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Manipulative experiments demonstrate how long-term soil moisture changes alter controls of plant water use

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2018-08

Grossiord , C , Sevanto , S , Limousin , J-M , Meir , P , Mencuccini , M , Pangle , R E , Pockman , W T , Salmon , Y , Zweifel , R & McDowell , N G 2018 , ' Manipulative experiments demonstrate how long-term soil moisture changes alter controls of plant water use ' , Environmental and Experimental Botany , vol. 152 , pp. 19-27 . <https://doi.org/10.1016/j.envexpbot.2017.12.010>

<http://hdl.handle.net/10138/307777>

<https://doi.org/10.1016/j.envexpbot.2017.12.010>

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21 **Abstract**

22 Tree transpiration depends on biotic and abiotic factors that might change in the future, including
23 precipitation and soil moisture status. Although short-term sap flux responses to soil moisture and
24 evaporative demand have been the subject of attention before, the relative sensitivity of sap flux to these
25 two factors under long-term changes in soil moisture conditions has rarely been determined
26 experimentally. We tested how long-term artificial change in soil moisture affects the sensitivity of tree-
27 level sap flux to daily atmospheric vapor pressure deficit (*VPD*) and soil moisture variations, and the
28 generality of these effects across forest types and environments using four manipulative sites in mature
29 forests. Exposure to relatively long-term (two to six years) soil moisture reduction decreases tree sap
30 flux sensitivity to daily *VPD* and relative extractable water (*REW*) variations, leading to lower sap flux
31 even under high soil moisture and optimal *VPD*. Inversely, trees subjected to long-term irrigation
32 showed a significant increase in their sensitivity to daily *VPD* and *REW*, but only at the most water-
33 limited site. The ratio between the relative change in soil moisture manipulation and the relative change
34 in sap flux sensitivity to *VPD* and *REW* variations was similar across sites suggesting common
35 adjustment mechanisms to long-term soil moisture status across environments for evergreen tree species.
36 Overall, our results show that long-term changes in soil water availability, and subsequent adjustments
37 to these novel conditions, could play a critical and increasingly important role in controlling forest water
38 use in the future.

39

40 **Keywords:** acclimation, climate change, drought, irrigation, sap flux, vapor pressure deficit, water use.

41

1 **Manipulative experiments demonstrate how long-term soil moisture changes alter controls of**
2 **plant water use**

3

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42 **Abbreviations**

43 F_D = mean daily sap flux density ($\text{g m}^{-2} \text{s}^{-1}$)

44 LAI = leaf area index ($\text{m}^2 \text{m}^{-2}$)

45 REW = relative extractable water (unitless)

46 VPD = vapor pressure deficit (kPa)

47

48 **Highlights**

- 49 • How long-term soil moisture change affects the sensitivity of transpiration to environmental
50 variability is unknown.
- 51 • Exposure to long-term soil moisture reduction decreased sap flux sensitivity to *VPD* and *REW*.
- 52 • Long-term irrigation increased sap flux sensitivity to *VPD* and *REW* but at highly water-limited
53 sites only.
- 54 • Relative sensitivity to *VPD* and *REW* is generalizable across forest types suggesting common
55 adjustment mechanisms to soil moisture status.
- 56

57 **1. Introduction**

58 Recent work has shown that plant transpiration could account for up to 90% of terrestrial
59 evapotranspiration (Jasechko et al., 2013), making vegetation a dominant force in the global water cycle.
60 Transpiration from forest ecosystems alone contributes between 50% and 70% to terrestrial
61 evapotranspiration (Schlesinger and Jasechko, 2014). Climate-related shifts in forest transpiration could
62 thus have large impacts on the global water cycle including modification in precipitation, groundwater
63 recharge, renewable fresh water, increasing soil erosion, and feedbacks on (micro) climate. Average
64 temperature at the world's surface has been steadily rising since the mid-50s (IPCC, 2014). Warmer air
65 increases vapor pressure deficit (*VPD*) and induces higher evaporation from land surfaces including
66 transpiration from plants (Hardwick et al., 2010). Simultaneously, the risk for severe and extended
67 droughts is increasing (Williams et al., 2013; Cook et al., 2015; Roderick et al., 2015).

68 At hourly to daily scales, tree-level sap flux primarily results from the driving force for
69 transpiration (radiation, *VPD* and wind), and hydraulic and stomatal conductance of the trees (Oren et
70 al., 1999). Without stomatal control, tree-level transpiration increases progressively with rising *VPD* as a
71 consequence of the atmosphere becoming less moisture saturated. As *VPD* increases, stomata respond
72 via an exponential decrease in stomatal conductance (Lange et al., 1971; Monteith, 1995). Consequently,
73 when high *VPD* is reached (≈ 2.5 kPa, species-specific), stomata start modulating the transpiration flux
74 and thus tree-level transpiration starts dropping due to partial stomatal closure. Therefore, the response
75 of tree-level transpiration to *VPD* can be expressed as a parabolic equation (Fig. 1) (Monteith, 1995). By
76 closing their stomata to avoid critically high sap flux rates and impacts on embolism, trees can avoid
77 excessive damages to their hydraulic system (Saliendra et al., 1995). At daily or longer time scales, tree-
78 level sap flux regulation is also largely controlled by soil moisture variation. Tree responses to soil water
79 availability are triggered by a chemical signal originating from the roots exposed to dry soil (see reviews

80 by Davies and Zhang, 1991; Davies et al., 1994). Strong relationships have already been established
81 between soil moisture (or water stress indexes) and tree-level sap flux enabling the incorporation of sap
82 flux responses to short-term precipitation change in several climate-vegetation models so far (e.g. Sala
83 and Tenhunen, 1996; Granier et al., 1999; Verhoef et al., 2014; De Cáceres et al., 2015).

84 Although rapid sap flux responses to soil moisture and evaporative demand have been the subject of
85 attention before, the relative sensitivity of sap flux to these two factors under long-term precipitation and
86 soil moisture change has rarely been determined experimentally (but see Grossiord et al., 2017).
87 However, to maintain integrity of water resources, and improve global predictions it is essential to
88 determine how these two drivers will influence forest transpiration in the long-term under projected
89 climate. Plants are known to have a wide range of active adjustment strategies to deal with long-term
90 exposure to changing soil moisture, including physiological and structural adjustments (Chaves et al.,
91 2002) directly influencing sap flux regulation. Under drier soil conditions, plants often undergo
92 adjustments that are directed toward a water saving strategy to limit excessive water loss (Ogaya and
93 Peñuelas, 2003; Leuzinger et al., 2005; Brando et al., 2008). For instance, trees may produce conducting
94 elements with reduced lumen diameters (Hacke and Sperry, 2004; Fonti and Jansen, 2012; Grossiord et
95 al., 2017), which can result in reduced sensitivity to short-term soil moisture variation (i.e. reduced sap
96 flux under both high and low soil water availabilities because of higher flow resistance, Fig. 1). Long-
97 term soil moisture reduction may also result in changes in stomata density (Luomala et al., 2005), or in
98 the synthesis of chemical signals inducing stomatal closure (Bartlett et al., 2012; Meinzer et al., 2014),
99 which can decrease the sensitivity of trees to evaporative demand and result in reduced sap flux, even
100 under optimal *VPD* (≈ 1.5 kPa) (Fig. 1) (Grossiord et al., 2017).

