Impacts of a partial rainfall exclusion in the field on growth and transpiration: consequences for leaf-level and whole-plant water-use efficiency compared to controlled conditions

Maxime Durand, Oliver Brendel, Cyril Buré, Pascal Courtois, Jean-Baptiste Lily, André Granier, Didier Le Thiec

1 **TITLE**

2 Impacts of a partial rainfall exclusion in the field on growth and transpiration: consequences for

3 leaf-level and whole-plant water-use efficiency compared to controlled conditions.

4 **AUTHORS**

5 Maxime Durand[1], Oliver Brendel[1], Cyril Buré[1], Pascal Courtois[1], Jean-Baptiste Lily[1], André

6 Granier[1] and Didier Le Thiec[1]

7 [1] Inra, Université de Lorraine, AgroParisTech, SILVA, F-54280 Champenoux, France

8 **AUTHOR FOR CORRESPONDANCE**

9 Didier Le Thiec

10 Email: didier.lethiec@inra.fr

11 Phone: (+33) 3 83 39 40 98

12 Address: UMR 1434 Silva, Centre Inra Grand-Est

13 Rue d'Amance, 54280 CHAMPENOUX, France.

14

No. of figures:	8 (figure 2 in colors)
No. of Tables:	1
No of Supporting Information files:	6 (4 figures, 2 tables)

15

ABBREVIATIONS

16 **ABBREVIATIONS**

17 WUE: water use efficiency

18 Wi: leaf intrinsic water use efficiency measured by leaf gas exchange (A/g_s)

19 TE: whole-plant transpiration efficiency (biomass production over water used, DM_T/WU)

20 Δ: carbon isotope discrimination

21 δ: carbon isotope composition

22 A: net CO_2 assimilation

23 C_i: internal CO_2 concentration

24 g_s: stomatal conductance to water vapour

25 WU: whole plant water use

26 E: daily whole-plant transpiration per unit area

27 VPD: vapour pressure deficit

28 Vc_{max}: maximum CO_2 carboxylation rate

29 J_{max}: maximum photosynthetic electron flux

30 A_{max}: net CO_2 assimilation measured under saturating atmospheric CO_2 concentration

31 SWC: soil water content

32 Ψ_p: predawn leaf water potential

33 H: tree height

34 DBH: diameter at 1.3 meters

35 DM_T: total aboveground dry mass

36 DM_L: leaf dry mass

37 DM_S: stem dry mass

38 TLA: total leaf area

39 LMA: leaf mass per unit area

40 SA: sapwood area

41 ABSTRACT

42   • Water use efficiency (WUE), oftentimes estimated as transpiration efficiency (TE): the amount

43   of biomass produced with regard to the water used, has not yet been used as a breeding trait to

44   select poplar genotypes with simultaneously high productivity and conservation of water. Before

45   its application as a selection target, evidence must be presented showing that WUE or its

46   estimators remain constant with age and across environmental conditions.

47   • We conducted a rainfall exclusion experiment in the field on two *Populus euramericana*

48   (Moench.) and two *Populus nigra* (L.) genotypes, and assessed leaf-level and whole-plant WUE

49   as well as their components and related traits. Then, we aimed to compare these results with the

50   same poplar genotypes grown in a glasshouse under contrasting water availability.

51   • Despite a reduction of soil water content and whole-plant transpiration, growth was stimulated

52   in the rainfall exclusion plot, likely as a result of an increased nitrogen assimilation. However,

53   TE values between the glasshouse and the field were similar, and genotype ranking remained

54   fairly constant for transpiration, carbon isotopic discrimination (Δ) and TE. Moreover, even

55   though the drivers of WUE in both experiments were different, increases of WUE measured as Δ

56   or TE was not associated with lower biomass production. Relatively good agreement was found

57   between Δ and TE in the field, absence of a similar correlation in the glasshouse is discussed.

58   • These results suggest that Δ may be a good proxy for TE, and could be used, both as a breeding

59   target for genotype selection in glasshouses without impacting biomass production when planted

60   in the field. However, reduced water availability modified the genotype ranking more

61   significantly than between the field/glasshouse experiments, suggesting a diversity of poplar

62   response to drought that should be considered in breeding strategies.

3

63 **KEYWORDS**

64 drought, Populus, stomatal conductance, transpiration, carbon isotopic discrimination

1. INTRODUCTION

Increased intensity and frequency of short and long-term droughts is an expected consequence of global changes (Dai, 2012; IPCC, 2014), as is predicted by current global climate change models (Sheffield and Wood, 2008). The amount of land exposed to droughts has risen significantly around the globe since 1950 (Dai, 2011). This will likely cause enduring environmental, economic and humanitarian consequences on societies and ecosystems (Touma et al., 2015). Since climate and forests are interconnected (Bonan, 2008), each influencing the other, the effect of a reduction of soil moisture on forests, while complex, is a key element in global change research.

A decrease in plant productivity is commonly found as a response to a decline in soil water availability (Ciais et al., 2005; Coopman et al., 2008; Monclus et al., 2006). A trend that is in contrast with the foreseen rise in wood demand, driven by economic growth in conjunction with energy and environmental policies (FAO, 2018). This divergence motivates plantation managers to extend their cultivation of trees species such as poplars away from their natural distribution in riparian ecosystems (Stobrawa, 2014), due to their high water requirements (Tschaplinski and Blake, 1989), to mesophyte habitats where soil water deficits are more frequent. Maintaining or increasing wood production while reducing water use appears therefore as a major challenge for the coming decades (Condon et al., 2004; Hamdy et al., 2003).

The concept of water-use efficiency (WUE) can be defined at the whole-plant scale as the transpiration efficiency (TE), the amount of biomass produced for a given amount of water used over a defined period of time (Condon et al., 2002). Since biomass production is linked at the leaf-level to CO_2 assimilation (A), and the majority of water is lost by leaf transpiration, driven by stomatal conductance (g_s) and the leaf-to-air vapour-pressure deficit (VPD), we can define

88   WUE at the leaf level intrinsically as W_i: the ratio of A over g_s (Ehleringer, 1993; Meinzer et al.,

89   1991).

90   Currently, commercial genotypes of hybrid poplars were selected mainly on a basis of high

91   productivity, resistance to pathogens and suitable wood properties but not for high WUE

92   (Monclus et al., 2006). If WUE is to be used as a breeding trait, reducing water use must not

93   come at the expense of wood production. Given the asymptotic relationship between A and g_s at

94   the leaf level, there is a range of high g_s for which A does not improve further (Wong et al.,

95   1979). Therefore, a reduction of g_s does not always cause a similar reduction of A, making

96   improvements of W_i possible.

97   At the whole-plant scale, methods for measuring TE demand a labor-intensive account of the

98   water used, often limiting its widespread use in large-scale experiments. This makes it not

99   realistically applicable to large screenings for cultivar improvement. Indirect estimations of

100   WUE by measuring the composition of stable carbon isotopes (δ, the ratio of ^{13}C to ^{12}C in a

101   biological sample relative to an international standard, Pee Dee Belemnite) enables the

102   comparisons of δ in the sample of interest relative to the one of the atmosphere (Δ). Differences

103   of δ between the two arise from processes of discriminations which provide insights into leaf gas

104   exchange processes and may be useful as a proxy to W_i (Farquhar et al., 1982). Δ has been

105   negatively related to W_i in C_3 crops (Anyia et al., 2007; Farquhar et al., 1989) and trees (Ponton

106   et al., 2002; Roussel et al., 2009b). In poplars Δ has been negatively linked with W_i and TE in

107   black and hybrid poplars (Rasheed et al., 2015; Rasheed et al., 2013). However, some studies

108   reported a lack of correlation with TE (Devi et al., 2011; Turner et al., 2007). Δ is a leaf-level

109   assimilation rate-weighted temporal integration of the A/g_s ratio. Unlike TE, Δ does therefore not

110   take into account possible carbon gains through photosynthetic stems, carbon losses by

6

111 respiration (apart from leaves during the day) and water losses at night or during the day in

112 organs other than leaves (Farquhar et al., 1989; Hubick and Farquhar, 1989). Because of the

113 different spatiotemporal integrations of WUE estimators (Δ, W_i, TE), understanding how and

114 under which circumstances they are related can be complex.

115 An important consideration regarding the usefulness of WUE in selecting genotypes for

116 improved WUE but similar or enhanced biomass production, is the stability of genotypic

117 differences across growing conditions (*i.e.* age and environmental conditions). Because TE is

118 laborious to measure, experiments are often conducted in pot experiments in glasshouses.

119 However, in controlled conditions the environment is widely different than in the field, with

120 plants rarely experiencing high winds or pathogens as well as often being limited in the range of

121 global radiation, temperature and humidity they are subjected to. Comparisons of WUE in

122 controlled and field conditions are scarce in the literature. Some studies were able to establish a

123 correlation or similar genotype ranking between growing conditions of Δ or WUE in grass

124 species (Johnson and Bassett, 1991) and crops (Anyia et al., 2007; Ehdaie et al., 1991) while

125 others reported a weak correlation (Lambrides et al., 2004) in sunflowers or not at all in three

126 wheat species (Frank et al., 1987). In oaks, field grown genotypes with high or low Δ (reflecting

127 low or high WUE, respectively) had a similar ranking in terms of Δ, A/g_s and TE when planted

128 in a glasshouse (Roussel et al., 2009a; Roussel et al., 2009b). To our knowledge and in general,

129 field estimations of TE are rare but extensive progress could be gained from understanding to

130 what degree WUE estimators from genotypes planted in glasshouses influence TE in the field.

131 In this context, we investigated the biomass production, water use, TE and Δ of poplar genotypes

132 grown in the field. Poplars were either irrigated or not, with the latter growing under a rainfall

133 exclusion system. Our aim was to compare this data with a glasshouse drought experiment using

134 the same poplar genotypes, previously published in Durand *et al.* (2019) in order to answer the

135 questions: (1) Is leaf-level and whole-plant WUE similar among well-watered poplar genotypes

136 when grown in a glasshouse or in the field? (2) Does soil water deficit modify our assessment of

137 the genotypic differences of leaf and whole-plant WUE under well-watered conditions? (3) What

138 are the underlying processes driving WUE at the leaf and whole-plant scale in controlled and

139 field conditions?

2. MATERIAL & METHOD

2.1 Plant material and experimental design

We used four poplar genotypes throughout the experiment. Carpaccio and I214 are commercially available *Populus deltoides × nigra* (Moench.) clones, while 6J29 and N38 are *Populus nigra* (L.) genotypes originating from natural populations in France (Drôme 6; FR-6) and Italy (La Zelata; IT1), respectively. Clones were selected to compare them with a previous glasshouse experiment (Durand et al., 2019) and for their diversity of drought tolerance, I214 and 6J29 being usually found to be drought-sensitive (Chen et al., 1997; Giovannelli et al., 2007; Muller and Lambs, 2009; Viger et al., 2016). The glasshouse drought experiment is explained in details in Durand *et al.* (2019). Shoot cuttings of similar diameter and length (30 cm), without roots, were used in the glasshouse and the field experiment. Briefly, 64 shoot cuttings were planted in 10 l pots filled with a sand/peat mixture (1/1, v/v) and complemented with fertilizers (1 g l^{-1} $CaMg(CO_3)_2$ and 20 g of Nutricote T100, 13:13:13:2 N/P/K/MgO and micronutrients, FERTIL S.A.S., Boulogne-Billancourt, France). Plants were grown in a fully automated glasshouse under natural light with a free range for air temperature between 15 and 25 °C at INRA-Grand-Est (48°45′09.3″N, 6°20′27.6″E; Champenoux, France). Volumetric soil water content (SWC) was maintained constant at 27.9% (85% of field capacity) using a weighting and watering robot at least three times a day. After 60 days, waterings were stopped on half the individuals until a SWC of 18.3% was reached (55% of field capacity). SWC was kept constant for 25 days using a pot-specific linear regression between SWC measured by Time Domain Reflectometry (HD2, IMKO, Ettlingen, Germany) and pot mass ($R^2 > 0.8$). Reference masses were adjusted each week to take the plant growth into account.

162   In the field, 144 shoot cuttings were planted in June 2nd 2014 by groups of three along six

163   parallel rows spaced 5 m apart. A single randomly selected genotype was use for each group of

164   three trees. Within and between groups on a row, poplars were separated by 1 m and 4 m,

165   respectively (Fig. S1). The plantation was setup in a nursery at INRA-Nancy (48°45′09.3″N,

166   6°20′27.6″E; Champenoux, France). The plot had a 6° slope southeastward. The studied site is

167   characterized by gray marls of the Jurassic inferior (Lotharingian) era, a deep homogenous

168   swelling heavy clay soil (52% silt, 40% clay, 8% sand). On average, the soil had a pH of 7.1, an

169   organic matter content of 33.3 g kg^{-1}, a total nitrogen content of 1.96 g kg^{-1} and a C/N ratio of

170   11.6.

171   *2.2 Rainfall exclusion setup*

172   Our objective was to establish a moderate drought, thus by design our rainfall exclusion is

173   partial, so that stomata would not be completely closed, preventing leaf gas exchanges. Each tree

174   was equipped with a water-dripping system, each dispensing 50 l per day. Waterings on the three

175   more elevated rows relative to the slope stopped in May 26th 2015, to limit surface runoff of

176   water to the rows below. In April 2016, covers 1.5 meters-wide were placed on the ground on

177   each side of the three rows where irrigation was stopped (Fig. S1), covering 85% of the total

178   ground area. Covers were two-fold, the one on top was green to mirror soil optical properties and

179   limit the rise of temperature under the covers (green canvas mulch, Triangle-outillage,

180   Ennevelin, France), the other was impervious to liquids but not to gases. Thus it prevented

181   rainfall to reach the ground without preventing soil evaporation (Berner France, Saint-Julien-du-

182   Sault, France). Gutters were placed on the downward side of each cover to evacuate water to the

183   bottom of the site (PVC, First Corp, Altare, Italy). The irrigated treatment was not covered by

184   canvas, thus received 100% of rainfalls, as well as water through irrigation.

