Species diversity and identity effects on the water consumption of tree sapling assemblages under ample and limited water supply

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Studies examining the influence of biodiversity on ecosystem functioning have rarely considered water turnover, the quantitatively most important biogeochemical flux in ecosystems and a process with high sensitivity to climate warming. With a tree sapling experiment consisting of three diversity levels (1, 3, 5 species), 11 different species combinations and two soil moisture levels (moist and dry), we examined the influence of tree species diversity and species identity on stand transpiration (T) under ample and restricted water supply. We further asked whether growth in mixture leads to adaptive responses in the hydraulic system and water loss regulation in plants with heterospecific neighbors compared to plants in monoculture. In moist soil, T was on average ~11% higher in the mixtures than in the monocultures (significant net diversity effect), which can mostly be attributed to a selection effect. Overyielding in T was highest in mixtures when *Tilia cordata* and/or *Fraxinus excelsior* were present. Both species developed larger leaf areas (LA) and sapwood areas (SA) in monocultures than the other species and furthermore increased LA and SA from the monocultures to the mixtures. Thus, inherent species differences in LA and hydraulics, but also neighbor effects on these traits determined T to a large extend. In dry soil, the diversity effect on T was not larger but slightly smaller, which is not in agreement with other published studies. We conclude that differences between pure and mixed sapling assemblages in stand water consumption and drought response are mainly caused by species identity effects, while species diversity seems to be less influential.

Ever rising human impact on ecosystems has raised concern about consequences of biodiversity loss for ecosystem functioning (Tilman 1999, Balvanera et al. 2006). Research into the relationship between biodiversity and ecosystem functioning (B-EF) has recently shifted to forests which are a key resource for humans to meet the wood and fuel demand and that harbor about two thirds of the world's terrestrial biodiversity (Millennium Ecosystem Assessment 2005). Theory predicts a positive effect of species richness on productivity and other ecosystem functions primarily through three mechanisms, niche complementarity which may reduce competition, facilitation and selection (or sampling) effects (Fridley 2001, Loreau and Hector 2001, Hooper et al. 2005). If valid on larger scales, more diverse forests should produce more timber and sequester more carbon than forests with lower tree species numbers. General evidence for a positive diversity–productivity relationship in tree communities is broadly provided (Zhang et al. 2012, Scherer-Lorenzen 2014). Another ecosystem function with possible dependence on species richness is plant water consumption, which determines deep seepage and groundwater recharge. Since enhanced productivity is often associated with increased water consumption (Law et al. 2002), more diverse forests could also transpire more water which can have implications for soil water availability and water management.

The effect of tree diversity on water consumption is much less studied than the diversity–productivity relationship. We are aware of only eight studies that compared the plot-level water consumption of monospecific and mixed stands of woody plants; the obtained results on diversity effects were mixed. Two of three experimental studies in tree plantations (boreal, temperate or tropical) showed increases in stand transpiration with higher diversity (Forrester et al. 2010, Kunert et al. 2012), while the third one did not (Grossiord et al. 2013). Higher water consumption of the mixtures was in all cases linked to higher productivity. Observational studies in mixed forests (temperate or Mediterranean) produced inconsistent results as well. Soil water depletion was greater under mixed stands of *Fagus sylvatica* and *Picea abies* than under the respective pure stands due to the deeper-reaching and more intense root system of beech (Schume et al. 2004). In old-growth 1-, 3- and 5-species stands with diluting beech abundance (*F. sylvatica*) in the Hainich forest (Germany), Gebauer et al. (2012) found in a sap flux study higher transpiration rates for the mixed stands than the pure beech stand in a moist summer, while no stand differences existed in a relatively dry summer. The latter observations are mirrored by patterns of soil water extraction conducted during the same period with a soil water balance modeling approach (Krämer and Hölscher 2010). Also in

the Hainich forest, Meißner et al. (2013) found no systematic differences in water consumption among small-sized tree clusters that differed in species composition and species richness (1–3 species). Different deuterium signatures in the xylem water of coexisting tree species in mixed stands pointed, however, at partitioning of soil water among species during a soil desiccation period and thus may indicate a certain complementarity in water use (Meißner et al. 2012). In drought-exposed Mediterranean mixed oak forests, interactions were driven by competition for water which reduced the transpiration of the inferior species (Grossiord et al. 2014a). In conclusion of the existing results, diversity may enhance plot-level water consumption when productivity increases with diversity, but it is unlikely to be higher in those cases where productivity is not enhanced by diversity.

Certain tree species such as *Abies alba* and *F. sylvatica* were found to be less sensitive to summer droughts when growing in mixtures as compared to monocultures (Lebourgeois et al. 2013, Pretzsch et al. 2013, Mölder and Leuschner 2014). Possible underlying mechanisms are hydraulic redistribution, complementary use of soil water and species differences in water consumption that result in reduced competition for water in mixed as compared to pure stands (Pretzsch 2013, Forrester 2014).

The diversity–water consumption relation may be influenced by climate, soil fertility, stand structure and the functional properties of the tree species (Forrester 2014). The dependence on resource availability is addressed by the stress-gradient hypothesis which predicts that the net outcome of biotic interactions shifts along gradients of limiting physical conditions. Positive diversity effects (facilitation) should be more common in severely resource-limited environments while negative interactions (competition) should dominate in richer and less-stressful environments (Callaway and Walker 1997). Since climate change scenarios predict increasing drought frequencies for various regions of Europe (IPCC 2013), facilitative interactions could become more important in forests that are increasingly affected by drought. This could result in a growing diversity effect on the carbon and water cycles in forests, because facilitation is one of the mechanisms that may drive a diversity effect. In contrast to this assumption, the modeling study by Morin et al. (2011) and some empirical evidence suggest that the benefit from mixtures could be greater in favorable and not in unfavorable and stressful environments (Dieler and Pretzsch 2013, Forrester et al. 2013). Thus, some uncertainty remains with respect to the importance of diversity effects under ample and limited soil moisture.

