Accepted Manuscript

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Please cite this article as: Grossiord, Charlotte, Sevanto, Sanna, Limousin, Jean-Marc, Meir, Patrick, Mencuccini, Maurizio, Pangle, Robert E., Pockman, William T., Salmon, Yann, Zweifel, Roman, McDowell, Nate G., Manipulative experiments demonstrate how long-term soil moisture changes alter controls of plant water use.Environmental and Experimental Botany https://doi.org/10.1016/j.envexpbot.2017.12.010

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

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Highlights

- How long-term soil moisture change affects the sensitivity of transpiration to environmental variability is unknown.

- Exposure to long-term soil moisture reduction decreased sap flux sensitivity to *VPD* and *REW*.

- Long-term irrigation increased sap flux sensitivity to *VPD* and *REW* but at highly water-limited sites only.

- Relative sensitivity to *VPD* and *REW* is generalizable across forest types suggesting common adjustment mechanisms to soil moisture status.

Abstract

Tree transpiration depends on biotic and abiotic factors that might change in the future, including precipitation and soil moisture status. Although short-term sap flux responses to soil moisture and evaporative demand have been the subject of attention before, the relative sensitivity of sap flux to these two factors under long-term changes in soil moisture conditions has rarely been determined experimentally. We tested how long-term artificial change in soil moisture affects the sensitivity of tree-level sap flux to daily atmospheric vapor pressure deficit (*VPD)* and soil moisture variations, and the generality of these effects across forest types and environments using four manipulative sites in mature forests. Exposure to relatively long-term (two to six years) soil moisture reduction decreases tree sap flux sensitivity to daily *VPD* and relative extractable water (*REW*) variations, leading to lower sap flux even under high soil moisture and optimal *VPD*. Inversely, trees subjected to long-term irrigation showed a significant increase in their sensitivity to daily *VPD* and *REW*, but only at the most water-limited site. The ratio between the relative change in soil moisture manipulation and the relative change

in sap flux sensitivity to *VPD* and *REW* variations was similar across sites suggesting common adjustment mechanisms to long-term soil moisture status across environments for evergreen tree species. Overall, our results show that long-term changes in soil water availability, and subsequent adjustments to these novel conditions, could play a critical and increasingly important role in controlling forest water use in the future.

Abbreviations

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

LAI = leaf area index (m^2 m^{-2})

REW = relative extractable water (unitless)

VPD = vapor pressure deficit (kPa)

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

1. Introduction

Recent work has shown that plant transpiration could account for up to 90% of terrestrial evapotranspiration (Jasechko et al., 2013), making vegetation a dominant force in the global water cycle. Transpiration from forest ecosystems alone contributes between 50% and 70% to terrestrial evapotranspiration (Schlesinger and Jasechko, 2014). Climate-related shifts in forest transpiration could thus have large impacts on the global water cycle including modification in precipitation, groundwater recharge, renewable fresh water, increasing soil erosion, and feedbacks on (micro) climate. Average temperature at the world's surface has been steadily rising since the mid-50s (IPCC, 2014). Warmer air

increases vapor pressure deficit (*VPD*) and induces higher evaporation from land surfaces including transpiration from plants (Hardwick et al., 2010). Simultaneously, the risk for severe and extended droughts is increasing (Williams et al., 2013; Cook et al., 2015; Roderick et al., 2015).

At hourly to daily scales, tree-level sap flux primarily results from the driving force for transpiration (radiation, *VPD* and wind), and hydraulic and stomatal conductance of the trees (Oren et al., 1999). Without stomatal control, tree-level transpiration increases progressively with rising *VPD* as a consequence of the atmosphere becoming less moisture saturated. As *VPD* increases, stomata respond via an exponential decrease in stomatal conductance (Lange et al., 1971; Monteith, 1995). Consequently, when high *VPD* is reached ($\approx$ 2.5 kPa, species-specific), stomata start modulating the transpiration flux and thus tree-level transpiration starts dropping due to partial stomatal closure. Therefore, the response of tree-level transpiration to *VPD* can be expressed as a parabolic equation (Fig. 1) (Monteith, 1995). By closing their stomata to avoid critically high sap flux rates and impacts on embolism, trees can avoid excessive damages to their hydraulic system (Saliendra et al., 1995). At daily or longer time scales, tree-level sap flux regulation is also largely controlled by soil moisture variation. Tree responses to soil water availability are triggered by a chemical signal originating from the roots exposed to dry soil (see reviews by Davies and Zhang, 1991; Davies et al., 1994). Strong relationships have already been established between soil moisture (or water stress indexes) and tree-level sap flux enabling the incorporation of sap flux responses to short-term precipitation change in several climate-vegetation models so far (e.g. Sala and Tenhunen, 1996; Granier et al., 1999; Verhoef et al., 2014; De Cáceres et al., 2015).

Although rapid sap flux responses to soil moisture and evaporative demand have been the subject of attention before, the relative sensitivity of sap flux to these two factors under long-term precipitation and soil moisture change has rarely been determined experimentally (but see Grossiord et al., 2017). However, to maintain integrity of water resources, and improve global predictions it is essential to

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determine how these two drivers will influence forest transpiration in the long-term under projected

climate. Plants are known to have a wide range of active adjustment strategies to deal with long-term

exposure to changing soil moisture, including physiological and structural adjustments (Chaves et al.,

2002) directly influencing sap flux regulation. Under drier soil conditions, plants often undergo

adjustments that are directed toward a water saving strategy to limit excessive water loss (Ogaya and

Peñuelas, 2003; Leuzinger et al., 2005; Brando et al., 2008). For instance, trees may produce conducting

elements with reduced lumen diameters (Hacke and Sperry, 2004; Fonti and Jansen, 2012; Grossiord et

al., 2017), which can result in reduced sensitivity to short-term soil moisture variation (i.e. reduced sap

flux under both high and low soil water availabilities because of higher flow resistance, Fig. 1). Long-

term soil moisture reduction may also result in changes in stomata density (Luomala et al., 2005), or in

the synthesis of chemical signals inducing stomatal closure (Bartlett et al., 2012; Meinzer et al., 2014),

which can decrease the sensitivity of trees to evaporative demand and result in reduced sap flux, even

under optimal VPD (≈ 1.5 kPa) (Fig. 1) (Grossiord et al., 2017).

Most of our knowledge on tree responses to climate variability is based on studies with potted plants

or seedlings under common garden conditions, which may not represent the true responses of mature

forests (Poorter et al., 2012; Rigling et al., 2013; Herzog et al., 2014). Manipulative field experiments on

mature individuals are one way to decipher ecosystem sensitivity to possible precipitation change, but

only a few experiments have been established in natural forests to date (Knapp et al., 2016). The lack of

experiments in natural conditions is partially related to the obvious technical limitations and the

financial costs associated with large-scale climate manipulation but also legal restrictions that can occur

in natural areas (Kayler et al., 2015). Such experiments have however great value as, contrary to studies

conducted along environmental gradients, they provide the ability to expose plants to environmental

conditions they would not experience otherwise (i.e. broadening of environmental conditions), isolate

mechanistic functions and identify threshold responses necessary for global predictions (Kayler et al.,

2015; Estiarte et al., 2016; Knapp et al., 2016).

