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1 **Evapotranspiration of a willow cultivar (*Salix miyabeana* SX67)**
2 **grown in a full-scale treatment wetland**

3

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22 **Declarations of interest:** none

23 **Abstract**

24 Since woody plants like willow are used increasingly in treatment wetlands, there is a
25 growing need to characterize their ecophysiology in these specific growing conditions. For
26 instance, potential evapotranspiration (ET) can be greatly increased in wetlands, due to
27 factors like high water availability as well as oasis and clothesline effects. Few studies
28 report willow ET rates measured in full-scale constructed wetland conditions, and fewer
29 still in a temperate North-American climate. The objective of this study was to measure and
30 model evapotranspiration of a commonly used willow cultivar, *Salix miyabeana* (SX67), to
31 provide the ET rates and crop coefficient for this species. During two growing seasons, we
32 studied a 48 m² horizontal subsurface flow willow wetland located in eastern Canada,
33 irrigated with pretreated wood preservative leachate. We found a mean monthly
34 evapotranspiration rate of 15 mm/day, for a seasonal cumulative ET value of 2785 mm and
35 a mean crop coefficient of 4.1. Both the evapotranspiration results and leaf area index
36 (LAI) were greater than most results reported for open field willow plantations. Maximal
37 stomatal conductance (\bar{G}_s) was higher than that expected for deciduous trees and even for
38 wetland plants, and mean values correlated well with temperature, solar radiation, relative
39 humidity and day of the year. We demonstrated that an ET model using \bar{G}_s , LAI and water
40 vapor pressure deficit (VPD) as parameters could predict the evapotranspiration rate of our
41 wetland. This simplification of traditional ET models illustrates the absence of
42 evapotranspiration limitations in wetlands. Furthermore, this study also highlights some
43 factors that can enhance ET in treatment wetlands. Our results should both improve the
44 design of treatment wetlands using fast growing willows, and provide a simple ET
45 predictive model based on major evapotranspiration drivers in wetlands.

46 **Keywords:** willow crop coefficient, wetland evapotranspiration, stomatal conductance,
47 willow leaf parameters, evapotranspiration modelling, zero-discharge wetlands

48

49 **1. Introduction**

50 Treatment wetlands, or vegetation filters, are now commonly used for treatment of various
51 types of wastewater (Valipour and Ahn, 2017). "Artificial" wetlands are generally planted
52 with herbaceous plants like *Phragmites*, *Typha*, graminoids or other aquatic and semi-
53 aquatic species (Kadlec and Wallace, 2008). More recently, woody species of the *Salix*
54 genus (willows), generally studied for biomass production, are being tested and used for
55 wastewater treatment purposes. *Salix* species are mostly hydrophilic and tolerate hypoxic
56 conditions and great water fluctuations well, have a high growth rate and develop a
57 vigorous root system (Kuzovkina *et al.*, 2008), making them good candidates for treatment
58 wetland purposes. Another advantage of using woody plants for water treatment is the
59 added value of biomass production that can be used for bioenergy and biofuel processes
60 (Duggan *et al.*, 2005). Consequently, there is growing interest in willow for use in
61 treatment of landfill leachate, domestic wastewater or other nitrogen rich wastewaters
62 (Białowiec *et al.*, 2003; Dimitriou and Aronsson, 2011; Guidi *et al.*, 2014). Fast growing
63 willows are also known for their great evapotranspiration (ET), which led to the
64 development of a new specific type of treatment wetlands called "zero-discharge wetlands"
65 (ZDWs; Dotro *et al.*, 2017). The design of ZDWs is based mainly on the ET capacity of the
66 plant selected. They operate without liquid effluent, immobilizing contaminants in the
67 wetland substrate and preventing any release of residual contamination in the environment.
68 Depending on the type of water contamination, ZDWs can function as the final step of a

69 treatment plant or as a secondary treatment. Such wetlands are now well implanted in
70 Scandinavian countries, mainly in Denmark, where the concept was first developed
71 (Gregersen and Brix, 2001; Brix and Arias, 2011), and Ireland (Curneen and Gill, 2014).
72 Conclusive tests have also been performed in Mongolia, under very cold climatic
73 conditions (Khurelbataar *et al.*, 2017), and zero-discharge wetlands are currently being
74 tested in other locations.

75 Sound scientific knowledge of the ET rate of the species used is an essential tool to design
76 a treatment wetland because of the direct impact it will have on the wetland hydraulics
77 (Kadlec and Wallace, 2008) and its removal performance (Białowiec *et al.*, 2014). It is even
78 more important for zero-discharge wetlands, where ET is the main "treatment" process,
79 ensuring that no liquid waste will flow out of the wetland. While many studies have been
80 published on willow ET, very few concern willows growing in full-scale treatment wetland
81 conditions. However, ET in artificial wetlands can differ greatly from ET measured in a
82 plantation, and can significantly surpass potential ET (Dotro *et al.*, 2017).

83 The willow species most studied for ET is *Salix viminalis*, its hybrids and their numerous
84 cultivars (Frédette *et al.* 2018). Although widely used in Europe, some long-term studies
85 have pointed out that, in North America, cultivars of *S. viminalis* are more prone to diseases
86 and insect attacks than other cultivars (Labrecque and Teodorescu, 2005; Nissim *et al.*,
87 2013). Instead, other cultivars from species like *Salix eriocephala*, *S. purpurea*, *S. nigra*
88 and *S. miyabeana* are frequently used (Smart and Cameron, 2008). In eastern Canada,
89 Nissim *et al.* (2013) concluded that *S. miyabeana* and some indigenous species were more
90 suited for plantation than *S. viminalis*. *Salix miyabeana* has also shown the highest biomass
91 production among cultivars (Labrecque and Teodorescu, 2005; Pitre *et al.*, 2010), good

92 phytoremediation capacity and high stress tolerance (Grenier *et al.*, 2015; Nissim *et al.*,
93 2014). Considering that this species and its cultivars have been proven to be well suited for
94 some regions of North America, there is now interest in using *S. miyabeana* for treatment
95 wetlands (Lévesque *et al.*, 2017, Grebenshchykova *et al.*, 2017), ET cover (Mirck and
96 Volk, 2009) and zero-discharge wetlands (Frédette *et al.*, 2017). However, we found a
97 single study that reported ET rates for this species, based on cultivars grown on a
98 contaminated site for leachate minimization in the north-eastern United States (Mirck and
99 Volk, 2009). For all species of willow combined, we found four studies reporting ET rates
100 in treatment wetland conditions, most of them conducted in Europe and none in the
101 Americas. There is thus a clear lack of knowledge regarding the ET capacity of
102 economically important North American willow cultivars, like *S. miyabeana*, growing in
103 treatment wetlands conditions.