101 Most of our knowledge on tree responses to climate variability is based on studies with potted plants
102 or seedlings under common garden conditions, which may not represent the true responses of mature

103 forests (Poorter et al., 2012; Rigling et al., 2013; Herzog et al., 2014). Manipulative field experiments on
104 mature individuals are one way to decipher ecosystem sensitivity to possible precipitation change, but
105 only a few experiments have been established in natural forests to date (Knapp et al., 2016). The lack of
106 experiments in natural conditions is partially related to the obvious technical limitations and the
107 financial costs associated with large-scale climate manipulation but also legal restrictions that can occur
108 in natural areas (Kayler et al., 2015). Such experiments have however great value as, contrary to studies
109 conducted along environmental gradients, they provide the ability to expose plants to environmental
110 conditions they would not experience otherwise (i.e. broadening of environmental conditions), isolate
111 mechanistic functions and identify threshold responses necessary for global predictions (Kayler et al.,
112 2015; Estiarte et al., 2016; Knapp et al., 2016).

113 Here we tested how long-term (multi-year) artificial change in incoming precipitation (i.e. increasing
114 or reducing long-term soil water availability) influences the sensitivity of tree sap flux to daily *VPD* and
115 soil moisture variations, and the generality of these effects across four forest types and environments
116 using four manipulative sites in natural and mature forests in the USA and Europe. We hypothesized
117 that:

- 118 (1) changes in long-term soil moisture would modify the sap flux sensitivity of trees to
119 environmental variability with trees subjected to reductions in soil water availability showing
120 decreased sap flux sensitivity to soil moisture and *VPD* variation while trees exposed to
121 increased soil moisture would show an enhanced sensitivity to the same factors, both because of
122 long-term adjustments in their physiology and structure (Fig. 1),
- 123 (2) changes in sap flux sensitivity to soil moisture and *VPD* variation would vary across climatic and
124 environmental conditions, and for various forest types as tree species have different inherent

125 responses to climatic variations (e.g. iso- and anisohydric strategies) and have adapted to their
126 local climate (e.g. long-term structural and physiological adjustments).

127 **2. Materials and methods**

128 **2.1 Experimental sites**

129 We used data collected from mature forest plots in four regions covering a large gradient of
130 environmental and climatic conditions and representing three major forest types (Fig. S1). The focal
131 regions are located in France (i.e. Mediterranean evergreen forest: the Puechabon site, PUE hereafter),
132 the United States (i.e. two semi-arid forest types: the SUMO and Sevilleta sites, SUM and SEV
133 hereafter, respectively) and Switzerland (i.e. dry Mountainous coniferous forest: the Pfywald site, PFY
134 hereafter). The Mediterranean evergreen forest is dominated by evergreen holm oak (*Quercus ilex* L.).
135 The dry Mountainous coniferous forest type is characterized by the dominance of Scots pine (*Pinus*
136 *sylvestris* L.) with pubescent oak (*Quercus pubescens* Willd.) mainly in the understory at the Swiss site.
137 The two semi-arid forest sites occur in high elevation deserts with the vegetation dominated by
138 evergreen junipers (*Juniperus monosperma* (Engelm.) Sarg.) and piñon pines (*Pinus edulis* Engelm.).
139 The target tree species pool varied depending on the site, going from one species in the PUE and PFY
140 sites to two species in the SEV and SUM sites (Table 1). Climatic conditions (rainfall, air temperature,
141 atmospheric humidity, solar radiation, wind speed) were measured continuously and recorded by a
142 weather station at each site (Fig. 2, see Table 1 for references). Climatic conditions were measured at 1-
143 3 m above ground in inter-canopy areas in all sites. Stand properties (leaf area index (*LAI*), basal area
144 (*BA*), tree height, soil depth, soil type) were taken from previous studies conducted at the same sites (see
145 Table 1 for more details on site characteristics).

146

147 **2.2 Soil moisture manipulation**

148 All sites were subjected to precipitation manipulation influencing available soil moisture (i.e.
149 reduction, addition or both) ranging from -90% to +80% (Table 1). A control treatment (i.e. ambient

150 precipitation) was included in each site and located near the soil moisture manipulation treatments (see
151 more details in Table 1). At the SUM site, the treatments could not be replicated because of logistical
152 and financial constraints, but the design is similar to other replicated large-scale ecosystem manipulation
153 experiments (e.g. Likens et al., 1970), whose strength is acknowledged, especially where large treatment
154 effects are expected (Pangle et al., 2012). In three sites (PUE, SEV and SUM), incoming precipitation
155 was reduced (-30% to -90%) by using systems of plastic panels and plastic-lined guttering installed at a
156 height between 0.5 and 2 m, depending on the site. In two sites (SEV and PFY), soil moisture was
157 artificially increased by +35% to + 80% using water applied via sprinklers at 1 m or 6 m above ground
158 for the PFY and SEV site, respectively (see Table 1 for site-specific references).

159

160 **2.3 Sap flux measurements**

161 At all sites tree sap flux density (F_D ; $\text{g m}^{-2} \text{s}^{-1}$) was measured with the thermal dissipation method
162 (Granier, 1987) during one growing season of different years (see Table 1 for year selection in each
163 site). Two probes (10- or 20-mm long depending on the site and the species) were installed under the
164 bark and the cambium at 0.8 to 1.3 m aboveground with a 10 cm vertical spacing between probes. This
165 height was necessary to minimize thermal disturbance from ground heating. All sensors were covered
166 with reflective insulation to reduce the risk of direct sunlight causing thermal gradients. F_D was
167 calculated following the equation proposed by Granier (1987). In each site between 11 and 42 dominant
168 trees were selected for the sap flux measurements (see Table 2 for tree characteristics). Methods used for
169 determining sapwood depth and appropriate corrections for radial variations in sap flux density at each
170 site are provided in Limousin et al., 2009; Pangle et al., 2015; Grossiord et al., 2017 and Mencuccini et
171 al., 2017.

172

173 2.4 Water balance modeling

174 As a measure of soil water availability, we estimated the daily relative extractable water (*REW*,
175 unitless, varying between 0 and 1) over the whole root zone at each site and for each treatment using the
176 forest water balance model BILJOU (Granier et al., 1999) (Fig. 3). This model predicts temporal
177 variations in soil water content and assesses the water stress conditions experienced by trees at a given
178 day. In this model, the input variables required are daily meteorological data (precipitation, global
179 radiation, air temperature and humidity, wind speed), soil water holding capacity, soil depth, soil bulk
180 density and leaf area index (*LAI*). The soil parameters were either directly measured or estimated at each
181 site and provided by the site managers. *REW* represents the ratio between available soil water and
182 maximum extractable water over the whole root zone and varies between 1 (i.e. field capacity) and 0
183 (i.e. permanent wilting point) (Granier et al., 1999). Although the BILJOU model has been validated at
184 the SUM site (Grossiord et al. 2016b), it is important to note that *REW* predictions are subject to
185 uncertainties at the SEV, PUE and PFY sites as the model has not been directly validated for these sites.
186 Simulations of *REW* with the BILJOU model were performed online
187 (<https://appgeodb.nancy.inra.fr/biljou/>, Fig. 3).