Deeper insights into the mechanisms, by which diversity or tree identity are influencing water turnover in forest ecosystems under both unlimited and resource-limited conditions, are urgently needed. This requires measurement of water consumption in monospecific and mixed stands at the stand and tree level and examination of plant traits underlying drought tolerance. The latter can reveal possible adaptive responses of leaf area and the water conducting system of the species to variable neighborhood constellations as are characteristic for mixed stands.

Here, we present the results of a diversity experiment with tree saplings aimed at investigating the diversity–water consumption relationship at the stand and tree levels, and

for moist and dry soil. The experiment was conducted with five temperate broad-leaved tree species (*Fraxinus excelsior, Acer pseudoplatanus, Carpinus betulus, Tilia cordata, Fagus sylvatica*), which are common in central European mixed forests and known to differ substantially with respect to their drought response (Köcher et al. 2009, Supplementary material Appendix 1 Table A1). *Fraxinus excelsior, T. cordata* and *C. betulus* are classified as more or less drought tolerant with respect to growth, while *F. sylvatica* and *A. pseudoplatanus* exhibit drought-sensitive properties (Zimmermann et al. 2015). We conducted a replicated outdoor growth experiment in large pots with 1- to 2-year-old saplings of the five species which consisted of three diversity levels (1-, 3- and 5-species), 11 species combinations and two soil moisture treatments (moist versus dry). Stand transpiration was measured by gravimetric quantification of the water balance of the pots. Six morphological and physiological plant traits related to tree water consumption (total leaf area, sapwood area in the stem, sapwood area-specific hydraulic conductivity, Huber value, stomatal conductance and leaf carbon isotope ratio) were measured to test the following hypotheses: 1) stand-level water consumption increases with tree species diversity, 2) diversity effects on stand-level water consumption are enhanced in dry soil due to the increasing importance of positive interactions, 3) more diverse stands reduce leaf conductance and stand transpiration upon drought to a lesser degree than the average monoculture due to the expansion of drought-adapted species in the mixtures (diversity–community resistance hypothesis), and 4) the water flux regulation and hydraulic properties (leaf conductance and xylem hydraulic conductivity) of individual trees in mixed stands are influenced by the water consumption of heterospecific neighbors.

Material and methods

Plant material and experimental design

In April 2011, a diversity experiment with saplings of sycamore maple *Acer pseudoplatanus*, European hornbeam *Carpinus betulus*, European beech *Fagus sylvatica*, European ash *Fraxinus excelsior* and small-leaved linden *Tilia cordata* was established in the Experimental Botanical Garden of Göttingen University (51°33′N, 9°57′E; 177 m a.s.l.). These species belong to five different families representing a broad variety of tree functional types differing in light demand and shade tolerance, canopy architecture, mycorrhizal type, drought tolerance and successional status (Köcher et al. 2013, Supplementary material Appendix 1 Table A1). The 1- to 2-year-old plants with an initial height of ~40–60 cm were obtained from a nursery close to Göttingen. They were cultivated outdoors under uniform conditions for 16 months to enable comparative measurements of water consumption, productivity, and a number of morphological and physiological parameters related to hydraulic architecture and growth. Five saplings were grown in each pot of 0.05 m^3 volume (height 0.30 m, diameter 0.58 m) filled with coarse-grained sand (98% sand, 1.8% silt, 0.2% clay) in a systematic planting scheme with equal distances between the plants (ca 17 cm; distance to pot walls: ca 12 cm). The sand had a

pH of 7.5 (Supplementary material Appendix 1 Table A2) for accommodating *F. excelsior*, *C. betulus* and *A. pseudoplatanus* which prefer neutral to base-rich soils (Ellenberg and Leuschner 2010). Four saplings were planted at the corners of a square placed on the pot surface and the fifth was positioned in the center. Experimental plants were selected from a larger number of plants according to similarity in plant height and number of leaf buds. We established three diversity levels (1, 3 and 5 species) and grew all five species either in monoculture (all five plants of the same species; five types of monocultures), in 3-species mixture (five of the ten possible three-species mixtures were established), or in 5-species mixture (all plants of different species identity; Table 1). In the 3-species mixtures, two species were present with each two plants and a third species with a single plant. The saplings were arranged in a pattern that minimized intraspecific competition (either two plants of the same species in opposite corners of the square, or a single plant in the center). The abundance of the species (one or two plants) and their spatial position differed among the replicates, i.e. three species reached on average equal abundances in the seven pots of a treatment and were exposed to comparable interspecific competition intensity.

The experiment consisted of a moist and a dry treatment, yielding 22 treatments in total (11 species combinations × 2 water levels), which were replicated 6 to 8-fold (Table 1). Drought was applied in the period July to September 2011 and May to August 2012. The volumetric soil water content (SWC) of each pot was kept in a constant range by adding every 3–5 days that amount of water which had been lost through evapotranspiration since the last adjustment. The target value of maximal SWC in the moist treatment was set to ~21% (95% of field capacity in the sandy soil) and ~12% for the dry treatment (i.e. 57% of field capacity). The difference between a pot's actual total and target weight in the respective treatment determined the amount of water to be added. The individual target weight was obtained from

the mass of dry soil in the pot (derived from soil bulk density and soil volume in the pot) and target SWC. Regular rewetting resulted in moderate fluctuation of SWC and soil matric potential (Ψ_{soil}) below the target value (Fig. 1). Estimates of Ψ_{soil} in the pots were obtained from volumetric soil moisture data with an empirically-derived soil moisture-soil water potential curve implemented in the LeachM model (British Soil Service, Hutson and Wagenet 1992) under consideration of grain size distribution, soil bulk density and soil organic carbon concentration. In the last phase of the experiment (summer 2012), the target values for the dry treatment were allowed to vary slightly between pots (11–14% SWC) according to the variable water consumption of the assemblages, in order to generate comparable stress intensities in all pots at the end of the measurement intervals.

