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1 **The decline of *Fraxinus angustifolia* Vahl in a Mediterranean salt meadow: Chlorophyll**  
2 **fluorescence measurements in long-term field experiment.**

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14

15 **Abstract:**

16 The ecological transition zones also known as ecotones are specific ecosystems but how global change  
17 will affect their biodiversity and functioning is poorly understood. In the case of the areas at the  
18 interface between riparian forest and salt meadows the biodiversity conservation remains a major  
19 issue. The aim of this work was to evaluate the effects of a salty environment on *Fraxinus angustifolia*  
20 a non-halophyte species. For this, the photochemistry of PSII was *in situ* explored during the summer  
21 between 2007 and 2016. The predawn maximum quantum efficiency of PSII (Fv/Fm) was always  
22 greater than 0.8 which indicates an absence of chronic photoinhibition induced by salinity. During  
23 these years, the light kinetic of the actual quantum yield of PSII ( $\Phi_{PSII}$ ) was studied. It appears that  
24 this yield is generally higher in salty environments than in the riparian forest. Multivariate analysis,  
25 showed that the dissipation regulated light energy fraction ( $\Phi_{NPQ}$ ), the photochemical quenching (qP)  
26 and the Fd/Fs ratio constitute the main explanatory variables of  $\Phi_{PSII}$  changes. The subsequent two-  
27 way-ANCOVA carried out demonstrated that  $\Phi_{NPQ}$  was weaker in saline environments, suggesting a  
28 better efficiency of photosystem collecting antennas. Meanwhile, the analysis of photosynthetic  
29 pigments did not exhibited a salt effect except the significant accumulation of chlorophyll a in 2016.  
30 In order to address the question of the salt-related toxicity, sodium ion contents in the leaves were  
31 found to be identical in the two sites, suggesting the establishment of an energy-dependent mechanism  
32 of salt exclusion/retention in roots. The growth rings of *F.angustifolia* in saline areas highly follows  
33 the annual water deficit changes. Between 2007 and 2016, the biomass in the salt meadow was higher  
34 with a peak in 2011 representing 60% of the biomass of control individuals. But, since 2011 a

35 continuous decline of growth was recorded. Water conditions seem to constitute a limiting factor even  
36 if the photosynthetic apparatus generates the excess metabolic energy necessary to cope with the saline  
37 toxicity. This study thus highlighted the plasticity of the photochemistry of the PSII depending to the  
38 environmental conditions of the salt meadows. It also shows the vulnerability of an ecotone through  
39 the decline in the productivity of a glycophyte with climate change. This augurs for changes in the  
40 composition of plant communities which is in favor of strict halophytes.

41 **Key words:** *Fraxinus angustifolia*; salt stress; PSII photochemistry; salt meadow; water  
42 deficit; ecophysiology.

43

## 44 1. Introduction

45 Global change projections of wetland ecosystems such as salt marshes include sea-level rise  
46 and extreme climatic events (Thorne et al., 2012). Salt meadows spread over narrow  
47 transitional areas between sea and terrestrial environments which are considered as high-risk  
48 zones subject to climate change impacts (Thorne et al., 2012). In these ecosystems, several  
49 taxonomic groups of plants coexist with salt requirements which are highly contrasted. They  
50 are divided into euhalophytes and facultative halophytes but typically an halophyte is defined  
51 as a plant that grows in soil conditions of more than 200 mM NaCl concentration (Flowers  
52 and Colmer, 2008) . Besides salinity conditions, biotic factors such as competition between  
53 individuals are also to be considered in terms of saltmarshes functioning (Pennings et al.,  
54 2005). Overall, it appears that the hydro-period regime that depends on specific precipitation  
55 and evaporation conditions is one of the main factors regulating the creation of niches for  
56 wetland plant species (Foti et al., 2012).

57 The question of the spatial distribution of species between the seashore and the boundary of  
58 non-halophytic terrestrial vegetation has been addressed by several authors (Pennings et al.,  
59 2005; Silvestri et al., 2005). For instance taking into account the elevation gradient, four  
60 clusters reflecting the composition of plant community were shown (Bang et al., 2018).  
61 However, the scarcity of studies focussing on the salt marsh-terrestrial boundaries was  
62 underlined (Traut, 2005; Veldkornet et al., 2015). Indeed, the border between the salt marsh  
63 and the adjacent upland (terrestrial vegetation) occupies a narrow surface also recognized as  
64 ecotone. These habitats are submitted to particular abiotic conditions leading to a plant  
65 community assembly that is specific to one or both of the adjacent ecosystems. In the  
66 Mediterranean region and in the Bouches-du-Rhône in particular, salt meadows develop at the

67 confluence of rivers and the Etang de Berre. This is the case for the river Arc which exhibits a  
68 steep fall of the presence of trees from the riparian forest in the upland. Among the riparian  
69 woody species only few specimens of *Fraxinus angustifolia* Vahl (narrow-leaved ash) were  
70 observed in the salt marsh upland transition zone possibly in the area so-called ecotone.  
71 Hence, information on species with narrow biotope tolerance might best characterize the  
72 effects of climate change (James and Zedler, 2000).

73  
74 *F. angustifolia* belongs to the family Oleaceae and is widespread across Southern Europe. In  
75 Mediterranean basin the plant is often encountered in riparian corridors. In Croatia for  
76 example, narrow-leaved ash is considered as one of the most important tree species of  
77 lowland floodplain forests and its sensitivity to drought was shown (Drvodelic et al., 2016).  
78 But, how *F. angustifolia* will cope with salinity and drought expected with climate change is  
79 poorly explored. More generally exploring the mechanisms of glycophytes adaptation to  
80 salinity is becoming a great challenge because soil salinization is recognized as a global  
81 problem resulting in soil degradation, arable land surface decrease and ecological  
82 environmental disturbance (Hu et al., 2019). By comparing two *Solanum* species, one  
83 glycophyte and the other salt-tolerant, it has been shown that ethylene induction plays a key  
84 role in salinity tolerance through an improved stomatal conductance, an efficient osmotic  
85 adjustment and the increase of Na<sup>+</sup> accumulation in shoots (Gharbi et al., 2017). In roots of  
86 the glycophyte *Arabidopsis thaliana* (L.) Heynh., salt stress triggered principally defence  
87 proteins as those involved in ROS scavenging whereas for the xero-halophyte *Zygophyllum*  
88 *xanthoxylum* Engl. Ex Dippel, Na<sup>+</sup> uptake was improved in addition with metabolic shifts  
89 towards *de novo* synthesis of glucose in order to sustain growth (Chai et al., 2019). Similarly,  
90 retention of sodium cations in roots thus preventing their translocation to shoots is considered  
91 as a key adaptive mechanism of glycophytes to salt stress (Assaha et al., 2017; Hasegawa,  
92 2013). Root sequestration of sodium cations not only improves the water balance by adequate  
93 osmotic adjustment but also preserves the photosynthetic machinery from ionic toxicity. Thus,  
94 from the xero-halophyte *Nitraria retusa* (Forssk.) Asch., PSII photochemistry and carotenoid  
95 content were not affected by salinity whereas a significant reduction in pigment  
96 concentrations and chlorophyll fluorescence parameters were observed from the glycophyte  
97 *Medicago arborea* L. (Boughalleb et al., 2009). This led to suggest that the decrease of the  
98 photosynthetic activity is likely the result of damages in the photosynthetic apparatus rather  
99 than factors driving stomatal closure. Other authors have also shown that the maximum  
100 quantum efficiency of PSII is highly reduced for rice salt-sensitive cultivars consecutively to

101 salt stress application as compared to salt-tolerant cultivars. Similarly, the minimum  
102 fluorescence intensity (F<sub>0</sub>), the actual quantum efficiency (PSII) and the Non-Photochemical  
103 Quenching (NPQ) were identified as the main fluorescence parameters that change depending  
104 on the intensity of salt stress (Tsai et al., 2019). Besides the ionic toxicity induced by soil  
105 salinity, water availability also constitutes a major constraint for glycophytes survival in  
106 saltmarshes. Although, chlorophyll a fluorescence has been used as a signal for monitoring  
107 plants response to drought in several reports (Dalberto et al., 2017; Vaz et al., 2016; Wang et  
108 al., 2016) few works deal with both drought and salinity effects. Moreover, plant *in situ* often  
109 experience multifactorial stress conditions.