188 Artificial manipulation of incoming precipitation based on the soil surface covered by the plastic
189 guttering or the percentage of water added relative to ambient precipitation was similar to the relative
190 changes in *REW* between ambient and treatment conditions (Fig. S2). This observation suggests that the
191 imposed treatments were reflected in actual reductions and additions of water experienced by the trees.
192 Only the PFY site diverged from the 1:1 line (Fig. S2). As soil moisture conditions were already high at
193 this site relative to other sites, an 80% precipitation addition resulted only in a 19 % increase in *REW*
194 relative to ambient conditions (Fig. S2).

195

196 2.5 Statistical analyses

197 All analyses were performed using the software R (3.2.1, R Development Core Team 2015). As
198 *REW* is estimated at daily time scales, we calculate the daily average in sap flux density (F_D) for each
199 tree and daily average in *VPD* by excluding nighttime values (i.e. values occurring when global
200 radiation $< 10 \text{ W m}^{-2}$).

201 To determine how soil moisture manipulation influenced the sensitivity of sap flux to evaporative
202 demand, several equations were tested to relate F_D to daily *VPD* variations including linear, exponential,
203 logarithmic and parabolic equations. Parabolic equations showed the best fit for all trees (i.e. highest
204 R^2), and were fitted to individual trees F_D and *VPD* relationships, similarly to Grossiord et al., (2017):

$$205 \quad F_D = aVPD^2 + bVPD \quad [1].$$

206 Adjustments to variations in *VPD* are expected to manifest as shifts in optimal *VPD* for transpiration to
207 maintain high carbon uptake under drier atmospheric conditions (Grossiord et al., 2017, corresponding
208 to the location of the vertex, VPD_{opt} , kPa). In contrast, adjustments to soil moisture changes, as
209 determined in this study, are expected to be reflected in the maximum F_D reached at optimal *VPD* (Fig.
210 1) (Grossiord et al., 2017, F_{DMax} , $\text{g m}^{-2} \text{ s}^{-1} / \text{kPa}$). F_{DMax} was thus extracted for individual trees (i.e.
211 ambient and manipulative treatments) from the fitted relationships of equation (1) by calculating the
212 location of the vertex (h) and inserting it back to equation [1]:

$$213 \quad h = -b / 2a \quad [2].$$

214 Analyzing how precipitation manipulation influenced the sensitivity of transpiration to soil
215 moisture was done by testing the same equations (i.e. linear, exponential, logarithmic and parabolic)
216 between F_D and daily *REW*. Linear equations showed the best R^2 for all trees and were fitted to
217 individual trees F_D and *REW* relationships following:

218
$$F_D = a + bREW \quad [3].$$

219 The sensitivity of F_D to REW variations, corresponding to the slope of the relationship (S_{REW} , $\text{g m}^{-2} \text{s}^{-1}$,
220 i.e. corresponding to the b factor), was extracted for individual trees from the fitted relationships. We
221 used different analyses for VPD and REW transpiration sensitivities because these two variables were
222 highly independent (correlation coefficient = 0.15). However it is important to note that effects related to
223 VPD and REW are not easily separable in natural manipulative experiments and thus results have to be
224 interpreted with caution.

225 All relationships were fitted using the package *nls*. Differences in F_{DMax} and S_{REW} between
226 treatments and sites were determined through two-way ANOVA where treatments, sites and their
227 interaction were used as fixed effects. For all tests, tree identity was treated as random effect. Post-hoc
228 analysis was performed with Tukey's HSD test to determine differences between treatments. An alpha
229 critical value of $\alpha=0.05$ was used to determine statistical significance.

230

231 3. Results

232 3.1 Climatic conditions

233 All four sites were characterized by contrasting soil water availability under ambient conditions with
234 the SEV site being the driest and the PFY site the wettest (mean yearly *REW*: SEV < SUM < PUE <
235 PFY, Fig. 3). Similarly, evaporative demand was highest at the SEV site (maximum daily average *VPD*
236 of 3.4 kPa) and lowest at the PFY site (maximum daily average *VPD* of 2.0 kPa) (mean yearly *VPD*:
237 SEV < SUM < PUE < PFY, Fig. 2). Climatic conditions in all sites were marked by a drying period
238 during the growing season that started earliest at the driest site (\approx DOY 90) and latest at the wettest site
239 (\approx DOY 140), and lasted between 144 and 90 days for the SEV and the PFY sites, respectively.

241 3.2 Effect of precipitation manipulation on F_D sensitivity to *VPD*

242 We observed a significant parabolic F_D response to daily variations in *VPD* in all sites and
243 treatments (Fig. 4). A significant effect of precipitation manipulation was found for the sap flux
244 sensitivity to *VPD*, i.e. maximum F_D at optimal *VPD* (F_{DMax} , optimal *VPD* \approx 2 kPa) ($P < 0.001$) with the
245 lowest F_{DMax} found under precipitation reduction and highest F_{DMax} found under precipitation addition
246 (Fig. 4). F_{DMax} significantly varied between sites ($P < 0.001$) (SUM < SEV < PUE < PFY) but the
247 treatment*site interaction was not significant suggesting that all sites responded similarly to
248 precipitation manipulation (Fig. 5). A strong relationship was found between the relative change in
249 F_{DMax} (i.e. F_{DMax} in the manipulative treatment / F_{DMax} in ambient conditions) and the relative change in
250 *REW* (*REW* in the manipulative treatment / *REW* in ambient conditions) across all sites (Fig. 6),
251 suggesting similar responses in sap flux sensitivity to *VPD* variations under long-term (two to 11 years)
252 precipitation manipulation for a large range of environmental, biotic and climatic conditions.

253

254 3.3 Effect of precipitation manipulation on F_D sensitivity to REW

255 We found strong linear F_D responses to daily REW variations in all sites and treatments (Fig. 4).
256 Precipitation manipulation significantly influenced the sensitivity of F_D to REW (S_{REW}) ($P < 0.001$) with
257 the lowest S_{REW} observed under precipitation reduction and the highest S_{REW} found under precipitation
258 addition (Fig. 4). S_{REW} also varied between sites ($P = 0.006$) (Fig. 5) but the treatment*site interaction
259 was not significant suggesting that F_D sensitivity to REW responded similarly to precipitation
260 manipulation in all sites. A strong relationship was found between the relative change in S_{REW} (i.e. S_{REW}
261 in the manipulative treatment / S_{REW} in ambient conditions) and the relative change in REW (REW in the
262 manipulative treatment / REW in ambient conditions) across all sites (Fig. 6), suggesting again similar
263 responses in sap flux sensitivity to REW variations for all the forest types and environmental conditions
264 included in this study.

265

266 4. Discussion

267 Forest transpiration is a major component of the global water cycle and plays a significant role in
268 trees' ability to store terrestrial carbon (Chapin et al., 1990). Long-term changes in precipitation could
269 largely impact forest services, distribution, and net primary production (Zhao and Running, 2010).
270 Several mechanisms have been proposed regarding how trees hydrologically respond to soil moisture
271 stress (McDowell et al., 2008; Adams et al., 2013) but no study has yet attempted to decipher how long-
272 term exposure to precipitation change, and subsequent adjustments to novel soil moisture conditions,
273 could alter the sensitivity of transpiration to environmental variability. Our analysis of sap flux
274 responses to artificial soil moisture manipulation in mature ecosystems highlights how long-term
275 adjustments to soil moisture status affect the sensitivity of trees to daily variations in evaporative
276 demand and soil water availability.