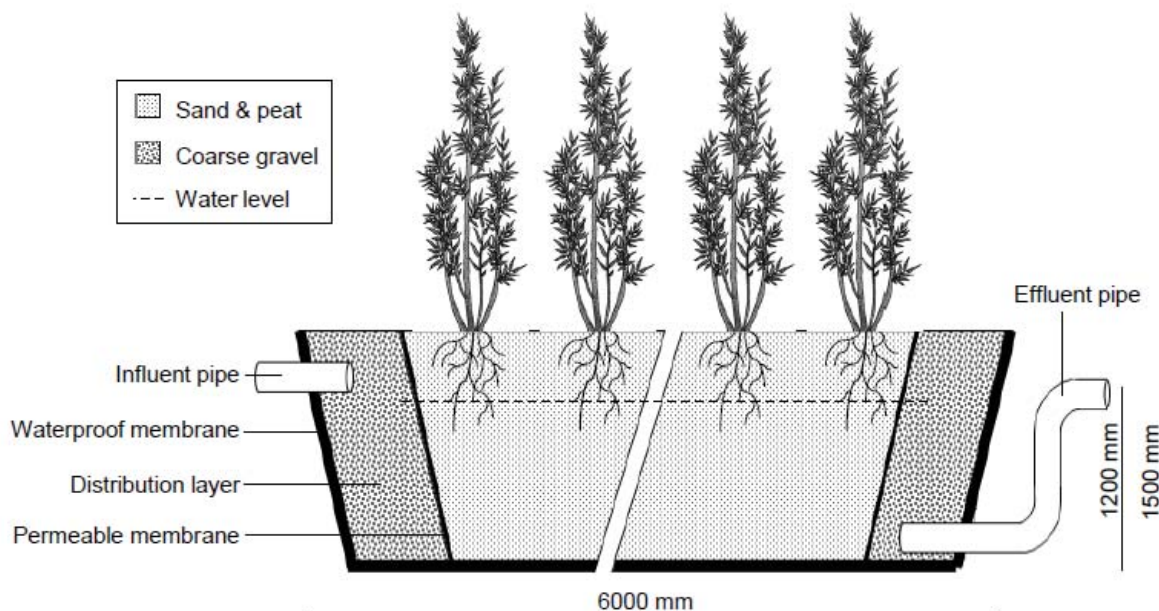
104 The first objective of our study was to measure the ET rate and provide a crop coefficient
105 (K_{ET}) for *Salix miyabeana* (SX67) grown in treatment wetland conditions in a sub-boreal
106 temperate climate. The second objective was to propose a predictive ET model, based on
107 simple meteorological and leaf parameters, which would be coherent with the wetland
108 growing conditions and physiology of fast growing willow species like *S. miyabeana*.

109 While the first objective would serve as a practical tool for development of a better
110 treatment wetland design and add to our knowledge of the ET of North American willow
111 cultivars, the predictive model would enable the transfer of our results to different climatic
112 scenarios and to other willow species that are physiologically similar but have different leaf
113 and phenological parameters.

114 **2. Material and methods**

115 **2.1 Study site**

116 The wetland studied is located in an industrial part of the city of Laval, Québec, where
 117 mean annual precipitation and temperature are 1000 mm and 6.8 °C, respectively, elevation
 118 is 91 m and the growing season is about 170 days. This willow wetland was established in
 119 2012 and serves as a final polishing step connected to a series of other constructed wetlands
 120 treating leachate contaminated with utility wood pole preservatives (chromated copper
 121 arsenate and pentachlorophenol). The treatment system is operated only during the growing
 122 season and when there is no risk of water freezing in the system, generally from May to
 123 December. More details about the experimental treatment project are provided in Levesque
 124 *et al.* (2017). The willow wetland is a horizontal subsurface flow wetland 8 m wide by 6 m
 125 long (Figure 1), lined with a waterproof membrane and filled with a mix of black peat
 126 (20%) and sand (80%) with a general porosity of 50%.



127

128 **Figure 1.** Section view of the horizontal subsurface flow wetland used to measure and model
 129 evapotranspiration of *S. miyabeana* in treatment wetland conditions.

130 Throughout this study, the average hydraulic loading rate of the willow wetland was about
 131 $55 \text{ L m}^{-2} \text{ d}^{-1}$ during the operating season, and the affluent contained a low concentration of
 132 contaminants (Table 1).

Table 1. Daily volume and general physical and chemical properties of the willow wetland influent, reported as the average value based on the entire growing season, in 2016 and 2017. Absence of values means that measured parameters were below detection limit in all samples.

Parameter	Unit	2016	2017
Daily volume	m^3	3.0	2.3
Hydraulic loading rate	$\text{L m}^{-2} \text{ d}^{-1}$	63	48
pH		7.64 ± 0.06	7.73 ± 0.12
DCO	mg/L	40 ± 1	-
PCCD/F	pg TEQ/L	0.32 ± 0.1	1.57 ± 0.46
Chlorinated phenols	$\mu\text{g/L}$	-	1.4 ± 1.4
As	$\mu\text{g/L}$	82 ± 16	160 ± 82
Cr	$\mu\text{g/L}$	11 ± 3	12 ± 6
Cu	$\mu\text{g/L}$	17 ± 7	22 ± 12

133 The wetland was fertilized in 2014, and again at the beginning of 2017 with a slow-acting
 134 fertilizer in (Acer 21-7-14). The shoots were cut back at the end of the 2014 season to
 135 maintain a juvenile state and high productivity (Nyland, 2016; Abrahamson *et al.*, 2002). A
 136 monitoring station (Campbell Scientific, various sensors) was present on site for basic
 137 meteorological data measurement (rainfall, temperature, relative humidity, solar radiation
 138 and wind speed).

139 **2.2 Plant material**

140 The wetland was planted with 112 stools of *S. miyabeana* SX67 at a planting density of 2.3
141 plants/m². *Salix miyabeana* is native to Asia and the cultivar SX67 was developed at the
142 University of Toronto, in Canada (Cameron *et al.*, 2007). It is usually grown from dormant
143 cuttings, and only male clones with no seed production are produced (Cameron *et al.*,
144 2007). Although it can reproduce vegetatively, it does not propagate laterally (*e.g.* stolon),
145 so the planting density does not change over time. However, the stools produce new stems
146 when they are cut back. They produce 6 stems on average (Tharakan *et al.*, 2005), ranging
147 from 2 to 12 (Fontana *et al.*, 2016). Tharakan *et al.* (2016) reported a mean leaf area index
148 of 4.9 for this cultivar at the end of a three-year rotation cycle. SX67 present stomata on
149 both abaxial and adaxial sides of leaves (amphistomatic) at the early development stage,
150 and adaxial stomatal density decreases as the leaves mature (Fontana *et al.*, 2017).