110 Understanding how a glycophyte may stabilize its photosynthesis activity also maintaining its  
111 ionic homeostasis under high soil salinity can help provide insight in terms of plant breeding  
112 and ecological management. In this report we evaluate the ecological fitness of a glycophyte  
113 *F. angustifolia* native to the saltmarsh/upland transition zone also termed ecotone upon a  
114 highly sensitive to global climatic change variables. Second, we hypothesized that the  
115 predicted increase warming and summer drought in Mediterranean basin would lead to more  
116 NaCl accumulation requiring additional metabolic energy to sustain growth and defence  
117 molecules biosynthesis. Finally we considered that the global change would drive profound  
118 changes in the assemblages of plant communities into the ecotones of the deltaic zones of the  
119 Mediterranean rivers.

## 120 2. Material and methods

### 121 2.1 The study site

122 The area that was the subject of this study is located in the Arc delta ecotone and in the  
123 riparian forest of the same river (Berre-l'Etang, France). The Arc delta occupies an area of  
124 190 ha. It is associated with hydromorphic soils covered with a halophilic vegetation, which  
125 extend about 700 m wide. Upstream the deltaic zone (1.4 km), the riparian forest is mainly  
126 constituted by ash, poplar and white willow. The ecotone was characterized by the occurrence  
127 of both euhalophytes and facultative halophytes as well as some specimens of *Fraxinus*  
128 *angustifolia*, namely: *Sueda vera* Forssk. ex J.F.Gmel., *Limonium narbonense* Mill.,  
129 *Limonium cuspidatum* (Delort) Erben, *Chenopodium chenopodioides* (L.) Aellen, *Tamarix*  
130 *gallica* L., *Obione portulacoides* (=Atriplex portulacoides L.) and *Plantago coronopus* L. The  
131 meteorological data were obtained from the nearest station namely Marignane which is

132 located at about 11 km away as the crow flies. Five to six trees (*F. angustifolia*) of similar  
133 height and diameter were chosen in each zone during this study.

## 134 2.2 Modulated chlorophyll fluorescence parameters monitoring

135 Fluorescence data were obtained using Hansatech fluorometer (FMS2). Leaves (3 per tree)  
136 were dark-adapted for 30 min using the dark-adaptation clip prior to the application of a low  
137 pulsed amber light intensity to determine the minimum fluorescence ( $F_0$ ) followed by the  
138 application of a saturating pulse for the maximum fluorescence measurement ( $F_m$ ) such that  
139 all primary quinone electron acceptors in PSII are closed ( $Q_A$  fully reduced). These data allow  
140 to calculate the maximum quantum efficiency through the ratio  $F_v/F_m$  where  $F_v$  ( $F_m - F_0$ )  
141 corresponds to the variable fluorescence. This ratio was determined at predawn, dawn and in  
142 diurnal period.

143 The diurnal course of chlorophyll parameters was monitored on 28<sup>th</sup> august 2007 from 9:00 h  
144 to 18:00 h by using successively the dark adaptation clip and the PAR/temperature leaf-clip  
145 which is designed for measurements under ambient light conditions. Thus additional variables  
146 were recorded from light acclimated leaves: minimum ( $F'_0$ ) maximum ( $F'_m$ ) and the steady  
147 state ( $F_s$ ) values of fluorescence. Through these data as well as  $F_m$  and  $F_0$  the following  
148 fluorescence parameters were determined: The photochemical efficiency in the light adapted  
149 state of photosystem II ( $\Phi_{PSII}$ ) calculated as  $(F'_m - F_s)/F'_m$  according to Genty et al. (1989), the  
150 non-photochemical quenching coefficient of chlorophyll fluorescence (NPQ), calculated as  
151  $(F_m - F'_m)/F'_m$  (Bilger and Björkman, 1990) which expresses the dissipation of excess energy  
152 as heat and the photochemical quenching (qP) coefficient, which was calculated from  $(F'_m -$   
153  $F_s)/(F'_m - F'_0)$  that gives an indication of the proportion of PSII reaction centers that are open  
154 (Maxwell and Johnson, 2000). An alternative non-photochemical quenching coefficient, QNP  
155  $= (F_m - F'_m)/(F_m - F_0)$  was also determined (Schreiber et al., 1986).

156 Light response kinetic measurements were carried out at dawn by selecting four incident  
157 photosynthetically active irradiance (PAR) intensities in order to simulate the half diurnal  
158 light radiation change: 300, 600, 900 and 1200  $\mu\text{mol Photons m}^{-2}\text{s}^{-1}$ . The light saturating pulse  
159 ( $8.000 \mu\text{mol Photons m}^{-2}\text{s}^{-1}$ ) was applied for each PAR intensity only when the steady-state  
160 fluorescence intensity ( $F_s$ ) was reached. This was checked prior to the design of a specific  
161 light kinetic script. In addition to the above-mentioned fluorescence parameters, other  
162 parameters were calculated for each actinic light application: the quantum efficiency of  
163 regulated  $\Delta\text{pH}$  and/or xanthophyll-dependent non-photochemical dissipation processes in the

164 antenna of PSII ( $\Phi_{NPQ} = F_s/F'_m - F_s/F_m$ ). The quantum efficiency of the constitutive non-  
165 photochemical fluorescence and energy dissipation ( $\Phi_{f,D} = F_s/F_m$ ) according to Hendrickson et  
166 al. (2005). The Chlorophyll fluorescence decrease ratio was calculated as:  $F_d/F_s = F_m - F_s/F_s$   
167 (Lichtenthaler et al., 2005). Also, the rate of the linear electron transport was determined:

168  $ETR = 0.5 \times PAR \times 0.87 \times \Phi_{PSII}$  assuming that the photon flux are equally distributed between  
169 the two photosystems.

### 170 *2.3 Simultaneous gas exchange and fluorescence monitoring*

171 The leaf gas exchange and fluorescence parameters were simultaneously determined using a  
172 gas exchange system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA) equipped with a  
173 fluorescence chamber head.  $CO_2$  assimilation rate ( $A$ ), internal  $CO_2$  ( $C_i$ ) and stomatal  
174 conductance ( $g_s$ ) were determined. All the measurements were carried out at  $25^\circ C$  and the  
175 relative humidity of the chamber was set at 70%, while the amount of blue light was set to  
176 10% of the photosynthetic photon flux density for optimized stomatal aperture. In light-  
177 adapted leaves, the steady state fluorescence yield ( $F_s$ ) was recorded and the maximum  
178 fluorescence ( $F'_m$ ) obtained after application of a saturating white light pulse ( $8.000 \mu mol$   
179  $photons\ m^{-2}\ s^{-1}$ , 0.8 s). The actinic light was then turned off and far-red illumination was  
180 applied ( $2 \mu mol\ photons\ m^{-2}\ s^{-1}$ ) to measure the light-adapted minimum fluorescence ( $F'_0$ ).  
181 Using these parameters,  $\Phi_{PSII}$ ,  $qP$  and the ratio  $F'_v/F'_m = (F'_m - F'_0)/F'_m$  which corresponds  
182 to the efficiency of the energy harvesting by the oxidized PSII reaction centers in light  
183 condition were determined.

184

### 185 *2.4 Predawn leaf water potential determination*

186 Water potential ( $\Psi$ ) was measured using a pressure chamber of Scholander type (Scholander  
187 et al., 1965), from mature fully expanded leaves of randomly selected plants. The  
188 measurements were carried out from five trees per site at 05:00 h then finished before sunrise.

### 189 *2.5 Soil and leaf sodium ions concentration*

190 Five trees and five soil samples were considered for each condition: salt meadow and the  
191 control. Plant samples was composed by a pool of five leaves per tree. Soil and leaf samples  
192 were ash transformed in a muffle furnace. The extracted solutions were analysed for  $Na^+$   
193 concentrations by using an Ion Chromatograph (Dionex Model DX 120, USA). The column



194 used was a CS16 with hydroxymethanesulfonic acid (26 mM) as eluent which a flow rate set  
195 at 1ml/min. Electrical conductivity was the mode of cations detection.

