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ORIGINAL ARTICLE

# Soil moisture and vapor pressure deficit controls of longleaf pine physiology: results from a throughfall reduction study

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## Abstract

***Key message*** **Longleaf pine demonstrated general resistance to reduced soil moisture and increased VPD, but results highlight the soil and atmospheric conditions that could trigger declines in longleaf pine function and productivity.**

**Abstract** Low soil moisture and high atmospheric vapor pressure deficit (VPD) independently limit tree function and forest productivity. However, questions remain about how large, established trees respond to dry soil and high VPD over longer time periods. We carried out a 3-year throughfall reduction experiment in a young (12–14-year-old) longleaf pine plantation in west Georgia (USA). We hypothesized that throughfall reduction would reduce soil moisture, leaf-scale stomatal conductance ($g_s$), and net photosynthesis ($P_{net}$), but increase intrinsic water-use efficiency (iWUE). We also hypothesized that throughfall reduction would reduce canopy conductance ($G_s$) at a reference VPD of 1 kPa and $G_s$ sensitivity to VPD. In addition, we used $G_s$ data collected across both treatments to identify breakpoints in the relative control of soil moisture and VPD on $G_s$. Throughfall reduction decreased soil moisture and caused small reductions in $g_s$ (−21%) and $P_{net}$ (−13%), but no change in iWUE. As expected, reduced throughfall decreased $G_s$ and $G_s$ sensitivity to VPD by 20 and 8%, respectively. Despite this, throughfall reduction had very little effect on tree growth or forest productivity. Importantly, $G_s$ sensitivity to VPD was similar at intermediate soil moisture, but highest and lowest at soil moistures above field capacity and below the permanent wilting point, respectively. Consequently, we could identify thresholds in the relative control of soil moisture and VPD over $G_s$. These results demonstrate the general resistance of longleaf pine plantations to reduced soil moisture and increased VPD but highlight the soil and atmospheric conditions that could trigger declines in longleaf pine function and productivity.

**Keywords** Plant water relations · Climate change adaptation · Drought · Stomatal conductance · Vapor pressure deficit

## Introduction

Worldwide, water availability strongly influences tree growth and function. Reduced soil water availability (i.e., drought) often leads to leaf water deficit, reduced hydraulic conductance, and lower rates of $CO_2$ and $H_2O$ exchange at the leaf- and canopy scale (Allen et al. 2010 ; Mitchell et al. 2016; Starr et al. 2016; Choat et al. 2018). Prolonged or repeated drought periods can lead to widespread and irreversible xylem embolism, and possibly tree death (Hubbard et al. 2001; Vilagrosa et al. 2003; Choat et al. 2008; Creek et al. 2020). Yet, relatively few experimental studies, in a handful of species and forest types, have examined how larger trees growing in the field respond to dry conditions and changing atmospheric conditions over longer time periods (e.g., warmer temperatures, lower relative humidity, higher vapor

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pressure deficit (VPD); Rowland et al. 2015, Ficklin and Novick 2017; Asbjornsen et al. 2021). Assessing this knowledge gap could provide further information on the diversity of tree and forest responses to dry conditions and potential interactive effects with prevailing atmospheric conditions.

Tree species exhibit a spectrum of water-use strategies and drought tolerance (or resistance) (Klein 2014). Species that are more anisohydric tolerate drought by maintaining stable rates of stomatal conductance ($g_s$) and transpiration (E) at low xylem water potentials (Ψ). This strategy allows for continued C fixation (photosynthesis) at the risk of cavitation (McDowell et al. 2008; Roman et al. 2015; Hochberg et al. 2017; Blackman et al. 2019). Species that are more isohydric show the opposite response to reduced soil moisture—decreased $g_s$ and lower E and net photosynthesis ($P_{net}$), yet greater homeostasis of leaf water potential and lower risk of cavitation (Hubbard et al. 2001; Vilagrosa et al. 2003; Choat et al. 2008, 2018; Creek et al. 2020). Although the iso/anisohydry concept may be overly simplistic, it provides a general framework for predicting species stomatal regulation, C fixation, and water use in the field under drying conditions.

Stomatal regulation of leaf Ψ also influences canopy-level conductance ($G_s$); a key regulator of ecosystem-scale E and important input parameter in models that predict $H_2O$ fluxes over large spatial scales (Siqueira et al. 2006; Novick et al. 2009). Stomatal conductance usually declines with reduced soil moisture at a threshold that depends on species and soil texture (Allen et al. 2010, Mitchell et al. 2016; Starr et al. 2016; Choat et al. 2018; Novick et al. 2009, 2016). Canopy $G_s$ is also sensitive to atmospheric demand for $H_2O$, quantified as atmospheric VPD (saturated vapor pressure minus actual atmospheric vapor pressure); $G_s$ generally declines non-linearly as VPD increases (Oren et al 1999; Novick et al. 2016). The rate at which $G_s$ declines per unit increase in VPD is a measure of stomatal sensitivity to VPD. In this way, $G_s$ shows dual sensitivity to both soil moisture and VPD. However, determining how soil moisture and VPD independently control $G_s$ is challenging since low soil moisture and high VPD conditions often co-occur. Moreover, for many species and forest types, the degree to which soil moisture influences $G_s$ sensitivity to VPD is unclear. There is some evidence that VPD, rather than soil moisture, is the dominant limitation of $G_s$ in mesic ecosystems, and stomatal sensitivity to VPD (and $G_s$ at reference VPD = 1 kPa) declines more steeply with decreasing soil moisture at drier sites than wetter sites (Novick et al. 2016). Throughfall reduction experiments in arid climates have also demonstrated that $G_s$ sensitivity to VPD declines steeply as drought conditions intensify (Grossiord et al. 2017). Nonetheless, the dominant limitation of $G_s$ (soil moisture versus VPD), and potential soil moisture thresholds for changes in $G_s$ sensitivity to VPD could differ strongly among forest types and species with different water-use strategies. New studies that manipulate water availability in the field could provide new information about the independent influence of soil moisture and VPD on $G_s$ and thresholds conditions where $G_s$ shifts from VPD dominated to soil moisture dominated.

The southeastern United States (U.S.) produces more wood annually than any other region in the U.S. or country in the world (Wear and Greis 2013). Average air temperatures in the region are expected to increase, coupled with increased VPD, greater evapotranspiration, and more intense and widely spaced precipitation events (IPCC 2013; Melillo et al. 2014; Samuelson et al. 2019). Longleaf pine (*Pinus palustris* Mill.) is considered one of the most drought-resistant southern pines. Despite a broad geographic distribution, the species often occurs on drier sites, including xeric sand hills and montane upland sites where water is limited, and related pine species (e.g., *Pinus taeda* L.) are less common or less productive. Longleaf pine has also demonstrated considerable resistance to drought through physiological and structural modifications that help reduce water use or demand (Gonzalez-Benecke et al. 2010; Starr et al. 2016; Samuelson et al. 2019). As a result, restoration of longleaf pine forests, or converting stands of less drought-resistant species to longleaf pine, is seen as one mechanism for adapting southern forests to drier, hotter conditions. Even so, new experimental studies, especially those conducted over longer time periods in the field, are needed to improve our basic understanding of longleaf responses to drought and VPD.

We carried out a 3-year (2017–2019) throughfall reduction experiment in a young longleaf plantation in west Georgia (USA) to determine the impacts of reduced water availability on longleaf pine leaf and canopy physiology, as well as canopy-scale $G_s$ sensitivity to VPD. This study builds upon the study by Samuelson et al. (2019), who examined the impacts of throughfall reduction on stand-level water use and growth in longleaf pine at the same site. This study, however, is more focused on detailing leaf-scale physiology and whole-tree scale responses to both soil moisture and VPD. We do report updated data for some of the same variables (water potential, sap flux, stand growth) presented in Samuelson et al. (2019), but these data are mainly presented to place the leaf-scale physiology and whole-tree VPD response data within the context of tree water relations and forest productivity. Each year, we repeatedly measured (approximately every 3–4 weeks) predawn and midday leaf water potential ($\Psi_{PD}$ and $\Psi_{MD}$, respectively) and midday leaf gas exchange in ambient throughfall and reduced (−40%) throughfall plots for three years (2017–2019). We also assessed potential changes in foliar nitrogen (N) and foliar $^{13}C$ isotopic composition [$\delta^{13}C$] (time-integrated measure of intrinsic water-use efficiency) with throughfall reduction. We used sap-flux measurements to estimate canopy conductance ($G_s$) and whole-tree hydraulic conductance

(K). We hypothesized that longleaf pine would employ a conservative (more isohydric) strategy and would reduce $g_s$ with reduced throughfall to help maintain relatively constant $\Psi_{MD}$. We expected that $P_{net}$ would also decrease with reduced throughfall, but less so than $g_s$, such that intrinsic water-use efficiency (iWUE, measured both instantaneously and isotopically) would increase under reduced throughfall. We also hypothesized that throughfall reduction would reduce $G_s$, $K$, and $G_s$ sensitivity to VPD. Using $G_s$ data collected across both treatments, we determined soil moisture conditions (quantiles for volumetric water content) where $G_s$ sensitivity to VPD differed. We expected that $G_s$ sensitivity to VPD would be high and relatively similar at high to intermediate soil moisture and would decline only at very low soil moisture.

