


# Complementary water uptake depth of *Quercus petraea* and *Pinus sylvestris* in mixed stands during an extreme drought

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## Abstract

**Aims** The growing demand from forest managers is to identify silvicultural practices to overcome projected water scarcity during the next decades. One solution is to mix tree species in the same stand, thereby increasing resource partitioning and minimizing competition for limited soil water. This study investigates the mixture approach for *Quercus petraea* (Matt.) Liebl. and *Pinus sylvestris* L. during an extreme summer drought event. **Methods** During the summer drought event in 2016, we analyzed the isotopic signatures of large- and small-tree xylem and soil water throughout the soil profile to assess the depth of water uptake for both tree species. We also measured predawn leaf water potentials (PLWP) to assess water availability for individual tree species. **Results** When grown in pure stands, both species primarily utilized soil water near the surface. In contrast, partial niche complementarity for limited water resources

between the two species in mixed stands resulted in less water constraint (i.e., less negative PLWP) for oak trees compared to pure stands, especially for small trees. **Conclusions** Results from this study show that contrasting water use strategies can change water availability for trees and could help some species, though not all, to cope with the water scarcity predicted in a changing climate.

**Keywords** Monoculture · Mixture · Stable isotopes · Resource partitioning · Predawn leaf water potential · Complementarity

## Abbreviations

PLWP	Predawn leaf water potential
VWC	Volumetric Water Content
SWC	Soil water content
$\delta^{18}\text{O}$	Isotopic signature of $^{18}\text{O}$ xygene
$\Psi_{\text{PLWP}}$	Predawn leaf water potential

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## Introduction

Mean annual temperatures as well as the frequency and intensity of summer drought events are predicted to increase in the coming decades in temperate regions (IPCC 2014). Trees growing in temperate forests are dependent on summer precipitation. Changes in the timing and amount of summer precipitation could therefore have severe effects on tree growth and increase mortality (Allen et al. 2010; Bigler et al. 2006). Several studies have examined how trees are able to cope with

changing climatic conditions when different silvicultural practices are applied, including changing composition through migration (Higgins et al. 2003) or assisting genetic migration by human selection (Aitken and Whitlock 2013). Other studies have investigated changes in stand density (Giuggiola et al. 2013; Trouvé et al. 2017) and mixing different tree species (del Río et al. 2016; Forrester and Bauhus 2016).

Mixing tree species in a forest stand may be favorable according to the idea of niche complementarity, which can occur as the result of resource partitioning (Loreau and Hector 2001) and result in increased tree growth in mixed forest stands compared to pure monoculture stands. However, there are contrasting results in the literature with regard to the benefits of growing trees in a mixture; some studies show greater tree growth in mixed stands (Liang et al. 2016), whereas other studies have revealed greater tree growth in monoculture stands (Richards et al. 2010). These contrasting results are likely coupled to variations in local abiotic conditions (Condés et al. 2013; Grossiord et al. 2014b; Lu et al. 2016; Toïgo et al. 2015), including the amount and duration of stress undergone by the stands. It is also important to point out that these previous studies focused primarily on tree growth (Condés et al. 2013; Forrester and Tang 2016) and, as a result, a mechanistic understanding of the observed differences is still lacking. However, this understanding is crucial for identifying appropriate silvicultural practices to maintain tree growth in the light of climate change.

The concept of niche complementarity may be extremely important for the co-existence of plant species in water limited ecosystems in which different plant species utilize different water sources either spatial or temporally. For instance, certain species may preferentially utilize soil water near the surface whereas other species may have the ability to access soil water at deeper depths, thereby minimizing competition for available soil water (Brinkmann et al. 2018; Grossiord et al. 2017; Loreau and Hector 2001; Martin-Gomez et al. 2017; Wu et al. 2016). Based on measurements of root distribution throughout the soil profile, there is some evidence for niche complementarity (Moreno et al. 2005; Rowe et al. 2006), though some studies have shown little evidence for niche complementarity (Hendriks and Bianchi 1995; Mommer et al. 2010; Schmid and Kazda 2002). However, the mere physical presence of roots does not necessarily parallel the area of active water uptake; considerable uncertainty therefore

exists when identifying the water sources used by plants based solely on root distribution within the soil profile.

Over the past 20 years, advances in stable isotope techniques have enabled researchers to more accurately determine water sources for individual plants (Brunel et al. 1995; Ehleringer and Dawson 1992; Hasselquist et al. 2010; White et al. 1985). It is generally assumed that there is no fractionation of water isotopes during water uptake by plant roots (Dawson and Ehleringer 1991; White et al. 1985); therefore, by comparing the isotopic signature of xylem water to the isotopic signature of different water sources throughout the soil profile, it is possible to determine the water uptake depth. In addition to stable isotope measurements to determine water sources for individual plants, measurements of pre-dawn leaf water potential are also useful to assess the plant's hydric state (Scholander et al. 1965) and to determine the hydric potential at equilibrium for the entire active rooting zone.

Under drought conditions, it is commonly thought that *Quercus petraea* (Matt) Liebl. trees utilize deeper water sources (Bréda et al. 1995; Epron and Dreyer 1990) than do *Pinus sylvestris* L. trees, which are more dependent on water sources near the soil surface (Michelot et al. 2012). It can therefore be assumed that growing a mixture of *Q. petraea* and *P. sylvestris* would reduce tree competition for water resources during drought events thanks to niche complementarity. In addition to interspecific competition, individuals of the same species could also be competing for soil water (i.e., intraspecific competition), and this competition could vary as trees mature. Some studies have shown that the growth of larger trees is more severely affected by drought (Castagneri et al. 2012; Zang et al. 2012), whereas other studies have shown a greater effect on smaller trees (Piutti and Cescatti 1997; Zang et al. 2012). Yet, the mechanism responsible for these contrasting results is not well understood, although it may be related to intraspecific competition for limited water sources.

The goal of this study was to assess sources of water uptake for *Q. petraea* and *P. sylvestris* trees when grown in either pure or mixed forest stands during an especially pronounced drought in the summer of 2016. Specifically, we addressed the following questions:

- (i) Do *Q. petraea* and *P. sylvestris* trees change where they access their water when grown in mixtures compared to pure stands?

- (ii) Do *Q. petraea* and *P. sylvestris* trees utilize different water sources when grown in mixed stands (i.e., interspecific niche complementarity)?
- (iii) Do small and large *Q. petraea* and *P. sylvestris* trees access different water sources when grown in pure stands (i.e., intraspecific niche complementarity)?

By addressing these questions, we aimed to provide insight into both intra- and interspecific relationship between *Q. petraea* and *P. sylvestris* trees in both pure and mixed stands in order to help identify effective silvicultural practices to mitigate projected water scarcity in a changing climate.

## Materials and methods

### Study site

This experiment was conducted at the OPTMix (Oak/Pine Tree Mixture, <https://optmix.irstea.fr/>) long-term experimental site located in the 35,000-ha Orléans state forest in central France (47°49'N2°29'E) (Korboulewsky et al. 2015). The OPTMix site is characterized by even-aged (60–80-year old) forest stands of pure *Pinus sylvestris*, pure *Quercus petraea* and mixed *P. sylvestris* / *Q. petraea* stands ( $N = 3$  per stand type) grown in two different stand densities (low and medium). The stands are either fenced or unfenced, and there is a total of 33 plots (0.5 ha each). For the current study, we selected plots which were as similar as possible to one another in terms of stand composition, stand age, number of trees per hectare and soil texture and profile (Fig. 1).

For this experiment, we choose the 0.5 ha unfenced plots with a medium stem density (Relative Density Index,  $RDI \approx 0.7$ , Appendix Table 2) (Reineke 1933). We selected the medium density treatment in order to maximize the probability of observing interaction effects between individual trees (Forrester et al. 2013). In the pure *P. sylvestris* and *Q. petraea* stands, the mean tree diameter was respectively 33.6 and 22.5 cm. In the mixed stands, mean tree diameter was 36.0 cm for *P. sylvestris* and 23.6 cm for *Q. petraea* (Appendix Table 2) (Korboulewsky et al. 2015). We had three replications in each of the three forest stands for a total of nine plots.

The experimental site is characterized by a temperate continental climate with an oceanic influence, a mean annual temperature of 10.8 °C and a mean annual rainfall of 729 mm (based on data from the SAFRAN and

ISBA analytical platforms between 1981 and 2010, Météo-France (Durand et al. 1993)). The soil is classified as a primary planosol (IUSS Working Group WRB 2015) with a pH of 4.5, less than 1% carbon and a carbon to nitrogen (C/N) ratio below 20. The upper soil horizon is loamy sand that overlies a more or less impermeable clay horizon at ca. 50 cm in depth (Fig. 1; Appendix Table 1), which leads to temporarily waterlogged conditions in winter and spring. Though some roots were found in this clayey layer, 80% of the roots were in the upper layer.

### Microclimate and edaphic measurements

We measured air temperature (CS215 Campbell Scientific®, Inc) and relative humidity (CS215 Campbell Scientific®, Inc) under the canopy in each plot at two locations at a height of 1.65 m. Temperature and relative humidity were measured every 3 mins and recorded as hourly averages (2 repetitions/plot making 6 repetitions per forest stand). Precipitation (ARG100 Campbell Scientific®, Inc.; mm) was measured in an open clearing at a height of 1.5 m. We also measured soil volumetric water content (VWC) with a probe (CS616 Campbell Scientific®, Inc) in each forest stand at depths of 20, 40 and 60 cm. The probe was comprised of two 30-cm-long stainless-steel rods connected to the measurement electronics, and used the time-domain method to measure VWC: the probe generates an electromagnetic pulse, then the elapsed travel time and pulse reflection are measured and used to calculate soil VWC.