151 **2.3 Physiological measurements**

152 To model transpiration of *S. miyabeana*, we measured two main physiological parameters,
153 *i.e.* stomatal conductance and leaf area index.

154 **2.3.1 Stomatal conductance**

155 Instant stomatal conductance (\bar{g}_s), representing the exchange rate of vapor water from leaf
156 to the boundary layer ($\text{mmol m}^{-2} \text{s}^{-1}$), was sampled on the abaxial side of leaves using a
157 steady state porometer (Decagon, SC-1). In 2016, we sampled \bar{g}_s on 34 days from May 15
158 to October 11, with measurements in the lower, middle and upper parts of the canopy, both
159 inside and at the border of the wetland, and from 6 AM to 9 PM, for a total of 4003
160 measurements. Data from 2016 allowed us to optimize sampling for the 2017 campaign,
161 with measurements performed from 10 AM to 2 PM, where mean values of \bar{g}_s were
162 observed, and only in middle and upper part of the canopy, because of the low influence of

163 the lower part in the general stomatal conductance (\bar{G}_s) of the wetland. In 2017, sampling
 164 took place on 43 days from May 11 to October 27, for a total of 3579 measurements. Also,
 165 because *S. miyabeana* presents amphistomatic characteristics (Fontana & *al.*, 2017), 150
 166 measurements were made on both adaxial and abaxial sides of the leaves (75 pairs of
 167 measurements, taken on four days from May to August 2017) to establish a ratio of
 168 transpiration occurring on the upper versus the lower side of the leaf.

169 2.3.2 Leaf area index

170 Leaf area index (LAI), which expresses the leaf area covering a given ground area (m^2
 171 leaf/ m^2 ground), was estimated once a month, in the middle of the month, from May to
 172 November and for both growing seasons. We calculated the LAI of the entire wetland
 173 based on extrapolation of individual willow leaf area and considering that there could be
 174 significant difference between leaf area of willows growing on the border and those
 175 growing in the center of the wetland:

$$176 \quad LAI = (N_{border}IA_{border} + N_{center}IA_{center})/A_{wetland} \quad (\text{Eq. 1})$$

177 Where N is the number of willows growing either on the border or in the center, and their
 178 respective mean individual leaf area (IA), and $A_{wetland}$ is the wetland area. IA was estimated
 179 for fifteen individual willows, seven growing on the border of the wetland and eight
 180 growing in the center, as follows:

$$181 \quad IA = A_{leaf}(S_{<1m}N_{leaf} + S_{1-3m}N_{leaf} + S_{>3m}N_{leaf}) \quad (\text{Eq. 2})$$

182 A_{leaf} is the average single leaf area and is measured each month based on 30 to 40 randomly
 183 collected leaves and using the software, Mesurim Pro v3.4.4.0. The number of stems (S)
 184 were counted on the individuals and divided in 3 height classes (<1m, 1-3m, >3m). Finally,

185 the average number of leaves (N_{leaf}) present on stems was estimated by direct counting on 5
 186 random stems of each class. Afterwards, we examined the spatial variation of the leaf area
 187 by comparing individual area of stools on the edge and stools in the center of the wetland.
 188 Because the leaf cover seemed to exceed the actual area of the wetland, we also calculated
 189 and adjusted value of LAI based on the projected canopy area (Allen *et al.*, 2011).

190 **2.4 Wetland evapotranspiration calculation**

191 To estimate actual ET of the willows, we used the water balance method, based on the
 192 following mathematical equation (Kadlec & Wallace, 2008):

$$193 \quad ET = \frac{Q_i + IQ_p + Q_r - Q_d - Q_o - \frac{dV}{dt}}{A} \quad (\text{Eq. 3})$$

194 Where ET is the ET rate, Q_i the inflow flowrate, Q_p the precipitation adjusted by a canopy
 195 interception factor (I), Q_r the flowrate of runoff entering the wetland, Q_d the underground
 196 drainage flowrate, Q_o the effluent flowrate, $\frac{dV}{dt}$ the variation of the volume of water
 197 contained in the wetland and A the wetland area. We considered an interception factor of
 198 25%, determined with an equation provided by Martin and Stephen (2005) and based on
 199 leaf area index (see section 2.2.2; $I = 3.01LAI + 1.12$), meaning that only 75% of the
 200 rainfall reaches the wetland substrate, the rest being evaporated directly from the leaf and
 201 thus not considered as tree ET *per se*. As we will demonstrate below, rapid closure of the
 202 wetland canopy makes this high interception factor very suitable. Because of the
 203 waterproof membrane and the highly permeable soil surrounding the wetland, it is assumed
 204 that Q_r and Q_d are equal to zero. The water volume variation in the wetland is calculated
 205 according to the water level and a measured soil porosity of 50%:

$$206 \quad \frac{dV}{dt} = \theta \cdot A \cdot \Delta L_{(t;t-1)} \quad (\text{Eq. 4})$$

207 Where θ represents the substrate porosity, A the wetland area and $\Delta L_{(t-1,t)}$ the water level
208 variation for a given period. Water level was measured hourly with two probes (Levellogger
209 Junior Edge, Solinst) placed at two points in the wetland, from May 27 to December 9 in
210 2016 and from April 21 to November 29 in 2017. Both influent and effluent volume of the
211 willow wetland were monitored with pulse meters (Omega, FTB8000B) throughout the
212 operating season (the system was completely shut down in winter) which represent 214 and
213 220 days for 2016 and 2017 respectively. Due to a malfunction of the flow meters, 2016
214 results are overestimated and late season results for both years (October and November
215 2016 and November 2017) are not presented. Finally, reference ET was calculated
216 according to the modified Penman-Monteith method (Allen & *al.*, 1998), and open water
217 evaporation estimated by pan evaporation.

218 ***2.5 Evapotranspiration modelling***

219 In a treatment wetland, there are few limitations on ET. Available energy is greater than
220 direct solar radiation because of both "oasis" and "clothesline" effects (Dotro *et al.*, 2017;
221 Kadlec and Wallace, 2008) that increase ET potential (Allen *et al.*, 1998). Oasis effect
222 provides a vertical energy transfer in the form of sensible heat from the air surrounding the
223 wetland because its moist condition and transpiration make it cooler than the ambient air.
224 The clothesline effect, resulting from the tall wetland plants being surrounded by smaller
225 vegetation, provides a horizontal energy transfer due to wind (Kirkham, 2014). Wind effect
226 is enhanced due to the small size of the wetland and constantly disturbs the boundary layer
227 of plant leaves (Kadlec and Wallace, 2008), meaning that water vapor excreted by the
228 leaves is automatically replaced with fresh air and transpiration potential increases.

