1	
E	NAD-ME	High VPDleaf (4-4.5 kPa)	ERSP - MUCU	-0.00469	0.000958	35.7	0.000191	***
E	NAD-ME	High VPDleaf (4-4.5 kPa)	ERSP - PAVI	-0.00184	0.000958	35.7	0.32375	
E	NAD-ME	High VPDleaf (4-4.5 kPa)	ERSP - SCPA	-0.00169	0.000883	31	0.331127	
E	NAD-ME	High VPDleaf (4-4.5 kPa)	MUCU - PAVI	0.002851	0.001008	43	0.052328	.
E	NAD-ME	High VPDleaf (4-4.5 kPa)	MUCU - SCPA	0.003003	0.000938	38.7	0.02165	*
E	NAD-ME	High VPDleaf (4-4.5 kPa)	PAVI - SCPA	0.000151	0.000938	38.7	0.999841	
E	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - ERSP	0.000932	0.000904	28.7	0.838678	
E	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - MUCU	-0.00187	0.000958	35.7	0.311637	
E	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - PAVI	-0.00033	0.000958	35.7	0.996639	
E	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - SCPA	-0.00026	0.000883	31	0.998244	
E	NAD-ME	Decreasing VPDleaf (3-1 kPa)	ERSP - MUCU	-0.0028	0.000958	35.7	0.044516	*
E	NAD-ME	Decreasing VPDleaf (3-1 kPa)	ERSP - PAVI	-0.00127	0.000958	35.7	0.678707	
E	NAD-ME	Decreasing VPDleaf (3-1 kPa)	ERSP - SCPA	-0.00119	0.000883	31	0.662231	
E	NAD-ME	Decreasing VPDleaf (3-1 kPa)	MUCU - PAVI	0.00153	0.001008	43	0.556884	
E	NAD-ME	Decreasing VPDleaf (3-1 kPa)	MUCU - SCPA	0.001604	0.000938	38.7	0.439719	
E	NAD-ME	Decreasing VPDleaf (3-1 kPa)	PAVI - SCPA	7.39E-05	0.000938	38.7	0.999991	
E	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - ERSP	0.000694	0.000952	34.8	0.948455	
E	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - MUCU	-0.00186	0.000997	41.2	0.35114	
E	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - PAVI	-0.00041	0.00108	52.8	0.995467	
E	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - SCPA	-0.00022	0.000926	36.8	0.999196	
E	NAD-ME	Steady State VPDleaf (1 kPa)	ERSP - MUCU	-0.00255	0.000964	36.6	0.081854	.
E	NAD-ME	Steady State VPDleaf (1 kPa)	ERSP - PAVI	-0.0011	0.001049	48.2	0.829867	
E	NAD-ME	Steady State VPDleaf (1 kPa)	ERSP - SCPA	-0.00092	0.00089	31.9	0.838817	

E	NAD-ME	Steady State VPDleaf (1 kPa)	MUCU - PAVI	0.001452	0.00109	54.6	0.672737	
E	NAD-ME	Steady State VPDleaf (1 kPa)	MUCU - SCPA	0.001637	0.000938	38.7	0.419651	
E	NAD-ME	Steady State VPDleaf (1 kPa)	PAVI - SCPA	0.000185	0.001025	51.4	0.999755	
g_{sw}	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - ERSP	0.056924	0.040361	26.1	0.626719	
g_{sw}	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - MUCU	-0.06422	0.042087	30.7	0.554201	
g_{sw}	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - PAVI	-0.00578	0.042087	30.7	0.999915	
g_{sw}	NAD-ME	Increasing VPDleaf (1-3 kPa)	BOGR - SCPA	-0.00269	0.039619	28.7	0.999995	
g_{sw}	NAD-ME	Increasing VPDleaf (1-3 kPa)	ERSP - MUCU	-0.12115	0.042087	30.7	0.052082	.
g_{sw}	NAD-ME	Increasing VPDleaf (1-3 kPa)	ERSP - PAVI	-0.0627	0.042087	30.7	0.57662	
g_{sw}	NAD-ME	Increasing VPDleaf (1-3 kPa)	ERSP - SCPA	-0.05961	0.039619	28.7	0.567958	
g_{sw}	NAD-ME	Increasing VPDleaf (1-3 kPa)	MUCU - PAVI	0.058445	0.043745	35.5	0.671076	
g_{sw}	NAD-ME	Increasing VPDleaf (1-3 kPa)	MUCU - SCPA	0.061536	0.041376	33.8	0.577521	
g_{sw}	NAD-ME	Increasing VPDleaf (1-3 kPa)	PAVI - SCPA	0.003091	0.041376	33.8	0.999993	
g_{sw}	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - ERSP	0.055066	0.040361	26.1	0.654836	
g_{sw}	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - MUCU	-0.05687	0.042087	30.7	0.662153	
g_{sw}	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - PAVI	0.007212	0.042087	30.7	0.999795	
g_{sw}	NAD-ME	High VPDleaf (4-4.5 kPa)	BOGR - SCPA	-0.00209	0.039215	27.6	0.999998	
g_{sw}	NAD-ME	High VPDleaf (4-4.5 kPa)	ERSP - MUCU	-0.11194	0.042087	30.7	0.08404	.
g_{sw}	NAD-ME	High VPDleaf (4-4.5 kPa)	ERSP - PAVI	-0.04785	0.042087	30.7	0.78583	
g_{sw}	NAD-ME	High VPDleaf (4-4.5 kPa)	ERSP - SCPA	-0.05716	0.039215	27.6	0.597289	
g_{sw}	NAD-ME	High VPDleaf (4-4.5 kPa)	MUCU - PAVI	0.064086	0.043745	35.5	0.591138	
g_{sw}	NAD-ME	High VPDleaf (4-4.5 kPa)	MUCU - SCPA	0.054781	0.040989	32.7	0.671026	
g_{sw}	NAD-ME	High VPDleaf (4-4.5 kPa)	PAVI - SCPA	-0.00931	0.040989	32.7	0.999378	
g_{sw}	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - ERSP	0.046289	0.040361	26.1	0.780429	
g_{sw}	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - MUCU	-0.08095	0.042087	30.7	0.326925	
g_{sw}	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - PAVI	-0.00108	0.042087	30.7	1	
g_{sw}	NAD-ME	Decreasing VPDleaf (3-1 kPa)	BOGR - SCPA	-0.01133	0.039215	27.6	0.998379	
g_{sw}	NAD-ME	Decreasing VPDleaf (3-1 kPa)	ERSP - MUCU	-0.12723	0.042087	30.7	0.037387	*
g_{sw}	NAD-ME	Decreasing VPDleaf (3-1 kPa)	ERSP - PAVI	-0.04737	0.042087	30.7	0.791963	
g_{sw}	NAD-ME	Decreasing VPDleaf (3-1 kPa)	ERSP - SCPA	-0.05762	0.039215	27.6	0.590005	
g_{sw}	NAD-ME	Decreasing VPDleaf (3-1 kPa)	MUCU - PAVI	0.079869	0.043745	35.5	0.375309	
g_{sw}	NAD-ME	Decreasing VPDleaf (3-1 kPa)	MUCU - SCPA	0.069613	0.040989	32.7	0.448909	

g_{sw}	NAD-ME	Decreasing VPDleaf (3-1 kPa)	PAVI - SCPA	-0.01026	0.040989	32.7	0.999088	
g_{sw}	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - ERSP	0.0705	0.041929	30.2	0.459951	
g_{sw}	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - MUCU	-0.08937	0.043389	34.4	0.260426	
g_{sw}	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - PAVI	-0.00141	0.046145	42.5	1	
g_{sw}	NAD-ME	Steady State VPDleaf (1 kPa)	BOGR - SCPA	-0.00674	0.040609	31.5	0.999819	
g_{sw}	NAD-ME	Steady State VPDleaf (1 kPa)	ERSP - MUCU	-0.15987	0.042297	31.3	0.005577	**
g_{sw}	NAD-ME	Steady State VPDleaf (1 kPa)	ERSP - PAVI	-0.07191	0.04512	39.3	0.510142	
g_{sw}	NAD-ME	Steady State VPDleaf (1 kPa)	ERSP - SCPA	-0.07724	0.03944	28.3	0.311454	
g_{sw}	NAD-ME	Steady State VPDleaf (1 kPa)	MUCU - PAVI	0.087965	0.04648	43.6	0.336486	
g_{sw}	NAD-ME	Steady State VPDleaf (1 kPa)	MUCU - SCPA	0.08263	0.040989	32.7	0.281169	
g_{sw}	NAD-ME	Steady State VPDleaf (1 kPa)	PAVI - SCPA	-0.00534	0.043896	41.5	0.999949	

Appendix Table A.3: Directional trends of mean A, g_{sw} , and E of C₄ subtypes across time within each VPD_{leaf} phase. Significance levels were computed with Walt z-tests.

Response	VPD _{leaf} Phase	Subtype	Slope	SE	df	LCL	UCL	test stat	p-value
A	Increasing (1-3 kPa)	NAD_ME	-0.807	0.0951	Inf	-0.9933	-0.6206	-8.48727	2.12E-17
A	Increasing (1-3 kPa)	NADP_ME	-1.1235	0.0865	Inf	-1.293	-0.954	-12.9909	1.38E-38
A	Increasing (1-3 kPa)	PCK	-1.1332	0.1008	Inf	-1.3309	-0.9356	-11.238	2.65E-29
A	High (4-4.5 kPa)	NAD_ME	-0.8866	0.0915	Inf	-1.066	-0.7072	-9.68636	3.45E-22
A	High (4-4.5 kPa)	NADP_ME	-0.7864	0.0842	Inf	-0.9515	-0.6214	-9.33992	9.64E-21
A	High (4-4.5 kPa)	PCK	-1.2491	0.0976	Inf	-1.4404	-1.0577	-12.7951	1.75E-37
A	Decreasing (3-1 kPa)	NAD_ME	-0.2106	0.1027	Inf	-0.4119	-0.0092	-2.04969	0.040395
A	Decreasing (3-1 kPa)	NADP_ME	-0.6331	0.0958	Inf	-0.8209	-0.4454	-6.61	3.84E-11
A	Decreasing (3-1 kPa)	PCK	-0.4126	0.1048	Inf	-0.6179	-0.2073	-3.93829	8.21E-05
A	Steady State (1 kPa)	NAD_ME	-1.1397	0.112	Inf	-1.3592	-0.9202	-10.1757	2.55E-24
A	Steady State (1 kPa)	NADP_ME	-0.7974	0.1068	Inf	-1.0067	-0.588	-7.46505	8.33E-14
A	Steady State (1 kPa)	PCK	-1.0678	0.1128	Inf	-1.2889	-0.8467	-9.46598	2.91E-21
g_{sw}	Increasing (1-3 kPa)	NAD_ME	-0.01295	0.000756	Inf	-0.01443	-0.01146	-17.1193	1.07E-65
g_{sw}	Increasing (1-3 kPa)	NADP_ME	-0.01225	0.000688	Inf	-0.01359	-0.0109	-17.8038	6.61E-71
g_{sw}	Increasing (1-3 kPa)	PCK	-0.01275	0.000802	Inf	-0.01432	-0.01118	-15.8966	6.69E-57
g_{sw}	High (4-4.5 kPa)	NAD_ME	-0.01162	0.000728	Inf	-0.01305	-0.0102	-15.9635	2.29E-57
g_{sw}	High (4-4.5 kPa)	NADP_ME	-0.0094	0.00067	Inf	-0.01071	-0.00808	-14.0307	1.01E-44
g_{sw}	High (4-4.5 kPa)	PCK	-0.01471	0.000776	Inf	-0.01623	-0.01319	-18.9484	4.55E-80
g_{sw}	Decreasing (3-1 kPa)	NAD_ME	-0.0041	0.000817	Inf	-0.0057	-0.0025	-5.02021	5.16E-07

g_{sw}	Decreasing (3-1 kPa)	NADP_ME	-0.00199	0.000762	Inf	-0.00348	-0.00049	-2.60876	0.009087
g_{sw}	Decreasing (3-1 kPa)	PCK	-0.00542	0.000833	Inf	-0.00705	-0.00379	-6.50232	7.91E-11
g_{sw}	Steady State (1 kPa)	NAD_ME	-0.00417	0.000891	Inf	-0.00591	-0.00242	-4.67918	2.88E-06
g_{sw}	Steady State (1 kPa)	NADP_ME	-0.00366	0.00085	Inf	-0.00533	-0.002	-4.31089	1.63E-05
g_{sw}	Steady State (1 kPa)	PCK	-0.00342	0.000897	Inf	-0.00518	-0.00166	-3.81325	0.000137
E	Increasing (1-3 kPa)	NAD_ME	0.9689	0.0222	Inf	0.9255	1.0124	43.70766	0
E	Increasing (1-3 kPa)	NADP_ME	0.7317	0.0202	Inf	0.6922	0.7712	36.28967	2.4E-288
E	Increasing (1-3 kPa)	PCK	1.038	0.0235	Inf	0.9919	1.084	44.14886	0
E	High (4-4.5 kPa)	NAD_ME	-0.3824	0.0213	Inf	-0.4242	-0.3405	-17.9179	8.55E-72
E	High (4-4.5 kPa)	NADP_ME	-0.3254	0.0196	Inf	-0.3639	-0.2869	-16.5747	1.06E-61
E	High (4-4.5 kPa)	PCK	-0.4236	0.0228	Inf	-0.4682	-0.379	-18.6111	2.61E-77
E	Decreasing (3-1 kPa)	NAD_ME	-0.93	0.024	Inf	-0.9769	-0.8831	-38.8297	0
E	Decreasing (3-1 kPa)	NADP_ME	-0.693	0.0223	Inf	-0.7367	-0.6492	-31.0308	2.1E-211
E	Decreasing (3-1 kPa)	PCK	-1.0684	0.0244	Inf	-1.1162	-1.0205	-43.7403	0
E	Steady State (1 kPa)	NAD_ME	-0.1042	0.0261	Inf	-0.1554	-0.0531	-3.992	6.55E-05
E	Steady State (1 kPa)	NADP_ME	-0.0775	0.0249	Inf	-0.1263	-0.0287	-3.1129	0.001853
E	Steady State (1 kPa)	PCK	-0.0759	0.0263	Inf	-0.1275	-0.0244	-2.88676	0.003892

Appendix Table A.4: Table showing directional trends of mean A, g_{sw}, and E of 15 C₄ grasses across time within each VPD_{leaf} phase. Significance levels were computed with Walt z-tests.