## Materials and methods

### Study site and experimental design

The study was established in an 11-year-old longleaf pine plantation in the Chattahoochee Fall Line Wildlife Management Area in Marion County, GA (32.5528° N, -84.776° W; site elevation of 210 m) in May 2016 (Samuelson et al. 2019). Trees were planted in early 2005 at an approximate spacing of 2.6 m × 2.6 m (density ~ 1479 trees $ha^{-1}$). Soils at the site are in the Lakeland Series (2–5% slopes), which are Thermic, coated Typic Quartzipsamments, consisting of very deep, permeable, excessively drained sands. Thirty-year mean (1981–2010) annual precipitation for Americus, GA (approximately 60 km from site) is 1245 mm, mean annual minimum and maximum air temperatures are 11.0 °C and 24.6 °C, and mean annual temperature at the site is 17.8 °C (https://www.ncdc.noaa.gov/cdo-web/datatools/normals, accessed February 2021).

The study utilized a randomized complete block design with three blocks (replicates), each containing two plots that were randomly assigned to one of the two throughfall treatments: ambient throughfall ($TR_0$) or an approximate 40% reduction of throughfall ($TR_{40}$). Each treatment plot was 21 m × 31 m (0.07 ha) in dimension with a central 11 m × 21 m (0.02 ha) measurement plot. The size of the treatment plot was determined by excavating and measuring one root from three trees adjacent to the project site. Roots were found to extend an average of 4.5 m from the base of the tree. By extending the treatment 5 m in all directions around the measurement plot, trees in the measurement plot would not receive water from outside of the treatment. Plots within each block were surrounded by two rows of buffer trees. Block selection was based on pre-treatment estimates of stand basal area, which did not differ between paired $TR_0$ and $TR_{40}$ plots (within each block) prior to treatment initiation. Pre-treatment means (± standard error) for basal area, density, DBH, and height were 18.2 ± 0.8 $m^2$ $ha^{-1}$, 1029 ± 33 trees $ha^{-1}$, 14.7 ± 0.3 cm, and 9.5 ± 0.1 m, respectively.

The 40% throughfall reduction was determined based on the 100-year mean annual precipitation for the area. The approximate 60% residual throughfall represented the 1st percentile of annual precipitation (i.e., 1- in 100 drought year) for Americus, Georgia (802 mm). To limit throughfall by approximately 40%, and thereby reduce soil moisture, sixteen 0.52-m throughfall exclusion troughs, constructed out of 12 mil polyethylene sheeting (Polyscrim 12, Americover Inc., Escondido, CA), were installed between rows in each $TR_{40}$ plot. Troughs were allocated in pairs and separated by a 50-cm gap (total of 8 pairs per plot). Troughs were an average height of 1.3 m and were supported by pressure-treated lumber and steel studded t-posts. A total of 16 troughs were installed per plot and covered 40% of the ground area in each plot. Throughfall was intercepted by the troughs and carried at least 20 m from the edge of the plots by large, corrugated pipe.

### Environmental data

A weather station was installed approximately 0.55 km from the site in a 0.65 ha clearing to measure wind-speed, rainfall, air temperature, and relative humidity (6152 Vantage Pro 2 Wireless Weather Station, Davis Instruments, Vernon Hills, Illinois). Relative humidity and air temperature used for VPD determination were measured by three sensors (HOBO U23 Pro v2 Temperature/Relative Humidity Logger, Onset Computer Corporation, Bourne, Massachusetts) under the canopy at approximately 2 m height at three locations between adjacent plots.

### Soil moisture

Volumetric soil moisture (θ, $cm^3$ water $cm^{-3}$ soil) was recorded every minute at 5 cm, 15 cm, 50 cm, and 100 cm depths in all plots ($\theta_5$, $\theta_{15}$, $\theta_{50}$, $\theta_{100}$, respectively) and the average recorded every 30 min with 10-cm length soil moisture sensors (10HS Large Soil Moisture Sensors, Decagon Devices, Meter Group Inc., Pullman, WA, USA). A soil specific calibration was calculated following Starr and Paltineanu (2002). Soil moisture sensors were linked to data loggers (HOBO Micro Station Data Logger, Onset Computer Corp, Bourne, MA, USA). Four sensors were located in the center of each treatment plot and the middle of a row, spaced 60 cm apart. Soil moisture sensors were located under troughs in the throughfall reduction plots. Consequently, the soil moisture sensors in the $TR_{40}$ plots measured the driest soil moisture levels in those plots (under troughs) and did not adequately represent the soil moisture variation between areas under and in between troughs. To better represent the mean volumetric water content in the $TR_{40}$ plots, soil moisture was estimated as the sum of soil moisture under the trough weighted by 40% (relative to plot area covered by troughs) and soil moisture in the companion ambient plot weighted by 60% (representing the soil moisture in the uncovered plot area). Note that we assumed that the soil moisture measured in each companion ambient plot represented soil moisture in the uncovered area in the drought plot (i.e., $TR_{40}$) within the same block. Volumetric water content at permanent wilting point (PWP) and field capacity (FCP) of the soil at the site were determined using data from in situ calibrated soil moisture sensors and soil retention curves (METER Group Inc., Pullman, WA, USA). For soils at our site, PWP is estimated to occur around $\theta = 0.032$ cm$^3$ cm$^{-3}$, while FCP is estimated to occur around $\theta = 0.074$ cm$^3$ cm$^{-3}$. After three years, we examined the overall distribution of θ at 15 cm depth. Following Novick et al. (2016), we grouped soil moisture data into six quantiles: 0–15% (0–0.032 cm$^3$ cm$^{-3}$), 15–30% (0.032–0.043 cm$^3$ cm$^{-3}$), 30 – 50% (0.043–0.052 cm$^3$ cm$^{-3}$), 50–70% (0.052–0.061 cm$^3$ cm$^{-3}$), 70–90% (0.061–0.074 cm$^3$ cm$^{-3}$), 90–100% (0.740–1.10 cm$^3$ cm$^{-3}$). We used these quantiles to determine where canopy stomatal conductance ($G_s$) transitions from being VPD dominated to soil moisture dominated (details on $G_s$ data collection below).

## Leaf physiology

Leaf gas exchange was measured to determine the effects of throughfall reduction on leaf physiology. Measurements were made between 1000 and 1400 h on sunny days and conducted approximately every 3–4 weeks in 2017, 2018, and 2019. Leaf gas exchange was measured using a portable photosynthesis system fitted with a 2 × 3 cm cuvette and a red/blue LED light source (LICOR 6400XT, Licor Inc., Lincoln, NE, USA). Measured variables included net photosynthesis ($P_{net}$, µmol m$^{-2}$ s$^{-1}$), stomatal conductance ($g_s$, mmol m$^{-2}$ s$^{-1}$), and intrinsic water-use efficiency (iWUE, µmol mol$^{-1}$), calculated as the ratio of $P_{net}$ to $g_s$. One scaffolding unit (6 m tall) was installed in each plot to facilitate access to the upper canopy of at least three measurement trees per plot. For each tree and on each measurement date, two fascicles (3 needles per fascicle) from two branches were sampled. The order in which blocks and plots within blocks were sampled was randomized across and within measurement dates. Foliage samples were chosen at random from the upper third of the canopy of each tree, ensuring that foliage was fully exposed to sunlight and from the most recent fully developed flush. Light intensity within the leaf chamber was maintained at 1800 µmol m$^{-2}$ s$^{-1}$ photosynthetic photon flux density (PPFD). The flow rate was held constant at 500 µmol s$^{-1}$, and chamber reference [$CO_2$] was set at 410 µmol mol$^{-1}$. Water vapor inside the leaf chamber was not scrubbed so that RH inside the cuvette approximated ambient conditions. Temperature was not controlled and was allowed to fluctuate with ambient conditions. Gas-exchange measurements were made on detached needles. Previous studies with detached pine needles (Aspinwall et al. 2011; Akalusi et al. 2021) and preliminary tests carried out on trees in our study indicated that leaf gas exchange was not sensitive to detachment if measurements were conducted within 15 min. Leaf gas-exchange data were recorded once rates reached steady state, which generally occurred within 5 min of sealing the needles in the cuvette. Total needle area (all-sided needle area) within the cuvette was calculated from measurement of fascicle diameter and needle length, following Samuelson et al. (2012). After measurements, sampled leaves were dried at 70 °C for 48 h and leaf dry mass per unit area (LMA, g m$^{-2}$) was calculated as the ratio of leaf dry mass to all-sided leaf area.