229 Frequent and high irrigation combined with a saturating water level in the wetland also
230 ensure high water availability and prevent limitation of ET due to water stress.

231 Based on these non-limited conditions, we hypothesized that transpiration of willows in a
232 treatment wetland should be highly correlated to stomatal conductance (*i.e.* water vapor
233 exchange rate between leaf and air; \bar{G}_s). \bar{G}_s is generally measured in a volume of water per
234 surface of leaf per time unit (*e.g.* $\text{mmol m}^{-2} \text{s}^{-1}$), meaning that leaf area capable of
235 transpiring (LAI_{active}) is also required for ET calculation. Because of the relatively constant
236 disturbance of the boundary layer by wind, transpiration rate should also be driven mainly
237 by water vapor pressure deficit (VPD) in the ambient air. Otherwise, the irrigation of the
238 wetland being below the surface, there is no open contact between water and the
239 atmosphere. According Shuttleworth and Wallace's energy partitioning model (1985), the
240 high average LAI of *S. miyabeana* ($> 4 \text{ m}^2$; Tharakan *et al.*, 2016) implies that most of the
241 energy available for ET is intercepted by the willows, reducing soil evaporation potential to
242 close to zero. Therefore, in this study, we assume that soil evaporation can be ignored and
243 that willow transpiration can be treated as ET. Daily ET of *S. miyabeana* grown in a
244 treatment wetland (mm/d) could then be estimated with the following leaf parameter based
245 equation:

$$246 \quad ET_{SX67} = \bar{G}_s \cdot LAI_{\text{active}} \cdot VPD \quad (\text{Eq. 5})$$

247 Active leaf area can be calculated throughout the season according to the seasonal leaf
248 development curve and the abaxial/adaxial ratio established by measurements presented in
249 section 2.3, and the vapor pressure deficit can be calculated with daily temperature and
250 relative humidity data. To estimate stomatal conductance, we chose an empirical approach
251 based on environmental parameters known to influence stomata openings (Buckley and

252 Mott, 2013). We wanted those parameters to be easily accessible, to allow the transpiration
 253 rate to be predicted with minimal resources. Through linear regressions, we tested the
 254 statistical relation between mean daily stomatal conductance measured on site and the
 255 following daily parameters: solar radiation, average and maximal air temperature, average
 256 and minimal relative humidity, wind speed and day of the year. Parameters presenting a
 257 significant relation with stomatal conductance ($p < 0.05$) were combined to predict canopy
 258 general conductance as follows:

$$259 \quad \bar{G}_s = \sum \alpha \bar{g}_s^x \quad (\text{Eq. 6})$$

260 Where partial stomatal conductance (\bar{g}_s) was calculated according to previously selected
 261 parameters (x) having their own relative influence (α) on the general stomatal conductance
 262 of the wetland canopy (\bar{G}_s). Finally, crop coefficient was calculated as follows (Kadlec and
 263 Wallace, 2008):

$$264 \quad K_{SX67} = ET_{SX67} / ET_0 \quad (\text{Eq. 7.})$$

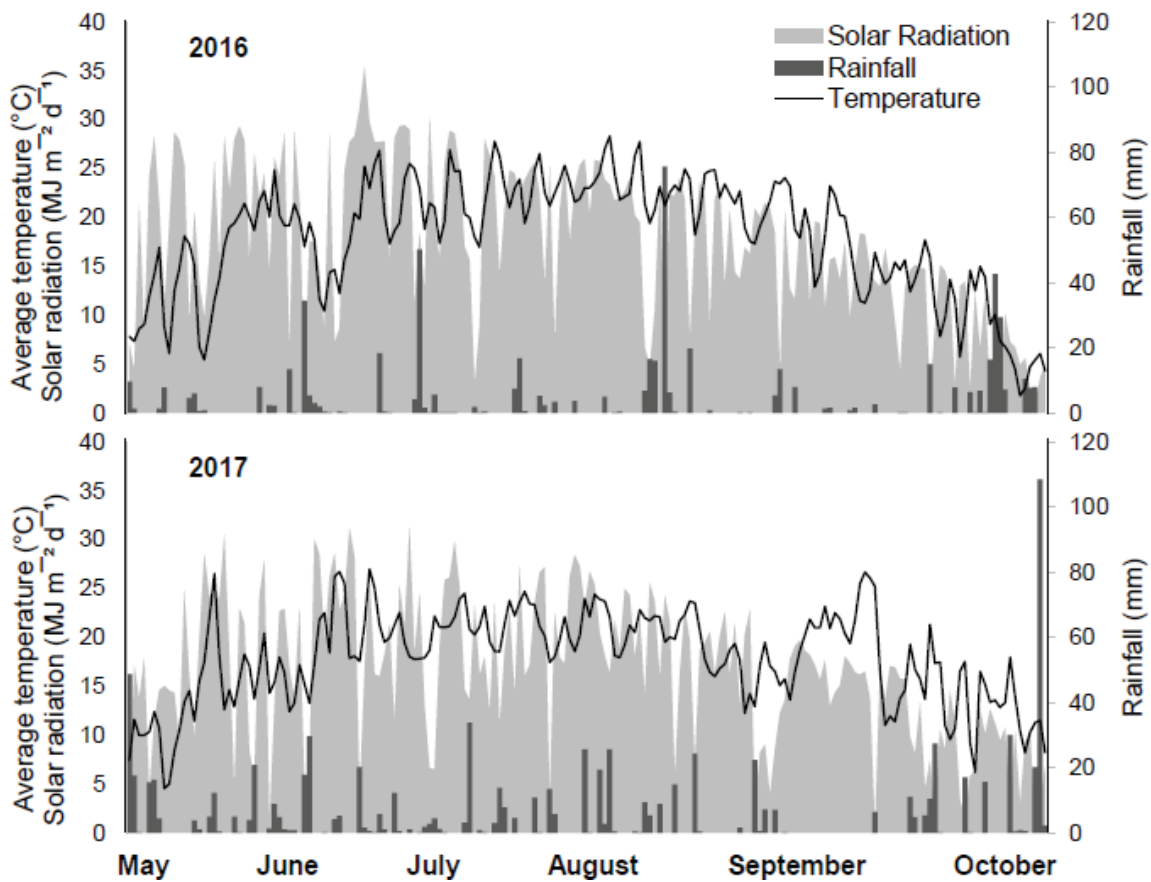
265 Where K_{SX67} is the crop, or plant, coefficient, ET_{SX67} is the modelled ET rate of the willow
 266 stand and ET_0 the reference crop ET.