277 Soil water content is a major limiting factor for tree sap flux on daily time-scales (Sperry et al.,
278 2002; Bréda et al., 2006). In the present study, we show that long-term soil moisture change could also
279 play a critical and increasingly important role in controlling forest water use in the future. Specifically,
280 exposure to relatively long-term (i.e. between two and six years) soil moisture reduction decreases the
281 sensitivity of trees to daily *VPD* and soil moisture variations (Fig. 5). As a result, trees are not able to
282 maintain sap flux rates similar to ambient conditions during the growing season, even under high soil
283 moisture and optimal *VPD* status (Fig. 4). Reduced sensitivity to daily environmental variability could
284 be the consequence of several mechanistic adjustments to long-term soil moisture limitation. For
285 instance, previous work conducted in one of the focal sites showed that trees shifted their water uptake
286 depth, sometimes to more superficial soil compartments, thereby exaggerating soil moisture constraints
287 on sap flux (Grossiord et al., 2016b). Other key attributes impacting tree water use rates and potentially
288 the sensitivity to *VPD* and soil moisture could be related to reductions in leaf area:sapwood area ratios

289 (Mencuccini and Grace, 1995; Limousin et al., 2012; Togashi et al., 2015), and reduced hydraulic
290 conductance (Pangle et al., 2015; Grossiord et al., 2016a; 2017; Salomón et al., 2017) resulting from
291 xylem embolism (Sperry and Tyree, 1988). One also cannot exclude legacy effects induced by
292 prolonged soil water limitations: artificial precipitation reduction was associated with a reduced ability
293 to assimilate new carbon in all experimental sites (Limousin et al., 2010; Limousin et al. 2013;
294 Grossiord et al. 2016a). Carbon resources are required for primary survival functions such as growth and
295 defense, thus long-term soil moisture reduction could alter the susceptibility of trees to extreme events
296 and likely increase their mortality risk during consecutive droughts (Waring, 1987). On a larger scale,
297 reduced sap flux sensitivity to environmental variations during the growing season could affect both the
298 global carbon and water cycles by limiting forest evapotranspiration rates and reducing carbon storage.

299 Precipitation addition, and thus increased long-term soil moisture, also significantly influenced
300 the sensitivity of trees to environmental variability. At the driest site (SEV), trees showed an enhanced
301 sensitivity to *VPD* and *REW* under a 35% precipitation increase (Fig. 5). As water is the main limiting
302 environmental factor for transpiration at this site, one could have expected this relatively strong
303 adjustment response to soil moisture addition (Valladares and Percy, 2002). Among other underlying
304 mechanisms, irrigation could have impacted tree allometry by shifting carbon investment between
305 below- and above-ground compartments: plants growing in mesic regions, or subjected to artificial
306 irrigation often develop a more dense and extended superficial rooting system relative to trees in arid
307 regions or subjected to soil moisture reduction (e.g. Togashi et al., 2015). This belowground adjustment
308 could have also occurred at the SEV site and could partially explain the high responsiveness of irrigated
309 trees to daily *REW* and *VPD* variations. In contrast, in the least water-limited site included in this study
310 (PFY), irrigation resulted in a modest increase of sap flux density (Fig. S3) and similar sensitivities to
311 *VPD* and *REW* variations relative to ambient conditions (Figs. 4 and 5). This response could be related

312 to the less dry conditions at this site relative to the SEV site (Table 1) as an 80% precipitation addition at
313 the PFY site did result in a less pronounced increase in soil moisture relative to ambient conditions than
314 in the SEV site (Fig. 2). Alternatively, the discrepancy between the two irrigation sites could be partially
315 related to differences in irrigation methods: while water was added at 1 m above ground at the PFY site
316 (only modifying soil moisture status), irrigation was applied at 6 m above ground at the SEV site thereby
317 changing soil moisture and microclimatic conditions in the crown simultaneously. Interestingly, earlier
318 observations at the PFY site found significant structural aboveground adjustments to irrigation including
319 increased needle and shoot length, stand *LAI* (Dobbertin et al., 2010; Timofeeva et al., 2017) and water-
320 use efficiency (i.e. $\delta^{13}\text{C}$, Eilmann et al., 2010) but a delay in fine root biomass production (Brunner et
321 al., 2009; Herzog et al., 2014), suggesting an immediate and a relatively higher investment in productive
322 functions rather than belowground root foraging in response to irrigation. Similar results were obtained
323 for holm oak trees along a precipitation gradient in Southern France, where the leaf area responded to
324 water availability, but not the fine root area (Martin-St Paul et al., 2013).

325 A key strength of the analysis conducted here is that it provides long-term sap flux adjustment
326 responses to soil moisture change across various temperate northern-hemisphere environments, and from
327 forest types composed of different tree species, but low species diversity. Contrary to our initial
328 hypothesis, we found a strong relationship between the relative change in soil moisture (from ambient
329 conditions) and the relative change in sap flux sensitivity to *VPD* and *REW* variations (from ambient
330 conditions), suggesting similar responses to soil water availability manipulation across all sites (Fig. 6).
331 Considering the large variability in climatic, environmental and biotic conditions among sites, this
332 observation is rather surprising. We know that sap flux regulation in response to evaporative demand
333 and soil moisture is species-specific (e.g. Zweifel et al., 2009; Matheny et al., 2014), and the limited
334 number of species investigated in each site did not allow us to separate species-specific responses. One

335 may expect that species with different functional strategies, particularly isohydric and anisohydric tree
336 species, would show differential responses to *VPD* and *REW* variations (e.g. Tardieu and Simonneau,
337 1998; Limousin et al., 2013; Garcia-Forner et al., 2016), and contrasting long-term adjustments in their
338 physiology and structure (Grossiord et al., 2016b; Grossiord et al., 2017). Furthermore, the sites were
339 subjected to soil moisture manipulation for different time spans before the measurements started,
340 enabling trees to adjust for more or less longer time periods to soil moisture manipulation. Trees that
341 have been subjected to longer treatment manipulation (e.g. PFY for 11 years) could have shown stronger
342 changes in sap flux sensitivity to *VPD* and *REW* than trees subjected to shorter soil moisture change
343 (e.g. SEV site for two years). Indeed, several studies have suggested that structural adjustments to
344 climate change, which are more likely to impact sap flux sensitivity to environmental variability, tend to
345 be more rapid than physiological ones (Valladares et al., 2000; Fisher et al., 2007; Grossiord et al.,
346 2016a). Nevertheless, as this study only included temperate-zone evergreen species, the results may
347 suggest common rapid adjustment mechanisms to long-term soil moisture status across environments for
348 leaf-persistent tree species. To further test this hypothesis, future work and manipulative experiments
349 should consider including more species from a large range of functional strategies and biomes.