## Leaf water potential

Predawn and midday measurements of Ψ ($\Psi_{PD}$ and $\Psi_{MD}$, respectively) were made on the same trees as leaf gas-exchange measurements, every 3–4 weeks (measured on same dates as leaf gas exchange), using a pressure chamber (1505D Pressure Chamber Instrument, PMS Instruments, Albany, WA, USA). $\Psi_{PD}$ samples were collected before sunrise when daily xylem water potential is highest (assumed to be near equilibrium with soil water potential) due to minimal nocturnal transpiration. $\Psi_{MD}$ samples were collected between 1100 to 1300 h. Scaffolding was used to collect five fascicles of recently mature, upper canopy foliage for $\Psi_{PD}$ and $\Psi_{MD}$ measurements. Upon collection, samples were placed in sealed plastic bags and stored in a cooler before measuring. Importantly, Samuelson et al., (2019) reported average $\Psi_{PD}$ and $\Psi_{MD}$ values for the same trees in both treatments for 2017 and 2018. We use the same $\Psi_{PD}$ and $\Psi_{MD}$ data from Samuelson et al. (2019), and $\Psi_{PD}$ and $\Psi_{MD}$ values from an additional year (2019), for calculating whole-tree hydraulic conductance (see below).

## Sap flux and whole-tree hydraulic conductance

A sap flow system with 30 mm thermal dissipation probes (TDP-30, Dynamax, Inc., Houston, Texas) was used to determine tree sap flow in 4–5 trees per plot (30 trees total). Trees were selected to represent the basal area distribution in each measurement plot (Čermák et al. 2004), and were also the same trees used for leaf gas exchange and water potential measurements. At the start of the study, DBH of the measurement trees ranged from 14.0 to 19.3 cm. Thermal dissipation probes were used to monitor temperature differences between a heated probe and a reference probe (FLGS-TDP Sap Velocity System Model XM1000, Dynamax, Inc., Houston, Texas). Probes were installed on selected trees at DBH (approximately 1.37 m). The outer bark was removed to install the probes, which were placed in two small holes spaced 9 cm apart vertically in each tree. Reflective insulation was wrapped around the probes and the stem around the probes to minimize temperature gradients. Measurements were taken every minute and 30- minute averages were recorded. To correct instances where the difference in maximum temperature was not attained at night, Baseliner (an open-source software for processing sap flow data) was used as described in Oishi et al. (2016). Sap flux density ($J_v$, mol $m^{-2}$ $s^{-1}$) was calculated according to Granier (1987). For each tree, $J_v$ was divided by the difference between the two water potential measures ($\Psi_{PD}$ and $\Psi_{MD,}$ here denoted as $\Delta\Psi$) to calculate whole-tree hydraulic conductance, K (mol $m^{-2}$ $s^{-1}$ $MPa^{-1}$).

## Canopy conductance

Calculations of $G_s$ (mmol $m^{-2}$ $s^{-1}$) followed Bartkowiak et al. (2015) – inverting the Penman–Monteith equation and assuming sap flow as transpiration. Values of $G_s$ were calculated when VPD $\geq$ 0.75 kPa to reduce possible effects of instrument error (Ewers and Oren 2000). Because VPD was below 0.75 kPa on many days during the winter months, $G_s$ was not calculated between November and February. Importantly, Samuelson et al. (2019) assessed the impacts of throughfall reduction on mean monthly midday $G_s$ in the same trees at the same site between 2016 and 2018. In this study, we focused on: (1) assessing the impacts of throughfall reduction on mean monthly $G_s$, and (2) the impacts of throughfall reduction on mean daytime $G_s$ sensitivity to VPD. We also determined how soil moisture (regardless of throughfall treatment) influenced the sensitivity of mean daytime $G_s$ to VPD. This allows us to determine soil moisture conditions where $G_s$ transitions from being VPD dominated to soil moisture dominated.

## Foliar $\delta^{13}$C, and carbon and nitrogen content

Foliar $\delta^{13}$C (‰), as well as carbon concentration (%C) and nitrogen concentration (%N), were measured on needles collected in Autumn (October/November) of 2017, 2018, and 2019. Values of $\delta^{13}$C provide a time-integrated measure of intrinsic water-use efficiency; higher (less negative) $\delta^{13}$C values indicate higher intrinsic water-use efficiency. Trees sampled were the same as those used for leaf gas exchange and leaf water potential measurements. Five needles from two terminal shoots (one primary and one secondary) of two branches were collected from three different trees in each treatment plot. Oven-dried samples were finely ground and homogenized with a ball mill grinder (Spex 8000, SPEX SamplePrep LCC, Metuchme, NJ, USA). $\delta^{13}$C, and %C and %N were determined using a continuous flow mass spectrometer (Thermo Finnigan Plus XL, Thermo Fisher Scientific, Waltham, MA, USA). Leaf N per unit area ($N_{area}$, g N $m^{-2}$) was also calculated as the product of %N content and LMA (LMA measured on the same trees and on the same date as %N). $\delta^{13}$C, %C, %N, and $N_{area}$ data were pooled and averaged by plot.

## Growth

Biometric data (basal area, height, DBH, stem volume) and aboveground biomass production (ANPP) data collected between 2016 and 2018 were previously presented in Samuelson et al. (2019). Using the same methods, we collected an additional year (2019) of biometric and aboveground productivity data. Shoot phenology and leaf area index (LAI) data were also collected between 2017 and 2019 and are reported in Mendonca et al. (2022).

## Data analysis

All statistical analyses were performed in SAS v9.3 (SAS Institute Inc. 2010, Cary, NC USA). Because 'plot' is the experimental unit, in most cases, we averaged data collected across measurement trees in each plot. A linear mixed model (PROC MIXED) was used to test the fixed effects of measurement date (i.e., time), treatment ($TR_0$ versus $TR_{40}$), and date × treatment interactions on the leaf- and canopy-scale physiological variables (e.g., $P_{net}$, K, mean monthly $G_s$). Block was considered a random effect. A linear mixed model was also used to test for year and treatment effects on $\delta^{13}$C, %C, and %N. In both models, block was considered a random effect. When appropriate, data were log-transformed to fulfill assumptions of normality.

We examined $G_s$ sensitivity to VPD and the influence of soil moisture in two ways. First, we determined whether the overall relationship between mean daytime $G_s$ and VPD differed between throughfall reduction treatments. Second, we determined whether the overall relationship between mean daytime $G_s$ and VPD differed between soil moisture quantiles ranging from above FCP to below the permanent PWP. In this case, data in the lowest soil moisture quantile frequently occurred in the throughfall reduction treatment, but this was not always the case, especially during periods when rainfall was high, and temperatures were low. Following Oren et al. (1999) the response of daytime $G_s$ to VPD was fit using the linear function: $G_s = bref + -m \cdot ln(\text{VPD})$, where $m$ quantifies the sensitivity of $G_s$ to increasing VPD (mmol $m^{-2}$ $s^{-1}$ $\ln(\text{kPa})^{-1}$), and $bref$ is the reference $G_s$ at VPD = 1 kPa. Analysis of covariance (ANCOVA) was used to test whether $m$ or $bref$ differed between throughfall treatments. In this analysis, VPD was a covariate and 'treatment' ($TR_0$, $TR_{40}$) was a factor. If treatment influenced the relationship between $G_s$ and VPD, a significant interaction between treatment and VPD was observed, and different *m* estimates were fit for each treatment. If treatment and VPD were both significant, but the interaction between treatment and VPD were not, equations with different *bref* estimates for each treatment, but a common *m* estimate, were fit to the data. If only the covariate was significant, one equation with a common *bref* and *m* estimate was fit to data from both treatments. The same approach (ANCOVA) was used to test whether *m* and *bref* differed among soil moisture quantiles.

## Results

### Environmental conditions

Annual precipitation at the site was 1234 mm in 2017, 1451 mm in 2018, and 1232 mm in 2019 (Fig. 1A). Annual precipitation in 2017 and 2019 was slightly lower than the long-term (30-year) mean annual precipitation for the location (1245 mm). Total precipitation in 2018 was approximately 17% higher than the long-term mean for the location. Under 40% throughfall reduction, total precipitation was estimated to be 740 mm in 2017, 871 mm in 2018, and 739 mm in 2019.

Mean daily average, maximum, and minimum temperatures were similar among years and averaged 18.3 °C, 24.9 °C, and 12.8 °C, respectively (Fig. 1B). However, daily maximum temperatures in 2017 and 2019 were sometimes above the average daily maximum temperature for the study area. Daily maximum vapor pressure deficit ($\mathrm{VPD}_{\max}$) was similar among years and ranged from 0.03 to 4.65 kPa (Fig. 1A).

