Research paper

Acclimation of leaf water status and stem hydraulics to drought and tree neighbourhood: alternative strategies among the saplings of five temperate deciduous tree species

Torben Lübbe[1], Bernhard Schuldt and Christoph Leuschner

Department of Plant Ecology and Ecosystems Research, Albrecht von Haller Institute for Plant Sciences, University of Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany;
[1]Corresponding author (tluebbe@gwdg.de)

Adjustment in leaf water status parameters and modification in xylem structure and functioning can be important elements of a tree's response to continued water limitation. In a growth trial with saplings of five co-occurring temperate broad-leaved tree species (genera *Fraxinus*, *Acer*, *Carpinus*, *Tilia* and *Fagus*) conducted in moist or dry soil, we compared the drought acclimation in several leaf water status and stem hydraulic parameters. Considering the extremes in the species responses, *Fraxinus excelsior* L. improved its leaf tissue hydration in the dry treatment through osmotic, elastic and apoplastic adjustment while *Fagus sylvatica* L. solely modified its xylem anatomy, which resulted in increased embolism resistance at the cost of hydraulic efficiency. Our results demonstrate the contrasting response strategies of coexisting tree species and how variable trait plasticity among species can be. The comparison of plants grown either in monoculture or in five-species mixture showed that the neighbouring species diversity can significantly influence a tree's hydraulic architecture and leaf water status regulation. Droughted *Carpinus betulus* L. (and to a lesser extent, *Acer pseudoplatanus* L.) plants developed a more efficient stem hydraulic system in heterospecific neighbourhoods, while that of *F. sylvatica* was generally more efficient in conspecific than heterospecific neighbourhoods. We conclude that co-occurring tree species may develop a high diversity of drought-response strategies, and exploring the full diversity of trait characteristics requires synchronous study of acclimation at the leaf and stem (and possibly also the root) levels, and consideration of physiological as well as morphological and anatomical modifications.

Keywords: drought tolerance, mixed tree assemblages, phenotypic plasticity, pressure–volume curve, tree hydraulics, turgor loss point, vulnerability to cavitation, wood anatomy.

Introduction

Climate change scenarios predict that most ecosystems will be exposed to more extreme environmental conditions in the future (IPCC 2013). Forests are particularly vulnerable to a rise in the frequency and intensity of drought events, which may reduce tree vitality, productivity and survival (Bréda et al. 2006, Allen et al. 2010, Zimmermann et al. 2015). The mechanisms underlying the tree drought response at the molecular, organ and tree level are not yet fully understood. Maintenance of hydraulic functioning in the root-to-leaf hydraulic pathway under water scarcity is certainly a key element of a successful drought tolerance strategy (Anderegg et al. 2011, Bartlett et al. 2012, Choat et al. 2012).

Whether forests can adapt to climatic change depends largely on which tree functional types are present, their specific trait characteristics and the species' phenotypic plasticity (Valladares et al. 2007, Nicotra et al. 2010, Bussotti et al. 2014). Even in the same environment, different tree species have developed contrasting drought acclimation strategies that involve modification of leaf, stem and root traits (Kozlowski and Pallardy 2002, Maseda and Fernández 2006). At the whole-plant level, trees

may enhance water acquisition through a variety of adjustments of plant morphology and C allocation patterns, including a rise in the root:shoot ratio, increase in fine-root surface area and deeper rooting (e.g., Brunner et al. 2015). Alternatively, the critical water demand can be lowered by reducing the transpiring leaf area (Le Dantec et al. 2000, Vilagrosa et al. 2003, Ogaya and Penuelas 2006).

Trees may also modify their water transport system in response to prolonged drought through physiological and structural adjustments in the root, stem and leaf xylem. At the leaf level, modification of cell water status through osmotic, elastic or apoplastic adjustments in response to water scarcity may help to maintain stomatal conductance when leaf water potential (Ψ_{leaf}) is dropping (Kozlowski and Pallardy 2002, Lenz et al. 2006, Bartlett et al. 2012). These adjustments directly influence the osmotic potential at turgor loss point (π_{tlp}) and the corresponding relative leaf water content (RWC_{tlp}), and may help to reduce the risk of hydraulic failure in the leaf tissue.

Adjustment in xylem anatomical properties can further help to tolerate Ψ_{leaf} reductions while maintaining hydraulic functioning (Sperry et al. 2008, Schuldt et al. 2016). Several studies have demonstrated considerable intraspecific plasticity in vascular traits across water availability gradients (Fonti et al. 2013, Schreiber et al. 2015, Schuldt et al. 2016), which might increase hydraulic safety at the cost of hydraulic efficiency (McDowell 2011). Many studies have confirmed that a vessel diameter decrease not only reduces hydraulic conductivity, but also lowers the risk of embolism caused by air-seeding both within and across species (e.g., Hacke and Sperry 2001, Sperry et al. 2006, Domec et al. 2010, Hajek et al. 2014). However, recent results suggest that the relation between conduit size and xylem safety is rather indirect and results from the close correlation between pit membrane thickness and embolism resistance (Lens et al. 2013, Li et al. 2016). Another modification leading to higher safety in the vascular system is the increase of vessel density (VD), which results in higher redundancy in the hydraulic pathway (Ewers et al. 2007, Schuldt et al. 2016). Plasticity in sapwood anatomical traits may thus enable plants to balance hydraulic safety with efficiency under conditions of water limitation (Vilagrosa et al. 2012, Fonti et al. 2013).

Although various studies have addressed species differences in the acclimation potential of either leaf hydration status (relating to cell wall properties and osmotic regulation), or stem and branch hydraulic traits in response to water deficits, studies considering both aspects and possible mutual interactions are scarce. While some recent results seem to indicate that the sensitivity of stomates to Ψ_{leaf} is related to xylem characteristics (Klein 2014), it remains unclear whether stem and leaf level responses are coordinated or not.

Soil water availability as a determinant of tree water status is certainly influenced by abiotic site characteristics and stand structural properties like tree size, plant density and species identity. However, the water budget of forests is further influenced by the tree community composition, the number and functional types of coexisting species and their degree of complementary resource use (Bravo-Oviedo et al. 2014, Forrester 2014, 2015). Spatial and temporal differences in the water consumption of neighbouring trees of different species identity may lead to reduced interspecific competition for water, but they can also result in higher water consumption of mixed than monospecific assemblages and thus greater soil desiccation (e.g., Grossiord et al. 2014a). Hence, the specific neighbourhood composition in mixed stands may affect the water status of target trees and this effect could feed back on the hydraulic architecture and leaf water status regulation of these individuals. In fact, several recent studies provided evidence that trees of certain species are less susceptible to drought and may even be more productive, when growing in mixture as compared with monoculture (del Río et al. 2013, Lebourgeois et al. 2013, Pretzsch et al. 2013, Mölder and Leuschner 2014, Neuner et al. 2015, Metz et al. 2016). This should be the case when intraspecific competition for water is more intense than interspecific competition. A recent meta-analysis on the carbon isotope signature of latewood from different European forest types showed that higher tree species diversity can enhance drought resistance through positive neighbourhood effects, but this effect was only evident in drought-prone environments and not under conditions of ample water supply (Grossiord et al. 2014b). The roles of conspecific or heterospecific neighbours have mostly been investigated with respect to productivity, while functional traits related to carbon gain or water use have only rarely been investigated (Forrester et al. 2010, Kunert et al. 2012, Pollastrini et al. 2014).

Here, we present the results of a tree sapling experiment on the drought acclimation of five temperate broad-leaved tree species that were exposed to either conspecific or heterospecific competition. We measured important pressure–volume traits in the leaf tissue and analysed the anatomy and embolism resistance of the stem xylem. These data are complementary to measurements on stem hydraulic conductivity, leaf conductance and above- and belowground productivity conducted in the same plants and presented by Lübbe et al. (2015, 2016). We tested the hypotheses that (i) species identity is a more important source of variation in morphological and physiological traits than trait modification in response to severe drought, (ii) different tree species that co-occur in a common habitat develop plasticity in response to soil desiccation either mostly at the foliage level (leaf water status regulation) or at the stem level (xylem anatomy and hydraulic properties), but normally do not modify both and (iii) morphological and physiological adjustments to drought depend in mixture, at least in certain species, on neighbour identity.