Here we tested how long-term (multi-year) artificial change in incoming precipitation (i.e. increasing

or reducing long-term soil water availability) influences the sensitivity of tree sap flux to daily *VPD* and

soil moisture variations, and the generality of these effects across four forest types and environments

using four manipulative sites in natural and mature forests in the USA and Europe. We hypothesized

that:

(1) changes in long-term soil moisture would modify the sap flux sensitivity of trees to

environmental variability with trees subjected to reductions in soil water availability showing

decreased sap flux sensitivity to soil moisture and *VPD* variation while trees exposed to

increased soil moisture would show an enhanced sensitivity to the same factors, both because of

long-term adjustments in their physiology and structure (Fig. 1),

(2) changes in sap flux sensitivity to soil moisture and *VPD* variation would vary across climatic and

environmental conditions, and for various forest types as tree species have different inherent

responses to climatic variations (e.g. iso- and anisohydric strategies) and have adapted to their

local climate (e.g. long-term structural and physiological adjustments).

2. Materials and methods

2.1 Experimental sites

We used data collected from mature forest plots in four regions covering a large gradient of

environmental and climatic conditions and representing three major forest types (Fig. S1). The focal

regions are located in France (i.e. Mediterranean evergreen forest: the Puechabon site, PUE hereafter),

the United States (i.e. two semi-arid forest types: the SUMO and Sevilleta sites, SUM and SEV

hereafter, respectively) and Switzerland (i.e. dry Mountainous coniferous forest: the Pfynwald site, PFY

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hereafter). The Mediterranean evergreen forest is dominated by evergreen holm oak (*Quercus ilex* L.).

The dry Mountainous coniferous forest type is characterized by the dominance of Scots pine *(Pinus*

sylvestris L.) with pubescent oak (*Quercus pubescens* Willd.) mainly in the understory at the Swiss site.

The two semi-arid forest sites occur in high elevation deserts with the vegetation dominated by

evergreen junipers (*Juniperus monosperma* (Engelm.) Sarg.) and piñon pines (*Pinus edulis* Engelm.).

The target tree species pool varied depending on the site, going from one species in the PUE and PFY

sites to two species in the SEV and SUM sites (Table 1). Climatic conditions (rainfall, air temperature,

atmospheric humidity, solar radiation, wind speed) were measured continuously and recorded by a

weather station at each site (Fig. 2, see Table 1 for references). Climatic conditions were measured at 1-

3 m above ground in inter-canopy areas in all sites. Stand properties (leaf area index (*LAI*), basal area

(*BA*), tree height, soil depth, soil type) were taken from previous studies conducted at the same sites (see

Table 1 for more details on site characteristics).

2.2 Soil moisture manipulation

All sites were subjected to precipitation manipulation influencing available soil moisture (i.e.

reduction, addition or both) ranging from -90% to +80% (Table 1). A control treatment (i.e. ambient

precipitation) was included in each site and located near the soil moisture manipulation treatments (see

more details in Table 1). At the SUM site, the treatments could not be replicated because of logistical

and financial constraints, but the design is similar to other replicated large-scale ecosystem manipulation

experiments (e.g. Likens et al., 1970), whose strength is acknowledged, especially where large treatment

effects are expected (Pangle et al., 2012). In three sites (PUE, SEV and SUM), incoming precipitation

was reduced (-30% to -90%) by using systems of plastic panels and plastic-lined guttering installed at a

height between 0.5 and 2 m, depending on the site. In two sites (SEV and PFY), soil moisture was

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artificially increased by +35% to + 80% using water applied via sprinklers at 1 m or 6 m above ground for the PFY and SEV site, respectively (see Table 1 for site-specific references).

2.3 Sap flux measurements

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

2.4 Water balance modeling

As a measure of soil water availability, we estimated the daily relative extractable water (*REW*, unitless, varying between 0 and 1) over the whole root zone at each site and for each treatment using the forest water balance model BILJOU (Granier et al., 1999) (Fig. 3). This model predicts temporal variations in soil water content and assesses the water stress conditions experienced by trees at a given day. In this model, the input variables required are daily meteorological data (precipitation, global radiation, air temperature and humidity, wind speed), soil water holding capacity, soil depth, soil bulk

density and leaf area index (*LAI*). The soil parameters were either directly measured or estimated at each

site and provided by the site managers. *REW* represents the ratio between available soil water and

maximum extractable water over the whole root zone and varies between 1 (i.e. field capacity) and 0

(i.e. permanent wilting point) (Granier et al., 1999). Although the BILJOU model has been validated at

the SUM site (Grossiord et al. 2016b), it is important to note that *REW* predictions are subject to

uncertainties at the SEV, PUE and PFY sites as the model has not been directly validated for these sites.

Simulations of *REW* with the BILJOU model were performed online

(https://appgeodb.nancy.inra.fr/biljou/, Fig. 3).

Artificial manipulation of incoming precipitation based on the soil surface covered by the plastic

guttering or the percentage of water added relative to ambient precipitation was similar to the relative

changes in *REW* between ambient and treatment conditions (Fig. S2). This observation suggests that the

imposed treatments were reflected in actual reductions and additions of water experienced by the trees.

Only the PFY site diverged from the 1:1 line (Fig. S2). As soil moisture conditions were already high at

this site relative to other sites, an 80% precipitation addition resulted only in a 19 % increase in *REW*

relative to ambient conditions (Fig. S2).

2.5 Statistical analyses

All analyses were performed using the software R (3.2.1, R Development Core Team 2015). As

REW is estimated at daily time scales, we calculate the daily average in sap flux density (F_D) for each

tree and daily average in *VPD* by excluding nighttime values (i.e. values occurring when global

radiation < 10 W m^{-2}).

To determine how soil moisture manipulation influenced the sensitivity of sap flux to evaporative

demand, several equations were tested to relate F_D to daily *VPD* variations including linear, exponential,

logarithmic and parabolic equations. Parabolic equations showed the best fit for all trees (i.e. highest R^2), and were fitted to individual trees F_D and VPD relationships, similarly to Grossiord et al., (2017):

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

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

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

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

$$F_D = a + bREW \qquad [3].$$

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

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

3. Results

3.1 Climatic conditions

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

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

We observed a significant parabolic F_D response to daily variations in *VPD* in all sites and treatments (Fig. 4). A significant effect of precipitation manipulation was found for the sap flux sensitivity to *VPD*, i.e. maximum F_D at optimal *VPD* (F_{DMax}, optimal $VPD \approx 2$ kPa) ($P < 0.001$) with the lowest F_{DMax} found under precipitation reduction and highest F_{DMax} found under precipitation addition (Fig. 4). F_{DMax} significantly varied between sites ($P < 0.001$) (SUM < SEV < PUE < PFY) but the

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treatment*site interaction was not significant suggesting that all sites responded similarly to precipitation manipulation (Fig. 5). A strong relationship was found between the relative change in F_{DMax} (i.e. F_{DMax} in the manipulative treatment/ F_{DMax} in ambient conditions) and the relative change in REW (REW in the manipulative treatment /REW in ambient conditions) across all sites (Fig. 6), suggesting similar responses in sap flux sensitivity to VPD variations under long-term (two to 11 years) precipitation manipulation for a large range of environmental, biotic and climatic conditions.

3.3 Effect of precipitation manipulation on F_D sensitivity to REW

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

4. Discussion

Forest transpiration is a major component of the global water cycle and plays a significant role in trees' ability to store terrestrial carbon (Chapin et al., 1990). Long-term changes in precipitation could largely impact forest services, distribution, and net primary production (Zhao and Running, 2010).