### Soil moisture

In both treatments, θ was frequently lower at deeper positions within the soil profile (Fig. 2A–D). Daily mean θ in the throughfall reduction treatment was often lower than in the ambient treatment (Fig. 2A–D). As soil depth increased, the difference in θ between $\mathrm{TR}_{40}$ and $\mathrm{TR}_0$ treatments generally decreased (Fig. 2A–D). Over time, θ at 5, 15, 50, and 100 cm depths were on average 47, 41, 32, and 34% lower, respectively, in the $\mathrm{TR}_{40}$ plots compared with the $\mathrm{TR}_0$ plots (Fig. 2A-D). Among years, monthly mean θ at 5 cm depth varied between 0.073 and 0.088 $\mathrm{cm}^3\ \mathrm{cm}^{-3}$ in the $\mathrm{TR}_0$ treatment and between 0.041 and 0.046 $\mathrm{cm}^3\ \mathrm{cm}^{-3}$ in the $\mathrm{TR}_{40}$

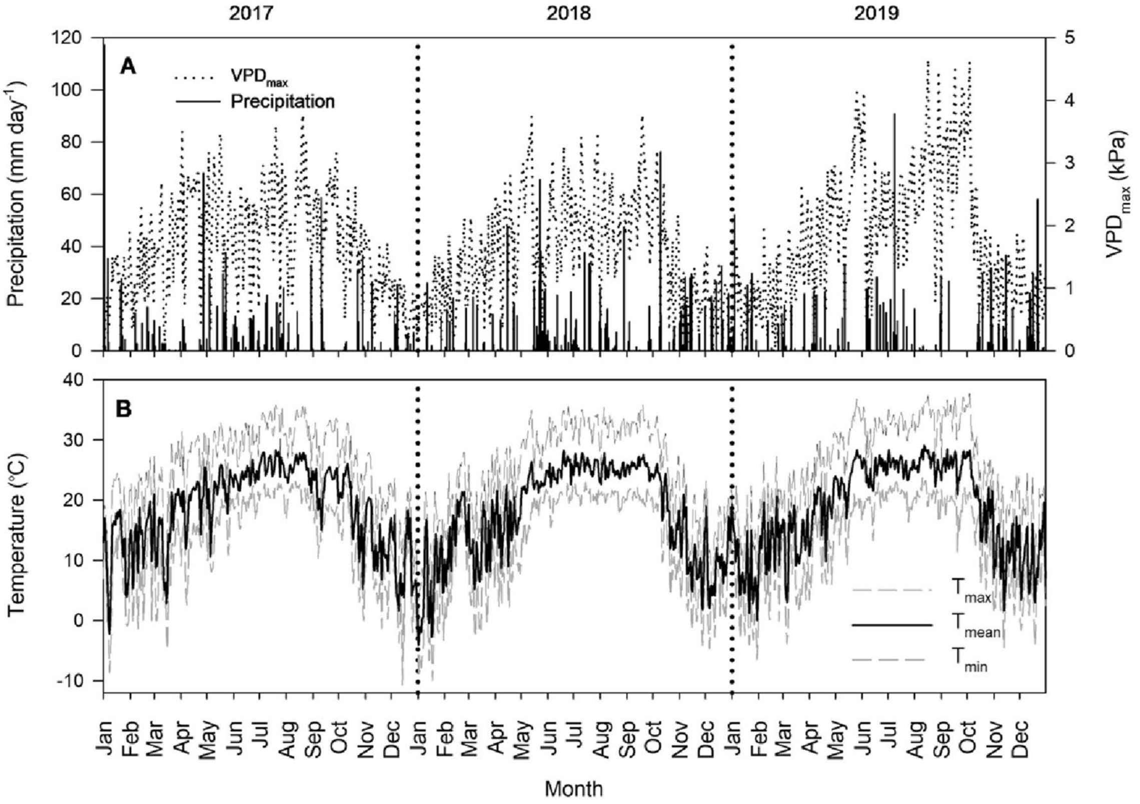

**Fig. 1** **A** Daily rainfall (bars) and daily maximum vapor pressure ($\mathrm{VPD}_{\max}$, dotted line), and **B** daily maximum, mean, and minimum temperature ($\mathrm{T}_{\max}$, $\mathrm{T}_{\mathrm{mean}}$, and $\mathrm{T}_{\min}$, respectively) in a longleaf pine plantation in Marion County, Georgia, USA

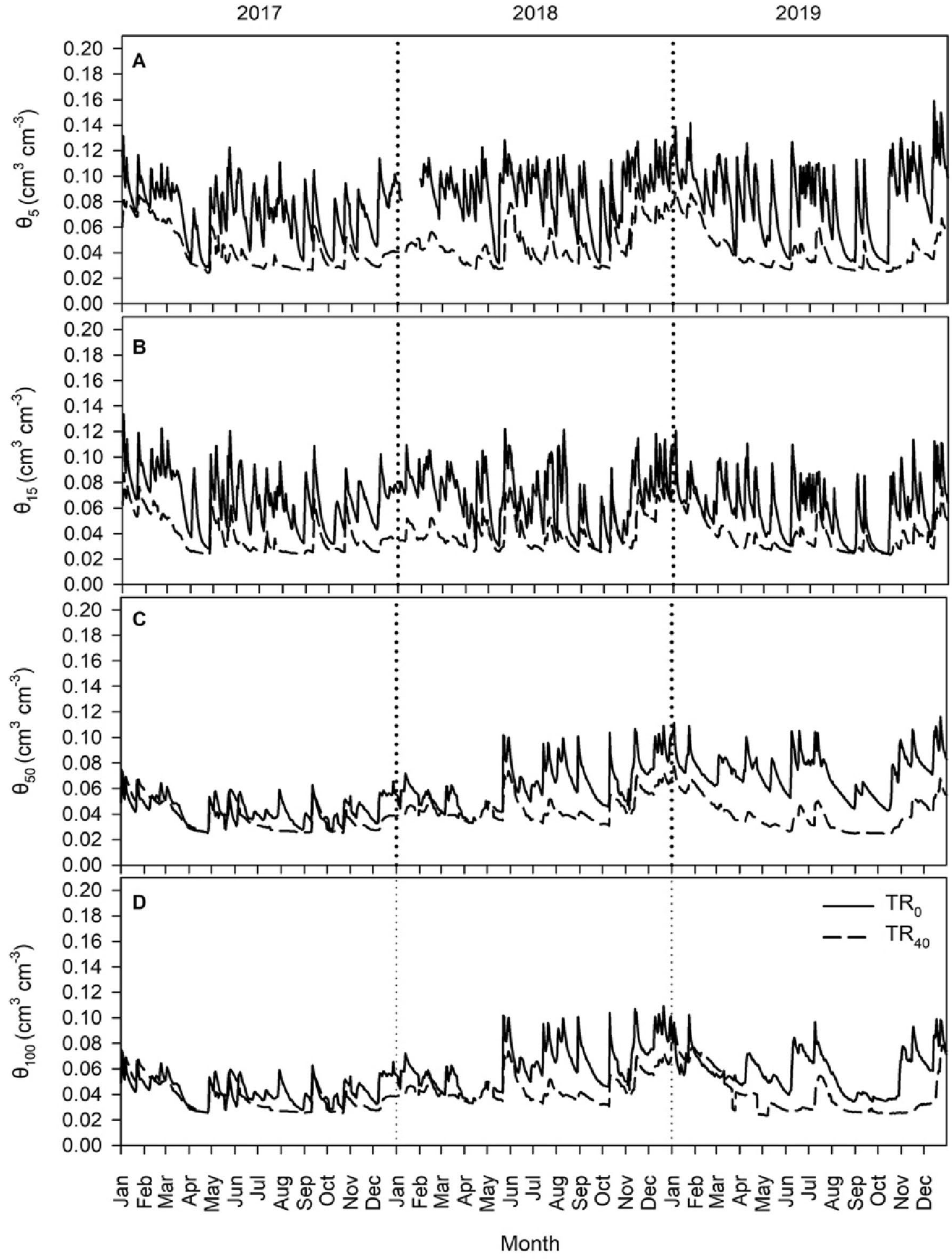

**Fig. 2** Mean daily soil volumetric water content at 5 cm ($\theta_5$) [**A**], 15 cm ($\theta_{15}$) [**B**], 50 cm ($\theta_{50}$) [**C**], and 100 cm ($\theta_{100}$) [**D**] depth in a longleaf pine plantation under an experimental 40% throughfall reduction treatment (TR$_{40}$) and ambient throughfall treatment (TR$_0$). Soil volumetric water content in the TR$_{40}$ was measured under throughfall exclusion trays

treatment. At 50 cm depth monthly mean θ varied between 0.043 and 0.065 $cm^3\ cm^{-3}$ in the $TR_0$ treatment and between 0.038 and 0.045 $cm^3\ cm^{-3}$ in the $TR_{40}$ treatment.

## Leaf-level gas-exchange responses

Averaged over time, throughfall reduction reduced $g_s$ by 21%, from 49.8 to 39.5 mmol $m^{-2}\ s^{-1}$ ($p = 0.047$; Table 1; Fig. 3B). Throughfall reduction caused a small reduction in $P_{net}$ (−13%); this effect was somewhat weak ($p = 0.063$, Table 1; Fig. 3a). In general, both treatments showed reduced $g_s$ and $P_{net}$ during drier periods (Fig. 3a–b). For instance, during a 20-day period between May and June of 2019 θ was as low as 4%, and values of $g_s$ and $P_{net}$ were much lower compared to wet periods (Fig. 3a–b).