Materials and methods

Experimental design

We conducted a replicated tree diversity experiment of 16 months duration (~450 days) from April 2011 to August 2012

in the Experimental Botanical Garden of the University of Göttingen (51°33′ N, 9°57′ E, 177 m altitude). Five temperate broad-leaved tree species (*Acer pseudoplatanus* L. (sycamore maple), *Carpinus betulus* L. (European hornbeam), *Fagus sylvatica* L. (European beech), *Fraxinus excelsior* L. (European ash) and *Tilia cordata* Mill. (small-leaved lime)) were investigated, which represent a broad variety of tree functional types differing in light demand and shade tolerance, canopy architecture, mycorrhiza type, drought tolerance and successional status (Köcher et al. 2013, Legner et al. 2013). The 2-year-old saplings had been reared from seed in a nursery close to Göttingen. The seed was collected from a few trees per species; this reduced the genetic variability of the experimental plants. The target plants were taken from a larger number of available plants and were selected on the basis of similarity in plant height and number of leaf buds. Nevertheless, the species differed slightly in initial height due to species differences in growth rate (height range: 40–60 cm, Lübbe et al. 2015). Five saplings each were planted in a large pot (0.05 m^3 volume, height 0.35 m, diameter 0.58 m) filled with coarse-grained sand (98% sand, 1.8% silt, 0.2% clay). To arrange the plants at equal distance (~17 cm), four saplings were planted at the corners of a square placed on the pot surface (distance to pot walls: ~12 cm) and the fifth was positioned in the centre.

For the purposes of this study, we selected monoculture pots (all five saplings of the same species) and five-species-mixture pots (all five saplings from different species) from a more comprehensive set of available species combinations as described in Lübbe et al. (2016). Each combination was replicated at least sixfold. In the five-species mixture, the position of the saplings of different species identity varied in the replicate pots in order to average over six different neighbourhood compositions.

The experiment consisted of a drought trial with two soil moisture levels (moist: ~21 vol. %, dry: ~12%) with target values of volumetric soil water content (SWC) equaling ~95 and ~57% maximum field capacity, respectively. These targets were maintained by adding every 3–5 days the amount of water that had been lost through evapotranspiration. Thus, soil moisture fluctuated moderately between two subsequent irrigation events below the target moisture values. Accordingly, lowest soil matrix potentials reached −84 kPa in the moist and −869 kPa in the dry treatment. Soil moisture content and thus the amount of required irrigation water were determined by regularly weighing the pots with a mobile digital hanging scale. To achieve similar drought stress intensities at the end of each irrigation interval, the target values of SWC varied slightly between the replicates of a moisture treatment in order to avoid soil water over-exploitation and critical soil desiccation in pots with larger trees and thus more water-consuming tree assemblages. For details of soil moisture determination and moisture control, see Lübbe et al. (2016).

The pots were installed under a light-transmitting roof, which excluded all precipitation but altered the microclimate much less than a glasshouse. Photosynthetically active radiation was reduced by ~30% compared with incident radiation. All pots were randomized and set up in rows to minimize the possible impact of microclimatic heterogeneity. Each pot was fertilized monthly with 4 ml Wuxal$^©$ solution (8.0% N, 8.0% P_2O_5, 6.0% K_2O) between May and September 2011 and with 6 ml from March to August 2012. Details on soil water dynamics, nutrient supply and climate conditions are given in Lübbe et al. (2015, 2016).

Tree sampling design

Six saplings per treatment (5 species × 2 soil moisture levels × 2 diversity levels × 6 replicates; 120 sampled plants in total) were investigated for leaf water status and hydraulic parameters by randomly selecting each one individual per species and pot. If possible, all physiological measurements were executed on the same tree individual. In case samples had to be dismissed, the measurements were repeated on another individual. The final harvest of the plants was carried out during 7 weeks in July and August 2012 by applying a rotating sampling scheme. In general, one replicate plant was sampled per treatment and week. Leaf samples for pressure–volume (*P*–*V*) curve analysis were taken 1–2 days before the date of harvest. Shoot samples for the measurement of xylem vulnerability to cavitation and wood anatomical structure were taken at the time of harvest. A list of all traits measured, the corresponding acronyms and units is given in Table 1.

P–V curve analysis

P–*V* curves were established by applying the pressure-chamber method according to Tyree and Hammel (1972). Fully developed single leaves or small terminal shoots were sampled early in the morning from the upper crown and the cut petioles or shoots were immediately placed in tap water, covered by plastic bags and allowed to rehydrate for 24–48 h at 5 °C. Subsequently, basal twig segments or petiole tips were re-cut with a razor blade, sample weight at full saturation determined at a precision of 0.1 mg (FM$_{sat}$) and samples inserted in a pressure chamber (M1000, PMS Instrument Co., Albany, OR, USA). After determining the initial leaf water potential (Ψ_{init}), only fully hydrated leaf samples ($\Psi_{init} > −0.3$ MPa) were processed. To reach higher pressure levels, samples were pressurized for 10 min and the petioles or twig segments were covered with Eppendorf cups containing cellulose strips to absorb and subsequently weigh the leaking fluid (L_t). Subsequently, samples were relaxed for 10 min by reducing the applied pressure by ~0.25 MPa before the actual leaf water potential was determined. Measurements started at 0.3 MPa and increased stepwise by 0.3 MPa up to 2.7 MPa, followed by 0.2 MPa steps, until the target pressure of 3.5 MPa was reached. Finally, the weight of the dehydrated samples was determined (FM$_{wilt}$) and the samples were dried to constant mass (DM) at 70 °C for 72 h in

Table 1. List of main variables with definition and units employed. The G_s, $\delta^{13}C$ and K_s data were taken from Lübbe et al. (2016).

Parameter	Unit	Description
Leaf hydration (pressure–volume curve traits)		
π_O	MPa	Leaf osmotic potential at full tugor
π_{tlp}	MPa	Leaf water potential at turgor loss point
RWC_{tlp}	%	Relative water content at turgor loss point
A_f	%	Apoplastic water fraction
ε_{max}	MPa	Maximum modulus of elasticity, calculated from symplastic water content
Leaf conductance		
G_s	mmol m^{-2} s^{-1}	Stomatal conductance
$\delta^{13}C$	‰	Foliar carbon isotope signature
Xylem structural traits (wood anatomical traits)		
A_{lumen}	%	Lumen fraction of cross-sectional sapwood area
VD	n mm^{-2}	Vessel density
D	µm	Mean vessel diameter
D_h	µm	Hydraulically weighted vessel diameter
Xylem hydraulic properties		
K_s	kg m^{-1} MPa^{-1} s^{-1}	Empirical hydraulic conductivity of xylem
K_p	kg m^{-1} MPa^{-1} s^{-1}	Potential hydraulic conductivity of xylem
P_{50}	MPa	Water potential inducing 50% loss of hydraulic conductance
P_{88}	MPa	Water potential inducing 88% loss of hydraulic conductance

order to calculate total leaf water content ($FM_{sat} - DM$). Water lost by transpiration during the measurement ($FM_{sat} - FM_{wilt} - \Sigma L_t$) was equally assigned to the pressure steps to correct the measured values. The water potential at turgor loss point (π_{tlp}) and the corresponding relative water content (RWC_{tlp}) were calculated according to the procedure described by Von Willert et al. (1995). The osmotic potential at full turgor (π_O) was estimated by extrapolating the linear segment of the curve by regression analysis to the y axis where RWC is 100%. The symplastic water fraction at saturation (S_f) was estimated as the x-intercept of the linear curve section with $\frac{1}{\Psi} = 0$, which separates the dynamic water fraction S_f and the assumedly constant apoplastic water fraction (A_f). To calculate the modulus of elasticity near full hydration (ε_{max}), $\Delta P/\Delta RWC'$ was obtained from the first four points of the P versus RWC relationship based on the symplastic water fraction. Following Bartlett et al. (2012), we primarily analysed the P–V curve parameters π_O, π_{tlp}, A_f, RWC_{tlp} and ε_{max}.