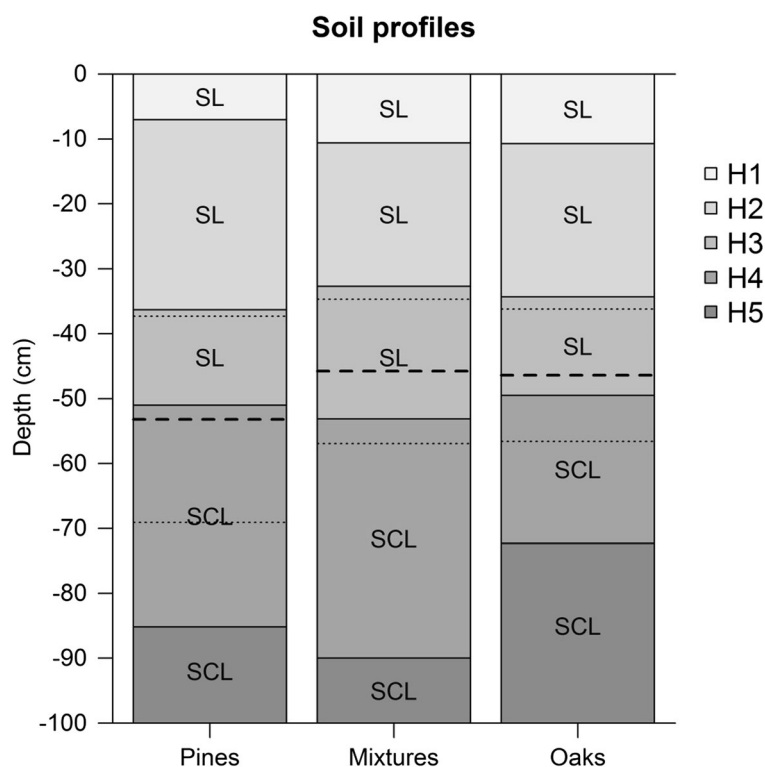
In each plot, VWC measurements were taken every half hour at three locations and recorded as 12 h averages ( $N = 9$  per forest stand). All micro-meteorological and volumetric water content data were recorded on CR1000/CR800 data loggers (Campbell Scientific®, Inc).

In order to compare values among forest stands, the data on volumetric water content was expressed as a percentage of the maximum volumetric water content, as follows:

$$\text{Relative VWC}_i = \frac{\text{VWC}_i}{\max(\text{VWC}_i)} \times 100, \quad (1)$$

where VWC represents the value of the volumetric water content for each  $i$  sensor, and  $\max(\text{VWC}_i)$  represents the maximum VWC for each sensor during the waterlogged period, when the VWC reached its highest plateau.

**Fig. 1** Soil profile horizons in the different forest plots studied in the experiment ( $N = 9$ ). Soil texture is shown for the whole soil profile (SL = Sandy Loam; SCL = Sandy Clay Loam); the appearance of the distinct clay layer is represented by a dashed line with the standard deviation shown as dotted lines



In addition, we also collected soil core samples in each plot (2.5 cm in diameter) to determine the gravimetric soil water content (SWC) and the isotopic signature of the soil water at different depths in the soil profile. For each soil sample ( $n = 1$  per plot), a sub-sample was taken at 5, 10, 20, 30, 40, 60 and 80 cm in depth to analyze the stable isotopes in the soil water (see below). One centimeter of soil above and below the target depth was removed from the core sample, placed in sealed vials and stored in a freezer ( $-20^{\circ}\text{C}$ ) until isotopic analysis. A second sub-sample was collected 2 cm above and below each target depth in order to determine SWC, which was calculated as  $[(\text{fresh weight} - \text{dry weight}) / \text{dry weight} \times 100]$  according to the difference in sample weight before and after thorough drying in an oven ( $105^{\circ}\text{C}$ , 48 h).

#### Root distribution throughout the soil profile

For both pine and oak trees, the distribution of roots in the soil profile was determined during the original set up of the OPTMix experiment (2013–2015). Within each plot, three soil pits were dug to a depth of 100 cm and the number of roots was measured in a vertical grid of  $40 \times 100$  cm. from which roots were counted in the

10 cm squares. The pits were dug in the autumn. We classified the roots as follows: (i) pine and oak roots (first group) were separated from all the other species (second group); (ii) roots were classified according to size based on diameter: fine roots ( $< 2$  mm) and thick roots ( $> 2$  mm); and (iii) we separated living roots from dead roots. In this study, we were especially interested in the physical distribution of fine roots in the soil profile and were less concerned about root activity at a precise date; therefore, root distribution was determined by counting fine roots, both living and dead.

#### Soil and plant sampling for isotopic analyses

In each plot, we selected two large trees ( $> 71\%$  of the stand DBH distribution) and two small trees ( $< 28\%$  of the stand DBH distribution) of each species for isotopic analyses of xylem water (stand characteristics in Appendix Table 2). In the mixed stands, species abundance was assessed inside a circle 10m in diameter. Then, individual trees were selected if the other species within the 10-m circle represented between 40 and 80% of the neighboring tree species. We sampled a total of 48 trees for isotopic analysis of xylem water (2 trees  $\times$  2

sizes  $\times$  2 species  $\times$  2 compositions (pure, mixed)  $\times$  3 replicates = 48 trees sampled).

On September 7, during the driest period of the 2016 summer drought (Fig. 2), suberized twigs were collected from the canopy of the selected trees. The outer bark and phloem were removed to eliminate potentially enriched isotope water sources before isotopic analysis. The twig samples were then stored in sealed glass vials in a freezer ( $-20\text{ }^{\circ}\text{C}$ ). At the same time we collected the twigs, we also took soil samples in each plot as described above, and stored those samples in a freezer ( $-20\text{ }^{\circ}\text{C}$ ) until isotopic analysis. Samples of groundwater were also collected 12 m below the soil surface from a well that was centrally located inside the OPTMix experimental site. We carried out three sampling campaigns (August 17th 2016, September 7th 2016, September 13th 2016), but present only the second one here, since the two other did not fulfill the basic conditions necessary to accurately interpret the soil water sources. The first campaign was removed because no gradient in isotopic signature was observed with depth (Appendix Fig. 8). The third campaign gave unreliable results since, the xylem water showed a  $\delta^{18}\text{O}$  value which was more enriched than the most enriched soil water source (campaign 3) (Appendix Fig. 8). Though the laboratory confirmed that all the water had been correctly extracted, the data could not be used. Such problems have been reported in other studies (Barbour 2007). This over-enrichment may have had several causes. First, due to time and budget constraints, we took only one soil core per campaign in each plot. This may have created a discrepancy in homogeneity between the isotopic signature of the soil core and the rooting zone. Second, the environmental conditions previous to sampling the twigs were conducive to water evaporation and this could have enriched the xylem isotopic signature, as per the Péclet effect (Farquhar and Lloyd 1993). Nevertheless, the sampled twigs should have been far enough away from the leaves to avoid this, and the transpiration flux was likely limited due to the drought. Third, some authors have discussed the possibility of isotopic fractionation when water is taken up by the roots (Lin and da S. L. Sternberg 1993). Nonetheless, we were unable to prove that any of these possible explanations were involved, and therefore, we removed campaign 3 from our data set.

Water was extracted from the frozen plant and soil samples with a cryogenic distillation line ( $90\text{ }^{\circ}\text{C}$ , 50 mtorr, 3 h) at the Basel Stable Isotope Ecology

Laboratory at the University of Basel, Switzerland. After complete extraction, the water from the individual samples was transferred to a non-combusted GC glass vial through a 4mm PTFE filter. Stable isotope analyses were conducted with a high-temperature elemental analyzer coupled to a Delta<sup>Plus</sup>V mass spectrometer (Thermo Electron corporation®, Bremen, Germany). Isotope ratios are reported using the standard delta notation in per mil (‰) relative to VSMOW. Analytical precision for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  measurements were 0.3‰ and 0.2‰, respectively (Newberry et al. 2017). For more details on this method, see Werner et al. (1999).

#### Predawn leaf water potential

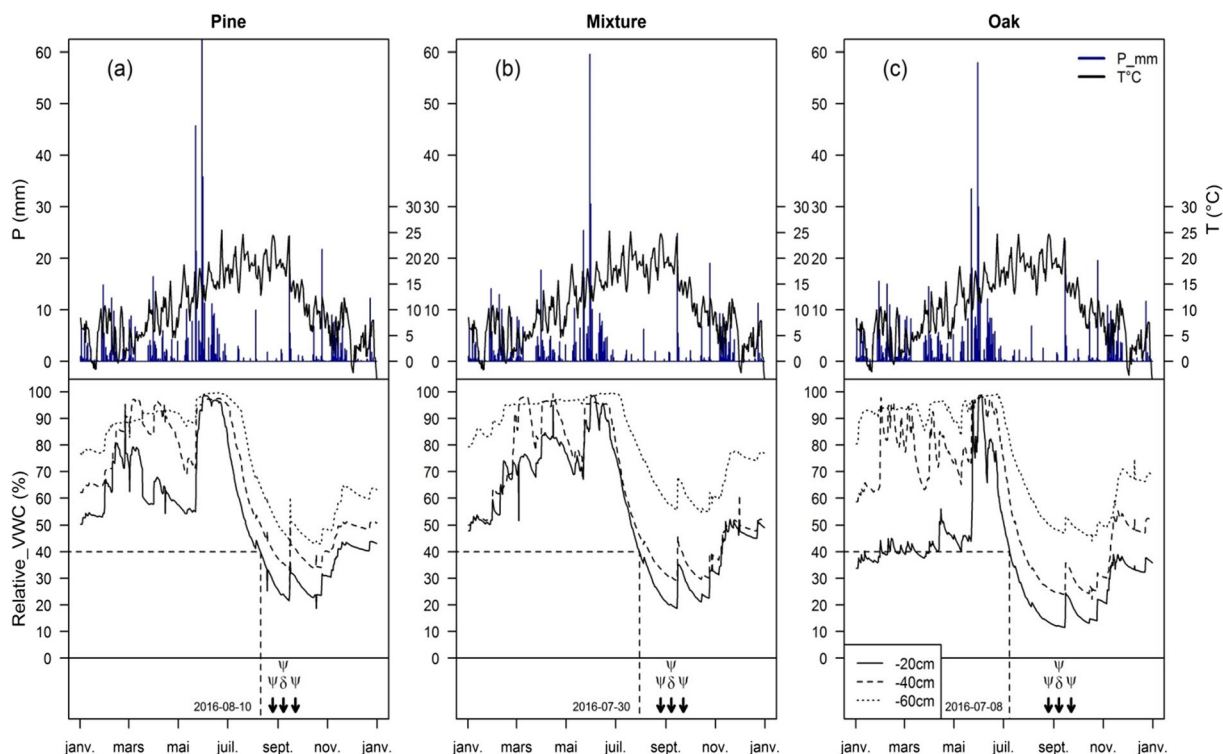
Predawn water potential was measured on the same trees from which xylem water was collected for the isotopic analyses ( $n=48$ ). We collected leafy twigs of both oak and pine from the canopy before sunrise and stored them in plastic bags in a cool box. The samples were transported to the laboratory within 3 h after collection and leaf water potential was immediately measured in a Scholander-type pressure chamber (Model 600, PMS Instrument Company, Oregon, USA) (Scholander et al. 1965). Measurements were taken on three different leafy twig samples from each individual tree to prevent a local effect. Then, the average of the two closest values was used as the predawn leaf water potential (PLWP) for the individual trees. PLWP measurements were carried out on three separate dates: during the drought, on August 25 and September 7; and after a rain event on September 25.