In general, iWUE increased during dry periods when $g_s$ was low and decreased during wet periods when $g_s$ was high. However, averaged over time, iWUE was similar between treatments ($p = 0.223$; Table 1; Fig. 3c). Foliar $\delta^{13}C$ (time-integrated estimate of water-use efficiency) differed among years (Table 1) and was −30.9 ± 0.3, −29.3 ± 0.3, and −27.9 ± 0.3 ‰ in 2017, 2018, and 2019, respectively. $\delta^{13}C$ did not differ between treatments ($p = 0.291$; Table 1).

Foliar %C was similar across years but %N and $N_{area}$ varied among measurement years (Table 1). %N averaged 1.3 ± 0.04, 1.1 ± 0.04, and 0.9 ± 0.04% in 2017, 2018, and 2019, respectively. $N_{area}$ averaged 1.07 ± 0.05, 0.87 ± 0.05, and 0.84 ± 0.05 g N $m^{-2}$ in 2017, 2018, and 2019, respectively. Although LMA varied among measurement dates (Table 1, Fig. 3d), and %C, %N, and $N_{area}$ varied among years, all four traits and were similar between treatments ($p > 0.436$; Table 1). No date × treatments interactions were detected for any trait ($p > 0.473$; Table 1).

## Leaf water potential

Predawn leaf water potential ($\Psi_{PD}$) showed a weak interaction between date × treatment ($p = 0.095$; Table 1, Fig. 4). Post hoc analysis indicated that throughfall reduction decreased $\Psi_{PD}$ at only three dates during the study period: June of 2017 ($p = 0.029$), February of 2018 ($p = 0.024$), and September of 2018 ($p < 0.001$) (Fig. 4). $\Psi_{MD}$ varied across sampling dates but did not differ between treatments ($p = 0.174$; Table 1, Fig. 4). Over time and across treatments, average $\Psi_{PD}$ and $\Psi_{MD}$ was -0.87 ± 0.02 MPa and −1.71 ± 0.03 MPa, respectively (Table 1, Fig. 4). In both treatments, $\Psi_{PD}$ and $\Psi_{MD}$ were lower during periods of low soil moisture. The difference between $\Psi_{PD}$ and $\Psi_{MD}$ measurements ($\Psi_{PD}$–$\Psi_{MD,}$ here referred as ΔΨ) was also analyzed. Overall, throughfall reduction had no effect on

**Table 1** Analysis of variance results for the effects of measurement date, throughfall treatment, and their interaction on leaf- and canopy-scale physiological traits in longleaf pine trees growing in a plantation in Georgia, United States

| Variable | Date | | Treatment | | Date × Treatment | | $TR_0$ | $TR_{40}$ |
|---|---|---|---|---|---|---|---|---|
| | *df* | *P* > *F* | *df* | *P* > *F* | *df* | *P* > *F* | | |
| $P_{net}$ (μmol $m^{-2}\ s^{-1}$) | 26,103 | **< 0.001** | 1,4 | 0.063 | 26,103 | 0.677 | 3.97 (± 0.1) | 3.45 (± 0.1) |
| $g_s$ (mmol $m^{-2}\ s^{-1}$) | 26,103 | **< 0.001** | 1,4 | **0.047** | 26,103 | 0.373 | 49.9 (± 2.6) | 39.5 (± 2.6) |
| iWUE (μmol $mol^{-1}$) | 26,103 | **< 0.001** | 1,4 | 0.223 | 26,103 | 0.486 | 92.0 (± 4.1) | 100.4 (± 4.1) |
| $\Psi_{PD}$ (MPa) | 26,103 | **< 0.001** | 1,4 | 0.157 | 26,103 | 0.095 | −0.84 (± 0.02) | −0.89 (± 0.02) |
| $\Psi_{MD}$ (MPa) | 26,103 | **< 0.001** | 1,4 | 0.174 | 26,103 | 0.983 | −1.67 (± 0.03) | −1.74 (± 0.03) |
| ΔΨ (MPa) | 26,103 | **< 0.001** | 1,4 | 0.600 | 26,103 | 0.741 | 0.82 (± 0.28) | 0.85 (± 0.28) |
| K (mol $m^{-2}\ s^{-1}\ MPa^{-1}$) | 23,90 | **< 0.001** | 1,4 | 0.235 | 23,90 | 0.076 | 2.18 (± 0.15) | 1.88 (± 0.15) |
| $G_s$ (mmol $m^{-2}\ s^{-1}$) | 24,93 | **< 0.001** | 1,4 | **0.021** | 24,93 | **0.031** | 69.9 (± 3.0) | 56.3 (± 3.0) |
| LMA (g $m^{-2}$) | 26,103 | **< 0.001** | 1,4 | 0.433 | 26,103 | 0.156 | 87.3 (± 1.9) | 85.0 (± 1.9) |
| $\delta^{13}C$ (‰) | 2,8 | **< 0.001** | 1,4 | 0.291 | 2,8 | 0.521 | −29.57 (± 0.23) | −29.19 (± 0.23) |
| %C (%) | 2,8 | 0.454 | 1,4 | 0.749 | 2,8 | 0.473 | 53.9 (± 1.0) | 54.4 (± 1.0) |
| $N_{area}$ (g N $m^{-2}$) | 2,8 | **0.002** | 1,4 | 0.962 | 2,8 | 0.491 | 0.93 (± 0.05) | 0.92 (± 0.05) |
| %N (%) | 2,8 | **< 0.001** | 1,4 | 0.436 | 2,8 | 0.869 | 1.10 (± 0.05) | 1.17 (± 0.05) |

Numerator and denominator degrees of freedom (*df*) and probability values (*p*-values) are provided for each factor. Mean and standard error (in parenthesis) values for leaf gas exchange, leaf water potential, whole-tree water flux traits, and leaf composition traits are shown for the ambient throughfall ($TR_0$) and reduced throughfall ($TR_{40}$) treatments

Parameter descriptions: $P_{net}$, net photosynthesis; $g_s$, leaf-level stomatal conductance; iWUE, intrinsic water-use efficiency; $\Psi_{PD}$, predawn leaf water potential; $\Psi_{MD}$ midday leaf water potentials; ΔΨ the difference between predawn and midday leaf water potentials; K, whole-tree hydraulic conductance; $G_{Smax}$, maximum canopy-level stomatal conductance; LMA, leaf specific mass per unit area; $\delta^{13}C$, foliar $^{13}C$ isotopic composition; %C, leaf carbon content; $N_{area}$, leaf nitrogen per unit area; and %N leaf nitrogen content. Average $\Psi_{PD}$ and $\Psi_{MD}$ used in this analysis were partially (2017–2018) provided by Samuelson et al., (2019)