Xylem resistance to cavitation

The Cavitron technique (Cochard et al. 2005) was applied to measure vulnerability to xylem cavitation in all species except for *F. excelsior*, which could not be processed with this technique due to the open-vessel artefact (Jansen et al. 2015). Immediately after harvest, samples of the terminal shoot were stored at 4 °C in a

MICROPUR® solution (Katadyn, Wallisellen, Switzerland) and processed within 7 days. Samples were first flushed with the Xyl'em apparatus at 120 kPa (Bronkhorst, Montigny-les-Cormeilles, France) in order to remove all potential emboli and to determine maximum hydraulic conductivity according to Sperry et al. (1988). Subsequently, the flushed samples were mounted in a custom-built rotor chamber of the Cavitron, which uses a commercially available centrifuge as the basis (Sorvall RC-5C, Thermo Fisher Scientific, Waltham, MA, USA). Spinning velocities were defined and recorded using the CaviSoft software (version 2.1, University of Bordeaux, Bordeaux, France). Measurements started at a pressure level of −0.37 MPa and were iteratively increased until the percentage loss of conductivity (PLC) reached at least 90%. Sigmoid functions (Pammenter and Van der Willigen 1998) were fitted for each branch segment to describe the relationship between PLC and xylem pressure (vulnerability curve) using the expression $PLC = 100/(1 + \exp(s/25 \times (P_i - P_{50})))$, where P_i is the initial pressure level, P_{50} (MPa) is the xylem pressure causing 50% loss of conductivity and s (% MPa^{-1}) is the slope of the curve at the inflexion point. The xylem pressures causing 88% loss of conductivity (P_{88}) were additionally calculated according to Domec and Gartner (2001).

Wood anatomical analysis

Transverse sections of the basipetal end of the shoot samples were stained with safranin (1% in 50% ethanol, Merck, Darmstadt, Germany) and cut with a sliding microtome (G.S.L.1, Schenkung Dapples, Zürich, Switzerland). The total transverse sections of the samples were digitalized at ×100 magnification using a stereomicroscope equipped with an automatic stage (SteREOV20, Carl Zeiss MicroImaging GmbH, Jena, Germany; Software: AxioVision v4.8.2, Carl Zeiss MicroImaging GmbH) and image processing was performed using Adobe Photoshop CS2 (Version 9.0, Adobe Systems Inc., San Jose, CA, USA) and the particle analysis function of ImageJ (v1.44p, http://rsb.info.nih.gov/ij). For all subsequent calculations, the complete xylem cross-section without pith and bark was analysed, yielding on average 3,578 (*Fraxinus*, moist) to 15,201 (*Tilia*, dry) measured vessels per branch sample. Measured parameters included relative vessel lumen area (A_{lumen}, %), i.e., the ratio of cumulative lumen area to sapwood area, VD (n mm^{-2}), the idealized vessel diameter (D, µm) obtained from major (a) and minor (b) vessel radii according to Lewis and Boose (1995) as $D = (32 \times (a \times b)^3/(a^2 + b^2))^{1/4}$, and hydraulically weighted vessel diameter (D_h, µm) according to Sperry et al. (1994) as $D_h = \Sigma D^5/\Sigma D^4$. Subsequently, the potential conductivity (K_p, kg m^{-1} MPa^{-1} s^{-1}) was calculated according to the Hagen–Poiseuille equation as $K_p = (((\pi \times \Sigma r^4)/8\eta) \times \rho)/A_{xylem}$, where η is the viscosity (1.002×10^{-9} MPa s) and ρ is the density of water (998.2 kg m^{-3}), both at 20 °C, and A_{xylem} (m^2) the corresponding xylem area without pith and bark.

Statistical analysis

Statistical analyses were executed in R version 3.0.2 (R Development Core Team 2013). We applied three-way analysis of variance (ANOVA) to test for effects of species identity in possible interactions with soil moisture and neighbour composition on physiological response variables. Tukey contrasts (*glht* procedure, *multcomp* package) were used for multiple comparisons to detect species differences in traits within each soil water treatment. Moreover, we used two-way ANOVA models to investigate the effects of drought and mixture on trait performance for each species individually. Model residuals were tested for normality (Shapiro–Wilk test) and equality of variances (Levene's test). In case of eminent violation of assumed homoscedasticity (as in the wood anatomy parameters), the ANOVAs were adjusted by applying a heteroscedasticity-corrected coefficient covariance matrix (*Anova*, *car* package). Pairwise comparisons among soil moisture treatments and diversity levels were carried out with Student's *t*-test, Welch's *t*-test or Mann–Whitney *U*-test depending on the data structure.

For comparing the plasticity between different sapwood- and leaf-related traits in response to soil drought, we calculated the response index (RI, or plasticity index) as the difference between the maximum and minimum mean value of a variable (i.e., the difference between moist and dry treatment), divided by the maximum mean value (Quero et al. 2006, Valladares et al. 2006). Six leaf-related and eight stem traits (leaf hydration traits, wood anatomical traits and xylem functional traits, respectively) were applied for this calculation. The relative values of RWC_{tlp} and A_f were replaced by absolute values in equivalent units, i.e., the amount of foliar water at turgor loss and the apoplastic water content per gram foliar dry mass, respectively. For assessing the plasticity in drought-response traits of a plant's foliar and woody tissue, we compared the plasticity indices of both tissue types.

Results

Tree species differences in leaf water status and xylem hydraulics

For all investigated leaf water status and stem hydraulic parameters, species identity was the most influential factor according to three-way ANOVA (see Table S1 available as Supplementary Data at *Tree Physiology* Online). The majority of species differences were significant and certain differences were large (Table 2). The modulus of leaf cell wall elasticity near maximum turgor (ε_{max}), for instance, was remarkably low in *T. cordata* (6.3 MPa, mean over all treatments) and at least twice as high in the other four species. *Carpinus betulus* had the lowest mean leaf osmotic potential at full turgor (π_O; −2.0 MPa) and also by far the lowest xylem pressures causing 50% (P_{50}; −5.0 MPa) or

88% loss of stem hydraulic conductivity (P_{88}; −6.0 MPa). The wood of ring-porous *F. excelsior* was characterized by very large hydraulically weighted vessel diameters (D_h; 75.5 μm, Table 2) despite a particularly small lumen-to-sapwood area ratio (A_{lumen}; 3.6%) and low VD (50.2 n mm^{-2}). *Fagus sylvatica* showed the highest potential hydraulic conductivity (K_p, 4.7 kg m^{-1} MPa^{-1} s^{-1}), which was about twice as high than that of *A. pseudoplatanus* and *C. betulus* (1.9 and 2.2 kg m^{-1} MPa^{-1} s^{-1}, respectively).

Drought adjustments at the stem and leaf level

Two-way ANOVA on *P–V* traits showed for *F. excelsior* significant osmotic and elastic adjustment under drought exposure (Table 3, see Table S3 available as Supplementary Data at *Tree Physiology* Online); π_O and leaf water potential at turgor loss point (π_{tlp}) decreased significantly from the moist to the dry treatment by 0.33 and 0.31 MPa, respectively ($P < 0.001$ and $P < 0.01$), while ε_{max} was by 41% larger in the droughted plants (13.3 vs 18.8 MPa, $P < 0.05$). None of the other four species showed significant osmotic or elastic adjustment at the leaf level. In *F. excelsior*, RWC_{tlp} was significantly higher in the dry treatment; this was not the case in the other species.

Fagus sylvatica was the species with most consistent modification of xylem-related properties in response to soil desiccation (Table 3, see Table S3 available as Supplementary Data at *Tree Physiology* Online). In this species, D (28.2 vs 26.7 μm; −5%) as well as D_h (38.9 vs 36.2 μm; −7%) decreased significantly ($P < 0.05$) in conjunction with an increase in embolism resistance (P_{50} and P_{88}) by 18% (P_{50}, reduction by 0.70 MPa) or 17% (P_{88}, reduction by 0.85 MPa), respectively. Similar to *F. sylvatica*, *C. betulus* showed a higher VD (by 20%) under dry than under moist conditions (147 vs 119 n mm^{-2}), and D_h decreased by 7% from 39.7 to 36.8 μm ($P < 0.05$). However, this was not mirrored in a significant decline in D, K_p or in P_{50} (Table 3, see Tables S2 and S3 available as Supplementary Data at *Tree Physiology* Online). In *T. cordata*, in contrast, xylem anatomical traits were not different between the two moisture treatments; only P_{50} was 10% more negative under dry conditions (reduction by 0.30 MPa, $P < 0.05$). In *A. pseudoplatanus*, significant effects of drought on xylem anatomical or vulnerability traits were lacking when considering the pooled sample. In contrast to all other species, *F. excelsior* increased D_h by 14% (70.7 vs 80.4 μm, $P < 0.01$) under dry conditions.

