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2022

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### Recommended Citation

Renninger HJ, Pitts JJ and RJ Rousseau. 2022. Comparisons of biomass, water use efficiency and water use strategies across five genomic groups of Populus and its hybrids. *Global Change Biology Bioenergy*. DOI: 10.1111/gcbb.13014

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**Comparisons of Biomass, Water Use Efficiency and Water Use Strategies  
Across Five Genomic Groups of *Populus* and its Hybrids**

Running Title: Water use metrics of *Populus* hybrids

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22 **ABSTRACT**

23 Genetic improvement and hybridization in the *Populus* genus have led to the  
24 development of genotypes exhibiting fast growth, high rooting ability and disease resistance.  
25 However, while large biomass production is important for bioenergy crops, efficient use of  
26 resources including water is also important in sites lacking irrigation and for maintaining  
27 ecosystem water availability. In addition, comparison of water use strategies across a range of  
28 growth rates and genetic variability can elucidate whether certain strategies are shared among the  
29 fastest growing and/or most water use efficient genotypes. We estimated tree water use  
30 throughout the second growing season via sapflow sensors of 48 genotypes from five *Populus*  
31 taxa; *P. deltoides* W. Bartram ex Marshall  $\times$  *P. deltoides* (D $\times$ D), *P. deltoides*  $\times$  *P. maximowiczii*  
32 A. Henry (D $\times$ M), *P. deltoides*  $\times$  *P. nigra* L. (D $\times$ N), *P. deltoides*  $\times$  *P. trichocarpa* Torr. & Gray  
33 (D $\times$ T) and *P. trichocarpa*  $\times$  *P. deltoides* (T $\times$ D) and calculated average canopy stomatal  
34 conductance ( $G_s$ ). We regressed  $G_s$  and atmospheric vapor pressure deficit (VPD) wherein the  
35 slope of the relationship represents stomatal sensitivity to VPD. At the end of the second  
36 growing season, trees were harvested, and their dry woody biomass was used to calculate whole  
37 tree water use efficiency ( $WUE_T$ ). We found that D $\times$ D and D $\times$ M genotypes exhibited differing  
38 water use strategies with D $\times$ D genotypes exhibiting high stomatal sensitivity while retaining  
39 leaves while D $\times$ M genotypes lost leaf area throughout the growing season but exhibited low  
40 stomatal sensitivity. Across measured taxa, biomass growth was positively correlated with  
41  $WUE_T$ , and genotypes representing each measured taxa except D $\times$ N and T $\times$ D had high two-year  
42 dry biomass of above 6 kg/tree. Overall, these data can be used to select *Populus* genotypes that  
43 combine high biomass growth with stomatal sensitivity and  $WUE_T$  to limit the negative impacts  
44 of bioenergy plantations on ecosystem water resources.

45 **KEYWORDS:** poplar; eastern cottonwood; short rotation woody crops (SRWCs); bioenergy;  
46 biofuel; sapflow; productivity

## 47 **INTRODUCTION**

48 Woody biomass has the potential to supply increasing amounts of renewable energy in  
49 the U.S. and abroad (U.S. Department of Energy, 2016) and interest from the various  
50 transportation sectors for renewable liquid fuels should increase investment in woody bioenergy  
51 fuels (Gegg, Budd, & Ison, 2014) in the future. *Populus* species and their hybrids have the  
52 potential to supply the increasing demand for renewable energy sources due to their historical  
53 use (and associated knowledge base and infrastructure) for pulpwood and low-cost hardwood  
54 timber (Ridge, Hinckley, Stettler, & Van Volkenburgh, 1986; Saxena, Adhikari, & Goyal, 2009;  
55 Spinelli & Hartsough, 2006; Stanton et al., 2002), ability to hybridize readily (Knox, Willing, &  
56 Pryor, 1972), adventitious rooting which allows for inexpensive stand establishment from  
57 unrooted cuttings and their demonstrated high yields (Stanton et al., 2002). Eastern cottonwood  
58 (*Populus deltoides* W. Bartram ex Marshall) is native to the southeastern U.S. with a distribution  
59 that extends from southern Canada to northeastern Mexico and is one of fastest growing species  
60 in the *Populus* genus (Isebrands, Richardson, & Ball, 2014). Genetic improvement programs  
61 have developed eastern cottonwood genotypes that exhibit high rootability, disease resistance  
62 and growth (Zalesny et al., 2016), and it tends to be one of the parent species in top performing  
63 hybrid poplar taxa. As such, greater potential gains for the southeastern U.S. might be possible  
64 through interspecific hybridization of selected eastern cottonwood with non-native *Populus*  
65 species including Japanese poplar (*Populus maximowiczii* A. Henry), black poplar (*Populus*  
66 *nigra* L.) and black cottonwood (*Populus trichocarpa* Torr. & Gray) particularly in drier upland  
67 areas where eastern cottonwood is less adapted. In most cases, these are F<sub>1</sub> hybrids as the

68 resulting genotypes will exhibit heterosis, whereas the F<sub>2</sub> generation shows greater variability of  
69 performance. Studies differ on which hybrid taxa perform best likely due to differing site  
70 conditions (Guo & Zhang, 2010; Heilman & Stettler, 1985; Trnka et al., 2008), but hybrid  
71 genotypes tend to perform better than either of their parental types (Coyle, Coleman, Durant, &  
72 Newman, 2006; Rousseau, Adams, & Wilkerson, 2013) in field trials on upland sites where  
73 *Septoria stem canker* (*Sphaerulina musiva* Peck formerly *Septoria musiva* Peck) is absent.  
74 However, eastern cottonwood can outperform hybrid taxa on alluvial sites and/or locations  
75 where *Septoria stem canker* is present (Rousseau, Herrin, & Ogunlolu, 2018).

76 Differing water use strategies influence characteristics of *Populus* genotypes including  
77 total water use, water use efficiency and response to drought, and in turn, their impacts on the  
78 water availability of an ecosystem (Bloemen et al., 2017; Maier et al., 2019; Sevigne et al.,  
79 2011). In general, water use strategies across species or individuals can be compared along an  
80 isohydricity spectrum. At one end of the spectrum, strictly isohydric species maintain constant  
81 midday leaf water potentials under both well-watered or drought conditions through tight control  
82 of stomatal conductance (i.e. high stomatal sensitivity to environmental conditions). At the other  
83 end of the spectrum, strictly anisohydric species exhibit lower stomatal sensitivity to  
84 environmental conditions causing their midday leaf water potentials to vary in response to the  
85 changing environment (Attia, Domec, Oren, Way, & Moshelion, 2015; Klein, 2014) and  
86 potentially risking hydraulic dysfunction (Tardieu & Simonneau, 1998). Each of these strategies  
87 possess pros and cons and can, therefore, be desirable based on environmental conditions and  
88 objectives. For example, genotypes with high stomatal sensitivity may perform better on sites  
89 that are more drought prone while greater water use from genotypes with lower stomatal  
90 sensitivity may be useful for phytoremediation purposes in terms of removing water-soluble

91 chemicals that could degrade surrounding water quality (O'Neill & Gordon, 1994). Particularly  
92 for woody bioenergy crops whose production should not compete with food crops and irrigation  
93 may not be financially feasible, future poplar plantations may occur on less fertile, more water-  
94 limited upland sites (U.S. Department of Energy, 2016) necessitating the selection of genotypes  
95 that exhibit conservative, drought-tolerant strategies (Monclus et al., 2006).

96         In addition to considering water use strategies and drought tolerance, water use efficiency  
97 or the amount of water used per unit biomass can vary widely across genotypes (Attia et al.,  
98 2015) and has the potential to greatly impact surrounding hydrology including groundwater  
99 recharge and streamflow (Petzold, Schwärzel, & Feger, 2011). For example, the large-scale  
100 production of genotypes which are efficient in their water use will likely place lower demands on  
101 local water sources and/or require lesser amounts of irrigation from outside sources than less  
102 efficient individuals (Petzold et al., 2011) while producing the same amount of harvestable  
103 biomass. Implementation of water use efficient genotypes, in turn, could reduce losses in local  
104 water budgets and potentially expand the profitable range where poplar can be grown for biofuel  
105 feedstocks into areas where lower annual rainfall levels are common. On the other hand, *Populus*  
106 genotypes that exhibit greater water use per unit aboveground biomass may rely on more  
107 extensive root systems and thereby achieve greater belowground carbon sequestration (Silim,  
108 Nash, Reynard, White, & Schroeder, 2009) in areas where ecosystem water budgets are not an  
109 issue. In addition, it is unclear if water use efficiency is correlated with overall aboveground  
110 productivity with some studies finding a positive correlation (King et al., 2013; Monclus et al.,  
111 2009; Renninger, Stewart, & Rousseau, 2021; Zhang, Zang, & Li, 2004) and other studies  
112 finding no correlation between water use efficiency and biomass production (Bonhomme et al.,

113 2008; Dillen et al., 2011; Marron et al., 2005; Monclus et al., 2005; Monclus et al., 2006;  
114 Monclus et al., 2009; Rae, Robinson, Street, & Taylor, 2004).

115 In this study, we sought to compare water use strategies across 48 different genotypes of  
116 *Populus* from eastern cottonwood (*P. deltoides* × *P. deltoides*; D×D) and hybrid poplar (*P.*  
117 *deltoides* × *P. maximowiczii* D×M; *P. deltoides* × *P. nigra* D×N; *P. deltoides* × *P. trichocarpa*  
118 D×T; *P. trichocarpa* × *P. deltoides* T×D) taxa. Specifically, we wanted to determine 1) if water  
119 use patterns in terms of canopy conductance differed among taxa across soil moisture and  
120 atmospheric vapor pressure deficit gradients throughout the growing season, 2) if water use  
121 strategies in terms of stomatal sensitivity vs. leaf area loss differed among taxa, 3) if parameters  
122 indicative of water use strategies were correlated with aboveground biomass production across  
123 taxa, and 4) which water use parameters were correlated with overall tree-level water use  
124 efficiency. We hypothesized that genotypes will vary in water use strategies based on their taxa  
125 and that genotypes within taxa can be identified that exhibit both high aboveground biomass as  
126 well as water use strategies that are indicative of water conservation and potential drought  
127 tolerance. On the other hand, genotypes that exhibit high water use can be identified and targeted  
128 for phytoremediation applications. Finally, identifying water use strategies and parameters for  
129 individual *Populus* genotypes can provide information for future breeding focused on the  
130 development of new genotypes suitable for biomass production in the southeastern U.S. The  
131 potential for defining and expanding the physiological strategies of *Populus* genotypes may  
132 provide the capacity to expand production into drier and more marginal areas as well as enable  
133 the ability of future *Populus* production to be adapted to changing climate conditions.