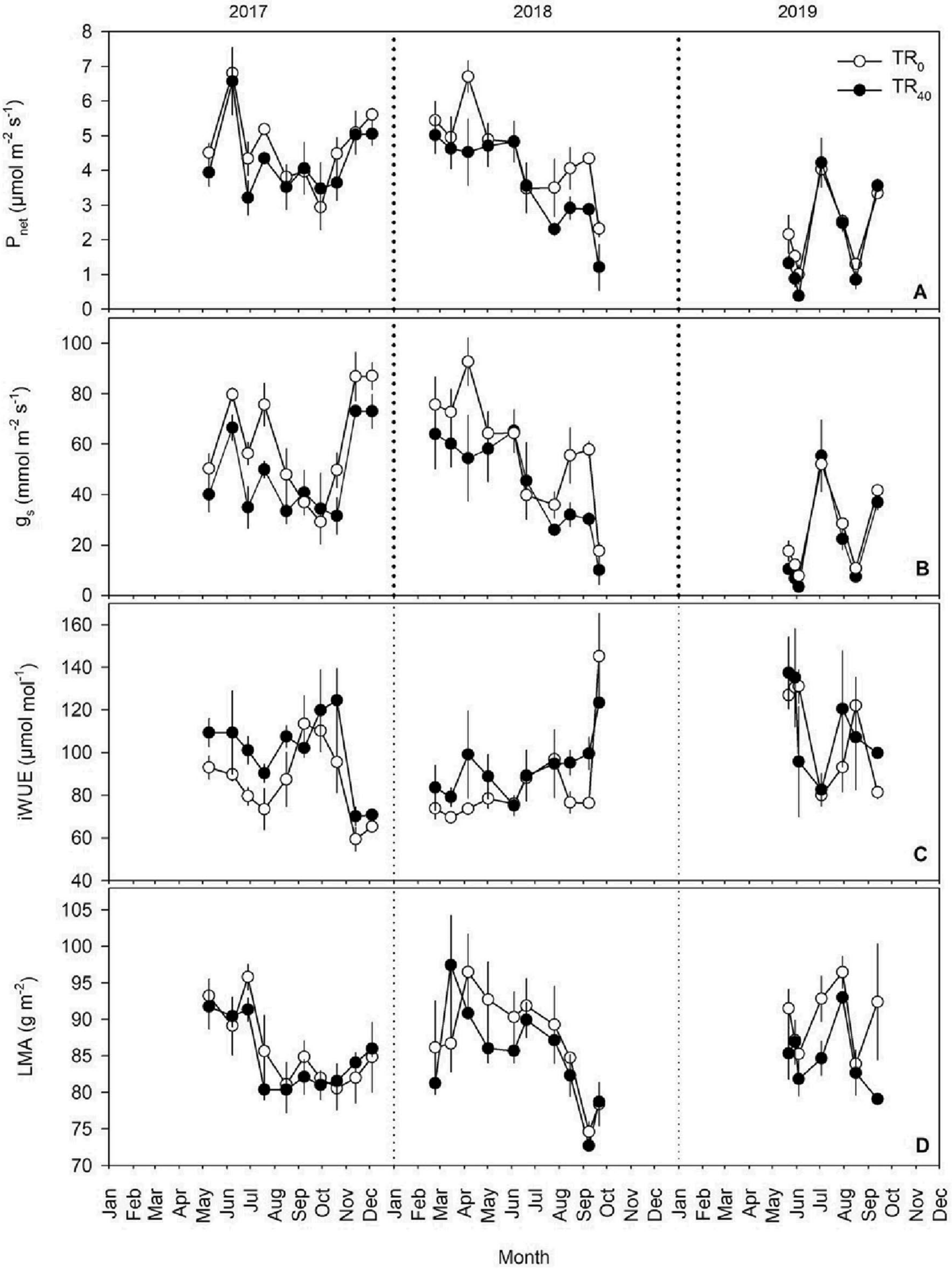

**Fig. 3** Mean (± standard error) values of net photosynthesis ($P_{net}$) [**A**], stomatal conductance ($g_s$) [**B**], intrinsic water-use efficiency (iWUE) [**C**], and leaf dry mass per unit area (LMA) [**D**] in ambient throughfall ($TR_0$) and 40% throughfall reduction ($TR_{40}$) treatments in a longleaf pine plantation

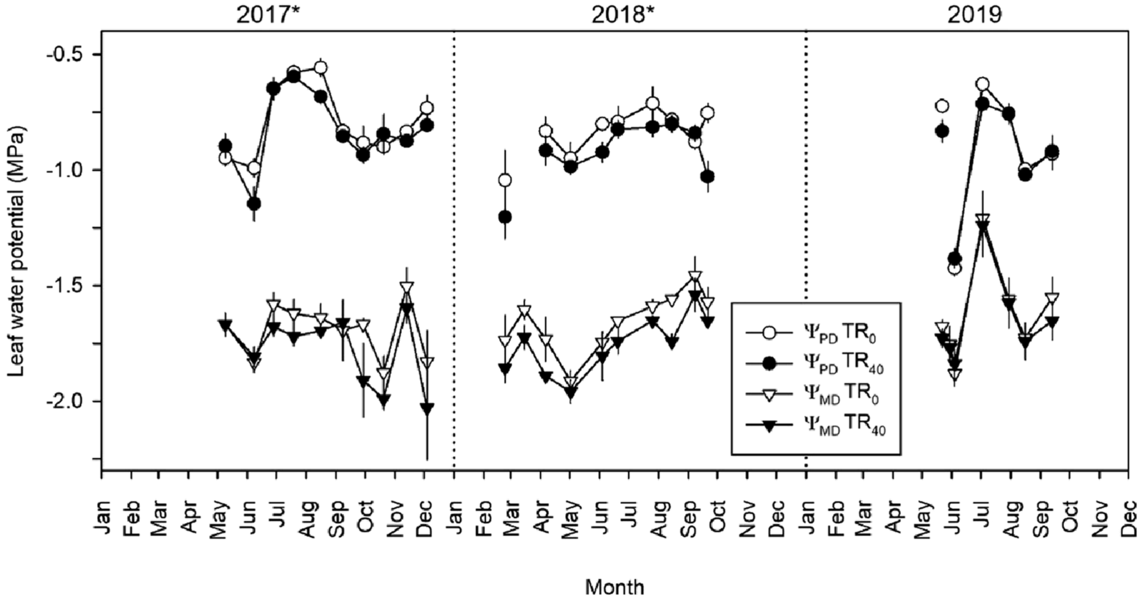

**Fig. 4** Mean (± standard error) values for predawn leaf water potential ($\Psi_{PD}$) and midday leaf water potential ($\Psi_{MD}$) in ambient throughfall ($TR_0$) and 40% throughfall reduction ($TR_{40}$) treatments in a longleaf pine plantation. *Data from 2017 and 2018 were previously reported in Samuelson et al. (2019)

$\Delta\Psi$ ($\Delta\Psi$ across treatments was $0.84 \pm 0.28$MPa; $p = 0.600$; Table 1). No date × treatments interactions were detected for $\Psi_{MD}$ and $\Delta\Psi$ ($p > 0.741$; Table 1).

## Whole-tree hydraulic conductance and canopy conductance

Whole-tree hydraulic conductance (K) showed a weak date × treatment interaction ($p = 0.076$, Table 1, Fig. 5A). Throughfall reduction decreased K at only two time points during the study period: June of 2018 ($p = 0.003$) and September of 2018 ($p = 0.002$) (Fig. 5A). Across both treatments, K decreases as θ decreases (Supplementary Material, Figure S1).

We observed a significant date × treatment interactions on for monthly $G_s$ (Table 1; Fig. 5B). The $TR_{40}$ treatment decreased mean monthly $G_s$ in twelve months between 2017 and 2019: July ($p = 0.002$), August ($p = 0.003$), and September ($p = 0.013$) in 2017; March ($p = 0.032$), April ($p < 0.001$), May ($p = 0.002$), August ($p < 0.001$), and September ($p = 0.014$) in 2018; and April ($p = 0.022$), May ($p = 0.035$), June ($p = 0.039$), and October ($p = 0.014$) in 2019. Decreases in mean monthly $G_s$ in response to the $TR_{40}$ treatment ranged from −15 to −45%; larger reductions in mean monthly daytime $G_s$ generally occurred after warm days with recent low levels of precipitation.

In both treatments, daytime $G_s$ declined as VPD increased. Although daytime $G_s$ declined with increasing VPD in both treatments, reduced water availability caused by throughfall reduction reduced daytime $G_s$ at VPD = 1 (*bref*) ($p < 0.001$; Fig. 6) and stomatal sensitivity (*m*) to increasing VPD ($p = 0.029$; Fig. 6) throughout the study period.

The relationship between daytime $G_s$ and VPD varied among soil moisture category (regardless of treatment). Across all categories, *bref* and *m* were highest in the highest soil moisture category (90–100% percentile of soil moisture) indicating that $G_s$ was highest and strongly influenced by VPD when soil moisture was at or above field capacity (Table 2; Fig. 7). Parameters *bref* and *m* were similar among the four intermediate soil moisture categories (15–30%, 30–50%, 50–70%, 70–90% percentiles of soil moisture), indicating that $G_s$ was high and very sensitive to VPD when soil moisture was within the range of plant available water, although less so than when soil moisture was at or above field capacity (Table 2; Fig. 7). Importantly, *bref* and *m* were lowest in the lowest soil moisture category (0–15% percentile), indicating that $G_s$ was very low and largely insensitive to VPD when soil moisture was near or below the permanent wilting point (Table 2; Fig. 7).

## Tree growth and forest productivity

Samuelson et al. (2019) reported growth and productivity data collected between 2016 and 2018. We updated the analysis with an additional year of data (2019). Averaged over time, stand volume was 21% lower under throughfall reduction than ambient throughfall ($p = 0.035$; Supplementary Material, Table S1). We also found a weak interaction