The pots were placed outdoors under a rain shelter made of transparent plexiglass, which excluded rainfall and thus allowed controlling water turnover. Although the flux density of photosynthetically active radiation (PAR) was reduced by approx. 30%, a pronounced greenhouse effect could be excluded. The pots were arranged randomly in a grid pattern for minimizing possible effects of environmental gradients. We fertilized all pots monthly between May and September 2011 with 4 ml solution (8.0% N, 8.0% P_2O_5, 6.0% K_2O), and with 6 ml between March and August 2012.

PAR was measured continuously at a weather station close to the experimental site using a quantum sensor. In close vicinity of the pots, relative air humidity and air temperature were continually recorded and 30-min means stored.

Transpiration measurement

Pot-specific transpiration was measured by weighing every 3–4 d with a digital hanging scale (resolution 1 g, max. load 150 kg; tot. error 0.02%, i.e. < 20 g for pots < 100 kg). The scale was fixed to a self-constructed mobile weighing vehicle made of steel tubes. The pots were placed on wooden boards equipped with suspension points for hooking and lifting the pots with an electric steel rope winch. The transducer measured the traction force on the rope during the lift. For every pot, we calculated pot total weight (dry soil plus pot weight) and pot target weight at 21% (moist treatment) or 12% SWC (dry treatment). Weight loss between two sequential measuring dates (time interval: 3–4 d) was assumed to represent water loss through transpiration and soil evaporation. According to percolation experiments, the 21%-target moisture in the moist treatment did not result in water percolation through the pot bottom. By covering the soil surface of the pots in certain measuring periods tightly with plastic sheets, soil evaporation could be minimized and the measured weight loss was caused nearly exclusively by transpirative water loss. This was confirmed by using control pots containing only bare soil (n = 8 in the moist and 7 in the dry treatment). In June 2012, soil sealing of these synchronously weighed control pots suppressed > 95% of evaporation, demonstrating the effective applicability of plastic coverage. Plastic sheets were only installed for the measuring periods of 3 to 4 d and subsequently removed (3 to 4 d) to enable unrestricted gas fluxes between soil and atmosphere in order to avoid hypoxia (Fig. 1). We neglected biomass

Table 1. Design of the experiment with diversity levels, species combination, the two moisture treatments (moist versus dry) and number of replicates. Each 50-l pot contained five saplings. Mono – monoculture, mix 3 – 3-species mixtures, mix 5 – 5-species mixture. A.p. – *Acer pseudoplatanus*, C.b. – *Carpinus betulus*, F.e. – *Fraxinus excelsior*, F.s. – *Fagus sylvatica*, T.c. – *Tilia cordata*.

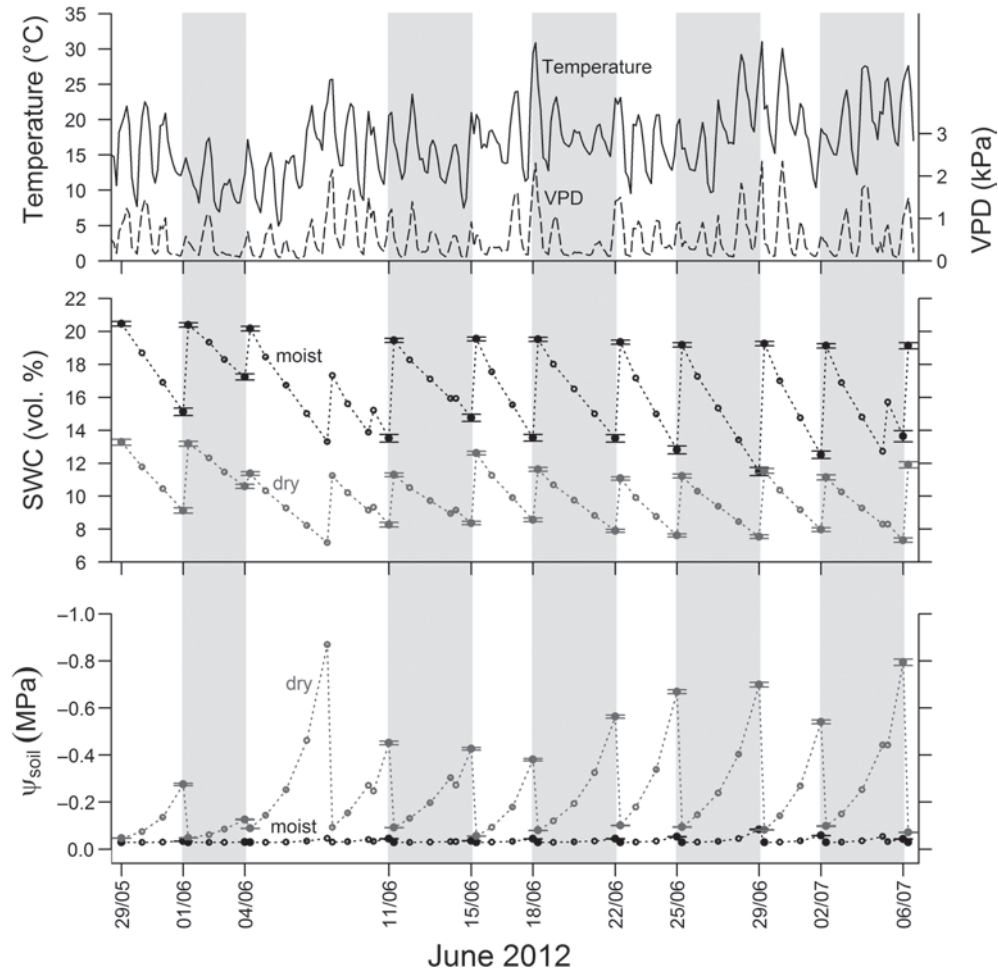| Diversity level	Species combination	Replicates Moist	Dry
mono			
	F. excelsior	7	7
	A. pseudoplatanus	7	7
	C. betulus	7	7
	T. cordata	7	7
	F. sylvatica	7	7
mix 3			
	A.p. – C.b. – F.e.	7	6
	A.p. – C.b. – T.c.	7	6
	A.p. – F.s. – F.e.	7	6
	C.b. – F.s. – T.c.	7	6
	F.e. – F.s. – T.c.	7	6
mix 5			
	A.c. – C.b. – F.s. – F.e. – T.c.	8	7