## 134 **MATERIALS AND METHODS**

### 135 **Genotypes, study area and environmental measurements**

136           The overall study consisted of 100 genotypes arranged in two-tree row plots (i.e., two  
137 ramets per genotype per block) in 12 replicated blocks with each block containing four sub-plots  
138 of specific taxa to provide similar, within taxa, competition in terms of rooting and crown  
139 architecture among genotypes. Genotypes of improved eastern cottonwood (*P. deltoides* × *P.*  
140 *deltoides*; D×D; 25 genotypes) and hybrid poplar taxa including *P. deltoides* × *P. maximowiczii*  
141 (D×M; 46 genotypes), *P. deltoides* × *P. nigra* (D×N; 11 genotypes), *P. deltoides* × *P.*  
142 *trichocarpa* (D×T; 13 genotypes) and *P. trichocarpa* × *P. deltoides* (T×D; 4 genotypes) were  
143 selected for planting. *Populus maximowiczii* has a native range from north-central China to east  
144 Siberia and northern Japan and is a rapidly growing, long-lived *Populus* species (Isebrands et al.,  
145 2014). *Populus nigra* is native to Europe, northern Africa and western Asia and can grow well in  
146 both riparian or well-drained sites as well as locations with dry summers but wetter spring and  
147 fall periods (Isebrands et al., 2014). *Populus trichocarpa* is native to the western U.S. and  
148 Canada is the largest *Populus* species in North America growing best in humid, coastal sites with  
149 limited drought conditions (Isebrands et al., 2014).

150           The study site was located on the Gulf Coastal Plain region in northeastern Mississippi  
151 (33°51'16"N 88°17'27"W). The climate of this area is humid subtropical, characterized by mild  
152 winters and long, hot summers. Mean annual temperature for the site was 18.7°C with a mean  
153 maximum temperature of 25.1°C and a mean minimum temperature of 12.4°C (National Oceanic  
154 and Atmospheric Administration & National Centers for Environmental Information, 2021).  
155 Rainfall is fairly evenly distributed throughout the year, with the region, on average, receiving  
156 about 144 cm of rainfall per year (National Oceanic and Atmospheric Administration & National  
157 Centers for Environmental Information, 2021). The soil of the site is characterized as a Prentiss



158 silty loam soil, which is moderately well-drained with a seasonal water table perched at 0.61-  
159 0.76 m (Natural Resources Conservation Service, 2021).

160 Prior to *Populus* planting in April, 2018, the site underwent limited management but was  
161 in peanut production. Before planting, the site was tilled and subsoiled to a depth of 45.7 cm  
162 along planted rows that were 2.74 m apart. Unrooted cuttings approximately 40.6 cm long for  
163 eastern cottonwoods and 22.9 cm long for hybrid poplars were planted in subsoiled rows  
164 approximately 1.83 m apart within rows. Dormant, unrooted hybrid poplar cuttings were  
165 provided by GreenWood Resources (Portland, OR, USA) and were placed in a walk-in freezer  
166 upon receipt. Eastern cottonwood cuttings were collected from the Mississippi State University  
167 Department of Forestry Cottonwood Cutting Orchard, soaked in water and insecticide (Admire  
168 Pro, Bayer Corp. Whippany, NJ, USA) and placed in plastic bags in a walk-in freezer until  
169 planting. Hybrid poplar cuttings were also soaked in Admire Pro insecticide approximately three  
170 days before planting. The entire test site was sprayed immediately after planting and prior to bud  
171 break with a preemergent Goal 2XL (Dow AgroSciences LLC, Indianapolis, IN, USA) and  
172 Pendulum 3.3EC (BASF, Florham Park, NJ, USA). Following the breakdown of the Goal and  
173 Pendulum, additional post emergent herbicide of Select (Valent USA LLC., Walnut Creek CA,  
174 USA) was applied to control johnson grass (*Sorghum halepense* (L.) Pers.). Combined  
175 mechanical weed control (tilling and hand weeding) was performed throughout the first growing  
176 season to control competition and provide “free to grow” conditions to the *Populus* cuttings. No  
177 competition control was needed during the second growing season. The test was periodically  
178 assessed for cottonwood leaf beetle (*Chrysomela scripta* Fabricius) feeding and received  
179 applications of Admire Pro (either ground applications or aerially depending on tree height) in  
180 August, 2018, July, 2019 and Sept. 2019.

181 Sensors measuring photosynthetic photon flux density (PPFD; LI190SB Quantum sensor;  
182 LI-COR Biosciences Inc., Lincoln, NE, USA), air temperature and relative humidity (HMP60  
183 temperature and relative humidity probe, Vaisala, Helsinki, FI), and rainfall (TE525MM tipping  
184 bucket rain gauge, Texas Electronics, Dallas, TX, USA) were located next to the *Populus*  
185 plantation in an open field. Eight soil moisture (30 cm long CS616 time domain reflectometry  
186 probes, Campbell Scientific Inc., Logan, UT, USA) sensors were installed vertically throughout  
187 the study site and located between subsoiled rows. Environmental sensors were installed shortly  
188 after planting in May, 2018 and connected to a Campbell Scientific CR1000 datalogger (and  
189 AM16/32B multiplexer (Campbell Scientific Inc.) for soil moisture sensors). Sensor data were  
190 collected every 30 seconds and half hourly averages were recorded by the datalogger. Air  
191 temperature and relative humidity were used to calculate atmospheric vapor pressure deficit  
192 (VPD).

### 193 **Sapflow and canopy conductance (Gs)**

194 In the beginning of the second growing season (May, 2019), laboratory made, thermal  
195 dissipation sap flow sensors (Granier, 1987) were inserted radially into the sapwood on the north  
196 side of 48 genotypes in two of the twelve replicate blocks for a total of 96 trees measured (two  
197 per genotype). Of the 48 genotypes measured, 12 were eastern cottonwood (D×D), 22 were D×M  
198 genotypes, 5 were D×N genotypes, 7 were D×T genotypes and 2 were T×D genotypes.  
199 Information about measured genotypes can be found in Table S1. In general, 2-cm-long sensors  
200 were used unless tree diameters were too small and required 1-cm-long sensors instead. Sensors  
201 were placed about 0.5 m above ground level and covered with reflective insulation. Thermal  
202 dissipation sensors were connected to one of three AM16/32B multiplexers that were each  
203 connected to a central datalogger (CR1000) which measured sensor data every 30 seconds and

204 logged averages every half hour. Sapflow sensors, dataloggers and environmental sensors were  
205 powered by marine deep cycle batteries and solar panels.

206 Sapflow sensor output (mV differential voltages) were converted to sapflow rates ( $J_s$ ; kg  
207  $m^{-2}$  sapwood area  $s^{-1}$ ) using Baseline 4 software (Oishi, Hawthorne, & Oren, 2016; Oishi, Oren, &  
208 Stoy, 2008) and the calibration equation described in Granier (1987). Missing data were  
209 gapfilled using data from an individual of the same genotype (if available) or data from another  
210 genotype from the same taxa. Half-hourly data were integrated to daily and monthly values and  
211 calculated on a per tree level by multiplying sapflow rates by the sapwood area of each  
212 individual. To determine sapwood areas, trees measured for sapflow in one replicate block were  
213 harvested in October of the second growing season (see below) and ratios of stem diameter to  
214 bark depth were determined for each taxa (Table S2). All wood was determined to be functional  
215 on these two-year-old trees. These taxa-specific regression equations were used to calculate  
216 sapwood areas from diameter measurements of unharvested trees (of the other replicate block)  
217 and from May, 2019 diameter measurements made when sensors were installed. Sapwood areas  
218 were assumed to increase linearly throughout the study period and all sapwood area was assumed  
219 to exhibit the same flow rate measured by the sapflow sensor (Samuelson, Stokes, & Coleman,  
220 2007).

221 To determine half-hourly canopy-averaged stomatal conductance ( $G_s$ ) tree-level sapflow  
222 rates ( $kg\ s^{-1}$ ) were divided by tree-specific leaf areas to determine leaf-specific transpiration rates  
223 ( $E_L$ ;  $kg\ m^{-2}$  leaf area  $s^{-1}$ ). Tree leaf areas were determined during biomass harvest (see below)  
224 and taxa-specific regression equations between tree basal area  $\times$  height and leaf area were  
225 developed (Table S2) and used to estimate leaf areas for unharvested trees as well as leaf areas  
226 for trees at the beginning of the sapflow measurement period (May, 2019). Because some D $\times$ T,

227 T×D and D×M taxa begin losing leaves in late July and August (well before biomass harvest  
228 occurred), the regression equation for D×D genotypes was used to estimate leaf areas in May for  
229 D×M, D×T, and T×D taxa. Previous research of leaf area index (LAI) measured in July found  
230 that a D×M and T×D genotypes had similar or slightly higher LAI than D×D genotypes  
231 (Renninger et al., 2021). A linear relationship between leaf areas estimated for May and leaf  
232 areas measured or estimated in October was assumed and differences between measured leaf  
233 areas in October and calculated leaf areas in May were used to estimate leaf area loss. For each  
234 individual and half-hourly transpiration measurement, average canopy stomatal conductance ( $G_s$ ;  
235  $\text{m s}^{-1}$ ) was calculated as follows:

$$236 \quad G_s = \frac{K_G \times E_L}{VPD}$$

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

240 Half-hourly  $G_s$  values for time periods when  $VPD > 0.6 \text{ kPa}$  were regressed vs.  
241 environmental parameters for each measured individual to determine genotype- and/or taxa-  
242 specific responses. Relationships between  $G_s$  and volumetric soil moisture content ( $\theta$ ;  $\text{cm}^3 \text{cm}^{-3}$ )  
243 were nonlinear, therefore, linear regressions were fitted separately for  $\theta < 0.22 \text{ cm}^3 \text{cm}^{-3}$  and  $\theta$   
244  $> 0.22 \text{ cm}^3 \text{cm}^{-3}$  and slopes of these regressions for each individual were estimated using  
245 Sigmaplot version 13 (Systat Software Inc. San Jose, California, USA). To estimate each  
246 individual's stomatal sensitivity to VPD (Oren et al., 1999), linear relationships were fitted  
247 between  $G_s$  and the natural log of VPD ( $\ln VPD$ ) with the sapflow dataset split into low soil  
248 moisture ( $< 0.22 \text{ cm}^3 \text{cm}^{-3}$ ) and average to high soil moisture ( $> 0.22 \text{ cm}^3 \text{cm}^{-3}$ ) categories to  
249 determine if stomatal sensitivity differs by soil moisture regime. For each  $G_s$  vs.  $\ln VPD$  linear

250 regression equation, parameters were estimated including  $G_{S,ref}$  (y-intercept or  $G_S$  at VPD = 1  
251 kPa), stomatal ( $G_S$ ) sensitivity (negative slope (m) of the regression line) and scaled  $G_S$   
252 sensitivity (negative slope/y-intercept).