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Several mechanisms have been proposed regarding how trees hydrologically respond to soil moisture stress (McDowell et al., 2008; Adams et al., 2013) but no study has yet attempted to decipher how long-term exposure to precipitation change, and subsequent adjustments to novel soil moisture conditions, could alter the sensitivity of transpiration to environmental variability. Our analysis of sap flux responses to artificial soil moisture manipulation in mature ecosystems highlights how long-term adjustments to soil moisture status affect the sensitivity of trees to daily variations in evaporative demand and soil water availability.

Soil water content is a major limiting factor for tree sap flux on daily time-scales (Sperry et al.., 2002; Bréda et al., 2006). In the present study, we show that long-term soil moisture change could also play a critical and increasingly important role in controlling forest water use in the future. Specifically, exposure to relatively long-term (i.e. between two and six years) soil moisture reduction decreases the sensitivity of trees to daily *VPD* and soil moisture variations (Fig. 5). As a result, trees are not able to maintain sap flux rates similar to ambient conditions during the growing season, even under high soil moisture and optimal *VPD* status (Fig. 4). Reduced sensitivity to daily environmental variability could be the consequence of several mechanistic adjustments to long-term soil moisture limitation. For instance, previous work conducted in one of the focal sites showed that trees shifted their water uptake depth, sometimes to more superficial soil compartments, thereby exaggerating soil moisture constraints on sap flux (Grossiord et al., 2016b). Other key attributes impacting tree water use rates and potentially the sensitivity to *VPD* and soil moisture could be related to reductions in leaf area:sapwood area ratios (Mencuccini and Grace, 1995; Limousin et al., 2012; Togashi et al., 2015), and reduced hydraulic conductance (Pangle et al., 2015; Grossiord et al., 2016a; 2017; Salomón et al., 2017) resulting from xylem embolism (Sperry and Tyree, 1988). One also cannot exclude legacy effects induced by prolonged soil water limitations: artificial precipitation reduction was associated with a reduced ability

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to assimilate new carbon in all experimental sites (Limousin et al., 2010; Limousin et al. 2013; Grossiord et al. 2016a). Carbon resources are required for primary survival functions such as growth and defense, thus long-term soil moisture reduction could alter the susceptibility of trees to extreme events and likely increase their mortality risk during consecutive droughts (Waring, 1987). On a larger scale, reduced sap flux sensitivity to environmental variations during the growing season could affect both the global carbon and water cycles by limiting forest evapotranspiration rates and reducing carbon storage.

Precipitation addition, and thus increased long-term soil moisture, also significantly influenced the sensitivity of trees to environmental variability. At the driest site (SEV), trees showed an enhanced sensitivity to *VPD* and *REW* under a 35% precipitation increase (Fig. 5). As water is the main limiting environmental factor for transpiration at this site, one could have expected this relatively strong adjustment response to soil moisture addition (Valladares and Percy, 2002). Among other underlying mechanisms, irrigation could have impacted tree allometry by shifting carbon investment between below- and above-ground compartments: plants growing in mesic regions, or subjected to artificial irrigation often develop a more dense and extended superficial rooting system relative to trees in arid regions or subjected to soil moisture reduction (e.g. Togashi et al., 2015). This belowground adjustment could have also occurred at the SEV site and could partially explain the high responsiveness of irrigated trees to daily *REW* and *VPD* variations. In contrast, in the least water-limited site included in this study (PFY), irrigation resulted in a modest increase of sap flux density (Fig. S3) and similar sensitivities to *VPD* and *REW* variations relative to ambient conditions (Figs. 4 and 5). This response could be related to the less dry conditions at this site relative to the SEV site (Table 1) as an 80% precipitation addition at the PFY site did result in a less pronounced increase in soil moisture relative to ambient conditions than in the SEV site (Fig. 2). Alternatively, the discrepancy between the two irrigation sites could be partially related to differences in irrigation methods: while water was added at 1 m above ground at the PFY site

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(only modifying soil moisture status), irrigation was applied at 6 m above ground at the SEV site thereby changing soil moisture and microclimatic conditions in the crown simultaneously. Interestingly, earlier observations at the PFY site found significant structural aboveground adjustments to irrigation including increased needle and shoot length, stand *LAI* (Dobbertin et al., 2010; Timofeeva et al., 2017) and water-use efficiency (i.e. $\delta^{13}C$, Eilmann et al., 2010) but a delay in fine root biomass production (Brunner et al., 2009; Herzog et al., 2014), suggesting an immediate and a relatively higher investment in productive functions rather than belowground root foraging in response to irrigation. Similar results were obtained for holm oak trees along a precipitation gradient in Southern France, where the leaf area responded to water availability, but not the fine root area (Martin-St Paul et al., 2013).

A key strength of the analysis conducted here is that it provides long-term sap flux adjustment responses to soil moisture change across various temperate northern-hemisphere environments, and from forest types composed of different tree species, but low species diversity. Contrary to our initial hypothesis, we found a strong relationship between the relative change in soil moisture (from ambient conditions) and the relative change in sap flux sensitivity to *VPD* and *REW* variations (from ambient conditions), suggesting similar responses to soil water availability manipulation across all sites (Fig. 6). Considering the large variability in climatic, environmental and biotic conditions among sites, this observation is rather surprising. We know that sap flux regulation in response to evaporative demand and soil moisture is species-specific (e.g. Zweifel et al., 2009; Matheny et al., 2014), and the limited number of species investigated in each site did not allow us to separate species-specific responses. One may expect that species with different functional strategies, particularly isohydric and anisohydric tree species, would show differential responses to *VPD* and *REW* variations (e.g. Tardieu and Simonneau, 1998; Limousin et al., 2013; Garcia-Forner et al., 2016), and contrasting long-term adjustments in their physiology and structure (Grossiord et al., 2016b; Grossiord et al., 2017). Furthermore, the sites were

subjected to soil moisture manipulation for different time spans before the measurements started, enabling trees to adjust for more or less longer time periods to soil moisture manipulation. Trees that have been subjected to longer treatment manipulation (e.g. PFY for 11 years) could have shown stronger changes in sap flux sensitivity to *VPD* and *REW* than trees subjected to shorter soil moisture change (e.g. SEV site for two years). Indeed, several studies have suggested that structural adjustments to climate change, which are more likely to impact sap flux sensitivity to environmental variability, tend to be more rapid than physiological ones (Valladares et al., 2000; Fisher et al., 2007; Grossiord et al., 2016a). Nevertheless, as this study only included temperate-zone evergreen species, the results may suggest common rapid adjustment mechanisms to long-term soil moisture status across environments for leaf-persistent tree species. To further test this hypothesis, future work and manipulative experiments should consider including more species from a large range of functional strategies and biomes.

The main results of the present study reinforces the idea that forest responses to climate change will depend on many biotic and abiotic factors, particularly the adjustment capacity of trees to novel climatic conditions. In summary, our results reveal that long-term adjustments to soil moisture will alter the sap flux sensitivity to daily variations in evaporative demand and soil moisture. Climate models commonly forecast an increase in precipitation in already moist regions in the next 100 years while water-limited regions may experience long-term precipitation reduction (IPCC, 2014). Based on our findings in dry ecosystems with experimentally lowered and increased soil water availability, this suggests that trees growing in moist regions may not experience significant changes in their sensitivity to environmental variability under projected precipitation change. Inversely, this work showed that trees growing in already highly water-limited regions may see significant reductions in their sensitivity to evaporative demand and soil moisture variations, highlighting the potential for stronger responses to precipitation change in water-limited regions. Milder and cooler environments where light, temperature

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or nutrients are the main limiting factor for tree functioning rather than soil moisture might show stronger responsiveness and adjustment capacities to other factors (Niinemets, 2010). This is in line with recent work showing that forest water use is mostly dominated by soil moisture limitations in dry regions and by evaporative demand in mesic ones (Novick et al., 2016). As our study only included soil moisture manipulation experiments, it is difficult to conclude how long-term adjustments to other climate-related stresses such as warming and subsequently rising evaporative demand may alter transpiration responses to environmental variability. New long-term experiments, in different climates (from dry to moist) and manipulating additional environmental factors such as temperature and CO_2, are urgently needed to help bring some light on tree functioning and on the longer-term adjustment potential of forest ecosystems to climate change.