Figure 1. Air temperature and vapor pressure deficit (VPD) at the experimental site together with volumetric soil water content (SWC) and soil matric potential (Ψ_{soil}) in the pots during the core measuring period from 29 May to 6 July 2012. Periods in grey indicate measuring intervals with soil surface sealing that were used to measure transpiration while excluding soil evaporation. Soil matrix potential was derived from soil water content using the soil moisture–matric potential relationship according to LeachM, British Soil Service, Hutson and Wagenet (1992).

increment in the short weighing intervals, because it was too small to significantly influence the water balance. Thus, we interpreted the entire weight reduction in the measuring interval as water loss caused by transpiration. Mean daily water loss (in mm d^{-1}) was taken as transpiration rate (T). The net biodiversity effect on the transpiration of mixed pots was calculated from Eq. 1 according to Loreau and Hector (2001),

$$NE = Y_O - Y_E = Y_O - \sum_i RY_{Ei} M_i \qquad (1)$$

as the deviation of observed (Y_O) and expected (Y_E) transpiration. RY_{Ei} determines the expected relative transpiration of species i in mixture (derived from the relative abundance of individuals of species i in the mixture at planting). M_i is the transpiration of the respective monoculture.

When calculating the temporal fluctuation of soil moisture in the pots from known soil dry weight and measured actual pot fresh weight, we accounted for long-term biomass increment by interpolating between plant biomass at harvest and biomass at the start of the main measuring

period (early May 2012). Plant biomass in early May before the start of soil desiccation was estimated from allometric equations established for all five species by harvesting each ten individuals of different size and regressing biomass on basal area and shoot height using multiple linear functions. This allowed estimating plant biomass in every pot at any time. For comparing water consumption among the species combinations, we focused on a core measuring period in June/July 2012, i.e. in the second summer after the experiment's implementation. In this five-week period (29 May – 6 July) before plant harvest in July/August 2012, the weather conditions were sufficiently stable and saplings had shown height growth and the formation of additional leaves, which indicates that they had successfully adapted to the specific water regimes since July 2011.

Tree physiological measurements

Stomatal conductance (g_s, mmol m^{-2} s^{-1}), foliar carbon isotope signature ($\delta^{13}C$, ‰) and stem sapwood area-specific hydraulic conductivity (k_s, kg m^{-1} MPa^{-1} s^{-1}) was measured

in selected plants of the five species in order to detect species differences and neighbor effects on the water loss regulation of target plants. During the core measuring period in June/July 2012, each six plants of the five species were investigated in monoculture and in 5-species mixture in the moist and dry treatment, yielding 120 plants ($6 \times 5 \times 2 \times 2$). To avoid pseudo-replication, plants from different pots were investigated as replicates for a species. Measurements of g_s were conducted with a porometer on each two fully developed leaves per plant in the upper canopy. The leaves were tagged and measurements repeated on four occasions in June 2012 at midday during conditions of bright sky with non-fluctuating radiation climate. Two of the four measuring dates refer to the days immediately before an irrigation event; the two others were placed immediately after irrigation.

The same leaves were sampled at the date of harvest in July/August 2012 for carbon isotope analysis in the Center for Stable Isotope Research and Analysis, University of Göttingen, using an isotope mass ratio spectrometer. Axial hydraulic conductivity (k_h, kg m MPa^{-1} s^{-1}) was measured with a XYL'EM apparatus and the software XylWin 3.0 according to Sperry et al. (1988) in the main shoot (segment length ca 30 cm), which was harvested at the experiment's end in July/August 2012. Appending branches and twigs were cut off and lateral scars sealed with quick-drying glue to prevent water loss. Immediately after cutting, the basal end of the shoot was air-tightly connected to the XYL'EM apparatus and hydraulic conductivity measured at low pressure (6 kPa) with filtered (0.20 μm) and degassed water containing 10 mM KCl and 1 mM CaCO$_3$. Maximum hydraulic conductivity was obtained after embolism removal by iterative flushing at high pressure (120 kPa) for 10 min until constant values were reached. For determining stem cross-sectional area (A_{cross}) and the corresponding xylem cross-sectional area without pith and bark (A_{xylem}) of the basal part of the shoot segment, we digitized transversal cuts using a stereo-microscope and analyzed the images with the software ImageJ (ver. 1.44p, <http://rsb.info.nih.gov/ij>). Empirical sapwood area-specific hydraulic conductivity (k_s, kg m^{-1} MPa^{-1} s^{-1}) was calculated for the mean diameter of the shoot segment by dividing k_h by A_{xylem}, which was derived from the specific ratio Across : Axylem.

Tree morphological analysis

All plants were harvested in a seven-week period in July and August 2012 in a rotating scheme with each one replicate pot of a treatment harvested every week, which guaranteed the same duration of the experimental treatment in all categories. Stem diameter at stem base was measured in two directions to calculate basal area (BA). The corresponding sapwood area (SA) at the stem base was extrapolated with the measured A_{cross}/A_{xylem} ratio from the stem segment that had been inspected microscopically. The specific leaf area (SLA, cm^2 g^{-1}) of fully expanded leaves in the upper crown was determined for all species in six selected treatment categories (three different diversity levels in the moist and dry treatment) with each six plants from different pots investigated per category. At least ten leaves per tree (depending on species) were sampled by random and leaf area (LA) analyzed on a flatbed scanner using WinFolia 2005b software.

