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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.

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15

16 **ABBREVIATIONS**

- 17 WUE: water use efficiency  
18  $W_i$ : 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  $\Delta$ : carbon isotope discrimination  
21  $\delta$ : 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  $V_{c_{max}}$ : 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  $\Psi_p$ : 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 ( $\Delta$ ) and TE. Moreover, even  
55 though the drivers of WUE in both experiments were different, increases of WUE measured as  $\Delta$   
56 or TE was not associated with lower biomass production. Relatively good agreement was found  
57 between  $\Delta$  and TE in the field, absence of a similar correlation in the glasshouse is discussed.

58 • These results suggest that  $\Delta$  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.

63 **KEYWORDS**

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

65        **1. INTRODUCTION**

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

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

83        The concept of water-use efficiency (WUE) can be defined at the whole-plant scale as the  
84        transpiration efficiency (TE), the amount of biomass produced for a given amount of water used  
85        over a defined period of time (Condon et al., 2002). Since biomass production is linked at the  
86        leaf-level to CO<sub>2</sub> assimilation (A), and the majority of water is lost by leaf transpiration, driven  
87        by stomatal conductance (g<sub>s</sub>) 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 ( $\delta$ , the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  in a  
101 biological sample relative to an international standard, Pee Dee Belemnite) enables the  
102 comparisons of  $\delta$  in the sample of interest relative to the one of the atmosphere ( $\Delta$ ). Differences  
103 of  $\delta$  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).  $\Delta$  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  $\Delta$  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).  $\Delta$  is a leaf-level  
109 assimilation rate-weighted temporal integration of the A/ $g_s$  ratio. Unlike TE,  $\Delta$  does therefore not  
110 take into account possible carbon gains through photosynthetic stems, carbon losses by

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 ( $\Delta$ ,  $W_i$ , 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  $\Delta$  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  $\Delta$  (reflecting  
127 low or high WUE, respectively) had a similar ranking in terms of  $\Delta$ ,  $A/g_s$  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  $\Delta$  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?

140        **2. MATERIAL & METHOD**

141        *2.1 Plant material and experimental design*

142        We used four poplar genotypes throughout the experiment. Carpaccio and I214 are commercially  
143        available *Populus deltoides* × *nigra* (Moench.) clones, while 6J29 and N38 are *Populus nigra*  
144        (L.) genotypes originating from natural populations in France (Drôme 6; FR-6) and Italy (La  
145        Zelata; IT1), respectively. Clones were selected to compare them with a previous glasshouse  
146        experiment (Durand et al., 2019) and for their diversity of drought tolerance, I214 and 6J29  
147        being usually found to be drought-sensitive (Chen et al., 1997; Giovannelli et al., 2007; Muller  
148        and Lambs, 2009; Viger et al., 2016). The glasshouse drought experiment is explained in details  
149        in Durand *et al.* (2019). Shoot cuttings of similar diameter and length (30 cm), without roots,  
150        were used in the glasshouse and the field experiment. Briefly, 64 shoot cuttings were planted in  
151        10 l pots filled with a sand/peat mixture (1/1, v/v) and complemented with fertilizers (1 g l<sup>-1</sup>  
152        CaMg(CO<sub>3</sub>)<sub>2</sub> and 20 g of Nutricote T100, 13:13:13:2 N/P/K/MgO and micronutrients, FERTIL  
153        S.A.S., Boulogne-Billancourt, France). Plants were grown in a fully automated glasshouse under  
154        natural light with a free range for air temperature between 15 and 25 °C at INRA-Grand-Est  
155        (48°45'09.3"N, 6°20'27.6"E; Champenoux, France). Volumetric soil water content (SWC) was  
156        maintained constant at 27.9% (85% of field capacity) using a weighting and watering robot at  
157        least three times a day. After 60 days, waterings were stopped on half the individuals until a  
158        SWC of 18.3% was reached (55% of field capacity). SWC was kept constant for 25 days using a  
159        pot-specific linear regression between SWC measured by Time Domain Reflectometry (HD2,  
160        IMKO, Ettlingen, Germany) and pot mass ( $R^2 > 0.8$ ). Reference masses were adjusted each week  
161        to take the plant growth into account.

162 In the field, 144 shoot cuttings were planted in June 2<sup>nd</sup> 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<sup>-1</sup>, a total nitrogen content of 1.96 g kg<sup>-1</sup> 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 26<sup>th</sup> 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 17<sup>th</sup>, June 23<sup>rd</sup>, July 18<sup>th</sup> and August  
193 22<sup>nd</sup> 2017. For the last three dates, predawn leaf water potentials ( $\Psi_p$ ) 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 11<sup>th</sup> 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 16<sup>th</sup> and sensors were removed on  
204 September 3<sup>rd</sup> 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.