350 The main results of the present study reinforces the idea that forest responses to climate change
351 will depend on many biotic and abiotic factors, particularly the adjustment capacity of trees to novel
352 climatic conditions. In summary, our results reveal that long-term adjustments to soil moisture will alter
353 the sap flux sensitivity to daily variations in evaporative demand and soil moisture. Climate models
354 commonly forecast an increase in precipitation in already moist regions in the next 100 years while
355 water-limited regions may experience long-term precipitation reduction (IPCC, 2014). Based on our
356 findings in dry ecosystems with experimentally lowered and increased soil water availability, this
357 suggests that trees growing in moist regions may not experience significant changes in their sensitivity

358 to environmental variability under projected precipitation change. Inversely, this work showed that trees
359 growing in already highly water-limited regions may see significant reductions in their sensitivity to
360 evaporative demand and soil moisture variations, highlighting the potential for stronger responses to
361 precipitation change in water-limited regions. Milder and cooler environments where light, temperature
362 or nutrients are the main limiting factor for tree functioning rather than soil moisture might show
363 stronger responsiveness and adjustment capacities to other factors (Niinemets, 2010). This is in line with
364 recent work showing that forest water use is mostly dominated by soil moisture limitations in dry
365 regions and by evaporative demand in mesic ones (Novick et al., 2016). As our study only included soil
366 moisture manipulation experiments, it is difficult to conclude how long-term adjustments to other
367 climate-related stresses such as warming and subsequently rising evaporative demand may alter
368 transpiration responses to environmental variability. New long-term experiments, in different climates
369 (from dry to moist) and manipulating additional environmental factors such as temperature and CO₂, are
370 urgently needed to help bring some light on tree functioning and on the longer-term adjustment potential
371 of forest ecosystems to climate change.

372

373 **Acknowledgments**

374 The SUMO and SEV experiments were funded by the US Department of Energy, Office of Science,
375 Biological and Environmental Research. The SEV experiment was also supported by the resources and
376 staff of the Sevilleta LTER (funded by NSF DEB 0620482), the Sevilleta Field Station at the University
377 of New Mexico, and the US Fish and Wildlife Service, who provided access to the Sevilleta National
378 Wildlife Refuge. The Puéchabon experimental site belongs to the SOERE F-ORE-T, which is supported
379 annually by Ecofor, Allenvi and the French national research infrastructure ANAEE. Evaluations were
380 based on data from the long-term irrigation experiment Pfywald, which is part of the Swiss Long-term
381 Forest Ecosystem Research programme LWF (www.lwf.ch) and the biological drought and growth
382 indicator network TreeNet (www.treenet.info). We are in particular grateful to Melissa Dawes and
383 Marcus Schaub who provided the soil moisture and forest structure data for the Pfywald site, and to
384 Jean-Marc Ourcival for Puéchabon ancillary data. CG was supported by a Director's Fellowship from
385 the Los Alamos National Laboratory. YS was funded by NERC (NE/I011749/1 to MM) and the
386 academy of Finland (1284701).

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594

Table 1: Characteristics of the study sites

<i>Forest name</i>	SUMO	Sevilleta	Puechabon	Pfynwald
<i>Country</i>	USA	USA	France	Switzerland
<i>Latitude/Longitude</i>	35.49°N, 106.18°W	34.23°N, 106.32°W	43.44°N, 3.35°E	46.19°N, 7.6°E
<i>Climate</i>	Semi-arid	Semi-arid	Mediterranean	Dry inner-Alpine
<i>Forest type</i>	Piñon-Juniper woodland	Piñon-Juniper woodland	Mediterranean oak evergreen	Mountainous Scots Pine evergreen
<i>MAT* (°C), MAP* (mm)</i>	9.2, 415	12.7, 358	13.2, 916	9.2, 657
<i>Topography</i>	Light steep	Flat to steep	Flat	Flat
<i>Soil depth (cm)</i>	55	20 to 100	450	500
<i>Elevation (m)</i>	2175	1911	270	615
<i>Number of target species</i>	2	2	1	1
<i>Percentage rainfall manipulation</i>	-90%	-45%, +35%	-30%	+80%
<i>Study year</i>	2016	2010	2009	2014
<i>Treatment duration before measurements</i>	4 years	2 years	6 years	11 years
<i>Mean LAI* (m² m⁻²)</i>	1.5	0.85	2	2.1
<i>References</i>	Adams et al., 2015 Grossiord et al., 2016a Grossiord et al., 2016b Garcia-Forner et al., 2016 Grossiord et al., 2017	Pangle et al., 2012 Pangle et al., 2015	Limousin et al., 2009 Limousin et al., 2010 Limousin et al., 2012	Mencuccini et al., 2017 Hartmann et al., 2017 Rigling et al., 2013 Sterck et al., 2012 Eilmann et al., 2010 Martinez-Vilalta et al., 2009

*MAP, mean annual sum of precipitation; MAT, mean annual temperature; LAI, leaf area index.

Table 2: Characteristics of the study trees.

Forest Name	Total number of measured trees	Number of treatments	Mean tree height (m)	Mean DBH* (cm)	Target species
SUMO	24	2	3.5	13.7	Piñon/Juniper
Sevilleta	42	3	4.0	27.1	Piñon/Juniper
Puechabon	11	2	4.5	11.5	Holm oak
Pfynwald	11	2	12.0	28	Scots pine

*DBH, diameter at breast height.

600 **Figure legends**

601 **Figure 1:** Hypothetical relationships between sap flux and daily soil moisture or vapor pressure deficit
602 (*VPD*) variation under different long-term soil moisture conditions (high, medium and low soil
603 moisture). The red arrow indicates changes in sap flux sensitivity to soil moisture and *VPD* resulting
604 from physiological and possible structural adjustments to soil moisture change (e.g. hydraulic resistance,
605 stomatal density, synthesis of chemicals inducing stomatal closure, rooting depth). Because of
606 adjustments to reduced soil moisture, trees would experience a decreased sensitivity to daily soil
607 moisture and *VPD* variation inducing lower sap flux under both high and low soil moisture status, and
608 lower maximum sap flux under optimal *VPD*. Sensitivity to soil moisture would thus be reflected
609 through changes in the slope of the linear relationship between sap flux and soil moisture while changes
610 in *VPD* sensitivity would be reflected in shifts of maximum sap flux at optimal *VPD* (location of the
611 vertex of the curve).

612 **Figure 2:** Daily variations in atmospheric temperature (°C) and vapor pressure deficit (*VPD*, kPa) at
613 each site for the selected years.

614 **Figure 3:** Daily relative extractable water (*REW*, unitless) simulated with the BILJOU water balance
615 model for each treatment at each site for the selected years (Table 1). The grey areas correspond to
616 periods where irrigation was ongoing at the SEV and PFY sites. Reduction in incoming precipitation
617 was ongoing all year long in all sites.

618 **Figure 4:** Relationships between mean daily sap flux density (F_D , $\text{g m}^{-2} \text{s}^{-1}$) (individual tree data), and
619 simulated relative extractable water (*REW*, unitless) or vapor pressure deficit (*VPD*, kPa) under ambient,
620 soil moisture addition and soil moisture reduction conditions across all sites. The bold lines represent the
621 fitted linear (i.e. for *REW*) and parabolic (i.e. for *VPD*) relationships for each treatment across all sites.
622 Individual sites are not identified for representation purposes.