185 1.6 and 0.9 meters-long neutron probe access tubes (ten of each, aluminum, closed at their base)

186 were installed, evenly spaced on the rows of the plot (Fig. S1), in order to quantify volumetric

187 soil water content every 10 cm in depth (TROXLER TX 4301, Research Triangle Park, NC,

188 USA). Calibration of each tube was performed following Normand (1974) by simultaneously

189 measuring at each depth the wet soil bulk density (Gamma probe, Campbell, CPN 501 DR), the

190 neutron counting speed of the soil (neutron probe) and the gravimetric soil moisture by taking

191 soil samples during the installation and subsequently measuring the ponderal water content.

192 Measurements were performed once each month, in May 17th, June 23rd, July 18th and August

193 22nd 2017. For the last three dates, predawn leaf water potentials (Ψ_p) were measured on the

194 same day (4:00 to 5:00 h, local time) on six randomly-chosen non-senescent leaves of each

195 genotype and treatment (*i.e.* 48 leaves) with a Scholander pressure bomb (SKPM-1400, Skye

196 Instruments LTD, Llandrindod Wells, UK).

197 *2.3 Sapflow*

198 Whole-tree water use (WU) was inferred from sapflow measurements using the thermal

199 dissipation technique. Probes 20 millimeters-long were installed on July 11th 2017 20 cm above

200 ground in the main stems in the North direction to estimate the sapflow per unit of sapwood area

201 (*i.e.* sap flux density), following Granier (1985; 1987). Six probes per genotype and treatment

202 were used (*i.e.* 48 probes). Probes were enclosed in aluminum sheets to protect them from direct

203 radiation and rainfall. Data collection started on July 16th and sensors were removed on

204 September 3rd 2017. WU per tree was then computed by scaling sap flux density from transversal

205 cross-section at sensor height since harvests revealed the absence of heartwood, which is

206 common in young trees.

2.4 Monitoring and harvests

208 Meteorological variables were measured in a meteorological station 190 m away from the

209 planted poplars. The station was equipped with a data logger (Campbell 21X, Campbell

210 Scientific Ltd), temperature and atmospheric relative humidity (Vaisala HMP45), global

211 radiation (pyranometer Kipp and Zonen CMP6), wind speed (contact anemometer, Campbell

212 Scientific A100) and rainfall sensors (tipping bucket rain gauge, Precis Mecanique 3030).

213 Diameter at 1.3 m (DBH) and height (H) of each tree were measured once each week during the

214 growing season (from budbreak to leaf fall) from 2015 to 2017. In April 18th 2016 and

215 September 4th 2017 harvests were conducted to measure the above-ground biomass (DM_T),

216 separated in leaves (DM_L) and stems (DM_S). The first harvest was done before bud-break so only

217 the woody biomass was measured. Every tree used for the sapflow experiment was used in the

218 second harvest. One of each group of three trees was used for each harvest (*i.e.* 48 trees). Leaves

219 and stems were oven-dried at 60°C until they reached a constant dry mass to estimate the

220 biomass in each compartment before the covers were installed and after the sapflow experiment.

221 Using an exponential relationship between H and woody biomass for each genotype (root-mean-

222 square error: RMSE < 26 g), we were able to compute the biomass in April 2016 for each tree

223 harvested in September 2017 (Fig. S2a). From this data, we fitted a second order polynomial

224 relationship between tree height and woody biomass for each combination of genotype and

225 treatment (RMSE < 229 g). This enabled us to estimate the woody biomass of each of the 48

226 trees used for sapflow measurements at the start of the sapflow experiment using their measured

227 height at the time (Fig. S2b). TE was estimated from estimation of above-ground biomass

228 between the start and the end of the sapflow experiment, divided by WU. As such it did not

229 integrate root biomass. TE from the glasshouse experiment was also re-calculated without the

230 root biomass to be able to consistently compare the two experiments.

231 Total leaf area (TLA) was estimated twice during the experiment. The first one was performed in

232 the first week of July 2017, right before the sapflow sensors installation. We fitted a third order

233 polynomial between the number of leaves and the total leaf area (estimated from leaf width, Fig.

234 S3) on each branch for two trees of each combination of genotype and treatment (*i.e.* eight trees).

235 Then we estimated the total leaf area on 19 trees by counting the number of leaves on each

236 branch and summing the computed leaf area of the tree. A second estimation of leaf area was

237 performed by fitting a linear relationship between leaf mass and area from 50 leaves per tree

238 harvested in September 2017 ($R^2 > 0.94$, Li-3000A, LI-COR, Lincoln, NE, USA). Using this

239 relationship we were able to estimate leaf mass per area (LMA), TLA from DM_L on the 48

240 harvested trees in September 4th and DM_L from the TLA estimated at the start of the sapflow

241 experiment (July 11th).

242 Since the sapwood area (SA), calculated from DBH measurements, was highly correlated with

243 TLA (using both dates for each genotype, $R^2 > 0.91$), we estimated TLA for each tree and each

244 day of the sapflow experiment from weekly measurements of SA, using a smoothing spline

245 function. Furthermore, by dividing for each day and each individual, their daily WU (from

246 sapflow measurements) by their daily estimated TLA, we were able to calculate daily whole-

247 plant   transpiration   per   unit   area   (E,   Fig.   1).
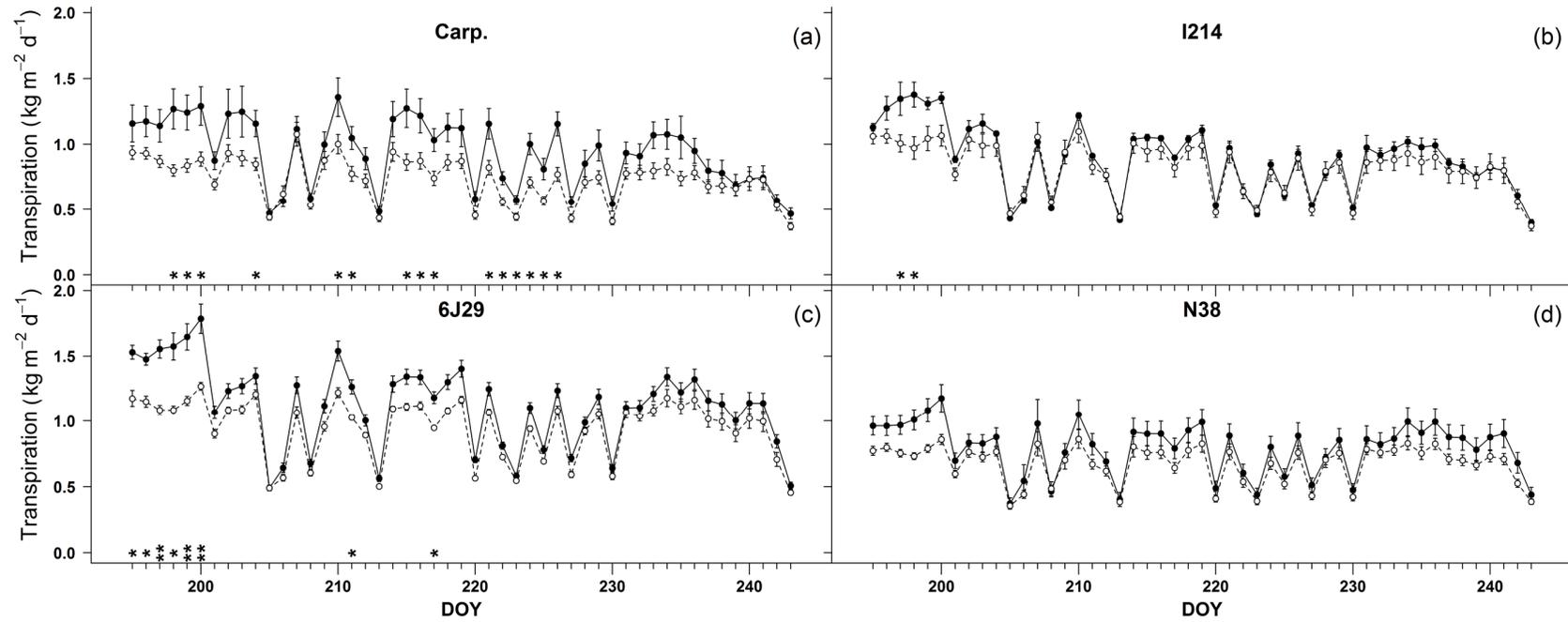
Figure 1. Daily transpiration per unit leaf area of two *Populus deltoides* × *nigra* Carpaccio (**a**), I214 (**b**) and two *Populus nigra* genotypes 6J29 (**c**), N38 (**d**) under irrigation or a rainfall exclusion setup in the field in 2017. Black and white circles are respectively for irrigated and rainfall exclusion trees (means ± standard error). Significant differences between water treatments for each date is shown (n = 6). *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001; ns, not significant.

253 *2.5 Leaf gas exchange*

254 At the end of July 2017, we assessed the photosynthetic capacity by measuring CO_2 assimilation

255 and internal CO_2 concentration (C_i) along discrete steps of [CO_2] using the LI-6800 portable

256 photosynthesis system (LI-COR, Lincoln, NE, USA). Conditions inside the leaf cuvette was as

257 follows: leaf temperature of 25°C, saturating irradiance of 326.1 W m^{-2}, leaf-to-air VPD of 1.6

258 kPa, fan speed of 10000 rpm, flowrate of 1000 µmol s^{-1} with an overpressure of 0.1 kPa. Before

259 starting, we waited for g_s to reach a steady-state. Infrared gas analysers were matched after every

260 step changes of [CO_2] which were in order: 400, 1800, 1500, 1200, 1000, 800, 600, 400, 250,

261 200, 150, 100, 50, 0 and 400 ppm. Maximum carboxylation rate (Vc_{max}), maximum electron-

262 transport rate (J_{max}) and maximum CO_2 assimilation at saturating C_i (A_{max}) were estimated using

263 the "plantecophys" R package (Duursma, 2015; R Core Team, 2019), using the Michaelis-

264 Menten constants for CO_2 (K_c), and O_2 (K_o) and the CO_2 photo-compensation point (Γ^*) of

265 Bernacchi *et al.* (2001).

266 On July 18th and August 22nd 2017 we monitored diurnal dynamics of gas exchange (A and g_s)

267 on a cloudless sky from 5:30 and 6:30 h to 15:30 and 16:30 h (universal time), respectively. To

268 avoid confusion, W_i in this article only refers to the ratio of A/g_s, measured by leaf gas exchange.

269 This was done on six replicates per genotype and treatment (*i.e.* 48 individuals) on sun leaves

270 facing the south-east direction using two intercalibrated portable photosynthesis systems (Li-

271 6200, LI-COR Inc., Lincoln, NE, USA).

2.6 Carbon isotope discrimination

273 Mature leaves were sampled in the field at the end of August 2015 and 2017 and in the

274 glasshouse during the leaf sampling described in Durand et al. (2019). In the field, leaves grew

275 under the rainfall exclusion setup, but they were fully mature before the start of the drought in

276 the glasshouse. All sampled leaves were oven-dried for 48 h at 70°C and grounded into a fine

277 powder. 1.0 ± 0.1 mg subsamples were weighed into tin capsules and δ was measured with a

278 continuous-flow elemental analyzer (Carlo Erba Analyzer-NA1500, Rodano, Italy) coupled with

279 an isotope ratio mass spectrometer (Thermo-Finnigan; Delta S, Bremen, Germany). $^{13}C/^{12}C$

280 ratios were computed relative to the Vienna Pee Dee Belemnite international standard. Isotopic

281 composition of the air in the glasshouse was -9.61 ‰, derived from isotopic compositions of two

282 Zea mays (L.) planted in the glasshouse at the time, following Marino & McElroy (1991). In the

283 field the isotopic composition of the air was -8.73 ‰, estimated from its evolution over the past

284 three centuries following Bonal et al. (2011). Isotopic discrimination was estimated as $\Delta = (\delta_{air} -$

285 $\delta_{leaf}) / (1 + \delta_{leaf} / 1000)$. Farquhar et al. (1982) showed that Δ is negatively related to the ratio of

286 internal to atmospheric CO_2 concentration (C_i/C_a), which at the same time reflects A/g_s and thus

287 W_i, when taking into account fractionation processes by CO_2 diffusion though stomata and at the

288 sites of carboxylation by Rubisco.

289 *2.7 Statistics*

290 Statistics were done using R 3.5.2 (R Core Team, 2019, data used is available in Table S2).

291 Significant differences among the four genotypes and the two treatments was tested with type

292 two Two-Way ANOVA. A similar but repeated ANOVA design with genotypes and treatments

293 as between-subjects factors and the time as within-subjects factor was used for diurnals gas

294 exchange and daily whole-plant transpiration (n = 5-6). Differences of soil water content was

295   tested with Student tests at every depth (n = 10). Correlations were computed by performing

296   linear regressions (n = 48), using genotype-treatment means when comparing field and

297   glasshouse data because pairing could not be established between trees (n = 8). Normality,

298   homoscedasticity and sphericity were checked by Shapiro-Wilk normality tests, Levene tests and

299   Mauchly tests, respectively. When sphericity was violated we used the Greenhouse-Geisser

300   correction. Significant differences between modalities of each factors were tested by post-hoc

301   contrast analysis and P values were adjusted to control for the false discovery rate. Significant

302   differences were considered at $P < 0.05$ for all tests.