### 253 **Biomass, leaf area and whole-tree water use efficiency**

254       Towards the end of the second growing season (October, 2019) but before significant leaf  
255 drop in genotypes that hold leaves until fall dormancy, biomass was determined for half (48  
256 individuals from one replicate block) of the individuals measured for sapflow. Ground line  
257 diameter (GLD) and diameter at breast height (DBH) were measured, then trees were cut and  
258 stem length was measured to estimate tree height. From the cut stem, sapwood diameter was  
259 estimated in two azimuthal directions. If trees were multi-stemmed, all stems greater than 2.53  
260 cm at their base were measured and harvested although only the height of the tallest stem was  
261 recorded. Small branches containing leaves were removed and placed into plastic bags for  
262 transport back to the lab. Defoliated branches and the main stem were weighed in the field to  
263 determine fresh green weight. A wood sample (with bark) from the main stem (about 10 cm  
264 long) was cut, its fresh field weight measured, and it was transported back to the lab. The  
265 volume of the wood sample was determined using volume displacement and it was placed in a  
266 drying oven at 105 °C and its dry weight was determined. For each individual, all collected  
267 leaves were removed from stems and their fresh weight was determined. A subsample of  
268 approximately 20 leaves was randomly collected and its fresh area (using a LI-COR 3100 leaf  
269 area meter; LI-COR Biosciences Inc., Lincoln, NE, USA) and fresh weight was obtained and  
270 used to calculate total leaf area from the total leaf fresh weight. Fresh weight of twigs with  
271 leaves removed were added to the weight of biomass samples measured in the field and dry  
272 biomass (kg) was estimated using the ratio of fresh to dry weight of the wood sample.

273 Whole-tree water use efficiency ( $WUE_T$ ; g biomass/kg water used) was calculated for  
274 each individual measured for sapflow for the period in which sapflow rates were measured.  
275 Monthly sapflow data for each individual were summed to calculate seasonal water use. For  
276 individuals that were not harvested as well as to determine biomass at the beginning of the  
277 sapflow period, linear or non-linear regression equations between dry biomass and basal area  $\times$   
278 height were determined for eastern cottonwood and hybrid poplars respectively (Table S2). The  
279 difference between dry biomass at the end and beginning of the sapflow period was divided by  
280 seasonal water use for each individual to calculate  $WUE_T$ .

### 281 **Statistical analysis**

282 Linear and non-linear regression relationships were fitted, and parameters for equations  
283 estimated, using Sigmaplot software. Biomass and water use parameters were compared across  
284 taxa using linear mixed effect models (nlme package; Pinheiro, Bates, DebRoy & Sarkar (2014))  
285 in R version 3.6.3 (R Core Team, 2014) including replicate block as a random effect in models.  
286 Tukey post-hoc tests were performed in R using the multcomp package (Hothorn, Bretz, &  
287 Westfall, 2008). Principal component analysis was performed in R using the prcomp function  
288 with all variables scaled and centered on the origin.

### 289 **RESULTS**

290 At low soil moistures ( $<0.22 \text{ cm}^3 \text{ cm}^{-3}$ ), canopy stomatal conductance ( $G_S$ ) increased at a  
291 faster rate for a given increase in soil moisture than that exhibited at average to high soil  
292 moistures ( $>0.22 \text{ cm}^3 \text{ cm}^{-3}$ ) with slope terms that were about five times greater under low vs.  
293 high soil moisture conditions (Table 1). At average to high soil moistures,  $G_S$  was much more  
294 consistent across a large soil moisture range with a low or near zero rate of change (Fig. 1).  
295 Taxa did not differ significantly in their responses of  $G_S$  to soil moisture exhibiting statistically

296 similar slope terms under both low and average to high soil moistures (Table 1) although both  
297 D×M and D×T/T×D genotypes exhibited a broader range in  $G_S$  at average to high soil moistures  
298 than D×N and D×D genotypes (Fig. 1). Given the different responses of  $G_S$  at low and average to  
299 high soil moistures, relationships between the natural log of vapor pressure deficit (lnVPD) and  
300  $G_S$  were examined under each soil moisture regime. For D×D, D×N and D×T/T×D genotypes,  
301 slopes (-m) of the relationship between  $G_S$  and lnVPD at low and average to high soil moistures  
302 did not differ significantly, while for D×M genotypes, slope terms were about 35% more  
303 negative ( $p < 0.001$ ) under average to high soil moisture compared with low soil moisture  
304 conditions (Fig. 2).

305         Because only one taxa exhibited significant differences based on soil moisture content,  
306 data were pooled across soil moisture categories to compare relationships between  $G_S$  and  
307 lnVPD across taxa and genotypes. D×M genotypes had the largest reference  $G_S$  ( $G_{S,ref}$ ) and  
308 greatest stomatal sensitivity (regression slope; -m) differing significantly from D×D and D×N  
309 genotypes (Table 1). Because individuals with larger  $G_{S,ref}$  will likely have greater calculated  
310 stomatal sensitivity given their larger conductances at low lnVPD, scaled stomatal sensitivity (-  
311  $m/G_{S,ref}$ ) was calculated to better compare across genotypes and taxa. D×D, D×T and T×D  
312 genotypes had scaled stomatal sensitivities that were significantly larger than the D×M  
313 genotypes, with D×N genotypes being statistically similar to all other taxa (Table 1). The D×D  
314 genotypes in October had significantly larger leaf areas than any of the hybrid taxa with the  
315 D×M genotypes also having significantly lower leaf areas than D×T genotypes (Table 1).  
316 Compared with calculated leaf areas based on October height and diameter, the D×M genotypes  
317 exhibited leaf losses that were about 1.9 times greater than D×N and D×T genotypes and about  
318 eight times greater than D×D genotypes.

319 At the end of the first and second growing seasons, D×D and D×M genotypes tended to  
320 be taller than D×N and D×T genotypes although only D×M genotypes differed significantly  
321 (Table 1). Dry biomass at the end of two growing seasons was statistically similar across D×D,  
322 D×M, D×T, and T×D genotypes with D×N genotypes having significantly lower biomass (Table  
323 1). Biomass growth in the second growing season did not differ significantly across taxa (Table  
324 1). Several D×M genotypes were at, or above, the 75<sup>th</sup> percentile in terms of two-year dry  
325 biomass including ‘7388’, ‘9711’, ‘9225’, ‘14591’ with ‘14507’, ‘13724’, ‘8002’ and ‘9709’  
326 being above the 90<sup>th</sup> percentile (Fig. 3a). For D×D genotypes, two-year dry biomass of both ‘6-  
327 5’ and ‘112107’ was at, or above, the 75<sup>th</sup> percentile and ‘6-4’ was near the 90<sup>th</sup> percentile (Fig.  
328 3a). For D×T genotypes, two-year dry biomass of both ‘7938’ and ‘10029’ was above the 75<sup>th</sup>  
329 percentile (Fig. 3a).

330 In terms of whole-tree water use efficiency ( $WUE_T$ ), D×M genotypes had significantly  
331 lower  $WUE_T$  than D×D, D×N or D×T genotypes (Table 1). For the D×M genotypes, only  
332 genotype ‘13700’ was above the 75<sup>th</sup> percentile in terms of  $WUE_T$  whereas D×D genotypes  
333 ‘S13C20’ and ‘6-1’ were at, or above, the 75<sup>th</sup> percentile and ‘112107’, ‘120-4’ and ‘6-4’ were  
334 above the 90<sup>th</sup> percentile in terms of  $WUE_T$  (Fig. 3). For D×N genotypes, ‘433’ and ‘11867’  
335 were above the 75<sup>th</sup> percentile and ‘11822’ was at the 90<sup>th</sup> percentile. For D×T genotypes,  
336 ‘10029’ and ‘8729’ were above the 75<sup>th</sup> percentile and ‘8717’ and ‘7903’ were at, or above, the  
337 90<sup>th</sup> percentile (Fig. 3).  $WUE_T$  did not exhibit a significant correlation with two-year dry  
338 biomass ( $p = 0.84$ ; Fig. 3a) but was significantly correlated with biomass growth occurring in the  
339 second growing season ( $p = 0.004$ ;  $r^2 = 0.13$ ) which is the year that seasonal water use was  
340 estimated (Fig. 3b). The D×M genotype ‘9189’, D×D genotypes ‘47-5’, ‘S13C20’ and ‘6-5’,  
341 D×N genotypes ‘433’ and ‘11822’, D×T genotype ‘8717’ and T×D ‘9755’ were above the 50<sup>th</sup>



342 percentiles in both two-year dry biomass and  $WUE_T$  (Fig. 3a). D×T genotype ‘10029’ was  
343 above the 75<sup>th</sup> percentile in both parameters and D×D genotypes ‘112107’ and ‘6-4’ were above  
344 the 90<sup>th</sup> percentile in  $WUE_T$  and above the 75<sup>th</sup> percentile in two-year dry biomass (Fig. 3a).

345 All measured parameters (Table 1) were incorporated into a principal component  
346 analysis to determine how parameters varied with one another and where individual genotypes  
347 were located based on multi-parameter principal components. PC1 captured the interaction  
348 between  $G_S$ , leaf area loss and  $WUE_T$  wherein trees with high PC1 scores had high reference  $G_S$   
349 ( $G_{S,ref}$ ) and stomatal sensitivity, high leaf area loss and low October leaf areas and  $WUE_T$  (Fig. 4;  
350 Table S3). Scaled  $G_S$  sensitivity exhibited a significant negative correlation with calculated leaf  
351 area loss of trees across taxa ( $p < 0.0001$ ;  $r^2 = 0.47$ ; Fig. 5) wherein trees that maintain leaf area  
352 throughout the growing season tend to exhibit high stomatal sensitivity. PC2 mainly captured  
353 variability in biomass parameters, scaled stomatal sensitivity, October leaf areas and changes in  
354  $G_S$  with soil moisture at low volumetric soil moistures ( $G_S$  slope, low  $\theta$ ). Trees with high PC2  
355 scores were smaller with low scaled stomatal sensitivity, October leaf areas and a low response  
356 of  $G_S$  to decreasing soil moisture levels (Fig. 4). D×M genotypes tended to cluster together in the  
357 upper right portion of the PC biplot (Fig. 4) exhibiting high leaf area losses, and low  $WUE_T$ ,  
358 October leaf areas and scaled stomatal sensitivity. D×D genotypes tended to cluster in the lower  
359 left portion of the PC biplot, opposite of D×M genotypes, with high  $WUE_T$ , October leaf areas  
360 and scaled stomatal sensitivity (Fig. 4). D×T/T×D genotypes were scattered across the biplot  
361 with some exhibiting high stomatal sensitivity (-m) and  $WUE_T$  (‘9552’ T×D, ‘9980’ D×T, ‘7903’  
362 D×T) and others exhibiting high biomass (‘10029’ D×T, ‘7938’ D×T; Fig. 4). Additionally,  
363 D×N genotypes did not cluster in any specific location in the biplot (Fig. 4).

