



Water Use, Efficiency, and Stomatal Sensitivity in Eastern Cottonwood and Hybrid Poplar Varietals on Contrasting Sites in the Southeastern United States

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The southeastern United States has wide-scale potential to achieve high productivity from elite eastern cottonwood and hybrid poplar varietals to produce renewable bioenergy and bioproducts. In order to determine how environmental drivers impact water use and growth so that individuals can maintain growth during drought periods, varietals that use water efficiently, and/or tolerate water stress conditions, are needed to make planting recommendations across a variety of sites. Additionally, inoculation with nitrogen-fixing endophytic bacteria may improve water stress tolerance. The goals of this research were (1) to determine water use strategies using measurements of diurnal sapflow and differences in leaf retention for three eastern cottonwood (*Populus deltoides*, ST66, S7C8, and 110412) and three hybrid poplar (two *P. deltoides* × *Populus maximowiczii*, 6329 and 8019, and one *Populus trichocarpa* × *P. deltoides*, 5077) varietals on contrasting field sites, (2) determine the physiological impact of endophyte inoculation, and (3) determine which physiological parameters were most highly correlated with aboveground biomass. We found that whole-tree water use efficiency (WUE) was similar across varietals at 5.2 g biomass per kg water used and that water use scaled with tree size. We found that water use strategies in terms of scaled stomatal sensitivity to vapor pressure deficit converged across varietals under stressful soil water conditions at both sites, but that varietals 8019 and 110412 tended to exhibit the highest plasticity in stomatal sensitivity exhibiting the largest range in scaled stomatal sensitivity under different soil moisture conditions. Endophyte inoculation increased growth and stomatal sensitivity at the nitrogen-limited site. Leaf area, whole-tree WUE, and plasticity in stomatal sensitivity were correlated with aboveground biomass production across sites and varietals. Overall, these data can be used to model hydrologic impacts of large-scale *Populus* biofuel production as well as recommend varietals with efficient water use and stomatal sensitivity under a range of soil and atmospheric moisture stress factors.

Keywords: biofuels, canopy conductance, endophytes, *Populus deltoides*, sapflow, short rotation woody crop, SRWC

INTRODUCTION

Short rotation woody crops (SRWCs) including *Populus* species gained popularity in the past as a prime feedstock for quality printing paper, however, *Populus* is now considered important as a renewable source of bioenergy and a wide array of bioproducts. The southeastern United States has the potential to provide substantial biofuel products to the United States and global customers due to its existing forestry infrastructure, land base and favorable climate. Both elite eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) as well as hybrid poplar varieties have been developed for superior growth, survival, disease resistance and wood characteristics (Ceulemans et al., 1992; Bandaru et al., 2015). Other traits including water use have been less commonly explored in these varieties, but may be important if water use is high and widespread planting occurs (Bloemen et al., 2017) as these fast-growing woody crops have the potential to deplete groundwater resources and alter hydrology (Rijtema and De Vries, 1994; Sevigne et al., 2011). However, water use impacts are likely site and variety specific with some studies finding that water use in hybrid poplars was similar to natural forests (Perry et al., 2001), and others finding that water use of hybrid poplar plantations exceeded annual precipitation (Jassal et al., 2013; Jones et al., 2017). On the other hand, high water use in varieties may be beneficial if SRWCs are planted to provide ecosystem services for phytoremediation or excessive nutrient removal for water quality improvement (Zalesny et al., 2006; Dimitriou et al., 2009).

In order for SRWC production to be financially viable, sites should be capable of high productivity, located near processing refineries, or an economical source of transportation (Stoof et al., 2015) and should not compete with existing food production (Dipesh et al., 2017). These factors may restrict planting locations to sites with more limited water availability than prime agricultural land (Zalesny et al., 2016) and irrigation may not be feasible depending on costs and profit margins (Coyle et al., 2013). Across the genus, poplars have been shown to exhibit either a conservative (isohydric) strategy in which they restrict water use and, in turn, productivity under water stress conditions [low soil moisture and/or high vapor pressure deficit (VPD)] or a more risky (anisohydric) strategy in which they continue to function under increasing water stress (Fichot et al., 2009; Silim et al., 2009; Attia et al., 2015; Navarro et al., 2020). Under a conservative water use strategy, trees restrict the loss of water by closing stomata under high water stress conditions which protects their water transport pathway, but limits photosynthesis by restricting CO₂ entry into leaves. In trees with an anisohydric strategy, stomata remain open during increasing water stress, risking hydraulic failure but maintaining photosynthetic rates (Tardieu and Simonneau, 1998; Klein, 2014). By analyzing the response of stomata to increasing VPD, stomatal sensitivity can be discerned and the water use strategy of *Populus* varieties can be placed on an isohydricity continuum (Schmidt-Walter et al., 2014). In addition to leaf-level responses, whole-tree responses to water stress may also play a critical role including more allocation to root growth and decreases in total leaf area due to leaf senescence during the growing season.

Finally, inoculation of trees with diazotrophic endophyte bacteria may improve water stress tolerance. In addition to improved disease resistance against *Melampsora* leaf rust and poplar stem canker (Raghavendra and Newcombe, 2013; Ren et al., 2013) and increased nutrient status through nitrogen fixation and improved soil acquisition (Kandel et al., 2017; Rho et al., 2018), endophytes may improve water stress tolerance (Rho et al., 2018). In *Populus deltoides* × *Populus nigra*, inoculation with endophytes increased total biomass by 28% and root biomass by 42%, and reduced damage by reactive oxygen species to provide potential drought tolerance (Rogers et al., 2012; Khan et al., 2016). Both choosing *Populus* varieties that exhibit the best water stress strategies for site conditions and potentially improving water stress tolerance with endophyte inoculation can increase the sites available for planting *Populus* SRWCs.

The water use strategies and total water use of *Populus* varieties over the growing season remain important for its influence on site hydrology, however the amount of water used per unit aboveground biomass produced, or whole-tree water use efficiency (WUE), is also important when comparing varieties as well as providing perspective for their water use. Attia et al. (2015) found a 10-fold difference in WUE for various *Populus* genotypes. The possibility of selecting genotypes with high productivity and high WUE would allow for short rotation *Populus* to be grown with lower impacts on surrounding hydrology, groundwater recharge and streamflow (Petzold et al., 2011). However, results are mixed in terms of the relationship between WUE and productivity with some studies finding that the most productive genotypes were also the most water use efficient (Zhang et al., 2004; Monclus et al., 2009; King et al., 2013), and other studies finding no relationship between productivity and WUE (Rae et al., 2004; Marron et al., 2005; Monclus et al., 2005, 2006; Bonhomme et al., 2008; Dillen et al., 2011). The lack of agreement in correlations between productivity and WUE may be due to limited variability in site and climate conditions (Prasolova et al., 2003; Rae et al., 2004) and/or the parameters used to estimate a tree's WUE. Water use efficiency can be estimated at differing scales including at the leaf-level as the ratio of photosynthesis and transpiration or from carbon isotope ratios and discrimination of various tissues which provide a proxy for temporally and spatially integrated WUE (Rae et al., 2004; Monclus et al., 2005, 2009; Bonhomme et al., 2008; Dillen et al., 2011; Broeckx et al., 2014) based on theory and equations from Farquhar et al. (1989). Although relatively easy to measure, carbon isotope discrimination needs to be interpreted with caution when inferring WUE (Broeckx et al., 2014) because other physiological parameters may be a larger driver of carbon isotopic differences than water use (Gilbert et al., 2011). These discrepancies may be resolved by presenting WUE, not at the leaf level or with carbon isotopes, but instead at the tree level where values of whole-tree biomass per unit water use across the growing season will provide more meaningful data and also capture tree-level responses to water stress including leaf area reductions (Monclus et al., 2006).

This research sought to answer the following research objectives: (1) Determine water use strategies for six *Populus* varieties (three eastern cottonwood and three hybrid poplars)

planted on research sites on the Upper Gulf Coastal Plain and the Lower Mississippi Alluvial Valley (LMAV) and determine if strategies differ depending on planting site, (2) determine if *Populus* varieties differ in their leaf retention in response to summer water stress and leaf pathogens, (3) determine if inoculation with diazotrophic endophyte bacteria impacts growth and water use strategies in these tested varieties and if site conditions impact the endophyte effect, and (4) determine 2-year aboveground biomass production for all varieties at both sites and identify physiological parameters that are most correlated with growth. Tested water use parameters include seasonal water use, and whole-tree WUE as well as canopy stomatal conductance and its correlation with environmental parameters including soil moisture, VPD and photosynthetic photon flux density (PPFD). Overall, this research provides information to better model the water use and productivity potential of the tested *Populus* varieties in the southeastern United States (Headlee et al., 2013a; Stanturf et al., 2019) to better predict ecosystem impacts of SRWCs and their productivity potential in the region. This research also provides additional information to match planted varieties to sites where they will perform optimally.