#### Statistical analyses

We used the IsoSource mixing model (freely available at <https://www.epa.gov/eco-research/stable-isotope-mixing-models-estimating-source-proportions>; Phillips and Gregg 2003) to determine the relative contribution of the different soil water sources (i.e., different depths) for both *P. sylvestris* and *Q. petraea*. This model is based solely on isotopic mass balance constraints and gives the distribution of feasible source proportions when there are a high number of potential water sources. We defined different water sources based on distinct  $\delta^{18}\text{O}$  signatures of the soil water found at different levels in the soil profile; we aggregated adjacent soil layers with similar mean  $\delta^{18}\text{O}$  signatures into a single source (Phillips et al. 2005). Thus, we



## Microclimate and soil moisture



**Fig. 2** Mean daily precipitation (histograms) and temperature (black line) (top panels) and mean Relative Soil Volumetric Water Content (VWC, bottom panels) at 20 cm (solid line), 40 cm (dashed line) and 60 cm (dotted line) in depth for pure pine (a), mixture (b) and pure oak (c) stands. Arrows in the bottom panels represent the

date of sampling for predawn leaf water potential ( $\Psi$ ) and isotope ( $\delta$ ) measurements, and the dashed straight lines point out the date when VWC at 20-cm-depth fell to 40%. Dates for VWC at 20 cm fell below 40% are given

obtained five distinct soil water sources (5, 10, 20, 30–45 and > 60 cm, which included groundwater). We used a source increment of 1% as suggested by Phillips and Gregg (2003). To account for the variability in our samples, we selected a mass balance tolerance of 0.25%, as suggested by Phillips and Gregg (2003):

$$t = 0.5 \times \text{increment} \\ \times \text{maximum difference between sources}$$

With sensitivity analyses, changes in source increment and mass balance tolerance do not change the mean contribution from each source and only moderately influence the range of contribution from individual sources (Phillips and Gregg 2003). All the other statistical analyses were performed with the R version 3.4.4 software (R\_Core\_Team 2018). We assessed the differences in SWC among the three different forest stands at each

depth with linear mixed models in the “nlme” package (Pinheiro et al. 2018). Plot was a random effect because three plots in one forest stand contained the same stand composition. Linear mixed models were also used to detect significant differences in PLWP among the three sampling dates, with plot as a random effect because two individuals were sampled from the same plot in each of the forest stands and were therefore not completely independent. We used a “varIdent” function to take into account the variability between the two tree sizes in the model. A significant difference was defined as  $\alpha = 0.05$ .

## Results

### Microclimate and soil water content

There was little variation in microclimatic conditions among the three forest stands (Fig. 2, Appendix Fig. 9).

Precipitation was intense during late May, where rainfall was greater than 50 mm a day and mean month precipitation was  $183.44 \pm 12.91$  mm (Fig. 2). After a rainy May, there was a considerable decrease in precipitation, with mean monthly precipitation falling to  $65.87 \pm 7.55$  mm in June,  $6.81 \pm 0.97$  mm in July, then recovering only slightly ( $10.7 \pm 0.65$  mm) in August. At the same time, there was a corresponding increase in mean daily temperature; a maximum of ca. 25 °C was reached at the end of July, then temperatures remained more or less steady until the middle of September (Fig. 2).

As a result of the heavy rains in May, relative soil volumetric water content for the three depths (20, 40 and 60 cm) was close to saturation (i.e., 100% VWC) during this period (Fig. 2, Appendix Figs. 11, 12, and 13). Then, starting in June, soil VWC continuously decreased to reach a minimum on September 14th, where relative VWC was only  $21.6 \pm 4.8\%$  for pure pine stands,  $18.7 \pm 1.2\%$  for mixed stands and  $11.5 \pm 0.7\%$  for pure oak stands (Fig. 2). It is worth noting that the decrease in relative VWC during July–August occurred more quickly in pure oak stands compared to the other two stand types. Indeed, relative VWC at 20 cm in depth fell below 40% in pure oak stands on July 8th, whereas in the mixed and pure pine stands this did not occur until July 30th and August 8th, respectively. On September 15, there was a three-day rain event leading to an increase in soil VWC at all three depths (Fig. 2).

Soil water content (SWC), as determined from collected soil samples, was not significantly ( $p > 0.05$ ) different between pure oak and the mixed stands, ranging between 3.0% in the upper soil layers (5–40 cm) to 9.4% at deeper soil depths in the pure oak stands, and between 3.2 and 9.4% in mixed stands. In contrast, soil SWC in pure pine stands was considerably higher than the other two stand types, especially at 10, 20 and 30 cm in depth ( $p = 0.048$ ,  $p = 0.017$ ,  $p = 0.047$ , respectively) (Fig. 3).

### Root distribution

In the pure stands of both tree species, the proportion of fine roots was greatest in the organic humus layer and then gradually decreased with soil depth. Very few fine roots were found at depths  $>90$  cm (Fig. 4a). The distribution of fine roots in the mixed stands did not reflect the addition of the two pure stands (Fig. 4a). Instead, the root density observed in the mixed stands was similar in magnitude to the root densities observed in each of the

two pure stand types (Fig. 4b). As in the pure stands, root density in the mixed stands was highest in the organic humus layer, then gradually decreased with soil depth (Fig. 4). Regardless of forest plot, around 70% of all fine roots were in the top 50 cm of the soil, though some were observed in the clay layer at ca. 50 cm depth in all three forest stands.

### Isotopic signature in xylem and soil water

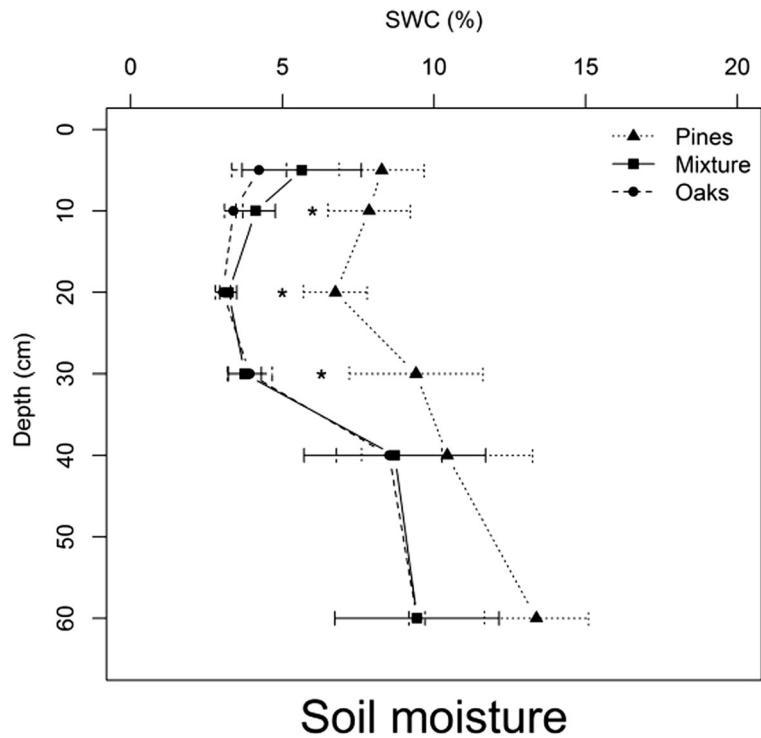
The  $\delta^{18}\text{O}$  signature of the groundwater collected at 12 m in depth from a centralized well at our study site was  $-7.81 \pm 0.70$  ‰. We used this value for all the studied stands (Fig. 5). In the pure pine stands, the mean  $\delta^{18}\text{O}$  signature of soil water ranged from  $-5.15 \pm 0.40$  ‰ near the soil surface to  $-7.38 \pm 0.12$  ‰ at 80 cm in depth (Fig. 5a). There was no difference in the  $\delta^{18}\text{O}$  signature of xylem water between small ( $-5.11 \pm 0.43$  ‰) and large ( $-4.97 \pm 0.35$  ‰) pine trees ( $p = 0.64$ ). In the pure oak stands, the range in the  $\delta^{18}\text{O}$  signature of soil water was greater than in the pure pine stands - from  $-3.09 \pm 0.43$  ‰ at 5 cm in depth to  $-8.04 \pm 0.85$  ‰ at 80 cm in depth (Fig. 5c). As in the pure pine stands, there was no significant difference in the  $\delta^{18}\text{O}$  signature of xylem water between small ( $-4.49 \pm 0.47$  ‰) and large ( $-3.70 \pm 1.04$  ‰) oak trees ( $p = 0.44$ ; Fig. 5c).

In mixed stands, we observed the greatest variation in soil water  $\delta^{18}\text{O}$  signatures in the soil profile; the mean ranged from  $-2.72 \pm 0.74$  ‰ near the soil surface to  $-7.74 \pm 0.31$  ‰ at 40 cm in depth. In general, the xylem water  $\delta^{18}\text{O}$  signature was slightly greater for pine trees ( $-4.03 \pm 0.77$  ‰) than for oak trees ( $-5.50 \pm 0.59$  ‰), although this difference was not significant ( $p = 0.12$ ). The xylem water  $\delta^{18}\text{O}$  signature was 2 ‰ greater in large pine trees than in large oak trees ( $p = 0.25$ ), whereas there was little difference between smaller pine and oak trees (Fig. 5b). Overall, there were no significant differences in xylem water  $\delta^{18}\text{O}$  signatures between small and large trees, regardless of species (Fig. 5b).