Response	subtype	VPD _{leaf} phase	Species	Slope	SE	df	LCL	UCL	test stat	p-value
A	NAD-ME	Increasing (1-3 kPa)	BOGR	-0.5132	0.1788	Inf	-0.8636	-0.1628	-2.87075	0.004095
A	NAD-ME	Increasing (1-3 kPa)	ERSP	-0.7972	0.188	Inf	-1.1657	-0.4287	-4.23988	2.24E-05
A	NAD-ME	Increasing (1-3 kPa)	MUCU	0.0574	0.2509	Inf	-0.4343	0.5491	0.228858	0.81898
A	NAD-ME	Increasing (1-3 kPa)	PAVI	-0.9835	0.2764	Inf	-1.5252	-0.4417	-3.55794	0.000374
A	NAD-ME	Increasing (1-3 kPa)	SCPA	-1.7222	0.1972	Inf	-2.1087	-1.3356	-8.73184	2.51E-18
A	NAD-ME	High (4-4.5 kPa)	BOGR	-0.9402	0.175	Inf	-1.2832	-0.5972	-5.3728	7.75E-08
A	NAD-ME	High (4-4.5 kPa)	ERSP	-0.5321	0.1837	Inf	-0.8921	-0.1722	-2.89744	0.003762
A	NAD-ME	High (4-4.5 kPa)	MUCU	-0.5795	0.248	Inf	-1.0655	-0.0934	-2.33652	0.019464
A	NAD-ME	High (4-4.5 kPa)	PAVI	-1.3432	0.2518	Inf	-1.8366	-0.8497	-5.33514	9.55E-08
A	NAD-ME	High (4-4.5 kPa)	SCPA	-1.099	0.1854	Inf	-1.4623	-0.7357	-5.9294	3.04E-09
A	NAD-ME	Decreasing (3-1 kPa)	BOGR	-0.4788	0.2068	Inf	-0.8841	-0.0736	-2.31592	0.020563
A	NAD-ME	Decreasing (3-1 kPa)	ERSP	-0.4401	0.2023	Inf	-0.8367	-0.0435	-2.17491	0.029637
A	NAD-ME	Decreasing (3-1 kPa)	MUCU	-0.7718	0.2561	Inf	-1.2738	-0.2699	-3.0136	0.002582
A	NAD-ME	Decreasing (3-1 kPa)	PAVI	-0.4286	0.2916	Inf	-1.0001	0.143	-1.46968	0.141649
A	NAD-ME	Decreasing (3-1 kPa)	SCPA	0.6135	0.2102	Inf	0.2015	1.0255	2.918348	0.003519
A	NAD-ME	Steady State (1 kPa)	BOGR	-0.4624	0.241	Inf	-0.9347	0.01	-1.91845	0.055053
A	NAD-ME	Steady State (1 kPa)	ERSP	-1.3711	0.2138	Inf	-1.7902	-0.9521	-6.4126	1.43E-10
A	NAD-ME	Steady State (1 kPa)	MUCU	-1.1649	0.2684	Inf	-1.6909	-0.6388	-4.3401	1.42E-05
A	NAD-ME	Steady State (1 kPa)	PAVI	-1.1002	0.3692	Inf	-1.8239	-0.3766	-2.98	0.002882
A	NAD-ME	Steady State (1 kPa)	SCPA	-1.5345	0.213	Inf	-1.952	-1.117	-7.20348	5.87E-13
A	NADP-ME	Increasing (1-3 kPa)	ANGE	-1.2494	0.1821	Inf	-1.6064	-0.8924	-6.85967	6.9E-12
A	NADP-ME	Increasing (1-3 kPa)	DICO	-0.5137	0.2496	Inf	-1.003	-0.0244	-2.05789	0.039601
A	NADP-ME	Increasing (1-3 kPa)	PAPU	-0.7171	0.1767	Inf	-1.0634	-0.3707	-4.05815	4.95E-05
A	NADP-ME	Increasing (1-3 kPa)	SCSC	-1.193	0.1844	Inf	-1.5544	-0.8315	-6.46949	9.83E-11
A	NADP-ME	Increasing (1-3 kPa)	SONU	-1.7196	0.1808	Inf	-2.0739	-1.3653	-9.5132	1.85E-21
A	NADP-ME	High (4-4.5 kPa)	ANGE	-0.7275	0.1753	Inf	-1.0711	-0.3839	-4.14966	3.33E-05
A	NADP-ME	High (4-4.5 kPa)	DICO	-0.2556	0.2463	Inf	-0.7383	0.2271	-1.03772	0.299401
A	NADP-ME	High (4-4.5 kPa)	PAPU	-0.7352	0.1756	Inf	-1.0794	-0.3909	-4.18596	2.84E-05
A	NADP-ME	High (4-4.5 kPa)	SCSC	-0.7942	0.1779	Inf	-1.1429	-0.4455	-4.46357	8.06E-06

A	NADP-ME	High (4-4.5 kPa)	SONU	-1.1587	0.1745	Inf	-1.5006	-0.8167	-6.64052	3.13E-11
A	NADP-ME	Decreasing (3-1 kPa)	ANGE	-0.9053	0.1985	Inf	-1.2944	-0.5162	-4.56	5.12E-06
A	NADP-ME	Decreasing (3-1 kPa)	DICO	-0.334	0.2658	Inf	-0.8549	0.1869	-1.25681	0.208822
A	NADP-ME	Decreasing (3-1 kPa)	PAPU	-0.0621	0.2007	Inf	-0.4554	0.3311	-0.30965	0.756827
A	NADP-ME	Decreasing (3-1 kPa)	SCSC	-0.6613	0.2077	Inf	-1.0684	-0.2542	-3.1838	0.001454
A	NADP-ME	Decreasing (3-1 kPa)	SONU	-1.1494	0.1997	Inf	-1.5408	-0.758	-5.75521	8.65E-09
A	NADP-ME	Steady State (1 kPa)	ANGE	-1.241	0.2228	Inf	-1.6777	-0.8043	-5.56953	2.55E-08
A	NADP-ME	Steady State (1 kPa)	DICO	-0.9722	0.2949	Inf	-1.5502	-0.3942	-3.29669	0.000978
A	NADP-ME	Steady State (1 kPa)	PAPU	-0.8454	0.2068	Inf	-1.2508	-0.4401	-4.08755	4.36E-05
A	NADP-ME	Steady State (1 kPa)	SCSC	-0.5678	0.2624	Inf	-1.082	-0.0536	-2.16422	0.030448
A	NADP-ME	Steady State (1 kPa)	SONU	-0.5047	0.219	Inf	-0.9339	-0.0754	-2.30425	0.021208
A	PCK	Increasing (1-3 kPa)	BOHI	1.1014	0.2536	Inf	0.6043	1.5984	4.34303	1.41E-05
A	PCK	Increasing (1-3 kPa)	CHVE	-1.072	0.1977	Inf	-1.4595	-0.6844	-5.42146	5.91E-08
A	PCK	Increasing (1-3 kPa)	MURA	-1.1169	0.2514	Inf	-1.6096	-0.6241	-4.44272	8.88E-06
A	PCK	Increasing (1-3 kPa)	SPPE	-2.4749	0.1842	Inf	-2.8359	-2.1139	-13.4374	3.65E-41
A	PCK	Increasing (1-3 kPa)	TRFL	-1.1621	0.2563	Inf	-1.6645	-0.6598	-4.53415	5.78E-06
A	PCK	High (4-4.5 kPa)	BOHI	-1.0817	0.2475	Inf	-1.5668	-0.5966	-4.37047	1.24E-05
A	PCK	High (4-4.5 kPa)	CHVE	-0.6942	0.1913	Inf	-1.0693	-0.3192	-3.6281	0.000286
A	PCK	High (4-4.5 kPa)	MURA	-0.8618	0.248	Inf	-1.3479	-0.3757	-3.47502	0.000511
A	PCK	High (4-4.5 kPa)	SPPE	-1.8687	0.1758	Inf	-2.2133	-1.524	-10.6269	2.23E-26
A	PCK	High (4-4.5 kPa)	TRFL	-1.5359	0.248	Inf	-2.022	-1.0498	-6.19293	5.91E-10
A	PCK	Decreasing (3-1 kPa)	BOHI	-0.9072	0.2503	Inf	-1.3978	-0.4165	-3.62395	0.00029
A	PCK	Decreasing (3-1 kPa)	CHVE	-0.6382	0.1957	Inf	-1.0217	-0.2546	-3.26122	0.001109
A	PCK	Decreasing (3-1 kPa)	MURA	-0.4163	0.2664	Inf	-0.9385	0.1058	-1.56283	0.118092
A	PCK	Decreasing (3-1 kPa)	SPPE	0.3388	0.2002	Inf	-0.0536	0.7313	1.692146	0.090618
A	PCK	Decreasing (3-1 kPa)	TRFL	-0.94	0.2773	Inf	-1.4834	-0.3965	-3.39025	0.000698
A	PCK	Steady State (1 kPa)	BOHI	-0.8373	0.2699	Inf	-1.3663	-0.3084	-3.10267	0.001918
A	PCK	Steady State (1 kPa)	CHVE	-0.704	0.2006	Inf	-1.0972	-0.3107	-3.50885	0.00045
A	PCK	Steady State (1 kPa)	MURA	-0.7535	0.304	Inf	-1.3492	-0.1577	-2.4789	0.013179
A	PCK	Steady State (1 kPa)	SPPE	-1.3096	0.2224	Inf	-1.7455	-0.8736	-5.88756	3.92E-09
A	PCK	Steady State (1 kPa)	TRFL	-1.953	0.2933	Inf	-2.528	-1.3781	-6.6582	2.77E-11
g _{sw}	NAD-ME	Increasing (1-3 kPa)	BOGR	-0.00869	0.001416	Inf	-0.01146	-0.00591	-6.1332	8.61E-10