303 **3. RESULTS**

304 *3.1 Water relations in the field*

305 Our experimental rainfall exclusion design proved successful in reducing the SWC. On May 17th,

306 the mean SWC ranged from 47 to 35.9% from 20 to 150 cm in depth with no significant

307 difference between the irrigated and the rainfall exclusion plot at any depth (data not shown).

308 Both plots showed a decrease of SWC on June 23rd, which was larger in the rainfall exclusion

309 plot. This led to significant differences of SWC between plots in most of the first 120 cm ($P <$

310 0.04, except at 70 and 80 cm, Fig. 2). During the following month SWC continued to decrease in

311 the rainfall exclusion plot causing larger differences between the two plots in the first 130 cm (P

312 < 0.007) on July 18th. Ψ_p was also significantly lower in the rainfall exclusion trees than in the

313 irrigated for all genotypes ($P < 0.02$). More rainfall during the month of August (see Fig. S4**c**

314 between DOY 200 and 233: July 19th to August 22nd 2017) led to a small increase of SWC on

315 both plots in the first 100 cm on August 22nd. Nonetheless, we found significant differences of

316 SWC between plots in the first 140 cm. Ψ_p was however similar between irrigated and rainfall

317 exclusion trees, except for Carpaccio which showed a more negative Ψ_p in the rainfall exclusion

318 trees than in the irrigated ones (Fig. 2).

319
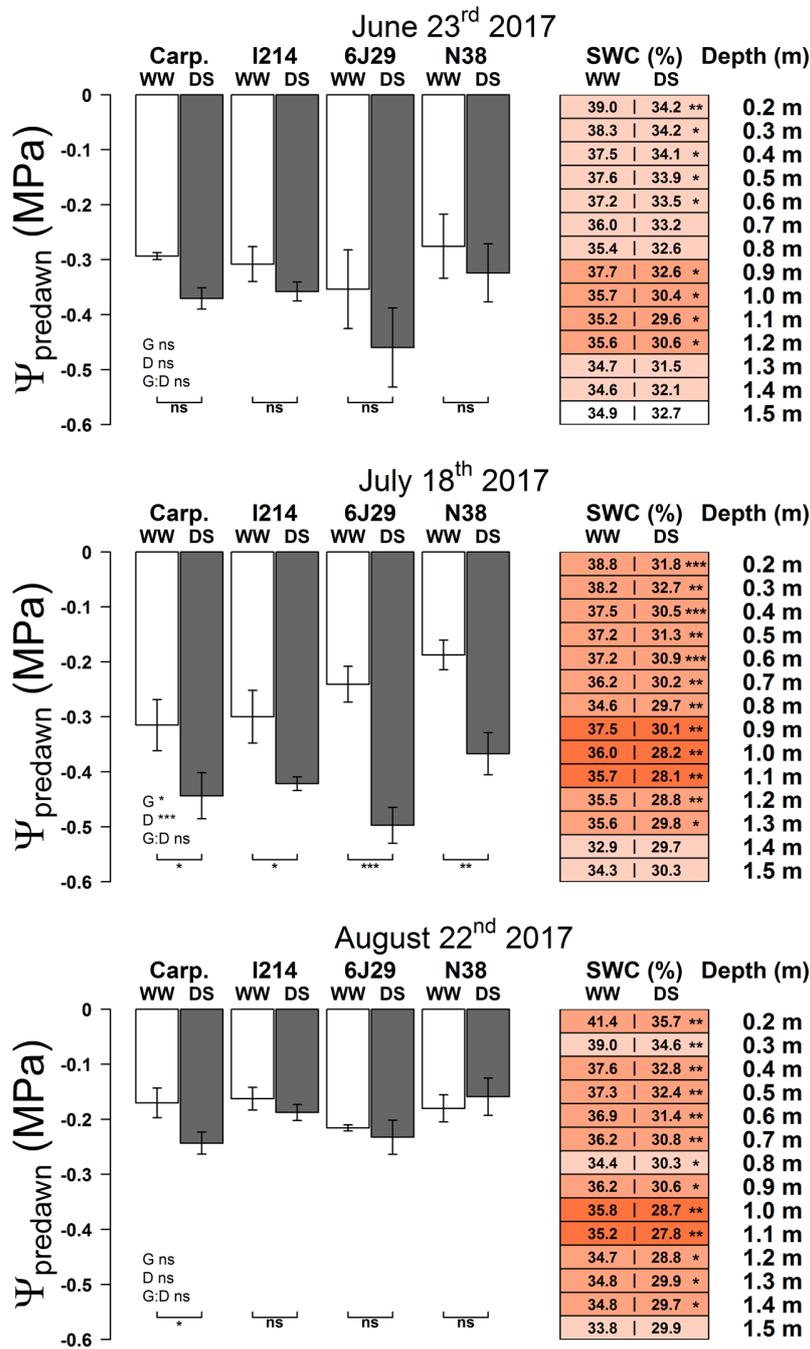
Figure 2. Predawn leaf water potential (left side) of two *Populus deltoides* × *nigra* (Carpaccio and I214) and two *Populus nigra* genotypes (6J29 and N38) and soil water content (right side) under irrigation or a rainfall exclusion setup in the field at three dates. Values reported are means ± standard error (n = 6). WW and DS is for irrigated and rainfall exclusion trees respectively (white and gray bars respectively). Mean soil water content (SWC) is shown for each 10cm in depth with stronger colors indicating stronger differences between treatments. Results of two-way ANOVA are given for main effects (G: genotype; D: water treatment) and interaction (G:D). Significant differences between water treatments are reported. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant.

329   Sapflow sensors recorded the flow of ascending sap in the main stem, from which we can infer

330   plant water use (WU), between July 14th and August 31st (Table 1). Whole-plant water use was

331   higher in the rainfall exclusion hybrid poplars than in the irrigated by 34 and 58% for Carpaccio

332   and I214 (P < 0.03). However, whole-plant daily transpiration per leaf area (E) was overall

333   significantly lower in rainfall exclusion trees. Post-hoc comparisons were able to detect

334   significant differences of E in Carpaccio and 6J29 but not in I214 and N38 (Table 1). When

335   investigating differences of E on specific days, transpiration in the irrigated trees was almost

336   always higher than in the rainfall exclusion trees for every genotype (Fig. 1). Despite this, we

337   were able to detect significant differences of transpiration between treatment only in 15, 2, 8 and

338   0 days for Carpaccio, I214, 6J29 and N38 respectively, because of the high individual variability

339   of whole-plant transpiration.

340   At the leaf level, diurnal gas exchange measurements on July 18th and August 22nd showed a

341   clear reduction of g_s in the rainfall exclusion plot compared to the irrigated one for every

342   genotype throughout the day except early in the morning (P < 0.001, Fig. 3**e-h**, Fig. 4**e-h**). On

343   July 18th, stomata of the rainfall exclusion trees, regardless of genotype, were completely closed

344   from 10:00 until the end of the day, while those of the irrigated trees continued to decrease

345   during the afternoon. Overall, g_s was similar between the four genotypes at this date (P = 0.45).

346   On August 22nd, Carpaccio rainfall exclusion trees were almost completely closed throughout the

347   day similarly to July 18th (Fig. 4**e**), while the other three genotypes showed different extents at

348   which g_s was reduced under rainfall exclusion with N38 exhibiting the second strongest and 6J29

349   the smallest reduction of g_s. Overall, g_s was also reduced in the rainfall exclusion trees but to a

350   smaller   degree   than   on   July   18th   (Fig.   4**e-h**).
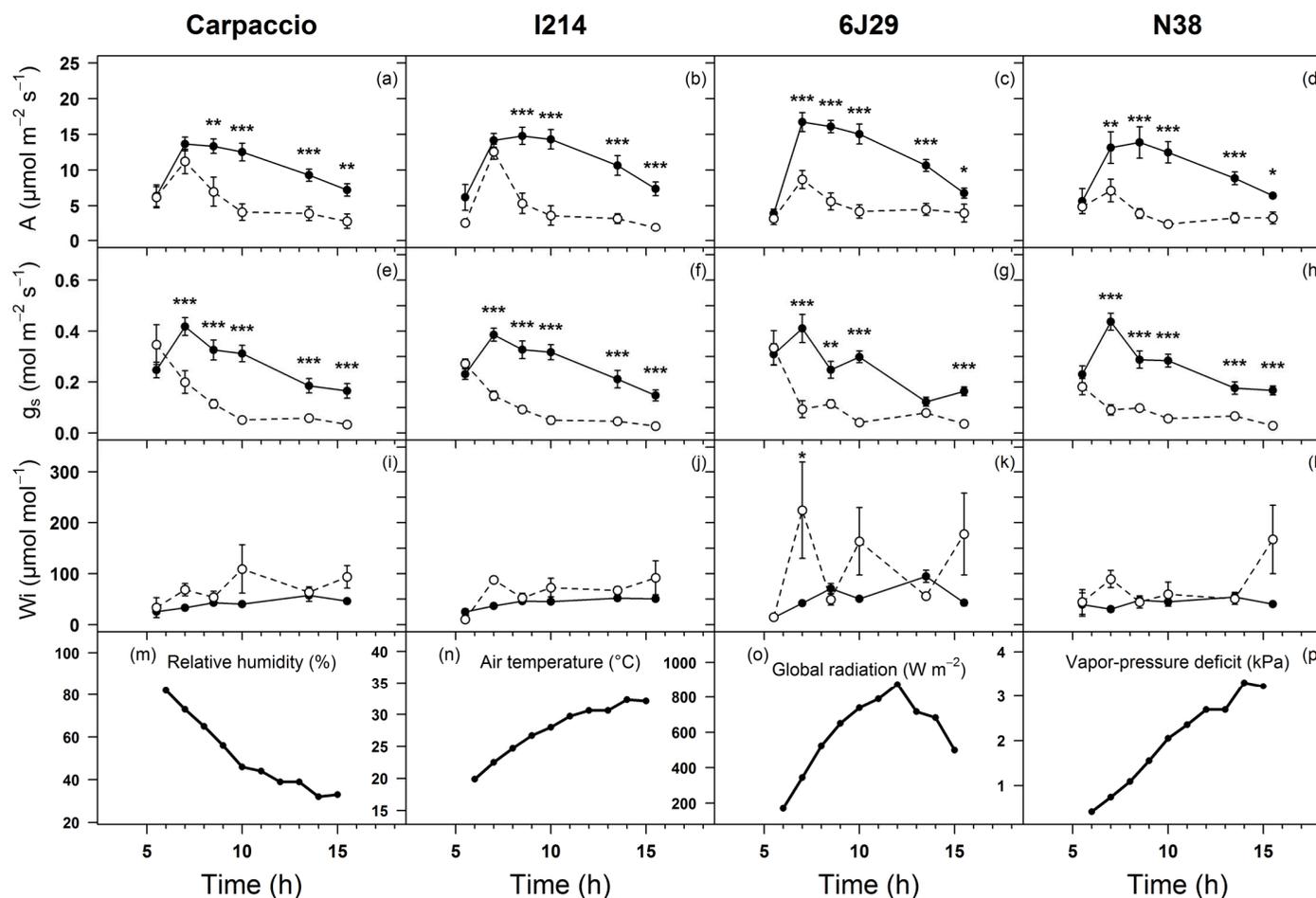
Figure 3. Gas-exchange dynamic on a cloudless sky of two *Populus deltoides* × *nigra* Carpaccio (**a**, **e** and **i**), I214 (**b**, **f** and **j**), and two *Populus nigra* genotypes 6J29 (**c**, **g** and **k**), N38 (**d**, **h** and **l**) under irrigation or a rainfall exclusion setup in the field. (**a**) to (**d**): net CO_2 assimilation (A), (**e**) to (**h**): stomatal conductance to water vapour (g_s) and (**i**) to (**l**): intrinsic water use efficiency (W_i), computed as the ratio of A/g_s. Relative humidity (**m**), air temperature (**n**), global radiation (**o**) and atmospheric vapour-pressure deficit (**p**) in the field are also reported. Black and white circles are respectively for irrigated and rainfall exclusion trees (means ± standard error). Time is displayed in universal time (local time –2h). All measurements were done on the 199st day of the year (July 18th), including the meteorological data. Significant differences between water treatments for each time is shown (n = 4-6). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