### Water partitioning

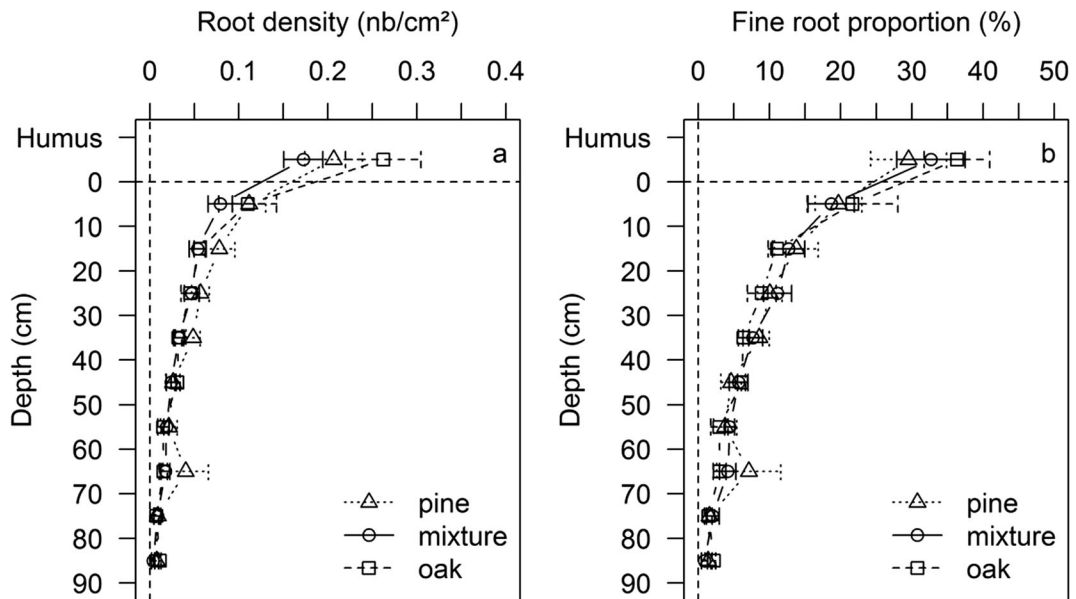
When grown in pure stands, both large and small pine trees primarily utilize soil water near the surface, with the relative contribution ranging between 56 and 75% (mean = 64.3%) for large trees and between 63 and 77% (mean = 68%) for small trees. The relative contribution from deeper water sources was considerably less and ranged from 0% to less than 40% (Fig. 6a), respectively.

**Fig. 3** Differences in soil water content in the soil profile on September 7, the same day samples that isotopic measurement of plant xylem was made: pure pine stands (dotted line with triangles), pure oak stands (dashed line with circles) and mixtures (solid line with squares). Symbols are on the mean and error bars show the stand error. An asterisk indicates a significant difference ( $P < 0.05$ ) between pure pine stands and the other two stand types



Similarly, in pure oak stands, the primary water source utilized by both large and small oak trees was near the soil surface, with the relative contribution of soil water

at 10 cm in depth ranging between 72 and 98% (mean = 83.7%) for large trees and between 65 and 87% (mean = 77.3%) small trees (Fig. 6c). The relative contribution



**Fig. 4** Root density (number observed/cm<sup>2</sup>; a) and proportion of total fine roots (b) found in the soil profile in the pure pine (triangles), mixed (circles) and pure oak (squares) stand types.

The symbol is on the mean and error bars show the standard error. Note that root density in the mixed stand represents total root density since we were unable to differentiate pine and oak roots



from deeper water sources was <25% (Fig. 6c) for both size classes.

In mixed stands, large pines appeared to primarily utilize two different water sources: ca. 55% from soil water near the soil surface (5 cm in depth) and 40% from soil water at 30–45 cm in depth. Similarly, small pine trees also appeared to primarily utilize the same two water sources, although small pine trees appeared to be more dependent than larger trees on deeper water sources. For small pine trees, the relative soil water contribution ranged from ca. 35% at 5 cm in depth to ca. 50% at 30–45 cm in depth (Fig. 6b). Oak trees grown in mixed stands utilized deeper primary water sources compared to when they were grown in pure stands (Fig. 6c and d). In mixtures, large oak trees primarily utilized soil water at 30–45 cm in depth, with a relative contribution ranging between 72 and 86% (mean = 79.6%). The relative contribution from shallower water sources was less than 10% (Fig. 6d). Small oak trees also mostly utilized soil water at 30–45 cm in depth when grown in mixed stands, with a relative contribution ranging between 66 and 72% (mean = 68.9%), even though small oak trees also continued to utilize some (ca. 20%) soil water near the surface (Fig. 6d).

#### Predawn leaf water potential (PLWP)

The first two dates PLWP was measured corresponded to strong drought conditions, whereas the last PLWP sampling date, September 22, occurred shortly after a rain event (Fig. 1). In general, there was no significant ( $p = 0.94$ ) difference in PLWP between large and small pine trees during the drought period:  $-0.61 \pm 0.09$  MPa and  $-0.62 \pm 0.18$  MPa, respectively (Fig. 7a, c and e); nor was there any difference ( $p = 0.88$ ) in PLWP for pine trees grown in mixed and pure stands (Fig. 7a, c and e). For oak trees, there was no significant difference ( $p = 0.44$ ) in PLWP between large and small trees, excepted during the second sampling date where the interaction with the stand composition was significant ( $p = 0.0031$ ) (i.e. when grown in pure versus mixed stands). Contrary to pine trees, there was a significant difference ( $p = 0.039$ ) in PLWP for oak trees growing in mixed stands and for those growing in pure stands (Fig. 7b, d and f).

On the first sampling date, pine trees had significantly ( $p = 1.10^{-4}$ ) less negative PLWP than did oak trees (Fig. 7a and b); yet, regardless of species, there was no difference ( $p = 0.69$ ) between large and small trees. Moreover, there was no difference ( $p = 0.45$ ) in PLWP

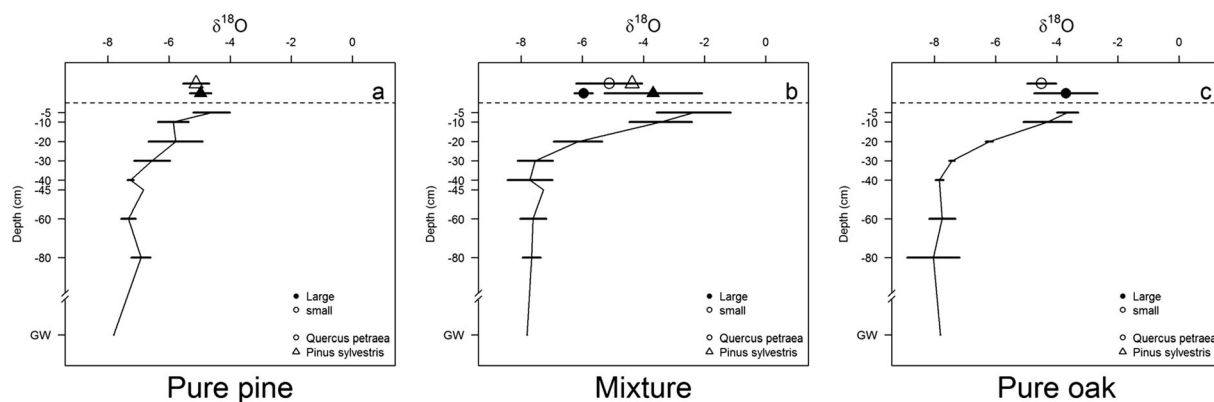
for pine trees grown in mixed and pure stands ( $-0.66 \pm 0.07$  MPa and  $-0.56 \pm 0.07$  MPa, respectively) (Fig. 7a). For oak trees, PLWP was considerably less negative for trees in pure stands compared to trees in mixed stands ( $-1.14 \pm 0.15$  MPa and  $-1.91 \pm 0.17$  MPa, respectively) (Fig. 7a and b), although the difference was only marginally significant ( $p = 0.075$ ).

On the second sampling date, the PLWP for oak trees grown in pure stands was ca. twice as low as for oak trees grown in mixtures ( $-1.41 \pm 0.24$  MPa and  $-0.63 \pm 0.06$  MPa for pure and mixed stands, respectively), although this difference was not significant ( $p = 0.49$ ). There was, however, a significant ( $p = 0.003$ ) interaction between tree size and stand composition (Fig. 7c and d). When grown in pure stands, small oak trees had a significantly ( $p = 0.015$ ) lower PLWP than did their larger counterparts ( $-1.91$  MPa  $\pm 0.38$  and  $-0.92$  MPa  $\pm 0.09$ , respectively; Fig. 7d). In contrast, there was no difference ( $p = 0.80$ ) between large and small oak trees growing in mixed stands (Fig. 7d).

On the last sampling date, shortly after a rain event, PLWP was globally and significantly ( $p = 2.1 \times 10^{-8}$ ) higher compared to the other two sampling dates (Fig. 7). At this date, there was no significant difference in PLWP between species ( $p = 0.75$ ), size classes ( $p = 0.78$ ) or stand composition types ( $p = 0.85$ ) (Fig. 7e and f).