196

197

## 198 *2.6 Photosynthetic pigments analysis*

199 Leaves (a pool of five leaves per tree from five trees in each site) were collected at predawn  
200 then wrapped in aluminum foil to avoid for degradation of pigments by light and finally  
201 stored at  $-20^{\circ}\text{C}$  until analysis in the laboratory. Chlorophylls and total carotenoids were  
202 extracted with acetone (80%) and filtered through Millipore membrane (0.2  $\mu\text{m}$  pore size).  
203 Quantification was performed with a spectrophotometer (BioMate<sup>TM</sup>3) by measuring their  
204 absorbance at 663, 646 and 470 nm (Lichtenthaler and Wellburn, 1983).

## 205 *2.7 Dendrochronology*

206 The sampling process took place during the spring 2017. We selected six dominant and  
207 mature ashes separated by at least 8 m from each other in the salt meadow; six other dominant  
208 ashes were selected as control trees. One core per tree at 1.3 m height was collected with a  
209 Swedish increment borer. In the laboratory, cores were prepared according to standard  
210 dendrochronological procedures (Fritts 1976). All cores were air-dried, glued onto groove  
211 boards, sanded with progressively finer grade sandpaper to produce smoothed surfaces on  
212 which the tree-ring boundaries were clearly visible and visually crossdated under a binocular  
213 to ensure that each ring was dated to the correct calendar year. Then, ring-width series were  
214 measured to the nearest 1/100 mm using a LINTAB6 measuring table (Rinntech®) and the  
215 TSAP-Win software (Rinn 2003). All ring-width series were crossdated graphically and  
216 crossdating was checked using the COFECHA software (Holmes, 1983) by assessing the  
217 consistency of the different ring-width series among trees.

218

## 219 *2.8 Statistical analysis*

220 A Student test was performed to compare the two sites for each interval of time regarding to  
221 the diurnal measurements of fluorescence parameters.

222 For the predawn physiological indicators ( $F_v/F_m$ , pigment content and  $\Psi$ ) a two-way ANOVA  
223 was designed to assess the relationship between the two independent variables (Site x Year)  
224 on each dependent variable followed by Tukey's HSD (Honestly Significant Differences)



225 tests. The PSII photochemistry parameters obtained from light kinetics were first treated by a  
226 multivariate analysis in order to determine the main explicative fluorescence parameters of  
227 the actual quantum yield kinetics. This was performed using R statistical software (R  
228 development Core Team, 2009) and the package “FactoMineR” Thus, covariance analyses  
229 (ANCOVA), with light intensities fixed as covariate were used to examine the effect of site  
230 and year on the pertinent explicative fluorescence variables. Curves fitting and statistical  
231 analyses were performed using Statgraphics Centurion XV software and R statistical  
232 software.

233

### 234 3. Results

#### 235 3.1 Annual changes in climatic conditions of the study site

236 The study area was located near the village of Mauran at 11 km of the nearest meteorological  
237 station which is that of Marignane (France). As shown in table 1, during this last decade,  
238 summer air temperature varied from 23 to 25°C. There was no significant difference between  
239 the years (one-way-ANOVA,  $F=2.12$ ,  $P=0.065$ ). However, the cumulated precipitation varied  
240 considerably (10-139 mm) resulting in similar changes in soil water deficit. For instance the  
241 highest water deficit was recorded in 2016 (-659 mm) while the lowest one was observed in  
242 2011 (-4.18 mm).

#### 243 3.2 Interannual radial growth

244 Analyzes of the cores collected from the individuals located at the two study sites allowed us  
245 to address their radial growth over the last ten years. The results clearly show that *F.*  
246 *angustifolia* grows better in salt meadows than the control site (Fig. 1). A peak of radial  
247 growth was recorded in 2014, where growth in the saline area was twice that of the riparian  
248 bank. However, since 2011, this growth has been falling to equal the radial growth of  
249 individuals in the control habitat.

#### 250 3.3 Leaf water and sodium ion status

251 *F. angustifolia* environmental conditions in both salt meadow and riparian forest bank were  
252 first characterized at the beginning of august 2007. Soil water availability was characterized  
253 by the measurement of leaf water potential in the absence of any transpiration flux as well as  
254 leaves and soil sodium chloride contents. Predawn water potential was higher in control stand

255 compared to salt marsh individuals. Leaves  $\text{Na}^+$  content were similar while soil  $\text{Na}^+$  content in  
256 salt meadow site was about 13-fold that of the riparian forest bank (Table 2).

257

258

### 259 *3.4 The diurnal variation of chlorophyll fluorescence parameters*

260 From 9:00 h to 18:00 h several chlorophyll fluorescence data were recorded (Fig. 2). The  
261 maximum quantum efficiency of PSII ( $F_v/F_m$ ) was significantly higher for samples of salt  
262 meadow site compared to those of the riparian forest only from 9:00 h to 13:00 h. For  
263 instance, at 13:00 h the ratios  $F_v/F_m$  were 0.739 and 0.614, respectively (Fig. 2A). When  
264 leaves were light acclimated, the actual efficiency of PSII photochemistry ( $\Phi_{\text{PSII}}$ ) decreases by  
265 late morning to 16:00 h then rises until 6:00 h pm in both cases (Fig. 2B). However at 9:00h  
266  $\Phi_{\text{PSII}}$  of *F. angustifolia* located in salty soils was about 2-fold that of none salty grown  
267 specimens. The exitonic energy used for the reduction of the plastoquinone pool, determined  
268 by the photochemical quenching coefficient (qP) exhibited similar patterns for both sites but  
269 with a significant difference obtained also only at 9:00 h am (Fig. 2C). Alternatively, the  
270 excess of absorbed excitation energy may be dissipated through non-photochemical  
271 processes referred as NPQ. No significant difference was found between the two sites in the  
272 morning (Fig. 2D).

273 Hence, it seems that *F. angustifolia* quantum yield in salty soil condition is higher than the  
274 control during the morning and this may be explained by both photochemical quenching  
275 mechanisms and PSII antenna efficiency. This led to further investigation regarding to the  
276 predawn maximum quantum efficiency and the plant response to light kinetic taking into  
277 account the interannual variability.

### 278 *3.5 Predawn $F_v/F_m$ and steady-state light response of chlorophyll fluorescence parameters in* 279 *field conditions*

#### 280 *3.5.1 Predawn $F_v/F_m$*

281 The analysis of the maximum quantum efficiency carried out between 2007 and 2016 showed  
282 that the factors site and year interact together ( $F=5.31$ ;  $p<0.001$ ). Hence, the predawn ratios  
283  $F_v/F_m$  of the sites were compared year by year. As a result (Fig. 3) *F. angustifolia* exhibited  
284 significant differences between sites in 2009 and 2016. For the former date, a lower ratio was  
285 obtained in salt meadow compare to the control; 0.819 and 0.886, respectively. For the latter,

286 an opposite response was shown because salt meadow Fv/Fm was about 0.05 units higher  
287 than the data recorded from control samples. The predawn ratio was also tested site by site for  
288 all the years which revealed that the ratio Fv/Fm tends to be lower in control with greater  
289 variability throughout the years (Fig. 4).