The susceptibility to drought as expressed by the response index to soil drought (RI) was generally higher for leaf hydraulic traits than for xylem properties in *F. excelsior* and *T. cordata*, while this ratio was reversed (higher susceptibility of xylem properties) in *A. pseudoplatanus*, *C. betulus* and *F. sylvatica* (Figure 1). The difference between the RI means of leaf and sapwood traits was significant in *F. sylvatica* ($P < 0.05$) and marginally significant in *F. excelsior* ($P < 0.10$).

Table 2. Physiological and structural plant traits of the five tree species for the two soil moisture treatments (moist and dry). Given values are means ± SE, capital letters indicate differences among species at a significance level of $P < 0.05$. Significant differences among moisture treatments are shown in Table 3.

	π_O				π_{tlp}				RWC_{tlp}			
	Dry		Moist		Dry		Moist		Dry		Moist	
F. excelsior	−2.12 ± 0.05	C	−1.79 ± 0.07	B	−2.52 ± 0.07	C	−2.21 ± 0.08	AB	0.86 ± 0.01	BC	0.82 ± 0.01	B
A. pseudopl.	−1.69 ± 0.03	A	−1.68 ± 0.05	AB	−2.01 ± 0.05	A	−2.01 ± 0.07	A	0.87 ± 0.01	C	0.86 ± 0.01	B
C. betulus	−2.06 ± 0.03	C	−2.02 ± 0.05	C	−2.59 ± 0.05	C	−2.59 ± 0.05	C	0.85 ± 0.01	BC	0.82 ± 0.02	B
T. cordata	−1.56 ± 0.04	A	−1.52 ± 0.03	A	−2.27 ± 0.07	B	−2.26 ± 0.07	AB	0.78 ± 0.01	A	0.75 ± 0.01	A
F. sylvatica	−1.90 ± 0.06	B	−1.79 ± 0.08	B	−2.49 ± 0.07	C	−2.35 ± 0.10	BC	0.82 ± 0.01	AB	0.83 ± 0.01	B
	A_f				ε_{max}				A_{Lumen}			
	Dry		Moist		Dry		Moist		Dry		Moist	
F. excelsior	0.13 ± 0.03	A	0.07 ± 0.02	A	18.81 ± 1.86	C	13.34 ± 1.08	B	3.67 ± 0.26	A	3.53 ± 0.29	A
A. pseudopl.	0.15 ± 0.02	A	0.14 ± 0.02	AB	14.77 ± 1.59	BC	16.74 ± 1.59	BC	5.49 ± 0.33	AB	6.00 ± 0.39	AB
C. betulus	0.28 ± 0.03	B	0.22 ± 0.05	AB	15.56 ± 0.90	BC	18.19 ± 2.00	C	7.50 ± 0.49	B	6.63 ± 0.27	B
T. cordata	0.30 ± 0.02	B	0.25 ± 0.04	B	6.55 ± 0.41	A	6.13 ± 0.49	A	13.70 ± 1.15	C	13.84 ± 1.52	C
F. sylvatica	0.24 ± 0.04	B	0.27 ± 0.06	B	12.44 ± 1.25	B	12.29 ± 1.41	B	12.97 ± 0.48	C	13.59 ± 0.82	C
	VD				D				D_h			
	Dry		Moist		Dry		Moist		Dry		Moist	
F. excelsior	56.19 ± 6.97	A	44.13 ± 3.92	A	24.91 ± 0.82	AB	25.82 ± 1.20	AB	80.41 ± 1.57	C	70.66 ± 2.13	C
A. pseudopl.	91.13 ± 5.41	B	90.83 ± 6.36	B	26.58 ± 0.36	BC	27.46 ± 0.61	B	36.46 ± 1.08	B	35.90 ± 0.93	AB
C. betulus	146.97 ± 9.35	C	119.21 ± 6.03	B	22.96 ± 0.56	A	23.66 ± 0.52	A	36.83 ± 1.74	B	39.65 ± 0.81	B
T. cordata	200.97 ± 13.86	D	207.87 ± 22.20	C	27.41 ± 0.26	C	27.78 ± 0.29	B	32.12 ± 0.58	A	32.71 ± 0.69	A
F. sylvatica	206.11 ± 7.26	D	185.54 ± 6.75	C	26.66 ± 0.46	BC	28.23 ± 0.59	B	36.21 ± 0.60	B	38.85 ± 0.82	B
	K_p				P_{50}				P_{88}			
	Dry		Moist		Dry		Moist		Dry		Moist	
F. excelsior	4.03 ± 0.35	B	3.65 ± 0.50	B	NA	−	NA	−	NA	−	NA	−
A. pseudopl.	1.81 ± 0.15	A	1.96 ± 0.17	A	−3.76 ± 0.13	A	−3.70 ± 0.10	B	−4.81 ± 0.22	B	−4.49 ± 0.10	A
C. betulus	2.14 ± 0.28	A	2.15 ± 0.11	A	−5.13 ± 0.09	B	−4.90 ± 0.15	C	−6.07 ± 0.15	C	−5.86 ± 0.18	B
T. cordata	3.73 ± 0.39	B	3.91 ± 0.48	BC	−3.56 ± 0.11	A	−3.22 ± 0.12	A	−4.17 ± 0.11	A	−3.94 ± 0.16	A
F. sylvatica	4.22 ± 0.24	B	5.25 ± 0.55	C	−3.83 ± 0.11	A	−3.13 ± 0.14	A	−5.06 ± 0.20	B	−4.22 ± 0.19	A

Neighbour effects on leaf water status and their interaction with soil moisture

The neighbourhood of a plant (i.e., conspecific vs heterospecific) had a significant influence on certain leaf water status parameters in three of the five species (Table 3). In *C. betulus*, π_{tlp} was 6% lower in plants grown with heterospecific neighbours as compared with conspecific neighbours (−2.51 vs −2.66 MPa, $P < 0.05$, Figure 2). The apoplastic water fraction (A_f) in *T. cordata* leaves was significantly higher (by 38%) in plants grown in mixed assemblages than in monocultures (22.7 vs 31.4%, $P < 0.05$). In *A. pseudoplatanus*, none of the tested P–V traits was influenced by the type of neighbourhood, but the significant interaction term 'Drought × Mixture' in ε_{max} (Table 3, $P < 0.05$) indicates that potential neighbourhood effects on P–V traits seem to act in opposite direction in the moist and dry treatments. In fact, ε_{max} was significantly higher (by 51%) in this species when grown with heterospecific neighbours (19.7 vs 13.0 MPa, $P < 0.05$) in the moist treatment (Figure 2, Table 4, see Table S3 available as Supplementary Data at *Tree Physiology* Online).

In xylem-related traits, direct mixture effects were observed in *C. betulus* and *F. excelsior* (Table 3); the latter species tended to increase D by 11% when grown with heterospecific neighbours (from 24.0 to 26.8 μm, $P < 0.10$). In *C. betulus*, not D but D_h was significantly increased in mixture (+10%; from 36.5 to 40.3 μm, $P < 0.01$), while the increase in P_{88} by 8% (from −6.2 to −5.7 MPa, $P < 0.05$) indicates higher vulnerability to drought (Figure 2). The frequent occurrence of significant interactions between soil moisture level and type of neighbourhood, especially in *A. pseudoplatanus* and *C. betulus*, indicates opposite effects of tree neighbourhood on xylem anatomy and hydraulics in the two moisture treatments (Table 3). D (21.4 vs 24.3 μm, $P < 0.01$), D_h (32.9 vs 41.8 μm, $P < 0.001$) and K_p (1.4 vs 2.8 kg m^{-1} MPa^{-1} s^{-1}, $P < 0.01$) all increased in mixture for *C. betulus* under dry conditions (Table 4, Figure 2). In parallel, the two measures of embolism resistance P_{50} (−5.4 vs −4.9 MPa, $P < 0.01$) and P_{88} (−6.4 vs −5.7 MPa, $P < 0.05$) increased in this species in response to heterospecific neighbourhood in the dry treatment. Similar to *C. betulus*, saplings of

Table 3. Significant effects of soil desiccation, species mixture (relative to the monoculture) and their interactions on the plasticity of plant structural and physiological traits according to two-way ANOVAs. The G_s, $\delta^{13}C$ and K_s data were taken from Lübbe et al. (2016). Arrows show direction of shift in mean values (upward: significant increase, downward: significant decrease, diagonal: increase/decrease at $P \leq 0.10$), significance level: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. For *F. excelsior*, no data on cavitation resistance are available.