The samples were subsequently oven-dried (70°C, 72 h) and weighed. The mean SLA of a treatment was used for calculating plant leaf area from leaf dry mass. The water comsumption of the tree assemblages was found to be significantly affected by tree size as well as by water-use related morphological properties LA and SA (Supplementary material Appendix 1 Fig. A1). To account for these size effects, daily transpiration rate was expressed per leaf area (T_{LA}) or sapwood area (T_{SA}) in the stem by dividing water loss per pot by the pot's total leaf area or cumulative sapwood area.

Statistical analysis

Statistical analyses were conducted with R software (ver. 3.0.0; <www.r-project.org>). A significance level of $p \leq 0.05$ was used in most tests; in a few cases, marginally significant results ($p \leq 0.1$) are also reported. All data sets were tested for normal distribution (Shapiro–Wilk test) and homogeneity of variances (Levene's test). The grand means of a net diversity effect on stand transpiration were tested against zero using a one-sample t-test to examine whether they differed significantly from the weighted average transpiration of the monocultures. To test for treatment effects and possible interactions between soil moisture level and species composition or tree diversity on transpiration rate (parameters T, T_{LA}, or T_{SA}), we applied two-way analysis of variance (ANOVA). When testing for diversity effects, two-way ANOVA was adjusted (type III sum of squares, 'Anova (), car' package) to account for unbalanced data. Tukey contrasts ('glht' procedure, 'multcomp' package) were used for multiple comparisons among different species combinations and diversity levels in the moist and dry treatment. Differences between the moist and dry treatments for the given species compositions or diversity levels were investigated with Student's t-test. Multiple comparisons among species or diversity levels of morphological or physiological parameters were conducted with one-way ANOVA followed by the Tukey contrasts procedure. Pairwise comparisons between the moist and dry treatments or the diversity levels (mono versus mix5 category) were conducted with Student's t-test, Welch's t-test or Mann–Whitney U-test depending on the structure of the data.

Data from this investigation are publically available in the Plant Trait Database (TRY) <www.try-db.org/TryWeb/Data.php#8;>

Results

Weather and soil moisture conditions

In the core measuring period (29 May to 6 July 2012), daily air temperature means varied between 9.5 and 22.8°C with an absolute maximum of 31.7°C at the end of June (Fig. 1: upper panel). Daily maximal vapor pressure deficit ranged between 0.21 kPa and 2.54 kPa and reached peak values > 1.5 kPa during ten days. Volumetric soil water content (SWC) typically fluctuated between 13.0 and 20.5% in the pots of the moist treatment and between 7.2 and 13.3% in the dry treatment with minima recorded before and maxima after irrigation events (Fig. 1: central panel). Soil matrix

potential (Ψ_{soil}) minima were calculated as –0.08 MPa in the moist and –0.86 MPa in the dry treatment immediately before irrigation events (Fig. 1: lower panel).

Stand-level water consumption: species diversity effects

In the five-week core measuring period, stand-level (pot-level) transpiration means (T; five plants each) ranged between 0.54 and 1.06 l d^{-1} in the moist treatment (expressed per pot surface area: means of 2.04–3.99 mm d^{-1}). A significant net diversity effect was found for the moist treatment (grand mean tested against zero: t = 4.98, p < 0.01). The transpiration rate increased on average from the monocultures to the 3-species mixtures by 11.0% and to the 5-species mixture by 11.8%. However, the transpiration means of the three diversity levels (3.02, 3.35 and 3.37 mm d^{-1}; Fig. 2) were not significantly different at p < 0.05 (ANOVA). Transpiration normalized to leaf area (T_{LA}; given as ml m^{-2} d^{-1} which is equivalent to 10^{-3} mm d^{-1}; for convenience, fluxes in mol m^{-2} d^{-1} are also given) was also not significantly different between the three diversity levels (moist treatment: 534 – 576 ml m^{-2} d^{-1}, i.e. 29.6–32.0 mol m^{-2} d^{-1}; Fig. 2: center panel).

In the dry treatment, average transpiration rate (T) was reduced to 59–62% of the corresponding water loss in the moist treatment (means of 0.39–0.67 l d^{-1}, equivalent to 1.46–3.54 mm d^{-1} per pot surface area). A significant net diversity effect on T existed (grand mean: t = 2.48, p < 0.05) with average increases to the 3-species and the 5-species mixtures by 6.8 and 11.6%, respectively, but the differences between the group means again were not significant. One-, 3- and 5-species pots showed very similar reductions of leaf area-specific transpiration in the dry treatment (to 401, 382 and 379 ml m^{-2} d^{-1} or 21.0–22.3 mol m^{-2} d^{-1}, Fig. 2: right part) with no diversity effect in the degree of reduction.