21
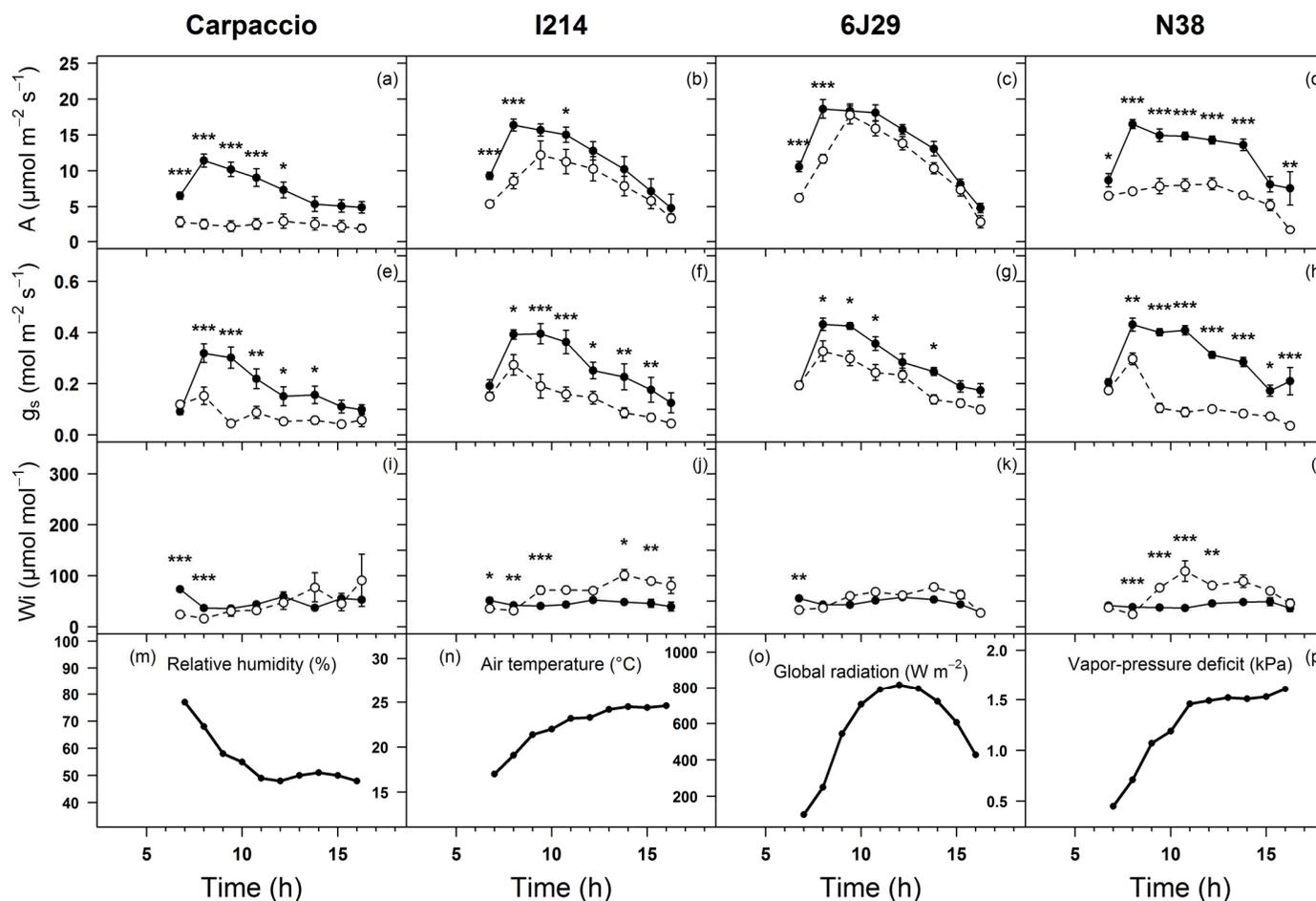
Figure 4. Gas-exchange dynamic on a cloudless sky of two *Populus deltoides* × *nigra* Carpaccio (**a**, **e** and **i**), I214 (**b**, **f** and **j**), and two *Populus nigra* genotypes 6J29 (**c**, **g** and **k**), N38 (**d**, **h** and **l**) under irrigation or a rainfall exclusion setup in the field. (**a**) to (**d**): net CO_2 assimilation (A), (**e**) to (**h**): stomatal conductance to water vapour (g_s) and (**i**) to (**l**): intrinsic water use efficiency (W_i), computed as the ratio of A/g_s. Relative humidity (**m**), air temperature (**n**), global radiation (**o**) and atmospheric vapour-pressure deficit (**p**) in the field are also reported. Black and white circles are respectively for irrigated and rainfall exclusion trees (means ± standard error). Time is displayed in universal time (local time −2h). All measurements were done on the 234st day of the year (August 22nd), including the meteorological data. Significant differences between water treatments for each time is shown (n = 4-6). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

3.2 Biomass and photosynthesis in the field

368 At the whole-plant scale at the end of the growth period in 2017, we found

369 H, DM_S and DM_L to be overall higher in the rainfall exclusion than in the irrigated trees ($P <$

370 0.001, Table S1). While post-hoc comparisons were not always able to show significant

371 genotypic differences, the lack of interaction between the genotype and treatment effect ($P >$

372 0.05) highlights this result is not dependent on genotype. In April 2016, there were no such

373 differences between treatments ($P > 0.19$). Growth and biomass accumulation in 2017 show

374 these differences were in part, established during the summer 2017 (Table 1). Growth was 23 to

375 60% higher for height and 43 to 154% for DBH in the rainfall exclusion than in the irrigated

376 trees, depending on genotype. DM_T and DM_S, although similar among genotypes, were also

377 more than doubled in the rainfall exclusion than in the irrigated trees with a mean total biomass

378 accumulation of 2.43 kg in the former and 1.04 kg in the latter. Moreover, even though LMA

379 was 8.4 to 12.6% higher in the rainfall exclusion than in the irrigated trees depending on

380 genotype ($P < 0.001$), both DM_L and TLA were overall higher in the rainfall exclusion trees as

381 well (DM_L: 203 and 446 g, TLA: 2.05 and 3.94 m^2, for irrigated and rainfall exclusion trees

382 respectively).

Genotype	Treatment	H (cm)	DBH (mm)	DM_T (g)	DM_S (g)	DM_L (g)	TLA (m²)	LMA (g mm⁻²)	WU (kg)	E (kg m⁻² d⁻¹)	TE (g kg⁻¹)	$\Delta^{13}C$ (‰)
Carpaccio	irrigated	53 ± 11 a	3.3 ± 0.1 a	911 ± 531 a	684 ± 425 a	239 ± 58 a	2.59 ± 0.71 ab	0.95 ± 0.05 a	429 ± 163 ab	1.00 ± 0.09 de	2.26 ± 0.79 a	21.57 ± 0.55 e
	rainfall excl.	85 ± 17 cde	8.4 ± 1.9 d	2571 ± 1125 b	1960 ± 742 b	622 ± 324 b	5.79 ± 3.59 b	1.03 ± 0.07 ab	574 ± 63 c	0.73 ± 0.12 ab	4.02 ± 1.61 bc	18.72 ± 0.70 b
I214	irrigated	56 ± 9 ab	5 ± 1.6 ab	954 ± 407 a	638 ± 343 a	190 ± 50 a	2.06 ± 0.66 ab	0.95 ± 0.09 a	346 ± 74 a	0.89 ± 0.04 bcd	2.55 ± 0.57 ab	21.09 ± 0.34 de
	rainfall excl.	88 ± 21 de	8.5 ± 1.9 d	2563 ± 798 b	2133 ± 706 b	446 ± 266 ab	3.99 ± 2.55 ab	1.06 ± 0.07 bc	545 ± 88 bc	0.82 ± 0.16 abc	5.07 ± 0.65 cd	18.66 ± 0.43 b
6J29	irrigated	80 ± 10 cd	5.5 ± 0.4 bc	1216 ± 506 a	928 ± 399 a	235 ± 139 a	2.18 ± 1.37 ab	0.95 ± 0.11 a	391 ± 99 a	1.13 ± 0.12 e	3.39 ± 1.55 ab	20.74 ± 0.38 d
	rainfall excl.	98 ± 11 e	8.5 ± 1.3 d	2437 ± 497 b	2021 ± 341 b	415 ± 223 ab	3.48 ± 1.97 ab	1.07 ± 0.03 bc	468 ± 52 abc	0.95 ± 0.05 cd	4.98 ± 1.17 cd	19.39 ± 0.41 c
N38	irrigated	71 ± 8 bc	4.9 ± 0.6 ab	1084 ± 360 a	802 ± 405 a	148 ± 117 a	1.36 ± 1.07 a	1.05 ± 0.03 b	345 ± 84 a	0.80 ± 0.19 abc	2.93 ± 0.69 ab	19.99 ± 0.42 c
	rainfall excl.	99 ± 10 e	7 ± 1.7 cd	2139 ± 558 b	1840 ± 591 b	300 ± 199 ab	2.50 ± 1.74 ab	1.14 ± 0.02 c	351 ± 71 a	0.67 ± 0.09 a	5.75 ± 0.41 d	17.95 ± 0.93 a
	Genotype	0.001	ns	ns	ns	ns	ns	0.001	0.002	< 0.001	0.049	< 0.001
P value	Treatment	< 0.001	< 0.001	< 0.001	< 0.001	0.001	0.005	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.014

383 **Table 1** Growth, biomass production and water use of two *Populus deltoides* × *nigra* (Carpaccio and I214) and two *Populus nigra* genotypes (6J29 and N38)
384 under irrigated or a rainfall exclusion setup in the field. Values reported are means ± standard deviation of the difference between the end and the start of the
385 sapflow experiment (July 16th to September 3rd, n = 6). ANOVA factors were considered significant when p < 0.05. Letters show significant differences by
386 post-hoc contrast among the eight groups (4 genotypes + 2 water treatments). H, plant height increment; D, main stem diameter increment at 1.3 m; DM_T, total
387 dry mass increment; DM_S, stem dry mass increment; DM_L, leaf dry mass increment; WU, cumulated water use; TLA, total leaf area increment; E, average
388 daily whole-plant transpiration per unit leaf area; TE, transpiration efficiency; $\Delta^{13}C$, carbon isotopic discrimination from bulk leaves; ns, not significant.

389   At the leaf level, photosynthetic capacity measurements showed a significant increase in

390   Vc_{max} by 32, 28 and 27% in Carpaccio, I214 and N38 rainfall exclusion trees compared the

391   irrigated ones(Fig. **5a**, P = 0.009, 0.006, 0.385, 0.007 for Carpaccio, I214, 6J29 and N38

392   respectively). J_{max} had a comparable overall increase in the rainfall exclusion trees but post-

393   hoc comparisons were not able to show genotypic-specific differences (Fig. **5b**). A_{max} did not

394   show any significant difference between treatments (Fig. **5c**, P = 0.67). Genotypic differences

395   were significant and similar among the three parameters tested (P = 0.002, 0.011 and 0.009

396   for Vc_{max}, J_{max} and A_{max} respectively). Carpaccio had the lowest Vc_{max}, J_{max} and A_{max} while

397   6J29 had the highest both under irrigated and rainfall exclusion conditions. Leaf nitrogen

398   content were also increased in the trees that were not irrigated in 2015 and under rainfall

399   exclusion in 2017, when compared to the irrigated ones (P < 0.001 in both cases, Fig. S5).

400   CO_2 assimilation during our gas exchange diurnals was significantly lower under rainfall

401   exclusion. On July 18th, A decreased in the rainfall exclusion compared to the irrigated trees

402   from 7:00 h onwards in the black poplars and from 8:30 h onwards in the hybrid poplars (Fig.

403   3**a-d**). On August 22nd, the reduction of A in the rainfall exclusion plants was still visible but

404   to a smaller degree depending on the concurrent decrease of g_s (Fig. 4**a-h**). I214 and 6J29

405   exhibited a decrease of A under rainfall exclusion, mostly in the early morning, which was

406   much smaller than in Carpaccio and N38.

407
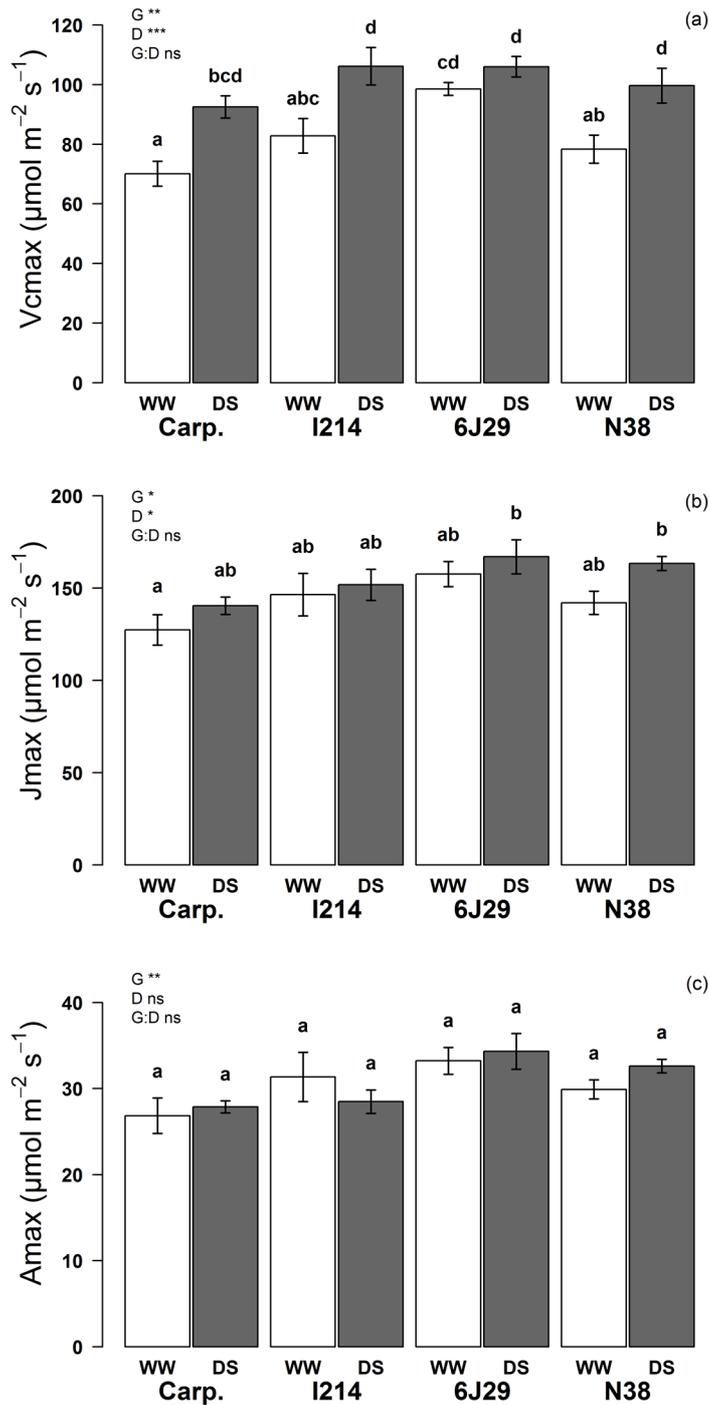
Figure 5. Photosynthetic capacity data of two *Populus deltoides* × *nigra* (Carpaccio and I214) and two *Populus nigra* genotypes (6J29 and N38) under irrigation or a water exclusion setup in the field. a) Maximum rate of rubisco carboxylation (V_{cmax}) b) maximum rate of electron transport (J_{max}) c) maximum CO_2 assimilation under saturating CO_2 concentration (A_{max}). Values reported are means ± standard error (n = 6). WW and DS is for irrigated and rainfall exclusion trees respectively (white and gray bars respectively). Results of two-way ANOVA are given for main effects (G: genotype; D: water treatment) and interaction (G:D). Significant differences between water treatments are reported. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant.