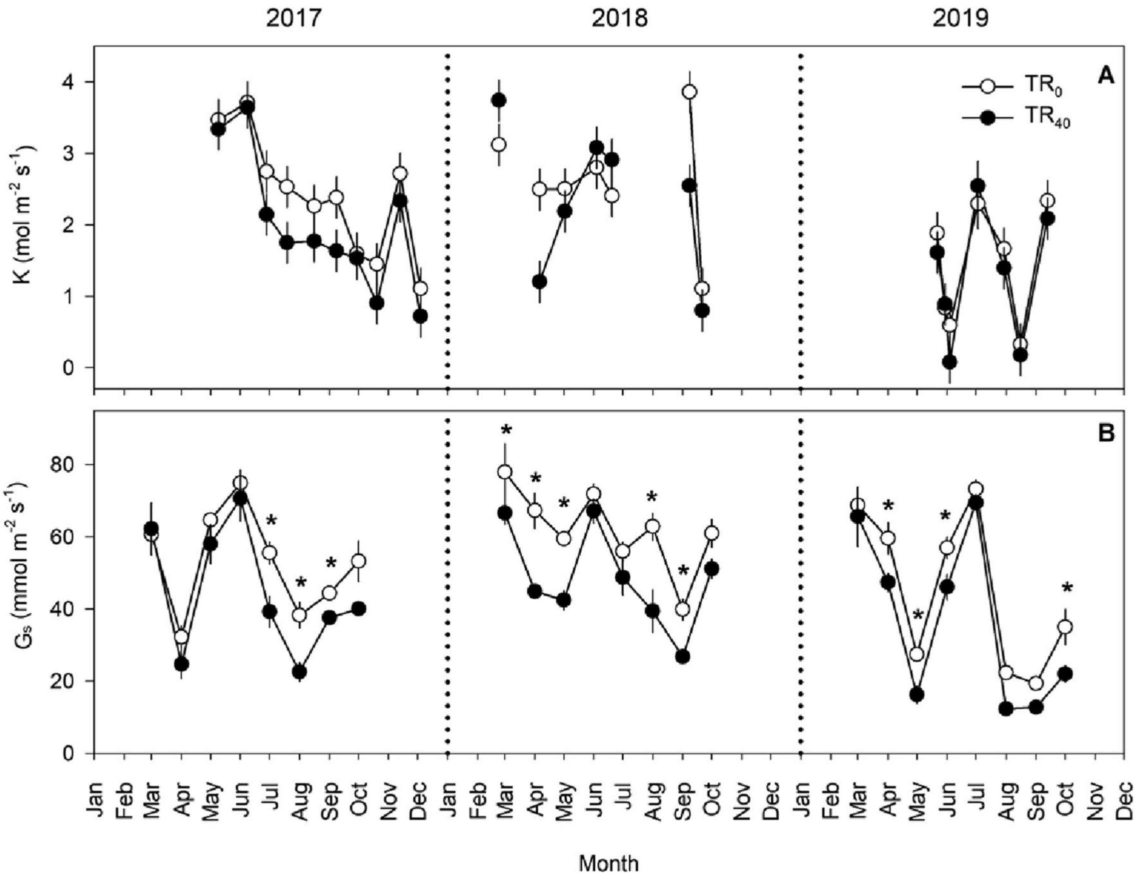

**Fig. 5** Mean (± standard error) values for mean monthly daytime canopy-level stomatal conductance ($G_S$) [**A**] and whole-tree hydraulic conductance (K) across the same dates as the leaf water potential measurements [**B**] in ambient throughfall (TR$_0$) and 40% throughfall reduction (TR$_{40}$) treatments in a longleaf pine plantation

**Fig. 6** The responses of mean daily daytime canopy-level stomatal conductance ($G_S$) to vapor pressure deficit (VPD) in longleaf pine trees growing under ambient throughfall (TR$_0$) and 40% throughfall reduction (TR$_{40}$) treatments

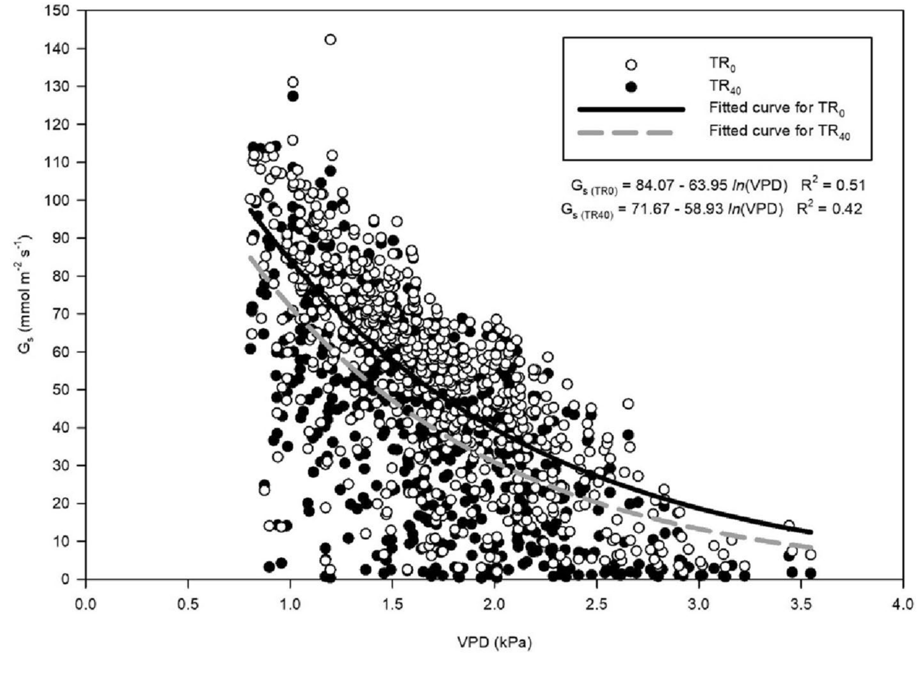

**Table 2** Probability values (*p*-values) for comparisons of canopy conductance ($G_s$) sensitivity to vapor pressure deficit (VPD) across different soil moisture categories

| Soil moisture category (%) | 0–15 | 15–30 | 30–50 | 50–70 | 70–90 | 90–100 |
|---|---|---|---|---|---|---|
| 0–15 | | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** |
| 15–30 | **< 0.001** | | 0.591 | 0.845 | 0.664 | **0.011** |
| 30–50 | **< 0.001** | 0.591 | | 0.522 | 0.406 | **0.019** |
| 50–70 | **< 0.001** | 0.845 | 0.522 | | 0.818 | **0.011** |
| 70–90 | **< 0.001** | 0.664 | 0.406 | 0.818 | | **0.009** |
| 90–100 | **< 0.001** | **0.011** | **0.019** | **0.011** | **0.009** | |

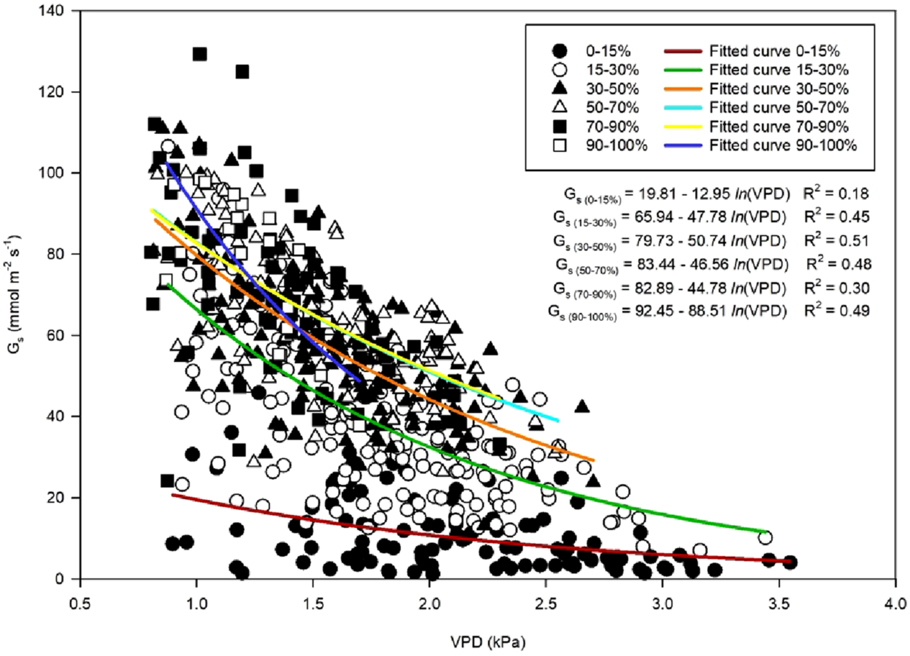

**Fig. 7** The responses of mean day daylight canopy-level stomatal conductance ($G_S$) to vapor pressure deficit (VPD) in longleaf pine trees at different levels of soil moisture categories

between date × treatment for DBH ($p = 0.066$; Supplementary Material, Table S1). However, post-hoc analysis indicated no differences in DBH between treatments in any individual year throughout the experiment. No other treatment effects or year × treatment effects were observed for tree height, basal area, mortality, aboveground primary production, or peak LAI ($p > 0.107$; Supplementary Material, Table S1).

## Discussion

We determined the impacts of reduced water availability on longleaf pine leaf and canopy physiology, as well as canopy-scale $G_s$ sensitivity to VPD. Over the study period, 40% throughfall reduction decreased soil moisture and caused small reductions in $g_s$ and $P_{\text{net}}$ but did not change instantaneous or isotopically determined leaf-scale water-use efficiency. Throughfall reduction also caused small reductions in K, mean monthly $G_s$, mean daytime $G_s$ at reference VPD = 1 kPa (*bref*), and $G_s$ sensitivity to VPD (*m*). Across treatments, *bref* and *m* showed separation among soil moisture categories; both parameters where highest under the highest soil moisture conditions, intermediate under soil moisture conditions representing the range of plant available water, and lowest when soil moisture was near or below the permanent wilting point. These results provide general support for our hypotheses about longleaf pine responses to throughfall reduction and soil moisture and VPD controls of $G_s$. Importantly, small effects of throughfall reduction on leaf and canopy-scale physiology were apparently not strong enough to significantly alter tree growth or forest productivity. We conclude that, even on sandy, well-drained sites, established longleaf pine trees may be relatively resistant to reduced rainfall, although $G_s$ and its sensitivity to VPD declines as soil moisture declines.