MATERIALS AND METHODS

Study Sites, Varieties and Experimental Design

Two field sites were planted in 2018, one on the Gulf Coastal Plain in northeastern Mississippi (“Coastal Plain”; 33°51′ N, 88°17′ W) and one in the “Delta” portion of Mississippi in the LMAV (“LMAV”, 33°24′ N, 90°11′ W). The Coastal Plain site consists of Prentiss fine silty loam soils that are moderately well drained (Natural Resources Conservation Service, 2021). Soils to a depth of 66 cm were reported to have an oven dry bulk density of 1.59 g cm⁻³, an available water capacity of 0.14 cm cm⁻¹, a clay content of about 13%, a sand content of about 54% and a silt content of about 33% (Natural Resources Conservation Service, 2021). This site did not receive intensive agricultural production prior to this study but was previously planted in peanuts. The LMAV site consists of somewhat poorly drained Tensas silty clay loam soils (Natural Resources Conservation Service, 2021) that tend to be saturated throughout the winter and early spring. Soils to a depth of 100 cm were reported to have an oven dry bulk density of about 1.62 g cm⁻³, an available water capacity of 0.17 cm cm⁻¹, a clay content of about 41%, a sand content of about 20%, and a silt content of about 39% (Natural Resources Conservation Service, 2021). This site has been in row crop agriculture for decades and had previously been planted in soybeans. Before planting, sites were tilled and subsoiled to a depth of about 35.5 cm. Six *Populus* varieties were planted at each site. Tested varieties consisted of three eastern cottonwood varieties (*P. deltoides* × *P. deltoides*, “D × D”) including 110412 (D × D), S7C8 (D × D), ST66 (D × D), and three hybrid poplar varieties, two of which were *P. deltoides* × *Populus maximowiczii* A. Henry crosses [“D × M”; 6329 (D × M); identification #: 284-93-6335 and 8019 (D × M); identification

#: 605-97-19163] and one *Populus trichocarpa* (Torr. & Gray ex Hook.) × *P. deltoides* cross [“T × D”; 5077 (T × D); identification #: 123-91-1903]. These hybrid poplar varieties were selected for study because they showed some resistance to *Septoria musiva* stem canker, a fungal pathogen prevalent in the southern United States, in previous studies. Eastern cottonwood varieties exhibit natural resistance to *S. musiva* (Newcombe and Ostry, 2001). Eastern cottonwood cuttings, approximately 40.6 cm long, were collected during winter, 2018 from the Mississippi State University, Department of Forestry Cottonwood Cutting Orchard, soaked in water and insecticide (Admire® Pro, Bayer Corp., Whippany, NJ, United States) for approximately 18 h and kept in plastic bags in a walk-in freezer at -2°C until planting. Dormant, unrooted hybrid poplar cuttings, approximately 30.5 cm long, were obtained from Greenwood Resources Inc. (Portland, OR, United States) and placed into the walk-in freezer upon receipt. Before planting, hybrid cuttings were soaked in a water/insecticide solution for approximately 3 to 4 days prior to planting.

Cuttings were planted in the subsoiled rows that were approximately 2.74 m apart with cuttings spaced every 1.83 m within rows. The Coastal Plain site was planted on April 18, 2018 and the LMAV site was planted on June 14, 2018 due to extended soil saturation that impeded site preparation. This late planting date likely impeded establishment survival and growth with survival at the LMAV site ranging from 10 to 50% across varieties and year one heights ranging from 1.6 to 2.4 m. At the Coastal Plain site survival ranged from 89 to 100% and year one heights ranged from 4 to 5.6 m. Each variety was planted in 15-tree variety plots (3 trees × 5 trees) replicated across five (at the LMAV site) or six (at the Coastal Plain site) replicate blocks depending of site size limitations. Twelve variety plots were contained within each replicate block and half received an inoculation of diazotrophic endophyte bacteria shortly after planting while the other half remained uninoculated. Endophytic bacteria isolated from *Populus* and *Salix* spp. were obtained from Intrinsyx Bio (Moffett Federal Airfield, Mountain View, CA, United States). Endophytes were encapsulated inside calcium alginate “beads” and were placed in approximately 25 cm deep holes next to the planted cuttings. Immediately after planting, the test site was broadcast treated with a preemergent combination of Goal 2XL (Dow AgroSciences LLC, Indianapolis, IN, United States) and Pendulum 3.3 EC (BASF, Florham Park, NJ, United States) to control both broadleaf and grass competition. During the first growing season, sites were maintained in “free to grow” conditions by controlling herbaceous competition through tilling, hand-weeding around trees and chemical herbicide applications. Sites were examined in the second growing season to determine if additional weed control was necessary and no herbaceous weed control was performed as trees had grown above herbaceous competition. Cottonwood leaf beetle (*Chrysomela scripta* Fabricius) larvae were found at the Coastal Plain site during the first and second growing seasons and were treated with a ground application of insecticide (Admire® Pro) in August, 2018 and aerial applications in July and September, 2019. A small amount of

cottonwood leaf beetle feeding was observed at the LMAV site, however, damage was not extensive enough to warrant an insecticide application.

At both sites, environmental monitoring sensors were installed in the beginning of the first growing season. Atmospheric conditions including air temperature and relative humidity were monitored with a sensor (HMP60 Temperature and Relative Humidity Probe; Vaisala, Helsinki, Finland) located about 1.8 m aboveground and these data were used to calculate VPD (Goff and Gratch, 1946). PPF (LI190SB Quantum Sensor; LI-COR Biosciences Inc., Lincoln, NE, United States) and rainfall (TE525MM Rain Gauge; Texas Electronics, Dallas, TX, United States) were measured near plots in an open location. Thirty-cm-long time domain reflectometry probes (CS 616 Time Domain Reflectometry Probe; Campbell Scientific Inc., Logan, UT, United States) were installed vertically to continuously measure volumetric soil moisture at four locations across each field site. At the LMAV site, soil moisture sensors were continually damaged by animals, therefore only one sensor located beneath the battery box was maintained continuously at that site. All environmental sensors were connected to a CR1000 datalogger (Campbell Scientific Inc., Logan, UT, United States) at each site that measured data every 30 s and recorded data averages every 30 min. The datalogger and sensors were powered by a solar panel and deep cycle batteries located at each site.

Water Use and Leaf Area Index Measurements

In order to continuously measure tree-level water use, laboratory-made, Granier-style, heat dissipation sapflow sensors (Granier, 1987) were installed in trees at the beginning of the second growing season (May, 2019). If trees were large enough in diameter, 2-cm-long sensors were installed and 1-cm-long sensors were installed in smaller diameter trees. Heat dissipation sensors consist of a pair of sensors installed radially into the sapwood about 10 cm vertically from one another and each containing an inner thermocouple. The upper sensor received constant heat and the lower sensor remained unheated. Water flow through the sapwood cools the heated sensor such that temperature differences between the sensor pair can estimate sap flow rates through an empirical equation (Granier, 1987). Sensor pairs were covered with reflective insulation to prevent sunflecks from affecting temperature measurements. At the Coastal Plain site, sensors were installed in one of the inner, middle trees of each 15-tree varietal plot being monitored for water use. Three trees per varietal per endophyte treatment (two for varietal 6329, endophyte inoculated) were monitored in plots located closest to the datalogger for a total of 35 trees monitored (due to space limitations in the datalogger). At the LMAV site, survival was low (see above), therefore two of the largest trees in each varietal/endophyte treatment group were selected for study for a total of 24 trees monitored at that site. The central CR1000 datalogger and AM16/32B multiplexer (Campbell Scientific Inc.) at each site measured sensor temperature differences every 30 s and averaged data every 30 min and sensors were powered by a solar panel and deep cycle batteries.

Half-hourly sensor temperature differences were converted to sapflow rates (J_S ; kg m^{-2} sapwood area s^{-1}) using the software program BaseLiner version 4 (Oishi et al., 2016). Missing half-hourly data were gapfilled from corresponding sensors in other trees of the same varietal/endophyte treatment group for each site. Half-hourly data were integrated into daily and monthly water use and determined on a tree-level basis by multiplying sapflow rates by stem sapwood areas. Stem diameters were measured after sensor installation and again at the end of the second growing season during stem harvest (see below). During stem harvest, the proportion of radius occupied by bark or sapwood was determined and a relationship between whole stem diameter and sapwood diameter ($\text{Diam}_{\text{sapwood}} = \text{Diam}_{\text{stem}} \times 0.9 - 0.107$; $r^2 = 0.89$) across both sites was used to calculate sapwood areas from earlier stem diameter measurements. Linear growth in sapwood areas was assumed throughout the second growing season and between measurement periods and all sapwood area was assumed to be functional with no radial adjustments in sapflow rate applied to the data (Samuelson et al., 2007). Leaf-specific transpiration rates (E_L ; kg m^{-2} leaf area s^{-1}) were then calculated by dividing these sapwood-area-scaled sapflow rates by tree leaf area. Leaf areas were determined during biomass harvest (see below) and varietal-specific linear relationships between tree diameter² × height (D^2H) and leaf area ($r^2 = 0.49$ to 0.96) were developed and used to calculate leaf areas at the beginning of the growing season. For hybrid varieties that experience leaf drop during the growing season due to leaf diseases and senescence, relationships between D^2H and leaf area for eastern cottonwood from biomass harvests were used to estimate leaf areas at the beginning of the growing season before significant leaf drop. A linear relationship between leaf area during the beginning and end of the growing season was assumed. Canopy-scaled stomatal conductance (canopy G_S ; m s^{-1}) was calculated as follows:

$$\text{Canopy } G_S = \frac{K_G \times E_L}{VPD}$$

where K_G is a coefficient calculated as $115.8 + 0.4226 \times$ air temperature (Phillips and Oren, 1998). Canopy stomatal conductance was further scaled to units of $\text{mol m}^{-2} \text{s}^{-1}$ by dividing values by the density of water vapor scaled based on air temperature [$0.0224 \times$ air temp(K)/27].

At the peak of the second growing season in June, 2019 during a sunny, cloud-free day, leaf area index (LAI) was estimated at three locations within each 15-tree plot at the Coastal Plain site using a LAI 2000 canopy analysis system (LI-COR Biosciences Inc., Lincoln, NE, United States). Due to low plot-level survival, LAI was not measured at the LMAV site. Measurements were made in the first tree-row of each plot between trees 1 and 2 and trees 4 and 5 of that row with the sensor facing plot center. A 90-degree view cap was placed on the sensor to block the view of surrounding plots and the outermost rings were excluded from analysis. Open sky conditions were measured repeatedly after six plots were measured and open sky data were interpolated between measurements. LAI for each 15-tree plot was calculated using FV2200 software version 2.1.1 from LI-COR Biosciences Inc.