26

416   *3.3 Water use efficiency and comparisons between the glasshouse and field experiment*

417   Regarding the intrinsic WUE (W_i) during the gas exchange diurnals on July 18th, we found

418   almost no significant differences between treatments (Fig. 3**i-l**, Fig. 4**i-l**). Similarly to A and

419   g_s, no genotypic difference of W_i was found at this date. On August 22nd, W_i tended to be

420   higher in the early morning in irrigated trees because of a similar g_s but higher Awhile the

421   reverse was found in the afternoon in I214 and N38, resulting from a larger decrease of g_s

422   than A when comparing them to the irrigated trees.

423   As a consequence of the respectively large increase in biomass accumulation and the

424   moderate increase in water use in the rainfall exclusion poplars when compared to the

425   irrigated ones, TE was overall 78% higher under rainfall exclusion than in irrigated conditions

426   (2.78 and 4.96 g kg^{-1}, for irrigated and rainfall exclusion respectively). On the contrary, Δ was

427   significantly reduced by 13, 12, 6.5 and 10% in the rainfall exclusion trees when comparing to

428   the irrigated trees, for Carpaccio, I214, 6J29 and N38 respectively. 6J29 showed the smallest

429   increase of TE (47%) and the smallest decrease of Δ (6.5%) under rainfall exclusion.

430   Furthermore, we found a negative correlation between of TE and Δ among treatments and

431   genotypes in the field ($P < 0.001$, $R^2 = 0.39$, Fig. 6**b**), driven mainly by the treatment. In the

432   glasshouse, TE and Δ were not significantly correlated ($P = 0.087$, $R^2 = 0.04$, Fig. 6**a**).

433   Components of TE were also different under controlled and field conditions. In the field, TE

434   was not correlated with water use ($P = 0.21$, Fig. 6**f**), but positively and strongly with biomass

435   accumulation ($P < 0.001$, $R^2 = 0.62$, Fig. 6**d**) while the reverse was true in the glasshouse

436   experiment. TE was negatively correlated with water use ($P < 0.001$, $R^2 = 0.40$, Fig. 6**e**) but

437   not with biomass accumulation ($P = 0.93$, Fig. 6**c**). Similar results were found when taking

438   the treatment into account using an ANCOVA statistical design.

439

440   Unexpectedly, despite this divergence we found TE values in the field to be close to the one

441   we found in the glasshouse experiment (Fig. 7**a**). Only the TE of N38 well-watered trees was

442   significantly lower in the field than in the glasshouse ($P = 0.02$) whereas the TE of water-

443   limited I214 and N38 were significantly higher in the field than in the glasshouse ($P < 0.004$).

444   By contrast, Δ in the field was always lower than in the glasshouse for every combination of

445   genotype and treatment ($P < 0.001$), by 11 and 19% on average for irrigated and rainfall

446   exclusion trees, respectively (Fig. 7**b**). This was similar to the lower E in the field than in the

447   glasshouse (Fig. 7**c**), also exhibited by every combination of genotype and treatment, by 24,

448   28, 34 and 49% for Carpaccio, I214, 6J29 and N38 respectively ($P < 0.001$).

449
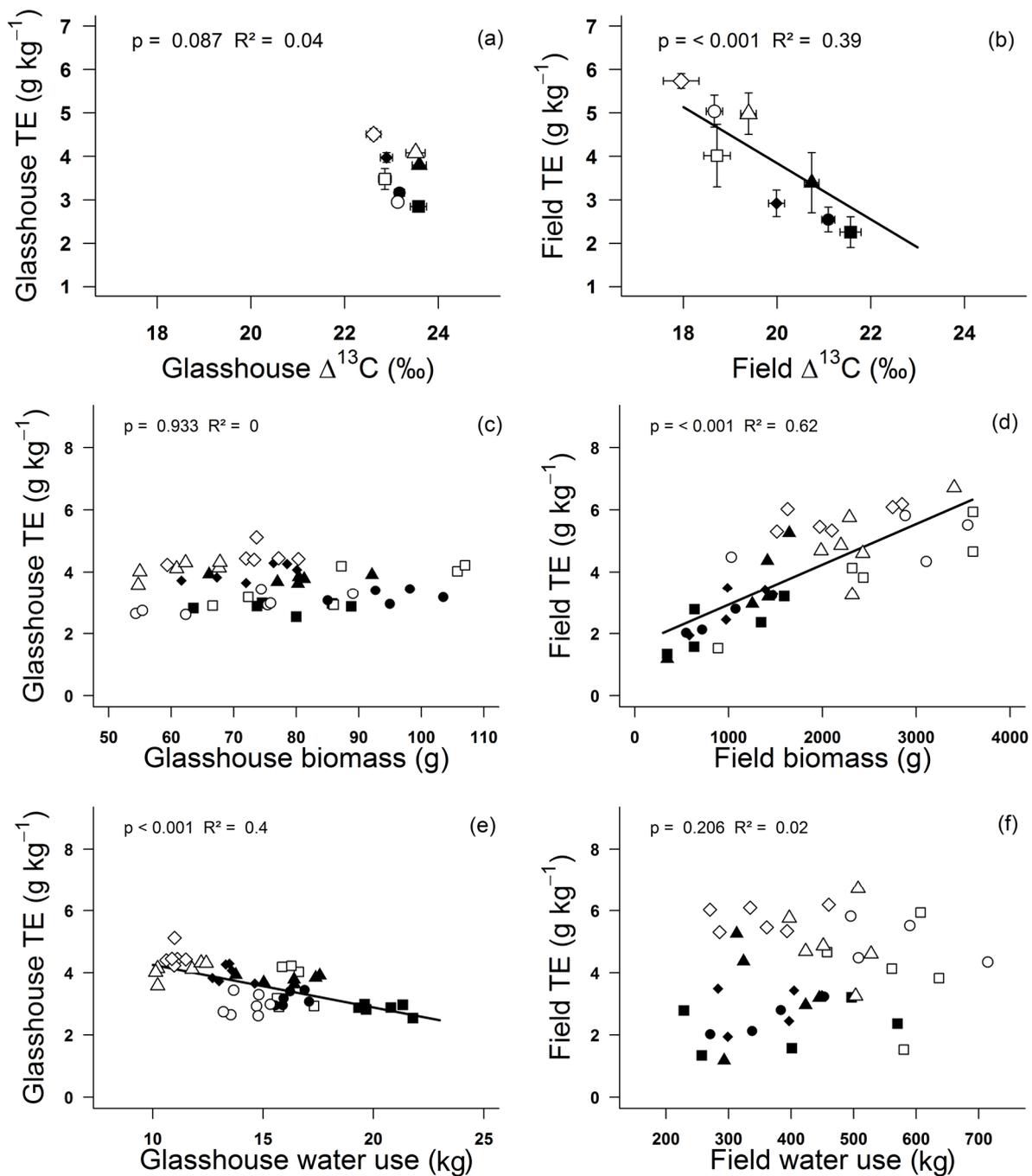
Figure 6. Correlations on two *Populus deltoides* × *nigra* (Carpaccio and I214) and two *Populus nigra* genotypes (6J29 and N38) under well-watered or reduced water availability either in a glasshouse or a field experiment. a) Transpiration efficiency and carbon isotopic discrimination in the glasshouse experiment b) Transpiration efficiency and carbon isotopic discrimination in the field experiment. c) Transpiration efficiency and biomass production in the glasshouse experiment d) Transpiration efficiency and and biomass production in the field e) Transpiration efficiency whole-plant water use in the glasshouse f) Transpiration efficiency and whole-plant water use in the field. For a) and b), values reported are means ± standard error (n = 6). Closed and open symbols are for well-watered and reduced water availability, respectively. Squares, circles, triangles and diamonds are for Carpaccio, I214, 6J29 and N38, respectively. *P* and R^2 values are shown.

460   Correlations of traits between the glasshouse and the field are difficult considering no pairing

461   can be established between trees. Correlations using genotypes and treatments means has the

462   effect of reducing the number of observations by a factor of 6 (equal to the number of

463   replicates in each conditions), thus reducing the statistical power to detect significant

464   correlations, even though each calculated mean is informed by six replicates. As a result,

465   these correlations of TE, Δ and E between the field and glasshouse experiment were not

466   statistically significant (P = 0.18, 0.05 and 0.14, respectively) despite the discernable positive

467   trends and the relatively strong correlation coefficients (R^2 = 0.15, 0.40 and 0.21,

468   respectively, Fig. 7). However, by randomly pairing greenhouse and field data by genotype

469   and treatment a million times (similar to a bootstrap with replacement), we found 92, 97 and

470   92% of P values under the 0.05 threshold and R^2 of 0.22, 0.23 and 0.19 for TE, Δ and E,

471   respectively (Fig. S6). Thus genotype ranking between the field and glasshouse experiment

472   was fairly maintained. Typically, only one genotype switched order between the field and

473   glasshouse conditions. For example, from lowest to highest TE in irrigated trees, the order

474   Carpaccio, I214 and 6J29 was similar in the field and in the glasshouse (Fig. 7**a**). Likewise in

475   rainfall exclusion trees, the order Carpaccio, 6J29 and N38 was found in both conditions.

476   Overall, N38 frequently had the highest TE and always had the lowest Δ in both conditions

477   and for both treatments (Fig. 7**a-b**). On the contrary, Carpaccio irrigated trees had the lowest

478   TE and the highest Δ in both conditions. 6J29 also had the highest E in both conditions for

479   both treatments (Fig. 7**c**).
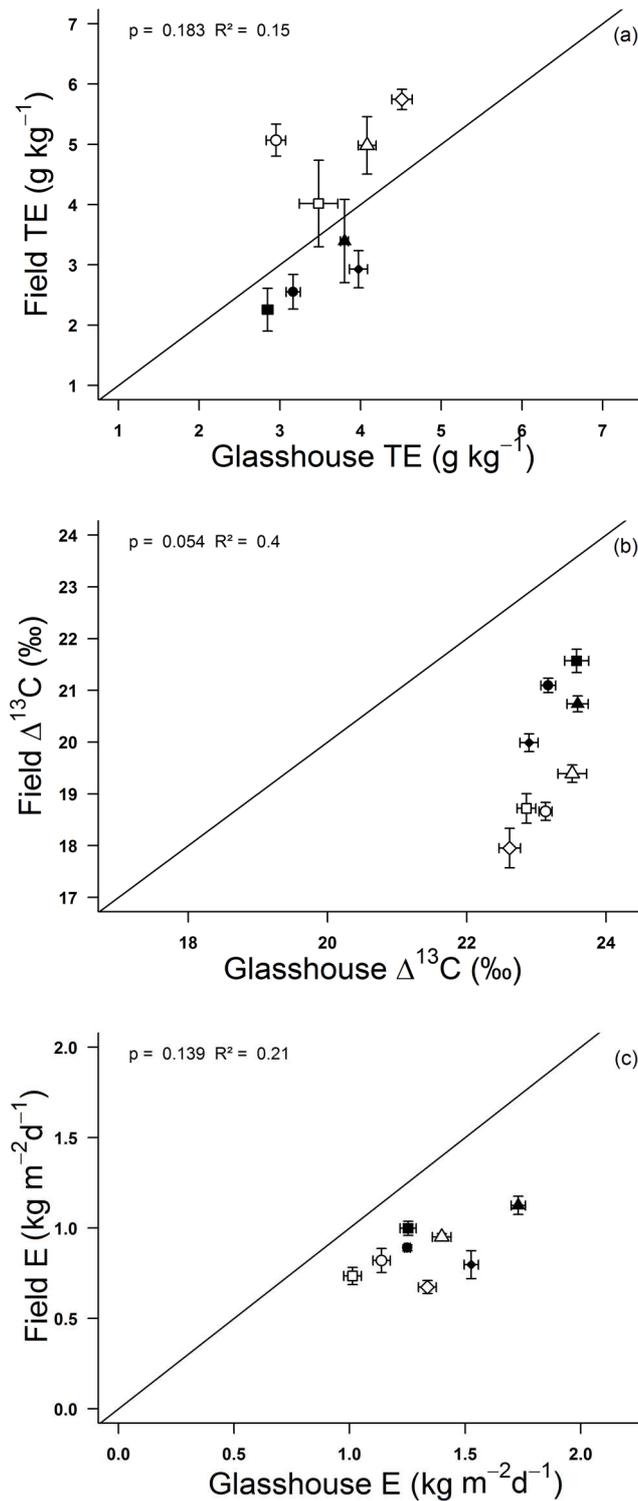
Figure 7. Correlations between a glasshouse and a field experiment on two *Populus deltoides* × *nigra* (Carpaccio and I214) and two *Populus nigra* genotypes (6J29 and N38) under well-watered or reduced water availability. a) Transpiration efficiency b) Carbon isotopic discrimination c) Mean daily whole-plant transpiration per unit leaf area. Closed and open symbols are for well-watered and reduced water availability, respectively. Squares, circles, triangles and diamonds are for Carpaccio, I214, 6J29 and N38, respectively. Values reported are means ± standard error (n = 6). *P* and R^2 values are shown. The straight continuous line shows the 1:1 regression line.