290

### 291 3.5.2 Dawn light kinetic fluorescence parameters measurements

292 Solar course during half a day was mimed by the implementation of a script where the  
293 intensity of the light increases from 300 to 1200  $\mu\text{mol. photon.m}^{-2}.\text{s}^{-1}$  in 4 steps. Fluorescence  
294 data were recorded during 6 years then first examined through the quantum yield kinetic.  
295 Two-way ANCOVA with light intensity as covariate was carried out. A significant effect of  
296 the factors year ( $F=61.54$   $p<0.001$ ) and site ( $F=9.38$   $p<0.001$ ) was shown and no interaction  
297 was found between them. Thus, the site effect on the dependent variable  $\Phi_{\text{PSII}}$  was tested year  
298 by year. As a result, the photochemical quantum yield decreases exponentially with light  
299 intensity (Fig. 5) with significant differences between the two sites except in 2014. The  
300 photosynthetic performance of individuals in salt meadows was higher than that of the  
301 controls. It is thus possible to summarize the functioning of the photosynthetic machinery in 2  
302 types of response. The first one is characterized by large variations in the actual quantum  
303 yield at low light intensities as in 2007, 2008 and 2016. The second type of response is  
304 manifested by an increasing difference as light intensities increase. This is the case in 2015  
305 and 2009 where the quantum yields were about 0.8. It appears that soil salinity contributes to  
306 increase *F. angustifolia* PSII quantum yield through physiological mechanisms that change  
307 depending to the year. This arises the question of the identification of the fluorescence  
308 parameters that explain these variations of the effective quantum yield. A multivariate  
309 analysis was performed in order to prioritize the fluorescence parameters involved. The factor  
310 map of the variables denotes a total variance of 89.46 % (Fig.6). Interestingly,  $\Phi_{\text{PSII}}$  is  
311 strongly correlated to axis 1 (correlation coefficient: 0.99) followed by  $\Phi_{\text{NPQ}}$  (correlation  
312 coefficient: 0.96) and qP (correlation coefficient: -0.87). As for axis 2, the main explanatory  
313 variable is Fd/Fs (correlation coefficient: 0.87). In summary the variations of  $\Phi_{\text{PSII}}$  are mainly  
314 explained by non-photochemical quenching mechanisms followed by the ratio Fd/Fs. For the  
315 former, the two-way ANCOVA performed showed a significant site ( $F=11.69$ ,  $p<0.001$ ) and  
316 year ( $F=50.35$ ,  $p<0.001$ ) effects, no interaction was found between the two factors. In these  
317 conditions,  $\Phi_{\text{NPQ}}$  was compared between sites, year by year. The results showed that  $\Phi_{\text{NPQ}}$  in  
318 the salty areas was lower than that of the control except in 2014, 2015 (Fig. 7).

319

320 *3.6 Gas exchange and fluorescence measurements*

321 Simultaneous measurements of gas exchanges and fluorescence data were carried out in 2009  
322 with a Licor 6400 equipped with the fluorescence chamber. The responses of *F. angustifolia*  
323 to salt meadow environmental conditions were examined through the measurements of leaf  
324 net photosynthesis (A), leaf internal CO<sub>2</sub> concentration (Ci), PSII antenna efficiency  
325 (Fv'/Fm'), the photochemical quenching qP and the quantum yield of PSII. As shown in table  
326 3 salt meadow plants exhibited a slight increase of the net photosynthesis (3.17 μmol. CO<sub>2</sub>.m<sup>-2</sup>.s<sup>-1</sup>)  
327 compared to the controls (2.93 μmol. CO<sub>2</sub>.m<sup>-2</sup>.s<sup>-1</sup>). Similar result was also obtained for  
328 leaf internal CO<sub>2</sub> content. However, no differences were found for the quantum yield and  
329 related fluorescence data.

330

331 *3.7 Photosynthetic pigments analysis*

332 The concentrations of chlorophylls and total carotenoids contents were measured from the  
333 two sites throughout six years. Based on the data obtained for chlorophyll a there was an  
334 interaction between the factors site and year (F=2.43; p<0.05). Sites were thus compared year  
335 by year but no significant difference was found except in 2016 where chlorophyll a contents  
336 from salt meadow samples were 3-fold higher compare to the controls (Fig. 8A). For the other  
337 pigments no interaction was ascertained between the two factors and significant changes were  
338 obtained only over the years. Hence, the highest contents of chlorophyll b were recorded in  
339 2008 whereas the lowest values were obtained in 2007 (Fig. 8B). Total carotenoids  
340 concentration (Fig. 8C) in 2008 was about 75 μg/g of DW which represents the highest values  
341 recorded during the years investigated while the lowest data were obtained in 2015 (27 μg/g  
342 of DW).

343

344 **4. Discussion**

345 In this report we showed for the first time a long term survey of the *in situ* adaptation of a  
346 glycophyte to salt march conditions based on chlorophyll fluorescence signals. The model  
347 used was *F. angustifolia* which occurs naturally in several Mediterranean deltaic zones unlike  
348 to most of riparian forest species.

349 During the last decade, the tree-rings of individuals growing in the salt marsh showed clearly  
350 two effects: on one hand, the biomass is greater than that of the individuals in the control area  
351 (Fig. 1) and on the other hand, this biomass varies according to the fluctuations of water  
352 deficit (Table 1). In particular, from the peak of 2011 the biomass has steadily decreased to  
353 reach that of the control individuals in 2017. A higher biomass could be the result of intense  
354 photosynthetic activity at the level of both light and enzymatic reactions but also the fact of a  
355 greater availability of nutrients. Indeed, river mouths are recognized as areas with high  
356 concentrations of mineral elements because of the importance of alluvial deposits. This is true  
357 for halophytes but glycophytes have to cope with salinity: 13-fold higher as found in this  
358 study (Table 2) suggesting the need of additional metabolic energy. Thus, the light-dependent  
359 reactions of photosynthesis were followed through several parameters based on the  
360 fluorescence of chlorophyll a. Measurements of the maximum quantum efficiency before  
361 sunrise did not show any particular stress since the values obtained were all greater than 0.75  
362 (Björkman and Demmig, 1987). This also indicates the lack of chronic photoinhibition or salt  
363 induced toxicity to PSII (Yan et al., 2015). However, for the year 2016, which was  
364 characterized by the greatest water deficit ( $P=10$  mm), the ratio  $F_v/F_m$  measured in the saline  
365 zone was significantly higher than of the control zone. Similarly, the levels of chlorophyll a  
366 were three times higher compared to individuals in the control zone (Fig.7A). This is an  
367 unexpected result because the ratio  $F_v/F_m$  trends to decrease with stress intensity as well as  
368 chlorophyll a contents (Manaa et al., 2019; Masondo et al., 2019). Nevertheless, the increase  
369 of chlorophyll a content under salinity was observed from individuals of *Phragmites karka*  
370 (Retz.) Trin ex Sternd (Shoukat et al., 2019) as such, the increase of chlorophyll content has  
371 been considered to be a marker of resistance to salinity (Acosta-Motos et al., 2017). This  
372 would mean that the increase in the  $F_v/F_m$  ratio corresponds to an adaptive response of *F.*  
373 *angustifolia* to both drought and salinity. Indeed chlorophyll a is present as a dimer in the  
374 reaction center of the photosystems, an increase in its concentration could result in an increase  
375 in the number of photosystems and consequently a better photosynthetic capacity of  
376 individuals to mitigate the environmental constraints.

377 Given the absence of chronic photoinhibition, fluorescence measurements were conducted in  
378 order to understand the dynamics of photosynthesis light reactions. Initially, the  
379 measurements made between 9:00 h and 18:00 h clearly showed that the actual photochemical  
380 yield ( $\Phi_{PSII}$ ) of individuals in salty environment was higher than those in the control area (Fig.  
381 2B). Subsequently, kinetics measurements were carried out by simulation of light intensity  
382 variations for half a day. During the six years of measurement the kinetic curves show that

383  $\Phi_{\text{PSII}}$  in saline medium is always higher than the control except during 2014. The water  
384 conditions alone do not explain this difference between the years because the water deficits  
385 between the years 2014 and 2015 were roughly the same (138 and 139). It seems that other  
386 environmental factors contributed to buffer  $\Phi_{\text{PSII}}$  variations in 2014. Nevertheless, the  
387 adaptive capacity of an invasive plant in salt-meadows, *Spartina patens* (Aiton) Muhl. was  
388 related to maintaining a high level of  $\Phi_{\text{PSII}}$  despite the increase in salinity (Duarte et al.,  
389 2015). We can therefore consider that maintaining a high level of  $\Phi_{\text{PSII}}$  of *F. angustifolia* in a  
390 salty environment is an adaptive trait.