Biomass Harvest and Whole Tree Water Use Efficiency

At the end of the second growing season but before autumn leaf drop (October 2019), trees measured for sapflow at each site were harvested to measure their biomass. Groundline and diameter at breast height were measured and trees were cut near their base. Sapwood diameter was measured in two perpendicular directions on the cut stump to compare with groundline diameter and determine the bark component. Once cut, the stem lengths were measured to estimate tree height. All stems containing leaves were removed in the field and placed in plastic bags for transport back to the lab. An approximately 10 cm long sample was cut from the stem and its green weight was determined before transport back to the lab. The remaining stem weight was measured in the field with a hanging balance. Once back in the lab, the volume of the fresh stem sample was obtained using water volume displacement and it was placed in a drying oven at 105°C until all water was expelled. The dry weight of the sample was obtained and specific gravity was calculated as sample dry weight divided by fresh volume divided by the density of water. All leaves were removed from remaining stems in the lab and the weight of these remaining stems was added to the weight of stems measured in the field. This total stem weight was converted to a dry wood biomass (kg) using the stem sample fresh weight/dry weight ratio for each tree. The fresh weight of all leaves was measured, then a random subsample of leaves was obtained from each tree. The fresh weight of the leaf subsample was measured as well as its cross-sectional area using a LI3100C area meter (LI-COR Biosciences Inc.). The area to weight ratio of the leaf subsample was multiplied by the total fresh leaf weight of each tree to determine total tree leaf area.

To estimate whole-tree WUE, an equation was developed to predict dry woody biomass from stem basal area (BA) \times tree height [Biomass = (BA \times H) \times 12.45 + 935.592; $r^2 = 0.87$] measured during the tree harvest. This equation was used to calculate dry woody biomass of each tree during sapflow sensor installation from height and diameter measurements taken during that time. Biomass during sensor installation was subtracted from biomass measured during the tree harvest to calculate aboveground biomass added during the 2019 growing season. This aboveground biomass growth was divided by the water used during that time period and estimated by the sapflow data to calculate whole-tree WUE (whole-tree WUE; g biomass/kg water used).

Data Analysis

For all reported parameters except LAI, individual trees measured for sapflow and harvested for biomass represent the experimental unit. LAI measurements were averaged at the plot level for the Coastal Plain site. Overall analysis of variance to test for significant differences in measured parameters based on site, varietal, endophyte inoculation as fixed effects and their interaction was performed in R version (R Core Team, 2014) using linear mixed effect models [nlme package; Pinheiro et al. (2014)] with replicate block included as a random effect in models. In addition, to compare varieties with one another at

each testing site, multiple comparison tests were accomplished for each site separately using Tukey contrasts in the multcomp package (Hothorn et al., 2008) in R.

Linear and non-linear relationships between canopy stomatal conductance (G_S) and environmental parameters were fitted with Sigmaplot version 13 (Systat Software Inc., San Jose, CA, United States). Canopy G_S was compared with the natural log of VPD (lnVPD) to produce a linear correlation between these parameters. Likewise, the natural log of canopy G_S was calculated to generate a linear relationship with soil moisture at the LMAV site. Relationships between canopy G_S and lnVPD or PPFD were estimated at both low soil moisture (low θ) and average to high soil moistures (high θ) by first splitting the datasets into low soil moisture conditions ($\theta < 0.2$ at the Coastal Plain site and $\theta < 0.55$ at the LMAV site), then averaging G_S by VPD or PPFD categories. These soil moisture thresholds were determined by identifying inflection points in the canopy G_S vs. soil moisture data. In order to determine if slope terms differed between varieties in linear regression relationships, linear mixed effect models were fitted using the lme package in R and multiple comparison tests of slope terms were analyzed using the phia package (De Rosario-Martinez, 2015) in R. To estimate stomatal sensitivity to VPD (Oren et al., 1999), parameters were estimated from the linear regression equations fitted to the canopy G_S vs. lnVPD relationships. The y-intercept of the equation represents the reference canopy G_S at VPD = 1 kPa ($G_{S,ref}$), the slope of the relationship (m) represents the rate of change between canopy G_S and lnVPD (G_S sensitivity), and the ratio of the slope and y-intercept represents the rate of change between canopy G_S and lnVPD per unit reference canopy G_S (scaled G_S sensitivity). Scaled G_S sensitivity at low vs. high soil moisture were compared for each varietal using paired t -tests (with measurements within each soil category paired within individuals) using R.

RESULTS

Biomass and Leaf Area Across Varietals and Sites

Two-year dry biomass was significantly greater at the Coastal Plain site compared with the LMAV site ($p = 0.008$; **Table 1**) averaging 6.7 ± 0.5 and 2.4 ± 0.4 kg per tree respectively across varieties. Varietals also differed significantly at each site with varietal 8019 (D \times M) and the eastern cottonwoods having the largest biomass at the Coastal Plain site. Varietal 8019 (D \times M) had significantly greater biomass than the other hybrid poplar varieties (**Table 2**). At the LMAV site, eastern cottonwoods again tended to have the greatest biomass with S7C8 (D \times D) having the largest biomass and 6329 (D \times M) having the smallest biomass. In contrast, specific gravity was significantly greater at the LMAV site ($p < 0.001$; **Table 1**) compared with the Coastal Plain site across varieties (0.44 ± 0.006 vs. 0.33 ± 0.006 g cm⁻³, respectively). At the Coastal Plain site, S7C8 (D \times D) and 6329 (D \times M) had the highest specific gravity and 110412 (D \times D) exhibited the lowest specific gravity (**Table 2**). At the LMAV site, S7C8 (D \times D) again had the largest specific gravity and ST66 (D \times D) had the lowest specific gravity. At the Coastal Plain

TABLE 1 | Analysis of variance results presenting significant effects of site (S), varietal (V) and endophyte inoculation (E), as well as their interaction (×) on productivity, water use, and canopy stomatal conductance (G_S) parameters under low and average to high soil moisture (θ).

Parameter	Site	Varietal	Endophyte	S × V	S × E	V × E	S × V × E
Biomass	**	**	–	–	*	–	–
Specific gravity	***	***	**	–	*	–	–
Leaf area	–	***	–	–	*	–	–
Leaf area index (LAI)	NA	***	–	NA	NA	–	NA
Seasonal water use	**	***	*	*	*	–	–
Whole-tree water use efficiency	–	–	–	–	–	–	–
G_{Sref} , low θ	–	***	**	*	–	–	*
G_S sensitivity, low θ	–	***	–	**	–	–	–
Scaled G_S sensitivity, low θ	–	*	***	**	***	**	–
G_{Sref} , high θ	–	**	–	**	–	–	–
G_S sensitivity, high θ	–	*	–	**	–	–	–
Scaled G_S sensitivity, high θ	–	**	*	**	–	–	–

Leaf area index (LAI) was only measured at the Coastal Plain site, therefore site effects could not be determined. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

site, for plot-level LAIs measured in July, 2019, D × M hybrids tended to exhibit the highest leaf areas with statistically larger LAI than 5077 (T × D) and eastern cottonwoods had intermediate LAI values (Figure 1A). In October at the Coastal Plain site, eastern cottonwoods S7C8 (D × D) and ST66 (D × D) tended to have the greatest leaf areas estimated from tree harvest with significantly more leaf area than hybrid poplars 8019 (D × M) and 5077 (T × D; Figure 1B).

Water Use and Canopy G_S vs. Soil Moisture and PPFD

Water use over the second growing season was significantly greater at the Coastal Plain site compared with the LMAV site ($p = 0.007$; Table 1) corresponding to the size differences of the trees at each site. Within the Coastal Plain site, 8019 (D × M) used the most water and differed significantly from 5077 (T × D) which used the least water (Table 2). Throughout the growing season, 8019 (D × M) used more water during the early portion of the growing season with water use declining after June (Supplementary Figure 1). Other varietals, including S7C8 (D × D), ST66 (D × D), and 6329 (D × M) increased water use during the growing season using the most water in August or used similar amounts of water for most months of the growing season (Supplementary Figure 1). At the LMAV site, S7C8 (D × D) used the most water differing significantly from all hybrid poplars which exhibited low water use (Table 1). In terms of monthly water use at the LMAV site, S7C8 (D × D) and 5077 (T × D) used the most water in the June and July, while other varietals either maintained or increased water use throughout the growing season (Supplementary Figure 1). Whole-tree WUE did not differ significantly between sites ($p = 0.34$) or varietals ($p = 0.51$; Table 1) and averaged 5.2 ± 0.3 g biomass per kg water used.