## Leaf-scale responses to throughfall reduction

Throughfall reduction resulted in lower soil moisture and a small decrease in $g_s$. Reduced $g_s$ was expected and is a typical response to soil water deficit, particularly in tree species that are generally considered to be more isohydric (Fuchs and Livingston 1996; Hubbard et al. 2001; Domec et al. 2009; Clark et al. 2012). Reductions in $g_s$ were coupled with small reductions in $P_{net}$. We hypothesize that stomatal rather than biochemical limitations were responsible for the small reductions in $P_{net}$ under throughfall reduction. We found that leaf *N*, on both a percent (mass) and leaf area basis, was not affected by throughfall reduction. Foliar N serves as a measure of the total enzyme (photosynthetic, respiratory) content of the foliage and often scales positively with the maximum capacity for Rubisco carboxylation (i.e., $V_{cmax}$, Atkin et al. 2015, Diaz-Espejo et al. 2007, Medlyn et al. 2002). If throughfall reduction did alter photosynthetic biochemistry, we might expect differences in leaf N between treatments (Lal et al. 1996; Pelloux et al. 2001; Parry et al. 2002). Droughts that are modest or short in duration typically have little effect on mesophyll conductance or photosynthetic biochemistry (Flexas and Medrano 2002; Flexas et al. 2004; Diaz-Espejo et al. 2007; Drake et al. 2016), and reduced $g_s$ is considered the primary limitation to net $CO_2$ uptake and plant production. Taken together, it is likely that stomatal limitation was the primary limitation of $P_{net}$ under 40% throughfall reduction, although photosynthetic $CO_2$-response measurements would help determine whether photosynthetic capacity was affected.iWUE was not increased under the throughfall reduction treatment as we expected. Likewise, the 40% throughfall reduction and subsequent reductions in $g_s$ and $P_{net}$ were not strong enough to change foliar $\delta^{13}C$, suggesting no increase in time-integrated foliar iWUE. Under marked reductions in $g_s$, increased foliar $\delta^{13}C$ is usually expected for trees under water stress, indicating higher iWUE (Helle and Schleser 2004; Shestakova et al. 2017; Castillo et al. 2018). These results reinforce our conclusion that throughfall reduction had relatively small effects on longleaf pine leaf physiology.

Pine species in the southern U.S. differ in their response to soil drying. In loblolly pine, Domec et al. (2009) showed that $\Psi_{PD}$ declines steadily in loblolly pine when relative extractable water (REW) drops below 40–50%. In longleaf pine, Samuelson et al. (2019) found that $\Psi_{PD}$ declines at a lower REW (~20%), indicating greater drought tolerance. During our study, there were many instances when REW dropped below 20% (volumetric water content ≤ ~0.04 $cm^3\ cm^{-3}$), but these declines were likely too brief to cause severe reductions in $\Psi_{PD}$ and leaf physiology. Samuelson et al. (2019) also found that $\Psi_{MD}$ at stomatal closure ($\Psi_{close}$) and the turgor loss point ($\Psi_{tlp}$), both indicators of drought resistance (Bartlett et al. 2012; Martin-StPaul et al. 2017), were as low as −2.9 and −3.0 MPa, respectively, in longleaf pine. These threshold $\Psi_{MD}$ values were never surpassed in our study, which probably explains the small effect of throughfall reduction on $g_s$. Similar to previous studies, substantial variation in K was observed over time in both treatments in response to changes in soil moisture (Oren et al. 2001; Addington et al. 2004). Yet, overall, throughfall reduction had little effect on K, suggesting that the 40% throughfall reduction treatment was not strong enough to severely impact tree hydraulic integrity during the study period.

We found that 40% throughfall reduction reduced stand volume (averaged over time), but otherwise had little effect on individual tree diameter, height, forest productivity, or tree mortality. This result is similar to that of Samuelson et al. (2019). In a previous study, we also found no effect of throughfall reduction on needle or shoot growth patterns or leaf area index (Mendonca et al. 2022). During our study, precipitation was similar among years and relatively frequent within years. The maximum period observed with no rain was 20 days, and the average interval between rain events was five days. Samuelson et al. (2019) found exceptionally low $\Psi_{PD}$ and $\Psi_{MD}$ values in the same longleaf pine trees (average lows of −2.9 and −3.6 MPa for $\Psi_{PD}$ and $\Psi_{MD}$, respectively) during an extreme natural drought event in late summer to early autumn 2016 which lasted 77 days with very little to no rain. During this natural drought, trees in both treatments were equally stressed and ceased transpiration at the canopy-scale. We conclude that reductions in total annual precipitation are unlikely to have strong negative impacts on established longleaf pine forests, even on xeric sites, unless reductions in average rainfall coincide with long dry spells (Phillips et al. 2016; Engelbrecht et al. 2017). Established longleaf pine may be resistant to dry surface soils, in part due to deep rooting. Using methods developed by Samuelson et al. (2016), we estimate that tap root depth was 3.0–3.2 m. Access to deeper soil moisture may have reduced throughfall reduction effects on tree function.

## Canopy-scale responses to soil moisture and VPD

Our canopy-scale responses to soil moisture and VPD generally supported our hypotheses. Throughfall reduction had small (but significant) effects on mean monthly $G_s$ and daytime $G_s$ at VPD = 1 kPa (*bref*), although mean monthly $G_s$ responded inconsistently to throughfall reduction over time. The reduction in *bref* coincided with reduced daytime $G_s$ sensitivity to VPD (*m*), which aligns with theory and previous observations in other species, including longleaf pine (Oren et al. 1999; Gonzalez-Benecke et al. 2011; Bartkowiak et al. 2015; Addington et al. 2006, 2004; Samuelson et al. 2019; Samuelson and Whitaker 2012). Our results indicated that trees growing under throughfall reduction were less sensitive to increasing VPD than trees growing under ambient, higher rainfall conditions. Throughfall reduction experiments with drought-resistant species growing in arid climates have also shown reduced $G_s$ and sensitivity to VPD (Grossiord et al. 2017). These results highlight the potential importance of site conditions and drought severity when considering tree responses to increasing VPD.

It is well known that soil moisture and VPD both influence $G_s$, but the independent influence of each factor has been difficult to assess. To address this, Novick et al. (2016) examined $G_s$ responses to VPD at different sites and different soil moisture percentiles within sites. They found that VPD dominates $G_s$ in mesic ecosystems, and $G_s$ shows larger reductions in sensitivity to VPD with declining soil moisture at dry sites than wet sites. We used the same approach with longleaf pine growing on a xeric site in a mesic region. We found that $G_s$ was highest and very sensitive to VPD when soil water was not limiting (i.e., when θ was at or above field capacity). Compared to very wet conditions, $G_s$ sensitivity to VPD dropped by 50% and was uniform at soil moisture levels within the range of plant available water. Therefore, $G_s$ was still strongly influenced by VPD, but soil moisture likely exerted some control over $G_s$. Interestingly, under very dry conditions when soil moisture was at or near the permanent wilting point, $G_s$ sensitivity to VPD declined by 73% relative to conditions when soil moisture was within the range of plant available water. This result indicates that soil moisture conditions near the permanent wilting point represent a major breakpoint in relative control of soil moisture and VPD on $G_s$. If soil moisture at the permanent wilting point is a good general predictor of $G_s$ transitioning from VPD dominated to soil moisture dominated, it could simplify our understanding and predictions of $G_s$ responses to VPD and soil moisture.

## Conclusion

Here, we studied how three years of reduced rainfall (throughfall reduction) impacted leaf- and canopy-scale physiology of established longleaf pine trees. We found that a 40% reduction in rainfall resulted in small reductions in leaf- and canopy-scale function, and these changes did not significantly impact growth. Previous findings from the same experiment showed that stress conditions only occur during prolonged dry spells with little or no rainfall. Therefore, changes in rainfall frequency or dry spell duration, especially during warmer seasons, could potentially have larger impacts on tree function than changes in total annual rainfall. Importantly, we identified soil moisture conditions associated with changes in $G_s$ responses to VPD. At soil moistures within and above the range for plant available water, $G_s$ is dominated by VPD. When soil moisture approaches to permanent wilting point, VPD no longer exerts a strong control over $G_s$, suggesting that this soil moisture is a breakpoint in the relative control of VPD and soil moisture over $G_s$. These results provide new information about the response of established longleaf pine trees to reduced soil moisture and general controls of $G_s$ which could inform both forest management under climate change and modeling of canopy water fluxes under hotter, drier conditions.

**Author contribution statement** LJS led the conception and design of the experiment. Questions and hypotheses were formulated by CCM, LJS, and MJA. CCM, TAS, and MRR collected the data. CCM, MJA, and LJS analyzed the data. CCM, MJA, and CGB wrote the manuscript with input from all authors.

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**Data availability** All data on the measured ecosystem variables that support this study's findings are included in this paper and its Supplementary Information files. The data supporting this study's findings are also available from the authors upon reasonable request.

## Declarations

**Competing interest** The authors declare that they have no competing interests.

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