364 Across all genotypes and taxa,  $WUE_T$  was negatively correlated with reference  $G_S$  ( $G_{S,ref}$ ;  
365  $p < 0.0001$ ;  $r^2 = 0.28$ ; Fig. 6a) and positively correlated with scaled stomatal sensitivity ( $p$   
366  $< 0.0001$ ;  $r^2 = 0.29$ ; Fig. 6b). In addition,  $WUE_T$  was positively and non-linearly correlated with  
367 leaf areas measured in October ( $p = 0.0002$ ;  $r^2 = 0.31$ ; Fig. 6c) and negatively correlated with  
368 calculated leaf area losses ( $p < 0.0001$ ;  $r^2 = 0.33$ ; Fig. 6d). Similarly to  $WUE_T$ , biomass growth  
369 in the second growing season was positively correlated with reference  $G_S$  ( $G_{S,ref}$ ;  $p = 0.002$ ;  $r^2 =$   
370  $0.18$ ; data not shown) and scaled stomatal sensitivity ( $p = 0.01$ ;  $r^2 = 0.12$ ) across genotypes and  
371 taxa (Fig. 7a). Genotypes ‘11822’ (D×N), ‘13724’ (D×M) and eastern cottonwood genotypes  
372 ‘47-5’ and ‘6-5’ were at, or above, the 75<sup>th</sup> percentile for biomass growth and above the 50<sup>th</sup>  
373 percentile for scaled  $G_S$  sensitivity. D×T genotypes ‘7938’ and ‘10029’ as well as eastern  
374 cottonwood genotypes ‘6-4’ and ‘112107’ were above the 90<sup>th</sup> percentile for biomass growth and  
375 at, or above, the 50<sup>th</sup> percentile in terms of scaled  $G_S$  sensitivity with D×D genotypes being  
376 above the 90<sup>th</sup> percentile in both categories (Fig. 7a). Biomass growth was positively correlated  
377 with changes in  $G_S$  with increasing soil moisture at low soil moisture contents ( $G_S$  slope, low  $\theta$ ;  $p$   
378  $= 0.02$ ;  $r^2 = 0.12$ ) across taxa and genotypes (Fig. 7b). However, genotypes that maintain  $G_S$   
379 with decreasing soil moisture (and hence exhibit low slope terms) could be considered to be  
380 more drought tolerant and several genotypes have both high biomass growth and lower changes  
381 in  $G_S$  with changes in soil moisture. For example, D×M genotypes ‘7388’ and ‘9225’ were  
382 above the 75<sup>th</sup> percentile in terms of biomass growth and below the 50<sup>th</sup> percentile in terms of  $G_S$   
383 response to low soil moisture while eastern cottonwood genotypes ‘112107’ and ‘6-4’ as well as  
384 D×M genotype ‘8002’ were above the 90<sup>th</sup> percentile in terms of biomass growth with a lower  
385 than average  $G_S$  response to soil moisture (Fig. 7b).

386 *Populus* taxa differed in their relationships between year two biomass growth and leaf  
387 area dynamics. For D×M genotypes, biomass growth was positively correlated with both  
388 October leaf areas ( $p = 0.009$ ;  $r^2 = 0.29$ ; Fig. 7c) and calculated leaf area losses ( $p = 0.003$ ;  $r^2 =$   
389  $0.37$ ; Fig. 7d). However, for D×D genotypes, biomass growth was positively correlated with  
390 October leaf areas ( $p < 0.0001$ ;  $r^2 = 0.85$ ; Fig. 7c) and marginally negatively correlated with leaf  
391 area losses ( $p = 0.05$ ;  $r^2 = 0.33$ ; Fig. 7d). D×N genotypes exhibited a positive correlation  
392 between biomass growth and October leaf areas ( $p = 0.01$ ;  $r^2 = 0.90$ ; Fig. 7c) and no correlation  
393 between biomass growth and leaf area loss ( $p = 0.50$ ; Fig. 7d). For D×T and T×D genotypes,  
394 neither October leaf areas ( $p = 0.34$ ; Fig. 7c) nor leaf area losses ( $p = 0.11$ ; Fig. 7d) were  
395 significantly correlated with biomass growth.

## 396 **DISCUSSION**

397 By selecting genotypes that exhibit high biomass and biomass growth, high water use  
398 efficiency, stomatal sensitivity and relatively low changes in canopy conductance ( $G_s$ ) with  
399 decreasing soil moisture, the most drought tolerant genotypes for the southeastern US can be  
400 selected for future testing and breeding. In addition, genotypes that maintain  $G_s$  at decreasing  
401 soil moisture may also sequester more belowground carbon (Sanchez et al., 2007; Stanton et al.,  
402 2002) in roots compared with individuals that are more responsive to changes in soil moisture.  
403 Two eastern cottonwood genotypes, ‘112107’ and ‘6-4’, scored high on all metrics being above  
404 the 90<sup>th</sup> percentile in terms of water use efficiency, scaled  $G_s$  sensitivity and were below average  
405 in terms of their  $G_s$  response to low soil moisture. Eastern cottonwood genotypes ‘47-5’ and ‘6-  
406 5’ also exhibited higher than average water use efficiency and stomatal sensitivity. Giovannelli  
407 et al. (2007) also found that an eastern cottonwood genotype was more drought tolerant than a  
408 D×N genotype. For hybrid poplar genotypes, D×N ‘11822’ was near the 90<sup>th</sup> percentile in terms

409 of water use efficiency and above the 50<sup>th</sup> percentile in terms of stomatal sensitivity, and D×T  
410 ‘10029’ had above average stomatal sensitivity and was above the 75<sup>th</sup> percentile in terms of  
411 water use efficiency. Bonhomme et al. (2008) found that one D×N genotype, ‘Soligo’, had high  
412 water use efficiency in both alluvial and non-alluvial sites compared with T×D genotypes. D×M  
413 genotypes tended to be below the 50<sup>th</sup> percentile in terms of water use efficiency, but genotypes  
414 ‘8002’, ‘7388’ and ‘9225’ were better than average in terms of smaller changes in  $G_s$  with  
415 decreasing soil moisture suggesting drought tolerance. In addition, if water is not limited or high  
416 water use (low water use efficiency) is desired in terms of phytoremediation (Bonhomme et al.,  
417 2008), several D×M genotypes exhibited high biomass growth (at or above the 75<sup>th</sup> percentile),  
418 and below average water use efficiency and low scaled  $G_s$  sensitivity including genotypes  
419 ‘9709’, ‘14507’, ‘9225’, ‘8002’ and ‘7388’.

420 In support of our hypothesis, we found that the tested *Populus* taxa exhibited differing  
421 water use strategies, with eastern cottonwood and D×M genotypes specifically differing from  
422 one another. While eastern cottonwoods tended to maintain high leaf areas and strong stomatal  
423 sensitivity to control water use and achieve high  $WUE_T$  (Muries et al., 2019), D×M genotypes  
424 exhibited larger leaf area losses with less stomatal sensitivity and lower  $WUE_T$ . Attia et al.  
425 (2015) found that anisohydric poplars that exhibit low stomatal control had lower water use  
426 efficiency than isohydric poplar genotypes that exhibited higher water use efficiency. Several  
427 other studies found that hybrid poplars exhibit leaf area loss as a drought avoidance strategy  
428 (Arango-Velez, Zwiazek, Thomas, & Tyree, 2011; Giovannelli et al., 2007; Monclus et al., 2006;  
429 Monclus et al., 2009) which could reallocate nutrients and resources to newly created leaves  
430 (Arango-Velez et al., 2011; Chaves, Maroco, & Pereira, 2003). We also found that leaf area  
431 losses and scaled stomatal sensitivity were inversely correlated across genotypes wherein trees

432 that lost significant leaf area throughout the growing season exhibited less stomatal sensitivity  
433 suggesting differing distinct strategies to deal with water stress.

434 D×N, D×T and T×D genotypes tended to fall between the eastern cottonwood and D×M  
435 genotypes in terms of leaf area losses and stomatal sensitivity. *P. trichocarpa* tends to be less  
436 drought tolerant and water use efficient compared with eastern cottonwood (Bassman & Zwier,  
437 1991) and hybrids of the two species should exhibit more drought tolerance than *P. trichocarpa*  
438 (Bassman & Zwier, 1991; Maier et al., 2019). In fact, Zalesny et al. (2019) found that eastern  
439 cottonwood genotypes exhibited relatively low WUE compared with backcross (T×D)×D  
440 genotypes which exhibited high WUE and Ghezehei et al. (2019) showed that growth of eastern  
441 cottonwood genotypes was more dependent on favorable site conditions compared with T×D and  
442 D×M genotypes likely because eastern cottonwood improved genotypes were selected from field  
443 trials on fertile, alluvial sites. Marron et al. (2007) reported that D×T genotypes were more  
444 productive than D×N genotypes, with our study also finding that D×N genotypes exhibited the  
445 lowest biomass and biomass growth at the southeastern U.S. site.

446 We found that while two-year biomass was not correlated with  $WUE_T$ , biomass growth in  
447 the second growing season was significantly, positively correlated with  $WUE_T$ . Several other  
448 studies report a positive correlation between biomass or growth rate and water use efficiency  
449 parameters (Maier et al., 2019; Monclus et al., 2009; Pilipović, Headlee, Zalesny Jr, Pekeč, &  
450 Bauer, 2022; Renninger et al., 2021) while other studies found no correlation (Dillen et al., 2011;  
451 Marron et al., 2005; Monclus et al., 2005; Monclus et al., 2006; Rae et al., 2004; Zhao, Jiang,  
452 Zhang, Jiang, & Cai, 2021). Across all genotypes, we found a mean  $WUE_T$  of about 4.14 g  
453 biomass/kg H<sub>2</sub>O with 10% of genotypes falling below 2.29 g/kg and 10% of genotypes falling  
454 above 5.98 g/kg. These  $WUE_T$  estimates compare well with other whole tree or whole stand

455 estimates of water use efficiency in *Populus* which range from 1.7 – 7.9 g biomass/kg water  
456 (assuming 50% C for studies that report gC/kg H<sub>2</sub>O) for studies that estimated water use  
457 efficiency using sapflow and biomass data (Kim, Oren, & Hinckley, 2008; Maier et al., 2019)  
458 and eddy covariance (Jones et al., 2017; Migliavacca et al., 2009; Xu et al., 2020) techniques.  
459 The similarity in ranges of WUE<sub>T</sub> across studies suggests that our whole tree water use estimates  
460 are not under- or overestimated and that assumptions of constant sapflow with sapwood depth  
461 are appropriate. However, for taxa including D×M, low WUE<sub>T</sub> may be due to an overestimation  
462 of water use based on the assumption of completely functional sapwood in the cross section and  
463 constant sapflow with sapwood depth. More research is needed to determine if sapflow rates  
464 decline with depth in D×M genotypes.