391

392 In order to highlight the mechanisms involved in the regulation of  $\Phi_{\text{PSII}}$ , a multivariate  
393 analysis was conducted. PCA analysis revealed that the main explanatory variables were the  
394 fraction of absorbed energy that is dissipated thermally by the  $\Delta\text{pH}$ - and the xanthophyll  
395 cycle-dependent quenching in PSII ( $\Phi_{\text{NPQ}}$ ), qP and Fd/Fs ratio. The one-way ANCOVA  
396 performed on  $\Phi_{\text{NPQ}}$  confirms significant differences between salty and control environments  
397 (Fig. 6) with the exception of the years 2014 and 2015. In particular, the high yields of  $\Phi_{\text{PSII}}$   
398 in saline environments may be explained by a better efficiency in the conversion of the light  
399 energy absorbed into reducing power since  $\Phi_{\text{NPQ}}$  is low. However, in another survey it has  
400 been reported that low  $\Phi_{\text{NPQ}}$  values indicate a lower photoprotection capacity in the salt marsh  
401 environmental condition (Arena et al., 2013). Photoinhibition mitigation was checked through  
402 the analysis of carotenoid contents but this did not show any site effect suggesting the need of  
403 further studies on individual xanthophylls characterization. Because no difference was found  
404 in leaves  $\text{Na}^+$  content it is likely that ash individuals in the meadow have evolved specific  
405 mechanisms of salt retention/exclusion at the root level. Indeed, several reports have shown  
406 that glycophytes may adapt to salt stress by sequestering sodium ions in their root  
407 compartment (Assaha et al., 2017; Chen et al., 2018). Among the mechanisms involved, the  
408 apoplastic barrier through the casparian strip seems to play a critical role in controlling  $\text{Na}^+$   
409 uptake and transport into the shoots. This is supported by the identification of suberin-forming  
410 key genes as *SbKCS11* (Chen et al., 2018).

411 Given the exclusion of salt from foliar cytoplasm, it appears that the major adaptive constraint  
412 of ash is water availability since the water deficit follows the growth pattern. Precisely, the  
413 greater the water deficit is the more saline concentration increases thus making the water less  
414 accessible to glycophytes. The concomitant fluorescence and gas exchange measurements  
415 carried out in 2009 showed that the net assimilation of  $\text{CO}_2$  was the same regardless of the  
416 difference of salinity between the two sites. It is likely that the higher values of  $\Phi_{\text{PSII}}$  obtained

417 in saline areas corresponds to a supplementary energy demand to sustain growth and  
418 adaptation to water/salt stress. When considering the radial growth of *F. angustifolia* that  
419 declines since 2011, both ATP and the anabolic coenzyme NADPH from photosynthesis  
420 light-dependent reactions and the carbon assimilated are rather allocated to defense  
421 processes. The tradeoffs between lignin biosynthesis and defense still remain challenging  
422 although jasmonic acid and phytochrome B signalling pathway was identified as a hub that  
423 coordinate plants growth and defense (Xie et al., 2018). Interestingly, differential analysis of  
424 genes expressed in response to salt stress in *Fraxinus velutina* showed that the up-regulated  
425 genes mainly concerned those of the biosynthetic pathway of alkaloids which are also known  
426 as defense components (Yan et al., 2019).

## 427 5. Conclusion

428 In conclusion, this work, carried out for 6 years between 2007 and 2016, highlighted some of  
429 the adaptive mechanisms of a glycophyte in salt meadows environmental condition. Since  
430 2011, a decline has been observed in the production of ash biomass in saline environments.  
431 This decline seems mainly related to recurrent water deficit. In the perspective of the climate  
432 change announced in the Mediterranean region, one could expect an extinction of ash in salt  
433 meadows. *F. angustifolia* resistance was shown by the establishment of an efficient  
434 photochemical yield both by a reduction of the fraction of the absorbed radiation which is  
435 dissipated as heat and also by increasing the concentration of chlorophyll a. However, non-  
436 native salt marsh species with a high tolerance to salinity and drought will be least affected by  
437 the sea-level increase and drought associated to global change. Further studies are needed to  
438 establish the metabolic network involved in the adaptive strategies of glycophytes in saline  
439 environments.

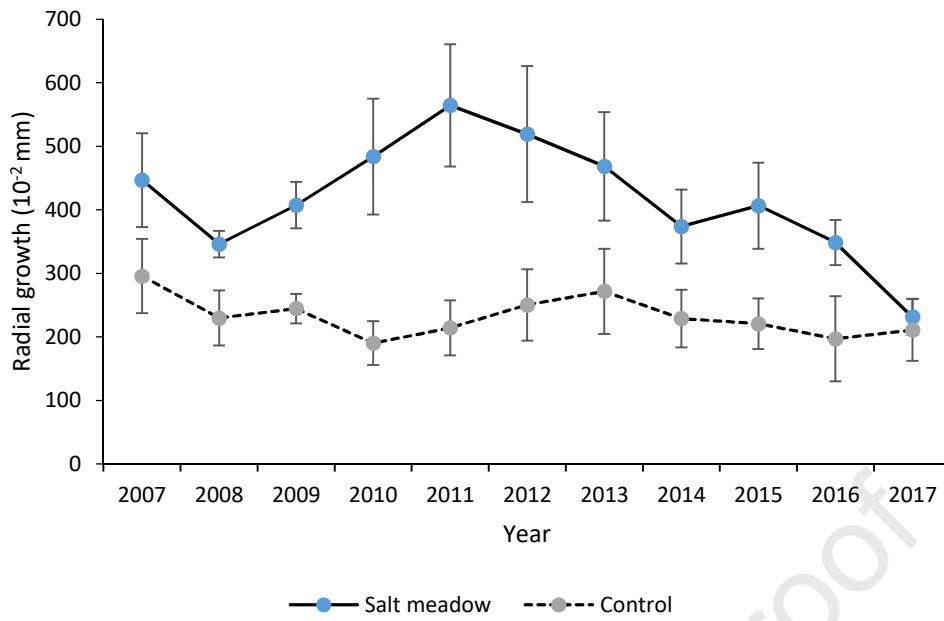
440

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442 Météo-France for providing the meteorological data. Dr Julien Ruffault is also acknowledged  
443 for graph fitting with R software.