Canopy-scaled stomatal conductance (canopy G_S) exhibited differing relationships with soil moisture depending on site (Figure 2A). At the Coastal Plain site, cubic functions best fit the data, with canopy G_S increasing to the greatest extent with increasing soil moisture under the drier soil moisture

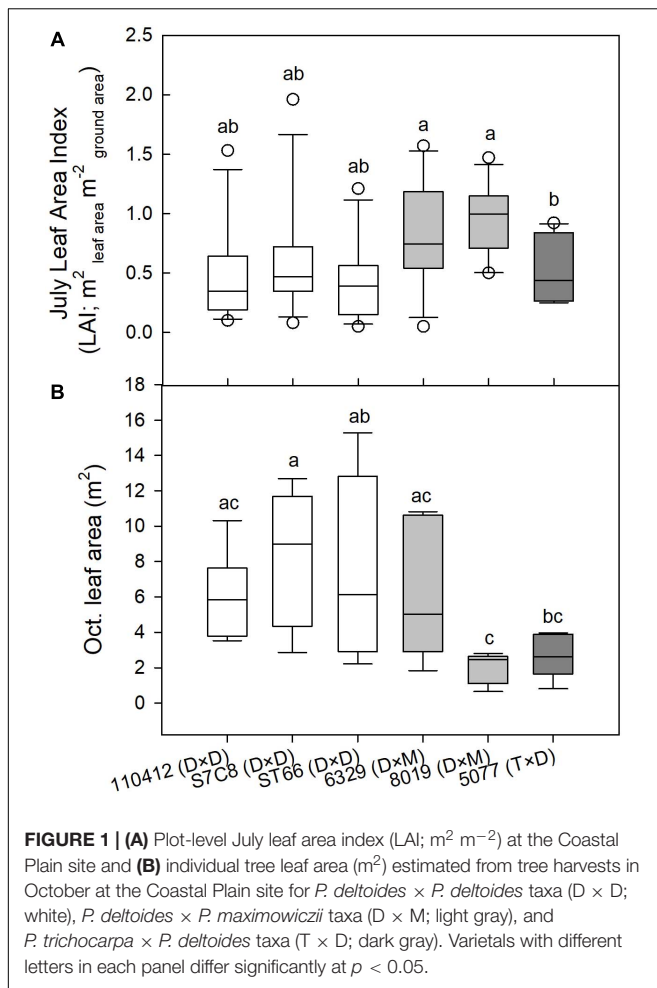
TABLE 2 | Productivity, water use, and water use efficiency (WUE) parameters (mean and SE in parentheses) for varietals growing on the Coastal Plain and LMAV sites.

	2-year dry biomass (kg tree ⁻¹)	Specific gravity	Seasonal water use (kg tree ⁻¹)	Whole-tree WUE (g biomass kg ⁻¹ water)
Coastal Plain				
110412 (D × D)	6.4 (1.0) ^{ac}	0.32 (0.007) ^c	710 (120) ^{ab}	5.9 (0.4) ^a
S7C8 (D × D)	8.3 (1.5) ^{ab}	0.35 (0.006) ^a	780 (110) ^{ab}	6.6 (1.0) ^a
ST66 (D × D)	6.2 (1.3) ^{ac}	0.33 (0.008) ^{bc}	850 (140) ^{ab}	5.2 (0.7) ^a
6329 (D × M)	5.8 (0.5) ^{bc}	0.35 (0.002) ^{ab}	850 (150) ^{ab}	4.4 (0.6) ^a
8019 (D × M)	9.1 (0.8) ^a	0.34 (0.003) ^{ac}	1100 (150) ^a	5.8 (0.4) ^a
5077 (T × D)	4.1 (0.6) ^c	0.34 (0.002) ^{ac}	400 (60) ^b	5.9 (1.6) ^a
LMAV				
110412 (D × D)	2.9 (0.6) ^{ab}	0.42 (0.01) ^{ab}	370 (50) ^{ab}	4.5 (1.0) ^a
S7C8 (D × D)	4.3 (1.5) ^a	0.47 (0.02) ^a	590 (120) ^a	3.7 (1.5) ^a
ST66 (D × D)	3.0 (0.8) ^{ab}	0.41 (0.008) ^b	410 (70) ^{ab}	4.9 (2.1) ^a
6329 (D × M)	0.9 (0.3) ^b	0.46 (0.02) ^{ab}	180 (20) ^b	2.9 (0.8) ^a
8019 (D × M)	1.3 (0.2) ^{ab}	0.46 (0.01) ^{ab}	150 (70) ^b	5.2 (1.3) ^a
5077 (T × D)	2.1 (0.4) ^{ab}	0.46 (0.008) ^{ab}	230 (40) ^b	6.2 (0.6) ^a

Varietals within sites with different superscript letters differ significantly from one another at $p < 0.05$.

range (0.14–0.2 cm³ cm⁻³). Under drier soil moistures, 8019 (D × M) exhibited the greatest increase in canopy G_S , while 6329 (D × M) exhibited the lowest increase. When soil moistures were above 0.2 cm³ cm⁻³, canopy G_S remained relatively consistent, with slight decreases seen in all varietals around 0.24 cm³ cm⁻³ (Figure 2A). At the LMAV site, canopy G_S increased exponentially with increasing soil moistures (Figure 2B). The natural log of canopy G_S resulted in a linear relationship, and the slope of the relationship between canopy G_S and soil moisture was significantly larger for all hybrid poplars compared with all eastern cottonwoods (Figure 2B).

At both sites, canopy G_S exhibited nonlinear relationships with PPFD under low and high soil moisture conditions, reaching



a maximum at low to mid PPFd values then decreasing again at the highest light levels (Supplementary Figure 2). At the Coastal Plain site under low soil moistures, all varietals reached maximum canopy G_S at PPFd around $825 \mu\text{mol m}^{-2} \text{s}^{-1}$. Under high soil moisture, canopy G_S reached a maximum at a lower light level of around $550 \mu\text{mol m}^{-2} \text{s}^{-1}$ in all varietals (Supplementary Figure 2). At the LMAV site, canopy G_S peaked at lower PPFd values under low soil moisture compared with higher soil moisture values (Supplementary Figure 2). Under low soil moisture, canopy G_S in 8019 (D × M) and S7C8 (D × D) peaked around $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ while all other varietals peaked around $550 \mu\text{mol m}^{-2} \text{s}^{-1}$. At high soil moistures, canopy G_S of all varietals reached a maximum at PPFd values around $750 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Supplementary Figure 2).

Stomatal Sensitivity to Vapor Pressure Deficit

In relationships between canopy G_S and $\ln\text{VPD}$ at low and average to high soil moistures, sites exhibited similar $G_{S,\text{ref}}$, G_S sensitivity and scaled G_S sensitivity, but varietals, as well as their ranking within sites (S × V), differed significantly (Table 1). At the Coastal Plain site (Figures 3A,B), both $G_{S,\text{ref}}$ (y intercept

of lines) and G_S sensitivity (slope of lines) exhibited the same ranking among varietals at low (Figure 3A) and high (Figure 3B) soil moistures (Table 3). Varietal 8019 (D × M) exhibited the largest $G_{S,\text{ref}}$ and G_S sensitivity, 110412 (D × D) and ST66 (D × D) were intermediate and S7C8 (D × D), 6329 (D × M), and 5077 (T × D) had the lowest $G_{S,\text{ref}}$ and G_S sensitivity regardless of soil moisture (Table 3). However, scaled G_S sensitivity (slope/y intercept) differed based on soil moisture regime with all varietals being statistically similar at low soil moisture and scaled G_S sensitivity averaging 0.71 ± 0.008 (Table 3). At average to high soil moistures, S7C8 (D × D) and 6329 (D × M) exhibited the highest scaled G_S sensitivity and differed significantly from 110412 (D × D) and 8019 (D × M) which exhibited lower scaled G_S sensitivity. Across soil moisture categories, all varietals at the Coastal Plain site exhibited significantly lower scaled G_S sensitivity at high soil moistures compared with low soil moisture conditions except varietal 6329 (D × M; Table 3).

At the LMAV site (Figures 3C,D), varietals differed in their rankings of $G_{S,\text{ref}}$ and G_S sensitivity and these rankings differed depending on if parameters were estimated at low (Figure 3C) or average to high (Figure 3D) soil moisture (Table 3). At low soil moistures (Figure 3C), D × M varietals had the highest $G_{S,\text{ref}}$, 5077 (T × D) was intermediate and the eastern cottonwoods tended to have the lowest $G_{S,\text{ref}}$ (Table 3). The D × M varietals also exhibited the highest G_S sensitivity, 5077 (T × D) and 110412 (D × D) were intermediate and S7C8 (D × D) had the lowest G_S sensitivity at low soil moistures. At low soil moistures, scaled G_S sensitivity was similar among varietals, although S7C8 (D × D) had significantly lower scaled G_S sensitivity compared with the other eastern cottonwood varietals. Under average to high soil moistures at the LMAV site (Figure 3D), all hybrid poplars had significantly greater $G_{S,\text{ref}}$ and G_S sensitivity than eastern cottonwoods with varietal 5077 (T × D) having significantly greater G_S sensitivity than varietal 8019 (D × M; Table 3). However, all varietals exhibited statistically similar scaled G_S sensitivity at high soil moistures at the LMAV site. Likewise, most varietals exhibited statistically similar scaled G_S sensitivity at low and high soil moistures at the LMAV site with only S7C8 (D × D) and 8019 (D × M) exhibiting significantly higher sensitivity at high soil moistures compared with low soil moisture conditions (Table 3).