4. DISCUSSION

Water use efficiency has a major potential to help select and breed new individuals with similar or increased biomass production and lower water consumption. However, estimating WUE is not an easy task. Further research is needed to better understand if and under which conditions different spatiotemporally-integrated estimators of WUE are related. In this study we investigated the water use efficiency and its components at the leaf and whole plant scale of four poplar genotypes in the field under irrigation or a rainfall exclusion setup. This data was also compared to a preceding study in a glasshouse (Table S3) in order to explore the links between leaf-level and whole-plant WUE in each condition and across them.

4.1 Rainfall exclusion effect in the field

A major and unexpected result of our study was the increased biomass and leaf area production in the rainfall exclusion trees as compared to the irrigated ones. The moderate but significant reduction of soil water content from June to August, and lower predawn leaf water potential in July, we found in the rainfall exclusion plot (Fig. 2) is usually associated with a reduced biomass production in poplar genotypes, driven by stomatal closure (Monclus et al., 2006). In our experiment at the leaf level, stomatal opening was substantially lower in the rainfall exclusion trees (Fig. 3-4), as theory predicts, decreasing both transpiration and CO_2 assimilation. Despite this, the higher biomass production was most likely amplified by the exponential growth with increased tree size, as larger trees have a higher total leaf area and thus a higher number of photosynthetic organs. The increased photosynthetic capacity (V_{cmax}), mitigating the reduced CO_2 assimilation prompted by stomatal closure may have also contributed, to a lower degree, to the higher biomass production in the rainfall exclusion plot compared to the irrigated one. The observed leaf-level lower transpiration per unit area (E) when water is limited was also seen at the whole plant scale, with different magnitude of

512   decrease depending on genotype and environmental conditions (Fig. 1). A number of factors

513   could explain these genotypic differences, such as leaf aging (Reich, 1984), differences of

514   stomatal density in newly developing leaves or with leaf position in the canopy (Ceulemans et

515   al., 1995) and differences in the physiology of shaded leaves compared to sun leaves

516   (Campany et al., 2016).

517   Another consideration is the increased nitrogen content in leaves in 2015 which was

518   maintained in 2017 (Fig. S5).While total soil nitrogen content was not significantly different

519   between plots in 2017, it does not rule out that nitrogen in other forms (NO_3^-, NH_4^+) may have

520   been present in different concentrations. Nitrogen availability in known to stimulate growth

521   (Gruber and Galloway, 2008), and has a complex relationship with soil water availability. As

522   the soil dries, the rate of both diffusion and mass flow to the roots is reduced leading to a

523   decrease of permeability of roots to nutrients (Oren and Sheriff, 1995), counteracted

524   somewhat by the increased concentration of the soil solution (Nye et al., 1979). On the other

525   hand mineralization and nitrification can be reduced under lower water availability (Larsen et

526   al., 2011). In maize roots, response to drought resulted in an overexpression of nearly all

527   genes involved in nitrogen uptake and assimilation (Wang et al., 2017). When plant growth is

528   manly limited by water, theory suggests that allocating more nitrogen per leaf area would

529   raise CO_2 assimilation when stomatal conductance and leaf area production declines

530   (Farquhar et al., 2002), which is in accordance with our results of higher nitrogen content and

531   photosynthetic capacity in the rainfall exclusion trees in the field. Mooney *et al.* (1978) found

532   an increased nitrogen content in eucalyptus species living in dryer habitats and other reports

533   show a similar results under drought in beech (Sánchez-Gómez et al., 2013) and willows

534   (Weih et al., 2011), driven by a higher leaf mass per area under drought, which is similar to

535   our findings (Table 1, Fig. S5). Higher LMA is often related to higher photosynthetic rates,

536   and higher nitrogen content per unit area and mass (Wright et al., 2004). In our experiment,

537   nitrogen content as a percent of leaf dry mass was higher in the rainfall exclusion trees, and

538   the leaves of these trees had a higher mass per leaf area, both contributing to the observed

539   higher photosynthetic capacity in the rainfall exclusion trees. This increased nitrogen content

540   was hypothesized to be a functional adaptation rather than a passive effect of reduced water

541   availability (Weih et al., 2011), potentially relevant as a breeding target for poplars.

542   This response may have interacted with the covers installed in the rainfall exclusion plot.

543   Placing covers permeable to water on the soil, like traditional mulch, is known to increase soil

544   water content (Walsh et al., 1996b), a rise in temperature of 1 to 2°C in the firsts 10 cm of soil

545   but not in the air 5 cm above the surface (Ham et al., 1993; Walsh et al., 1996b), and an

546   increased nitrate content in the soil because of the lower weed competition and lower leaching

547   (Walsh et al., 1996a). This results in an increased growth and biomass production when

548   compared to grass or mixed flora covers. In our setup the soil water content was reduced

549   under the covers (Fig. 2), which may have been promoted by increased soil evaporation

550   induced by the increase in soil surface temperature. However the amount of reflected light and

551   rise in temperature caused by the covers is largely dependent on the cover color (Hostetler et

552   al., 2007) which was green in our case, similar to the grass cover in the irrigated plot.

553   Poplar roots can have associations with ectomycorrhizal and with vesicular-arbuscular

554   mycorrhizal fungi but can be limited by high soil nitrogen and temperature (Block et al.,

555   2006), and by low water availability (Nickel et al., 2017). However, root systems were found

556   to be related to the main stem diameter in poplars, depending on soil fertility (Fortier et al.,

557   2015). Following this assumption would lead to a probable higher root biomass in the rainfall

558   exclusion trees than in the irrigated ones, even if mycorrhization may be limited under the

559   canvas. Carbon allocation to roots was found to be increased both under drought

560   (Tschaplinski et al., 1998) and under low soil nitrogen (Fortier et al., 2015). Furthermore,

561   under high nitrogen but low water availability, root proliferation was enhanced in different

562   poplar hybrids (Ibrahim et al., 1998), increasing levels of carotenoids, proline, ABA and

563   consequently, drought tolerance (Song et al., 2019). This has the additional result of

564   decreasing leaf gas exchange (Liu and Dickmann, 1996; Song et al., 2019). These

565   mechanisms may have strengthened the drought tolerance of the rainfall exclusion trees

566   subjected to repeated summer droughts.

567   *4.2 Carbon isotope discrimination as a leaf-level estimator of transpiration efficiency*

568   Numerous studies have found a negative relationship between Δ and WUE in C_3 crops and

569   trees species, in accordance with theory (see: Brugnoli and Farquhar, 2000; Roussel et al.,

570   2009b). More specifically, Δ measured in soluble sugars was negatively correlated with TE in

571   six hybrid poplar genotypes (Rasheed et al., 2013) and several black poplar genotypes

572   (Bogeat-Triboulot et al., 2019; Durand et al., 2019; Rasheed et al., 2015) grown in a

573   glasshouse. This is in agreement with our study showing the negative relationship between Δ

574   and TE across hybrid and black poplar genotypes in the field (Fig. 6**b**). In the glasshouse, our

575   lack of correlation may be explained by a number of factors. First, the sampled leaves were

576   already mature at the start of the drought so that leaf gas exchange measurements could be

577   performed on the same leaves (Durand et al., 2019). Thus, a significant part of the carbon

578   analyzed (*e.g.* carbon used for cell walls) was assimilated under well-watered conditions,

579   before the drought, and dampens the drought signal integrated into short turnover molecules

580   such as sugars and starch. This likely led to the observed small range (2 ‰) of Δ values in the

581   glasshouse. The trend may have been driven by leaf starch and soluble sugars as they are

582   known to more closely reflect the daily assimilation-weighted average of C_i over atmospheric

583   CO_2 concentrations (C_i/C_a; Brugnoli *et al.*, 1988) . Second, Δ and TE involve different spatial

584 and temporal integration. At the leaf level the link between Δ and C_i/C_a could be modified by

585 cuticular and mesophyll conductance (Brugnoli and Farquhar, 2000), fractionation during

586 respiration and photorespiration (Gillon and Griffiths, 1997), leaf physiology (*e.g.* sun/shade)

587 and leaf age causing different metabolisms and chemical compositions (Gutierrez and

588 Meinzer, 1994). All of which are susceptible to have genotypic-specific variations and/or be

589 altered under stress. At the whole-plant level, Δ does not include carbon lost through

590 respiration by non-photosynthetic organs during the day, or at night by the whole plant. It

591 does not take into account the water losses during the night through stomata or through other

592 organs during the day (Farquhar et al., 1989; Hubick and Farquhar, 1989). 6J29 appears to

593 deviate to the upper right in Fig. 6 both in controlled and field conditions by having the

594 highest combined TE and Δ, the latter being usually associated with lower WUE. A possible

595 explanation may be that the higher photosynthetic capacity found in this genotype may result

596 from differences of mesophyll conductance compared with the other genotypes, shifting the

597 relation between Δ and TE as well.

598 For WUE to be considered as a breeding trait, the optimization of plant water use should not

599 be at the cost of biomass production. In our glasshouse experiment TE was negatively

600 correlated with water use but not with biomass production (Fig. 6**c-e**), suggesting genotypes

601 with higher TE associated with reduced stomatal conductance without a concurrent and

602 proportional reduction in assimilation rate. The rainfall exclusion carried out likely shifted

603 within the asymptotic part of the A-g_s relationship (Wong et al., 1979) thereby decreasing

604 water use at a trivial cost of CO_2 assimilation. Many studies reported a positive (Bogeat-

605 Triboulot et al., 2019; Rasheed et al., 2013; Voltas et al., 2006) or an absence of correlation

606 (Marron et al., 2005; Monclus et al., 2005; Monclus et al., 2006; Rasheed et al., 2015)

607 between WUE estimators and biomass production in black and hybrid poplars as well as in

608   other species (Hubick et al., 1986; Virgona and Farquhar, 1996), both enabling the possibility

609   of selecting genotypes with improved TE and productivity. Negative correlations between

610   WUE and productivity are somewhat counter intuitive since for a given WU, the higher the

611   productivity, the higher WUE. For the correlation to be negative, a higher productivity has to

612   be met with an even higher WU so that WUE gets lower despite the higher productivity. Thus

613   it predicates upon a negative correlation between TE and water use so that increments of

614   biomass production would require a larger increment in water use. Such cases exists, often

615   under drought (Ray et al., 1999; Read et al., 1991), or when comparing genotypes with

616   contrasting growth adaptation to dryer climates (Zhang et al., 2004), but not necessarily

617   (Ehdaie and Waines, 1993). However in the field, the more productive trees were also under

618   reduced water availability, hence under stomatal control (Fig. 3-4) which led to higher TE and

619   lower Δ when compared with the not water-limited but less productive trees.

620   *4.3 Stability of transpiration efficiency across growing conditions*

621   Genotypic means of TE were mostly similar between in the field and the glasshouse

622   experiment. This is in agreement with investigations on the stability of poplar genotype

623   ranking with age (Rasheed et al., 2011) and different VPD treatments (Rasheed et al., 2015),

624   which make part of the distinctions between our glasshouse and field experiment. For black

625   poplars specifically, N38 when grown in a glasshouse and in the field tend to have a lower TE

626   and a higher Δ than 6J29, in agreement with previous drought experiments (Bogeat-Triboulot

627   et al., 2019; Durand et al., 2019). These findings of relatively stable transpiration efficiency

628   across wildly different environmental conditions and age suggests that selecting poplar

629   genotypes with higher WUE and higher productivity in glasshouses may be viable for poplar

630   plantation in the field. By contrast Δ, while finding significants correlations by combinatory

631   methods (Fig. S6), was systematically and largely higher in the glasshouse than in the field,

632   similar to previous studies on wheat (Ehdaie et al., 1991) and barley (Anyia et al., 2007). This

633   difference is not likely to be related to differences in the isotopic composition of the air as it

634   was 1‰ higher in the field (δ = -9.61 and -8.73‰ for glasshouse and field air respectively).

635   Accurate estimations of air δ are difficult, and soil respiration may have interacted with the air

636   to further modify δ depending on species and environmental conditions (Ghashghaie et al.,

637   2001; Xu et al., 2004). Apart from differences of leaf sampling between experiments, these

638   differences of Δ may be rooted in differences of g_s between the two environments. Genotype

639   ranking of whole-plant daily transpiration was also fairly maintained, except for N38 between

640   the glasshouse and the field experiment. While comparisons of VPD experienced between the

641   two growing conditions are difficult, diurnals gas exchange data performed in similar

642   instrumental conditions, and during solar noon in summer on a cloudless sky, suggests a

643   lower g_s in the field (Fig. 3-4) as compared to the glasshouse (Durand et al., 2019), further

644   reduced by the decreased water availability. Overall, the sustained growth and low reduction

645   in predawn water potential measured under rainfall exclusion compared to trees under

646   irrigation, suggests that the reduced water availability in the field was moderate (Granier et

647   al., 1999). This is similar to our glasshouse experiment because the relative extractable water

648   was higher than the 40% threshold. The lower g_s in the field would contribute to a higher W_i

649   and thus a lower Δ, in agreement with our findings. Little is known about the stability of

650   WUE between field and glasshouse experiment, with only a handful of studies showing either

651   a strong (Ehdaie et al., 1991), a weak (Lambrides et al., 2004), a trend (Johnson and Bassett,

652   1991) or no correlation at all (Frank et al., 1987).