Species/ parameter	Drought					Mixture					Interaction				
	F. excelsior	*A. pseudopl.*	*C. betulus*	*T. cordata*	*F. sylvatica*	*F. excelsior*	*A. pseudopl.*	*C. betulus*	*T. cordata*	*F. sylvatica*	*F. excelsior*	*A. pseudopl.*	*C. betulus*	*T. cordata*	*F. sylvatica*
π_0	↓***														
π_{tlp}	↓**							↓*							
RWC_{tlp}	↑**			↗											
A_f	↗								↑*						
ε_{max}	↑*											*			
G_s	↓**	↓*	↓***	↓*	↓***	↑**									
$\delta^{13}C$	↑***	↑*	↑***	↑*	↗	↓*				↘					
A_{Lumen}												*			
VD		↑*			↗							*			
D					↓*	↗							***		
D_h	↑**	↓*			↓*				↑**			*	***		○
K_s		↘	↓**		↓*	↑*			↘				***		
K_p					↘						**	**			
P_{50}				↓*	↓**									**	
P_{88}					↓**			↑*							

A. pseudoplatanus growing in mixture in the dry treatment showed larger D (25.8 vs 27.2 µm, $P < 0.05$), D_h (33.6 vs 38.4 µm, $P < 0.05$) and K_p values (1.4 vs 2.1 kg m^{-1} MPa^{-1} s^{-1}, $P < 0.01$) than in monoculture. In the moist treatment, only tendencies were visible. In addition, tree mixture effects were detected in *F. sylvatica* and *T. cordata* in the moist treatment (Table 4). In *F. sylvatica*, P_{88} significantly decreased (from −3.8 to −4.6 MPa, $P < 0.05$), while the striking decline in K_p by 29% (from 6.2 to 4.4 kg m^{-1} MPa^{-1} s^{-1}, Table 4, Figure 2) was not significant. *Tilia cordata* had a 17% less negative P_{50} value in mixture than in monoculture (−3.5 to −2.9 MPa, $P < 0.01$) in the moist treatment.

Discussion

Species differences in plant physiological traits

In accordance with our first hypothesis, species identity was by far the most influential factor explaining the variance of leaf water status and stem hydraulic traits. Although the five species are co-occurring in mixed forests in Central Europe (Leuschner et al. 2009), they represent a fairly broad spectrum of functional types with respect to growth strategy (early-/mid-successional vs late-successional), wood anatomy (diffuse-porous vs ring-porous) and drought tolerance (sensitive to relatively insensitive; Köcher et al. 2009, Ellenberg and Leuschner 2010). The high cell sap concentration of osmotic substances in *C. betulus* as visible in a leaf osmotic potential at full turgor (π_0) value less than −2.0 MPa and the relatively low turgor loss point

($\pi_{tlp} < -2.5$ MPa) in combination with high resistance to xylem cavitation ($P_{50} < -4.8$ MPa) indicate that this species was the most drought resistant in our sample. In fact, all other species had higher (more positive) values of π_0, π_{tlp}, P_{50} and P_{88}. In contrast, the accumulation of osmolytes in leaf tissue was much lower and the cavitation resistance in the stem sapwood weaker in the saplings of *A. pseudoplatanus* and *T. cordata* (the latter species also showed the steepest growth decline in response to water scarcity, Lübbe et al. 2015) and are thus considered as the most drought-vulnerable species of our sample. In the majority of investigated physiological and anatomical traits, the drought treatment was (besides species identity) the second-most important factor causing trait variation. An exception was hydraulically weighted vessel diameter (D_h) and the derived potential conductivity (K_p), which were more influenced by the type of neighbourhood (or an interaction with this factor) than by the drought treatment itself.

Drought responses at the leaf and stem levels

To cope with drought stress, trees may improve drought tolerance by forming smaller leaves with thicker cuticle or by producing new sapwood with smaller conduit diameters, or postpone tissue dehydration by elastic or osmotic adjustment in the existing leaves or roots (e.g., Bartlett et al. 2012, Fonti et al. 2013). It is likely that some drought-induced modifications of the tree's anatomy, morphology and physiology occur synchronously at leaf, stem and root levels, but it is not well known how these processes interact in trees under drought stress, and whether there
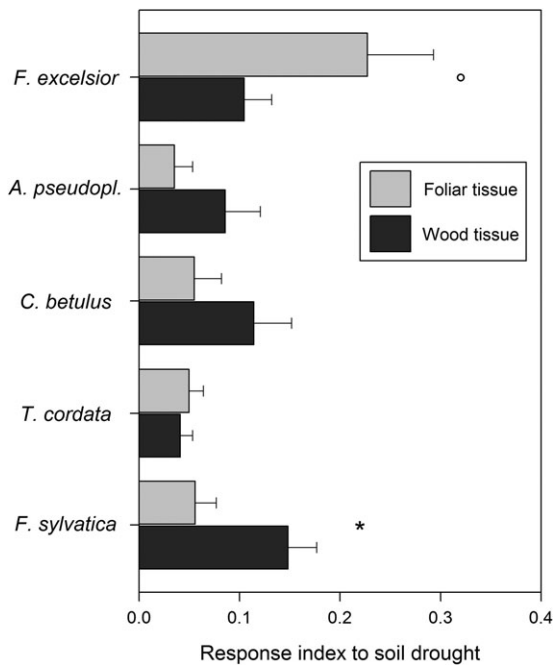
Figure 1. Mean values ± SE of RIs to the drought treatment for six leaf-related (leaf hydration including $\delta^{13}C$) and eight stem-related traits (wood anatomy and xylem functioning) of the five tree species. Symbols mark significant differences between RI at the leaf and the wood level (°$P \leq 0.1$, *$P \leq 0.05$). Please note that for *F. excelsior* no data on cavitation vulnerability were available.

is a trade-off between leaf and stem response, or if certain species mostly restrict a plastic response to one of these organs.

In our sample, only *F. excelsior* showed a plastic response to water scarcity at the leaf level, while *F. sylvatica* and *C. betulus* modified their stem wood tissue upon drought exposure but not the foliar *P–V* parameters. Despite the long controversy about the roles of osmotic, elastic and apoplastic adjustment and their importance for leaf water status regulation (Kozlowski and Pallardy 2002, Lenz et al. 2006), it appears that both leaf water potential (π_{tlp}) and the relative water content at turgor loss (RWC$_{tlp}$) are traits controlling the drought tolerance of plants (Bartlett et al. 2012). While π_{tlp} is closely related to π_O (Bartlett et al. 2012), elastic adjustment (in combination with apoplastic adjustment) was shown to be the main factor that maintains a high relative water content at turgor loss point, thus preventing cell dehydration below a dangerous threshold.

In *F. excelsior*, all important leaf water status parameters were markedly altered by water limitation, revealing pronounced osmotic, elastic and also apoplastic adjustment. Increased solute concentration in the symplast as well as enhanced cell wall stiffness under dry conditions is well documented for this species and seems to represent a key element of its drought tolerance strategy (Guicherd et al. 1997, Peltier and Marigo 1999, Marigo et al. 2000). Increased ε_{max} values of drought-treated *F. excelsior* saplings in conjunction with an increase in RWC$_{tlp}$ relative to the moist treatment are thus in line with the cell water

conservation hypothesis (e.g., Cheung et al. 1975), which also agrees with the interpretation of Peltier and Marigo (1999).