#### Discussion

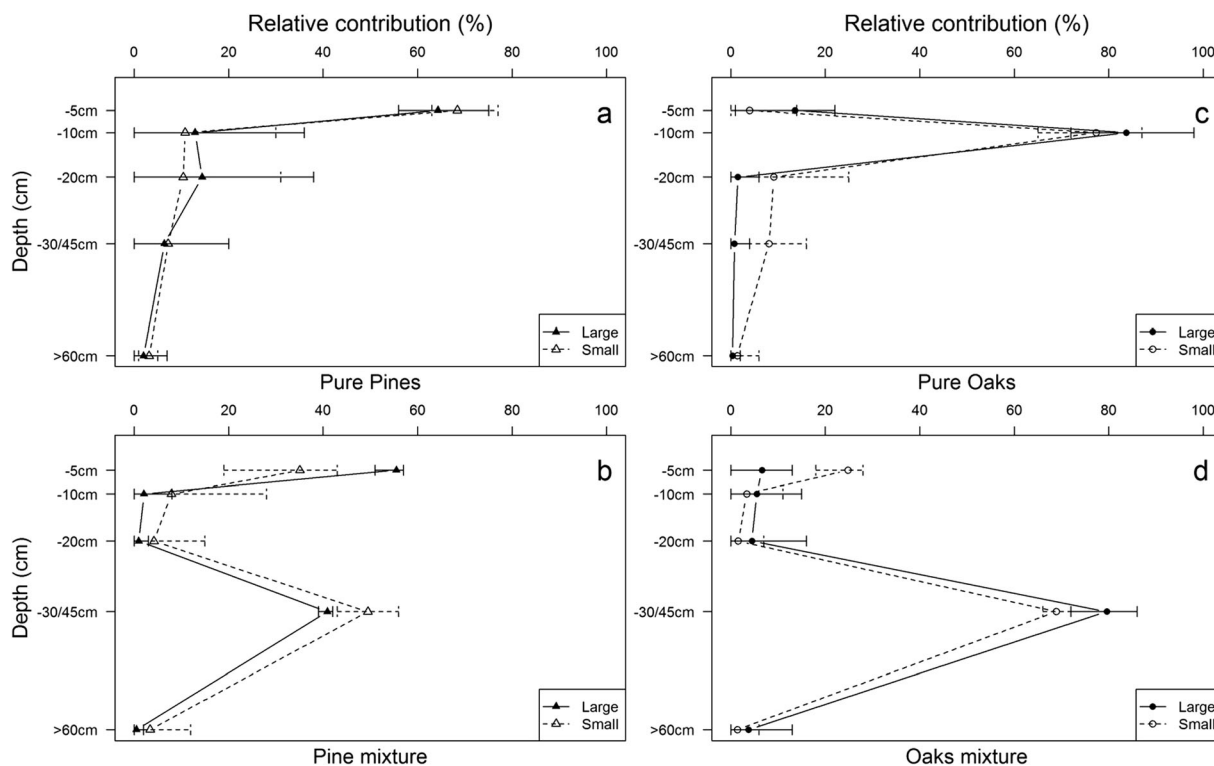
The major finding of this study is that when *Q. petraea* and *P. sylvestris* trees grow in mixed stands, both species appear to utilize deeper water sources compared to when they grow in pure stands (Fig. 6). This downward shift in water source utilization was especially pronounced for oak trees. Despite similar amounts of precipitation and similar soil moisture content in the soil profile in both the pure oak and oak/pine mixed stands (Fig. 3), soil water was depleted faster in pure oak stands than in the mixtures (Fig. 2). In addition, the oak trees in the pure stands relied more heavily on soil moisture near the surface (Fig. 6) and showed signs of tricky water uptake - as indicated by more negative PLWP values (Grossiord et al. 2017; Scholander et al. 1965) in pure than in mixed stands. This was especially true for small oak trees (Fig. 7d). These differences in pure and mixed oak stands result from both intra- and interspecific competition, which is due to different water use strategies between



**Fig. 5** Mean ( $\pm$ SE, ‰)  $\delta^{18}\text{O}$  signatures of xylem water and soil water found from the soil profile in pure pine (a), pure oak (b) and mixed stands (c). Circles represent oak trees and triangles represent pine trees. Full and empty symbols represent large and small trees, respectively

the two species. It is well known that pine trees implement a water conservative strategy (i.e., isohydric species) when exposed to drought conditions (Irvine et al. 1998; Sturm et al. 1998), which results in reduced transpiration and a subsequent reduction in plant water

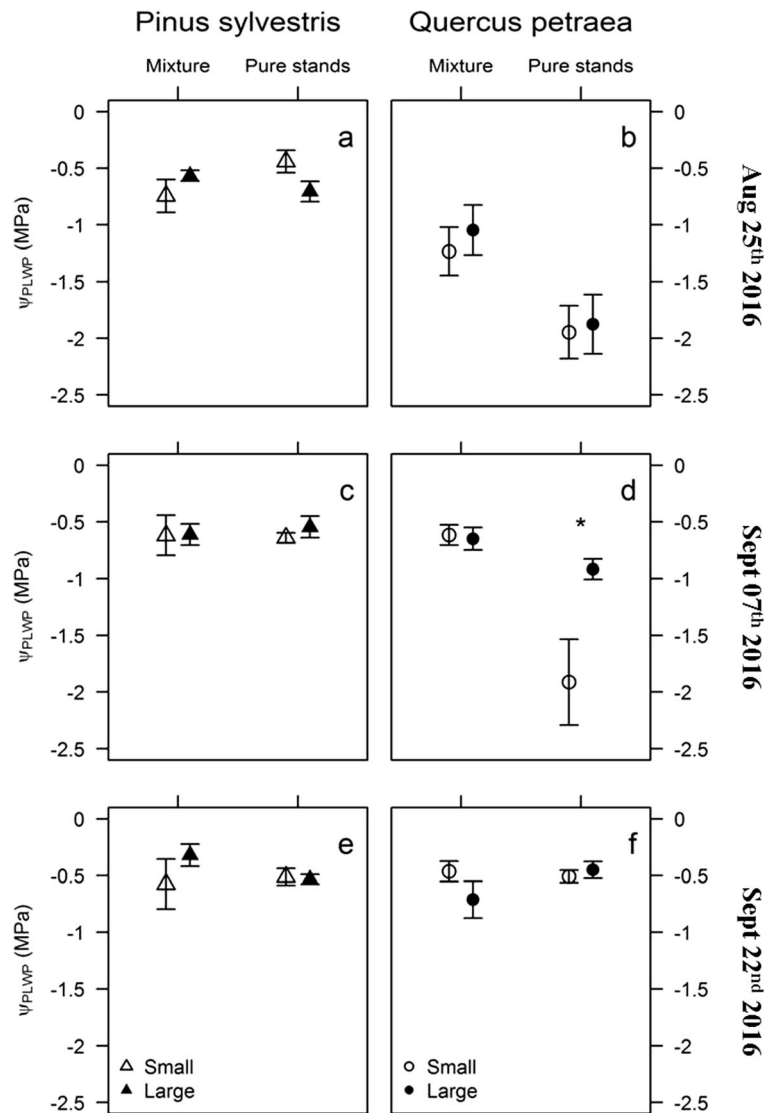
uptake (Buckley 2005). In contrast, oak trees do not display strong stomatal regulation during drought events (i.e., anisohydric species) (Renninger et al. 2015; Zweifel et al. 2007); they continue to consume soil water long after isohydric species have reduced their



**Fig. 6** Relative contribution of different water sources used by small trees (open symbols and dashed lines) and large trees (filled symbols and solid lines) in pure pine stands (a), pines in mixed

stands (b), pure oak stands (c) and oaks in mixed stands (d). Symbols are on the mean and the bars represent the 1st and the 99th distribution percentiles

**Fig. 7** Mean ( $\pm$ SE) predawn leaf water potential (PLWP) for large (filled triangles) and small (open triangles) *Pinus sylvestris* trees and large (filled circles) and small (open circles) *Quercus petraea* trees when grown in pure (left side) and mixed stands (right side). PLWP measurements were conducted on August 25, 2016 (a and b), September 7, 2016 (c and d) and September 22, 2016 (e and f). An asterisk indicates a significant difference between large and small oak trees when grown in mixed stands



water consumption (Buckley 2005; Martinez-Sancho et al. 2018). When grown in pure stands, oak trees are surrounded by co-specific anisohydric neighbors. The trees in the stand therefore have only limited stomatal control (Buckley 2005; Martinez-Sancho et al. 2018), water is depleted faster, water uptake is shallow and greater water issues occur in times of drought. In contrast, when grown in mixed stands, oak trees shift their water sources downward and primarily utilize deeper, more reliable sources; they are therefore less susceptible to water stress. These findings suggest that, for oak trees, intraspecific competition for limited water sources is stronger than interspecific competition; therefore, oak

growth may be enhanced when trees are grown in mixed stands, particularly in a future climate where drought events are predicted to be more frequent and more intense (Bello et al. 2019).

We also observed a downward shift in water source utilization in pine trees, but to a lesser extent than in oak trees. In fact, even when grown in mixed stands, pine trees - especially large trees - still derived nearly 50% of their water from shallow sources (< 10 cm depth) (Fig. 6b). This shift, though partial, is consistent with previous studies that have shown low root system plasticity in pine trees, which tend to keep most of their fine roots near the soil surface (Curt and Prevosto 2003). In any case, SWC

was not enough (Al Majou et al. 2008) to support high transpiration rates; all pine trees showed signs of reduced growth during this drought event (Bello et al. 2019). In pure pine stands, soil SWC was higher than in the two other stands (Fig. 3) and some water was still available at these values for this soil type (pure pine stands) (Al Majou et al. 2008). It seems likely that pine trees decreased their stomatal conductance in order to overcome water limitations during the 2016 drought event (Irvine et al. 1998; Sturm et al. 1998), making sufficient soil water available to support low transpiration rates; this would explain the observed PLWP values, which indicate little water stress (Pena and Grace 1986).

Our study addressed the question of whether or not there is complementarity in water source use between *Q. petraea* and *P. sylvestris*. Indeed, it is commonly thought that oak trees are capable of utilizing deeper water sources compared to pine trees (Kolb and Stone 2000), based on previous studies assessing the vertical distribution of roots in the soil profile (Hendriks and Bianchi 1995; Schmid and Kazda 2002). Nevertheless, our results do not provide evidence that there is physical complementarity in root distribution in the mixed stands since we did not distinguish pine roots from oak roots. We were able to evaluate potential competition from overall root density data, however. Root density in the soil profile in mixed stands was no different from the root density profile in the pure stands (Fig. 4b), suggesting that inter-specific competition between oak and pine trees may function in a similar way to intra-specific competition in pure stands (Curt and Prevosto 2003). This lack of physical complementarity in root distribution at our site may result from the presence of a distinct clay layer at 50 cm in depth; this layer acted as a physical barrier for root penetration (Meinen et al. 2009) and may explain why nearly 70% of all fine roots were between 5 and 50 cm in depth.