Endophyte Effects

While year two biomass at the Coastal Plain site was statistically similar ($p = 0.19$) between trees receiving the endophyte treatment and control trees, at the LMAV site, endophyte-treated trees differed significantly ($p = 0.04$) from control trees having about 70% more biomass across all varietals (Figure 4A). Endophyte inoculated trees at the LMAV site also used significantly more water than non-inoculated trees given their larger size and biomass, however, whole-tree WUE was marginally, significantly greater ($p = 0.057$) for trees receiving the endophyte treatment compared with control trees at the LMAV site. Under low soil moisture conditions, scaled G_S sensitivity was marginally, significantly greater in endophyte-treated vs. control trees at the Coastal Plain site ($p = 0.056$) and significantly greater at the LMAV site ($p < 0.001$; Figure 4B). At the Coastal Plain

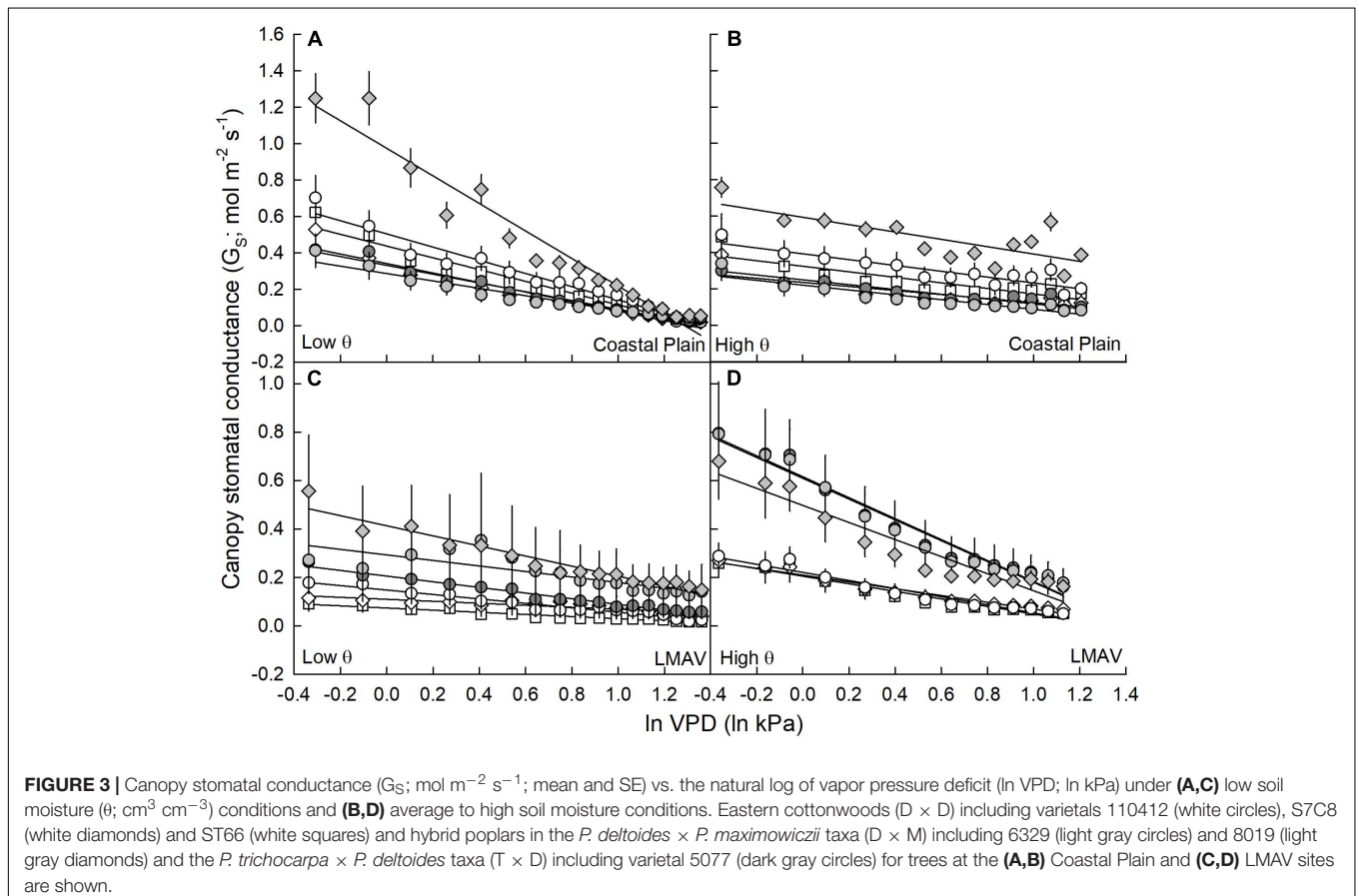
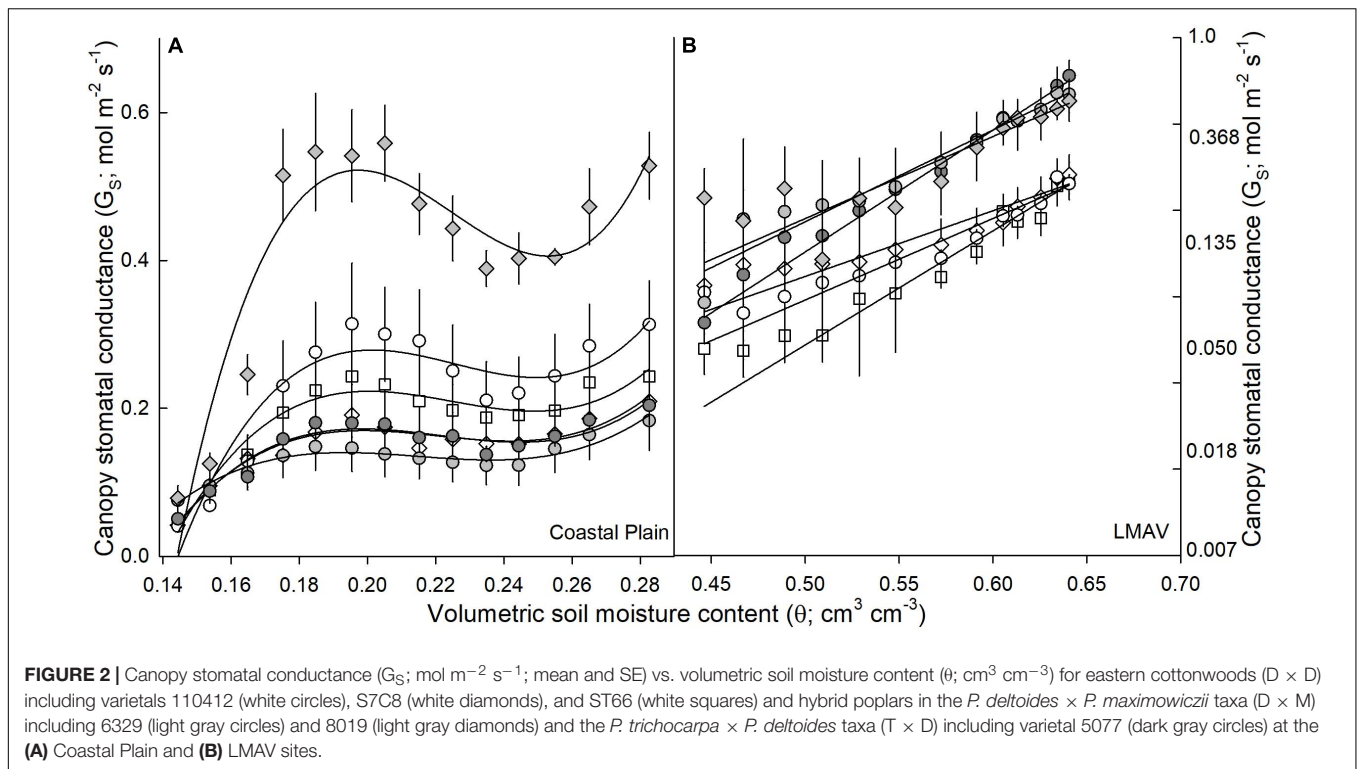


TABLE 3 | Parameters for varieties growing in the Coastal Plain and LMAV sites estimated from linear relationships between canopy stomatal conductance (G_S) and the natural log of vapor pressure deficit ($\ln VPD$; **Figure 3**) estimated under low vs. average to high soil moisture (θ ; $\text{cm}^3 \text{cm}^{-3}$) conditions including G_{Sref} ($\text{mol m}^{-2} \text{s}^{-1}$; y-intercept; or G_S when $VPD = 1 \text{ kPa}$), G_S sensitivity ($\text{mol m}^{-2} \text{s}^{-1} \ln \text{kPa}^{-1}$; slope of line) and scaled G_S sensitivity (slope/y-intercept).

		Low soil moisture (θ)		Scaled G_S sensitivity	High soil moisture (θ)		Scaled G_S sensitivity	Low vs. high θ
		G_{Sref}	G_S sensitivity		G_{Sref}	G_S sensitivity		
Coastal Plain	110412 (D × D)	0.49 (0.09) ^b	0.33 (0.05) ^b	0.69 (0.03) ^a	0.39 (0.08) ^b	0.16 (0.04) ^b	0.41 (0.03) ^{bc}	0.003
	S7C8 (D × D)	0.33 (0.07) ^c	0.24 (0.05) ^c	0.73 (0.01) ^a	0.25 (0.05) ^c	0.13 (0.02) ^c	0.55 (0.04) ^a	0.007
	ST66 (D × D)	0.43 (0.08) ^b	0.30 (0.05) ^b	0.71 (0.01) ^a	0.33 (0.05) ^b	0.16 (0.03) ^b	0.49 (0.05) ^{ab}	0.008
	6329 (D × M)	0.28 (0.06) ^c	0.19 (0.04) ^c	0.67 (0.02) ^a	0.22 (0.05) ^c	0.13 (0.04) ^c	0.60 (0.04) ^a	0.11
	8019 (D × M)	0.95 (0.11) ^a	0.70 (0.08) ^a	0.74 (0.004) ^a	0.59 (0.03) ^a	0.20 (0.02) ^a	0.34 (0.02) ^c	<0.001
	5077 (T × D)	0.33 (0.02) ^c	0.23 (0.02) ^c	0.69 (0.02) ^a	0.24 (0.02) ^c	0.12 (0.02) ^c	0.48 (0.030) ^{ab}	0.001
LMAV	110412 (D × D)	0.15 (0.04) ^{cd}	0.088 (0.02) ^{bc}	0.63 (0.04) ^a	0.22 (0.02) ^b	0.15 (0.02) ^c	0.70 (0.01) ^a	0.11
	S7C8 (D × D)	0.11 (0.05) ^{cd}	0.045 (0.02) ^{cd}	0.42 (0.05) ^b	0.21 (0.05) ^b	0.13 (0.03) ^c	0.65 (0.02) ^a	0.04
	ST66 (D × D)	0.07 (0.01) ^d	0.042 (0.007) ^d	0.60 (0.06) ^a	0.20 (0.04) ^b	0.15 (0.04) ^c	0.70 (0.02) ^a	0.21
	6329 (D × M)	0.29 (0.19) ^{ab}	0.11 (0.05) ^a	0.48 (0.10) ^{ab}	0.60 (0.14) ^a	0.40 (0.10) ^{ab}	0.66 (0.01) ^a	0.2
	8019 (D × M)	0.41 (0.17) ^a	0.20 (0.09) ^a	0.47 (0.04) ^{ab}	0.49 (0.10) ^a	0.33 (0.09) ^b	0.66 (0.04) ^a	0.05
	5077 (T × D)	0.20 (0.09) ^{bc}	0.11 (0.06) ^{bc}	0.50 (0.07) ^{ab}	0.61 (0.16) ^a	0.40 (0.09) ^a	0.67 (0.01) ^a	0.12