653   In conclusion, even though the environmental conditions and age of the poplars in the

654   glasshouse and the field experiment were largely different, the absolute values of transpiration

655   efficiency were similar, and the genotype ranking was fairly maintained. There was good

656   agreement between TE and Δ in the field, suggesting that leaf processes are the main drivers

657   of whole-plant TE. Thus, measurements of Δ could be used to estimate WUE instead of the

658   more laborious measurement of TE. Moreover, while TE was driven by water used in the

659   glasshouse experiment and biomass production in the field, both results suggest that

660   genotypes could be selected for both higher biomass production and lower water use. More

661   research is needed, for example in other poplar genotypes, to investigate the effect of the

662   environment during growth on WUE in poplars so that a consensus can be reached. Other

663   factors may play a major role. For example differences of stomatal dynamics along the day

664   has been found to impact whole-plant transpiration in poplar genotypes, partly linked to

665   variation in stomatal density and sizes (Durand et al., 2019). Under field and glasshouse

666   conditions, stomatal density, size and speed and their relation to transpiration and WUE may

667   be different. The higher wind speed in the field than in the glasshouse may lead to widely

668   different boundary layer conductance of the leaf, changing the relation between variations of

669   environmental conditions and leaf transpiration. This highlights the need for future studies to

670   investigate the drivers behind these contrasting patterns so that the opportunity to breed plants

671   for improved water use does not remain beyond our reach forever.

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.

AUTHOR CONTRIBUTION

MD, CB, JBL, PC and DLT contributed to the data collection. MD, OB, AG and DLT contributed to the experimental design. MD, OB, DLT, JBL and AG contributed to data analysis and interpretation. All contributors were involved in the writing of the manuscript.

690 **REFERENCES**

691 Anyia, A.O., Slaski, J.J., Nyachiro, J.M., Archambault, D.J. and Juskiw, P., 2007.
692   Relationship of carbon isotope discrimination to water use efficiency and productivity
693   of barley under field and greenhouse conditions. J. Agron. Crop Sci., 193(5): 313-323.
694   10.1111/j.1439-037X.2007.00274.x

695 Bernacchi, C.J., Singsaas, E.L., Pimentel, C., Portis, A.R. and Long, S.P., 2001. Improved
696   temperature response functions for models of Rubisco-limited photosynthesis. Plant
697   Cell Environ., 24(2): 253-259. 10.1046/j.1365-3040.2001.00668.x

698 Block, R.M.A., Rees, K.C.J. and Knight, J.D., 2006. A review of fine root dynamics in
699   Populus plantations. Agrofor. Syst., 67(1): 73-84. 10.1007/s10457-005-2002-7

700 Bogeat-Triboulot, M.B., Buré, C., Gérardin, T., Chuste, P.A., Le Thiec, D., Hummel, I.,
701   Durand, M., Wildhagen, H., Douthe, C., Molins, A., Galmès, J., Smith, H.K., Flexas,
702   J., Polle, A., Taylor, G. and Brendel, O., 2019. Additive effects of high growth rate
703   and low transpiration rate drive differences in whole plant transpiration efficiency
704   among black poplar genotypes. Environ. Exp. Bot., In press.
705   10.1016/j.envexpbot.2019.05.021

706 Bonal, D., Ponton, S., Le Thiec, D., Richard, B., Ningre, N., Hérault, B., Ogée, J., Gonzalez,
707   S., Pignal, M., Sabatier, D. and Guehl, J.-M., 2011. Leaf functional response to
708   increasing atmospheric CO2 concentrations over the last century in two northern
709   Amazonian tree species: a historical δ13C and δ18O approach using herbarium
710   samples. Plant Cell Environ., 34(8): 1332-1344. 10.1111/j.1365-3040.2011.02333.x

711 Bonan, G.B., 2008. Forests and climate change: forcings, feedbacks, and the climate benefits
712   of forests. Science, 320(5882): 1444-1449. 10.1126/science.1155121

713 Brugnoli, E. and Farquhar, G.D., 2000. Photosynthetic fractionation of carbon isotopes. In:
714   R.C. Leegood, T.D. Sharkey and S. von Caemmerer (Editors), Photosynthesis:
715   physiology and metabolism. . Kluwer Academic Publishers, Dordrecht, pp. 399-434.

716 Brugnoli, E., Hubick, K.T., von Caemmerer, S., Wong, S.C. and Farquhar, G.D., 1988.
717   Correlation between the carbon isotope discrimination in leaf starch and sugars of C3
718   plants and the ratio of intercellular and atmospheric partial pressures of carbon
719   dioxide. Plant Physiol., 88(4): 1418-1424. 10.1104/pp.88.4.1418

720 Campany, C.E., Tjoelker, M.G., von Caemmerer, S. and Duursma, R.A., 2016. Coupled
721   response of stomatal and mesophyll conductance to light enhances photosynthesis of

shade leaves under sunflecks. Plant Cell Environ., 39(12): 2762-2773. 10.1111/pce.12841

Ceulemans, R., Praet, L. and Jiang, X.N., 1995. Effects of CO2 enrichment, leaf position and clone on stomatal index and epidermal cell density in poplar (Populus). New Phytol., 131(1): 99-107. 10.1111/j.1469-8137.1995.tb03059.x

Chen, S.L., Wang, S.S., Altman, A. and Huttermann, A., 1997. Genotypic variation in drought tolerance of poplar in relation to abscisic acid. Tree Physiol., 17(12): 797-803. 10.1093/treephys/17.12.797

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, C., Carrara, A., Chevallier, F., De Noblet, N., Friend, A.D., Friedlingstein, P., Grunwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D., Vesala, T. and Valentini, R., 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nat., 437(7058): 529-533. 10.1038/nature03972

Condon, A.G., Richards, R., Rebetzke, G. and Farquhar, G., 2002. Improving Intrinsic Water-Use Efficiency and Crop Yield. 42: 122-131. 10.2135/cropsci2002.0122

Condon, A.G., Richards, R.A., Rebetzke, G.J. and Farquhar, G.D., 2004. Breeding for high water-use efficiency. J. Exp. Bot., 55(407): 2447-2460. 10.1093/jxb/erh277

Coopman, R.E., Jara, J.C., Bravo, L.A., Sáez, K.L., Mella, G.R. and Escobar, R., 2008. Changes in morpho-physiological attributes of Eucalyptus globulus plants in response to different drought hardening treatments. Electron. J. Biotechnol., 11(2): 30-39. 10.2225/vol11-issue2-fulltext-9

Dai, A., 2012. Increasing drought under global warming in observations and models. Nat. Clim. Change, 3: 52. 10.1038/nclimate1633

Dai, A.G., 2011. Drought under global warming: a review. Wiley Interdiscip. Rev. Clim. Change, 2(1): 45-65. 10.1002/wcc.81

Devi, M.J., Bhatnagar-Mathur, P., Sharma, K.K., Serraj, R., Anwar, S.Y. and Vadez, V., 2011. Relationships between transpiration efficiency and its surrogate traits in the rd29A:DREB1A transgenic lines of groundnut. J. Agron. Crop Sci., 197(4): 272-283. 10.1111/j.1439-037X.2011.00464.x

754 Durand, M., Brendel, O., Buré, C. and Le Thiec, D., 2019. Altered stomatal dynamics induced
755   by changes in irradiance and vapour-pressure deficit under drought: impact on the
756   whole plant transpiration efficiency of poplar genotypes. New Phytol., 222: 1789-
757   1802. 10.1111/nph.15710

758 Duursma, R.A., 2015. Plantecophys - An R package for analysing and modelling leaf gas
759   exchange data. PLos One, 10(11). 10.1371/journal.pone.0143346

760 Ehdaie, B., Hall, A.E., Farquhar, G.D., Nguyen, H.T. and Waines, J.G., 1991. Water-use
761   efficiency and carbon isotope discrimination in Wheat. Crop Sci., 31(5): 1282-1288.
762   10.2135/cropsci1991.0011183X003100050040x

763 Ehdaie, B. and Waines, J.G., 1993. Variation in water-use efficiency and its components in
764   Wheat .1. Well-watered pot experiment. Crop Sci., 33(2): 294-299.
765   10.2135/cropsci1993.0011183X003300020016x

766 Ehleringer, J.R., 1993. 1 - Introduction: Water Use in Relation to Productivity. In: J.R.
767   Ehleringer, A.E. Hall and G.D. Farquhar (Editors), Stable Isotopes and Plant Carbon-
768   water Relations. Academic Press, San Diego, pp. 3-8.

769 FAO, 2018. The state of the world's forests, Rome, Italy.

770 Farquhar, G.D., Buckley, T. and Miller, J., 2002. Optimal stomatal control in relation to leaf
771   area and nitrogen content. Silva Fennica, 36(3). 10.14214/sf.530

772 Farquhar, G.D., Ehleringer, J.R. and Hubick, K.T., 1989. Carbon isotope discrimination and
773   photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. , 40: 503-537.
774   10.1146/annurev.pp.40.060189.002443

775 Farquhar, G.D., O'Leary, M.H.O. and Berry, J., 1982. On the relationship between carbon
776   isotope discrimination and the intercellular carbon dioxide concentration in leaves.
777   Aust. J. Plant Physiol., 9: 121-137. 10.1071/PP9820121

778 Fortier, J., Truax, B., Gagnon, D. and Lambert, F., 2015. Plastic allometry in coarse root
779   biomass of mature hybrid poplar plantations. Bioenergy Res., 8(4): 1691-1704.
780   10.1007/s12155-015-9621-2

781 Frank, A.B., Barker, R.E. and Berdahl, J.D., 1987. Water-use efficiency of grasses grown
782   under controlled and field conditions. Agron. J., 79.
783   10.2134/agronj1987.00021962007900030028x

784 Ghashghaie, J., Duranceau, M., Badeck, F.W., Cornic, G., Adeline, M.T. and Deleens, E.,
785   2001. $\delta 13C$ of CO_2 respired in the dark in relation to $\delta 13C$ of leaf metabolites:

786   comparison between Nicotiana sylvestris and Helianthus annuus under drought. Plant
787   Cell Environ., 24(5): 505-515. 10.1046/j.1365-3040.2001.00699.x

788 Gillon, J.S. and Griffiths, H., 1997. The influence of (photo)respiration on carbon isotope
789   discrimination in plants. Plant Cell Environ., 20(10): 1217-1230. 10.1046/j.1365-
790   3040.1997.d01-24.x

791 Giovannelli, A., Deslauriers, A., Fragnelli, G., Scaletti, L., Castro, G., Rossi, S. and
792   Crivellaro, A., 2007. Evaluation of drought response of two poplar clones (Populus x
793   canadensis Monch 'I-214' and P-deltoides Marsh. 'Dvina') through high resolution
794   analysis of stem growth. J. Exp. Bot., 58(10): 2673-2683. 10.1093/jxb/erm117

795 Granier, A., 1985. A new method of sapflow measurement in tree stems. Ann. Sci. For.,
796   42(2): 193-200. 10.1051/forest:19850204

797 Granier, A., 1987. Evaluation of transpiration in a Douglas-fir stand by means of sapflow
798   measurements. Tree Physiol., 3(4): 309-319. 10.1093/treephys/3.4.309

799 Granier, A., Breda, N., Biron, P. and Villette, S., 1999. A lumped water balance model to
800   evaluate duration and intensity of drought constraints in forest stands. 116(2-3): 269-
801   283. 10.1016/s0304-3800(98)00205-1

802 Gruber, N. and Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen
803   cycle. Nat., 451: 293. 10.1038/nature06592

804 Gutierrez, M.V. and Meinzer, F.C., 1994. Carbon isotope discrimination and photosynthetic
805   gas exchange in coffee hedgerows during canopy development. Funct. Plant Biol., 21:
806   207-219. 10.1071/PP9940207

807 Ham, J.M., Kluitenberg, G.J. and Lamont, W.J., 1993. Optical-properties of plastic mulches
808   affect the field temperature regime. J. Am. Soc. Hortic. Sci., 118(2): 188-193.
809   10.21273/JASHS.118.2.188

810 Hamdy, A., Ragab, R. and Scarascia-Mugnozza, E., 2003. Coping with water scarcity: water
811   saving and increasing water productivity. Irrig. Drain., 52(1): 3-20. 10.1002/ird.73

812 Hostetler, G.L., Merwin, I., Brown, M.G. and Padilla-Zakour, O., 2007. Influence of
813   geotextile mulches on canopy microclimate, yield, and fruit composition of cabernet
814   franc. Am. J. Enol. Vitic., 58: 431-442.

815 Hubick, K.T. and Farquhar, G.D., 1989. Carbon isotope discrimination and the ratio of carbon
816   gained to water lost in Barley cultivars. Plant Cell Environ., 12(8): 795-804.
817   10.1111/j.1365-3040.1989.tb01641.x

818 Hubick, K.T., Farquhar, G.D. and Shorter, R., 1986. Correlation between water-use efficiency
819   and carbon isotope discrimination in diverse peanut (arachis) germplasm. Aust. J.
820   Plant Physiol., 13(6): 803-816. 10.1071/pp9860803

821 Ibrahim, L., Proe, M.F. and Cameron, A.D., 1998. Interactive effects of nitrogen and water
822   availabilities on gas exchange and whole-plant carbon allocation in poplar. Tree
823   Physiol., 18(7): 481-487. 10.1093/treephys/18.7.481

824 IPCC, 2014. Climate change 2014: Synthesis report. Contribution of working groups I, II and
825   III to the fifth assessment report of the intergovernmental panel on climate change,
826   Cambridge University Press, Geneva, Switzerland.