g_{sw}	NAD-ME	Increasing (1-3 kPa)	ERSP	-0.01737	0.00149	Inf	-0.02029	-0.01445	-11.6594	2.06E-31
g_{sw}	NAD-ME	Increasing (1-3 kPa)	MUCU	-0.00801	0.001988	Inf	-0.0119	-0.00411	-4.02857	5.61E-05
g_{sw}	NAD-ME	Increasing (1-3 kPa)	PAVI	-0.0123	0.00219	Inf	-0.01659	-0.008	-5.61399	1.98E-08
g_{sw}	NAD-ME	Increasing (1-3 kPa)	SCPA	-0.01742	0.001563	Inf	-0.02048	-0.01435	-11.145	7.57E-29
g_{sw}	NAD-ME	High (4-4.5 kPa)	BOGR	-0.01242	0.001387	Inf	-0.01514	-0.0097	-8.95606	3.36E-19
g_{sw}	NAD-ME	High (4-4.5 kPa)	ERSP	-0.01	0.001455	Inf	-0.01285	-0.00715	-6.87214	6.32E-12
g_{sw}	NAD-ME	High (4-4.5 kPa)	MUCU	-0.01095	0.001965	Inf	-0.0148	-0.0071	-5.57246	2.51E-08
g_{sw}	NAD-ME	High (4-4.5 kPa)	PAVI	-0.01305	0.001995	Inf	-0.01696	-0.00914	-6.54321	6.02E-11
g_{sw}	NAD-ME	High (4-4.5 kPa)	SCPA	-0.01179	0.001469	Inf	-0.01467	-0.00891	-8.02854	9.86E-16
g_{sw}	NAD-ME	Decreasing (3-1 kPa)	BOGR	-0.00777	0.001638	Inf	-0.01098	-0.00456	-4.74488	2.09E-06
g_{sw}	NAD-ME	Decreasing (3-1 kPa)	ERSP	-0.00813	0.001603	Inf	-0.01128	-0.00499	-5.07334	3.91E-07
g_{sw}	NAD-ME	Decreasing (3-1 kPa)	MUCU	0.003985	0.002029	Inf	0.000008	0.007962	1.96374	0.04956
g_{sw}	NAD-ME	Decreasing (3-1 kPa)	PAVI	-0.00413	0.00231	Inf	-0.00866	0.000398	-1.78779	0.073811
g_{sw}	NAD-ME	Decreasing (3-1 kPa)	SCPA	-0.00286	0.001666	Inf	-0.00612	0.000409	-1.71464	0.086411
g_{sw}	NAD-ME	Steady State (1 kPa)	BOGR	-0.00278	0.00191	Inf	-0.00652	0.000961	-1.45645	0.145269
g_{sw}	NAD-ME	Steady State (1 kPa)	ERSP	-0.00674	0.001694	Inf	-0.01006	-0.00342	-3.97685	6.98E-05
g_{sw}	NAD-ME	Steady State (1 kPa)	MUCU	0.002599	0.002126	Inf	-0.00157	0.006767	1.222394	0.221558
g_{sw}	NAD-ME	Steady State (1 kPa)	PAVI	-0.00348	0.002925	Inf	-0.00921	0.002257	-1.18843	0.234663
g_{sw}	NAD-ME	Steady State (1 kPa)	SCPA	-0.00646	0.001688	Inf	-0.00977	-0.00316	-3.83005	0.000128
g_{sw}	NADP-ME	Increasing (1-3 kPa)	ANGE	-0.01905	0.001443	Inf	-0.02188	-0.01622	-13.2026	8.48E-40
g_{sw}	NADP-ME	Increasing (1-3 kPa)	DICO	-0.00317	0.001978	Inf	-0.00705	0.000702	-1.60483	0.108531
g_{sw}	NADP-ME	Increasing (1-3 kPa)	PAPU	-0.00989	0.0014	Inf	-0.01263	-0.00714	-7.06317	1.63E-12
g_{sw}	NADP-ME	Increasing (1-3 kPa)	SCSC	-0.00872	0.001461	Inf	-0.01159	-0.00586	-5.9701	2.37E-09
g_{sw}	NADP-ME	Increasing (1-3 kPa)	SONU	-0.01635	0.001432	Inf	-0.01915	-0.01354	-11.4136	3.58E-30
g_{sw}	NADP-ME	High (4-4.5 kPa)	ANGE	-0.00866	0.001389	Inf	-0.01138	-0.00593	-6.23166	4.62E-10
g_{sw}	NADP-ME	High (4-4.5 kPa)	DICO	-0.00795	0.001951	Inf	-0.01177	-0.00412	-4.07217	4.66E-05
g_{sw}	NADP-ME	High (4-4.5 kPa)	PAPU	-0.0086	0.001391	Inf	-0.01133	-0.00587	-6.1788	6.46E-10
g_{sw}	NADP-ME	High (4-4.5 kPa)	SCSC	-0.00846	0.00141	Inf	-0.01122	-0.0057	-5.99958	1.98E-09
g_{sw}	NADP-ME	High (4-4.5 kPa)	SONU	-0.01247	0.001382	Inf	-0.01518	-0.00976	-9.0221	1.85E-19
g_{sw}	NADP-ME	Decreasing (3-1 kPa)	ANGE	-0.00252	0.001573	Inf	-0.0056	0.000563	-1.60215	0.109121
g_{sw}	NADP-ME	Decreasing (3-1 kPa)	DICO	0.000261	0.002106	Inf	-0.00387	0.004388	0.123749	0.901514
g_{sw}	NADP-ME	Decreasing (3-1 kPa)	PAPU	-0.00095	0.00159	Inf	-0.00407	0.002167	-0.59702	0.550496

g _{sw}	NADP-ME	Decreasing (3-1 kPa)	SCSC	-0.00024	0.001646	Inf	-0.00346	0.002988	-0.14432	0.885245
g _{sw}	NADP-ME	Decreasing (3-1 kPa)	SONU	-0.00594	0.001582	Inf	-0.00904	-0.00284	-3.75269	0.000175
g _{sw}	NADP-ME	Steady State (1 kPa)	ANGE	-0.00444	0.001765	Inf	-0.0079	-0.00098	-2.51619	0.011863
g _{sw}	NADP-ME	Steady State (1 kPa)	DICO	-0.00903	0.002337	Inf	-0.01361	-0.00446	-3.8665	0.00011
g _{sw}	NADP-ME	Steady State (1 kPa)	PAPU	-0.00192	0.001639	Inf	-0.00513	0.001297	-1.16846	0.24262
g _{sw}	NADP-ME	Steady State (1 kPa)	SCSC	-0.00108	0.002079	Inf	-0.00516	0.002991	-0.52105	0.602333
g _{sw}	NADP-ME	Steady State (1 kPa)	SONU	-0.00416	0.001735	Inf	-0.00756	-0.00076	-2.39697	0.016531
g _{sw}	PCK	Increasing (1-3 kPa)	BOHI	-0.01915	0.002009	Inf	-0.02309	-0.01522	-9.53253	1.54E-21
g _{sw}	PCK	Increasing (1-3 kPa)	CHVE	-0.01688	0.001567	Inf	-0.01995	-0.01381	-10.7743	4.55E-27
g _{sw}	PCK	Increasing (1-3 kPa)	MURA	0.003497	0.001992	Inf	-0.00041	0.007401	1.755822	0.079119
g _{sw}	PCK	Increasing (1-3 kPa)	SPPE	-0.01613	0.001459	Inf	-0.01899	-0.01327	-11.0563	2.04E-28
g _{sw}	PCK	Increasing (1-3 kPa)	TRFL	-0.01049	0.002031	Inf	-0.01447	-0.00651	-5.16318	2.43E-07
g _{sw}	PCK	High (4-4.5 kPa)	BOHI	-0.01512	0.001961	Inf	-0.01897	-0.01128	-7.71078	1.25E-14
g _{sw}	PCK	High (4-4.5 kPa)	CHVE	-0.0134	0.001516	Inf	-0.01638	-0.01043	-8.8407	9.51E-19
g _{sw}	PCK	High (4-4.5 kPa)	MURA	-0.00962	0.001965	Inf	-0.01347	-0.00576	-4.89359	9.9E-07
g _{sw}	PCK	High (4-4.5 kPa)	SPPE	-0.01893	0.001393	Inf	-0.02166	-0.0162	-13.5878	4.73E-42
g _{sw}	PCK	High (4-4.5 kPa)	TRFL	-0.01338	0.001965	Inf	-0.01723	-0.00953	-6.80966	9.78E-12
g _{sw}	PCK	Decreasing (3-1 kPa)	BOHI	0.003118	0.001983	Inf	-0.00077	0.007006	1.572188	0.115907
g _{sw}	PCK	Decreasing (3-1 kPa)	CHVE	-0.00484	0.00155	Inf	-0.00788	-0.0018	-3.12175	0.001798
g _{sw}	PCK	Decreasing (3-1 kPa)	MURA	-0.01688	0.002111	Inf	-0.02102	-0.01274	-7.99752	1.27E-15
g _{sw}	PCK	Decreasing (3-1 kPa)	SPPE	-0.00055	0.001587	Inf	-0.00366	0.002564	-0.34374	0.731044
g _{sw}	PCK	Decreasing (3-1 kPa)	TRFL	-0.01423	0.002197	Inf	-0.01853	-0.00992	-6.47612	9.41E-11
g _{sw}	PCK	Steady State (1 kPa)	BOHI	-0.00026	0.002138	Inf	-0.00446	0.003927	-0.1233	0.901869
g _{sw}	PCK	Steady State (1 kPa)	CHVE	-0.00216	0.00159	Inf	-0.00527	0.000961	-1.35543	0.175279
g _{sw}	PCK	Steady State (1 kPa)	MURA	-0.0026	0.002408	Inf	-0.00732	0.002122	-1.07904	0.280569
g _{sw}	PCK	Steady State (1 kPa)	SPPE	-0.00388	0.001762	Inf	-0.00734	-0.00043	-2.20329	0.027574
g _{sw}	PCK	Steady State (1 kPa)	TRFL	-0.0089	0.002324	Inf	-0.01346	-0.00435	-3.82969	0.000128
E	NAD-ME	Increasing (1-3 kPa)	BOGR	1.009	0.0408	Inf	0.9289	1.089	24.71738	7E-135
E	NAD-ME	Increasing (1-3 kPa)	ERSP	0.5154	0.0429	Inf	0.4313	0.5996	12.00575	3.31E-33
E	NAD-ME	Increasing (1-3 kPa)	MUCU	1.7186	0.0573	Inf	1.6063	1.8309	30.00181	9.3E-198
E	NAD-ME	Increasing (1-3 kPa)	PAVI	1.171	0.0631	Inf	1.0473	1.2947	18.55353	7.64E-77
E	NAD-ME	Increasing (1-3 kPa)	SCPA	0.8298	0.045	Inf	0.7415	0.9181	18.42543	8.21E-76

E	NAD-ME	High (4-4.5 kPa)	BOGR	-0.3997	0.04	Inf	-0.4781	-0.3214	-10.0037	1.47E-23
E	NAD-ME	High (4-4.5 kPa)	ERSP	-0.3433	0.0419	Inf	-0.4255	-0.2611	-8.18614	2.7E-16
E	NAD-ME	High (4-4.5 kPa)	MUCU	-0.3919	0.0566	Inf	-0.5029	-0.2809	-6.92017	4.51E-12
E	NAD-ME	High (4-4.5 kPa)	PAVI	-0.4762	0.0575	Inf	-0.5889	-0.3635	-8.2836	1.2E-16
E	NAD-ME	High (4-4.5 kPa)	SCPA	-0.3564	0.0423	Inf	-0.4394	-0.2735	-8.42215	3.7E-17
E	NAD-ME	Decreasing (3-1 kPa)	BOGR	-1.0052	0.0472	Inf	-1.0977	-0.9127	-21.2918	1.4E-100
E	NAD-ME	Decreasing (3-1 kPa)	ERSP	-0.6117	0.0462	Inf	-0.7022	-0.5211	-13.2388	5.24E-40
E	NAD-ME	Decreasing (3-1 kPa)	MUCU	-1.146	0.0585	Inf	-1.2607	-1.0314	-19.5966	1.65E-85
E	NAD-ME	Decreasing (3-1 kPa)	PAVI	-1.0639	0.0666	Inf	-1.1944	-0.9334	-15.979	1.79E-57
E	NAD-ME	Decreasing (3-1 kPa)	SCPA	-1.0017	0.048	Inf	-1.0957	-0.9076	-20.8676	1.05E-96
E	NAD-ME	Steady State (1 kPa)	BOGR	-0.0302	0.055	Inf	-0.138	0.0777	-0.54826	0.583512
E	NAD-ME	Steady State (1 kPa)	ERSP	-0.1286	0.0488	Inf	-0.2243	-0.0329	-2.63336	0.008454
E	NAD-ME	Steady State (1 kPa)	MUCU	-0.2202	0.0613	Inf	-0.3403	-0.1001	-3.59284	0.000327
E	NAD-ME	Steady State (1 kPa)	PAVI	-0.152	0.0843	Inf	-0.3172	0.0133	-1.80261	0.07145
E	NAD-ME	Steady State (1 kPa)	SCPA	-0.0719	0.0486	Inf	-0.1673	0.0234	-1.47863	0.13924
E	NADP-ME	Increasing (1-3 kPa)	ANGE	0.6994	0.0416	Inf	0.6179	0.7809	16.81665	1.84E-63
E	NADP-ME	Increasing (1-3 kPa)	DICO	0.9888	0.057	Inf	0.8771	1.1005	17.34676	2.09E-67
E	NADP-ME	Increasing (1-3 kPa)	PAPU	0.5398	0.0403	Inf	0.4607	0.6189	13.3792	8E-41
E	NADP-ME	Increasing (1-3 kPa)	SCSC	0.7302	0.0421	Inf	0.6477	0.8128	17.34335	2.21E-67
E	NADP-ME	Increasing (1-3 kPa)	SONU	0.8305	0.0413	Inf	0.7496	0.9114	20.12239	4.7E-90
E	NADP-ME	High (4-4.5 kPa)	ANGE	-0.3098	0.04	Inf	-0.3882	-0.2313	-7.73816	1.01E-14
E	NADP-ME	High (4-4.5 kPa)	DICO	-0.1809	0.0562	Inf	-0.2912	-0.0707	-3.21721	0.001294
E	NADP-ME	High (4-4.5 kPa)	PAPU	-0.3084	0.0401	Inf	-0.387	-0.2298	-7.68998	1.47E-14
E	NADP-ME	High (4-4.5 kPa)	SCSC	-0.3127	0.0406	Inf	-0.3923	-0.2331	-7.69678	1.4E-14
E	NADP-ME	High (4-4.5 kPa)	SONU	-0.4346	0.0398	Inf	-0.5127	-0.3565	-10.909	1.04E-27
E	NADP-ME	Decreasing (3-1 kPa)	ANGE	-0.7947	0.0453	Inf	-0.8835	-0.7058	-17.5292	8.58E-69
E	NADP-ME	Decreasing (3-1 kPa)	DICO	-0.8538	0.0607	Inf	-0.9728	-0.7349	-14.0702	5.79E-45
E	NADP-ME	Decreasing (3-1 kPa)	PAPU	-0.3232	0.0458	Inf	-0.413	-0.2334	-7.05311	1.75E-12
E	NADP-ME	Decreasing (3-1 kPa)	SCSC	-0.6465	0.0474	Inf	-0.7395	-0.5536	-13.6317	2.6E-42
E	NADP-ME	Decreasing (3-1 kPa)	SONU	-0.934	0.0456	Inf	-1.0234	-0.8447	-20.4823	3.1E-93
E	NADP-ME	Steady State (1 kPa)	ANGE	-0.1092	0.0509	Inf	-0.209	-0.0095	-2.14716	0.031781
E	NADP-ME	Steady State (1 kPa)	DICO	-0.1214	0.0673	Inf	-0.2534	0.0105	-1.8034	0.071326