In contrast, *F. sylvatica* showed the highest plasticity in xylem anatomical and hydraulic properties in response to drought. This species reduced its vessel diameter (*D*) in the dry treatment, while VD was increased causing a decline in specific conductivity (K_s) and thus in hydraulic efficiency (Lübbe et al. 2016). This confirms earlier results on the marked influence of water availability on conduit size in *F. sylvatica* (Sass and Eckstein 1995, Caquet et al. 2009). Both anatomical adjustments are supposed to enhance the resistance to drought-induced xylem cavitation through increased capillary forces in narrower vessels; these adjustments act in combination with increased VD and hence pathway redundancy (Fonti et al. 2013, Schuldt et al. 2016). Correspondingly, drought-exposed *F. sylvatica* saplings in fact developed a more drought-resistant xylem structure of the stem wood. A similar hydraulic acclimation was found in mature beech trees along a precipitation gradient (Schuldt et al. 2016), in accordance with the high phenotypic plasticity in cavitation resistance observed in *F. sylvatica* in earlier studies (Herbette et al. 2010, Wortemann et al. 2011). The reduction in growth performance and hydraulic efficiency of *F. sylvatica* saplings in dry soil (Lübbe et al. 2015) confirms the hypothesized trade-off in hydraulic functioning, by which higher safety is achieved at the cost of efficiency when water is scarce (McDowell 2011). Although we could confirm this trade-off for our sample, a linear relation between hydraulic safety and efficiency could not be demonstrated at the global scale (Gleason et al. 2016).

A similar, but less pronounced, drought adjustment was found for the xylem properties of *C. betulus* with a VD increase and a concomitant reduction in D_h. The latter was partially reflected in the large K_p decrease (by 40%) in the monoculture. However, this anatomical modification did not lead to increased cavitation resistance in *C. betulus* under dry conditions. In comparison with the other species, *A. pseudoplatanus* showed only minor or no physiological adjustments in the dry treatment. Cavitation resistance tended to increase and K_p decreased by 40% in response to drought in the monoculture, which is consistent with the observed reduction in K_s (Lübbe et al. 2016).

In conclusion, the five investigated tree species differed markedly in their capability of physiological drought acclimation. In agreement with our second hypothesis, three species (*F. excelsior*, *F. sylvatica*, *C. betulus*) showed a plastic response either at the stem or at the leaf level, while the two other species (*A. pseudoplatanus*, *T. cordata*) revealed no clear pattern. It appears that at least some of the species develop high plasticity in those organs where trait adjustment provides the largest benefit in terms of plant fitness. However, we cannot generalize that evidence for plasticity in one organ is precluding plasticity in another. A coordination of drought responses across different organs is also likely, which makes it advisable to investigate plasticity within a set of multiple traits (Kleyer and Minden 2015).
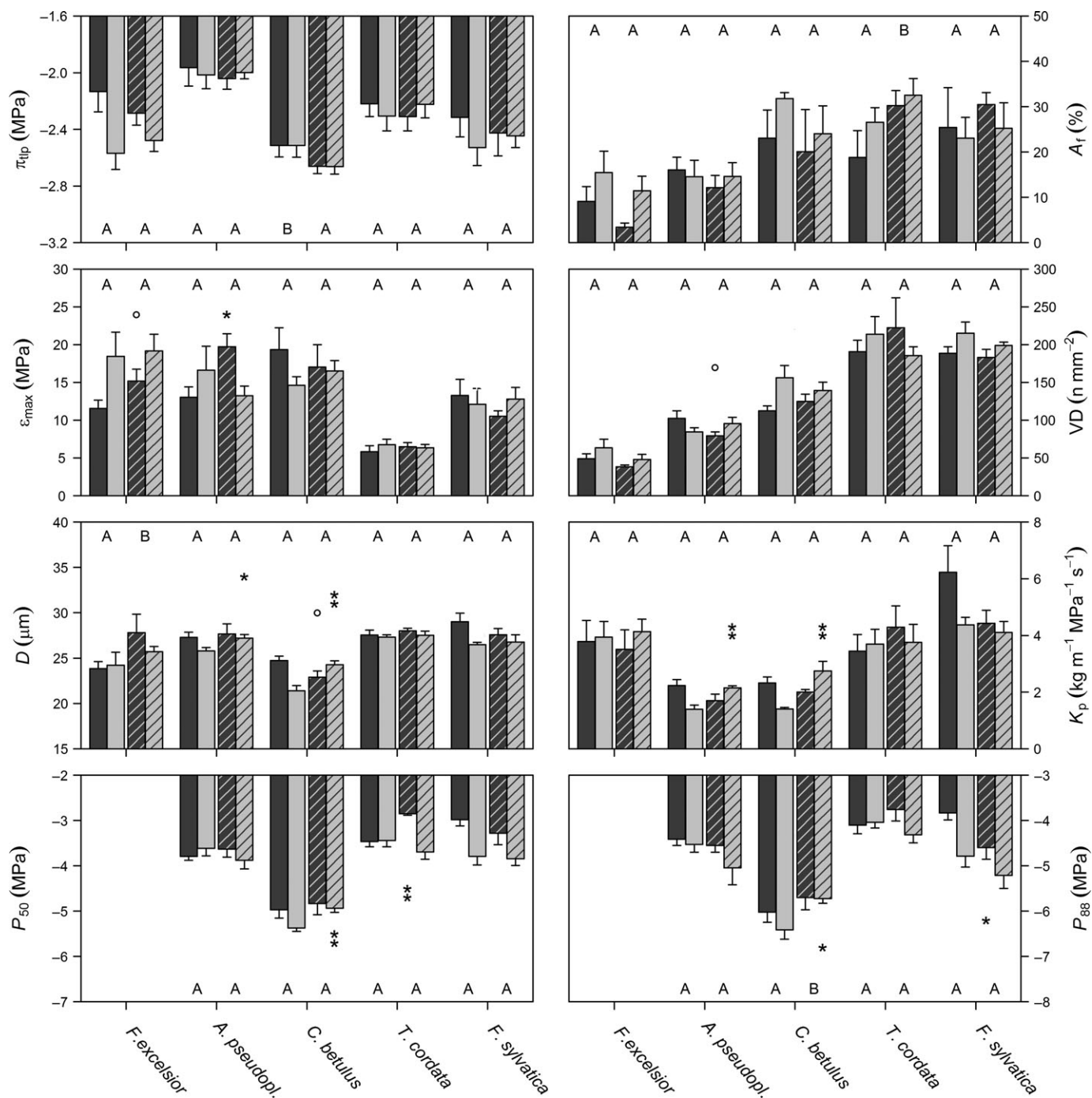
Figure 2. Means ± SE of selected traits in the monoculture (solid bars) and mixture (hatched bars) and the moist (dark bars) and dry treatment (light bars) for the five species. Different capital letters indicate differences between plants of a species grown in monoculture or in tree mixture across both levels of soil water availability significant at $P < 0.1$. Symbols mark significant tree mixture effects for a species within the same soil moisture treatment. Significance levels: $^{\circ}P \leq 0.10$, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$. Differences between the moist and dry treatment that were significant only in the monocultures or the mixtures are given in Table S2 available as Supplementary Data at *Tree Physiology* Online.

A main finding of our study is that the drought-induced plasticity of relevant traits varies largely across the co-occurring tree species within a habitat, and within a given tree functional type. Even if co-occurring species possess many similar functional properties, they can respond to drought with different trait plasticity.

Effects of species mixing on leaf water status and hydraulic traits

Two species of our sample, *C. betulus* and *A. pseudoplatanus*, achieved enhancement in xylem hydraulic functioning under dry conditions when heterospecific neighbours were present. Both species increased vessel diameter, and empirical or theoretical

Table 4. Significance of mixture effects on the physiological plasticity of the five tree species in the two soil moisture treatments according to pairwise comparisons with Student's t-test or Mann–Whitney U-test. The G_s, δ^{13}C and K_s data were taken from Lübbe et al. (2016). Arrows show direction of shift in mean values (upward: significant increase, downward: significant decrease, diagonal: increase/decrease at $P \leq 0.10$), significance level: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. For $F.\ excelsior$, no data on cavitation resistance are available.