Two-way ANOVA with transpiration rate (three different parameters) as dependent and moisture treatment, species composition, and the interaction of moisture treatment × species composition as independent variables revealed a highly significant effect of moisture treatment (moist versus dry) and a less influential effect of species composition on water consumption. Species composition effects were detected when different sets of combinations were pooled (all 11 combinations, all monocultures, or all 3-species combinations) but not when the three diversity levels were compared indicating that species identity, but not diversity, influenced water consumption. Significant interaction effects (moisture treatment × species composition) were detected mainly in case of T_{LA} as dependent variable (Table 2).
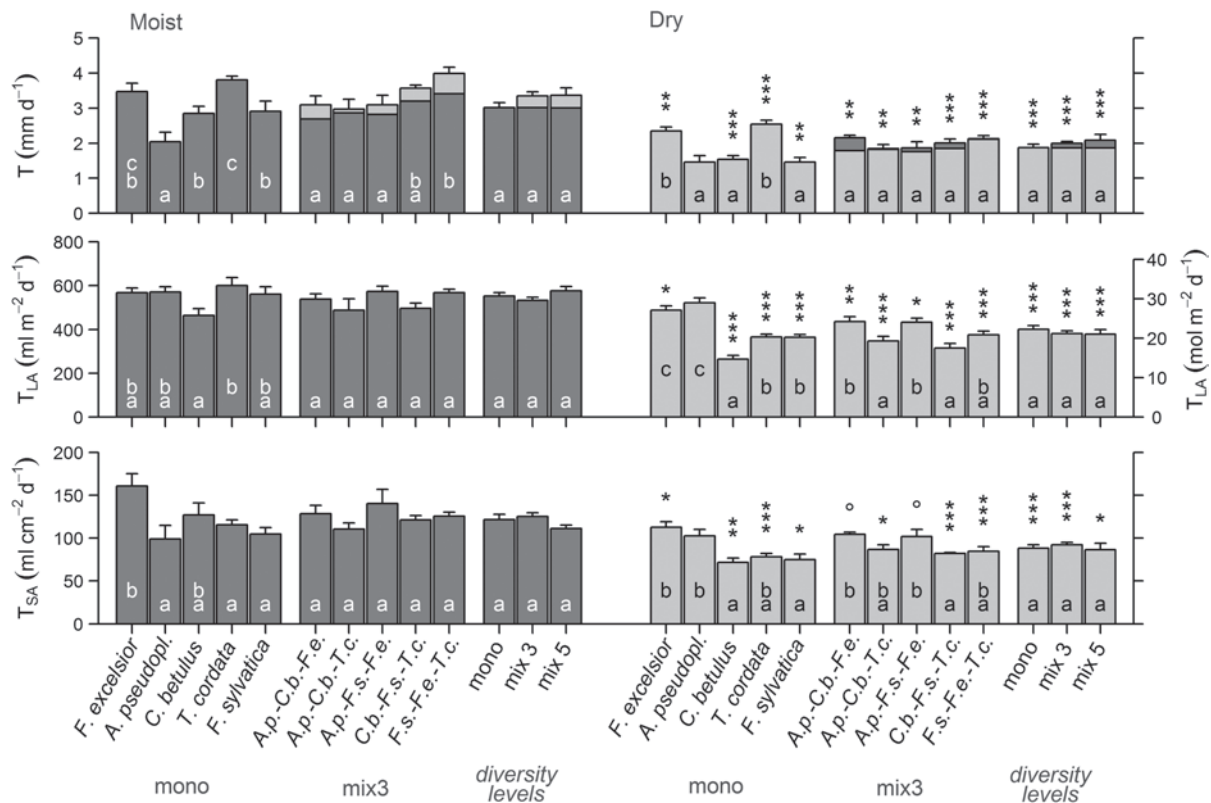

Figure 2. Transpiration rate per pot (T, top) and transpiration rate normalized to leaf area (T_{LA}, middle) or to cumulative sapwood area (T_{SA}, bottom) in the five monospecific culture types (first group), the five 3-species mixtures (second group), and the averages of the 1-species, 3-species and the 5-species combinations (third group) in the moist (left part of Fig.) and dry treatment (right part of Fig.). The unit of T (mm d^{-1}) is equivalent to l m^{-2} d^{-1}; the small light or dark tops of the bars display the fraction of stand transpiration assignable to a net biodiversity effects in that mixture. T_{LA} is given in ml m^{-2} s^{-1} (left y axis) or, for convenience, in mol m^{-2} d^{-1} (right y axis) for both the moist and dry treatments. Different small letters indicate significant differences at p < 0.05 between the species combinations or diversity levels in a group. Asterisks indicate significant differences between soil moisture treatments at the levels:°: p ≤ 0.1, *: p ≤ 0.05, **: p ≤ 0.01, ***: p ≤ 0.001.

Table 2. Summary of results of two-way ANOVAs testing for effects of the moisture treatment (moist versus dry) and species composition or diversity level and their interaction on three transpiration parameters. The ANOVAs were calculated for different samples (group of comparison: all species combinations, only monocultures, only 3-species mixtures, and data pooled at the diversity level). Transpiration is given per pot surface area (T), per leaf area (T_{LA}) and per sapwood area (T_{SA}). Significance levels: *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

Group of comparison	Transpiration	Moisture treatment			Species composition			Interaction		
		DF	F	Signif.	DF	F	Signif.	DF	F	Signif.
Diversity levels (3)	T	1,148	73.51	***	2,147	0.52		2,144	0.45	
	T_{LA}		51.63	***		0.49			0.47	
	T_{SA}		19.20	***		0.65			1.09	
All species combinations (11)	T	1,148	241.23	***	10,139	10.31	***	10,128	1.50	
	T_{LA}		212.55	***		9.21	***		2.74	**
	T_{SA}		71.87	***		4.71	***		1.60	
Monoculture types (5)	T	1,68	92.29	***	4,65	19.18	***	4,60	1.63	
	T_{LA}		94.89	***		16.70	***		5.49	***
	T_{SA}		28.84	***		7.12	***		2.76	*
3-specics mixture types (5)	T	1,63	125.13	***	4,60	4.19	**	4,55	1.80	
	T_{LA}		81.77	***		5.17	**		0.91	
	T_{SA}		38.05	***		2.70	*		0.52	

Stand-level water consumption: species identity effects

The monocultures of *Tilia cordata* and *Fraxinus excelsior* had the highest, those of *Fagus sylvatica* and *Carpinus betulus* intermediate, and that of *Acer pseudoplatanus* the lowest T means in the moist treatment (Fig. 2). The five investigated 3-species combinations differed by up to 25% in T in the moist treatment. A particularly high transpiration rate was recorded for the *Fagus–Fraxinus–Tilia* mixture (3.99 mm d^{-1}) which differed significantly from most of the other mixtures and represented the highest degree of 'overyielding' in terms of water consumption (by 0.58 mm d^{-1}, i.e. + 17.0% deviation from the expected T; Fig. 2: upper panel). In contrast, the diversity effect was rather small for the *Acer–Carpinus–Tilia* mixture (+ 3.7% overyielding).