E	NADP-ME	Steady State (1 kPa)	PAPU	-0.0552	0.0472	Inf	-0.1477	0.0374	-1.16815	0.242748
E	NADP-ME	Steady State (1 kPa)	SCSC	-0.0703	0.0599	Inf	-0.1877	0.0471	-1.1736	0.240554
E	NADP-ME	Steady State (1 kPa)	SONU	-0.118	0.05	Inf	-0.216	-0.02	-2.35891	0.018329
E	PCK	Increasing (1-3 kPa)	BOHI	1.6061	0.0579	Inf	1.4926	1.7196	27.73675	2.5E-169
E	PCK	Increasing (1-3 kPa)	CHVE	0.9358	0.0451	Inf	0.8473	1.0243	20.72692	1.98E-95
E	PCK	Increasing (1-3 kPa)	MURA	1.024	0.0574	Inf	0.9115	1.1365	17.83843	3.56E-71
E	PCK	Increasing (1-3 kPa)	SPPE	0.7583	0.0421	Inf	0.6759	0.8407	18.03081	1.12E-72
E	PCK	Increasing (1-3 kPa)	TRFL	1.154	0.0585	Inf	1.0393	1.2687	19.71917	1.48E-86
E	PCK	High (4-4.5 kPa)	BOHI	-0.4385	0.0565	Inf	-0.5493	-0.3278	-7.75922	8.55E-15
E	PCK	High (4-4.5 kPa)	CHVE	-0.2801	0.0437	Inf	-0.3657	-0.1945	-6.41072	1.45E-10
E	PCK	High (4-4.5 kPa)	MURA	-0.309	0.0566	Inf	-0.42	-0.198	-5.4571	4.84E-08
E	PCK	High (4-4.5 kPa)	SPPE	-0.5799	0.0402	Inf	-0.6586	-0.5012	-14.4415	2.84E-47
E	PCK	High (4-4.5 kPa)	TRFL	-0.4551	0.0566	Inf	-0.5661	-0.3441	-8.03606	9.28E-16
E	PCK	Decreasing (3-1 kPa)	BOHI	-1.4727	0.0572	Inf	-1.5847	-1.3607	-25.7643	2.2E-146
E	PCK	Decreasing (3-1 kPa)	CHVE	-1.036	0.0447	Inf	-1.1236	-0.9485	-23.1873	6.1E-119
E	PCK	Decreasing (3-1 kPa)	MURA	-1.0107	0.0608	Inf	-1.1299	-0.8915	-16.6158	5.36E-62
E	PCK	Decreasing (3-1 kPa)	SPPE	-0.8471	0.0457	Inf	-0.9367	-0.7575	-18.5268	1.26E-76
E	PCK	Decreasing (3-1 kPa)	TRFL	-1.0939	0.0633	Inf	-1.218	-0.9698	-17.2796	6.7E-67
E	PCK	Steady State (1 kPa)	BOHI	-0.0072	0.0616	Inf	-0.128	0.1135	-0.11759	0.90639
E	PCK	Steady State (1 kPa)	CHVE	-0.0329	0.0458	Inf	-0.1227	0.0569	-0.71818	0.472649
E	PCK	Steady State (1 kPa)	MURA	-0.1228	0.0694	Inf	-0.2588	0.0133	-1.76877	0.076933
E	PCK	Steady State (1 kPa)	SPPE	-0.0679	0.0508	Inf	-0.1674	0.0316	-1.33685	0.181273
E	PCK	Steady State (1 kPa)	TRFL	-0.2569	0.067	Inf	-0.3882	-0.1257	-3.83625	0.000125

Appendix Table A.5: Table showing pairwise contrasts of normalized stomatal sensitivity (m/g_{ref}) between C_4 subtypes.

transition	contrast	estimate	SE	df	t-ratio	p-value
1 $\rightarrow$ 2 kPa	NADP-ME - PCK	0.331	0.139	83.9	2.38	0.0507
1 $\rightarrow$ 2 kPa	NADP-ME – NAD-ME	-0.019	0.127	56.7	-0.15	0.988
1 $\rightarrow$ 2 kPa	PCK – NAD-ME	-0.35	0.142	74.6	-2.46	0.0422
2 $\rightarrow$ 1 kPa	NADP-ME - PCK	0.081	0.077	61.1	1.04	0.553
2 $\rightarrow$ 1 kPa	NADP-ME – NAD-ME	0.113	0.08	59.2	1.42	0.337
2 $\rightarrow$ 1 kPa	PCK – NAD-ME	0.033	0.079	56.2	0.41	0.911
2 $\rightarrow$ 3 kPa	NADP-ME - PCK	-0.004	0.068	77	-0.05	0.998
2 $\rightarrow$ 3 kPa	NADP-ME – NAD-ME	-0.014	0.066	64.5	-0.21	0.976
2 $\rightarrow$ 3 kPa	PCK – NAD-ME	-0.01	0.071	71.9	-0.14	0.989
3 $\rightarrow$ 2 kPa	NADP-ME - PCK	-0.043	0.06	78.6	-0.71	0.76
3 $\rightarrow$ 2 kPa	NADP-ME – NAD-ME	-0.043	0.059	65.5	-0.73	0.746
3 $\rightarrow$ 2 kPa	PCK – NAD-ME	0	0.062	70.4	0	1
3 $\rightarrow$ 4 kPa	NADP-ME - PCK	0.055	0.092	71.9	0.6	0.823
3 $\rightarrow$ 4 kPa	NADP-ME – NAD-ME	0.096	0.088	65.5	1.09	0.522
3 $\rightarrow$ 4 kPa	PCK – NAD-ME	0.041	0.098	70.1	0.42	0.906
4 $\rightarrow$ 3 kPa	NADP-ME - PCK	-0.009	0.066	79.2	-0.13	0.991
4 $\rightarrow$ 3 kPa	NADP-ME – NAD-ME	-0.038	0.062	62.7	-0.61	0.816
4 $\rightarrow$ 3 kPa	PCK – NAD-ME	-0.029	0.069	72.8	-0.42	0.906
4 $\rightarrow$ 4.5 kPa	NADP-ME - PCK	0.379	0.469	13.9	0.81	0.704
4 $\rightarrow$ 4.5 kPa	NADP-ME – NAD-ME	0.474	0.452	11.5	1.05	0.563
4 $\rightarrow$ 4.5 kPa	PCK – NAD-ME	0.095	0.499	12.2	0.19	0.98
4.5 $\rightarrow$ 4 kPa	NADP-ME - PCK	-0.194	0.335	11	-0.58	0.834
4.5 $\rightarrow$ 4 kPa	NADP-ME – NAD-ME	-0.202	0.287	11	-0.7	0.766
4.5 $\rightarrow$ 4 kPa	PCK – NAD-ME	-0.008	0.346	11	-0.02	1

Appendix Table A.6: Pairwise contrasts of microanatomical traits of C₄ subtypes.

Response	contrast	estimate	SE	df	t-ratio	p-value
Major bundle sheath cell area	NAD - NADP	141.316	39.4	71	3.59	0.00183
Major bundle sheath cell area	NAD - PCK	-15.917	37.566	71	-0.42	1
Major bundle sheath cell area	NADP - PCK	-157.233	35.638	71	-4.41	0.000107
Minor bundle sheath cell area	NAD - NADP	165.012	37.408	71	4.41	0.000108
Minor bundle sheath cell area	NAD - PCK	40.282	35.667	71	1.13	0.788
Minor bundle sheath cell area	NADP - PCK	-124.731	33.836	71	-3.69	0.00132
Major BSC:MSC	NAD - NADP	0.036	0.009	70	3.85	0.000767
Major BSC:MSC	NAD - PCK	0.001	0.009	70	0.16	1
Major BSC:MSC	NADP - PCK	-0.035	0.008	70	-4.09	0.000344
Minor BSC:MSC	NAD - NADP	0.028	0.009	70	3.15	0.00727
Minor BSC:MSC	NAD - PCK	0.002	0.008	70	0.27	1
Minor BSC:MSC	NADP - PCK	-0.026	0.008	70	-3.2	0.00628
Interveinal distance	NAD - NADP	31.029	8.54	77	3.63	0.00151
Interveinal distance	NAD - PCK	20.579	8.54	77	2.41	0.0551
Interveinal distance	NADP - PCK	-10.45	7.639	77	-1.37	0.526

Appendix Table A.7: Tukey-adjusted pairwise contrasts of stomatal traits across C₄ subtypes.

Trait	contrast	estimate	SE	df	t-ratio	p-value
SD _{ab}	NADP - PCK	-67.5478	24.27098	71	-2.78307	0.018679
SD _{ab}	NADP - NAD	-58.4577	24.5225	71	-2.38384	0.051201
SD _{ab}	PCK - NAD	9.090087	24.5225	71	0.370684	0.927138
SD _{ad}	NADP - PCK	-96.9691	25.03838	66	-3.87282	0.000722
SD _{ad}	NADP - NAD	-139.521	25.29737	66	-5.51525	1.86E-06
SD _{ad}	PCK - NAD	-42.5521	24.75277	66	-1.71909	0.205726
SL _{ab}	NADP - PCK	0.004858	0.001924	71	2.52464	0.036384
SL _{ab}	NADP - NAD	0.007421	0.001944	71	3.816982	0.000826
SL _{ab}	PCK - NAD	0.002563	0.001944	71	1.318236	0.389841
SL _{ad}	NADP - PCK	0.006538	0.00166	68	3.939281	0.000566
SL _{ad}	NADP - NAD	0.006235	0.001644	68	3.793402	0.000919
SL _{ad}	PCK - NAD	-0.0003	0.001607	68	-0.18833	0.980642

Appendix Table A.8: Model estimated pairwise contrasts for $\delta^{13}\text{C}$ and leaf C:N across the C_4 subtypes.

Response	Effect	contrast	estimate	SE	df	t-ratio	lower CL	upper CL	p-value
$\delta^{13}\text{C}$	subtype	NAD - NADP	-1.2846	0.5806	71	-2.21	-2.6744	0.1052	0.0759
$\delta^{13}\text{C}$	subtype	NAD - PCK	0.1613	0.5866	71	0.27	-1.2429	1.5655	0.959
$\delta^{13}\text{C}$	subtype	NADP - PCK	1.4459	0.5866	71	2.46	0.0417	2.8501	0.0421
C:N	subtype	NAD - NADP	2.6423	2.382	71	1.11	-3.0598	8.3445	0.512
C:N	subtype	NAD - PCK	5.4395	2.4067	71	2.26	-0.3217	11.2008	0.0682
C:N	subtype	NADP - PCK	2.7972	2.4067	71	1.16	-2.964	8.5585	0.48

Appendix Table A.9: Model estimated pairwise contrasts for $\delta^{13}\text{C}$ across 15 species of C₄ grass.