267 **2.6 Statistical analysis**

268 The relation between meteorological parameters and \bar{G}_s was tested with either linear,
 269 quadratic and power regressions. The influence of parameters on a given variable (*e.g.*
 270 influence of leaf face on \bar{G}_s variation) was tested with two-way ANOVAs analysis with a
 271 0.05 significance threshold ($\alpha = 0.05$). Tukey's post-hoc statistical test was used when
 272 necessary to better interpret the results of the analysis of variance ($\alpha = 0.05$). All statistical
 273 analysis were done using R 3.5.1 software.

274 **3. Results**

275 The summer of 2016 was hot and dry, with a mean temperature of 18.0 °C (± 6.0) and 569
 276 mm of rainfall from May to October. Mean temperature was similar in 2017 (17.9 °C
 277 ± 4.8), but with less days on which maximum temperature rose above 30 °C. Also, 2017
 278 saw much higher rainfall, with 819 mm for the same period. A summary of solar radiation,
 279 rainfall and daily mean temperature for both growing seasons is shown in Figure 2.



280

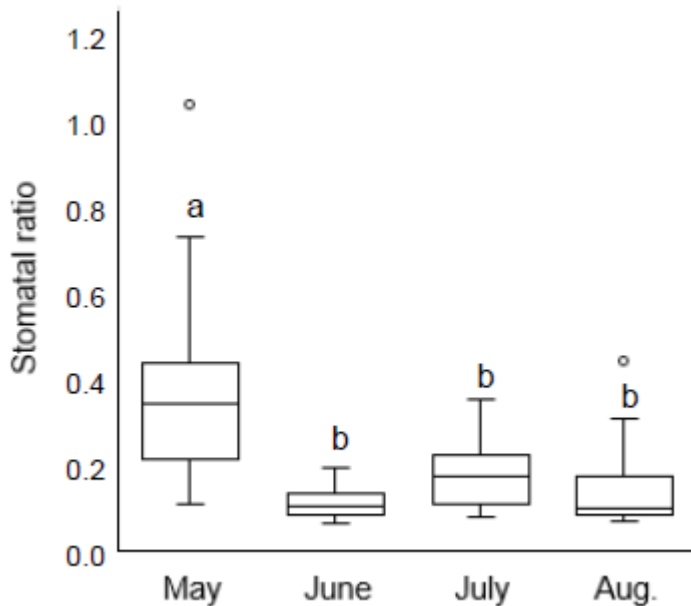
281 **Figure 2.** Summary of the meteorological conditions at the experimental site for the 2016 and 2017
 282 growing seasons.

283 Average reference crop ET was 4.5 mm/d in 2016 and 3.2 mm/d in 2017, for a total of 808
 284 mm and 750 mm respectively, from May to November. Pan evaporation measured in 2017
 285 represented 81% of reference ET. For the willow wetland, we calculated a mean daily ET

286 rate of 30.9 mm/d and a seasonal total ET of 4536 mm from May 9 to September 30 in
 287 2016, and 16.6 mm/d and a seasonal total of 2906 mm from May 15 to October 31 in 2017.

288 **3.2 Physiological measurements**

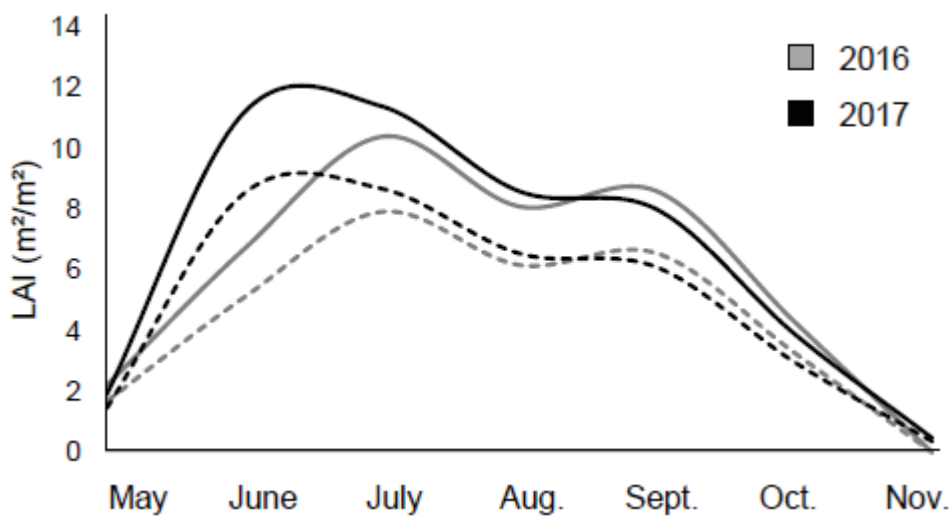
289 Stomatal conductance values were generally higher and more variable in the 2016 season,
 290 with a mean value of $418 (\pm 124)$ $\text{mmol m}^{-2} \text{s}^{-1}$ compared to $309 (\pm 59)$ $\text{mmol m}^{-2} \text{s}^{-1}$ in
 291 2017. The adaxial/abaxial stomatal conductance ratio was relatively high (0.33 ± 0.17) and
 292 variable in the early season, decreasing to relatively constant and low values (0.14 ± 0.06)
 293 for the rest of the summer (Figure 3).



294
 295 **Figure 3.** Adaxial/abaxial stomatal conductance ratio of *S. miyabeana* growing in treatment wetland
 296 conditions for the 2017 summer season. Different letters represent statistically different values.

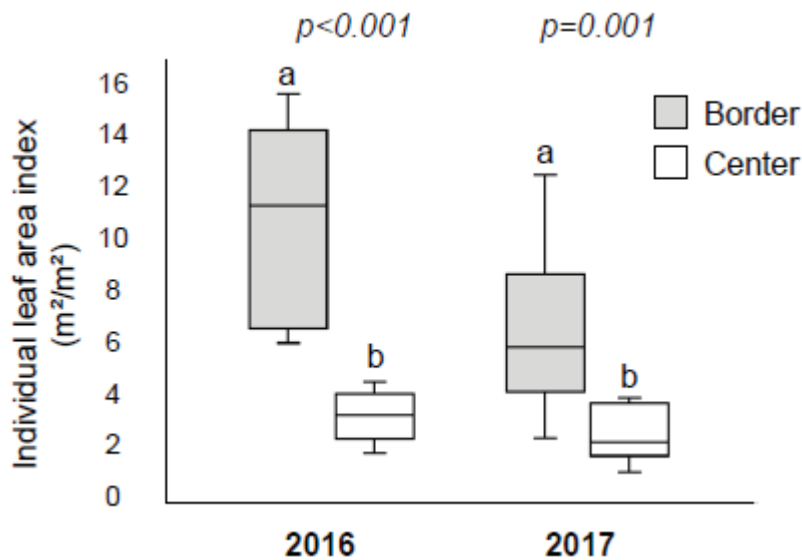
297 Thus, overall seasonal transpiration occurring on the upper part (adaxial) of the leaf
 298 represents about 20% of the lower side (abaxial) transpiration, and actual stomatal
 299 conductance equals approximately 120% of the values measured on the abaxial side of the
 300 leaf only. In both the 2016 and 2017 seasons, leaf cover established rapidly, attaining its

301 highest value in July, with 10.4 and 11.4 m² of leaves per m² of soil respectively. The
 302 canopy extended beyond the wetland borders by about 50 cm meter on each side, for a
 303 projected canopy area of 63 m² compared to the actual wetland area of 48 m². Peak LAI
 304 measured using the projected canopy area was 7.9 in 2016 and 8.7 in 2017. In 2017, the
 305 global leaf area was a little higher than in 2016, attained its maximal value earlier and
 306 retained active foliage later in the season (Figure 4).