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445



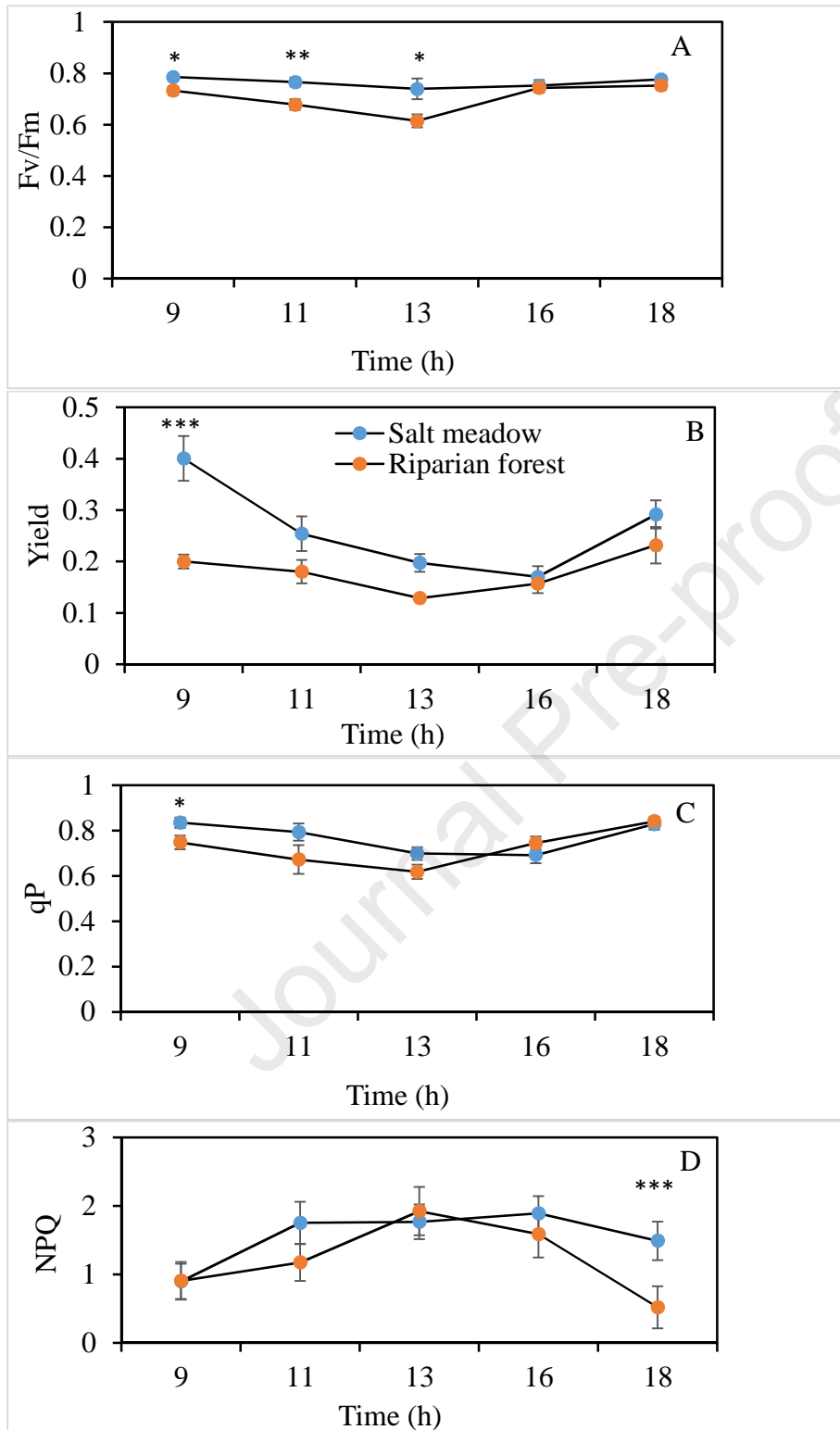


446

447 Fig. 1: Annual radial growth of *Fraxinus angustifolia* from trees of salt meadow ecotone and  
448 the control riparian forest from 2007-2017.  $n=6$ , means  $\pm$  SE)

449

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451 Fig. 2 : Diurnal course of summer fluorescence measurements from *Fraxinus angustifolia* in  
 452 salt meadow and riparian forest sites: The ratio Fv/Fm (A), the actual quenching yield (B), the  
 453 photochemical quenching qP (C), and the Non-Photochemical Quenching NPQ (D). A student  
 454 test was applied to compare the sites at each interval of time.\*-  $P < 0.05$ , \*\*-  $P < 0.01$ , \*\*\*-  
 455  $P < 0.001$ .  $n = 6$ , means  $\pm$  SE

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457 Mevy et al

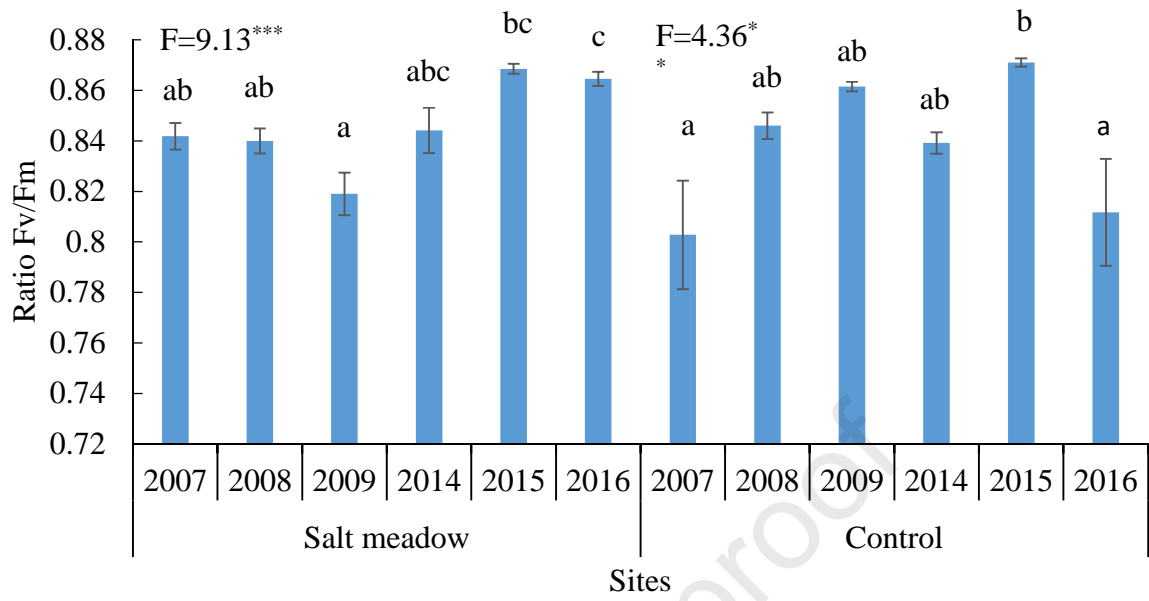


470

471 Fig.3: Predawn Fv/Fm ratio of *Fraxinus angustifolia* from salt meadow and the control  
 472 riparian forest between 2007 and 2016. The test was performed year by year (one-way-  
 473 ANOVA, \*-  $p < 0.05$ , ns- not significant,  $n = 18$ , means  $\pm$  SD).

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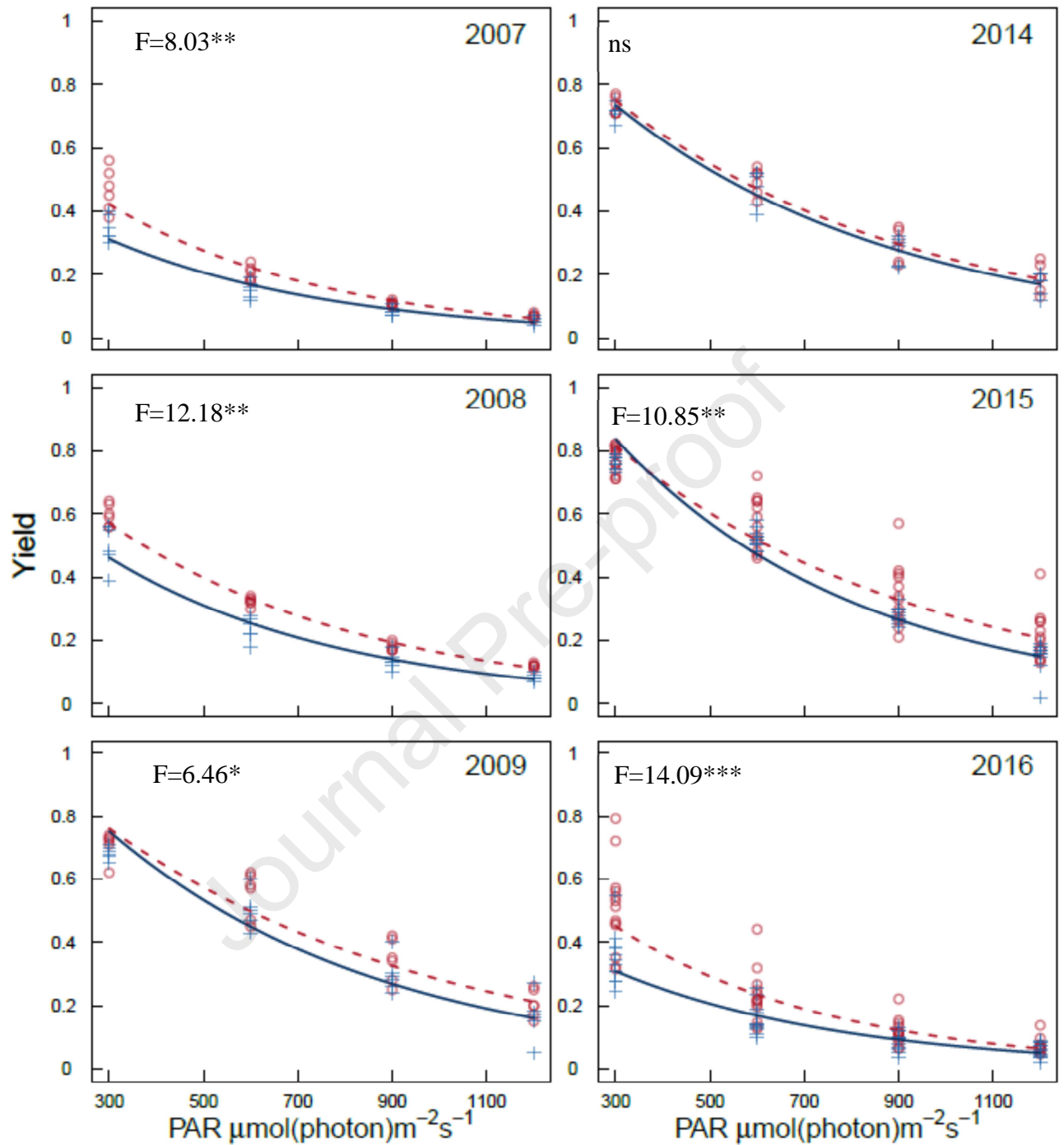
475 Mevy et al



476  
 477 Fig. 4: Predawn Fv/Fm ratio of *Fraxinus angustifolia* from salt meadow and the control  
 478 riparian forest between 2007 and 2016. The test was performed site by site (one-way-  
 479 ANOVA, \*\* $-p < 0.001$ , ns- not significant,  $n = 18$ , means  $\pm$  SD).