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References

Adams, H. D., Germino, M. J., Breshears, D. D., Barron- Gafford, G. A., Guardiola- Claramonte, M., Zou, C. B., & Huxman, T. E., 2013. Nonstructural leaf carbohydrate dynamics of Pinus edulis during drought- induced tree mortality reveal role for carbon metabolism in mortality mechanism. New Phyt. 197, 1142-1151.

Adams, H. D., Collins, A. D., Briggs, S. P., Vennetier, M., Dickman, L. T., Sevanto, S. A., Garcia-Forner, N., McDowell, N. G., 2015. Experimental drought and heat can delay phenological development and reduce foliar and shoot growth in semiarid trees. Glob. Chan. Biol., 21, 4210-4220.

Bartlett, M. K., Scoffoni, C., Sack, L., 2012. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta‐analysis. Ecol. Lett. 15, 393-405.

Brando, P. M., Nepstad, D. C., Davidson, E. A., Trumbore, S. E., Ray, D., Camargo, P., 2008. Drought effects on litterfall, wood production and belowground carbon cycling in an Amazon forest: results of a throughfall reduction experiment. Philosophical Transactions of the Royal Society of London B: Biol. Scie. 363, 1839-1848.

Bréda, N., Huc, R., Granier, A., Dreyer, E., 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Ann. For. Sci. 63, 625-644.

Brunner, I., Graf-Pannatier, E., Frey, B., Rigling, A., Landolt, W., Dobbertin, M., 2009. Morphological and physiological responses of Scots pine fine roots to water supply in a climatic dry area in Switzerland. Tree Physiol. 29, 541-550.

Chapin, F. S., Schulze, E. D., Harold, A. M., 1990. The ecology and economics of storage in plants. Ann. Rev. Ecol. Syst. 21, 423-447.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T. Pinheiro, C., 2002. How plants cope with water stress in the field? Photosynthesis and growth. Ann. Bot. 89, 907-916.

Cook, B. I., Ault, T. R., Smerdon, J. E., 2015. Unprecedented 21st century drought risk in the American Southwest and Central Plains. Sci. Adv. 1, e1400082.

Davies, W. J., Zhang, J., 1991. Root signals and the regulation of growth and development of plants in drying soil. Ann. Rev. Plant Biol. 42, 55-76.

Davies, W. J., Tardieu, F., & Trejo, C. L., 1994. How do chemical signals work in plants that grow in drying soil?. Plant Physiol. 104, 309.

De Cáceres, M., Martínez-Vilalta, J., Coll, L., Llorens, P., Casals, P., Poyatos, R., Pausas, J.G., Brotons, L., 2015. Coupling a water balance model with forest inventory data to predict drought stress: the role of forest structural changes vs. climate changes. Agri. For. Meteor. 213, 77-90.

Dobbertin, M., Eilmann, B., Bleuler, P., Giuggiola, A., Graf Pannatier, E., Landolt, W., Schleppi, P., Rigling, A., 2010. Effect of irrigation on needle, shoot and stem growth in natural drought exposed Pinus sylvestris forests. Tree Physiol. 30, 346-360.

Eilmann, B., Buchmann, N., Siegwolf, R., Saurer, M., Cherubini, P., Rigling, A., 2010. Fast response to improved water availability of tree-ring width and d13C in Scots pine. Plant, Cell & Envir. 33,1351-1360.

Estiarte, M., Vicca, S., Peñuelas, J., Bahn, M., Beier, C., Emmett, B.A., Fay, P.A., Hanson, P.J., Hasibeder, R., Kigel, J., Kröel- Dulay, G., 2016. Few multiyear precipitation–reduction experiments find a shift in the productivity–precipitation relationship. Glob. Change Biol. 22, 2570-2581.

Fisher, R.A., Williams, M., Costa, D., Lola, A., Malhi, Y., da Costa, R.F., Almeida, S., Meir, P., 2007. The response of an Eastern Amazonian rain forest to drought stress: results and modelling analyses from a throughfall exclusion experiment. Glob. Change Biol. 13, 2361-2378.

Fonti, P., Jansen, S., 2012. Xylem plasticity in response to climate. New Phyt. 195, 734-736.

Garcia- Forner, N., Adams, H.D., Sevanto, S., Collins, A.D., Dickman, L.T., Hudson, P.J., Zeppel, M.J., Jenkins, M.W., Powers, H., Martínez- Vilalta, J. Mcdowell, N.G., 2016. Responses of two semiarid conifer tree species to reduced precipitation and warming reveal new perspectives for stomatal regulation. Plant, Cell & Envir. 39, 38-49.

Granier, A., 1987. Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. Tree Physiol. 3, 309-320.

Granier, A., Bréda, N., Biron, P., Villette, S., 1999. A lumped water balance model to evaluate duration and intensity of drought constraints in forest stands. Ecol. Model. 116, 269-283.

Grossiord, C., Sevanto, S., Adams, H. D., Collins, A. D., Dickman, L. T., McBranch, N., Michaletz, S. T., Stockton, E. A., Vigil, M., McDowell, N. G., 2016a. Precipitation, not air temperature, drives functional responses of trees in semi- arid ecosystems. Jour. Ecol. 105, 163-175.

Grossiord, C., Sevanto, S., Dawson, T. E., Adams, H. D., Collins, A. D., Dickman, L. T., Newman, B. D., Stockton, E. A., McDowell, N. G., 2016b. Warming combined with more extreme precipitation regimes modifies the water sources used by trees. New Phytol. 213, 584-596.

Grossiord, C., Sevanto, S., Borrego, I., Chan, A. M., Collins, A. D., Dickman, L. T., Hudson, P. J., McBranch, N., Michaletz, S. T., Pockman, W. T., Ryan, M., Vilagrosa, A., McDowell, N., 2017. Tree water dynamics in a drying and warming world. Plant, Cell & Environ. DOI: 10.1111/pce.12991

Hacke, U. G., Sperry, J. S., Pittermann, J., 2004. Analysis of circular bordered pit function II. Gymnosperm tracheids with torus-margo pit membranes. Amer. Jour. Bot. 91, 386-400.

Hanson, P.J., 2000. Large-scale water manipulations. Chapter 23 in Methods in Ecosystem Science (eds SalaOE, JacksonRB, MooneyHA, HowarthRW), pp. 341–352. Springer-Verlag, New York.

Hardwick, J., Westra, S., Sharma, A., 2010. Observed relationships between extreme sub- daily precipitation, surface temperature, and relative humidity. Geophys. Res. Lett. 37.