307
 308 **Figure 4.** Evolution of the leaf area index of a 48 m² wetland (solid line) planted with *S.*
 309 *miyabeana* throughout 2 successive growing seasons, and the corresponding values
 310 adjusted for a 63 m² projected canopy area (dashed line).

311 Trees on the edge of the wetland had up to three times more leaf area than those in the
 312 center (Figure 5).



313

314 **Figure 5.** Leaf area, measured in the month of July, of 15 individuals of *S. miyabeana* growing
 315 either at the border or in the center of a 48 m² constructed wetland. Different letters represent
 316 statistically different values.

317 3.3 Evapotranspiration modelling

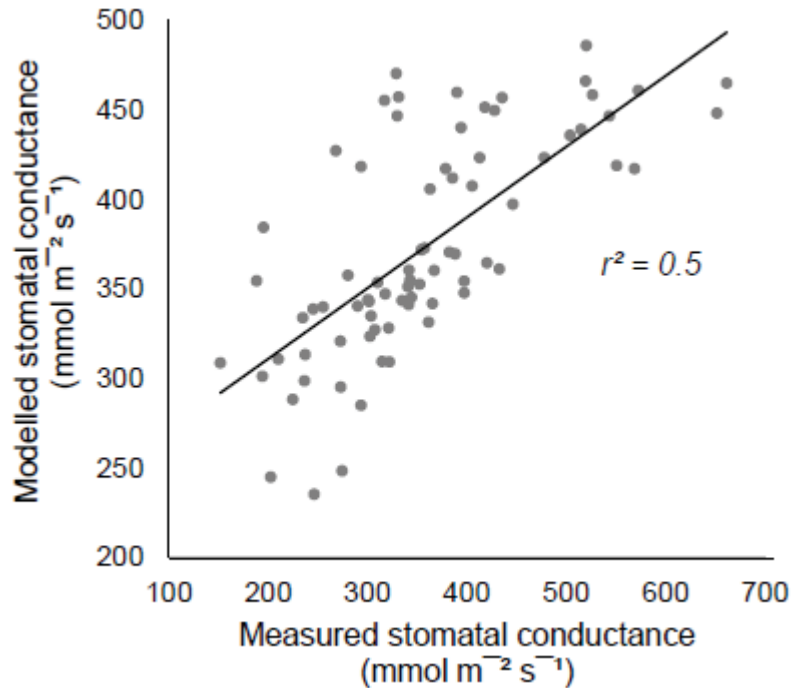
318 We found a significant effect of temperature, solar radiation, relative humidity and day of
 319 the year on stomatal conductance (Table 2), but no effect of wind speed.

Table 2. Parameters of the relations found between stomatal conductance of *S. miyabeana* and temperature (T), day of year (DOY), solar radiation (Rad) and relative humidity (RH). Parameter importance (α) and predictive equations used for stomatal conductance modelling are presented.

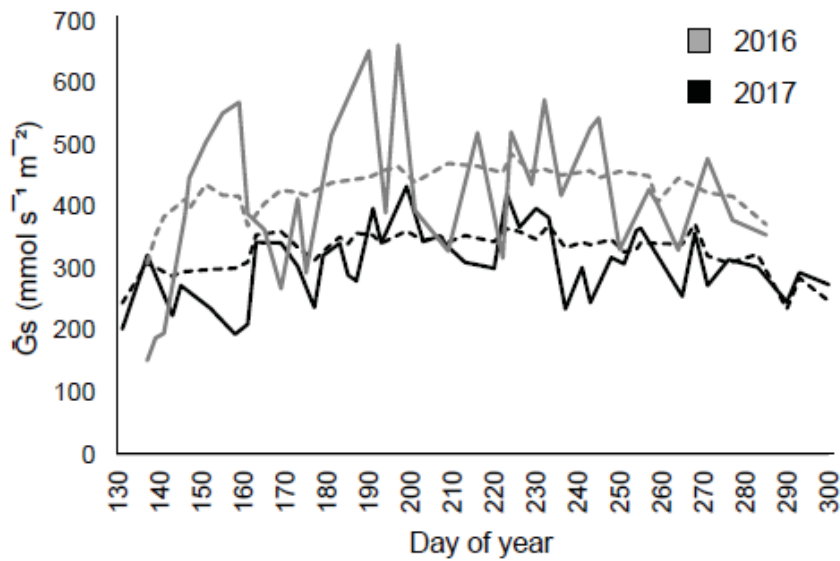
Parameter	Type of relation	p _{value}	R ²	α	Equation
T	Power	<0.001	0.21	0.48	$88.4x^{0.5}$
DOY	Quadratic	0.002	0.13	0.30	$-0.02x^2 + 9x - 572$
Rad	Quadratic	0.05	0.05	0.11	$-0.005x^2 + 2x - 177$
RH	Linear	0.03	0.05	0.11	$2.9x + 168$

320 For temperature and relative humidity, mean daily values were better predictors than
 321 maximum and minimum values respectively. Correlation between \bar{G}_s and each factor

322 separately was relatively weak (r^2 from 0.05 to 0.21), but together they explained half of
323 stomatal conductance variation throughout the season (Figure 6.), which can be considered
324 satisfying due to the many other factors driving this parameter but not measured here
325 (Buckley and Mott, 2013).



326
327 **Figure 6.** Results of \bar{G}_s modelling, based on temperature, solar radiation, relative humidity and day
328 of year, over \bar{G}_s measured on the field under the same parameters.
329 The model was good at predicting mean \bar{G}_s , with a predicted mean seasonal value of 428
330 mmol m⁻² s⁻¹ over 418 mmol m⁻² s⁻¹ measured in 2016, and 329 mmol m⁻² s⁻¹ predicted over
331 309 mmol m⁻² s⁻¹ measured in 2017. Daily variation was captured more accurately in 2017
332 than in 2016 (Figure 7).