465         In this study we found that the most water use efficient genotypes had the lowest  
466 reference canopy conductance and highest stomatal sensitivity and tended to have high leaf areas  
467 that were retained throughout the growing season. Marron et al. (2005) reported that carbon  
468 isotope discrimination, an indirect measure of water use efficiency, was also correlated with leaf  
469 area in terms of the total numbers of leaves on trees. Genotypes exhibiting high WUE may be  
470 less susceptible to drought stress compared with genotypes with low WUE (Yin, Wang, Duan,  
471 Luo, & Li, 2005) however genotypes may have other strategies for coping with drought stress  
472 including leaf area reductions (Monclus et al., 2006). We determined that biomass growth was  
473 also positively correlated with stomatal sensitivity and the rate of change in canopy conductance  
474 with changes in soil moisture content under low soil moistures. Maier et al. (2019) also reported  
475 that slow growing *Populus* genotypes tended to exhibit lower stomatal sensitivity compared with  
476 more productive genotypes. However, Attia et al. (2015) found that anisohydric genotypes  
477 which potentially exhibit lower stomatal sensitivity were more productive than more sensitive,

478 isohydric genotypes under well-watered conditions. In response to low soil moisture or soil  
479 water stress conditions, we showed that genotypes that were more responsive in terms of  
480 stomatal closure to decreasing soil moisture had greater biomass growth. However, Arango-  
481 Velez et al. (2011) reported that stomatal responsiveness to drought conditions did not explain  
482 differences in growth for measured hybrid poplars and balsam poplars although a smaller  
483 number of genotypes were tested.

484 We found that biomass growth was positively correlated with leaf area measured in  
485 October for both D×D and D×M genotypes even though these taxa exhibited differing water use  
486 strategies with D×D genotypes having higher stomatal sensitivity and  $WUE_T$  and D×M  
487 genotypes having greater leaf area loss and lower sensitivity. Several other studies also reported  
488 that leaf area was positively correlated with biomass (Marron et al., 2005; Monclus et al., 2005;  
489 Pellis, Laureysens, & Ceulemans, 2004; Pregitzer, Dickmann, Hendrick, & Nguyen, 1990; Rae  
490 et al., 2004). However, D×D and D×M genotypes had opposing relationships between biomass  
491 growth and calculated leaf area losses throughout the growing season, wherein there was a  
492 negative correlation for D×D genotypes, but a positive correlation in D×M genotypes. Similar to  
493 D×D genotypes, Verlinden et al. (2013) showed that leaf area duration was positively correlated  
494 with biomass production in 12 hybrid poplar genotypes. For D×M genotypes, this negative  
495 relationship may be driven by the tendency for larger trees with the potential for greater biomass  
496 growth to have more leaf area to lose compared with less productive genotypes.

## 497 **CONCLUSIONS**

498 Overall, we found that taxa differed in their water use strategies, particularly D×D and  
499 D×M genotypes, however certain D×D, D×M and D×T genotypes exhibited high two-year  
500 biomass (around 6 kg/tree) and biomass growth across the two-year study period. D×D

501 genotypes exhibited high stomatal sensitivities and water use efficiency and held leaves longer  
502 into the growing season while D×M genotypes were less water use efficient, lost significant leaf  
503 area throughout the growing season, and tended to exhibit less stomatal sensitivity. Across all  
504 taxa, genotypes with lower reference canopy conductance, higher scaled stomatal sensitivity,  
505 large October leaf areas and low leaf area loss throughout the growing season tended to be the  
506 most water use efficient on a whole-tree level. Across taxa, biomass growth during the second  
507 growing season was positively correlated with whole tree water use efficiency, scaled stomatal  
508 sensitivity, October leaf area and the rate of change in  $G_S$  with decreases in soil moisture under  
509 dry conditions. Taken together, these data support our hypothesis and shed light on genotypes  
510 that may show promise on drier sites given their high biomass growth coupled with stomatal  
511 sensitivity, water use efficiency and low stomatal response under dry soil conditions. Likewise,  
512 genotypes with high biomass growth coupled with high water use and low stomatal sensitivity  
513 may be optimal for wetter sites and/or for phytoremediation purposes. However, future research  
514 is necessary to determine the longer-term viability of identified genotypes in terms of continued  
515 high productivity, disease resistance (particularly to *Septoria* stem canker; Kaczmarek, Coyle &  
516 Coleman (2013)), coppice production potential and disease resistance following coppice.  
517 Overall, genotype-specific data on water use strategies across individuals with large genetic  
518 variability provide ranges for *Populus* physiological parameters which can be used to refine  
519 model estimates of *Populus* production and ecosystem impacts under a variety of climate  
520 conditions (Wang, LeBauer, & Dietze, 2013) and inform future *Populus* breeding efforts.

## 521 **ACKNOWLEDGEMENTS**

522 The authors would like to thank C. James, M. Murphy, T. Bowling, H. Stapleton, W. Booth and  
523 T.Y. Kyaw for fieldwork assistance This research was supported by grants from the National



524 Institute of Food and Agriculture, U.S. Department of Agriculture (2018-68005-27636 and  
525 McIntire-Stennis project MISZ- 067050) and the United States Department of Energy (DE-  
526 EE0009280). This publication is a contribution of the Forest and Wildlife Research Center,  
527 Mississippi State University.

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728 **TABLE 1:** Height, biomass and physiological parameters related to water use strategies for *Populus deltoides* W. Bartram ex  
729 Marshall (D) as well as hybrids of *P. deltoides* crossed (×) with *P. maximowiczii* A. Henry (M), *P. nigra* L. (N) and *P. trichocarpa*  
730 Torr. & Gray (T). Ref.  $G_S$  = reference canopy conductance ( $G_S$ ) at vapor pressure deficit (VPD) = 1 kPa;  $G_S$  sensitivity = slope of  
731 regression between  $G_S$  and  $\ln$ VPD; scaled  $G_S$  sensitivity =  $G_S$  sensitivity/ref.  $G_S$ ;  $G_S$  slope low/high  $\theta$  = slope of regression between  
732  $G_S$  and soil moisture ( $\theta$ ) under low or high  $\theta$ ; WUE = water use efficiency. Means with different superscript letters differed  
733 significantly at  $p < 0.05$ .

<b>Parameter</b>	<b>D×D</b>	<b>D×M</b>	<b>D×N</b>	<b>D×T</b>	<b>T×D</b>
$G_S$ slope, low $\theta$	2.95 (0.4) <sup>a</sup>	2.34 (0.4) <sup>a</sup>	2.17 (0.6) <sup>a</sup>	3.12 (0.7) <sup>a</sup>	1.78 (0.24) <sup>a</sup>
$G_S$ slope, high $\theta$	0.37 (0.1) <sup>a</sup>	0.68 (0.1) <sup>a</sup>	0.43 (0.1) <sup>a</sup>	0.47 (0.2) <sup>a</sup>	0.60 (0.2) <sup>a</sup>
Ref. $G_S$ ( $G_{S,ref}$ ; mol m <sup>-2</sup> s <sup>-1</sup> )	0.15 (0.01) <sup>b</sup>	0.27 (0.01) <sup>a</sup>	0.16 (0.02) <sup>b</sup>	0.21 (0.03) <sup>ab</sup>	0.16 (0.02) <sup>ab</sup>
$G_S$ sensitivity (-m; mol m <sup>-2</sup> s <sup>-1</sup> kPa <sup>-1</sup> )	0.11 (0.008) <sup>b</sup>	0.17 (0.009) <sup>a</sup>	0.10 (0.02) <sup>b</sup>	0.14 (0.01) <sup>ab</sup>	0.12 (0.02) <sup>ab</sup>
Scaled $G_S$ sensitivity (m/ $G_{S,ref}$ )	0.72 (0.009) <sup>a</sup>	0.60 (0.01) <sup>b</sup>	0.65 (0.03) <sup>ab</sup>	0.67 (0.01) <sup>a</sup>	0.74 (0.02) <sup>a</sup>
Oct. leaf area (m <sup>2</sup> )	4.2 (0.3) <sup>a</sup>	1.2 (0.1) <sup>c</sup>	1.7 (0.4) <sup>bc</sup>	2.2 (0.1) <sup>b</sup>	2.3 (0.4) <sup>bc</sup>
Leaf area loss (m <sup>2</sup> )	0.4 (0.2) <sup>c</sup>	3.2 (0.1) <sup>a</sup>	1.7 (0.3) <sup>b</sup>	1.7 (0.2) <sup>b</sup>	1.3 (0.3) <sup>bc</sup>
Year 1 height (m)	4.1 (0.1) <sup>ab</sup>	4.4 (0.1) <sup>a</sup>	3.8 (0.3) <sup>b</sup>	3.8 (0.2) <sup>b</sup>	3.7 (0.3) <sup>ab</sup>
Year 2 height (m)	6.6 (0.2) <sup>ab</sup>	6.7 (0.2) <sup>a</sup>	5.9 (0.4) <sup>b</sup>	6.1 (0.2) <sup>ab</sup>	6.0 (0.4) <sup>ab</sup>
Year 2 dry biomass (kg)	5.1 (0.3) <sup>ab</sup>	5.2 (0.3) <sup>a</sup>	3.5 (0.6) <sup>b</sup>	5.1 (0.5) <sup>ab</sup>	4.1 (0.6) <sup>ab</sup>
Year 2 biomass growth (kg)	2.3 (0.2) <sup>a</sup>	2.2 (0.1) <sup>a</sup>	1.9 (0.3) <sup>a</sup>	2.8 (0.3) <sup>a</sup>	1.6 (0.4) <sup>a</sup>
Seasonal water use (kg/tree)	678 (59) <sup>b</sup>	944 (64) <sup>a</sup>	534 (97) <sup>b</sup>	769 (98) <sup>ab</sup>	570 (90) <sup>ab</sup>

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Whole-tree WUE (WUE <sub>T</sub> ; g/kg)	4.79 (0.4) <sup>a</sup>	3.26 (0.2) <sup>b</sup>	4.88 (0.6) <sup>a</sup>	5.43 (0.7) <sup>a</sup>	3.71 (0.5) <sup>ab</sup>
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735 **FIGURE LEGENDS**

736 **FIGURE 1:** Canopy-scaled stomatal conductance ( $G_s$ ;  $\text{mol m}^{-2} \text{s}^{-1}$ ) vs. volumetric soil moisture  
737 content ( $\text{cm}^3 \text{cm}^{-3}$ ) in the top 30 cm of soil for **a)** *Populus deltoides* W. Bartram ex Marshall  $\times$  *P.*  
738 *deltoides* (D $\times$ D), **b)** *P. deltoides*  $\times$  *P. maximowiczii* A. Henry (D $\times$ M), **c)** *P. deltoides*  $\times$  *P. nigra*  
739 L. (D $\times$ N) and **d)** *P. deltoides*  $\times$  *P. trichocarpa* Torr. & Gray (D $\times$ T)/ *P. trichocarpa*  $\times$  *P. deltoides*  
740 (T $\times$ D). Linear regression equations were fitted in both low ( $<0.22 \text{ cm}^3 \text{ cm}^{-3}$ ) and average to high  
741 ( $>0.22 \text{ cm}^3 \text{ cm}^{-3}$ ) volumetric soil moisture content and include best fit lines (solid), 95%  
742 confidence intervals (dashed) and 95% prediction intervals (dotted lines).