Hartmann, M., Brunner, I., Hagedorn, F., Bardgett, R.D., Stierli, B., Herzog, C., Chen, X., Zingg, A., Graf-Pannatier, E., Rigling, A., Frey, B., 2017. A decade of irrigation transforms the soil microbiome of a semi-arid pine forest. Molecul. Ecol. doi:10.1111/mec.13995

Herzog, C,. Steffen, J., Graf Pannatier, E., Hajdas, I., Brunner, I., 2014. Nine Years of Irrigation Cause Vegetation and Fine Root Shifts in a Water-Limited Pine Forest. PLoS ONE 9, e96321. DOI: 10.1371/journal.pone.0096321

IPCC., 2014: *Climate Change 2014: Synthesis Report*. *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jasechko, S., Sharp, Z. D., Gibson, J. J., Birks, S. J., Yi, Y., Fawcett, P. J., 2013. Terrestrial water fluxes dominated by transpiration. Nature 496, 347-350.

Kayler, Z.E., De Boeck, H.J., Fatichi, S., Grünzweig, J.M., Merbold, L., Beier, C., McDowell, N. Dukes, J.S., 2015. Experiments to confront the environmental extremes of climate change. Front. Ecol. Environ. 13, 219-225.

Knapp, A. K., Avolio, M. L., Beier, C., Carroll, C. J., Collins, S. L., Dukes, J. S., Fraser, L.H., Griffin‐Nolan, R.J., Hoover, D.L., Jentsch, A., Loik, M. E., 2016. Pushing precipitation to the extremes in distributed experiments: recommendations for simulating wet and dry years. Glob. Change Biol. 23, 1774-1782.

Lange, O.L., Losch, R., Schulze, E.-D., Kappen, L., 1971. Responses of stomata to changes in humidity. Planta 100, 76-86.

Leuzinger, S., Zotz, G., Asshoff, R., Körner, C., 2005. Responses of deciduous forest trees to severe drought in Central Europe. Tree Physiol. 25, 641-650.

Likens, G. E., Bormann, F. H., Johnson, N. M., Fisher, D. W., Pierce, R. S., 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed‐ ecosystem. Ecol. Monogr. 40, 23-47.

Limousin, J. M., Rambal, S., Ourcival, J. M., Rocheteau, A., Joffre, R., Rodríguez‐ Cortina, R., 2009. Long‐ term transpiration change with rainfall decline in a Mediterranean Quercus ilex forest. Global Change Biol. 15, 2163-2175.

Limousin, J.M., Misson, L., Lavoir, A.V., Martin, N.K., Rambal, S. 2010. Do photosynthetic limitations of evergreen Quercus ilex leaves change with long-term increased drought severity ? Plant Cell and Environ. 33, 863-875

Limousin, J.M., Rambal, S., Ourcival, J.M., Rodriguez-Calcerrada, J., Pérez-Ramos, I.M., Rodriguez-Cortina, R., Misson, L., Joffre, R. 2012. Morphological and phenological shoot plasticity in a Mediterranean evergreen oak facing long-term increased drought. Oecologia 169, 565-577.

Limousin, J. M., Bickford, C.P., Dickman, L.T., Pangle, R.E., Hudson, P.J., Boutz, A.L., Gehres, N., Osuna, J.L., Pockman, W.T., McDowell, N.G., 2013. Regulation and acclimation of leaf gas exchange in a pinon–juniper woodland exposed to three different precipitation regimes. Plant, Cell & Environ. 36, 1812-1825.

Luomala, E., Laitinen, K., Sutinen, S., Kellomäki, S., Vapaavuori, E., 2005. Stomatal density, anatomy and nutrient concentrations of Scots pine needles are affected by elevated CO2 and temperature. Plant, Cell & Environ. 28, 733-749.

Matheny, A.M., Bohrer, G., Vogel, C.S., Morin, T.H., He, L., Frasson, R.P.D.M., Mirfenderesgi, G., Schäfer, K.V., Gough, C.M., Ivanov, V.Y. Curtis, P.S., 2014. Species- specific transpiration responses to intermediate disturbance in a northern hardwood forest. Journ. Geophys. Res.: Biogeosci. 119, 2292-2311.

Martinez-Vilalta, J., Cochard, H., Mencuccini, M., Sterck, F.J., Herrero, A., Korhonen, J.F.J., Llorens, P., Nikinmaa, E., Poyatos, R., Ripullone, F., Sass-Klaassen, U., Zweifel, R., 2009. Hydraulic adjustment of Scots pine across Europe. New Phytol. 184, 353–364.

Martin-StPaul, N.K., Limousin, J.M., Vogt-Schilb, H., Rodriguez-Calcerrada, J., Rambal, S., Longepierre, D., Misson, L. 2013. The temporal response to drought in a Mediterranean evergreen tree : comparing a regional precipitation gradient and a throughfall exclusion experiment. Global Change Biol. 19, 2413-2426.

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G. Yepez, E.A., 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought?. New phytol 178, 719-739.

Meinzer, F. C., Woodruff, D. R., Marias, D. E., McCulloh, K. A., Sevanto, S., 2014. Dynamics of leaf water relations components in co‐occurring iso‐ and anisohydric conifer species. Plant, Cell & Environ. 37, 2577-2586.

Mencuccini, M., Grace, J., 1995. Climate influences the leaf area/sapwood area ratio in Scots pine. Tree Physiol. 15, 1-10.

Mencuccini, M., Salmon, Y., Mitchell, P., Holtta, T., Choat, B., Meir, P., O'Grady, A., Tissue, D., Zweifel, R., Sevanto, S., Pfautsch, S., 2017. An empirical method that separates irreversible stem radial growth from bark water content changes in trees: theory and case studies. Plant, Cell & Environ. 40, 290-303.

Monteith, J. L., 1995. A reinterpretation of stomatal responses to humidity. Plant, Cell & Environ. 18, 357-364.

Niinemets, Ü., 2010. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. For. Ecol. Manag. 260,1623-1639.

Novick, K. A., Ficklin, D. L., Stoy, P. C., Williams, C. A., Bohrer, G., Oishi, A. C., Papuga, S.A., Blanken, P.D., Noormets, A., Sulman, B.N. Scott, R.L., 2016. The increasing importance of atmospheric demand for ecosystem water and carbon fluxes. Nature Climate Change 6, 1023-1027.

Ogaya, R., Peñuelas, J., 2003. Comparative field study of Quercus ilex and Phillyrea latifolia: photosynthetic response to experimental drought conditions. Env. Exp. Bot. 50, 137-148.

Oren, R., Sperry, J. S., Katul, G. G., Pataki, D. E., Ewers, B. E., Phillips, N., Schäfer, K. V. R., 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. Plant, Cell & Envir. 22, 1515-1526.

Pangle, R. E., Hill, J. P., Plaut, J. A., Yepez, E. A., Elliot, J. R., Gehres, N., Pockman, W. T., 2012. Methodology and performance of a rainfall manipulation experiment in a piñon–juniper woodland. Ecosphere 3, 1-20.

Pangle R.E., Limousin J-M, Plaut J.A., Yepez E.A., Hudson P.J., Boutz A.L., Gehres N., Pockman W.T., McDowell N.G., 2015. Prolonged experimental drought reduces plant hydraulic conductance and transpiration and increases mortality in a piñon-juniper woodland. *Ecol. Evol.* DOI: 10.1002/ece3.1422.