Response	contrast	estimate	SE	df	t-ratio	lower CL	upper CL	p-value
$\delta^{13}\text{C}$	ANGE - BOGR	4.4432	1.1771	59	3.77	0.2776	8.6088	0.026
$\delta^{13}\text{C}$	ANGE - BOHI	3.4486	1.1771	59	2.93	-0.717	7.6143	0.213
$\delta^{13}\text{C}$	ANGE - CHVE	1.5382	1.1771	59	1.31	-2.6274	5.7039	0.992
$\delta^{13}\text{C}$	ANGE - DICO	0.6375	1.1771	59	0.54	-3.5281	4.8031	1
$\delta^{13}\text{C}$	ANGE - ERSP	0.9419	1.1771	59	0.8	-3.2238	5.1075	1
$\delta^{13}\text{C}$	ANGE - MUCU	1.9515	1.1771	59	1.66	-2.2141	6.1172	0.937
$\delta^{13}\text{C}$	ANGE - MURA	2.7186	1.1771	59	2.31	-1.447	6.8842	0.588
$\delta^{13}\text{C}$	ANGE - PAPU	0.7622	1.1771	59	0.65	-3.4034	4.9279	1
$\delta^{13}\text{C}$	ANGE - PAVI	-0.3162	1.1771	59	-0.27	-4.4818	3.8494	1
$\delta^{13}\text{C}$	ANGE - SCPA	1.4721	1.1771	59	1.25	-2.6935	5.6378	0.994
$\delta^{13}\text{C}$	ANGE - SCSC	0.5059	1.1771	59	0.43	-3.6597	4.6716	1
$\delta^{13}\text{C}$	ANGE - SONU	0.1638	1.1771	59	0.14	-4.0019	4.3294	1
$\delta^{13}\text{C}$	ANGE - SPPE	0.2653	1.1771	59	0.23	-3.9003	4.4309	1
$\delta^{13}\text{C}$	ANGE - TRFL	1.1953	1.2485	59	0.96	-3.223	5.6136	1
$\delta^{13}\text{C}$	BOGR - BOHI	-0.9946	1.1771	59	-0.84	-5.1602	3.1711	1
$\delta^{13}\text{C}$	BOGR - CHVE	-2.905	1.1771	59	-2.47	-7.0706	1.2607	0.478
$\delta^{13}\text{C}$	BOGR - DICO	-3.8057	1.1771	59	-3.23	-7.9713	0.3599	0.109
$\delta^{13}\text{C}$	BOGR - ERSP	-3.5013	1.1771	59	-2.97	-7.667	0.6643	0.194
$\delta^{13}\text{C}$	BOGR - MUCU	-2.4917	1.1771	59	-2.12	-6.6573	1.674	0.719
$\delta^{13}\text{C}$	BOGR - MURA	-1.7246	1.1771	59	-1.47	-5.8902	2.441	0.977
$\delta^{13}\text{C}$	BOGR - PAPU	-3.681	1.1771	59	-3.13	-7.8466	0.4846	0.139
$\delta^{13}\text{C}$	BOGR - PAVI	-4.7594	1.1771	59	-4.04	-8.925	-0.5938	0.0117
$\delta^{13}\text{C}$	BOGR - SCPA	-2.9711	1.1771	59	-2.52	-7.1367	1.1946	0.44
$\delta^{13}\text{C}$	BOGR - SCSC	-3.9373	1.1771	59	-3.34	-8.1029	0.2284	0.0827
$\delta^{13}\text{C}$	BOGR - SONU	-4.2794	1.1771	59	-3.64	-8.4451	-0.1138	0.0384
$\delta^{13}\text{C}$	BOGR - SPPE	-4.1779	1.1771	59	-3.55	-8.3435	-0.0123	0.0486
$\delta^{13}\text{C}$	BOGR - TRFL	-3.2479	1.2485	59	-2.6	-7.6662	1.1704	0.39
$\delta^{13}\text{C}$	BOHI - CHVE	-1.9104	1.1771	59	-1.62	-6.076	2.2553	0.946
$\delta^{13}\text{C}$	BOHI - DICO	-2.8111	1.1771	59	-2.39	-6.9767	1.3545	0.533

$\delta^{13}\text{C}$	BOHI - ERSP	-2.5067	1.1771	59	-2.13	-6.6724	1.6589	0.71
$\delta^{13}\text{C}$	BOHI - MUCU	-1.4971	1.1771	59	-1.27	-5.6627	2.6685	0.993
$\delta^{13}\text{C}$	BOHI - MURA	-0.73	1.1771	59	-0.62	-4.8957	3.4356	1
$\delta^{13}\text{C}$	BOHI - PAPU	-2.6864	1.1771	59	-2.28	-6.852	1.4792	0.607
$\delta^{13}\text{C}$	BOHI - PAVI	-3.7648	1.1771	59	-3.2	-7.9305	0.4008	0.118
$\delta^{13}\text{C}$	BOHI - SCPA	-1.9765	1.1771	59	-1.68	-6.1421	2.1891	0.931
$\delta^{13}\text{C}$	BOHI - SCSC	-2.9427	1.1771	59	-2.5	-7.1083	1.2229	0.456
$\delta^{13}\text{C}$	BOHI - SONU	-3.2849	1.1771	59	-2.79	-7.4505	0.8808	0.28
$\delta^{13}\text{C}$	BOHI - SPPE	-3.1833	1.1771	59	-2.7	-7.349	0.9823	0.328
$\delta^{13}\text{C}$	BOHI - TRFL	-2.2533	1.2485	59	-1.8	-6.6717	2.165	0.887
$\delta^{13}\text{C}$	CHVE - DICO	-0.9007	1.1771	59	-0.77	-5.0664	3.2649	1
$\delta^{13}\text{C}$	CHVE - ERSP	-0.5964	1.1771	59	-0.51	-4.762	3.5693	1
$\delta^{13}\text{C}$	CHVE - MUCU	0.4133	1.1771	59	0.35	-3.7523	4.5789	1
$\delta^{13}\text{C}$	CHVE - MURA	1.1803	1.1771	59	1	-2.9853	5.346	0.999
$\delta^{13}\text{C}$	CHVE - PAPU	-0.776	1.1771	59	-0.66	-4.9417	3.3896	1
$\delta^{13}\text{C}$	CHVE - PAVI	-1.8544	1.1771	59	-1.58	-6.0201	2.3112	0.957
$\delta^{13}\text{C}$	CHVE - SCPA	-0.0661	1.1771	59	-0.06	-4.2318	4.0995	1
$\delta^{13}\text{C}$	CHVE - SCSC	-1.0323	1.1771	59	-0.88	-5.1979	3.1333	1
$\delta^{13}\text{C}$	CHVE - SONU	-1.3745	1.1771	59	-1.17	-5.5401	2.7911	0.997
$\delta^{13}\text{C}$	CHVE - SPPE	-1.2729	1.1771	59	-1.08	-5.4386	2.8927	0.999
$\delta^{13}\text{C}$	CHVE - TRFL	-0.343	1.2485	59	-0.27	-4.7613	4.0754	1
$\delta^{13}\text{C}$	DICO - ERSP	0.3044	1.1771	59	0.26	-3.8613	4.47	1
$\delta^{13}\text{C}$	DICO - MUCU	1.314	1.1771	59	1.12	-2.8516	5.4796	0.998
$\delta^{13}\text{C}$	DICO - MURA	2.0811	1.1771	59	1.77	-2.0846	6.2467	0.901
$\delta^{13}\text{C}$	DICO - PAPU	0.1247	1.1771	59	0.11	-4.0409	4.2903	1
$\delta^{13}\text{C}$	DICO - PAVI	-0.9537	1.1771	59	-0.81	-5.1193	3.2119	1
$\delta^{13}\text{C}$	DICO - SCPA	0.8346	1.1771	59	0.71	-3.331	5.0002	1
$\delta^{13}\text{C}$	DICO - SCSC	-0.1316	1.1771	59	-0.11	-4.2972	4.034	1
$\delta^{13}\text{C}$	DICO - SONU	-0.4738	1.1771	59	-0.4	-4.6394	3.6919	1
$\delta^{13}\text{C}$	DICO - SPPE	-0.3722	1.1771	59	-0.32	-4.5378	3.7934	1
$\delta^{13}\text{C}$	DICO - TRFL	0.5578	1.2485	59	0.45	-3.8606	4.9761	1

$\delta^{13}\text{C}$	ERSP - MUCU	1.0097	1.1771	59	0.86	-3.156	5.1753	1
$\delta^{13}\text{C}$	ERSP - MURA	1.7767	1.1771	59	1.51	-2.3889	5.9423	0.97
$\delta^{13}\text{C}$	ERSP - PAPU	-0.1797	1.1771	59	-0.15	-4.3453	3.986	1
$\delta^{13}\text{C}$	ERSP - PAVI	-1.2581	1.1771	59	-1.07	-5.4237	2.9076	0.999
$\delta^{13}\text{C}$	ERSP - SCPA	0.5302	1.1771	59	0.45	-3.6354	4.6959	1
$\delta^{13}\text{C}$	ERSP - SCSC	-0.4359	1.1771	59	-0.37	-4.6016	3.7297	1
$\delta^{13}\text{C}$	ERSP - SONU	-0.7781	1.1771	59	-0.66	-4.9438	3.3875	1
$\delta^{13}\text{C}$	ERSP - SPPE	-0.6766	1.1771	59	-0.57	-4.8422	3.4891	1
$\delta^{13}\text{C}$	ERSP - TRFL	0.2534	1.2485	59	0.2	-4.1649	4.6717	1
$\delta^{13}\text{C}$	MUCU - MURA	0.7671	1.1771	59	0.65	-3.3986	4.9327	1
$\delta^{13}\text{C}$	MUCU - PAPU	-1.1893	1.1771	59	-1.01	-5.3549	2.9763	0.999
$\delta^{13}\text{C}$	MUCU - PAVI	-2.2677	1.1771	59	-1.93	-6.4334	1.8979	0.83
$\delta^{13}\text{C}$	MUCU - SCPA	-0.4794	1.1771	59	-0.41	-4.645	3.6862	1
$\delta^{13}\text{C}$	MUCU - SCSC	-1.4456	1.1771	59	-1.23	-5.6112	2.72	0.995
$\delta^{13}\text{C}$	MUCU - SONU	-1.7878	1.1771	59	-1.52	-5.9534	2.3779	0.968
$\delta^{13}\text{C}$	MUCU - SPPE	-1.6862	1.1771	59	-1.43	-5.8519	2.4794	0.981
$\delta^{13}\text{C}$	MUCU - TRFL	-0.7563	1.2485	59	-0.61	-5.1746	3.6621	1
$\delta^{13}\text{C}$	MURA - PAPU	-1.9564	1.1771	59	-1.66	-6.122	2.2093	0.936
$\delta^{13}\text{C}$	MURA - PAVI	-3.0348	1.1771	59	-2.58	-7.2004	1.1308	0.405
$\delta^{13}\text{C}$	MURA - SCPA	-1.2465	1.1771	59	-1.06	-5.4121	2.9192	0.999
$\delta^{13}\text{C}$	MURA - SCSC	-2.2127	1.1771	59	-1.88	-6.3783	1.953	0.853
$\delta^{13}\text{C}$	MURA - SONU	-2.5548	1.1771	59	-2.17	-6.7205	1.6108	0.683
$\delta^{13}\text{C}$	MURA - SPPE	-2.4533	1.1771	59	-2.08	-6.6189	1.7123	0.739
$\delta^{13}\text{C}$	MURA - TRFL	-1.5233	1.2485	59	-1.22	-5.9416	2.895	0.996
$\delta^{13}\text{C}$	PAPU - PAVI	-1.0784	1.1771	59	-0.92	-5.244	3.0872	1
$\delta^{13}\text{C}$	PAPU - SCPA	0.7099	1.1771	59	0.6	-3.4557	4.8755	1
$\delta^{13}\text{C}$	PAPU - SCSC	-0.2563	1.1771	59	-0.22	-4.4219	3.9093	1
$\delta^{13}\text{C}$	PAPU - SONU	-0.5985	1.1771	59	-0.51	-4.7641	3.5672	1
$\delta^{13}\text{C}$	PAPU - SPPE	-0.4969	1.1771	59	-0.42	-4.6626	3.6687	1
$\delta^{13}\text{C}$	PAPU - TRFL	0.4331	1.2485	59	0.35	-3.9853	4.8514	1
$\delta^{13}\text{C}$	PAVI - SCPA	1.7883	1.1771	59	1.52	-2.3773	5.954	0.968