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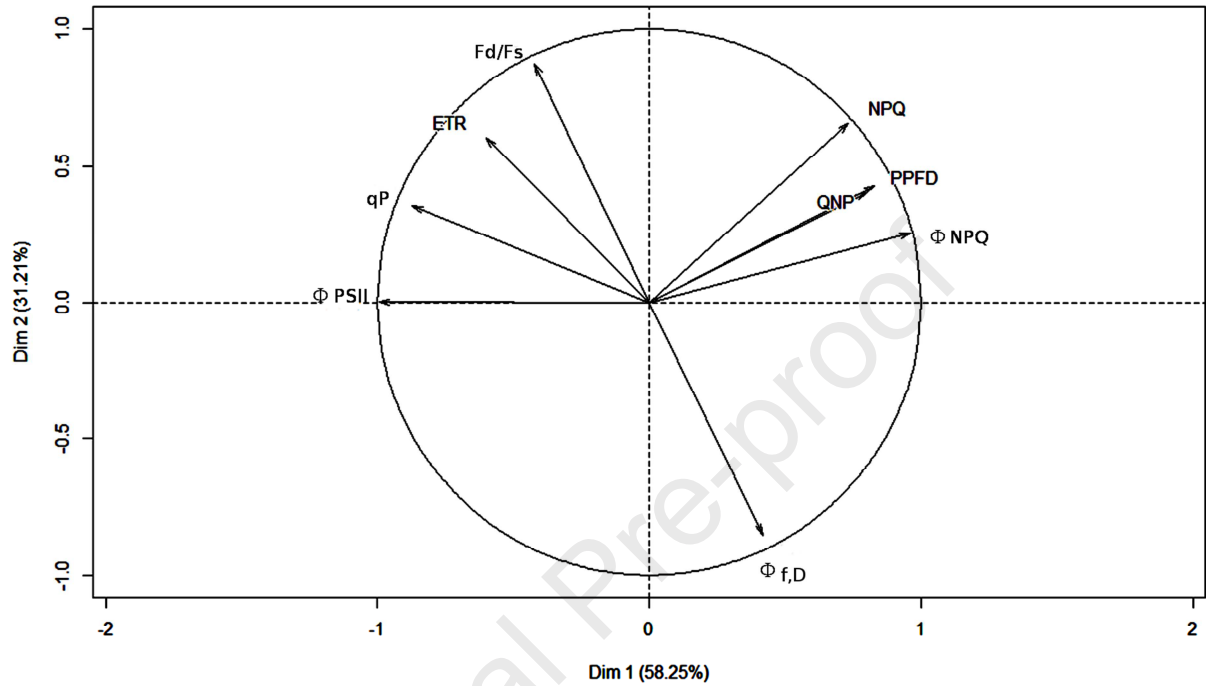
484 Fig. 5: Light response of PSII actual quantum yield of *Fraxinus angustifolia* in salt meadow  
 485 (red dotted line) and riparian forest sites (blue line). one-way-ANCOVA. \*-  $P < 0.05$ , \*\* -  
 486  $P < 0.01$ , \*\*\*-  $P < 0.001$ .

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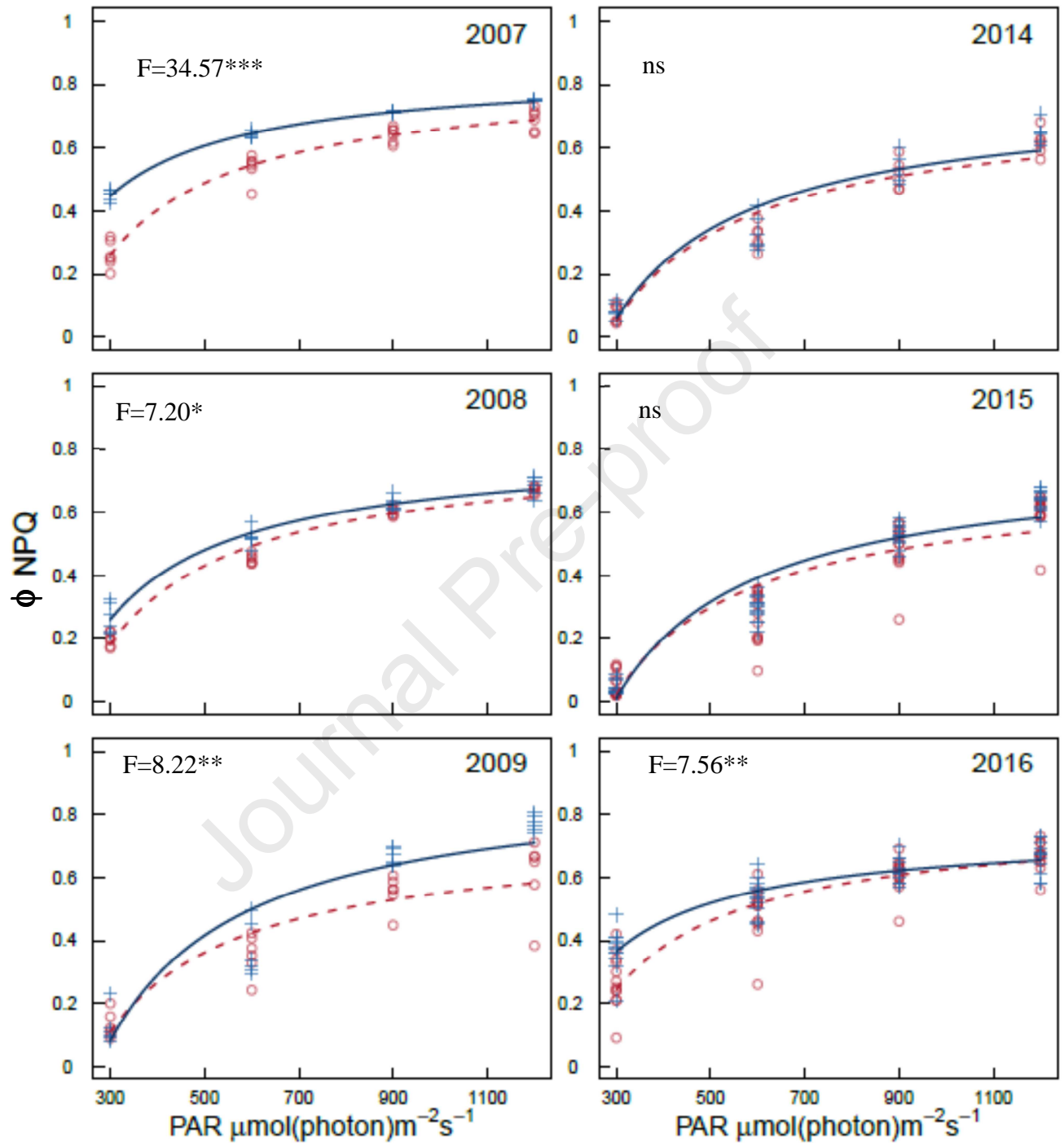
491

492 Fig. 6: Principal Component Analysis (PCA) of the fluorescence variables map on axes 1 and  
 493 2 plus the correlation circle: The photochemical efficiency in the light adapted state of  
 494 photosystem II ( $\Phi_{PSII}$ ), the non-photochemical quenching coefficient (NPQ), the  
 495 photochemical quenching coefficient (qP), the regulated  $\Delta pH$  and/or xanthophyll-dependent  
 496 non-photochemical dissipation processes ( $\Phi_{NPQ}$ ), the constitutive non-photochemical  
 497 fluorescence and energy dissipation ( $\Phi_{f,D}$ ), the ratio Fd/Fs, the Photosynthetic Photon Flux  
 498 Density (PPFD), the Electron Transport Rate (ETR) and the alternative non-photochemical  
 499 quenching coefficient, QNP.