Varietals within sites with different superscript letters differ significantly from one another at $p < 0.05$. "Low vs. high θ " represents p -values (<0.05 in bold) for paired t -tests comparing scaled G_S sensitivity between low and high θ conditions.

site, endophyte inoculated trees had about 4% higher scaled G_S sensitivity compared with control trees, whereas at the LMAV site, the endophyte treatment resulted in about 35% higher scaled stomatal sensitivity compared with control trees.

Physiological Parameters Predicting Growth

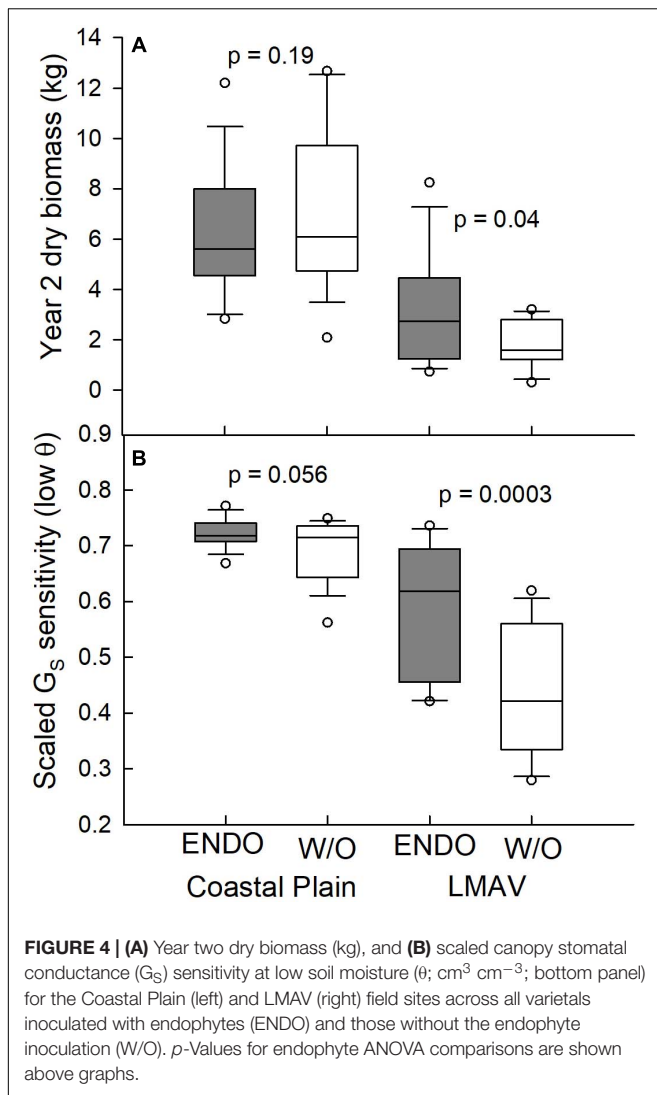
Across varieties and sites, tree leaf area was significantly ($r^2 = 0.29$; $p < 0.0001$), positively correlated with aboveground woody biomass (**Figure 5A**). Whole-tree WUE also exhibited a statistically significant, positive correlation ($r^2 = 0.23$; $p = 0.0002$) with biomass across all sites and varieties (**Figure 5B**). For relationships between biomass and scaled G_S sensitivity, results differed depending on soil moisture conditions. Under low soil moisture conditions, trees that exhibited greater scaled G_S sensitivity had greater biomass ($r^2 = 0.49$; $p < 0.0001$ for the relationship between the log of biomass and scaled G_S sensitivity) with this relationship driven largely by trees at the LMAV site (**Figure 5C**). Under average to high soil moisture conditions, trees with lower scaled G_S sensitivity had higher biomass ($r^2 = 0.33$; $p < 0.0001$ for the relationship between the log of biomass and scaled G_S sensitivity) and this relationship was largely driven by trees at the Coastal Plain site (**Figure 5D**).

DISCUSSION

Water Use Strategies – Whole Tree Water Use and Efficiency

For the measured *Populus* varieties at two sites, we found that water use mirrored tree sizes in terms of biomass with larger varieties [8019 (D × M)] at the better quality, Coastal Plain site using the most water at around 1200 kg water per tree during the measurement period (May–September) or about 450 mm of

water over the growing season (April–October) based on this tree spacing. Smaller trees at the more marginal LMAV site ranged in water use from 170 to 730 kg water used per tree from May to September with eastern cottonwoods using more water than hybrids. The lowest water user and smallest trees at the Coastal Plain site, variety 5077 (T × D) used about 400 kg water per tree during the measurement period or about 150 mm of water over the growing season. This differs from findings of Bassman and Zwier (1991) who found that T × D hybrids had higher transpiration rates than *P. deltoides* varieties. However, Zalesny et al. (2019) found that varieties from an F1 backcross population [(T × D) × *P. deltoides*] tended to use less water than eastern cottonwood varieties. Eastern cottonwoods at the Coastal Plain site were intermediate and used between 600 and 800 kg water per tree during the measurement period or 320–360 mm of water over the growing season. During the same time period (April–October), the Coastal Plain site received about 980 mm of rainfall in 2019 and generally receives about 740 mm of rainfall based on a 30-year average (Arguez et al., 2010). Therefore, if single varieties were planted on a hectare scale at the spacing of the present study, transpiration from these plantations would account for 20% [for 5077 (T × D)] to 61% [for 8019 (D × M)] of rainfall during the growing season based on the 30-year average rainfall amount. This differs from other studies which found that *Populus* evapotranspiration exceeded precipitation for the same time period in a hybrid poplar plantation in Canada (Jassal et al., 2013), a D × N plantation (Clone I-214) in northern Italy (Migliavacca et al., 2009), and D × N varieties growing near Beijing, China (Xu et al., 2018). Of course, these studies estimated total evapotranspiration which includes interception losses as well as soil evaporation in addition to transpiration. Song et al. (2021) found that transpiration in *Populus simonii* × *P. nigra* varieties growing in China was about 45% of total precipitation over the same time period which is similar to our transpiration/rainfall proportion. In addition,



water use may increase as trees get larger, are planted on a closer spacing or after coppice, therefore these data represent water use for young, pre-coppice stands.

Daily water use at the Coastal Plain site averaged about 0.9 (5077 T × D) to 2.3 (8019 D × M) mm day^{-1} across varieties throughout the growing season which is similar to reported average daily water use of 1.5 mm day^{-1} for *P. simonii* × *P. nigra* growing in China (Song et al., 2021), 1.8 mm day^{-1} for varietal I214 (D × N) in southwestern France (Muller and Lambs, 2009), 2.2 mm day^{-1} for *P. maximowiczii* × *P. nigra* varieties in Germany (Petzold et al., 2011), and 2.3–3.1 mm day^{-1} for hybrid poplars growing in Germany (Schmidt-Walter et al., 2014). However, this water use is lower than the 3.6 mm day^{-1} for a T × D hybrid poplar growing in Washington State (Hinckley et al., 1994) and 3.4 mm day^{-1} for an irrigated T × D varietal growing on the east side of the Cascade Mountains in Washington (Kim et al., 2008). Overall, these higher daily water usage values compared to our study may be due to the drier atmospheric growing conditions (i.e., higher VPDs) at these sites

during the growing season coupled with the availability of soil moisture or irrigation conditions which allow trees to use higher amounts of water.

Overall, both sites and all varieties in our study exhibited a statistically similar whole-tree WUE of around 5.2 g biomass per kg water used during the second growing season. This suggests that these varieties exhibited similar strategies in terms of whole tree WUE and further highlights the positive relationship between WUE and biomass. Likewise, since these tested varieties were chosen for their high productivity in previous studies, this suggests that our WUE represent the upper range for high performing *Populus* grown in natural environmental conditions. For example, our calculated whole tree WUE is higher than found by Kim et al. (2008) who calculated WUE of about 2.3 g C per kg water used (or about 4.6 g biomass per kg water assuming 50% C content) in 3-year-old irrigated T × D trees, as well as Souch and Stephens (1998) for 1 to 2-year old T × D and D × N hybrids grown in a greenhouse which exhibited WUE of 4.4 g biomass kg^{-1} and 3.5 g biomass kg^{-1} , respectively. The lower reported WUE of trees in these studies is likely the result of supplemental watering in either greenhouse or irrigated conditions. Additionally, our measured WUE is about double what was found by Maier et al. (2019) for eastern cottonwood and hybrid poplars across a range of growth performance in North Carolina, United States. Because Maier et al. (2019) included varieties across a range of growth rates, their reported WUE is likely lower than in the varieties tested in our study which were selected in part due to their higher growth rates.