Poorter, H., Fiorani, F., Stitt, M., Schurr, U., Finck, A., Gibon, Y., Pons, T. L., 2012. The art of growing plants for experimental purposes: a practical guide for the plant biologist. Funct. Plant Biol. 39, 821-838.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rigling, A., Bigler, C., Eilmann, B., Mayer, P., Ginzler, C., Vacchiano, G., Weber, P., Wohlgemuth, T., Zweifel, R., Dobbertin, M., 2013. Driving factors of a vegetation shift from Scots pine to pubescent oak in dry Alpine forests. Global Change Biol. 19, 229-240. DOI:10.1111/gcb.12038

Roderick, M.L., Greve, P., Farquhar, G.D., 2015. On the assessment of aridity with changes in atmospheric CO2. Water Resour. Res. 51, 5450-5463.

Sala, A., J. D. Tenhunen., 1996. Simulations of canopy net photosynthesis and transpiration in Quercus ilex L. under the influence of seasonal drought. Agricul. For. Met. 78, 203-222.

Saliendra, N. Z., Sperry, J. S., Comstock, J. P., 1995. Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in Betula occidentalis. Planta 196, 357-366.

Salomón, R.L., Limousin, J.M., Ourcival, J.M., Rodríguez- Calcerrada, J. Steppe, K., 2017. Stem hydraulic capacitance decreases with drought stress: implications for modelling tree hydraulics in the Mediterranean oak *Quercus ilex*. Plant, Cell & Envir., DOI: 10.1111/pce.12928.

Schlesinger, W. H., Jasechko, S., 2014. Transpiration in the global water cycle. Agri. For. Met. 189, 115-117.

Sperry, J.S., Tyree, M.T., 1988. Mechanism of water stress-induced xylem embolism. Plant Physiol. 88, 581–587.

Sperry, J.S., Hacke, U.G., Oren, R., Comstock, J.P., 2002. Water deficits and hydraulic limits to leaf water supply. Plant, Cell and Environ. 25, 251-263

Sterck, F.J., Martinez-Vilalta, J., Mencuccini, M., Cochard, H., Gerrits, P., Zweifel, R., Herrero, A., Korhonen, J.F.J., Llorens, P., Nikinmaa, E., Nole, A., Poyatos, R., Ripullone, F., Sass-Klaassen, U., 2012. Understanding trait interactions and their impacts on growth in Scots pine branches across Europe. Funct. Ecol. 26, 541-549.

Tardieu, F. Simonneau, T., 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. Jour. Exp. Bot. 419-432.

Togashi, H.F., Prentice, I.C., Evans, B.J., Forrester, D.I., Drake, P., Feikema, P., Brooksbank, K., Eamus, D. Taylor, D., 2015. Morphological and moisture availability controls of the leaf area- to-sapwood area ratio: analysis of measurements on Australian trees. Ecol. Evol. 5, 1263-1270.

Timofeeva, G., Treydte, K., Bugmann, H., Rigling, A., Schaub, M., Siegwolf, R., Saurer, M., 2017. Long-term effects of drought on tree-ring growth and carbon isotope variability in Scots pine in a dry environment. Tree Physiol., 1-14.

Valladares, F., Wright, S. J., Lasso, E., Kitajima, K., Pearcy, R. W., 2000. Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. Ecology 81, 1925-1936.

Valladares, F., Pearcy, R.W., 2002. Drought can be more critical in the shade than in the sun: a field study of carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year. Plant, Cell and Environ. 25, 749–759.

Verhoef, A., Egea, G., 2014. Modeling plant transpiration under limited soil water: Comparison of different plant and soil hydraulic parameterizations and preliminary implications for their use in land surface models. Agri. For. Met. 191, 22-32.

Waring, R. H., 1987. Characteristics of trees predisposed to die. Bioscience 37, 569-574.

Williams, A.P., Allen, C.D., Macalady, A.K., Griffin, D., Woodhouse, C.A., Meko, D.M., Swetnam, T.W., Rauscher, S.A., Seager, R., Grissino-Mayer, H.D., Dean, J.S., McDowell N.G., 2013. Temperature as a potent driver of regional forest drought stress and tree mortality. Nature Climate Change 3, 292–297

Zhao, M., Running S.W., 2010. Drought-induced reduction in global terrestrial net primary production from 2000 through 2009. Science 329, 940–943

Zweifel, R., Rigling, A. Dobbertin, M., 2009. Species‐specific stomatal response of trees to drought‐a link to vegetation dynamics? Jour. Veg. Sci. 20, 442-454.

Table 1: Characteristics of the study sites

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

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.

Garcia-Forner et al., 2016		Eilmann et al., 2010
Grossiord et al., 2017		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.

Figure legends

Figure 1: Hypothetical relationships between sap flux and daily soil moisture or vapor pressure deficit (*VPD*) variation under different long-term soil moisture conditions (high, medium and low soil moisture). The red arrow indicates changes in sap flux sensitivity to soil moisture and *VPD* resulting from physiological and possible structural adjustments to soil moisture change (e.g. hydraulic resistance, stomatal density, synthesis of chemicals inducing stomatal closure, rooting depth). Because of adjustments to reduced soil moisture, trees would experience a decreased sensitivity to daily soil moisture and *VPD* variation inducing lower sap flux under both high and low soil moisture status, and

lower maximum sap flux under optimal *VPD*. Sensitivity to soil moisture would thus be reflected through changes in the slope of the linear relationship between sap flux and soil moisture while changes in *VPD* sensitivity would be reflected in shifts of maximum sap flux at optimal *VPD* (location of the vertex of the curve).

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

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

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

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 moisture addition and soil moisture reduction conditions in each site (PFY = Mountainous coniferous forest, PUE = Mediterranean evergreen forest, SEV and SUM = semi-arid forest types). Sites are ordered along the x-axis by soil moisture manipulation intensity going from the highest irrigation to the highest soil moisture reduction site.
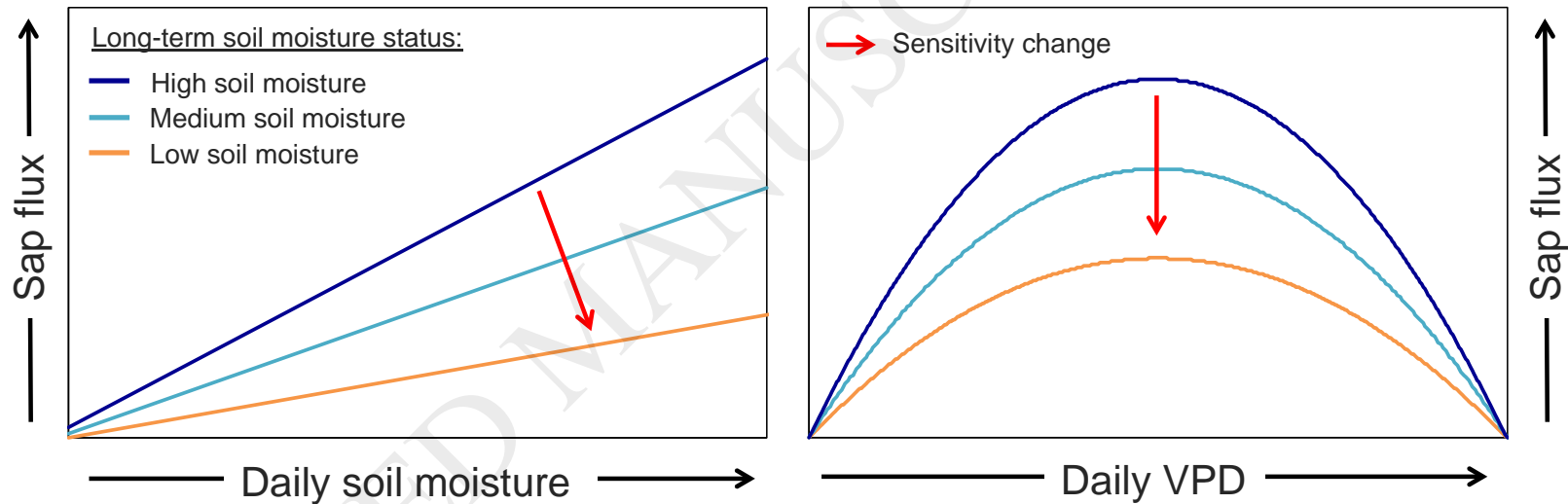
Figure 6: Relative change in the sensitivity of F_D to simulated *REW* between ambient and treatment conditions (S_{REW} in the manipulative treatment / $S_{REW\_Ambient}$), and relative change in the maximum sap