333

334 **Figure 7.** Stomatal conductance (\bar{G}_s) field measurements (solid line) and modelling results
 335 (dashed line) over the 2016 and 2017 growing seasons.

336 Using the general stomatal conductance predicted with this model and the previously
 337 established leaf area parameters, we calculated the ET rate (Eq. 5) and the corresponding
 338 crop coefficient (Eq. 7; Table 3). Willow ET was higher in 2016, as was reference ET, with
 339 a mean daily rate of 17.1 mm/d compared to 12.9 mm/d in 2017 (Table 3). Calculated
 340 seasonal ET was 3170 mm in 2016 and 2400 mm in 2017. Crop coefficients were constant
 341 in both years, with an average of 4.1, and values slightly above 5 times the reference ET for
 342 the months of July, August and September (Table 3). Highest ET rates were calculated in
 343 August 2016 (44.8 mm/d on August 13) and in July 2017 (34.3 mm/d). Modelled crop
 344 coefficients are very close to those calculated with the water balance for most of the 2017
 345 season, but lower than water balance ET in 2016, probably due to the overestimation of
 346 actual ET for this season (section 2.4).

Table 3. Mean daily Penman-Monteith reference evapotranspiration (ET_0), estimated active leaf area index of

the 48 m² treatment wetland (LAI), modelled willow evapotranspiration (ET_{SX67}) and crop coefficient (K_{SX67}) presented as monthly and seasonal averages, for the 2016 and 2017 growing seasons.

	2016				2017			
	ET ₀	LAI _{active}	ET _{SX67}	K _(SX67)	ET ₀	LAI _{active}	ET _{SX67}	K _(SX67)
May	5.2 ± 0.9	3.3 ± 1.3	4.3 ± 5.1	0.8 ± 0.7	4.0 ± 2.0	3.4 ± 1.9	3.9 ± 3.5	1.0 ± 0.7
June	5.5 ± 0.9	8.2 ± 1.4	15.5 ± 10.4	2.8 ± 1.0	3.9 ± 1.9	12.1 ± 2.0	16.5 ± 8.7	4.3 ± 1.1
July	5.4 ± 0.6	11.6 ± 0.5	26.8 ± 9.5	5.0 ± 0.9	3.8 ± 1.4	13.3 ± 0.5	19.4 ± 5.6	5.1 ± 1.1
August	5.0 ± 0.5	10.1 ± 0.3	27.4 ± 9.8	5.5 ± 0.9	3.5 ± 1.1	10.7 ± 0.7	17.8 ± 4.3	5.1 ± 0.7
Sept.	3.9 ± 0.6	9.5 ± 0.9	20.0 ± 5.8	5.2 ± 1.2	2.6 ± 1.1	9.1 ± 0.8	13.4 ± 4.6	5.1 ± 1.1
October	1.8 ± 0.5	4.5 ± 1.9	8.7 ± 3.8	4.8 ± 1.3	1.4 ± 0.9	4.8 ± 1.4	6.4 ± 2.3	4.4 ± 0.8
Average	4.5 ± 2.0	7.9 ± 3.2	17.1 ± 12.0	4.0 ± 1.9	3.2 ± 1.8	8.9 ± 3.9	12.9 ± 8.6	4.2 ± 1.6

347 4. Discussion

348 The mean monthly ET rate measured for *Salix miyabeana* in treatment wetland conditions
 349 ranged from 3.9 to 27.4 mm/d, with a mean seasonal cumulative ET of 2785 mm. Although
 350 ET was greater in 2016 than in 2017, crop coefficients were similar for both years, ranging
 351 from 0.8 to 5.5 with a mean value of 4.1 times the Penman-Monteith reference ET. These
 352 ET results differ from those reported in the very few studies conducted in comparable
 353 conditions (Curneen and Gill, 2014; Gregersen and Brix, 2001; Brix and Arias, 2005;
 354 Kučerová *et al.*, 2001), although crop coefficients are similar (Table 4). On the other hand,
 355 LAI is very high compared to the only study we found for another cultivar of *S. miyabeana*
 356 (SX64; Mirck and Volk, 2009; Table 4), grown in open field plantation, with low water
 357 input and soil contamination.

Table 4. Evapotranspiration results obtained for fast growth willow cultivars in treatment wetland conditions (A) compared to results obtained for a plantation of Japanese willow (B)

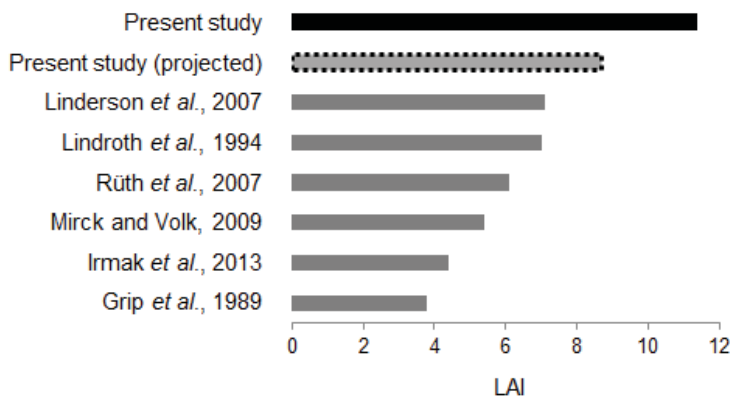
Species (cultivar)	Country	Seasonal	Peak	Seasonal	Annual	Ref.
		ET	K _{ET}	K _{ET}	K _{ET}	
<i>S. miyabeana</i> (SX67)	Canada	2785 mm	5.5	4.1	2.5	1
<i>S. viminalis</i> (Bjorn, Tora, Jorr)	Denmark	1113 mm	-	-	2.5	2
<i>S. viminalis</i>	Ireland	669 mm	5.1	3.0	-	3
<i>S. cinereal</i>	Belgium	-	6.7	-	-	4
<i>S. miyabeana</i> (9882-34, 9870-23, SX61, SX64)	USA	515 mm	1.4	1.2	-	5

Note: 1: present article; 2: Gregersen & Brix, 2001; 3: Curneen & Gill, 2014, 4: Kučerová *et al.*, 2001; 5: Mirck & Volk, 2009.