In the dry treatment, the species ranking was comparable to the moist monocultures with *F. sylvatica*, *C. betulus* and *A. pseudoplatanus* reaching relatively low T means. The five 3-species mixtures had more similar T means that differed by not more than 15% among the different species combinations (Fig. 2). However, the deviation from the expected water consumption was large in the *Acer–Carpinus–Fraxinus* mixture (+ 20.9% overyielding) while it was negligible in the *Acer–Carpinus–Tilia* (–1.3%) and *Fagus–Fraxinus–Tilia* mixtures (+ 0.7%).

Stand-level water consumption scaled to leaf area and sapwood area

Transpiration scaled to leaf area (T_{LA}) differed less between the species in the monospecific pots than transpiration per ground area (T), but a broadly similar species ranking appeared (*T. cordata* > *F. excelsior*, *A. pseudoplatanus*, *F. sylvatica* > *C. betulus*; Fig 2: left part). *T. cordata* (higher) and *C. betulus* (lower) differed significantly in their T_{LA} means in the moist treatment. In contrast to stand-level transpiration (T), T_{LA} was not significantly different between the five 3-species combinations in the moist treatment (489–574 ml m^{-2} d^{-1}; Fig. 2: left part).

In the dry treatment, the species ranking was partly reversed due to strong transpiration reductions in *T. cordata*, *C. betulus* and *F. sylvatica* but only weak responses in *A. pseudoplatanus* and *F. excelsior*, leading to a ranking regarding T_{LA} in the sequence *A. pseudoplatanus* > *F. excelsior* > *T. cordata*, *F. sylvatica* > *C. betulus* (Fig. 2: right part). The significant interaction between the variables 'species composition' and 'moisture treatment' in the two-way ANOVA ($F_{4,60} = 5.49$, $p < 0.001$) for the monocultures reflects these species differences in transpiration reduction upon soil desiccation (Table 2). The two 3-species mixtures, in which both *A. pseudoplatanus* and *F. excelsior* were present, had significantly higher leaf area-specific water losses than the other three combinations. These species reduced transpiration only little in the monocultures and also in the mixtures. Lowest T_{LA} rates in dry soil were measured in the only mixture (*Carpinus–Fagus–Tilia*) where *A. pseudoplatanus* and *F. excelsior* were absent (Fig. 2: right part).

Transpiration scaled to sapwood area (T_{SA}) was for both soil moisture treatments highest in ring-porous *F. excelsior* with significant differences to *A. pseudoplatanus*, *F. sylvatica* and *T. cordata* (moist treatment) and to *C. betulus* and *F. sylvatica* (dry treatment) (Fig. 2). The drought-induced reduction in T_{SA} differed markedly among the species and was most pronounced in *T. cordata* but lacking in *A. pseudoplatanus*. The contrasting species ranking in the moist and dry treatments with respect to T_{SA} is reflected by the significant interaction term 'species composition' × 'moisture treatment' in the two-way ANOVA ($F_{4,60} = 2.76$, $p = 0.03$; Table 2).

Stand-level transpiration as influenced by leaf and sapwood area

Due to different inherent growth rates, the five species developed different leaf areas during the 16-month period between planting and harvest. In general, *T. cordata* had a larger total leaf area per plant than *C. betulus* and *F. excelsior*. *A. pseudoplatanus* and *F. sylvatica* developed the smallest leaf areas with less than half of that of *T. cordata* (Table 3).

Table 3. Species differences in six morphological or physiological traits (sapwood area-specific hydraulic conductivity of the stem, k_s; stomatal conductance of sun leaves, g_s; carbon isotope ratio of sun leaf mass, $\delta^{13}C$; total leaf area, LA; sapwood area in the stem, SA; Huber value (ratio LA : SA) of the five species (means ± SE over all species combinations) in the moist and dry treatments. Different capital letters indicate significant ($p < 0.05$) differences between the species, asterisks significant differences between the species in the moist and dry treatment at:° : $p \leq 0.10$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

Species	Sample size [n] Moist	Dry	k_s [kg m⁻¹ MPa⁻¹ s⁻¹] Moist	Dry	g_s [mmol m⁻² s⁻¹] Moist	Dry	$\delta^{13}C$ [‰] Moist	Dry
F. excelsior	12	12	2.28 ± 0.33 BC	1.97 ± 0.25 B	254.0 ± 14.5 AB	189.9 ± 10.3 C**	− 28.09 ± 0.20 AB	− 25.99 ± 0.24 A***
A. pseudopl.	12	12	1.04 ± 0.13 A	0.72 ± 0.08 A°	277.7 ± 12.6 B	205.1 ± 18.4 BC*	− 27.56 ± 0.16 A	− 26.77 ± 0.20 A*
C. betulus	12	12	1.25 ± 0.12 AB	0.87 ± 0.09 A*	201.9 ± 12.9 A	94.9 ± 8.1 A***	− 29.25 ± 0.12 C	− 28.23 ± 0.21 B**
T. cordata	12	12	1.92 ± 0.20 BC	1.79 ± 0.22 B	240.8 ± 14.6 AB	165.1 ± 22.3 BC**	− 29.31 ± 0.25 BC	− 28.37 ± 0.21 B*
F. sylvatica	12	12	2.13 ± 0.17 C	1.61 ± 0.14 B*	253.6 ± 15.5 AB	148.4 ± 9.5 B***	− 28.69 ± 0.14 BC	− 28.17 ± 0.16 B