207 *2.4 Monitoring and harvests*

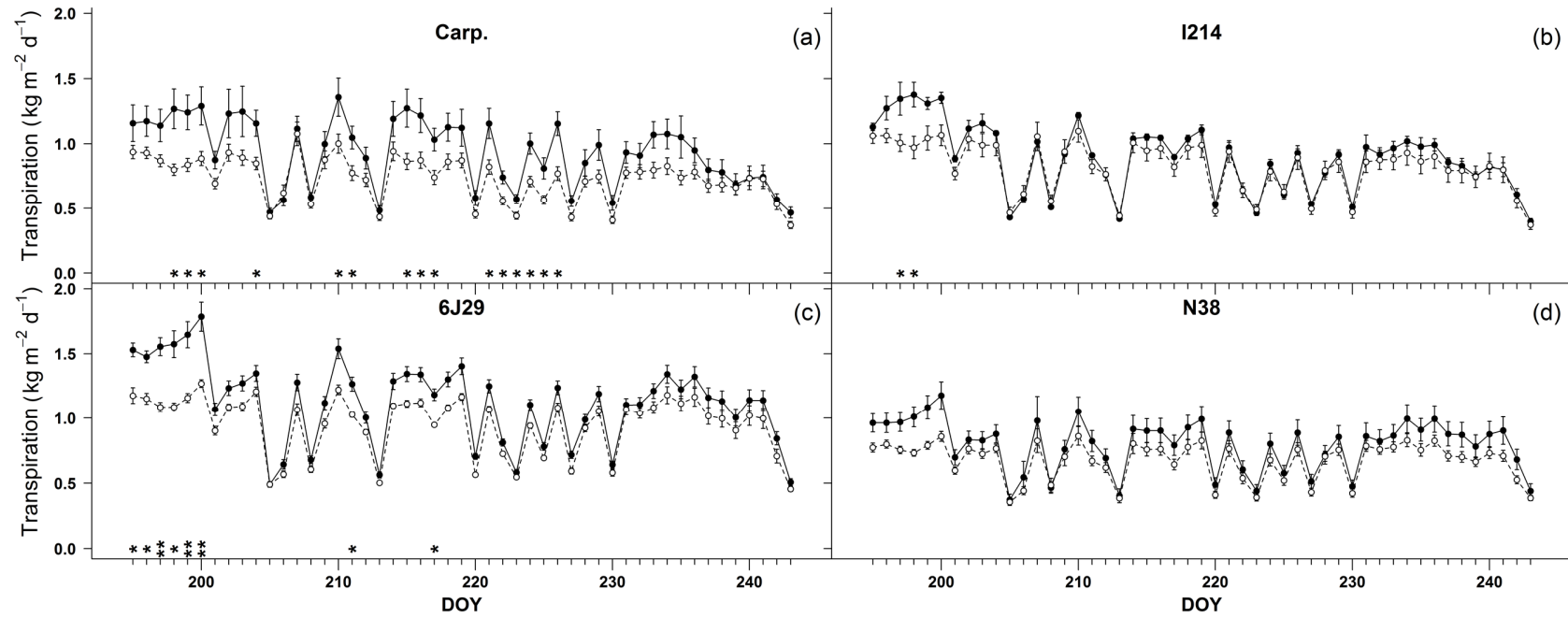
208 Meteorological variables were measured in a meteorological station 190 m away from the  
209 planted poplars. The station was equipped with a data logger (Campbell 21X, Campbell  
210 Scientific Ltd), temperature and atmospheric relative humidity (Vaisala HMP45), global  
211 radiation (pyranometer 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 18<sup>th</sup> 2016 and  
215 September 4<sup>th</sup> 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 4<sup>th</sup> and  $DM_L$  from the TLA estimated at the start of the sapflow  
241 experiment (July 11<sup>th</sup>).

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).



248

249 **Figure 1.** Daily transpiration per unit leaf area of two *Populus deltoides* × *nigra* Carpaccio (a), I214 (b) and two *Populus nigra* genotypes 6J29  
 250 (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  
 251 exclusion trees (means ± standard error). Significant differences between water treatments for each date is shown (n = 6). \*, P < 0.05; \*\*, P <  
 252 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<sub>2</sub> assimilation  
255 and internal CO<sub>2</sub> concentration (C<sub>i</sub>) along discrete steps of [CO<sub>2</sub>] 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<sup>-2</sup>, leaf-to-air VPD of 1.6  
258 kPa, fan speed of 10000 rpm, flowrate of 1000 μmol s<sup>-1</sup> with an overpressure of 0.1 kPa. Before  
259 starting, we waited for g<sub>s</sub> to reach a steady-state. Infrared gas analysers were matched after every  
260 step changes of [CO<sub>2</sub>] 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 (V<sub>cmax</sub>), maximum electron-  
262 transport rate (J<sub>max</sub>) and maximum CO<sub>2</sub> assimilation at saturating C<sub>i</sub> (A<sub>max</sub>) were estimated using  
263 the “plantecophys” R package (Duursma, 2015; R Core Team, 2019), using the Michaelis-  
264 Menten constants for CO<sub>2</sub> (K<sub>c</sub>), and O<sub>2</sub> (K<sub>o</sub>) and the CO<sub>2</sub> photo-compensation point (Γ\*) of  
265 Bernacchi *et al.* (2001).

266 On July 18<sup>th</sup> and August 22<sup>nd</sup> 2017 we monitored diurnal dynamics of gas exchange (A and g<sub>s</sub>)  
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<sub>i</sub> in this article only refers to the ratio of A/g<sub>s</sub>, 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).



## 272 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.  $1.0 \pm 0.1$  mg subsamples were weighed into tin capsules and  $\delta$  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).  $^{13}\text{C}/^{12}\text{C}$   
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  $\Delta = (\delta_{\text{air}} -$   
285  $\delta_{\text{leaf}}) / (1 + \delta_{\text{leaf}} / 1000)$ . Farquhar et al. (1982) showed that  $\Delta$  is negatively related to the ratio of  
286 internal to atmospheric  $\text{CO}_2$  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  $\text{CO}_2$  diffusion through 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 diurnal gas  
294 exchange and daily whole-plant transpiration ( $n = 5-6$ ). Differences of soil water content was