827 Johnson, R.C. and Bassett, L.M., 1991. Carbon isotope discrimination and water use
828   efficiency in four cool-season grasses. Crop Sci., 31.
829   10.2135/cropsci1991.0011183X003100010036x

830 Lambrides, C.J., Chapman, S.C. and Shorter, R., 2004. Genetic variation for carbon isotope
831   discrimination in sunflower: association with transpiration efficiency and evidence for
832   cytoplasmic inheritance. Crop Sci., 44. 10.2135/cropsci2004.1642

833 Larsen, K.S., Andresen, L.C., Beier, C., Jonasson, S., Albert, K.R., Ambus, P., Arndal, M.F.,
834   Carter, M.S., Christensen, S., Holmstrup, M., Ibrom, A., Kongstad, J., van der Linden,
835   L., Maraldo, K., Michelsen, A., Mikkelsen, T.N., Pilegaard, K., Prieme, A., Ro-
836   Poulsen, H., Schmidt, I.K., Selsted, M.B. and Stevnbak, K., 2011. Reduced N cycling
837   in response to elevated CO2, warming, and drought in a Danish heathland:
838   Synthesizing results of the CLIMAITE project after two years of treatments. Glob.
839   Change Biol., 17(5): 1884-1899. 10.1111/j.1365-2486.2010.02351.x

840 Liu, Z.J. and Dickmann, D.I., 1996. Effects of water and nitrogen interaction on net
841   photosynthesis, stomatal conductance, and water-use efficiency in two hybrid poplar
842   clones. Physiol. Plant., 97(3): 507-512. 10.1111/j.1399-3054.1996.tb00510.x

843 Marino, B.D. and McElroy, M.B., 1991. Isotopic composition of atmospheric CO2 inferred
844   from carbon in C4 plant cellulose. Nat., 349(6305): 127-131. 10.1038/349127a0

845 Marron, N., Villar, M., Dreyer, E., Delay, D., Boudouresque, E., Petit, J.M., Delmotte, F.M.,
846   Guehl, J.M. and Brignolas, F., 2005. Diversity of leaf traits related to productivity in
847   31 Populus deltoides x Populus nigra clones. Tree Physiol., 25(4): 425-435.
848   10.1093/treephys/25.4.425

849 Meinzer, F.C., Ingamells, J.L. and Crisosto, C., 1991. Carbon isotope discrimination
850   correlates with bean yield of diverse coffe seedling populations. Hortsci., 26(11):
851   1413-1414. 10.21273/HORTSCI.26.11.1413

852 Monclus, R., Dreyer, E., Delmotte, F.M., Villar, M., Delay, D., Boudouresque, E., Petit, J.M.,
853   Marron, N., Brechet, C. and Brignolas, F., 2005. Productivity, leaf traits and carbon
854   isotope discrimination in 29 Populus deltoides x P-nigra clones. New Phytol., 167(1):
855   53-62. 10.1111/j.1469-8137.2005.01407.x

856 Monclus, R., Dreyer, E., Villar, M., Delmotte, F.M., Delay, D., Petit, J.M., Barbaroux, C., Le
857   Thiec, D., Brechet, C. and Brignolas, F., 2006. Impact of drought on productivity and
858   water use efficiency in 29 genotypes of Populus deltoides x Populus nigra. New
859   Phytol., 169(4): 765-777. 10.1111/j.1469-8137.2005.01630.x

860 Mooney, H.A., Ferrar, P.J. and Slatyer, R.O., 1978. Photosynthetic capacity and carbon
861   allocation patterns in diverse growth forms of Eucalyptus. Oecologia, 36(1): 103-111.
862   10.1007/bf00344575

863 Muller, E. and Lambs, L., 2009. Daily variations of water use with vapor pressure deficit in a
864   plantation of I214 poplars. Water, 1(1): 32. 10.3390/w1010032

865 Nickel, U.T., Winkler, J.B., Mühlhans, S., Buegger, F., Munch, J.C. and Pritsch, K., 2017.
866   Nitrogen fertilisation reduces sink strength of poplar ectomycorrhizae during recovery
867   after drought more than phosphorus fertilisation. Plant Soil, 419(1): 405-422.
868   10.1007/s11104-017-3354-2

869 Normand, M., 1974. Méthode d'étalonnage d'un humidimètre à neutrons utilisant les mesures
870   de densité du densimètre gamma associé.: 53-69.

871 Nye, P.H., Tinker, P.B. and Boast, C.W., 1979. Solute movement in the soil-root system. Soil
872   Sci., 127(4): 254. 10.1038/272564b0

873 Oren, R. and Sheriff, D.W., 1995. Water and nutrient acquisition by roots and canopies. In:
874   W.K. Smith and T.M. Hinckley (Editors), Resource physiology of conifers.
875   acquisition, allocation, and Utilization,. Academic Press, Inc., San Diego, United
876   States, pp. 39-74.

877 Ponton, S., Dupouey, J.-L., Nathalie, B. and Dreyer, E., 2002. Comparison of water-use
878   efficiency of seedlings from two sympatric oak species: Genotype x environment
879   interactions. Tree Physiol., 22: 413-22. 10.1093/treephys/22.6.413

880 Rasheed, F., Dreyer, E., Richard, B., Brignolas, F., Brendel, O. and Le Thiec, D., 2015.
881   Vapour pressure deficit during growth has little impact on genotypic differences of

882 transpiration efficiency at leaf and whole-plant level: an example from Populus nigra
883 L. Plant Cell Environ., 38(4): 670-684. 10.1111/pce.12423

884 Rasheed, F., Dreyer, E., Richard, B., Brignolas, F., Montpied, P. and Le Thiec, D., 2013.
885 Genotype differences in C-13 discrimination between atmosphere and leaf matter
886 match differences in transpiration efficiency at leaf and whole-plant levels in hybrid
887 Populus deltoides x nigra. Plant Cell Environ., 36(1): 87-102. 10.1111/j.1365-
888 3040.2012.02556.x

889 Rasheed, F., Richard, B., Le Thiec, D., Montpied, P., Paillassa, E., Brignolas, F. and Dreyer,
890 E., 2011. Time course of delta C-13 in poplar wood: genotype ranking remains stable
891 over the life cycle in plantations despite some differences between cellulose and bulk
892 wood. Tree Physiol., 31(11): 1183-1193. 10.1093/treephys/tpr108

893 Ray, I.M., Townsend, M.S., Muncy, C.H. and Henning, J.A., 1999. Heritabilities of water-use
894 efficiency traits and correlations with agronomic traits in water-stressed alfalfa. Crop
895 Sci., 39(2): 494-498. 10.2135/cropsci1999.0011183X0039000200032x

896 Read, J.J., Johnson, D.A., Asay, K.H. and Tieszen, L.L., 1991. Carbon isotope discrimination,
897 gas-exchange, and water-use efficiency in crested Wheatgrass clones. Crop Sci.,
898 31(5): 1203-1208. 10.2135/cropsci1991.0011183X003100050025x

899 Reich, P.B., 1984. Loss of stomatal function in ageing hybrid poplar leaves. Ann. Bot., 53(5):
900 691-698. 10.1093/oxfordjournals.aob.a086734

901 Roussel, M., Dreyer, E., Montpied, P., Le-Provost, G., Guehl, J.M. and Brendel, O., 2009a.
902 The diversity of C-13 isotope discrimination in a Quercus robur full-sib family is
903 associated with differences in intrinsic water use efficiency, transpiration efficiency,
904 and stomatal conductance. J. Exp. Bot., 60(8): 2419-2431. 10.1093/jxb/erp100

905 Roussel, M., Le Thiec, D., Montpied, P., Ningre, N., Guehl, J.-M. and Brendel, O., 2009b.
906 Diversity of water use efficiency among Quercus robur genotypes: contribution of
907 related leaf traits. Ann. For. Sci., 66(4): 408-408. 10.1051/forest/2009010

908 Sánchez-Gómez, D., Robson, T.M., Gascó, A., Gil-Pelegrín, E. and Aranda, I., 2013.
909 Differences in the leaf functional traits of six beech (Fagus sylvatica L.) populations
910 are reflected in their response to water limitation. Environ. Exp. Bot., 87: 110-119.
911 10.1016/j.envexpbot.2012.09.011

912 Sheffield, J. and Wood, E.F., 2008. Projected changes in drought occurrence under future
913 global warming from multi-model, multi-scenario, IPCC AR4 simulations. Clim.
914 Dyn., 31(1): 79-105. 10.1007/s00382-007-0340-z

915   Song, J.Y., Wang, Y., Pan, Y.H., Pang, J.Y., Zhang, X., Fan, J.F. and Zhang, Y., 2019. The
916   influence of nitrogen availability on anatomical and physiological responses of
917   Populus alba x P-glandulosa to drought stress. BMC Plant Biol., 19. 10.1186/s12870-
918   019-1667-4

919   Stobrawa, K., 2014. Poplars (Populus spp.): ecological role, applications and scientific
920   perspectives in the 21st century, 20, 204-213 pp.

921   Touma, D., Ashfaq, M., Nayak, M.A., Kao, S.-C. and Diffenbaugh, N.S., 2015. A multi-
922   model and multi-index evaluation of drought characteristics in the 21st century. J.
923   Hydrol., 526: 196-207. 10.1016/j.jhydrol.2014.12.011

924   Tschaplinski, T.J. and Blake, T.J., 1989. Water relations, photosynthetic capacity, and root
925   shoot partitioning of photosynthates as determinants of productivity in hybrid poplar.
926   Can. J. Bot., 67(6): 1689-1697. 10.1139/b89-213

927   Tschaplinski, T.J., Tuskan, G.A., Gebre, G.M. and Todd, D.E., 1998. Drought resistance of
928   two hybrid Populus clones grown in a large-scale plantation. Tree Physiol., 18(10):
929   653-658. 10.1093/treephys/18.10.653

930   Turner, N.C., Palta, J.A., Shrestha, R., Ludwig, C., Siddique, K.H.M. and Turner, D.W.,
931   2007. Carbon isotope discrimination is not correlated with transpiration efficiency in
932   three cool-season grain legumes (pulses). J. Integr. Plant Biol., 49(10): 1478-1483.
933   10.1111/j.1672-9072.2007.00557.x

934   Viger, M., Smith, H.K., Cohen, D., Dewoody, J., Trewin, H., Steenackers, M., Bastien, C. and
935   Taylor, G., 2016. Adaptive mechanisms and genomic plasticity for drought tolerance
936   identified in European black poplar (Populus nigra L.). Tree Physiol., 36(7): 909-928.
937   10.1093/treephys/tpw017

938   Virgona, J.M. and Farquhar, G.D., 1996. Genotypic variation in relative growth rate and
939   carbon isotope discrimination in sunflower is related to photosynthetic capacity. Aust.
940   J. Plant Physiol., 23(2): 227-236. 10.1071/pp9960227

941   Voltas, J., Serrano, L., Hernandez, M. and Peman, J., 2006. Carbon isotope discrimination,
942   gas exchange and stem growth of four euramerican hybrid poplars under different
943   watering regimes. New For., 31(3): 435-451. 10.1007/s11056-005-0879-7

944   Walsh, B.D., MacKenzie, A.F. and Buszard, D.J., 1996a. Soil nitrate levels as influenced by
945   apple orchard floor management systems. Can. J. Soil Sci., 76(3): 343-349.
946   10.4141/cjss96-041

947 Walsh, B.D., Salmins, S., Buszard, D.J. and MacKenzie, A.F., 1996b. Impact of soil
948   management systems on organic dwarf apple orchards and soil aggregate stability,
949   bulk density, temperature and water content. Can. J. Soil Sci., 76(2): 203-209.
950   10.4141/cjss96-028

951 Wang, H., Yang, Z., Yu, Y., Chen, S., He, Z., Wang, Y., Jiang, L., Wang, G., Yang, C., Liu,
952   B. and Zhang, Z., 2017. Drought enhances nitrogen uptake and assimilation in maize
953   roots. Agron. J., 109(1): 39-46. 10.2134/agronj2016.01.0030

954 Weih, M., Bonosi, L., Ghelardini, L. and Rönnberg-Wästljung, A.C., 2011. Optimizing
955   nitrogen economy under drought: increased leaf nitrogen is an acclimation to water
956   stress in willow (Salix spp.). Ann. Bot., 108(7): 1347-1353. 10.1093/aob/mcr227

957 Wong, S.C., Cowan, I.R. and Farquhar, G.D., 1979. Stomatal conductance correlates with
958   photosynthetic capacity. Nat., 282(5737): 424-426. 10.1038/282424a0

959 Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-
960   Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom,
961   P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J.,
962   Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L.,
963   Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. and Villar,
964   R., 2004. The worldwide leaf economics spectrum. 428(6985): 821-827.
965   10.1038/nature02403

966 Xu, C.y., Lin, G.h., Griffin, K.L. and Sambrotto, R.N., 2004. Leaf respiratory CO_2 is 13C-
967   enriched relative to leaf organic components in five species of C3 plants. New Phytol.,
968   163(3): 499-505. 10.1111/j.1469-8137.2004.01153.x

969 Zhang, X., Zang, R. and Li, C., 2004. Population differences in physiological and
970   morphological adaptations of Populus davidiana seedlings in response to progressive
971   drought stress. Plant Sci., 166(3): 791-797. 10.1016/j.plantsci.2003.11.016

972

Glasshouse

Field

Whole-plant transpiration efficiency

Whole-plant transpiration efficiency

Growing conditions

Upscaling

Upscaling

Drought impact on poplar genotypes

Leaf-level water use efficiency

Growing conditions

Leaf-level water use efficiency