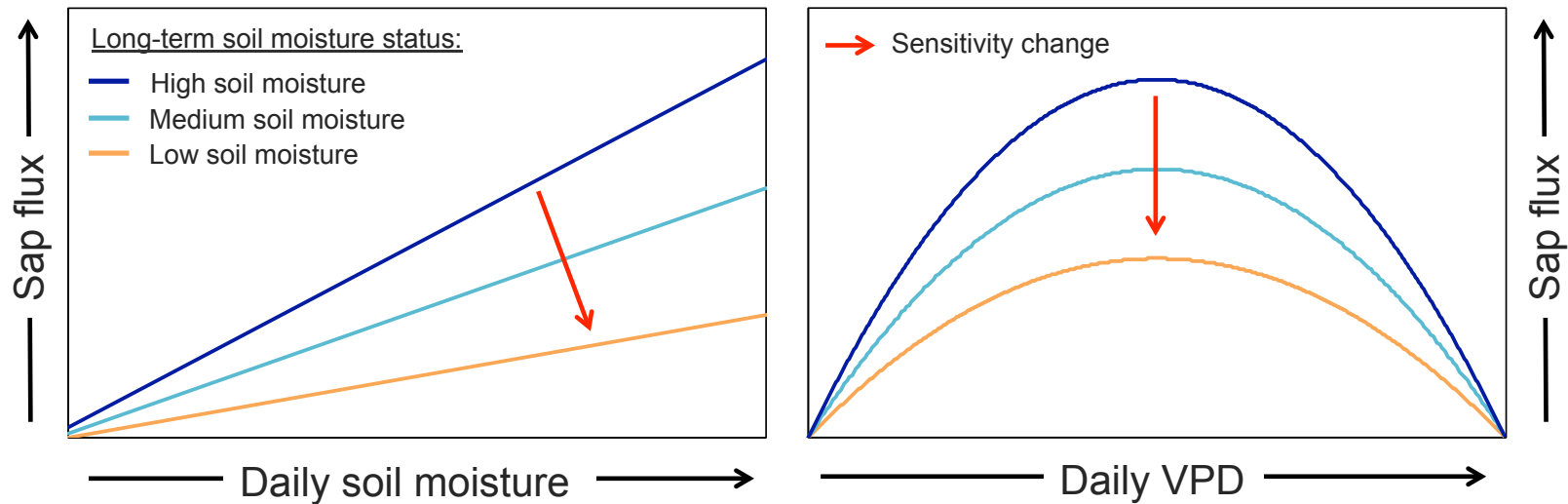
623

624 **Figure 5:** Sensitivity of F_D to simulated REW (S_{REW}) and to VPD (F_{DMax}) with standard errors of the
625 mean under ambient, soil moisture addition and soil moisture reduction conditions in each site (PFY =
626 Mountainous coniferous forest, PUE = Mediterranean evergreen forest, SEV and SUM = semi-arid
627 forest types). Sites are ordered along the x-axis by soil moisture manipulation intensity going from the
628 highest irrigation to the highest soil moisture reduction site.

629 **Figure 6:** Relative change in the sensitivity of F_D to simulated REW between ambient and treatment
630 conditions (S_{REW} in the manipulative treatment / $S_{REW_Ambient}$), and relative change in the maximum sap
631 flux density at optimum VPD between ambient and treatment conditions (F_{DMax} in the manipulative
632 treatment / $F_{DMax_Ambient}$) as a function of the relative change in relative extractable water between
633 ambient and treatment conditions during the measurements (REW in the manipulative treatment
634 / $REW_Ambient$). Every data point represents the difference between mean ambient conditions (for each site)
635 and an individual tree under treatment conditions (at the same site).

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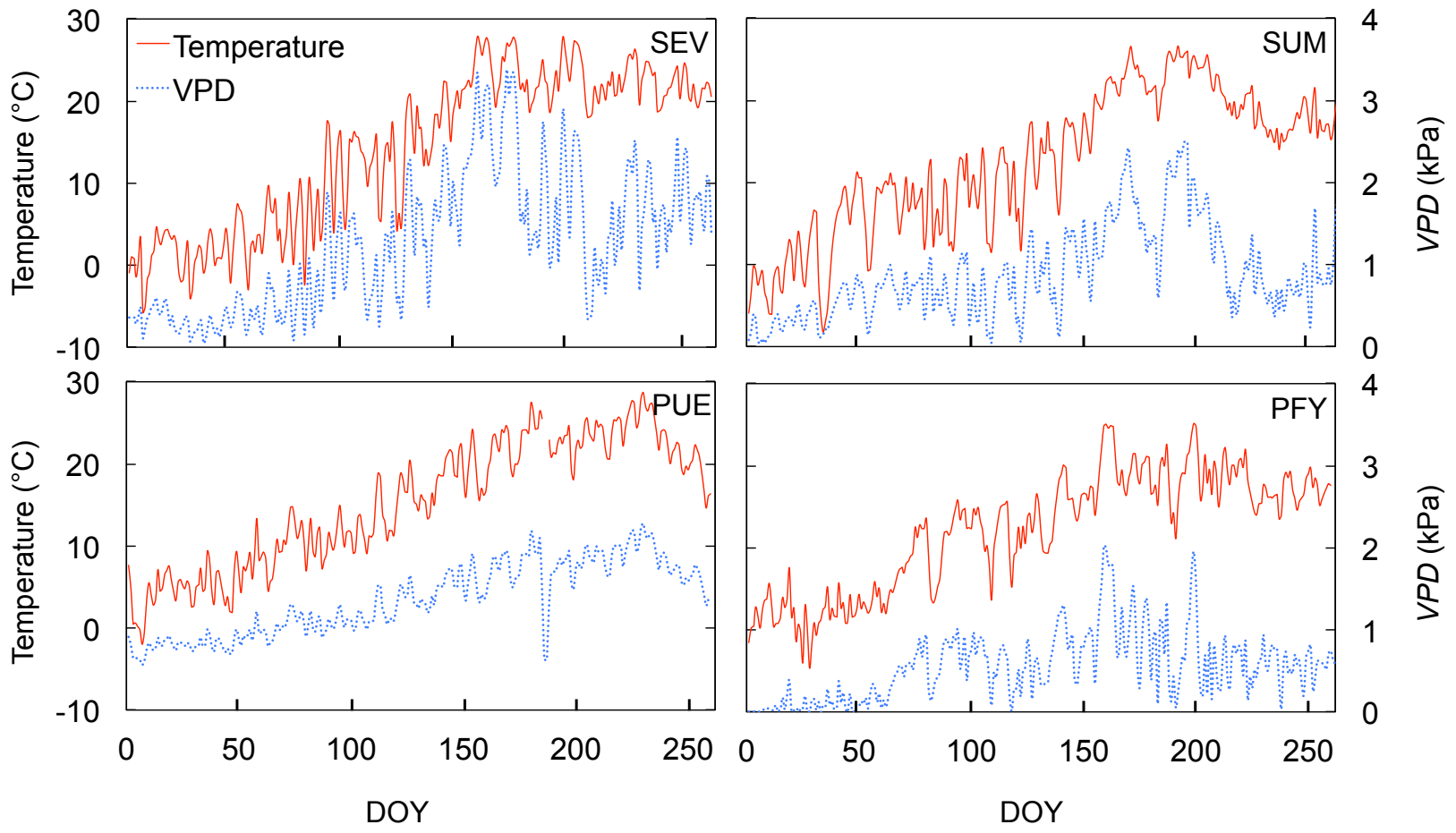
637 **Figures**



638

639 **Figure 1:** Hypothetical relationships between sap flux and daily soil moisture or vapor pressure deficit (*VPD*) variation under different
640 long-term soil moisture conditions (high, medium and low soil moisture). The red arrow indicates changes in sap flux sensitivity to
641 soil moisture and *VPD* resulting from physiological and possible structural adjustments to soil moisture change (e.g. hydraulic
642 resistance, stomatal density, synthesis of chemicals inducing stomatal closure, rooting depth). Because of adjustments to reduced soil
643 moisture, trees would experience a decreased sensitivity to daily soil moisture and *VPD* variation inducing lower sap flux under both
644 high and low soil moisture status, and lower maximum sap flux under optimal *VPD*. Sensitivity to soil moisture would thus be
645 reflected through changes in the slope of the linear relationship between sap flux and soil moisture while changes in *VPD* sensitivity
646 would be reflected in shifts of maximum sap flux at optimal *VPD* (location of the vertex of the curve).