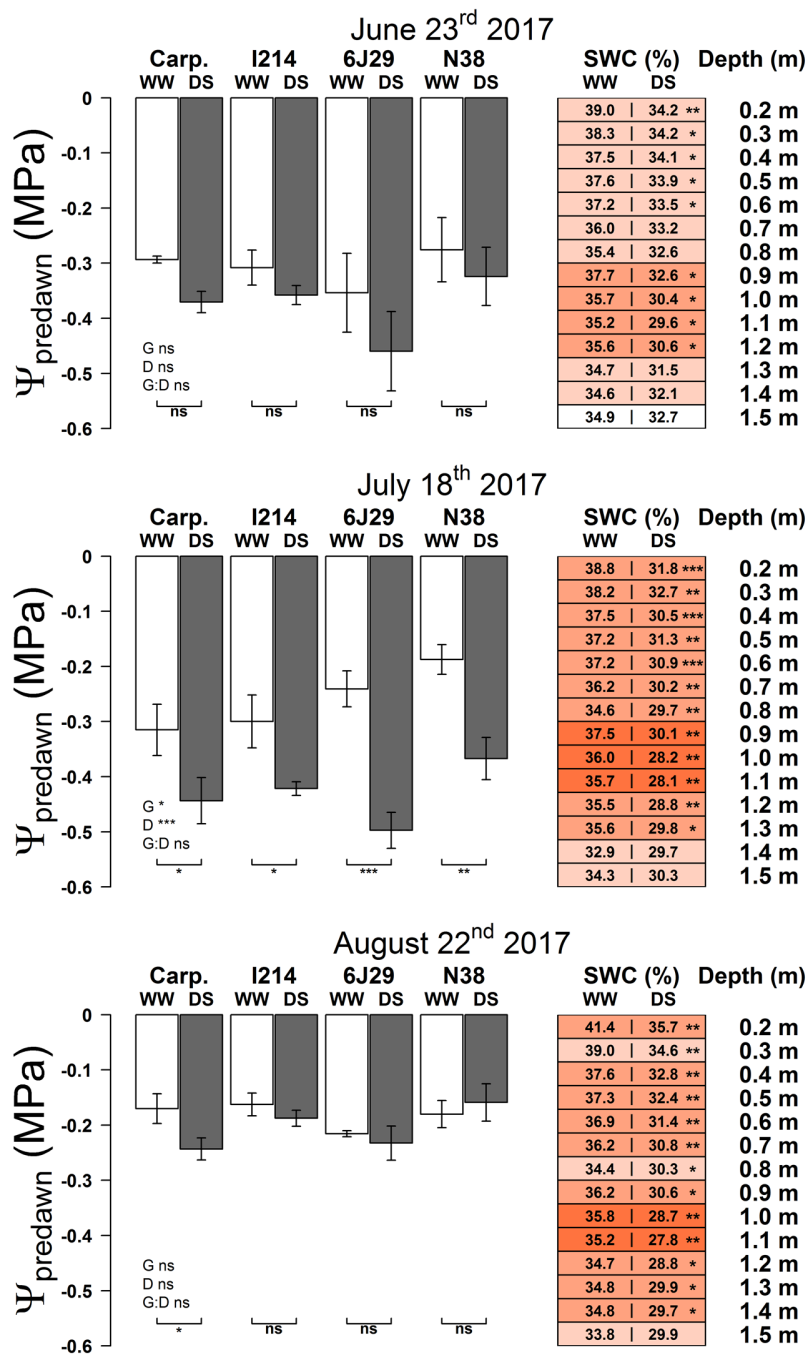
295 tested with Student tests at every depth ( $n = 10$ ). Correlations were computed by performing  
296 linear regressions ( $n = 48$ ), using genotype-treatment means when comparing field and  
297 glasshouse data because pairing could not be established between trees ( $n = 8$ ). Normality,  
298 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 17<sup>th</sup>,  
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 23<sup>rd</sup>, 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 18<sup>th</sup>.  $\Psi_p$  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. S4c  
314        between DOY 200 and 233: July 19<sup>th</sup> to August 22<sup>nd</sup> 2017) led to a small increase of SWC on  
315        both plots in the first 100 cm on August 22<sup>nd</sup>. Nonetheless, we found significant differences of  
316        SWC between plots in the first 140 cm.  $\Psi_p$  was however similar between irrigated and rainfall  
317        exclusion trees, except for Carpaccio which showed a more negative  $\Psi_p$  in the rainfall exclusion  
318        trees than in the irrigated ones (Fig. 2).