743 **FIGURE 2:** Canopy-scaled stomatal conductance ( $G_s$ ;  $\text{mol m}^{-2} \text{s}^{-1}$ ) vs. the natural log of vapor  
744 pressure deficit (ln kPa) under dry (light symbols) and average to high soil moisture (dark  
745 symbols for **a)** *Populus deltoides* W. Bartram ex Marshall  $\times$  *P. deltoides* (D $\times$ D), **b)** *P. deltoides*  
746  $\times$  *P. maximowiczii* A. Henry (D $\times$ M), **c)** *P. deltoides*  $\times$  *P. nigra* L. (D $\times$ N) and **d)** *P. deltoides*  $\times$  *P.*  
747 *trichocarpa* Torr. & Gray (D $\times$ T)/ *P. trichocarpa*  $\times$  *P. deltoides* (T $\times$ D). Linear regression  
748 equations were fitted to both low (gray lines) and average to high soil moisture (black lines) and  
749 include best fit lines (solid) and 95% confidence intervals (dashed).

750 **FIGURE 3:** Whole tree water use efficiency ( $\text{WUE}_T$ ; g biomass/kg water) vs. **a)** two-year dry  
751 woody biomass on a per tree level (kg/tree; left axis) and per hectare level (Mg/ha; right axis  
752 assuming 100% survival and study spacing) and **b)** biomass growth during the second growing  
753 season ( $y = 1.66x + 0.97$ ) on a per tree (left axis) and per hectare (right axis) level. Genotypes are  
754 shown in the *Populus deltoides* W. Bartram ex Marshall  $\times$  *P. deltoides* (D $\times$ D; red), *P. deltoides*  
755  $\times$  *P. maximowiczii* A. Henry (D $\times$ M; green), *P. deltoides*  $\times$  *P. nigra* L. (D $\times$ N; blue), *P. deltoides*  
756  $\times$  *P. trichocarpa* Torr. & Gray (D $\times$ T; purple) and *P. trichocarpa*  $\times$  *P. deltoides* (T $\times$ D; dark  
757 purple) taxa.

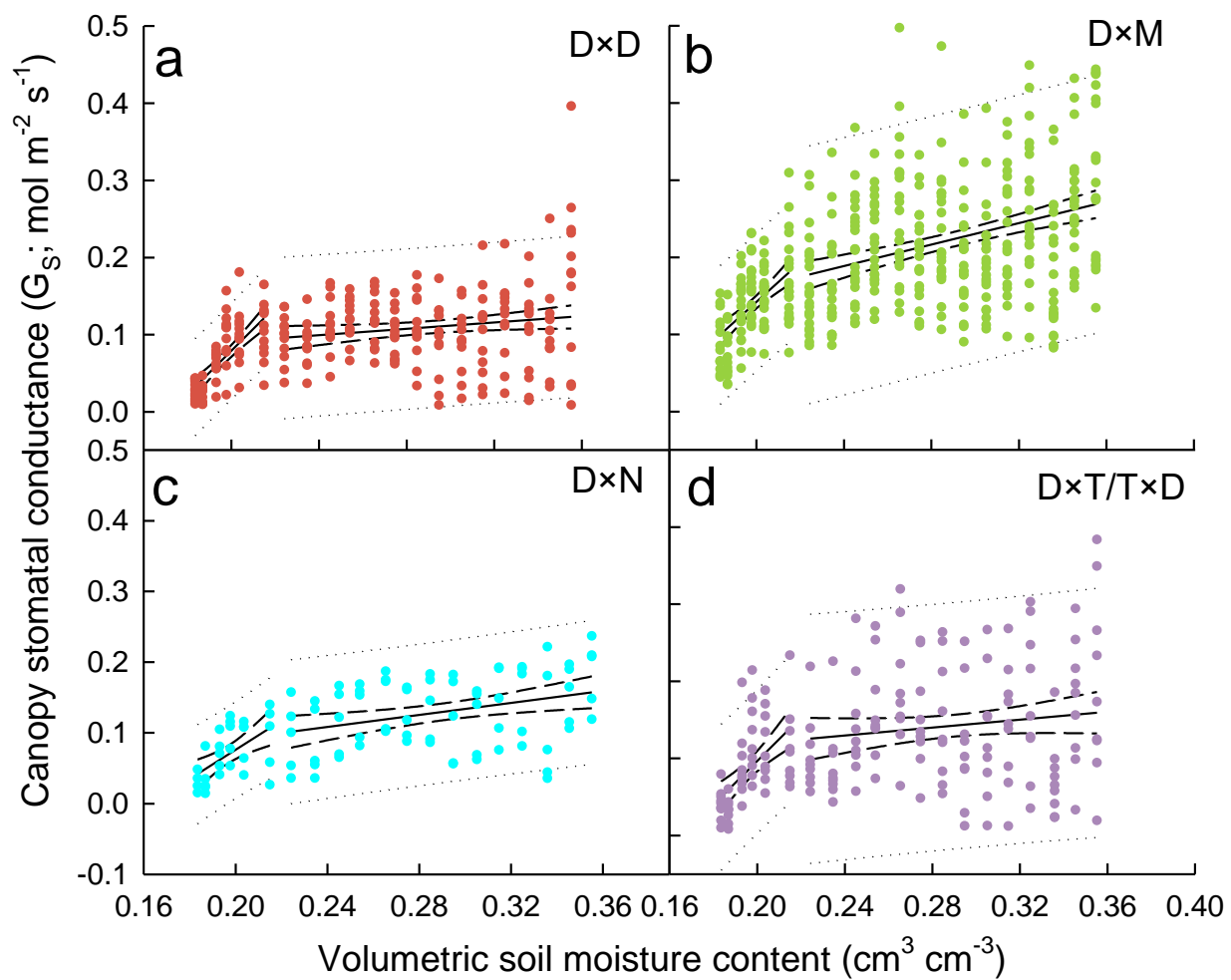
758 **FIGURE 4:** Principal component analysis (PCA) biplot showing the loading of physiological  
759 parameters (black arrows) in relation to PC1 and PC2 and the PC scores for each genotype in the  
760 *Populus deltoides* W. Bartram ex Marshall  $\times$  *P. deltoides* (D $\times$ D; red), *P. deltoides*  $\times$  *P.*  
761 *maximowiczii* A. Henry (D $\times$ M; green), *P. deltoides*  $\times$  *P. nigra* L. (D $\times$ N; blue), *P. deltoides*  $\times$  *P.*  
762 *trichocarpa* Torr. & Gray (D $\times$ T; light purple) and *P. trichocarpa*  $\times$  *P. deltoides* (T $\times$ D; dark  
763 purple) taxa.  $G_{S,ref}$  = reference canopy conductance ( $G_S$ ) at vapor pressure deficit (VPD) = 1 kPa;  
764  $m$  = stomatal sensitivity or slope of regression between  $G_S$  and  $\ln$ VPD; low/high  $\theta$  slope = slope  
765 of regression between  $G_S$  and soil moisture ( $\theta$ ) under low/high  $\theta$ ;  $WUE_T$  = tree level water use  
766 efficiency.

767 **FIGURE 5:** Relationship between scaled sensitivity of canopy conductance ( $G_S$ ) to vapor  
768 pressure deficit (VPD; slope of relationship ( $m$ )/reference  $G_S$ ) and calculated leaf area losses  
769 ( $m^2$ ) occurring between May and October ( $y = -0.037x + 0.73$ ) for *Populus deltoides* W. Bartram  
770 ex Marshall  $\times$  *P. deltoides* (D $\times$ D; red), *P. deltoides*  $\times$  *P. maximowiczii* A. Henry (D $\times$ M; green),  
771 *P. deltoides*  $\times$  *P. nigra* L. (D $\times$ N; blue), *P. deltoides*  $\times$  *P. trichocarpa* Torr. & Gray (D $\times$ T; light  
772 purple) and *P. trichocarpa*  $\times$  *P. deltoides* (T $\times$ D; dark purple) taxa.

773 **FIGURE 6:** Whole-tree water use efficiency ( $WUE_T$ ; g biomass/kg water used) vs. **a)** reference  
774 canopy conductance ( $G_S$ ; mol  $m^{-2} s^{-1}$  estimated at vapor pressure deficit (VPD) = 1 kPa), **b)**  
775 scaled  $G_S$  sensitivity to VPD (slope of relationship/ reference  $G_S$ ), **c)** leaf areas measured in  
776 October ( $m^2$ ), and **d)** differences between leaf areas calculated for May and measured in October  
777 ( $m^2$ ) for *Populus deltoides* W. Bartram ex Marshall  $\times$  *P. deltoides* (D $\times$ D; red), *P. deltoides*  $\times$  *P.*  
778 *maximowiczii* A. Henry (D $\times$ M; green), *P. deltoides*  $\times$  *P. nigra* L. (D $\times$ N; blue), *P. deltoides*  $\times$  *P.*  
779 *trichocarpa* Torr. & Gray (D $\times$ T; light purple) and *P. trichocarpa*  $\times$  *P. deltoides* (T $\times$ D; dark  
780 purple) taxa.