Nevertheless, the studies mentioned above showed that partial niche complementarity for water source utilization during droughts between pine and oak trees in mixtures can result from root plasticity. Both species utilize deeper water sources in mixtures than in pure stands, and while large oaks primarily utilize soil water at deeper depths, half of the water sources used by large pine trees remains in the shallow horizon. This indicates that partial niche complementarity is at play between large pine and oak trees in the case of limited water sources. The downward shift in water sources must involve associative relationships between tree roots and

certain mycorrhizas. Indeed, ectomycorrhizas are important in plant water uptake, helping to sustain photosynthesis and whole plant performance, especially during drought (Khalvati et al. 2005; Lehto and Zwiazek 2011). Differences in underground plant communities in mixed and pure stands could favor these relationships in mixed stands. Nevertheless, the role mycorrhizas play is poorly known, especially during long-term or repeated droughts (Lehto and Zwiazek 2011) and in the deeper soil horizons. Moreover, allelopathy (mutual interactions between organisms through the excretion of matter into the environment) is known to play an important role in forest ecosystems, and could affect root distribution in mixed stands. Although, allelopathy has been widely proven in germination and seedling growth, few studies have investigated its role in the root development of adult trees or in water uptake. A recent study by Fruleux et al. (2016) on beech, pine and oak seedlings found that developmental, morphological and anatomical root traits responded only weakly to competition and that there was no clear shift in soil exploitation under well-watered conditions. However, under drought conditions, the authors found a positive interaction between beech and oak, and none with pine. Further investigations should be made to understand the causes, which may very well be multifactorial.

Interestingly, only oak seems to benefit from mixture (less negative PLWP values). Water uptake from shallow sources are generally found in pure stands in temperate regions under favorable conditions; then when water availability decreases, some species are able to shift to deeper water sources while others are not (Brinkmann et al. 2018). Similarly, Grossiord et al. 2014a, showed that managing mixed-species stands in Mediterranean forests would only alleviate the effect of water stress during summer droughts for the deepest-rooted species like *Q. faginea* and *P. nigra*, to the detriment to shallow-rooted trees such as *P. sylvestris*. In addition, they showed that *P. sylvestris* decreased its sap flux density more in mixed stands than when interacting with conspecific neighbors in pure stands. This confirms our finding that mixing effect is species specific, and that for pine, interspecific competition is greater than intraspecific competition. The situation is the opposite for oak where a beneficial mixture effect is clear. It is also worth pointing out that even larger pine trees received ca. 40% of their water from the 30–45 cm depth, which is also the primary water source for large oak trees. This results in competition between large oak

and large pine trees for the limited water sources at this depth. Whereas the oak root systems are capable of accessing deeper soil layers (Kolb and Stone 2000), in our study, we found the highest root density in the top 50 cm. The presence of a distinct clay layer at 50 cm in depth discouraged deeper root growth (Meinen et al. 2009), and created more competition between the two species for the water available at 30–45 cm in depth. Additionally, small pore sizes are associated with clay, and this means that water moves much more slowly through clay than through other soil textures (Al Majou et al. 2008). This in turn could have caused soil water to pool above the clay layer, which could partially explain the relatively high SWC around 40 cm in depth. To sum up, not only is the identity of the species present in the direct neighborhood of a given tree important, the pedological characteristics of the site also determine the influence of tree species mixtures on water availability and on how co-existing tree species use the available water during summer droughts.

Interestingly, in pure stands, we found that small oak trees had significantly more water uptake challenges than did large oak trees, as indicated by their lower PLWP values on the second sampling date. One possible explanation is that, under water stress conditions, larger oak trees may be able to utilize deeper water sources. However, our  $\delta^{18}\text{O}$  signature data did not confirm this hypothesis. Both small and larger oak trees in pure stands primarily utilized soil water near the surface (Fig. 6c). An alternative explanation for in the large oak trees' better resistance to water stress may involve the light rain which occurred 3 days prior to the second PLWP sampling date. This rain may have been better intercepted by the large trees since they have a larger crown and leaf surface compared to small trees. Larger trees may also have greater stemflow (Van Stan et al. 2014) leading to a higher soil moisture near the soil surface, thereby reducing water stress of large oak trees.

## Conclusion

In light of the predicted changes in the intensity and frequency of rain events in temperate regions, growing different tree species in a mixed stands may enhance trees' resistance to drought. This is based on the niche complementarity concept: different tree species utilize different soil resources. Isotopic measurements of xylem water and the soil water found at different

levels in the soil profile show that both pine and oak trees primarily utilize soil water near the surface, when growing in pure stands. However, when the two species are together in mixed stands, we observed a downward shift in access to soil water, especially for oak trees. Although we did also observe a downward shift in water utilization for pine trees in mixed stands, their primary water source remained near the soil surface. These findings suggest only partial niche complementarity for drought-limited water resources between *Q. petraea* and *P. sylvestris* in mixed stands during a drought event. Small oak trees, in particular, were benefitted (easier water uptake - i.e., less negative PLWP values) by the downward shift in water sources and mixing species with two different water regulation strategies (anisohydric, isohydric). Larger oak trees also benefitted, though less so than the small oak trees (Fig. 7b,d). Finally, the results from this study show that growing a mixture of tree species can change water availability for some trees and could help certain species, though not all, to cope with the water scarcity forecast in a changing climate.

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## Appendices

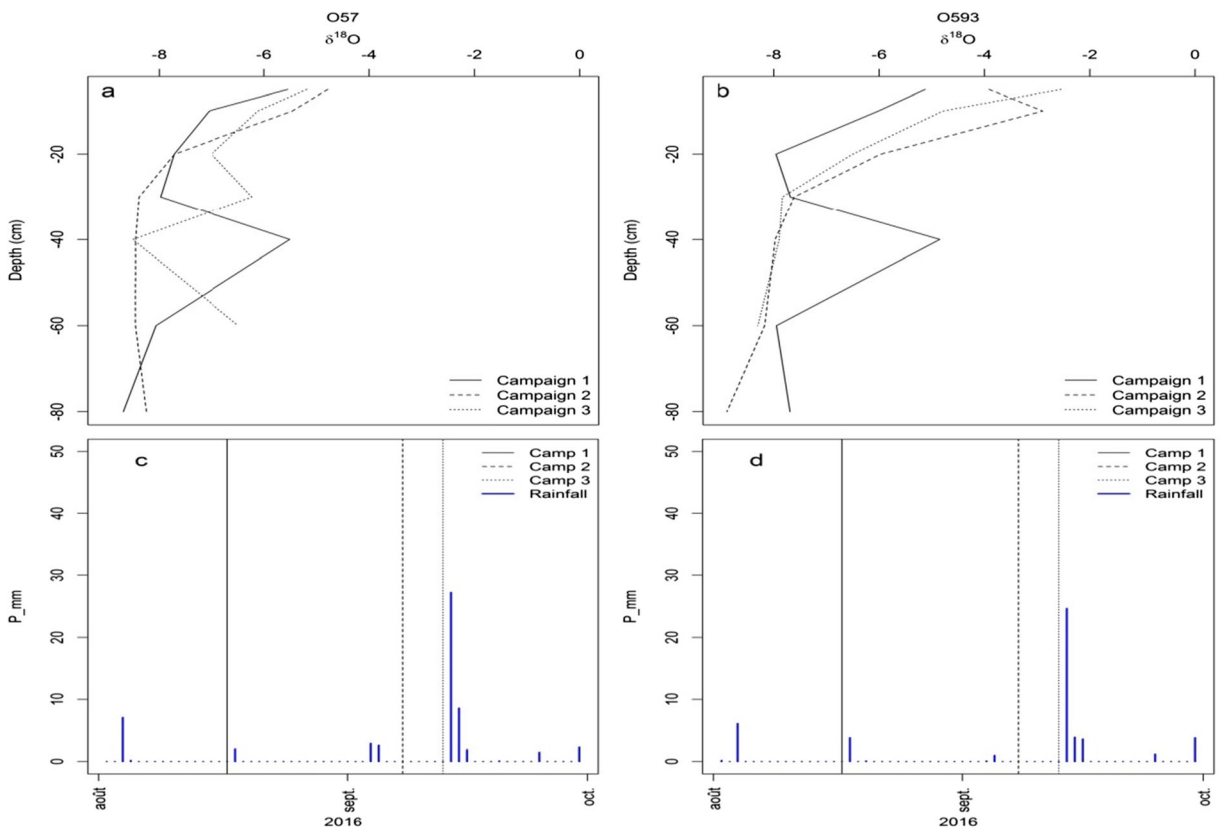
**Table 1** Description of the thickness, depth and sand, silt and clay content of the soil horizons in the different stand types and the depth of the clay layer

	Horizon	Thickness (cm ± sd)	Depth (cm ± sd)	Sand (%)	Silt (%)	Clay (%)	Depth of clay layer (cm + sd)
Pine	H1	7.0 ± 1.8	7.0 ± 1.8	64.3 ± .	20.4 ± .	9.5 ± .	
	H2	29.3 ± 14.7	36.3 ± 13.5	76.2 ± 7.8	15.9 ± 6.8	6.9 ± 0.7	
	H3	14.7 ± 7.3	51.0 ± 14.7	70.8 ± 18.3	15.5 ± 9.0	12.8 ± 13.5	53.2 ± 15.9
	H4	34.2 ± 18.3	85.2 ± 18.6	56.5 ± 17.7	9.9 ± 2.3	32.8 ± 15.6	
	H5	32.7 ± 10.0	106.7 ± 5.2	9.3 ± .	26.5 ± .	63.5 ± .	
Mixture	H1	10.6 ± 6.5	10.6 ± 6.5	.	.	.	
	H2	22.1 ± 6.0	32.7 ± 5.4	69.2 ± 8.5	21.7 ± 6.0	7.6 ± 2.4	
	H3	20.4 ± 13.2	53.1 ± 13.0	65.8 ± 11.3	17.9 ± 4.8	15.4 ± 6.4	45.8 ± 11.1
	H4	36.9 ± 21.8	90.0 ± 21.1	54.5 ± 10	11.5 ± 5.1	33.3 ± 8.8	
	H5	26.5 ± 17.3	98.3 ± 3.5	72.6 ± .	5.5 ± .	21.2 ± .	
Oak	H1	10.7 ± 2.1	10.7 ± 2.1	72.2 ± .	14.4 ± .	7.5 ± .	
	H2	23.6 ± 6.8	34.2 ± 8.2	72.8 ± 6.1	18.3 ± 4.2	7.4 ± 1.6	
	H3	15.2 ± 6.1	49.4 ± 11.7	68.6 ± 4.3	19.6 ± 2.1	11.1 ± 2.8	46.4 ± 10.2
	H4	22.8 ± 11.1	72.2 ± 21.7	58.0 ± 8.2	9.4 ± 4.2	31.9 ± 11.7	
	H5	36.7 ± 9.3	95.0 ± 5.5	58.4 ± 4.3	8.4 ± 5.4	32.5 ± 1.1	