319

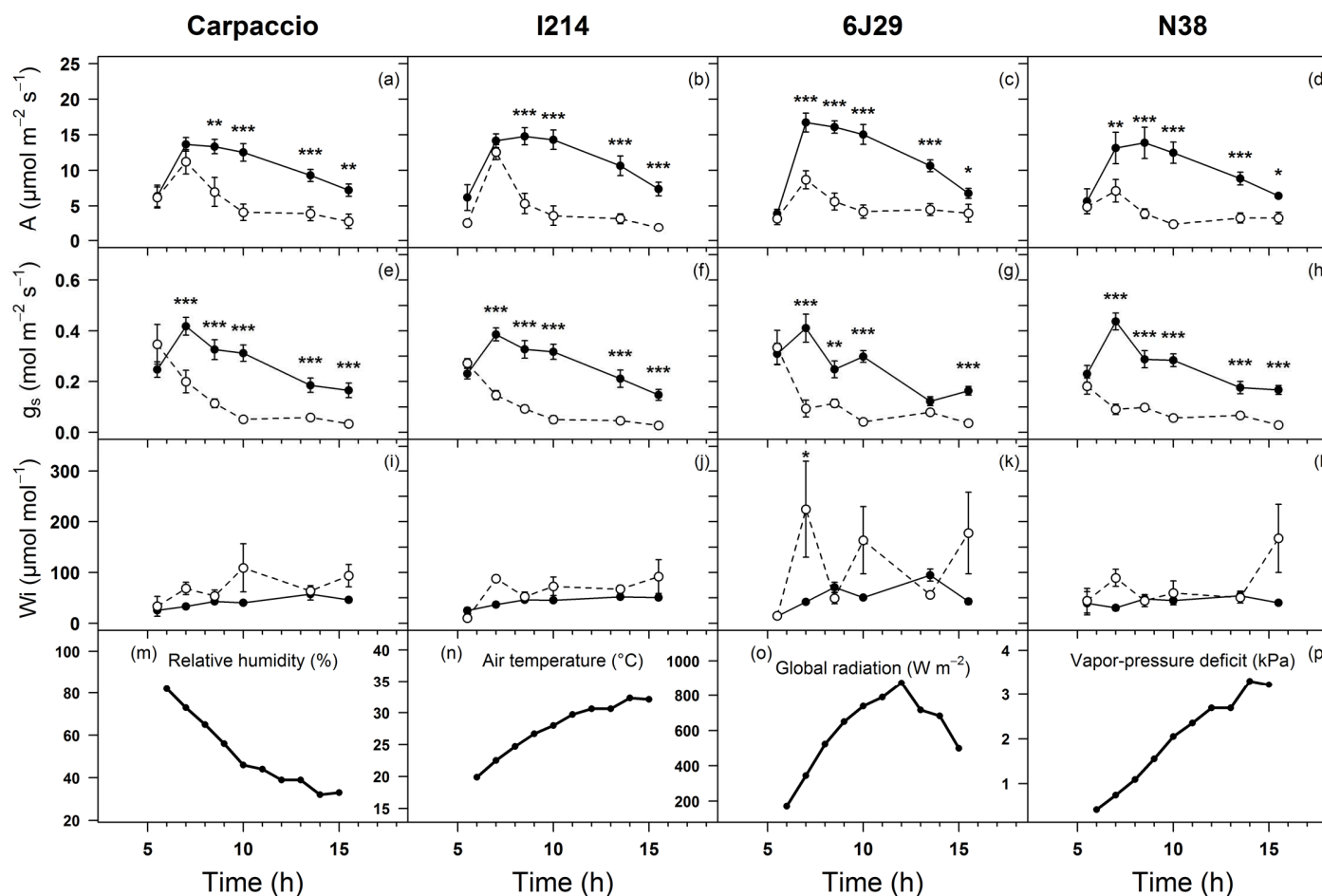


320

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

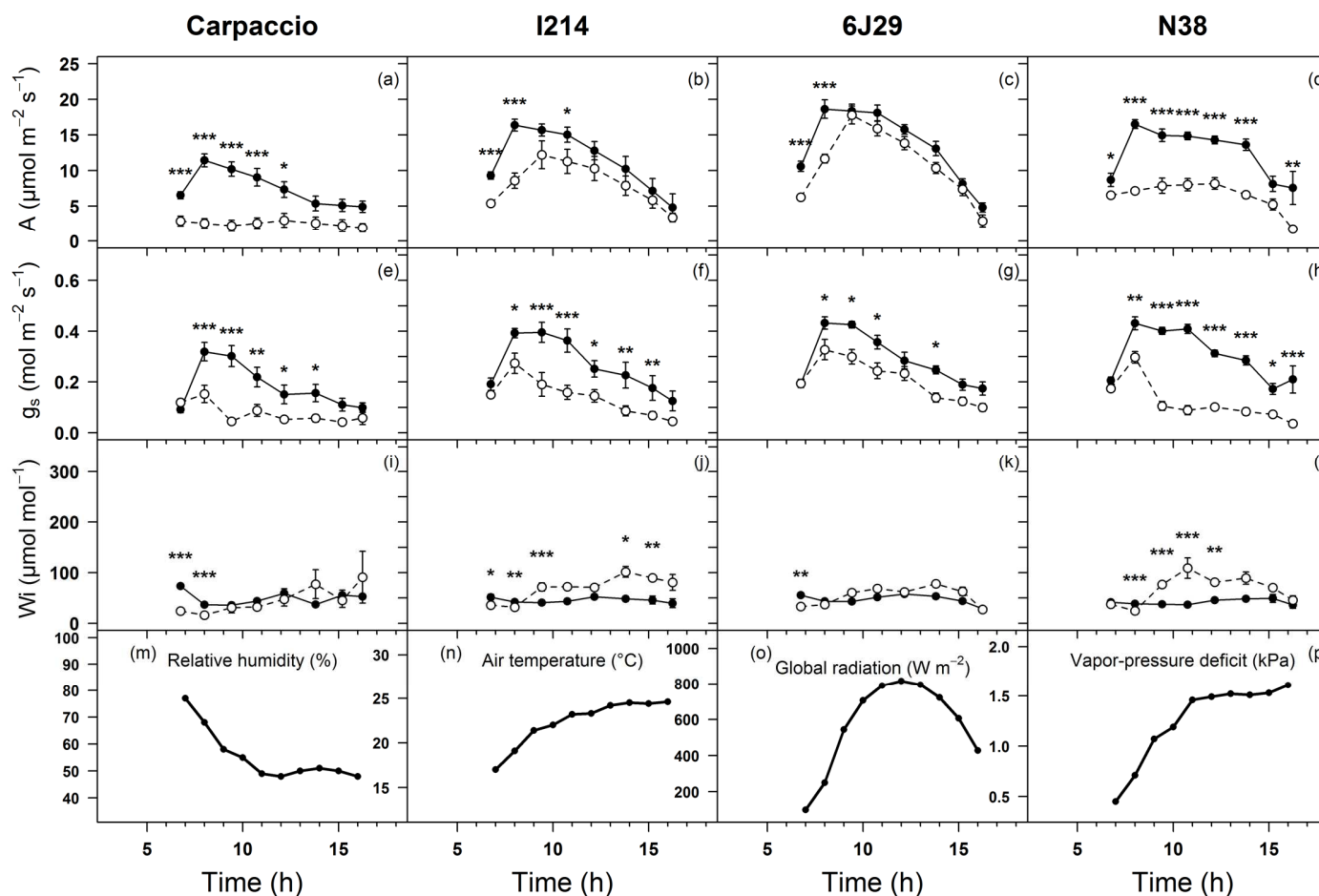
329 Sapflow sensors recorded the flow of ascending sap in the main stem, from which we can infer  
330 plant water use (WU), between July 14<sup>th</sup> and August 31<sup>st</sup> (Table 1). Whole-plant water use was  
331 higher in the rainfall exclusion hybrid poplars than in the irrigated by 34 and 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 18<sup>th</sup> and August 22<sup>nd</sup> 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. 3e-h, Fig. 4e-h). On  
343 July 18<sup>th</sup>, 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 22<sup>nd</sup>, Carpaccio rainfall exclusion trees were almost completely closed throughout the  
347 day similarly to July 18<sup>th</sup> (Fig. 4e), 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 18<sup>th</sup> (Fig. 4e-h).