Species/parameter	Moist					Dry				
	F. excelsior	*A. pseudopl.*	*C. betulus*	*T. cordata*	*F. sylvatica*	*F. excelsior*	*A. pseudopl.*	*C. betulus*	*T. cordata*	*F. sylvatica*
π_0										
π_{tlp}										
RWC$_{tlp}$										
A_f										
ε_{max}	↗	↑*								
G_s						↑*	↘			
δ^{13}C	↓*					↓*		↘		↓*
A_{Lumen}		↘								
VD		↘								
D			↘				↑*	↑**		
D_h					↘		↑*	↑***		
K_s	↗		↓*					↑*		
K_p							↑**	↑**		
P_{50}				↑**				↑**		
P_{88}					↓*			↑*		

hydraulic conductivity in comparison with plants grown in monocultures. In the case of *C. betulus*, increased hydraulic efficiency was achieved at the cost of xylem safety. However, our results point to less intense interspecific than intraspecific competition for those species when water is scarce. The superior performance of *C. betulus* in water-stressed mixed assemblages is also mirrored by the largest relative growth rate increase from the monoculture to the mixture among the five species (see Figure S1 available as Supplementary Data at *Tree Physiology* Online, Lübbe et al. 2015). In contrast, both species seem to be less competitive under ample soil water supply as indicated by significant interaction terms between soil drought and tree mixture effects in wood anatomical or hydraulic traits. The inferiority of *A. pseudoplatanus* in well-watered mixed species assemblages due to more intense crowding is not only documented by low productivity (see Table S1 available as Supplementary Data at *Tree Physiology* Online), but also by reduced foliar tissue elasticity (higher ε_{max}) in comparison with plants from monocultures, which indicates conditions of increased competition by heterospecific neighbours.

Our assumption that neighbouring species diversity affects the magnitude of modifications to drought in certain species (third hypothesis) was confirmed by the physiological adjustments of *C. betulus* and *A. pseudoplatanus* in response to tree neighbourhood. The observed modifications were exclusively related to xylem structural properties and functionality, but provided distinct benefits to the xylem hydraulic pathway and growth performance. In contrast, saplings of *F. sylvatica* obtained the highest hydraulic efficiency when other tree species were excluded. The xylem structure and hydraulic functioning seemed to be equally impaired by reduced soil water availability and the admixture of heterospecific neighbours. The inferior competitive ability of beech in this experiment is also expressed by low biomass production, especially in the dry treatment (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). The relatively slow growth of young beech trees is well known (Lei et al. 2012, Beyer et al. 2013, Lübbe et al. 2015), which contrasts with the relatively high productivity and competitive ability in the adult stage (Ellenberg and Leuschner 2010). The other two species, *F. excelsior* and *T. cordata*, showed no clear physiological response to alterations in tree neighbourhood, at least when water was scarce. However, *F. excelsior* exhibited enhanced leaf hydraulic conductance (G_s) by species mixture under dry conditions (Lübbe et al. 2016), which is in line with the supposedly high drought tolerance and thus competitiveness of this species (Köcher et al. 2009).

Conclusions

Our drought trial with five common Central European broadleaved tree species suggests that drought acclimation at the leaf and the stem level may often represent alternatives rather than interacting components of a drought-response strategy. Across different species, a given drought regime is unlikely to trigger a similarly plastic drought response in all species that coexist in a habitat. In our species sample, no species showed significant

adjustment at both the leaf and the stem level. However, high trait plasticity in the face of drought in one plant organ does not necessarily preclude plasticity in other organs, which suggests that understanding drought-response strategies of trees requires studying more than one organ (e.g., leaves, stem and roots).

As in all experiments with juvenile trees, sapling data cannot be extrapolated to adult trees due to ontogenetic changes in many relevant traits. Furthermore, other factors than leaf water status regulation and modification of xylem hydraulic properties should be considered that can also determine a tree's drought sensitivity, among them stem water storage and root system acclimation to drought.

The finding that certain species modify their stem hydraulic system in mixture in response to heterospecific neighbours as compared with monoculture produces evidence that the specific neighbourhood of a tree can have a significant impact on functional traits beyond productivity. The results also show that mixtures can have positive, as well as negative, effects on the performance of trees in comparison with monocultures, which should be taken into account in the recent debate about assumed positive effects of diversity on forest ecosystem functioning.

Supplementary Data

Supplementary data for this article are available at *Tree Physiology* Online.

Acknowledgments

Conflict of interest

None declared.

Funding

References

Allen CD, Macalady AK, Chenchouni H et al. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manage 259:660–684.

Anderegg WRL, Berry JA, Smith DD, Sperry JS, Anderegg LDL, Field CB (2011) The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. Proc Natl Acad Sci USA 109:233–237.

Bartlett MK, 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.

Beyer F, Hertel D, Jung K, Fender A-C, Leuschner C (2013) Competition effects on fine root survival of *Fagus sylvatica* and *Fraxinus excelsior*. For Ecol Manage 302:14–22.

Bravo-Oviedo A, Pretzsch H, Ammer C et al. (2014) European Mixed Forests: definition and research perspectives. For Syst 23:518–533.

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, Herzog C, Dawes MA, Arend M, Sperisen C (2015) How tree roots respond to drought. Front Plant Sci 6:547.

Bussotti F, Pollastrini M, Holland V, Brüggemann W (2014) Functional traits and adaptive capacity of European forests to climate change. Environ Exp Bot 111:91–113.

Caquet B, Barigah TS, Cochard H, Montpied P, Collet C, Dreyer E, Epron D (2009) Hydraulic properties of naturally regenerated beech saplings respond to canopy opening. Tree Physiol 29: 1395–1405.

Cheung Y, Tyree M, Dainty J (1975) Water relations parameters on single leaves obtained in a pressure bomb and some ecological interpretations. Can J Bot 53:1342–1346.

Choat B, Jansen S, Brodribb TJ et al. (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–755.

Cochard H, Damour G, Bodet C (2005) Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. Physiol Plant 124: 410–418.

del Río M, Schütze G, Pretzsch H (2013) Temporal variation of competition and facilitation in mixed species forests in Central Europe. Plant Biol 16:166–176.

Domec J-C, Gartner BL (2001) Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. Trees 15:204–214.

Domec J-C, Schäfer K, Oren R, Kim HS, McCarthy HR (2010) Variable conductivity and embolism in roots and branches of four contrasting tree species and their impacts on whole-plant hydraulic performance under future atmospheric CO_2 concentration. Tree Physiol 30:1001–1015.

Ellenberg H, Leuschner C (2010) Vegetation Mitteleuropas mit den Alpen, 6th edn. Ulmer, Stuttgart.

Ewers FW, Ewers JM, Jacobsen AL, Lopez-Portillo J (2007) Vessel redundancy: modeling safety in numbers. IAWA J 28:373–388.

Fonti P, Heller O, Cherubini P, Rigling A, Arend M (2013) Wood anatomical responses of oak saplings exposed to air warming and soil drought. Plant Biol (Stuttg) 15:210–219.

Forrester DI (2014) The spatial and temporal dynamics of species interactions in mixed-species forests: from pattern to process. For Ecol Manage 312:282–292.

Forrester DI (2015) Transpiration and water-use efficiency in mixed-species forests versus monocultures: effects of tree size, stand density and season. Tree Physiol 35:289–304.

Forrester DI, Theiveyanathan S, Collopy JJ, Marcar NE (2010) Enhanced water use efficiency in a mixed *Eucalyptus globulus* and *Acacia mearnsii* plantation. For Ecol Manage 259:1761–1770.

Gleason SM, Westoby M, Jansen S et al. (2016) Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. New Phytol 209:123–136.

Grossiord C, Granier A, Gessler A, Jucker T, Bonal D (2014a) Does drought influence the relationship between biodiversity and ecosystem functioning in boreal forests? Ecosystems 17:394–404.

Grossiord C, Granier A, Ratcliffe S et al. (2014b) Tree diversity does not always improve resistance of forest ecosystems to drought. Proc Natl Acad Sci USA 111:14812–14815.

Guicherd P, Peltier J, Gout E, Bligny R, Marigo G (1997) Osmotic adjustment in Fraxinus excelsior L: malate and mannitol accumulation in leaves under drought conditions. Trees 11:155–161.

Hacke U, Sperry J (2001) Functional and ecological xylem anatomy. Perspect Plant Ecol Evol Syst 4:97–115.

Hajek P, Leuschner C, Hertel D, Delzon S, Schuldt B (2014) Trade-offs between xylem hydraulic properties, wood anatomy and yield in Populus. Tree Physiol 34:744–756.