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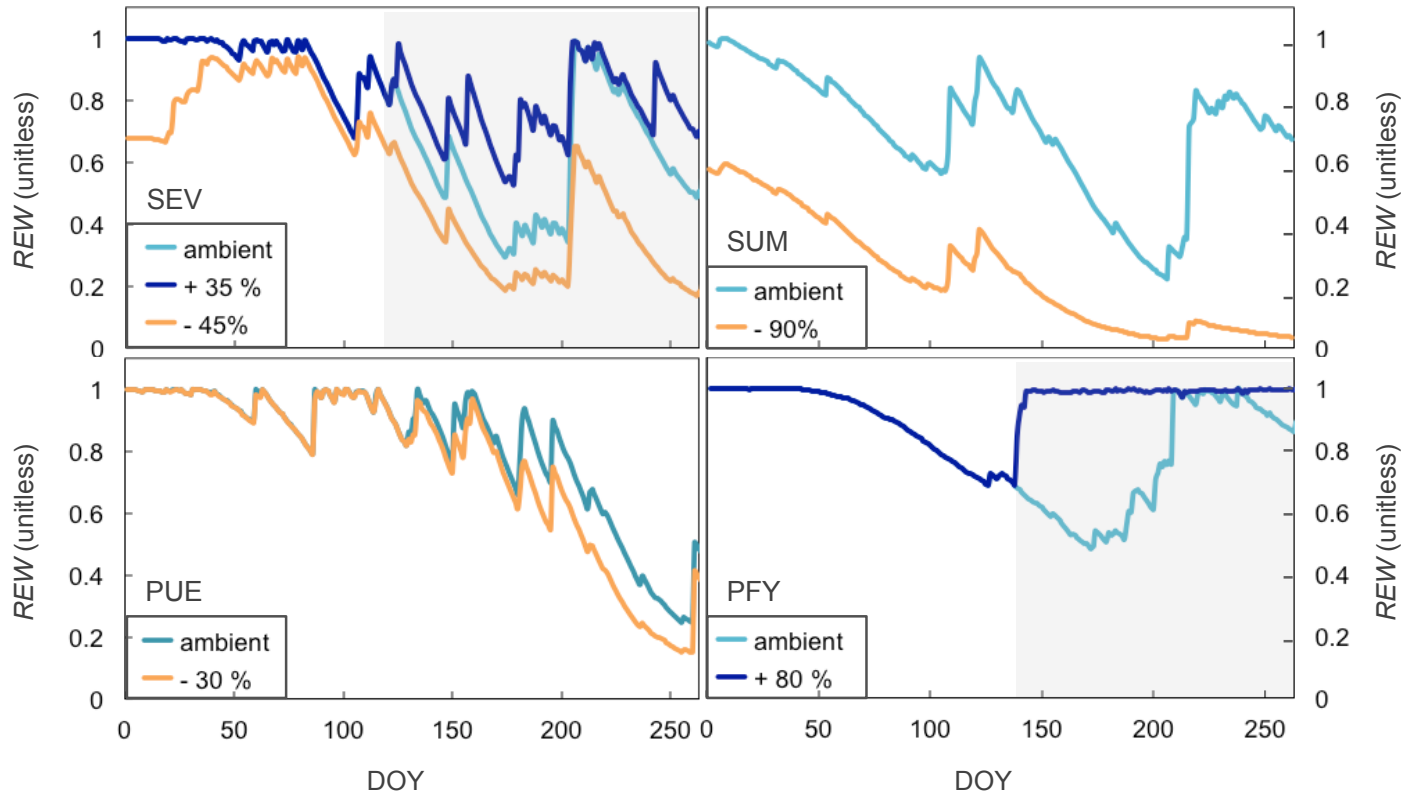
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649 **Figure 2:** Daily variations in atmospheric temperature (°C) and vapor pressure deficit (VPD, kPa) at each site for the selected years.

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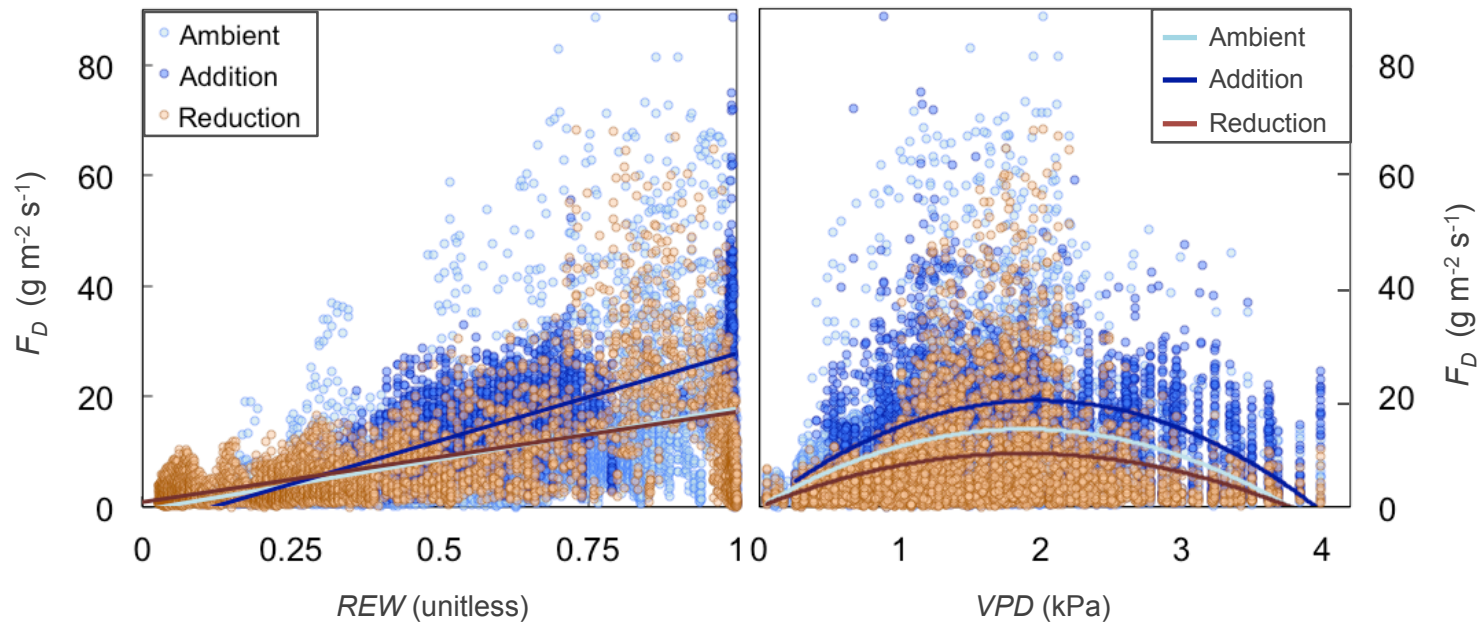


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655 **Figure 3:** Daily relative extractable water (*REW*, unitless) simulated with the BILJOU water balance model for each treatment at each
656 site for the selected years (Table 1). The grey areas correspond to periods where irrigation was ongoing at the SEV and PFY sites.