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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)
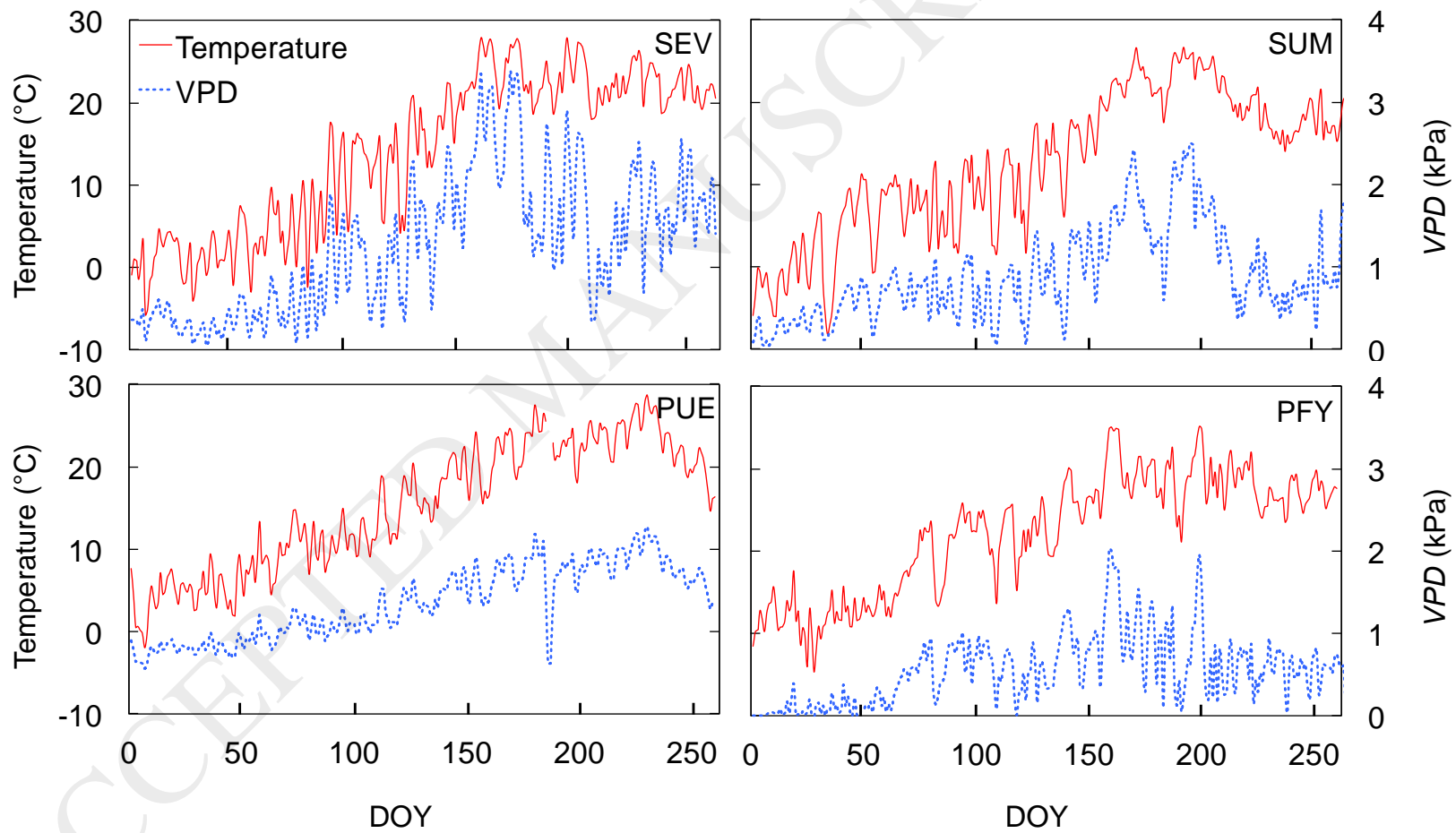
and an individual tree under treatment conditions (at the same site).
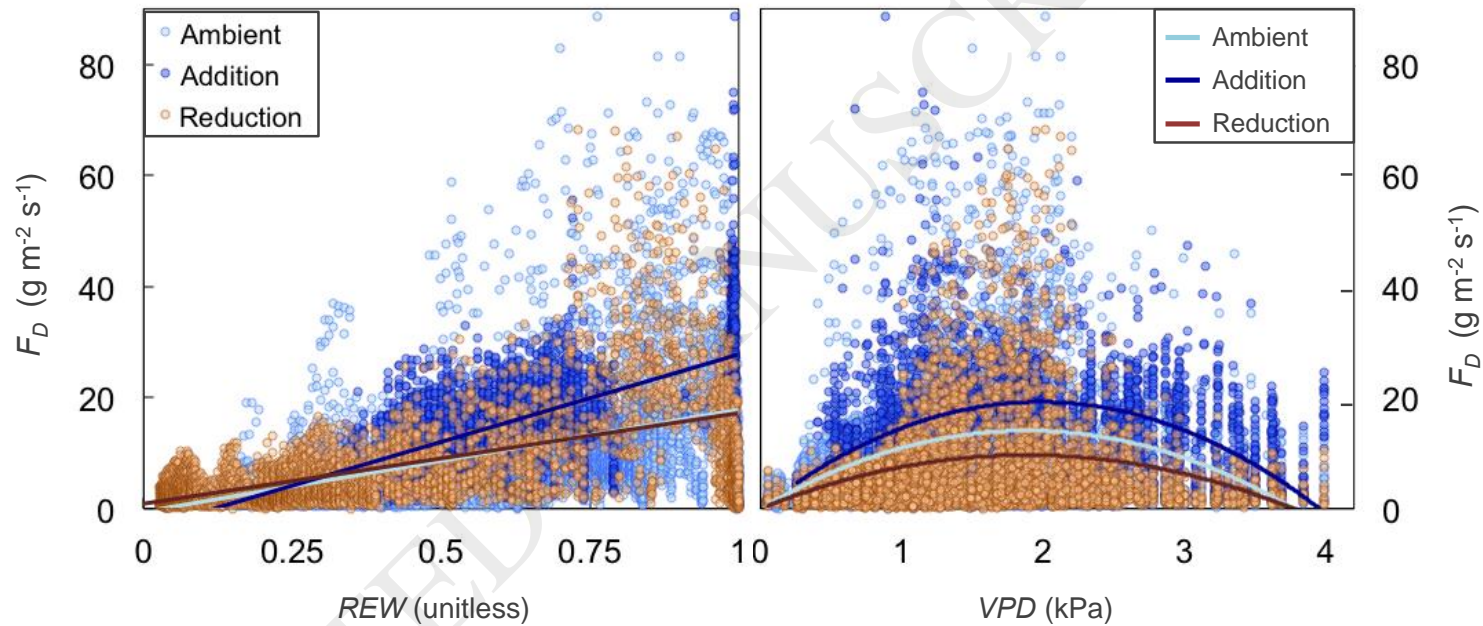
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Figures