Herbette S, Wortemann R, Awad H, Huc R, Cochard H, Barigah TS (2010) Insights into xylem vulnerability to cavitation in Fagus sylvatica L: phenotypic and environmental sources of variability. Tree Physiol 30:1448–1455.

IPCC (2013) Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA, p 1535.

Jansen S, Schuldt B, Choat B (2015) Current controversies and challenges in applying plant hydraulic techniques. New Phytol 205: 961–964.

Klein T (2014) The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. Funct Ecol 28:1313–1320.

Kleyer M, Minden V (2015) Why functional ecology should consider all plant organs: an allocation-based perspective. Basic Appl Ecol 16:1–9.

Köcher P, Gebauer T, Horna V, Leuschner C (2009) Leaf water status and stem xylem flux in relation to soil drought in five temperate broad-leaved tree species with contrasting water use strategies. Ann For Sci 66:1.

Köcher P, Horna V, Leuschner C (2013) Stem water storage in five coexisting temperate broad-leaved tree species: significance, temporal dynamics and dependence on tree functional traits. Tree Physiol 33: 817–832.

Kozlowski T, Pallardy S (2002) Acclimation and adaptive responses of woody plants to environmental stresses. Bot Rev 68:270–334.

Kunert N, Schwendenmann L, Potvin C, Hölscher D (2012) Tree diversity enhances tree transpiration in a Panamanian forest plantation. J Appl Ecol 49:135–144.

Lebourgeois F, Gomez N, Pinto P, Mérian P (2013) Mixed stands reduce Abies alba tree-ring sensitivity to summer drought in the Vosges mountains, western Europe. For Ecol Manag 303:61–71.

Le Dantec V, Dufrêne E, Saugier B (2000) Interannual and spatial variation in maximum leaf area index of temperate deciduous stands. For Ecol Manage 134:71–81.

Legner N, Fleck S, Leuschner C (2013) Low light acclimation in five temperate broad-leaved tree species of different successional status: the significance of a shade canopy. Ann For Sci 70:557–570.

Lei P, Scherer-Lorenzen M, Bauhus J (2012) Belowground facilitation and competition in young tree species mixtures. For Ecol Manage 265:191–200.

Lens F, Tixier A, Cochard H, Sperry JS, Jansen S, Herbette S (2013) Embolism resistance as a key mechanism to understand adaptive plant strategies. Curr Opin Plant Biol 16:287–292.

Lenz TI, Wright IJ, Westoby M (2006) Interrelations among pressure-volume curve traits across species and water availability gradients. Physiol Plant 127:423–433.

Leuschner C, Jungkunst HF, Fleck S (2009) Functional role of forest diversity: pros and cons of synthetic stands and across-site comparisons in established forests. Basic Appl Ecol 10:1–9.

Lewis AM, Boose ER (1995) Estimating volume flow rates through xylem conduits. Am J Bot 82:1112–1116.

Li S, Lens F, Espino S, Karimi Z, Klepsch M, Schenk J, Schmitt M, Schuldt B, Jansen S (2016) Intervessel pit membrane thickness as a key determinant of embolism resistance in angiosperm xylem. IAWA J 37: 152–171.

Lübbe T, Schuldt B, Leuschner C (2015) Species identity and neighbor size surpass the impact of tree species diversity on productivity in experimental broad-leaved tree sapling assemblages under dry and moist conditions. Front Plant Sci 6:1–12.

Lübbe T, Schuldt B, Coners H, Leuschner C (2016) Species diversity and identity effects on the water consumption of tree sapling assemblages under ample and limited water supply. Oikos 125:86–97.

Marigo G, Peltier J-P, Girel J, Pautou G (2000) Success in the demographic expansion of Fraxinus excelsior L. Trees 15:1–13.

Maseda PH, Fernández RJ (2006) Stay wet or else: three ways in which plants can adjust hydraulically to their environment. J Exp Bot 57: 3963–3977.

McDowell NG (2011) Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. Plant Physiol 155:1051–1059.

Metz J, Annighöfer P, Schall P, Zimmermann J, Kahl T, Schulze ED, Ammer C (2016) Site-adapted admixed tree species reduce drought susceptibility of mature European beech. Glob Chang Biol 22: 903–920.

Mölder I, Leuschner C (2014) European beech grows better and is less drought sensitive in mixed than in pure stands: tree neighbourhood effects on radial increment. Trees 28:777–792.

Neuner S, Albrecht A, Cullmann D et al. (2015) Survival of Norway spruce remains higher in mixed stands under a dryer and warmer climate. Glob Chang Biol 21:935–946.

Nicotra AB, Atkin OK, Bonser SP et al. (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 15:684–692.

Ogaya R, Penuelas J (2006) Contrasting foliar responses to drought in Quercus ilex and Phillyrea latifolia. Biol Plant 50:373–382.

Pammenter N, Van der Willigen C (1998) A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. Tree Physiol 18:589–593.

Peltier J-P, Marigo G (1999) Drought adaptation in Fraxinus excelsior L: physiological basis of the elastic adjustment. J Plant Physiol 154: 529–535.

Pollastrini M, Holland V, Brüggemann W, Koricheva J, Jussila I, Scherer-Lorenzen M, Berger S, Bussotti F (2014) Interactions and competition processes among tree species in young experimental mixed forests, assessed with chlorophyll fluorescence and leaf morphology. Plant Biol (Stuttg) 16:323–331.

Pretzsch H, Schütze G, Uhl E (2013) Resistance of European tree species to drought stress in mixed versus pure forests: evidence of stress release by inter-specific facilitation. Plant Biol 15:483–495.

Quero J, Villar R, Marañón T, Zamora R (2006) Interactions of drought and shade effects on seedlings of four Quercus species: physiological and structural leaf responses. New Phytol 170:819–834.

R Development Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sass U, Eckstein D (1995) The variability of vessel size in beech (Fagus sylvatica L.) and its ecophysiological interpretation. Trees 2: 247–252.

Schreiber SG, Hacke UG, Hamann A (2015) Variation of xylem vessel diameters across a climate gradient: insight from a reciprocal transplant experiment with a widespread boreal tree. Funct Ecol 29: 1392–1401.

Schuldt B, Knutzen F, Delzon S, Jansen S, Müller-Haubold H, Burlett R, Clough Y, Leuschner C (2016) How adaptable is the hydraulic system of European beech in the face of climate change-related precipitation reduction? New Phytol 210:443–458.

Sperry J, Donnelly J, Tyree M (1988) A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ 11: 35–40.

Sperry J, Nichols K, Sullivan J, Eastlack S (1994) Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. Ecology 75:1736–1752.

Sperry J, Hacke U, Pittermann J (2006) Size and function in conifer tracheids and angiosperm vessels. Am J Bot 93:1490–1500.

Sperry JS, Meinzer FC, McCulloh KA (2008) Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. Plant Cell Environ 31:632–645.

Tyree M, Hammel H (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J Exp Bot 23:267–282.

Valladares F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J Ecol 94:1103–1116.

Valladares F, Gianoli E, Gómez J (2007) Ecological limits to plant phenotypic plasticity. New Phytol 176:749–763.

Vilagrosa A, Bellot J, Vallejo VR, Gil-Pelegrin E (2003) Cavitation, stomatal conductance, and leaf dieback in seedlings of two co-occurring Mediterranean shrubs during an intense drought. J Exp Bot 54:2015–2024.

Vilagrosa A, Chirino E, Barigah T, Cochard H, Gil-Pelegrin E (2012) Plant responses to drought stress. In: Aroca R (ed) Plant responses to drought. Springer, Berlin, Heidelberg, p 426.

Von Willert D, Matyssek R, Herppich W (1995) Experimentelle Pflanzenökologie: Grundlagen und Anwendungen. Thieme-Verlag, Stuttgart.

Wortemann R, Herbette S, Barigah TS, Fumanal B, Alia R, Ducousso A, Gomory D, Roeckel-Drevet P, Cochard H (2011) Genotypic variability and phenotypic plasticity of cavitation resistance in *Fagus sylvatica* L across Europe. Tree Physiol 31:1175–1182.

Zimmermann J, Hauck M, Dulamsuren C, Leuschner C (2015) Climate warming-related growth decline affects *Fagus sylvatica*, but not other broad-leaved tree species in Central European mixed forests. Ecosystems 18:560–572.