358 Average seasonal ET rates reported for other fast growing willow cultivars grown in field
 359 plantation are also generally much lower than our results (1.4 mm/d, Linderson *et al.*, 2007;
 360 3.0 mm/d, Lindroth *et al.*, 1994; 2.9 mm/d, Personn, 1995; 1.0 mm/d, Mata-Gonzalez; 3.1
 361 mm/d, Budny and Bencotter, 2012). In comparison, similar rates (from 10 to 23 mm/d)
 362 were measured for young *S. babylonica* grown in water saturated conditions in the north-
 363 eastern United States (Pauliukonis *et al.*, 2001). Such high ET rates can be explained by
 364 both enhancing factors linked to the treatment wetland itself (*i.e.* oasis and clothesline
 365 effect, high water availability, important border effect) and by *S. miyabeana* ecophysiology
 366 (*i.e.* high stomatal conductance and leaf area index).
 367 In this study, a simple model based mainly on two leaf parameters was sufficient to model
 368 ET. As expected, the model ET results were lower than the water balance results in 2016
 369 (see section 2.4). However, 2017 simulation results closely resembled water balance
 370 results. The fact that our simplified ET model yielded conclusive results supports our

371 premise that typical ET limiting factors are greatly attenuated in small wetlands. Other
372 studies presenting ET modelling methods for willows often include several limiting factors
373 (Irmak *et al.*, 2013, Iritz *et al.*, 2001), ignore heat advection effect (Přibáň and Ondok,
374 1986) or focus on soil hydrology (Personn, 1995, Hartwich *et al.*, 2016; Borek *et al.*, 2010)
375 or complex physiological processes (Tallis *et al.*, 2013). Although based on sound scientific
376 assumptions, those models hardly apply in treatment wetland conditions where water level
377 is constant, limitations are attenuated and heat advection effect is very important. The few
378 input parameters required for the operation of the model also represent an opportunity for
379 managers working with treatment wetlands to easily include ET estimation in their planning
380 activities. However, to be used for other taxa, a basic knowledge of the LAI dynamic and
381 general stomatal conductance for the species is needed, and could require additional \bar{g}_s
382 measurement in the field to adjust the model.

383 Regarding ET related characteristics specific to *S. miyabeana*, we found that mean stomatal
384 conductance ($0.4 \text{ mol m}^{-2} \text{ s}^{-1}$) was consistent with published results for other willows (0.4
385 $\text{mol m}^{-2} \text{ s}^{-1}$, Budny and Benscoter, 2016; $0.2\text{-}0.7 \text{ mol m}^{-2} \text{ s}^{-1}$, Hall *et al.*, 1998) or higher
386 ($0.2 \text{ mol m}^{-2} \text{ s}^{-1}$, Kučerová *et al.*, 2001). Leaf area index values were higher than those
387 reported in the literature for other willow cultivars, even when using the projected canopy
388 area for the calculation (Figure 8).



389

390 **Figure 8.** Maximal leaf area index (LAI) reported for willow stands (different cultivars) in various
 391 studies including the present results, and the corresponding value adjusted with projected canopy
 392 area.

393 As for stomatal conductance, it is also interesting to note that the highest mean daily value
 394 measured ($661 \text{ mmol m}^{-2} \text{ s}^{-1}$) is much higher than the values proposed for deciduous trees
 395 and even plants from wet habitats (Jones, 2013). The ratio between the conductance of the
 396 upper and lower side of the leaf is consistent with the literature predicting higher adaxial
 397 activity or adaxial stomatal density in younger leaves (Fontana *et al.*, 2017). Meteorological
 398 factors could only explain about half of the stomatal conductance values and variability.
 399 Stomatal aperture is also driven by many biochemical and environmental factors (Buckley
 400 and Mott, 2013) that were not studied here. Aging of the willows, or negative effects of
 401 contaminant accumulation in the substrate are also factors affecting long term variability of
 402 \bar{G}_s in a wetland that should be considered. A sampling campaign (data not shown)
 403 conducted in June of 2017 in Denmark on *S. viminalis* clones used for zero-discharge
 404 wetlands showed significantly greater stomatal conductance in willows recently coppiced,
 405 compared to older individuals growing in the exact same conditions, which supports the
 406 aging hypothesis. Those factors should be investigated thoroughly in the future. Leaf area

407 of the willow wetland attained its maximal value (complete canopy closure) with two-year-
408 old shoots, peaking in July at around 12 m² of leaves per m² of ground. Planting density
409 and methodological differences could partially explain why LAI of our wetland was very
410 high compared to findings reported in the literature. Furthermore, all results presented in
411 Figure 8 are based on field plantation or natural river bands of much greater size than our
412 wetland and the effect of increased leaf area at the border is negligible. Our finding
413 comparing individual leaf area at the edges versus in the center of the wetland is also
414 interesting because it means we could modulate ET rate directly in the wetland design.
415 Indeed, if ET is directly related to LAI as demonstrated here, adjusting the edge or aspect
416 ratio of the surface area of a wetland could enhance (higher ratio) or limit (lower ratio) ET
417 per ground unit, according to management objectives. Fertilization applied at the beginning
418 of 2017 seemed to have accelerated the establishment of the leaf cover but did not
419 significantly increase maximal LAI. Since the fertilizer used consisted of solid granules
420 applied directly on the soil, with degradation regulated by rainfall and temperature, it is
421 possible that rapid closure of the canopy and high rain interception by willows prevented
422 the fertilizer from appropriately degrading and penetrating the substrate. This hypothesis is
423 supported by the absence of nitrogen in the wetland effluent throughout the season (result
424 not shown). In 2016, the canopy already seemed completely closed by mid-season and it is
425 possible that maximum leaf area index was already attained. Indeed, in 2017, stems grew
426 higher but there was little or no leaf development at the bottom of the stems (as was
427 observed in 2016), probably because canopy closure was achieved and all available light
428 was intercepted in the upper part of the trees. Therefore, we conclude that maximal LAI

429 was achieved with two-year-old shoots, without a need for fertilization, and that coppicing
430 should be scheduled on a two-year basis.

431 **5. Conclusions**

432 *S. miyabeana* ET in treatment wetland condition was very high throughout this study. We
433 highlighted several factors related to treatment wetlands that can significantly increase
434 potential ET. Because there are few limitations on ET in wetlands, a model exclusively
435 based on leaf parameters successfully predicted ET values and calculated crop coefficients
436 for the studied willow wetland. Because these results are based on a full-scale wetland, they
437 can be used as design parameters for treatment wetlands using *S. miyabeana*, and the
438 equation presented for ET calculation can be adjusted for other fast-growing willow species
439 used in similar growing conditions. We also presented a strategy to optimize ET per ground
440 area by changing the aspect ratio of the wetland, and consequently its leaf area index, as
441 well as regularly coppicing the stems. In the future, other parameters possibly affecting ET
442 in treatment wetlands such as tree aging, substrate type and contaminant toxicity could be
443 investigated. This study is a first step towards better ecophysiological characterization of
444 woody plants used in treatment wetlands.

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