$\delta^{13}\text{C}$	PAVI - SCSC	0.8221	1.1771	59	0.7	-3.3435	4.9878	1
$\delta^{13}\text{C}$	PAVI - SONU	0.48	1.1771	59	0.41	-3.6857	4.6456	1
$\delta^{13}\text{C}$	PAVI - SPPE	0.5815	1.1771	59	0.49	-3.5841	4.7471	1
$\delta^{13}\text{C}$	PAVI - TRFL	1.5115	1.2485	59	1.21	-2.9068	5.9298	0.996
$\delta^{13}\text{C}$	SCPA - SCSC	-0.9662	1.1771	59	-0.82	-5.1318	3.1994	1
$\delta^{13}\text{C}$	SCPA - SONU	-1.3084	1.1771	59	-1.11	-5.474	2.8573	0.998
$\delta^{13}\text{C}$	SCPA - SPPE	-1.2068	1.1771	59	-1.03	-5.3725	2.9588	0.999
$\delta^{13}\text{C}$	SCPA - TRFL	-0.2768	1.2485	59	-0.22	-4.6952	4.1415	1
$\delta^{13}\text{C}$	SCSC - SONU	-0.3422	1.1771	59	-0.29	-4.5078	3.8235	1
$\delta^{13}\text{C}$	SCSC - SPPE	-0.2406	1.1771	59	-0.2	-4.4063	3.925	1
$\delta^{13}\text{C}$	SCSC - TRFL	0.6893	1.2485	59	0.55	-3.729	5.1077	1
$\delta^{13}\text{C}$	SONU - SPPE	0.1015	1.1771	59	0.09	-4.0641	4.2672	1
$\delta^{13}\text{C}$	SONU - TRFL	1.0315	1.2485	59	0.83	-3.3868	5.4498	1
$\delta^{13}\text{C}$	SPPE - TRFL	0.93	1.2485	59	0.74	-3.4883	5.3483	1

Appendix Table A.10: Median, upper and lower quartiles, upper and lower whiskers, and sample size for calculated stomatal sensitivity (m) across C₄ subtypes at each stepwise transition of VPD_{leaf}.

treatment	subtype	transition	n	q25	median	q75	low whisker	high whisker
Increasing	NADP-ME	1 → 2 kPa	40	-0.31875	53.55484	106.5924	-71.6089	253.8206
Increasing	NADP-ME	2 → 3 kPa	43	-9.09152	13.09051	66.95546	-77.4968	138.7179
Increasing	NADP-ME	3 → 4 kPa	44	60.03433	101.1969	132.4463	6.044533	224.0919
Increasing	NADP-ME	4 → 4.5 kPa	6	109.6311	123.8171	183.5881	107.0909	223.8324
Increasing	PCK	1 → 2 kPa	26	-24.9709	29.4482	58.19333	-94.3414	120.5222
Increasing	PCK	2 → 3 kPa	33	-5.63487	21.50474	58.61656	-52.0487	150.2386
Increasing	PCK	3 → 4 kPa	30	85.74551	117.0725	169.1734	30.69628	247.4789
Increasing	PCK	4 → 4.5 kPa	4	172.3799	201.8946	239.9114	97.18382	340.6137
Increasing	NAD-ME	1 → 2 kPa	36	19.52839	60.46626	104.7234	-35.1441	206.6064
Increasing	NAD-ME	2 → 3 kPa	36	-10.7701	22.28834	69.05131	-94.8337	139.9738
Increasing	NAD-ME	3 → 4 kPa	34	64.62113	92.39467	179.1055	-20.9845	305.088
Increasing	NAD-ME	4 → 4.5 kPa	7	100.5683	176.4114	190.7203	40.73457	316.158
Decreasing	NADP-ME	2 → 1 kPa	27	-42.4488	-23.5781	-14.3177	-67.8375	23.72697
Decreasing	NADP-ME	3 → 2 kPa	40	-43.2759	-14.9391	-7.15997	-93.4699	44.50592
Decreasing	NADP-ME	4.5 → 4 kPa	6	-18.3987	10.86756	38.69381	-82.1149	41.41092
Decreasing	NADP-ME	4 → 3 kPa	45	7.365483	26.69947	45.68793	-45.2229	94.48499
Decreasing	PCK	2 → 1 kPa	28	-70.0821	-31.3323	6.922286	-117.508	98.38851
Decreasing	PCK	3 → 2 kPa	33	-44.6574	-18.8961	14.49586	-106.259	99.54704
Decreasing	PCK	4.5 → 4 kPa	3	85.61454	89.04138	117.9158	82.1877	146.7903
Decreasing	PCK	4 → 3 kPa	31	7.384491	38.67255	82.60783	-40.1071	128.1343
Decreasing	NAD-ME	2 → 1 kPa	26	-57.2729	-39.942	-15.4314	-108.432	20.1763
Decreasing	NAD-ME	3 → 2 kPa	36	-40.7678	-24.1732	-0.54157	-99.5799	46.69204
Decreasing	NAD-ME	4.5 → 4 kPa	6	18.85379	51.42806	133.5621	8.863061	241.4658
Decreasing	NAD-ME	4 → 3 kPa	37	1.447299	35.9936	69.21145	-31.4572	161.9696

Appendix B - Chapter 3 Supplemental

Appendix Table B.1: Pairwise comparisons of A, g_{sw} , and E responses of C4 subtypes in each treatment across the 10-day drought period.

Drought	Response	Treatment	Contrast	estimate	SE	df	t-ratio	p-value
VPD_{leaf}	A	Control	NAD - NADP	-4.72125	5.309316	3.386089	-0.88924	0.678955
VPD_{leaf}	A	Control	NAD - PCK	-2.37231	5.349985	3.485174	-0.44342	0.900231
VPD_{leaf}	A	Control	NADP - PCK	2.348939	5.343319	3.468324	0.439603	0.901821
VPD_{leaf}	A	Treatment	NAD - NADP	-7.09923	5.302612	3.369518	-1.33882	0.460551
VPD_{leaf}	A	Treatment	NAD - PCK	-4.16584	5.30686	3.380259	-0.78499	0.734115
VPD_{leaf}	A	Treatment	NADP - PCK	2.933385	5.305328	3.376366	0.552913	0.851706
VPD_{leaf}	g_{sw}	Control	NAD - NADP	-0.01523	0.027958	4.888138	-0.5447	0.853672
VPD_{leaf}	g_{sw}	Control	NAD - PCK	-0.02274	0.028509	5.24585	-0.79758	0.719878
VPD_{leaf}	g_{sw}	Control	NADP - PCK	-0.00751	0.028422	5.18435	-0.26422	0.962522
VPD_{leaf}	g_{sw}	Treatment	NAD - NADP	-0.05643	0.02787	4.82874	-2.02484	0.204287
VPD_{leaf}	g_{sw}	Treatment	NAD - PCK	-0.06763	0.027925	4.866555	-2.42172	0.129108
VPD_{leaf}	g_{sw}	Treatment	NADP - PCK	-0.01119	0.027905	4.85289	-0.40114	0.916592
VPD_{leaf}	E	Control	NAD - NADP	0.000202	0.000573	6.34696	0.352322	0.934553
VPD_{leaf}	E	Control	NAD - PCK	-0.00051	0.000592	7.090682	-0.86841	0.675379
VPD_{leaf}	E	Control	NADP - PCK	-0.00072	0.000589	6.952999	-1.21551	0.482037
VPD_{leaf}	E	Treatment	NAD - NADP	-0.00045	0.000569	6.215909	-0.78658	0.723842
VPD_{leaf}	E	Treatment	NAD - PCK	-0.00197	0.000571	6.301372	-3.43915	0.029822
VPD_{leaf}	E	Treatment	NADP - PCK	-0.00152	0.000571	6.270365	-2.65859	0.080606
$VPD_{leaf} \times SM$	A	Control	NAD - NADP	-8.62291	5.752631	3.643069	-1.49895	0.38987
$VPD_{leaf} \times SM$	A	Control	NAD - PCK	-2.85834	5.819258	3.799014	-0.49119	0.879435
$VPD_{leaf} \times SM$	A	Control	NADP - PCK	5.764571	5.816594	3.792061	0.991056	0.621689
$VPD_{leaf} \times SM$	A	Treatment	NAD - NADP	-7.89917	5.749899	3.636164	-1.37379	0.440321
$VPD_{leaf} \times SM$	A	Treatment	NAD - PCK	-4.41879	5.751828	3.641044	-0.76824	0.741617
$VPD_{leaf} \times SM$	A	Treatment	NADP - PCK	3.48038	5.750517	3.63773	0.605229	0.825874
$VPD_{leaf} \times SM$	g_{sw}	Control	NAD - NADP	-0.02089	0.033189	4.278073	-0.62931	0.812462
$VPD_{leaf} \times SM$	g_{sw}	Control	NAD - PCK	-0.06401	0.03413	4.714997	-1.87555	0.244324
$VPD_{leaf} \times SM$	g_{sw}	Control	NADP - PCK	-0.04313	0.034095	4.69592	-1.26486	0.473943

$VPD_{leaf} \times SM$	g_{sw}	Treatment	NAD - NADP	-0.06171	0.033153	4.259617	-1.86122	0.257206
$VPD_{leaf} \times SM$	g_{sw}	Treatment	NAD - PCK	-0.06164	0.033179	4.272675	-1.85791	0.257844
$VPD_{leaf} \times SM$	g_{sw}	Treatment	NADP - PCK	6.23E-05	0.033161	4.263816	0.001879	0.999998
$VPD_{leaf} \times SM$	E	Control	NAD - NADP	-0.00016	0.00057	7.880871	-0.27998	0.957937
$VPD_{leaf} \times SM$	E	Control	NAD - PCK	-0.00145	0.000612	9.637081	-2.36447	0.093731
$VPD_{leaf} \times SM$	E	Control	NADP - PCK	-0.00129	0.00061	9.541843	-2.10887	0.139878
$VPD_{leaf} \times SM$	E	Treatment	NAD - NADP	-0.00201	0.000568	7.79165	-3.53862	0.019361
$VPD_{leaf} \times SM$	E	Treatment	NAD - PCK	-0.00147	0.000569	7.854998	-2.5773	0.076449
$VPD_{leaf} \times SM$	E	Treatment	NADP - PCK	0.000543	0.000569	7.81219	0.95546	0.623453

Appendix Table B.2: Model estimated slopes of C₄ subtypes in each treatment for A, g_{sw}, and E responses across the 10-day drought treatment.

Response	treatment	subtype	slope	SE	df	lower CL	upper CL	p-value
A	Control	NAD	0.8492	0.1599	850.4221	0.5354	1.1631	1.39E-07
A	Control	NADP	1.4897	0.1573	841.8398	1.181	1.7985	2.73E-20
A	Control	PCK	1.0629	0.1961	855.877	0.678	1.4479	7.79E-08
A	Treatment	NAD	0.0638	0.1572	840.4651	-0.2448	0.3725	0.685
A	Treatment	NADP	0.311	0.1553	840.4304	0.0061	0.6159	0.0456
A	Treatment	PCK	0.3011	0.1591	840.4631	-0.0112	0.6133	0.0587
g _{sw}	Control	NAD	0.0063	0.0014	850.7789	0.0035	0.009	9.33E-06
g _{sw}	Control	NADP	0.0071	0.0014	842.0322	0.0044	0.0098	3.91E-07
g _{sw}	Control	PCK	0.0082	0.0017	856.7741	0.0049	0.0116	2.07E-06
g _{sw}	Treatment	NAD	-0.0013	0.0014	840.692	-0.0041	0.0014	0.33
g _{sw}	Treatment	NADP	0.0014	0.0014	840.6526	-0.0013	0.0041	0.305
g _{sw}	Treatment	PCK	-0.0021	0.0014	840.6875	-0.0049	0.0006	0.132
E	Control	NAD	0.000156	3.85E-05	850.4803	8.06E-05	0.000232	5.43E-05
E	Control	NADP	0.000164	3.8E-05	842.2688	8.9E-05	0.000238	1.86E-05
E	Control	PCK	0.000185	4.74E-05	858.4246	9.22E-05	0.000278	9.96E-05
E	Treatment	NAD	-1.9E-05	3.8E-05	840.9848	-9.4E-05	5.52E-05	0.611
E	Treatment	NADP	0.000305	3.75E-05	840.9332	0.000232	0.000379	1.45E-15
E	Treatment	PCK	-0.00011	3.84E-05	840.9734	-0.00019	-3.5E-05	0.00406

Appendix Table B.3: Model estimated slopes of C₄ subtypes for A, g_{sw}, E, and midday leaf water potential (Ψ) responses across the growing season. Slopes were calculated as the magnitude of change between the start day and end day of each sampling interval.