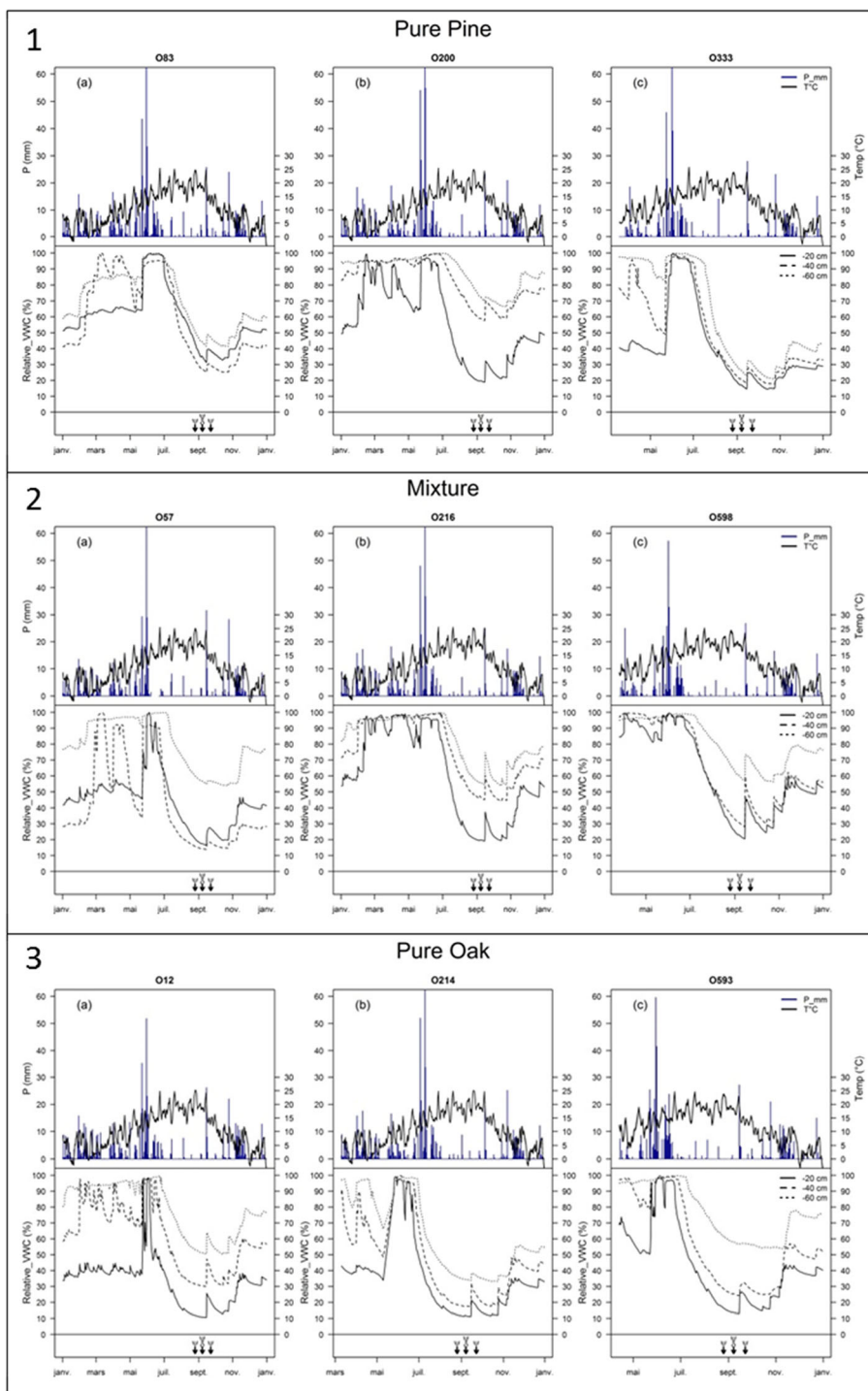
**Table 2** Dendrometrical characteristics of the three stands in 2015

Density	Composition	Species	Dg (cm)	Basal area (m <sup>2</sup> /ha)	Number of stems (/ha)	RDI
Medium	Pure oak	Oak	22.5 (2.4)	20.8 (1.6)	530 (75)	0.57 (0.05)
	Mixture	Oak	23.6 (1.9)	10.4 (1.1)	245 (60)	0.29 (0.03)
		Pine	36.0 (3.3)	15.7 (2.8)	154 (19)	0.35 (0.06)
	Pure Pine	Pine	33.6 (1.7)	30.7 (1.7)	348 (23)	0.68 (0.04)

For the mixture, Basal area, Number of stems and RDI are partial values by species. The total value for the stand is the sum of the two partial values. Standard deviations are given in parentheses

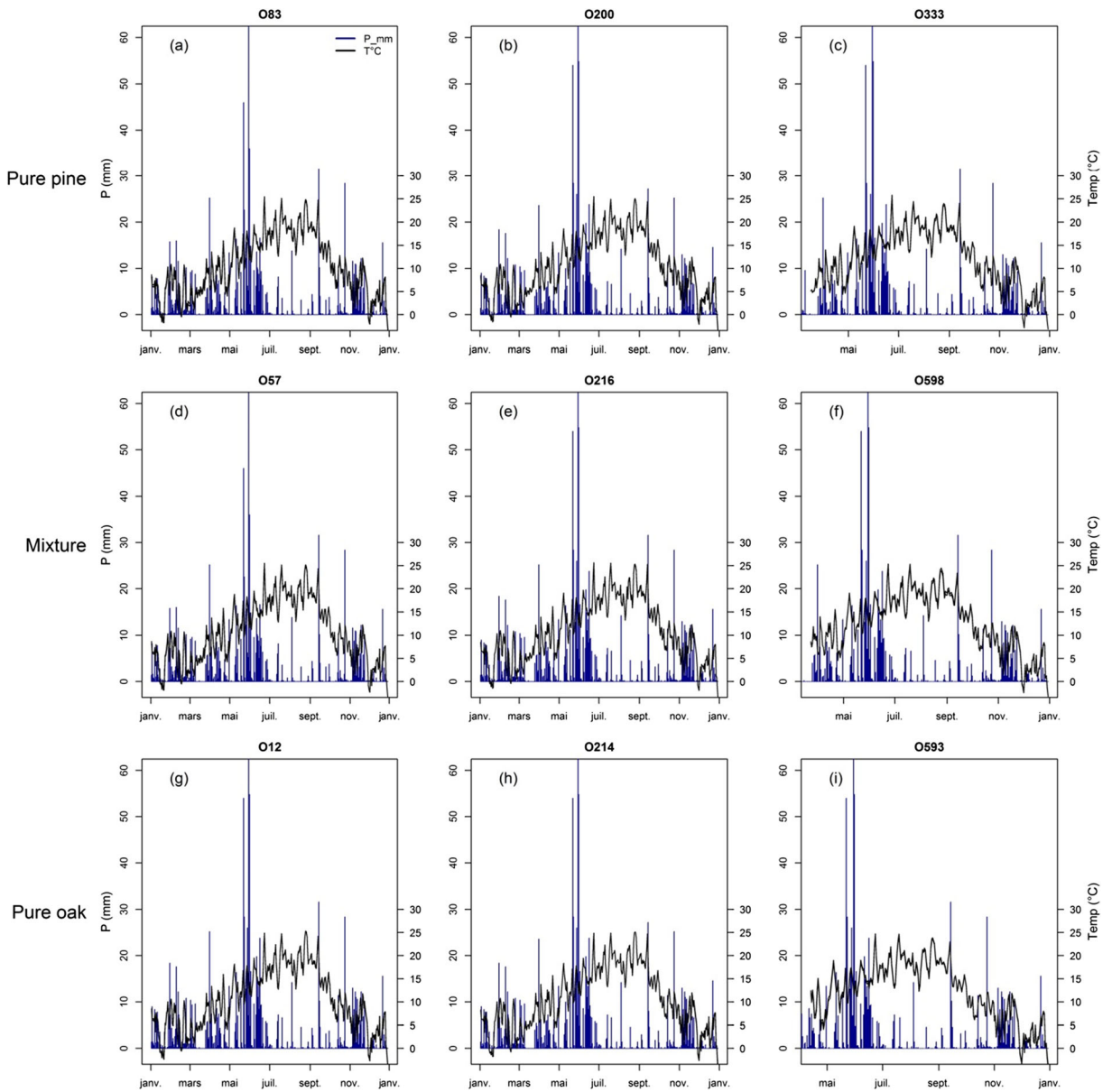


**Fig. 8** Isotopic signature of the soil samples from plots O57 (a) and O593 (b) for the three isotope campaigns (a and b) and soil depth and precipitation (mm) measured in these two plots (c and d). The three campaigns are represented by the vertical lines in c and d