351

352 **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*  
 353 *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<sub>2</sub> assimilation (A),  
 354 (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  
 355 humidity (m), air temperature (n), global radiation (o) and atmospheric vapour-pressure deficit (p) in the field are also reported. Black and white  
 356 circles are respectively for irrigated and rainfall exclusion trees (means ± standard error). Time is displayed in universal time (local time –2h). All  
 357 measurements were done on the 199<sup>th</sup> day of the year (July 18<sup>th</sup>), including the meteorological data. Significant differences between water  
 358 treatments for each time is shown (n = 4-6). \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001; ns, not significant.



359

360 **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*  
 361 *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<sub>2</sub> assimilation (A),  
 362 (e) to (h): stomatal conductance to water vapour (g<sub>s</sub>) and (i) to (l): intrinsic water use efficiency (W<sub>i</sub>), computed as the ratio of A/g<sub>s</sub>. Relative  
 363 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  
 364 measurements were done on the 234<sup>st</sup> day of the year (August 22<sup>nd</sup>), including the meteorological data. Significant differences between water  
 365 treatments for each time is shown (n = 4-6). \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001; ns, not significant.  
 366

367 *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<sub>S</sub> and DM<sub>L</sub> 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<sub>T</sub> and DM<sub>S</sub>, 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<sub>L</sub> and TLA were overall higher in the rainfall exclusion trees as  
381 well (DM<sub>L</sub>: 203 and 446 g, TLA: 2.05 and 3.94 m<sup>2</sup>, for irrigated and rainfall exclusion trees  
382 respectively).