657 Reduction in incoming precipitation was ongoing all year long in all sites.



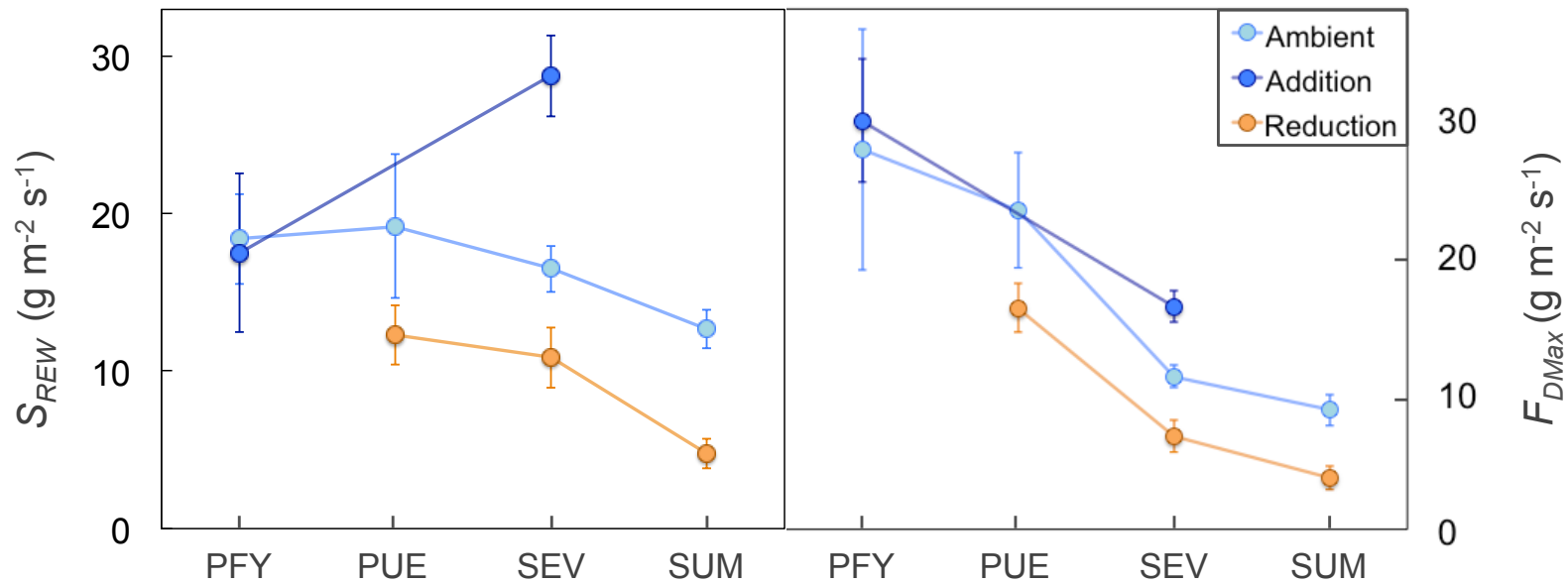
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659 **Figure 4:** Relationships between mean daily sap flux density (F_D , $\text{g m}^{-2} \text{s}^{-1}$) (individual tree data), and simulated relative extractable
 660 water (REW , unitless) or vapor pressure deficit (VPD , kPa) under ambient, soil moisture addition and soil moisture reduction
 661 conditions across all sites. The bold lines represent the fitted linear (i.e. for REW) and parabolic (i.e. for VPD) relationships for each
 662 treatment across all sites. Individual sites are not identified for representation purposes.

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668 **Figure 5:** Sensitivity of F_D to simulated REW (S_{REW}) and to VPD (F_{DMax}) with standard errors of the mean under ambient, soil
669 moisture addition and soil moisture reduction conditions in each site (PFY = Mountainous coniferous forest, PUE = Mediterranean
670 evergreen forest, SEV and SUM = semi-arid forest types). Sites are ordered along the x-axis by soil moisture manipulation intensity
671 going from the highest irrigation to the highest soil moisture reduction site.

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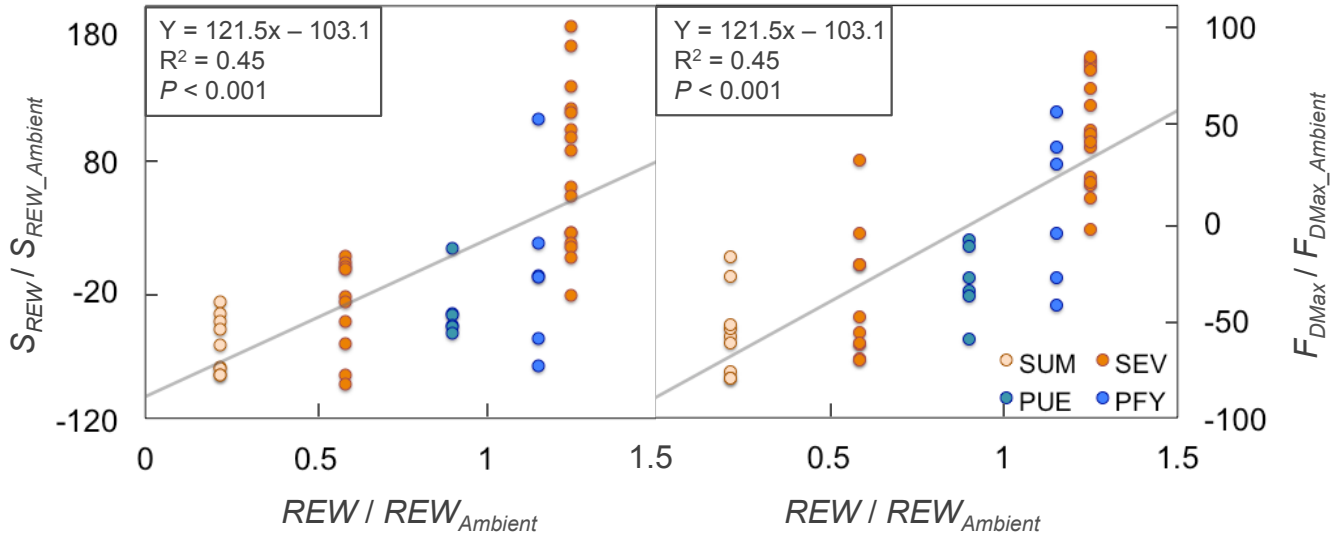


Figure 6: Relative change in the sensitivity of F_D to simulated REW between ambient and treatment conditions (S_{REW} in the manipulative treatment / $S_{REW_Ambient}$), and relative change in the maximum sap flux density at optimum VPD between ambient and treatment conditions (F_{DMax} in the manipulative treatment / $F_{DMax_Ambient}$) as a function of the relative change in relative extractable water between ambient and treatment conditions during the measurements (REW in the manipulative treatment / $REW_{Ambient}$). Every data point represents the difference between mean ambient conditions (for each site) and an individual tree under treatment conditions (at the same site).