781 **FIGURE 7:** Biomass growth (kg/tree) in the second growing season vs. **a)** scaled canopy  
782 conductance ( $G_s$ ) sensitivity to vapor pressure deficit (VPD; slope of relationship/ reference  $G_s$ ;  
783 y-intercept), **b)** slope of regression between  $G_s$  and soil moisture ( $\theta$ ) under low  $\theta$  conditions, **c)**  
784 tree leaf areas ( $m^2$ ) measured in October and **d)** differences between leaf areas calculated for  
785 May and measured in October ( $m^2$ ) for *Populus deltoides* W. Bartram ex Marshall  $\times$  *P. deltoides*  
786 ( $D \times D$ ; red), *P. deltoides*  $\times$  *P. maximowiczii* A. Henry ( $D \times M$ ; green), *P. deltoides*  $\times$  *P. nigra* L.  
787 ( $D \times N$ ; blue), *P. deltoides*  $\times$  *P. trichocarpa* Torr. & Gray ( $D \times T$ ; light purple) and *P. trichocarpa*  
788  $\times$  *P. deltoides* ( $T \times D$ ; dark purple) taxa.  
789

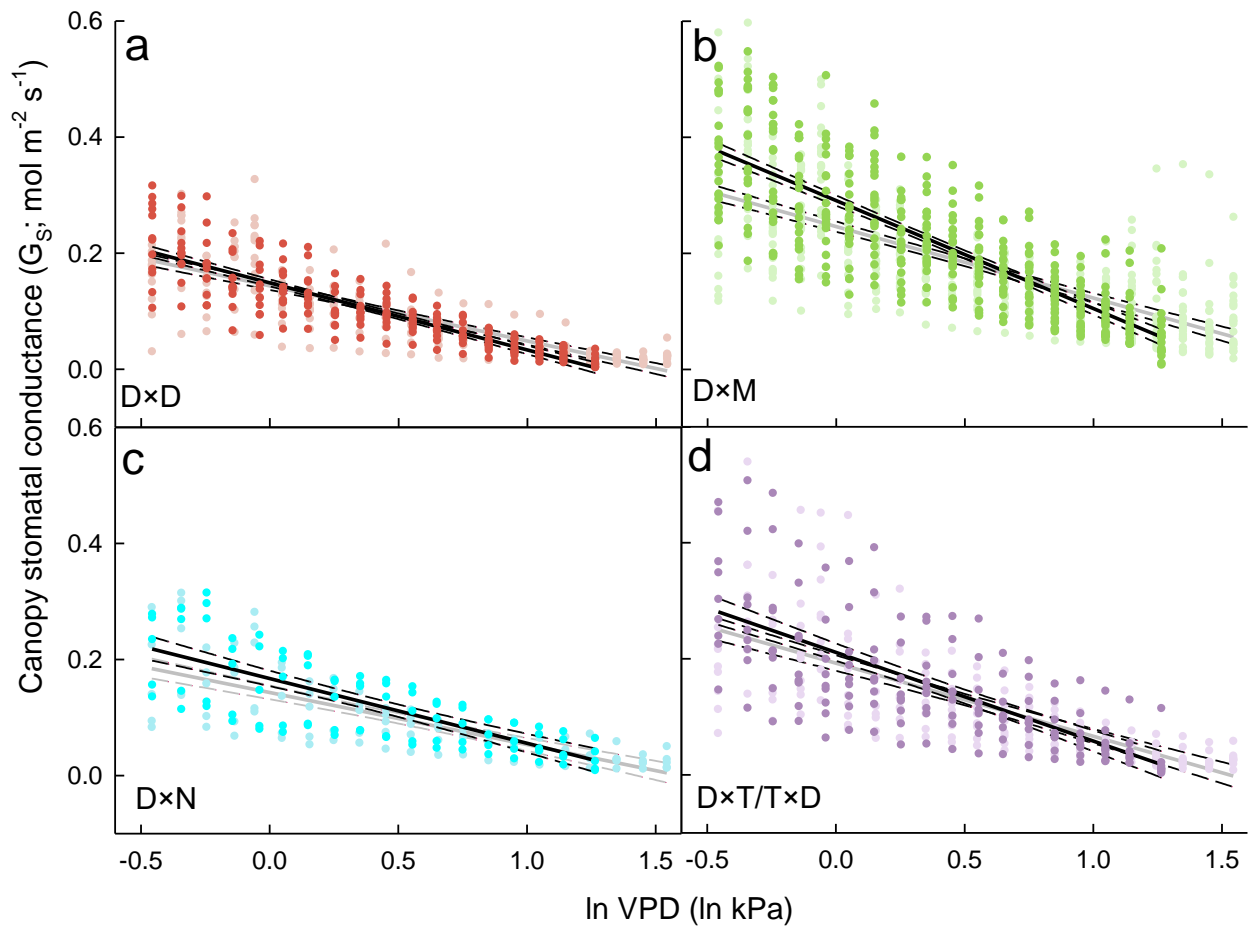
790 **FIGURE 1:**



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793 **FIGURE 2**

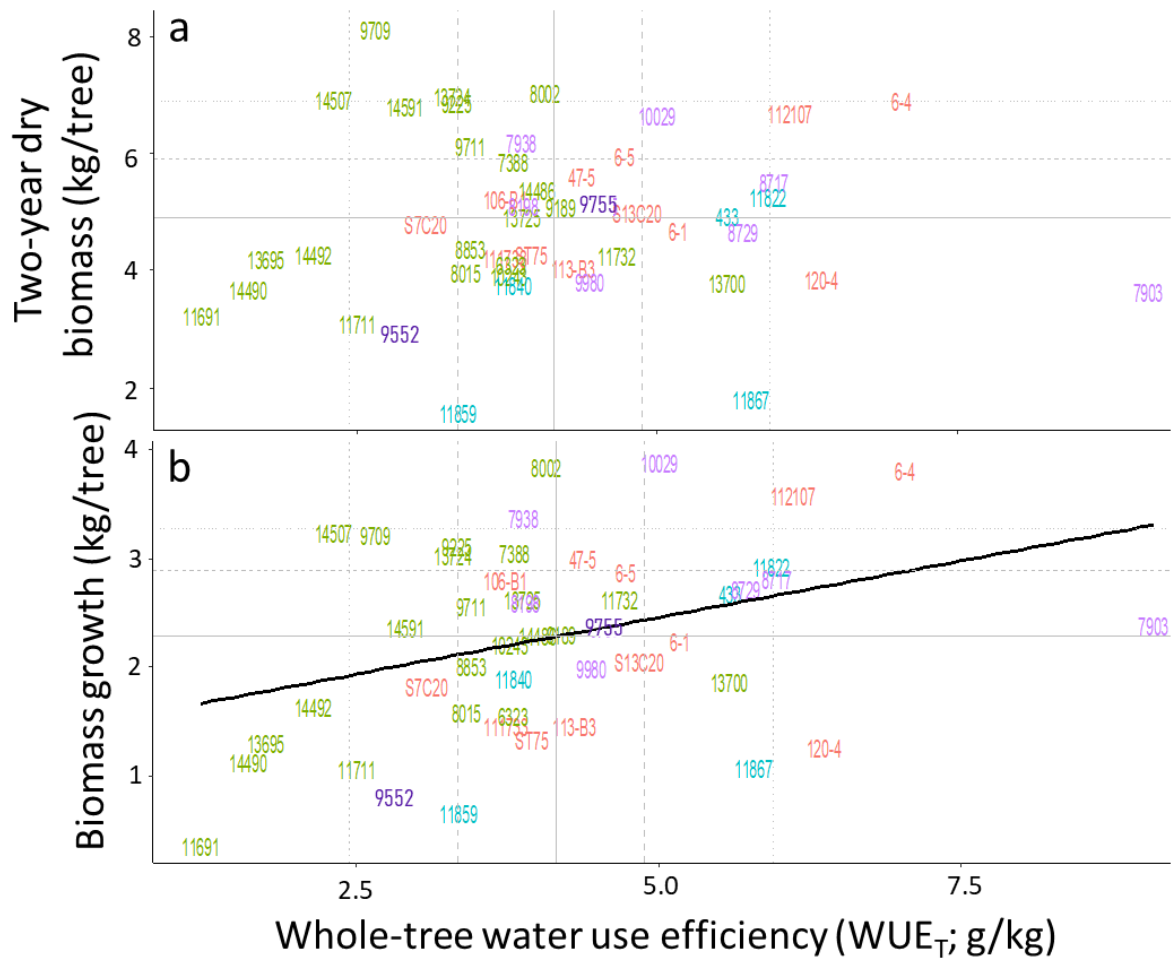


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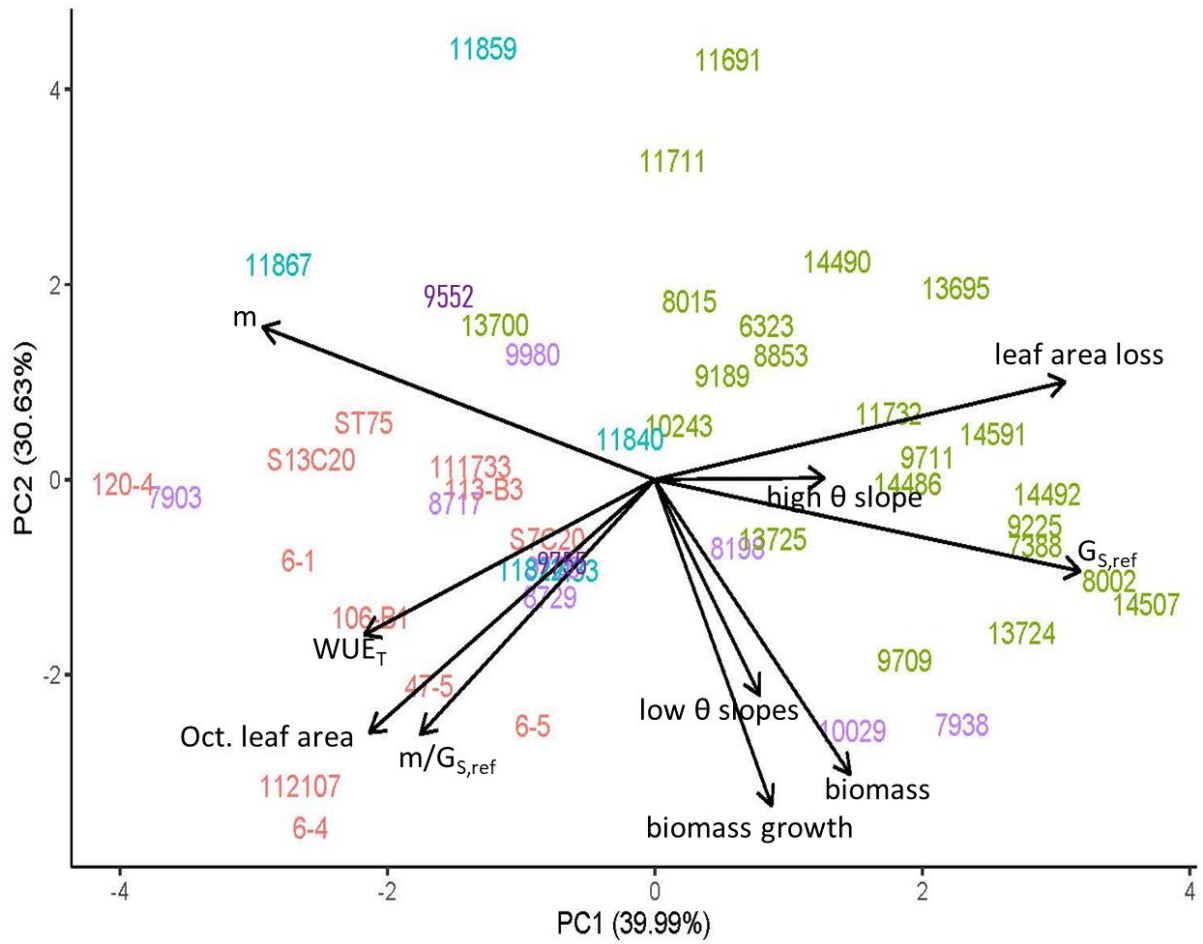
796 **FIGURE 3**