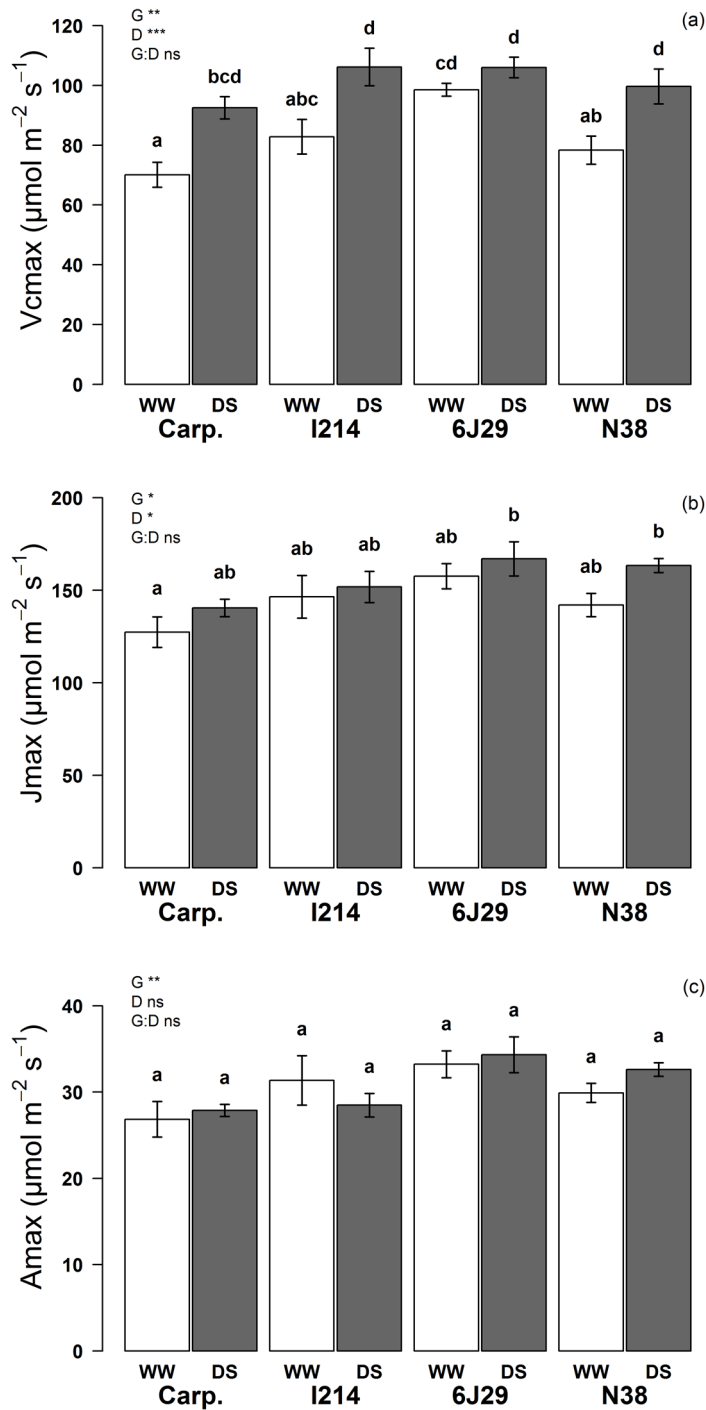


Genotype	Treatment	H (cm)	DBH (mm)	DM <sub>T</sub> (g)	DM <sub>S</sub> (g)	DM <sub>L</sub> (g)	TLA (m <sup>2</sup> )	LMA (g mm <sup>-2</sup> )	WU (kg)	E (kg m <sup>-2</sup> d <sup>-1</sup> )	TE (g kg <sup>-1</sup> )	Δ <sup>13</sup> C (‰)
Carpaccio	irrigated	53 ± 11 a	3.3 ± 0.1 a	911 ± 531 a	684 ± 425 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	<b>0.001</b>	ns	ns	ns	ns	ns	<b>0.001</b>	<b>0.002</b>	<b>&lt; 0.001</b>	<b>0.049</b>	<b>&lt; 0.001</b>
<i>P</i> value	Treatment	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>0.001</b>	<b>0.005</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
	Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	<b>0.014</b>

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 16<sup>th</sup> to September 3<sup>rd</sup>, 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<sub>T</sub>, total  
387 dry mass increment; DM<sub>S</sub>, stem dry mass increment; DM<sub>L</sub>, 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; Δ<sup>13</sup>C, carbon isotopic discrimination from bulk leaves; ns, not significant.

389 At the leaf level, photosynthetic capacity measurements showed a significant increase in  
390  $V_{c_{max}}$  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  $V_{c_{max}}, J_{max}$  and  $A_{max}$  respectively). Carpaccio had the lowest  $V_{c_{max}}, J_{max}$  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 18<sup>th</sup>,  $A$  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 3a-d). On August 22<sup>nd</sup>, 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. 4a-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

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

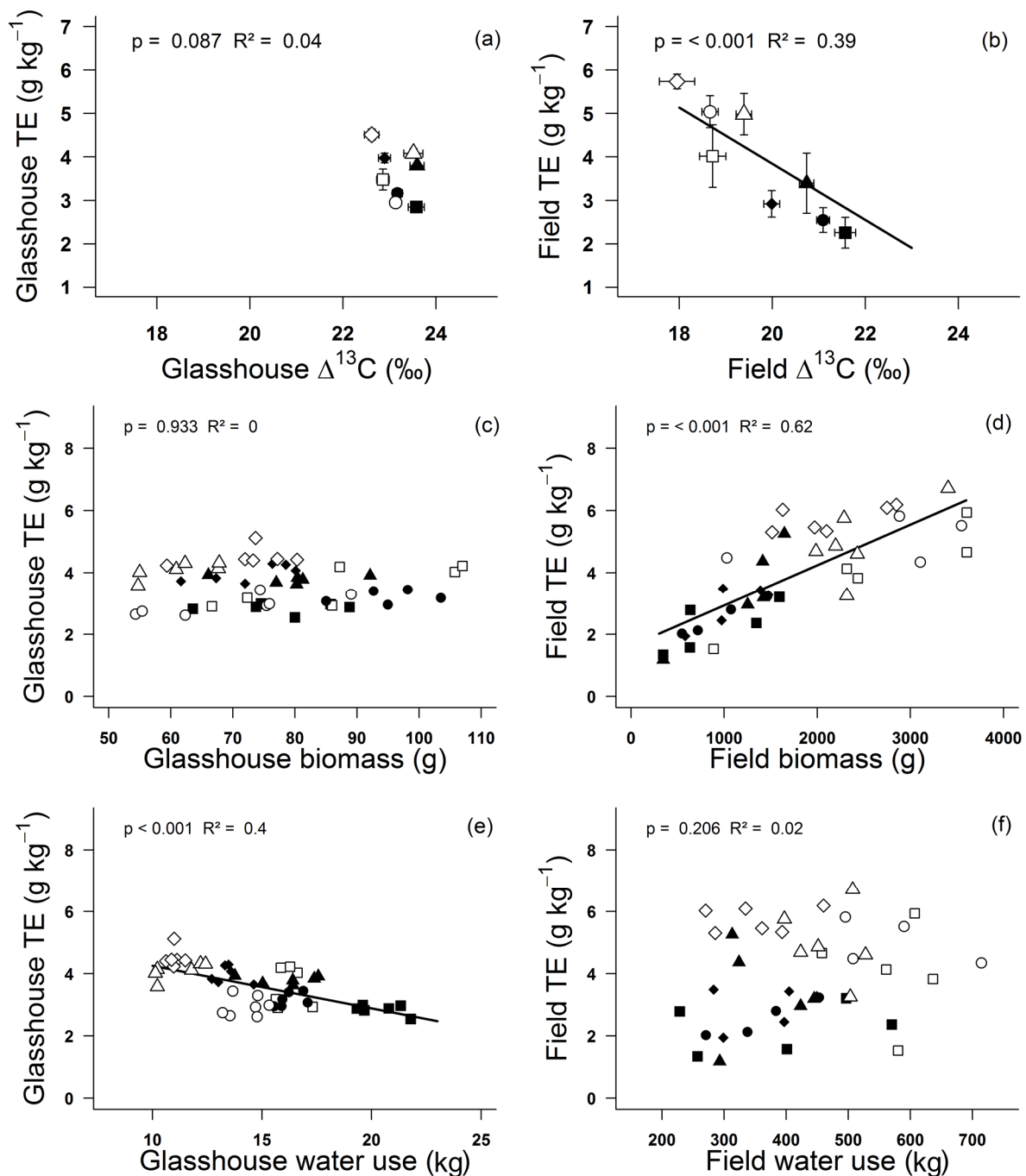
416 *3.3 Water use efficiency and comparisons between the glasshouse and field experiment*