Water Use Strategies – Stomatal Sensitivity

Although whole tree WUE was statistically similar across varieties, scaled stomatal sensitivity differed between sites, across varieties and between soil moisture levels (low soil moisture vs. average to high soil moisture) suggesting that differing strategies under different environmental conditions and in different varieties can lead to similarly high WUE. At the more well drained, Coastal Plain site, varieties converged at a high scaled G_s sensitivity of around 0.7 under low soil moistures suggesting similar moisture stress among varieties during these low soil moisture conditions as well as a similar ability to increase G_s sensitivity at low soil moistures. At average to high soil moistures, scaled G_s sensitivity decreased in every varietal except 6329 (D × M) with 8019 (D × M) and 110412 (D × D) exhibiting the lowest scaled G_s sensitivity around 0.38 at high soil moistures signifying a high degree of plasticity in G_s sensitivity in these varieties depending on soil moisture conditions. Therefore, varieties 8019 (D × M) and 110412 (D × D) which exhibited the greatest plasticity in scaled G_s sensitivity may be the most adaptable to a range of growing conditions and able to best match their stomatal functioning to soil moisture conditions. Song et al. (2021) measured a similar plasticity in G_s sensitivity among years in a *P. simonii* × *P. nigra* varietal in Northeast China which decreased stomatal regulation from 1 year to another. Schmidt-Walter et al. (2014) found that scaled G_s sensitivity changed by month during the growing season, generally becoming more

sensitive in the later months of the growing season. In this study, varieties S7C8 (D × D), ST66 (D × D), and 5077 (T × D) exhibited intermediate G_S sensitivity of around 0.51 at high soil moistures suggesting some degree of stomatal adjustment based on soil moisture conditions. Varietal 6329 (D × M) exhibited the highest sensitivity at ample soil moisture conditions, but also the lowest (although not statistically different) stomatal sensitivity at low soil moisture conditions suggesting relatively isohydric water use and potentially the most conservative water use strategy.

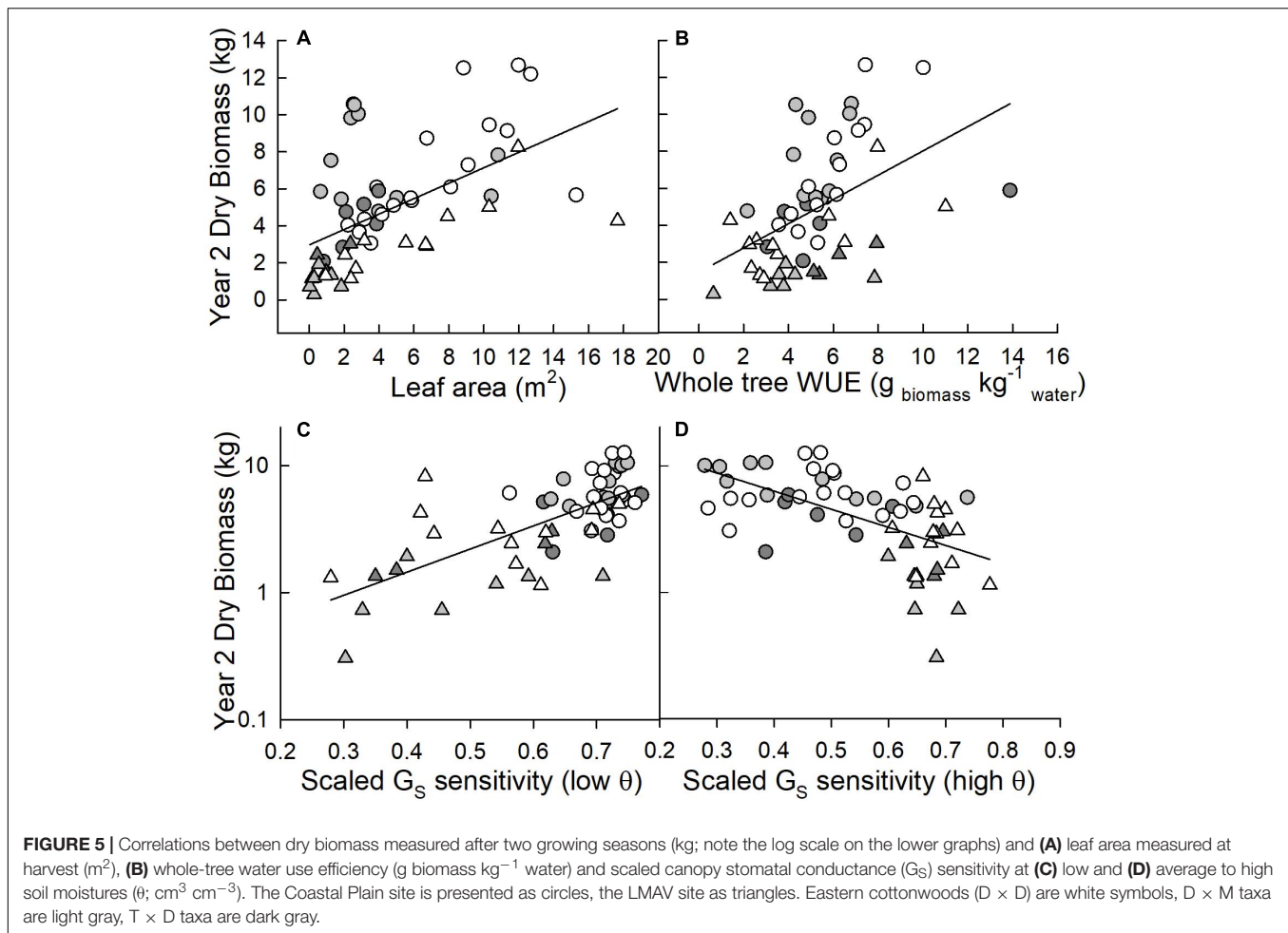
At the LMAV site with more poorly drained, clay soils, scaled G_S sensitivity converged across varieties under high soil moistures at around 0.67. This relatively high G_S sensitivity suggests stress under high soil moisture conditions potentially due to anaerobic conditions in the soil at high moisture levels. Under low soil moistures, scaled G_S sensitivity tended to be slightly lower in all varieties, but only differed significantly in S7C8 (D × D) and 8019 (D × M). Migliavacca et al. (2009) shared similar results for a D × N variety in northern Italy where trees exhibited less G_S sensitivity under low soil moisture conditions. S7C8 (D × D) had higher biomass at the LMAV site suggesting that its lower stomatal regulation at lower soil moistures allowed it to take in more CO₂ and exhibit greater productivity. On the other hand, 8019 (D × M) had relatively low productivity at the LMAV but also exhibited high plasticity at the Coastal Plain site suggesting high stomatal plasticity as a trait for variety 8019 across sites. Overall, scaled G_S sensitivity averaged 0.59 across all study individuals which is very close to the estimated sensitivity of 0.6 across a variety of species and sites (Oren et al., 1999) and similar to findings of Kim et al. (2008) who reported a G_S sensitivity of 0.64 for an irrigated T × D plantation in eastern Washington, Maier et al. (2019) who reported stomatal sensitivities of around 0.63 for T × D, D × D, and D × M varieties in North Carolina, United States, but higher than Xu et al. (2018) who reported G_S sensitivities of 0.36–0.48 for D × N hybrids growing near Beijing, China.

At the two field sites, we found that canopy G_S responded differently to increases in soil moisture with canopy G_S mainly increasing at low soil moistures (below 20%) at the Coastal Plain site, but increasing exponentially with increasing soil moisture at the LMAV site. This is likely related to the differing soil types and soil moisture release curves at each site with the Coastal Plain site occurring on silty loam soils and the LMAV site occurring on silty clay loam soils (Roy et al., 2018). These relationships differ from those identified by Song et al. (2021) who found linear relationships between canopy G_S and soil moisture for poplars growing on sandy soils although soil moisture explained a relatively small proportion of canopy G_S compared with VPD and PPFD in that study. However, Maier et al. (2019) found similar relationships between canopy G_S and relative extractable soil water as our Coastal Plain site for their site located on sandy loam soils with canopy G_S increasing until relative extractable water reached about 20% then remaining relatively constant at higher extractable water contents. We also found that soil moisture affected the relationships between canopy G_S and PPFD or VPD. Especially at the Coastal Plain site, relationships between canopy G_S and PPFD had higher r^2 values at high soil moistures while relationships between canopy G_S and VPD had higher r^2

values at lower soil moisture suggesting that VPD is a stronger driver of canopy G_S at low soil moisture while PPFD is a stronger driver at high soil moistures. We also found that canopy G_S peaked at lower PPFD levels at high soil moistures compared with lower soil moistures at the Coastal Plain site whereas the opposite was true at the LMAV site. We expected that, at lower soil moistures, canopy G_S would peak at lower PPFD values, however, the opposite findings at the Coastal Plain site could suggest other interacting factors between environmental parameters are causing lower peak PPFD values at high soil moistures (Xu et al., 2017). Overall, these findings can aid in modeling of water use in these *Populus* varieties under the interacting environmental drivers of soil moisture, VPD and incoming radiation.