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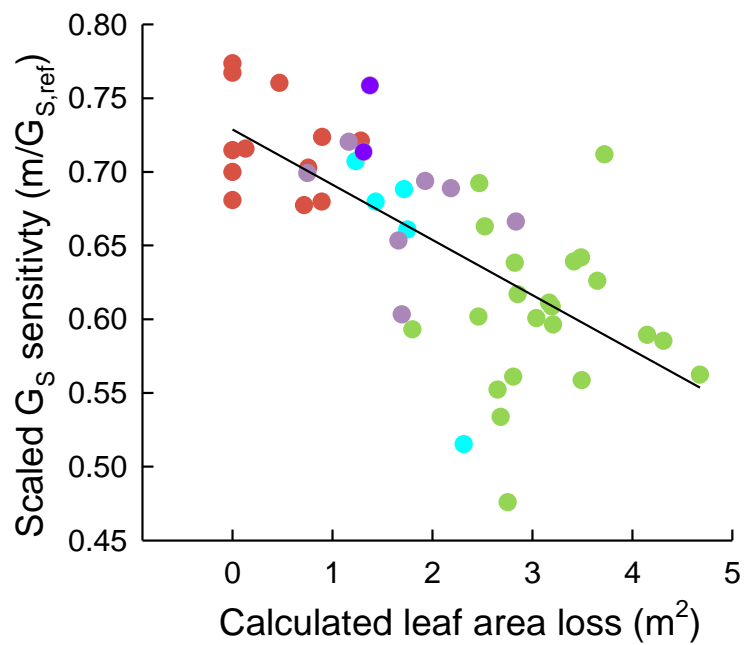
799 **FIGURE 4**



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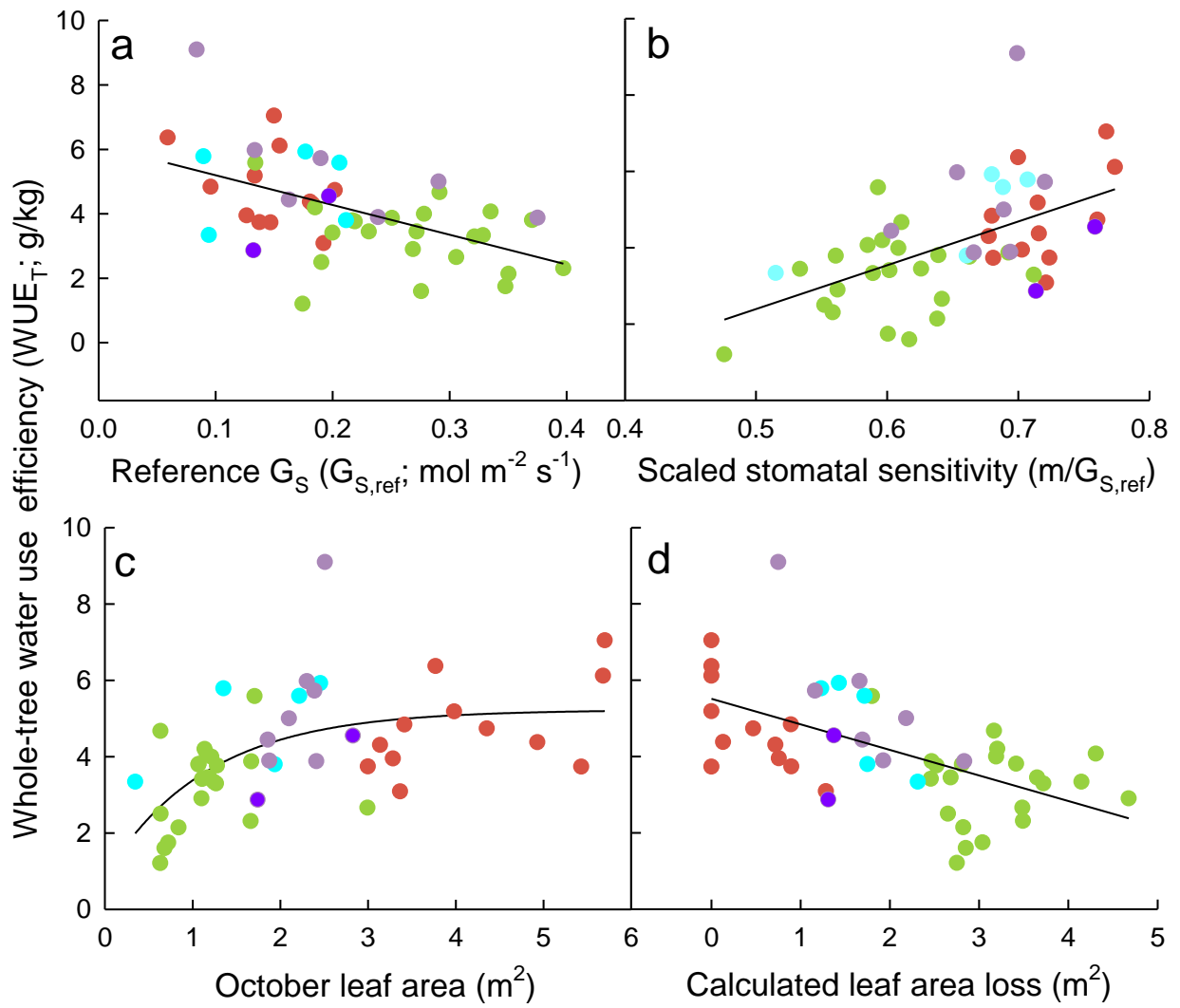
802 **FIGURE 5**



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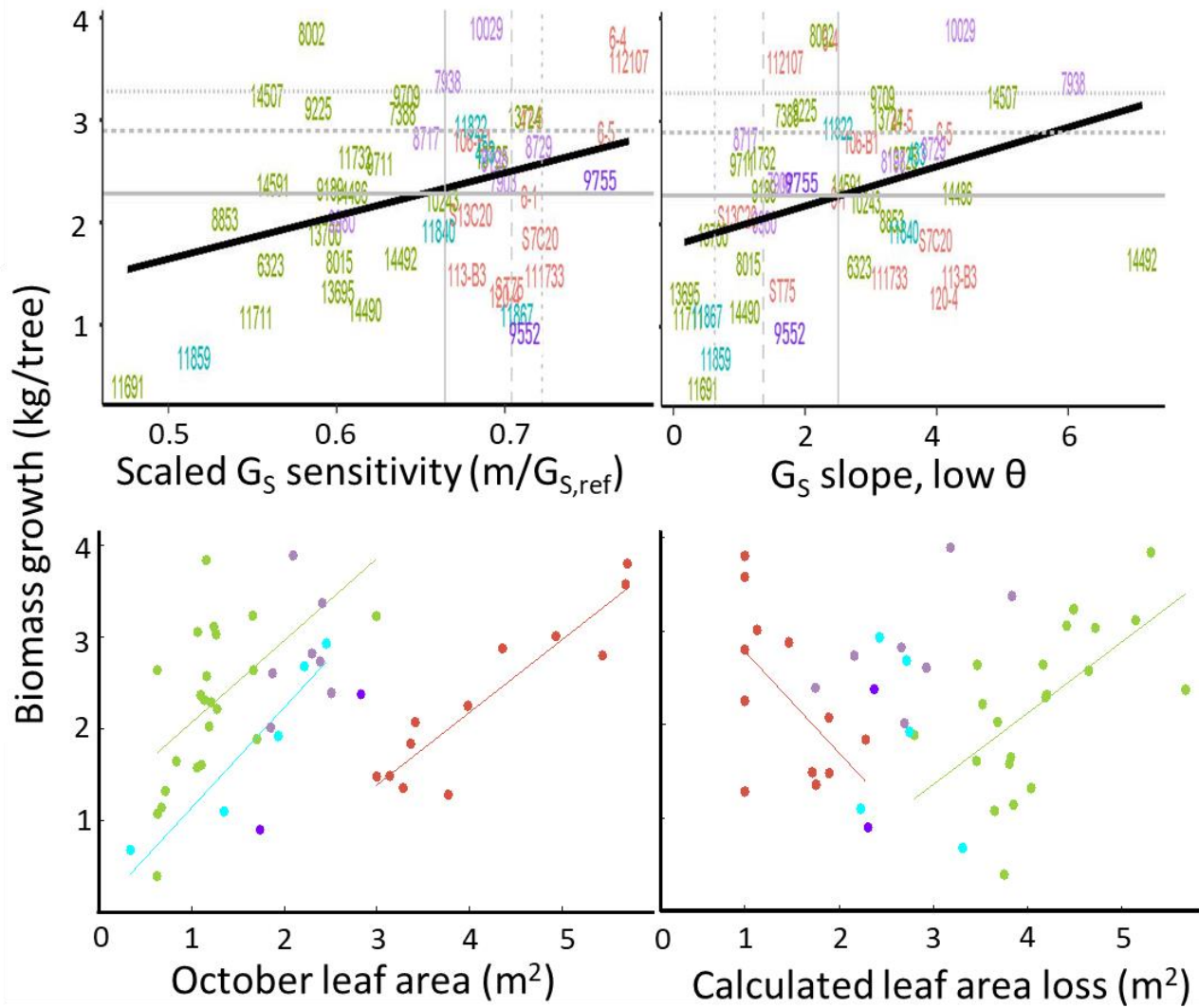
805 **FIGURE 6:**



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808 **FIGURE 7:**



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