417 Regarding the intrinsic WUE ( $W_i$ ) during the gas exchange diurnals on July 18<sup>th</sup>, we found  
418 almost no significant differences between treatments (Fig. 3i-l, Fig. 4i-l). Similarly to A and  
419  $g_s$ , no genotypic difference of  $W_i$  was found at this date. On August 22<sup>nd</sup>,  $W_i$  tended to be  
420 higher in the early morning in irrigated trees because of a similar  $g_s$  but higher A while 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<sup>-1</sup>, for irrigated and rainfall exclusion respectively). On the contrary,  $\Delta$  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  $\Delta$  (6.5%) under rainfall exclusion.  
430 Furthermore, we found a negative correlation between of TE and  $\Delta$  among treatments and  
431 genotypes in the field ( $P < 0.001$ ,  $R^2 = 0.39$ , Fig. 6b), driven mainly by the treatment. In the  
432 glasshouse, TE and  $\Delta$  were not significantly correlated ( $P = 0.087$ ,  $R^2 = 0.04$ , Fig. 6a).  
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. 6f), but positively and strongly with biomass  
435 accumulation ( $P < 0.001$ ,  $R^2 = 0.62$ , Fig. 6d) 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. 6e) but  
437 not with biomass accumulation ( $P = 0.93$ , Fig. 6c). Similar results were found when taking  
438 the treatment into account using an ANCOVA statistical design.

439

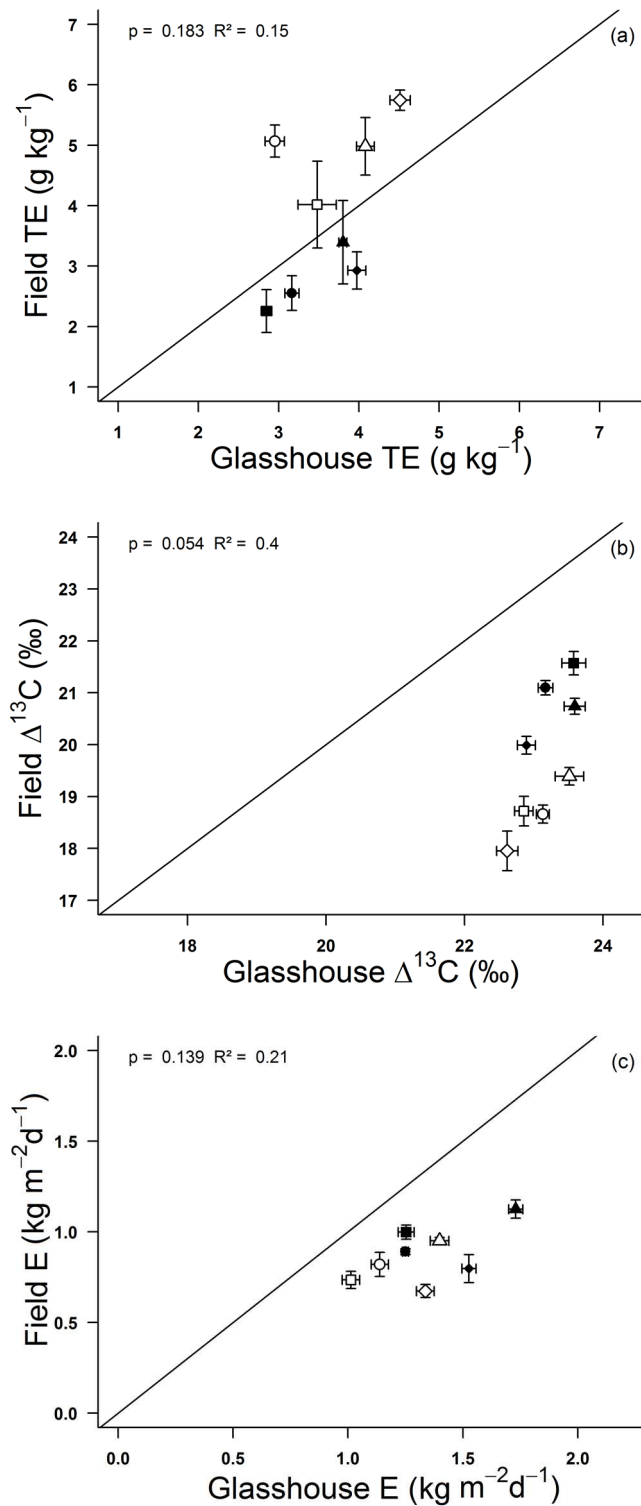
440 Unexpectedly, despite this divergence we found TE values in the field to be close to the one  
441 we found in the glasshouse experiment (Fig. 7a). Only the TE of N38 well-watered trees was  
442 significantly lower in the field than in the glasshouse ( $P = 0.02$ ) whereas the TE of water-  
443 limited I214 and N38 were significantly higher in the field than in the glasshouse ( $P < 0.004$ ).  
444 By contrast,  $\Delta$  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. 7b). This was similar to the lower E in the field than in the  
447 glasshouse (Fig. 7c), 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