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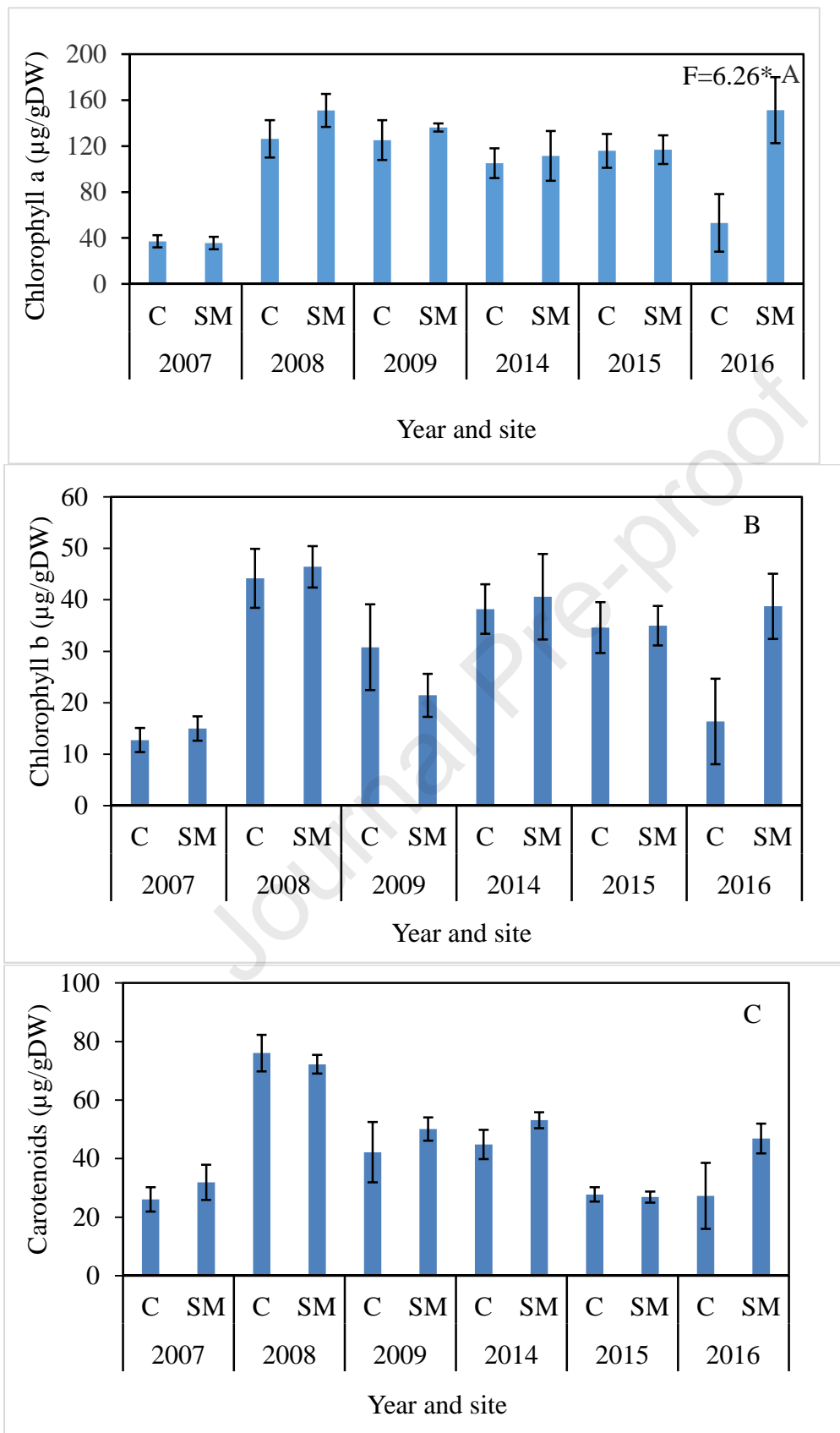
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505 Fig.7: Light response of PSII Non-Photochemical Quenching yield of *Fraxinus angustifolia* in  
 506 salt meadow (red dotted line) and riparian forest sites (blue line). one-way-ANCOVA.

507





509 Fig. 8: Interannual variations of photosynthetic pigments from *Fraxinus angustifolia* in salt  
 510 meadow (SM) and riparian forest (C) stands ( $n=5$  means  $\pm$  SE). A; Chlorophyll a content.

511 Sites are compared year-by-year, one-way ANOVA, \* -  $P < 0.05$ . B; Chlorophyll b content (no  
512 site effect,  $P > 0.05$ ). C; Carotenoids content (no site effect,  $P > 0.05$ ).

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Sampling date	Air temperature (°C)	Precipitations (P ; mm)	Potential evapotranspiration (PET ; mm)	Water deficit (mm)
Summer 2007	23.53±1.10	36.8	635.7	-598.9
Summer 2008	23.87±2.28	27.6	649.1	-621.5
Summer 2009	24.88±1.79	109.8	691.3	-581.5
Summer 2011	22.86±1.81	191.2	609.6	-418.4
Summer 2014	23.53±0.99	138.5	646.0	-507.5
Summer 2015	25.14±1.66	139.0	675.8	-536.8
Summer 2016	24.42±1.80	10.0	669.8	-659.8

513

514 Table 1: Annual variations and climatic variables of Marignane (France) station close to the  
515 study area. Climatic data for 3 months in summer before each sampling date. Average air  
516 temperature ( $\pm$  SD) of decadal data in each year ( $n = 9$ ). Cumulative values (P and PET) are  
517 calculated from daily data for each summer ( $n = 90$ ). Water deficit: P-PET. Air temperature  
518 test: One-way-ANOVA,  $F=2.12$ ,  $P=0.065$ ).

	Predawn leaf water potential (MPa)	Leaf cation Na <sup>+</sup> content (g/g of ash)	Soil Na <sup>+</sup> content (g/l of soil solution)
Salt meadow	-4.61 (0.24)	0.74 (0.23)	20.74 (8.50)
Riparian forest	-4.13 (0.21)	0.76 (0.23)	1.63 (0.09)

519

520 Table 2: Predawn leaf water potential, leaf and soil Na<sup>+</sup> contents from Salt meadow and  
521 riparian forest of *Fraxinus angustifolia* in august 2007.

522

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Site	Net photosynthesis (A; $\mu\text{mol CO}_2$ $\text{m}^{-2}\cdot\text{s}^{-1}$ )	$\Phi_{\text{PSII}}$	Fv'/Fm'	qP	Ci ( $\mu\text{mol CO}_2$ $\text{mol}^{-1}$ )
Control	2.93 (0.53)	0.24 (0.01)	0.44 (0.01)	0.54 (0.03)	261.70 (25.82)
Salt meadow	3.17 (0.84)	0.24 (0.03)	0.42 (0.01)	0.55 (0.06)	273.98 (26.58)

524

525 Table 3: Gas exchange and fluorescence measurements from leaves of *Fraxinus angustifolia*  
 526 in salt meadow and riparian forest stands. ( $n=12$ , means  $\pm$ SD).

527

528

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**Highlights**

Adaptation of a glycophyte as *Fraxinus angustifolia* to salt meadow is determined by:

- An increase of the actual quantum yield of PSII photochemistry
- An increase of chlorophyll a contents
- A lower fraction of absorbed energy that is dissipated thermally by the  $\Delta pH$ - and the xanthophyll cycle-dependent quenching in PSII ( $\Phi_{NPQ}$ )
- A higher photochemical quenching (qP) and the ratio Fd/Fs

*Fraxinus angustifolia* in salt meadow is more sensitive to global climate change. Since 2011 a decrease of the tree-rings growth was shown suggesting changes in the specific composition in these areas.

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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