Response	subtype	start day	end day	midpoint	interval slope
A	NADP-ME	162	179	170.5	0.00335
A	PCK	162	179	170.5	-0.20462
A	NAD-ME	162	179	170.5	0.16381
A	NADP-ME	179	210	194.5	0.099714
A	PCK	179	210	194.5	-0.01055
A	NAD-ME	179	210	194.5	-0.18416
A	NADP-ME	210	226	218	-0.77114
A	PCK	210	226	218	-0.34655
A	NAD-ME	210	226	218	0.045017
A	NADP-ME	226	247	236.5	-0.26581
A	PCK	226	247	236.5	-0.23836
A	NAD-ME	226	247	236.5	-0.231
g _{sw}	NADP-ME	162	179	170.5	-0.00119
g _{sw}	PCK	162	179	170.5	0.002333
g _{sw}	NAD-ME	162	179	170.5	0.004814
g _{sw}	NADP-ME	179	210	194.5	0.002926
g _{sw}	PCK	179	210	194.5	-0.00101
g _{sw}	NAD-ME	179	210	194.5	-0.00207
g _{sw}	NADP-ME	210	226	218	-0.00281
g _{sw}	PCK	210	226	218	-0.00096
g _{sw}	NAD-ME	210	226	218	-0.00039
g _{sw}	NADP-ME	226	247	236.5	-0.00681
g _{sw}	PCK	226	247	236.5	-0.00241
g _{sw}	NAD-ME	226	247	236.5	-0.00144
E	NADP-ME	162	179	170.5	-5.1E-05
E	PCK	162	179	170.5	0.00011
E	NAD-ME	162	179	170.5	0.000211
E	NADP-ME	179	210	194.5	0.000135
E	PCK	179	210	194.5	-4.5E-05
E	NAD-ME	179	210	194.5	-9.3E-05
E	NADP-ME	210	226	218	-0.00013
E	PCK	210	226	218	-5.1E-05
E	NAD-ME	210	226	218	-2.4E-05
E	NADP-ME	226	247	236.5	-0.00033
E	PCK	226	247	236.5	-0.00014
E	NAD-ME	226	247	236.5	-9.1E-05
Ψ	NADP-ME	162	179	170.5	-0.10784
Ψ	PCK	162	179	170.5	-0.13725
Ψ	NAD-ME	162	179	170.5	-0.15686

Ψ	NADP-ME	179	210	194.5	-0.12097
Ψ	PCK	179	210	194.5	-0.00538
Ψ	NAD-ME	179	210	194.5	0.10484
Ψ	NADP-ME	210	226	218	0.38021
Ψ	PCK	210	226	218	-0.39583
Ψ	NAD-ME	210	226	218	-0.03646
Ψ	NADP-ME	226	247	236.5	-0.19048
Ψ	PCK	226	247	236.5	0.15476
Ψ	NAD-ME	226	247	236.5	-0.23413

Appendix Table B.4: Pairwise contrasts of the C₄ subtypes for A, g_{sw}, E, and midday leaf water potential (Ψ) across the growing season.

Response	contrast	day of year	estimate	SE	df	t-ratio	p-value
A	NAD - NADP	162	-4.06323	2.977201	44.24741	-1.36478	0.367867
A	NAD - PCK	162	-8.38734	2.977201	44.24741	-2.81719	0.019371
A	NADP - PCK	162	-4.32411	2.977201	44.24741	-1.45241	0.323445
A	NAD - NADP	179	-1.33535	2.977201	44.24741	-0.44853	0.895308
A	NAD - PCK	179	-2.12402	2.977201	44.24741	-0.71343	0.756884
A	NADP - PCK	179	-0.78867	2.977201	44.24741	-0.2649	0.962088
A	NAD - NADP	210	-10.1353	2.977201	44.24741	-3.40432	0.003979
A	NAD - PCK	210	-7.50576	2.977201	44.24741	-2.52108	0.039998
A	NADP - PCK	210	2.629577	2.977201	44.24741	0.883238	0.653597
A	NAD - NADP	226	2.649757	3.080608	47.70407	0.860141	0.66777
A	NAD - PCK	226	-1.24068	2.977201	44.24741	-0.41673	0.908917
A	NADP - PCK	226	-3.89044	3.080608	47.70407	-1.26288	0.422882
A	NAD - NADP	247	3.654347	2.977201	44.24741	1.227444	0.443565
A	NAD - PCK	247	-1.08614	2.977201	44.24741	-0.36482	0.929382
A	NADP - PCK	247	-4.74049	2.977201	44.24741	-1.59226	0.259576
g _{sw}	NAD - NADP	162	-0.07514	0.033231	73.68691	-2.26117	0.067816
g _{sw}	NAD - PCK	162	-0.06612	0.033231	73.68691	-1.98965	0.121912
g _{sw}	NADP - PCK	162	0.009023	0.033231	73.68691	0.271521	0.960197
g _{sw}	NAD - NADP	179	0.026911	0.033231	73.68691	0.809821	0.698162
g _{sw}	NAD - PCK	179	-0.02393	0.033231	73.68691	-0.72004	0.75245
g _{sw}	NADP - PCK	179	-0.05084	0.033231	73.68691	-1.52986	0.282989
g _{sw}	NAD - NADP	210	-0.12784	0.033231	73.68691	-3.84697	0.00073
g _{sw}	NAD - PCK	210	-0.05669	0.033231	73.68691	-1.70592	0.209812
g _{sw}	NADP - PCK	210	0.071149	0.033231	73.68691	2.14105	0.088597
g _{sw}	NAD - NADP	226	-0.08924	0.034925	73.78672	-2.55525	0.033514
g _{sw}	NAD - PCK	226	-0.04753	0.033231	73.68691	-1.43017	0.330819
g _{sw}	NADP - PCK	226	0.041715	0.034925	73.78672	1.19444	0.460237
g _{sw}	NAD - NADP	247	0.023529	0.033231	73.68691	0.708046	0.759518
g _{sw}	NAD - PCK	247	-0.02705	0.033231	73.68691	-0.81396	0.69561
g _{sw}	NADP - PCK	247	-0.05058	0.033231	73.68691	-1.52201	0.28659
E	NAD - NADP	162	-0.00321	0.001512	73.50423	-2.12353	0.092042
E	NAD - PCK	162	-0.00278	0.001512	73.50423	-1.83823	0.164399
E	NADP - PCK	162	0.000431	0.001512	73.50423	0.285297	0.956151
E	NAD - NADP	179	0.001248	0.001512	73.50423	0.825405	0.688548
E	NAD - PCK	179	-0.00105	0.001512	73.50423	-0.69738	0.765761
E	NADP - PCK	179	-0.0023	0.001512	73.50423	-1.52279	0.286247
E	NAD - NADP	210	-0.00582	0.001512	73.50423	-3.8484	0.000728
E	NAD - PCK	210	-0.00255	0.001512	73.50423	-1.68585	0.217431
E	NADP - PCK	210	0.00327	0.001512	73.50423	2.162552	0.084551
E	NAD - NADP	226	-0.00412	0.001589	73.66014	-2.59337	0.030442

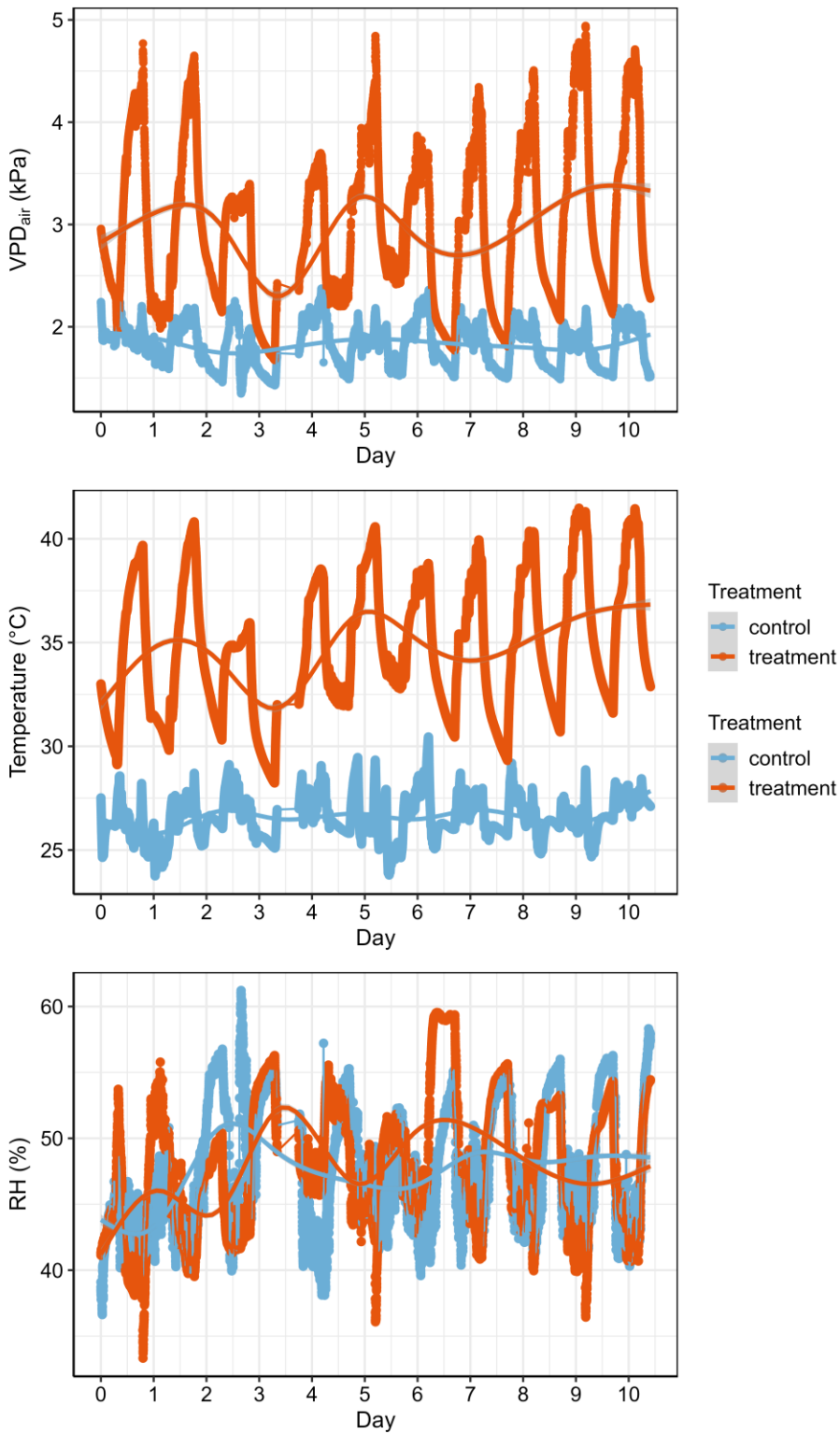
E	NAD - PCK	226	-0.00211	0.001512	73.50423	-1.3959	0.348296
E	NADP - PCK	226	0.00201	0.001589	73.66014	1.264956	0.419402
E	NAD - NADP	247	0.000982	0.001512	73.50423	0.649483	0.793244
E	NAD - PCK	247	-0.00106	0.001512	73.50423	-0.7016	0.763299
E	NADP - PCK	247	-0.00204	0.001512	73.50423	-1.35108	0.37188
Ψ	NAD - NADP	162	-0.75	1.854105	73.9534	-0.40451	0.913867
Ψ	NAD - PCK	162	1.5	1.854105	73.9534	0.809016	0.698653
Ψ	NADP - PCK	162	2.25	1.854105	73.9534	1.213524	0.449021
Ψ	NAD - NADP	179	-1.58333	1.854105	73.9534	-0.85396	0.67081
Ψ	NAD - PCK	179	1.166667	1.854105	73.9534	0.629234	0.804556
Ψ	NADP - PCK	179	2.75	1.854105	73.9534	1.483195	0.304797
Ψ	NAD - NADP	210	5.416667	1.854105	73.9534	2.921446	0.012682
Ψ	NAD - PCK	210	4.583333	1.854105	73.9534	2.471992	0.041191
Ψ	NADP - PCK	210	-0.83333	1.854105	73.9534	-0.44945	0.894798
Ψ	NAD - NADP	226	-1.23153	1.949198	73.96872	-0.63182	0.803124
Ψ	NAD - PCK	226	10.33333	1.854105	73.9534	5.573219	1.15E-06
Ψ	NADP - PCK	226	11.56487	1.949198	73.96872	5.933142	2.65E-07
Ψ	NAD - NADP	247	-2.16667	1.854105	73.9534	-1.16858	0.475583
Ψ	NAD - PCK	247	2.166667	1.854105	73.9534	1.168578	0.475583
Ψ	NADP - PCK	247	4.333333	1.854105	73.9534	2.337156	0.056888

Appendix Table B.5: Pairwise contrasts of the C₄ subtypes for $\delta^{13}\text{C}$ and leaf C:N across the growing season.