450 **Figure 6.** Correlations on two *Populus deltoides* × *nigra* (Carpaccio and I214) and two *Populus nigra*  
 451 genotypes (6J29 and N38) under well-watered or reduced water availability either in a glasshouse or a  
 452 field experiment. a) Transpiration efficiency and carbon isotopic discrimination in the glasshouse  
 453 experiment b) Transpiration efficiency and carbon isotopic discrimination in the field experiment. c)  
 454 Transpiration efficiency and biomass production in the glasshouse d) Transpiration efficiency and  
 455 biomass production in the field e) Transpiration efficiency whole-plant water use in the glasshouse f)  
 456 Transpiration efficiency and whole-plant water use in the field. For a) and b), values reported are  
 457 means ± standard error (n = 6). Closed and open symbols are for well-watered and reduced water  
 458 availability, respectively. Squares, circles, triangles and diamonds are for Carpaccio, I214, 6J29 and  
 459 N38, respectively. *P* and *R*<sup>2</sup> 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,  $\Delta$  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,  $\Delta$  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. 7a). 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  $\Delta$  in both conditions  
477 and for both treatments (Fig. 7a-b). On the contrary, Carpaccio irrigated trees had the lowest  
478 TE and the highest  $\Delta$  in both conditions. 6J29 also had the highest E in both conditions for  
479 both treatments (Fig. 7c).



480

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



488 **4. DISCUSSION**

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

497 *4.1 Rainfall exclusion effect in the field*

498 A major and unexpected result of our study was the increased biomass and leaf area  
499 production in the rainfall exclusion trees as compared to the irrigated ones. The moderate but  
500 significant reduction of soil water content from June to August, and lower predawn leaf water  
501 potential in July, we found in the rainfall exclusion plot (Fig. 2) is usually associated with a  
502 reduced biomass production in poplar genotypes, driven by stomatal closure (Monclus et al.,  
503 2006). In our experiment at the leaf level, stomatal opening was substantially lower in the  
504 rainfall exclusion trees (Fig. 3-4), as theory predicts, decreasing both transpiration and CO<sub>2</sub>  
505 assimilation. Despite this, the higher biomass production was most likely amplified by the  
506 exponential growth with increased tree size, as larger trees have a higher total leaf area and  
507 thus a higher number of photosynthetic organs. The increased photosynthetic capacity ( $V_{cmax}$ ),  
508 mitigating the reduced CO<sub>2</sub> assimilation prompted by stomatal closure may have also  
509 contributed, to a lower degree, to the higher biomass production in the rainfall exclusion plot  
510 compared to the irrigated one. The observed leaf-level lower transpiration per unit area ( $E$ )  
511 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 ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ) may have  
520 been present in different concentrations. Nitrogen availability is 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 mainly limited by water, theory suggests that allocating more nitrogen per leaf area would  
529 raise  $\text{CO}_2$  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  $\Delta$  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,  $\Delta$  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  $\Delta$   
574 and TE across hybrid and black poplar genotypes in the field (Fig. 6b). 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  $\Delta$  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,  $\Delta$  and TE involve different spatial

584 and temporal integration. At the leaf level the link between  $\Delta$  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,  $\Delta$  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  $\Delta$ , 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  $\Delta$  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. 6c-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<sub>2</sub> 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 predicated 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  $\Delta$  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  $\Delta$  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  $\Delta$ , while finding significant 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 ( $\delta = -9.61$  and  $-8.73\%$  for glasshouse and field air respectively).  
635 Accurate estimations of air  $\delta$  are difficult, and soil respiration may have interacted with the air  
636 to further modify  $\delta$  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  $\Delta$  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, diurnal 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  $\Delta$ , 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  $\Delta$  in the field, suggesting that leaf processes are the main drivers  
657 of whole-plant TE. Thus, measurements of  $\Delta$  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.



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683 **CONFLICT OF INTEREST**

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

686 **AUTHOR CONTRIBUTION**

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

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973 **SUPPORTING INFORMATION**

974 **Table S1** Growth and biomass production of the four poplar genotypes, Carpaccio, I214, 6J29  
975 and N38 before and after the rainfall exclusion experiment.

976 **Table S2** Dataset used for statistical analysis (see separate Excel file).

977 **Table S3** Summary of the conditions during the measurement period in the glasshouse and  
978 field experiment.

979 **Fig. S1** Experimental design for the poplar plantation in the field.

980 **Fig. S2** Allometries for wood biomass estimation in irrigated or rainfall exclusion Carpaccio  
981 trees (*Populus deltoides* × *nigra*) in the field

982 **Fig. S3** Allometries for total leaf area estimation in irrigated or rainfall exclusion Carpaccio  
983 trees (*Populus deltoides* × *nigra*) in the field.

984 **Fig. S4** Weather data during the 2017 growth season, from April 1<sup>st</sup> to August 31<sup>st</sup> 2017.

985 **Fig. S5** Soil and leaf nitrogen content.

986 **Fig. S6** Distribution of R<sup>2</sup> and *P* values from linear regression between glasshouse and field  
987 values of TE, Δ and E randomly paired for each genotype and treatment.

# Glasshouse

# Field

Whole-plant  
transpiration efficiency

Whole-plant  
transpiration efficiency

Growing conditions