Interactions Between Water Use and Leaf Retention

We found that varieties also differed in their water use patterns throughout the growing season, which corresponds to leaf area differences measured in the peak of the growing season (July) vs. the late growing season (October). Variety 8019 (D × M) at the Coastal Plain site exhibited peak water use in June with water use declining every month thereafter. This variety had among the highest leaf areas in July but the lowest leaf areas in October, suggesting its declining water use was due to successive leaf losses throughout the growing season, likely due to Septoria leaf spot. In contrast, at the Coastal Plain site, eastern cottonwoods ST66 and S7C8 increased water use throughout the growing season exhibiting peak water use in August. All eastern cottonwood varieties also tended to have lower crown leaf areas based on LAI estimates than the hybrid poplars in July, but among the highest crown leaf areas (based on tree harvest) in October suggesting increased water use was due to increasing leaf areas throughout the growing season. It should be noted that, because eastern cottonwoods tend to hold their leaves more vertically while hybrid poplars hold their leaves more horizontally, LAI measurements based on understory light levels (i.e., from the LAI 2000) may have underestimated leaf areas for eastern cottonwoods compared with hybrid poplar. However, overall, similar results were found by Ceulemans et al. (1992) who found that hybrid poplars had the most leaves early in the growing season, while eastern cottonwoods had more leaves in the middle of the growing season. Leaf losses from hybrid poplars may relate to both premature leaf drop as a result of water stress (Souch and Stephens, 1998; Giovannelli et al., 2007) and effects of leaf pathogens which may cause leaf drop (Pellis et al., 2004; Coyle et al., 2006; Elena et al., 2014). In terms of resistance to leaf pathogens, tested eastern cottonwoods in this study are native to the region (110412 and ST66) or from Texas (S7C8) meaning they may exhibit relatively more resistance to native pathogens (Land and Jeffreys, 2006) particularly Septoria and Marsonnia leaf spot compared with hybrid poplars. However, eastern cottonwoods have still been shown to be vulnerable to *Melampsora medusae* foliar rust. Hybrid poplar 6329 (D × M) exhibited the lowest relative differences in crown leaf area among varieties between July and October suggesting higher leaf-level resistance to leaf pathogens than other hybrid poplar taxa.



Endophyte Inoculation Impacts

We found that the endophyte inoculation increased biomass across varieties at the LMAV site but not at the Coastal Plain site. Other results demonstrate that nitrogen availability limited growth at the LMAV site, while water use limited growth at the Coastal Plain site (Stewart, 2020). We also found greater scaled G_s sensitivity at low soil moistures leading to marginally higher WUE in addition to the greater biomass of inoculated trees at the LMAV site. Khan et al. (2016) found that stomatal conductance was lower during drought conditions in endophyte inoculated D \times N individuals compared with control plants, but inoculated plants maintained higher growth signifying higher WUE. Endophyte inoculated Arizona fescue (*Festuca arizonica* Vasey) also exhibited significantly less negative leaf water potentials, lower stomatal conductances and transpiration rates than non-inoculated plants which conferred increased drought tolerance of inoculated individuals (Morse et al., 2002). Khan et al. (2016) also found that endophytes produced several plant hormones including indole-3-acetic acid, jasmonic acid, gibberellin-3-acid and abscisic acid which have been shown to be involved in plant stress tolerance as well as growth, specifically for roots, which may confer additional tolerance to drought stress conditions. Overall, these findings suggest that

endophytes can alter the drought tolerance of *Populus* varieties by improving the G_s sensitivity of trees under soil moisture stress conditions.

Biomass and Physiological Parameters That Predict Growth

Two-year biomass was almost three times higher at the Coastal Plain site compared with the LMAV site. Other studies have reported good growth of eastern cottonwood in the LMAV (Stanturf et al., 2019), however, our site may have been more marginal in terms of soil inundation and potential nutrient limitations. Likewise, the biomass average across all varieties in the LMAV site is decreased by the low biomass of the hybrid poplars at the LMAV site. Hybrid poplars are generally not planted in the LMAV due to susceptibility to Septoria stem canker (Robison et al., 2006), however, the planted varieties displayed some Septoria resistance. The Coastal Plain site exhibited high biomass growth for the eastern cottonwoods and hybrid poplar 8019 (D \times M) but lower growth for 5077 (T \times D). In contrast, Zalesny et al. (2019) found that eastern cottonwood produced greater biomass than T \times D and D \times M varieties on a Coastal Plain site, but hybrids, particularly T \times D varieties, performed better at a mountain site. At a better quality site

similar to our Coastal Plain site, Ghezehei et al. (2019) found that eastern cottonwood varieties exhibited better growth than hybrids (T × D and D × M). Similar to our study Ghezehei et al. (2019) also found that site affected specific gravity likely due to differences in growth rate. We found some overarching trends, specifically with S7C8 (D × D) which tended to have higher specific gravity than other eastern cottonwoods at both sites. Overall, our specific gravity measurements ranged from 0.32 to 0.47 which is similar to ranges reported in Headlee et al. (2013b) and Ghezehei et al. (2019). Assuming high initial survival (which is generally seen in hybrid poplar) and similar growth as this study, 8019 (D × M) in the first 2 years of growth produced about $18 \pm 1.6 \text{ Mg ha}^{-1}$ of dry woody biomass (assuming the spacing of the current study) or about $9 \text{ Mg ha}^{-1} \text{ year}^{-1}$. This early productivity compares favorably with other studies being larger than *P. deltoides* varieties (ST66 and S7C15) growing with irrigation and fertilization in South Carolina [$3.1\text{--}6.3 \text{ Mg ha}^{-1} \text{ year}^{-1}$; (Coyle and Coleman, 2005; Coyle et al., 2013)], *P. deltoides* growing in the Great Lakes region [$2.5 \text{ Mg ha}^{-1} \text{ year}^{-1}$ (Netzer et al., 2002)], and in the Mississippi River Valley [$6\text{--}7.7 \text{ Mg ha}^{-1} \text{ year}^{-1}$ (Switzer et al., 1976; Francis and Baker, 1981)]. However, the biomass seen in the first 2 years of growth in 8019 (D × M) was slightly lower than T × D hybrids growing in the Pacific Northwest [$9.7 \text{ Mg ha}^{-1} \text{ year}^{-1}$ (Heilman and Zie, 1993)] and lower than estimates for hybrid poplar productivity ($9.5\text{--}11.9 \text{ Mg ha}^{-1} \text{ year}^{-1}$) reported in Zalesny et al. (2016) and about half the productivity of the most superior selections ($20 \text{ Mg ha}^{-1} \text{ year}^{-1}$) reported in Zalesny et al. (2009) although these growth rates may be for older, more established plantations.

We found that, across varieties and sites, leaf area and whole-tree WUE were positively correlated with biomass. Coyle et al. (2016) found that net primary productivity was driven by both leaf and fine root production in eastern cottonwoods ST66 and S7C15. Similarly, Maier et al. (2019) also found that WUE was positively correlated with growth and suggested that this relationship was controlled to a greater extent by larger photosynthetic rates as opposed to lower stomatal conductances. Our positive correlation between biomass and tree leaf area also supports the suggestion that increased photosynthesis is playing a larger role in achieving WUE as opposed to a minimization of water use. However, Jones et al. (2017) found a significant correlation between annual gross primary productivity and stand level evapotranspiration rates estimated from eddy covariance in hybrid poplars growing in Canada suggesting higher water use was associated with greater growth. In addition, we found contrasting effects of scaled G_s sensitivity under differing soil moisture conditions where trees with greater stomatal sensitivity under low soil moisture but less stomatal sensitivity under high soil moisture conditions exhibited greater biomass. Although studies differ on whether the most productive *Populus* varieties are relatively drought tolerant (Giovannelli et al., 2007) or drought sensitive (Monclus et al., 2006), we found that individuals exhibiting plasticity in G_s sensitivity depending on site and soil moisture conditions were the most productive.

CONCLUSION

In this study, we found that overall water use was related to tree size with larger trees (across varieties and sites) using more water than smaller trees and daily water use in this study being similar to studies in other non-irrigated *Populus* systems. In addition, across sites and varieties whole-tree WUE was about 5.2 g biomass per kg water which was slightly higher than WUE for *Populus* reported in the literature suggesting that high whole tree WUE is necessary for the high productivity. Although varieties displayed statistically similar whole-tree WUE, they used water differently throughout the growing season and exhibited different water use strategies in terms of canopy G_s and its sensitivity to VPD. Varietal 8019 (D × M) used more water early in the growing season while eastern cottonwoods ST66 and S7C8 increased water use throughout the growing season likely reflecting differences in leaf phenology for these varieties. Varietal 8019 (D × M) along with 110412 (D × D) also exhibited the lowest scaled G_s sensitivity under average to high soil moistures at the Coastal Plain site which differed significantly from G_s sensitivity at low soil moisture suggesting a high amount of plasticity in their water use strategies corresponding to soil conditions. This plasticity in stomatal sensitivity may allow varieties 8019 (D × M) and 110412 (D × D) to exhibit higher adaptability to a range of growing sites. Across varieties, scaled G_s sensitivity converged under low soil moistures at the Coastal Plain site and under high soil moistures at the LMAV site suggesting water use strategies of varieties converge during stressful conditions. We found that inoculation with endophytes caused significant impacts at the more N limited, LMAV site leading to increased biomass production and scaled G_s sensitivity under low soil moistures. Across sites and varieties, whole-tree WUE was positively correlated with woody biomass, while plasticity in scaled G_s sensitivity was important with lower sensitivity under high soil moisture as well as higher sensitivity under low soil moisture being positively correlated with biomass. Overall, these data shed light on the water use strategies of eastern cottonwood and hybrid poplar varieties to aid in planting recommendations and the modeling of their environmental impacts.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HR and RR conceived of, planned and received funding for the research, and revised the manuscript. HR, LS, and RR carried out the experiments and analyzed the data. HR led the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2021.704799/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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