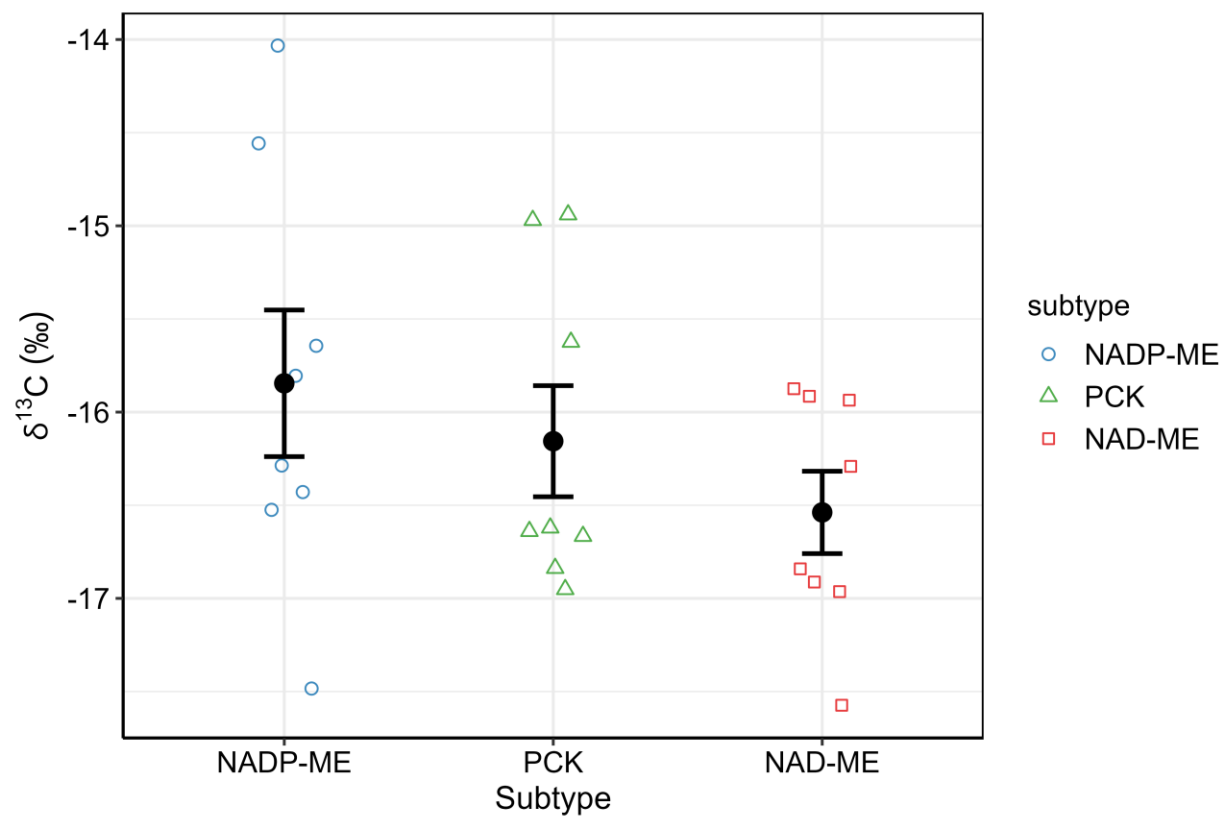
Response	contrast	day of year	estimate	SE	df	t-ratio	p-value
$\delta^{13}\text{C}$	NAD - NADP	162	-0.38833	0.521988	33.32624	-0.74395	0.739325
$\delta^{13}\text{C}$	NAD - PCK	162	1.05	0.521988	33.32624	2.011539	0.125096
$\delta^{13}\text{C}$	NADP - PCK	162	1.438333	0.521988	33.32624	2.755489	0.024902
$\delta^{13}\text{C}$	NAD - NADP	179	-0.8	0.521988	33.32624	-1.5326	0.288965
$\delta^{13}\text{C}$	NAD - PCK	179	0.813333	0.521988	33.32624	1.558145	0.277638
$\delta^{13}\text{C}$	NADP - PCK	179	1.613333	0.521988	33.32624	3.090746	0.010912
$\delta^{13}\text{C}$	NAD - NADP	210	-1.19167	0.521988	33.32624	-2.28294	0.072223
$\delta^{13}\text{C}$	NAD - PCK	210	0.071667	0.521988	33.32624	0.137296	0.989665
$\delta^{13}\text{C}$	NADP - PCK	210	1.263333	0.521988	33.32624	2.420233	0.053713
$\delta^{13}\text{C}$	NAD - NADP	226	-1.035	0.521988	33.32624	-1.9828	0.132193
$\delta^{13}\text{C}$	NAD - PCK	226	-0.495	0.521988	33.32624	-0.9483	0.614081
$\delta^{13}\text{C}$	NADP - PCK	226	0.54	0.521988	33.32624	1.034506	0.560724
$\delta^{13}\text{C}$	NAD - NADP	247	-0.94833	0.521988	33.32624	-1.81677	0.179679
$\delta^{13}\text{C}$	NAD - PCK	247	0.381667	0.521988	33.32624	0.731178	0.74689
$\delta^{13}\text{C}$	NADP - PCK	247	1.33	0.521988	33.32624	2.54795	0.040372
C:N	NAD - NADP	162	4.382573	4.296239	61.85156	1.020095	0.567103
C:N	NAD - PCK	162	7.319399	4.296239	61.85156	1.703676	0.211954
C:N	NADP - PCK	162	2.936826	4.296239	61.85156	0.683581	0.773908
C:N	NAD - NADP	179	3.938499	4.296239	61.85156	0.916732	0.631863
C:N	NAD - PCK	179	10.65564	4.296239	61.85156	2.480225	0.041425
C:N	NADP - PCK	179	6.717137	4.296239	61.85156	1.563493	0.269067
C:N	NAD - NADP	210	7.878617	4.296239	61.85156	1.833841	0.167118
C:N	NAD - PCK	210	12.49366	4.296239	61.85156	2.908046	0.013786
C:N	NADP - PCK	210	4.615043	4.296239	61.85156	1.074206	0.533542
C:N	NAD - NADP	226	6.910874	4.296239	61.85156	1.608587	0.249686
C:N	NAD - PCK	226	15.76294	4.296239	61.85156	3.66901	0.001458
C:N	NADP - PCK	226	8.852067	4.296239	61.85156	2.060423	0.10662
C:N	NAD - NADP	247	7.957623	4.296239	61.85156	1.85223	0.161402
C:N	NAD - PCK	247	12.82887	4.296239	61.85156	2.98607	0.011127
C:N	NADP - PCK	247	4.871247	4.296239	61.85156	1.13384	0.497124

Appendix Table B.6: Pairwise comparisons of microanatomical traits between C₄ subtypes.

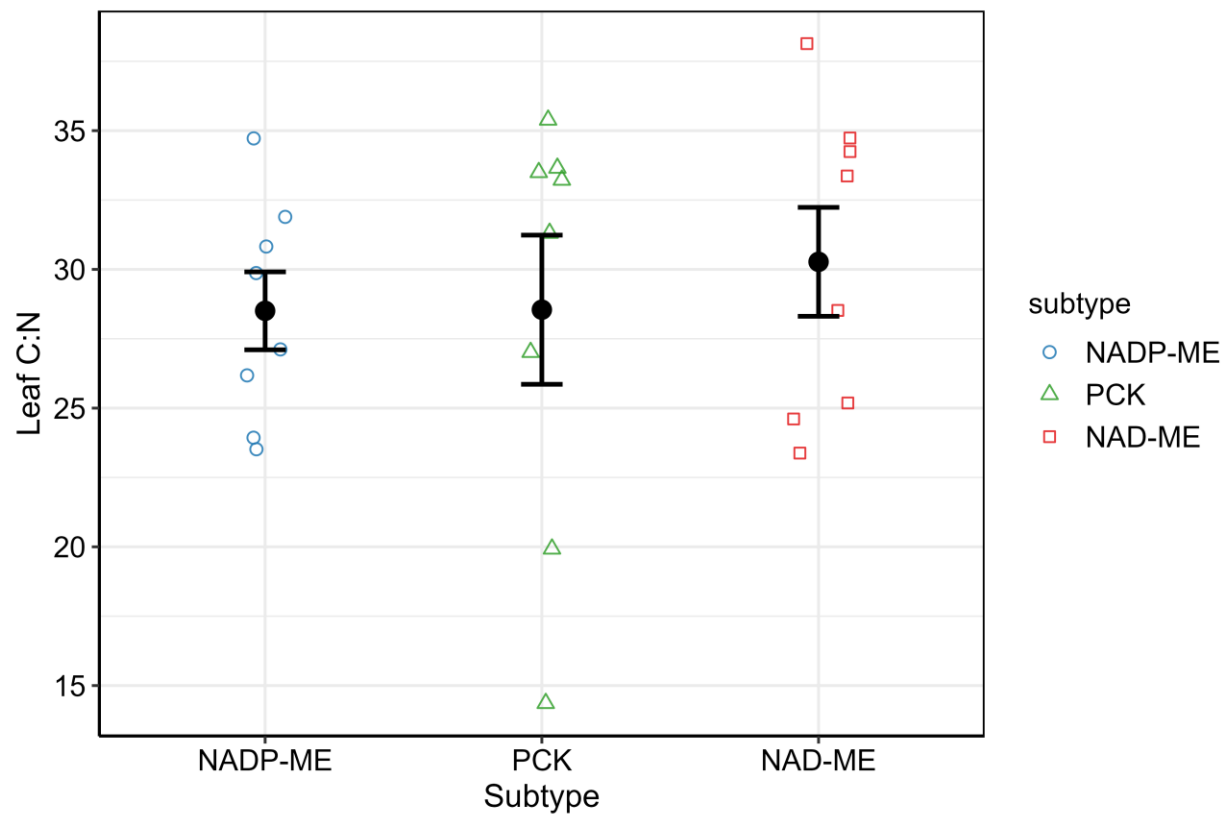
Trait	contrast	estimate	SE	df	t.ratio	p.value
Xylem area (%)	NADP-ME - PCK	0.83	0.2715	20	3.0576	0.01637
Xylem area (%)	NADP-ME - NAD-ME	0.6614	0.281	20	2.354	0.07106
Xylem area (%)	PCK - NAD-ME	-0.1686	0.281	20	-0.5999	0.8217
Vessel area (%)	NADP-ME - PCK	-0.6575	1.6256	20	-0.4045	0.9142
Vessel area (%)	NADP-ME - NAD-ME	-2.9327	1.6827	20	-1.7429	0.2144
Vessel area (%)	PCK - NAD-ME	-2.2752	1.6827	20	-1.3521	0.3841
Bundle sheath area (%)	NADP-ME - PCK	-14.2688	1.811	20	-7.8791	<0.0001
Bundle sheath area (%)	NADP-ME - NAD-ME	-10.3693	1.8745	20	-5.5317	<0.0001
Bundle sheath area (%)	PCK - NAD-ME	3.8995	1.8745	20	2.0802	0.1195
Mesophyll area (%)	NADP-ME - PCK	-0.4462	1.8411	20	-0.2424	0.9682
Mesophyll area (%)	NADP-ME - NAD-ME	-0.3045	1.9058	20	-0.1598	0.986
Mesophyll area (%)	PCK - NAD-ME	0.1418	1.9058	20	0.0744	0.997
Abaxial bulliform area (%)	NADP-ME - PCK	11.2994	1.2568	17	8.9907	<0.0001
Abaxial bulliform area (%)	NADP-ME - NAD-ME	6.5763	1.5392	17	4.2724	0.001417
Abaxial bulliform area (%)	PCK - NAD-ME	-4.7232	1.5392	17	-3.0685	0.01811
Adaxial bulliform area (%)	NADP-ME - PCK	0.0187	0.9572	20	0.0196	0.9998
Adaxial bulliform area (%)	NADP-ME - NAD-ME	-0.66	0.9908	20	-0.6661	0.7855
Adaxial bulliform area (%)	PCK - NAD-ME	-0.6788	0.9908	20	-0.6851	0.7748
Extra bulliform area (%)	NADP-ME - PCK	-0.9329	1.1326	18	-0.8236	0.6936
Extra bulliform area (%)	NADP-ME - NAD-ME	-0.9429	1.1326	18	-0.8324	0.6883
Extra bulliform area (%)	PCK - NAD-ME	-0.01	1.1326	18	-0.0088	1
Total bulliform area (%)	NADP-ME - PCK	10.5013	3.4907	20	3.0084	0.01822
Total bulliform area (%)	NADP-ME - NAD-ME	11.0446	3.6132	20	3.0568	0.0164
Total bulliform area (%)	PCK - NAD-ME	0.5434	3.6132	20	0.1504	0.9876



Appendix Figure B.1: Plots showing VPD_{air} , temperature, and relative humidity in control (blue) and treatment (orange) conditions.



Appendix Figure B.2: Mean $\pm$ SE of $\delta^{13}\text{C}$ for each of the C_4 subtypes in the 10-day drought treatment.



Appendix Figure B.3: Mean $\pm$ SE of leaf C:N for each of the C₄ subtypes in the 10-day drought treatment.