Figure 1: Hypothetical relationships between sap flux and daily soil moisture or vapor pressure deficit (*VPD*) variation under different long-term soil moisture conditions (high, medium and low soil moisture). The red arrow indicates changes in sap flux sensitivity to soil moisture and *VPD* resulting from physiological and possible structural adjustments to soil moisture change (e.g. hydraulic resistance, stomatal density, synthesis of chemicals inducing stomatal closure, rooting depth). Because of adjustments to reduced soil moisture, trees would experience a decreased sensitivity to daily soil moisture and *VPD* variation inducing lower sap flux under both high and low soil moisture status, and lower maximum sap flux under optimal *VPD*. Sensitivity to soil moisture would thus be reflected through changes in the slope of the linear relationship between sap flux and soil moisture while changes in *VPD* sensitivity would be reflected in shifts of maximum sap flux at optimal *VPD* (location of the vertex of the curve).
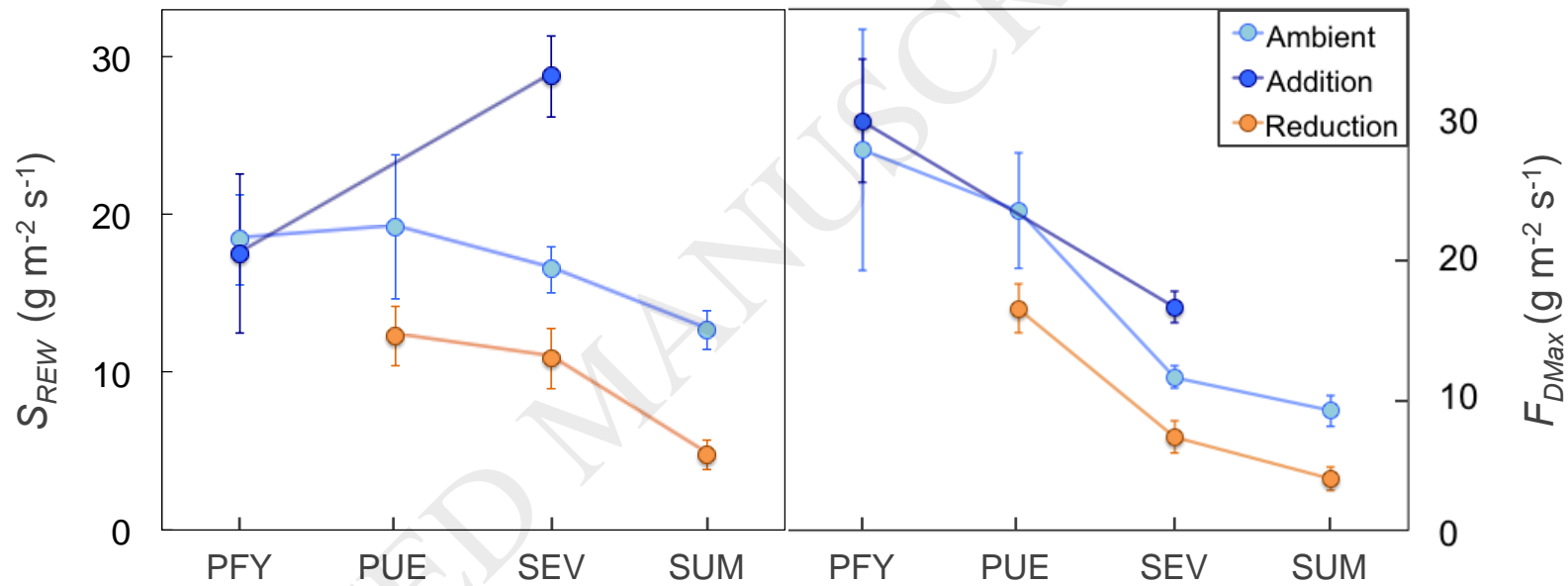
Figure 2: Daily variations in atmospheric temperature (°C) and vapor pressure deficit (*VPD*, kPa) at each site for the selected years.
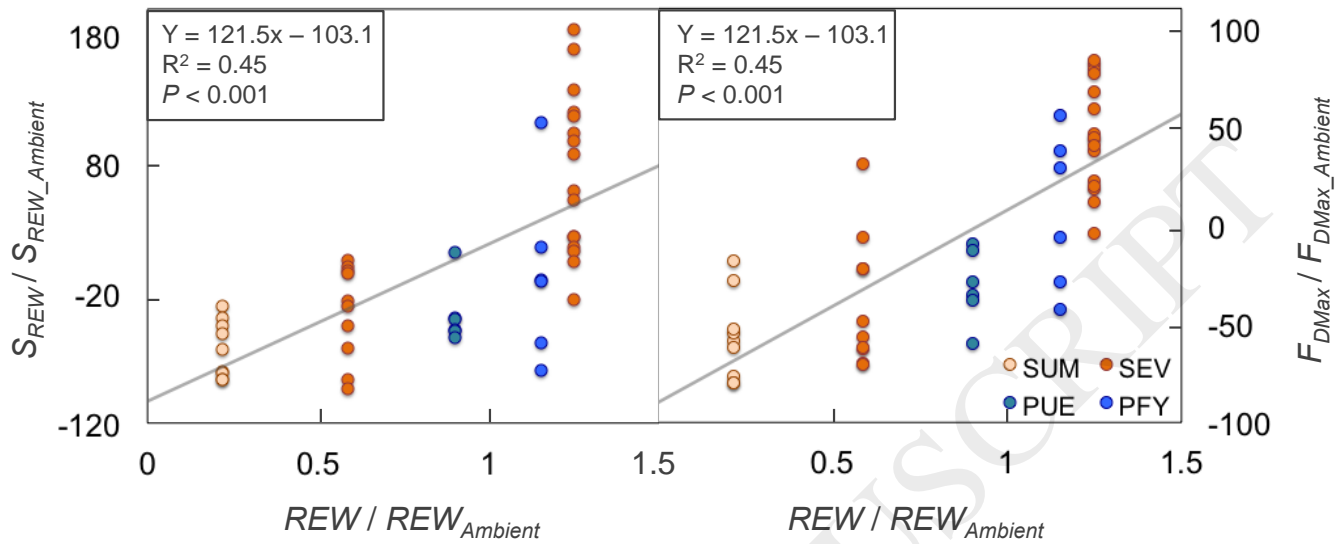
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Figure 3: Daily relative extractable water (*REW*, unitless) simulated with the BILJOU water balance model for each treatment at each site for the selected years (Table 1). The grey areas correspond to periods where irrigation was ongoing at the SEV and PFY sites. Reduction in incoming precipitation was ongoing all year long in all sites.

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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

moisture addition and soil moisture reduction conditions in each site (PFY = Mountainous coniferous forest, PUE = Mediterranean

evergreen forest, SEV and SUM = semi-arid forest types). Sites are ordered along the x-axis by soil moisture manipulation intensity

going from the highest irrigation to the highest soil moisture reduction site.

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).

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