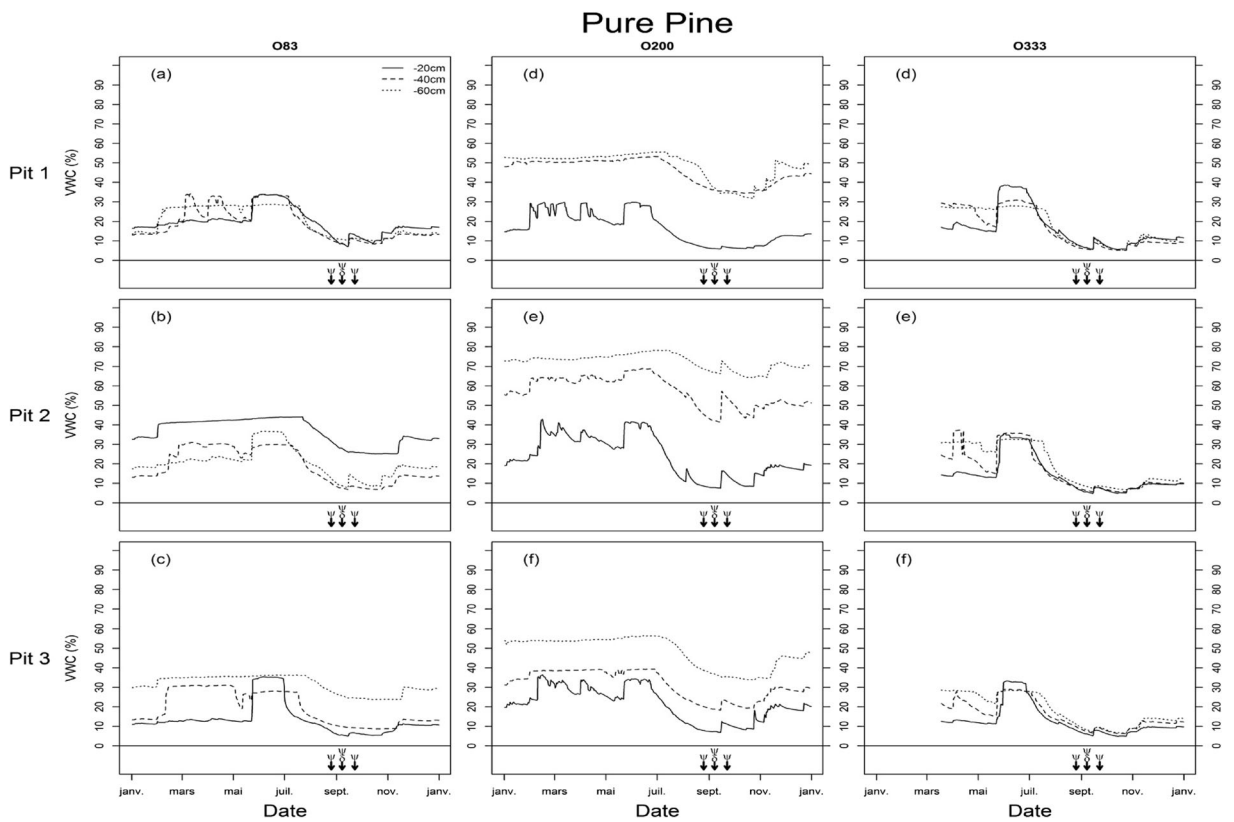


**Fig. 9** Daily precipitation (blue bars, top windows) and temperature (black line, top windows) and Relative Soil Volumetric Water Content (bottom windows) at 20cm (solid line), 40cm (dashed line) and 60cm (dotted line) in depth in pure pine (1), mixed (2)

and pure oak (3) stands. Arrows represent the sampling date for predawn leaf water potential ( $\Psi$ ) and isotope ( $\delta$ ) measurements. The triple-graph (a, b and c) corresponds to the three plot repetitions for the three stand composition types



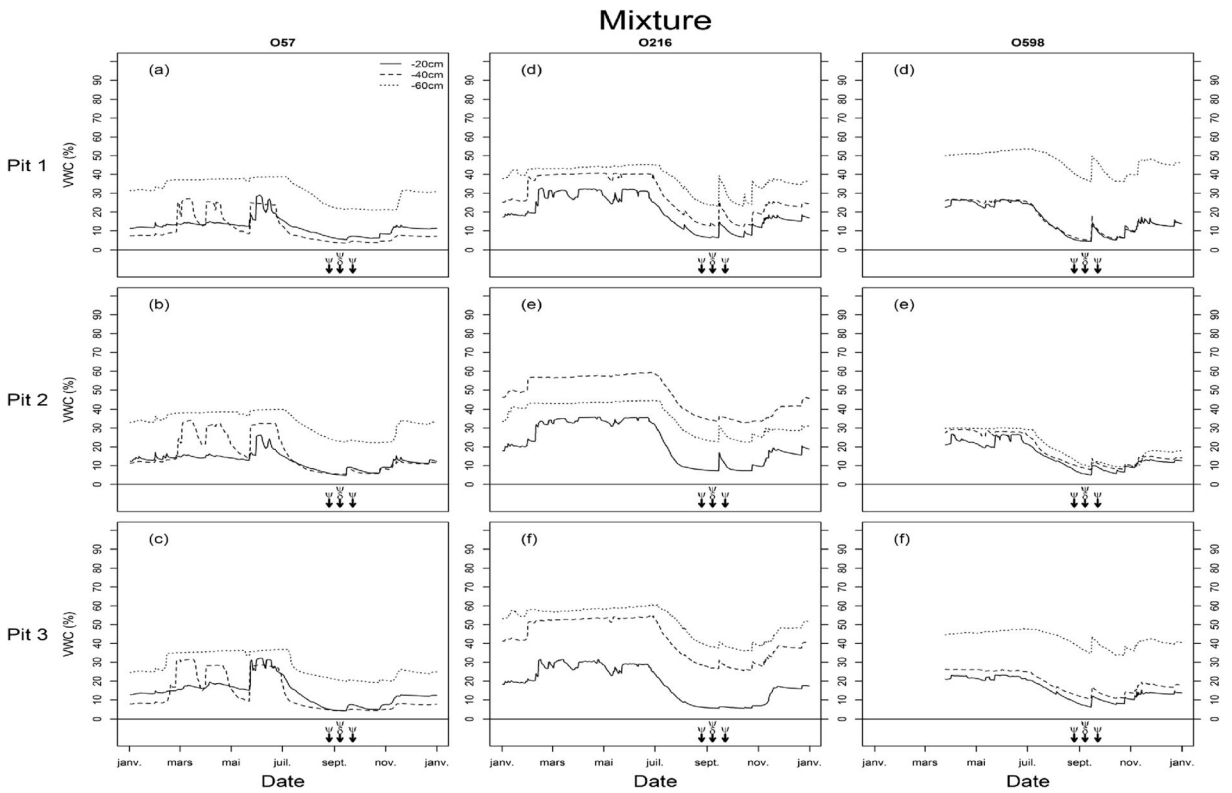
**Fig. 10** Daily precipitation (blue bars) and mean temperature (black line) for each plot in pure pine (a,b and c), mixed (d, e and f) and pure oak (g, h and i) stands



**Fig. 11** Volumetric Water Content (VWC) for each soil pit at 20 cm (solid line), 40 cm (dashed line) and 60 cm (dotted line) in depth for the three pure pine stands (O83, O200 and O333).

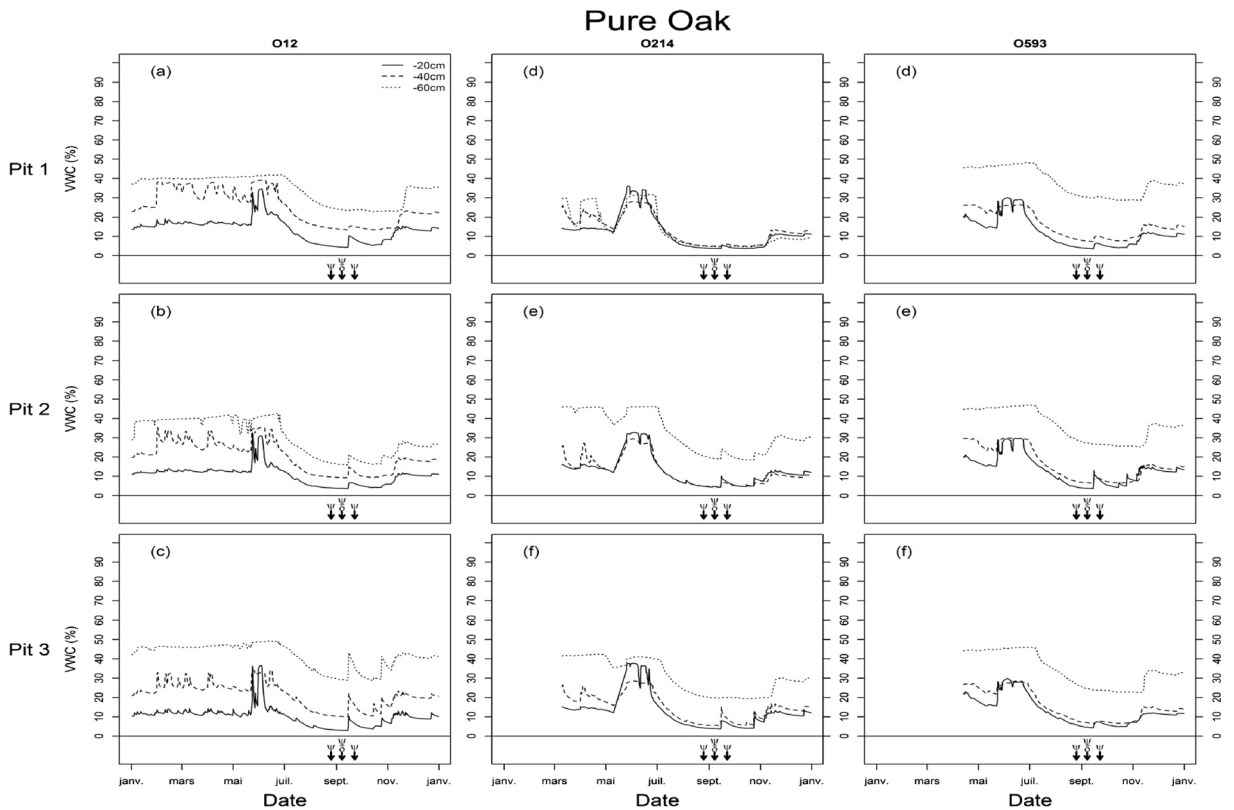
Arrows indicate the sampling date for predawn leaf water potential ( $\Psi$ ) and isotope ( $\delta$ ) measurements





**Fig. 12** Volumetric Water Content (VWC) for each soil pit at 20 cm (solid line), 40 cm (dashed line) and 60 cm (dotted line) in depth for the three mixed stands (O57, O216 and O598).

Arrows indicate the sampling date for predawn leaf water potential ( $\Psi$ ) and isotope ( $\delta$ ) measurements



**Fig. 13** Volumetric Water Content (VWC) for each soil pit at 20 cm (solid line), 40 cm (dashed line) and 60 cm (dotted line) in depth for the three pure oak stands (O12, O214 and O593).

Arrows indicate the sampling date for predawn leaf water potential ( $\Psi$ ) and isotope ( $\delta$ ) measurements

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