Species	Sample size [n] Moist	Dry	LA [m²] Moist	Dry	SA [cm²] Moist	Dry	Huber value [m² cm⁻²] Moist	Dry
F. excelsior	35	32	0.39 ± 0.03 B	0.31 ± 0.03 B*	1.77 ± 0.13 C	1.27 ± 0.07 C***	0.22 ± 0.01 AB	0.24 ± 0.01 AB
A. pseudopl.	36	32	0.17 ± 0.02 A	0.16 ± 0.02 A	0.86 ± 0.05 A	0.72 ± 0.05 A*	0.20 ± 0.01 A	0.22 ± 0.01 A
C. betulus	34	32	0.35 ± 0.02 B	0.33 ± 0.02 BC	1.33 ± 0.07 B	1.19 ± 0.06 C	0.26 ± 0.01 B	0.27 ± 0.01 B
T. cordata	36	31	0.54 ± 0.03 C	0.42 ± 0.03 C**	2.51 ± 0.13 D	1.81 ± 0.09 D***	0.21 ± 0.01 A	0.22 ± 0.01 A
F. sylvatica	35	31	0.21 ± 0.01 A	0.20 ± 0.01 A	1.03 ± 0.05 A	0.90 ± 0.04 B	0.20 ± 0.01 A	0.21 ± 0.01 A

Corresponding to the leaf area differences, the five species showed considerable differences in sapwood area in the stem xylem (SA, cm²) with highest SA values in *T. cordata*, intermediate ones in *F. excelsior* and *C. betulus*, and lowest values in *F. sylvatica* and *A. pseudoplatanus* (Table 3). This species sequence existed also in the dry treatment but SA was by 10–20% smaller (Table 3). The Huber value (plant leaf area per stem sapwood area; unit: m² cm⁻²) was similar among the species (most values between 0.20 and 0.27 m² cm⁻²) except for a higher mean in *C. betulus* (Table 3). Upon soil desiccation, the ratio was not significantly altered.

Stand-level transpiration as influenced by leaf conductance and stem hydraulic conductivity

In the moist treatment, measurements of stomatal conductance (g_s) showed highest species means for *A. pseudoplatanus* and lowest for *C. betulus* (difference significant; Table 3). In the dry treatment, g_s was particularly high in *A. pseudoplatanus* and *F. excelsior* and low in *C. betulus* (difference significant). This pattern matches the species ranking with respect to the drought-induced reduction in leaf conductance (largest reduction in *C. betulus*: –53%, smallest reduction in *A. pseudoplatanus*: –26%). All species showed less negative $\delta^{13}C$ values of sun leaf mass in the dry than in the moist treatment (significant in all species except for *F. sylvatica*) reflecting the significant reduction in leaf conductance (Table 3).

Empirically determined hydraulic conductivity in the stem xylem (k_s) was in general lower in the dry than in the moist treatment (difference significant in three species).

Performance of target species in pure and mixed stands: neighbor effects on leaf area, hydraulics and water consumption

In the moist treatment, leaf area (LA), sapwood area (SA) and Huber value were significantly higher in *T. cordata* plants grown in mixture than in monoculture. *F. excelsior* exhibited larger LA in the 3-species mixtures than in the 5-species mixture and larger SA in the 3-species mixtures than in the monoculture. In contrast, the *F. sylvatica* plants achieved higher LA and SA in monoculture than in the 3-species mixtures (Table 4).

Stomatal conductance (g_s) tended to be higher for all species in the 5-species mixtures than in the monocultures in the moist treatment, but differences were not significant. In the dry treatment, g_s of *F. excelsior* was significantly higher in mixture than in monoculture (Table 5), which was also observed when pooled across both water levels ($p < 0.05$). This trend was also reflected in the $\delta^{13}C$ signatures with a significantly lower mean in *F. excelsior* (moist and dry treatment) and *F. sylvatica* (dry treatment) grown in 5-species mixture as compared to monoculture (Table 5). A similar tendency existed also for *C. betulus* in the dry treatment. For *C. betulus*, we also measured significantly higher k_s in 5-species mixture than in monoculture in the dry treatment, but the opposite relation in the moist treatment (Table 5). A similar pattern was observed for *F. excelsior* in the moist treatment were k_s increased marginally significantly in mixture; this tendency was significant when both moisture treatments were pooled ($p < 0.05$).

Discussion

Diversity effects on stand-level water consumption

We observed a significant net biodiversity effect on water consumption across all mixtures for the moist treatment in support of our first hypothesis, but the effect size was small. Transpiration per pot surface area (T) was on average ~11% higher in the 3- and 5-species mixtures than in the monocultures. The transpiration increase most likely bases on a selection effect, because 1) the species' transpiration rates differed considerably in the monocultures, and 2) the presence of intensively transpiring species (*Tilia cordata* and *Fraxinus*

Table 4. Total leaf area (LA), stem sapwood area (SA) and Huber value (ratio LA : SA) of plants of the five species grown either in monoculture (mono), 3-species mixture (mix3) or 5-species mixture (mix5) in the moist and dry treatments (means ± SE of n replicate pots). Different small letters indicate significant (p < 0.05) differences between the plants in 1-species, 3-species or 5-species combinations of a species.

Species	Diversity level	Sample size [n]		LA [m²]		SA [cm²]		Huber value [m² cm⁻²]	
		Moist	Dry	Moist	Dry	Moist	Dry	Moist	Dry
F. excelsior	mono	7	7	0.34 ± 0.04 ab	0.25 ± 0.01 a	1.37 ± 0.14 a	1.17 ± 0.11 a	0.25 ± 0.02 a	0.23 ± 0.02 a
	mix3	21	18	0.44 ± 0.05 b	0.33 ± 0.04 a	2.00 ± 0.19 b	1.29 ± 0.09 a	0.22 ± 0.02 a	0.25 ± 0.02 a
	mix5	7	7	0.27 ± 0.05 a	0.29 ± 0.04 a	1.49 ± 0.21 ab	1.34 ± 0.22 a	0.18 ± 0.02 a	0.23 ± 0.03 a
A. pseudopl	mono	7	7	0.19 ± 0.02 a	0.15 ± 0.02 a	0.91 ± 0.10 a	0.69 ± 0.08 a	0.21 ± 0.01 a	0.21 ± 0.01 a
	mix3	21	18	0.18 ± 0.02 a	0.16 ± 0.02 a	0.85 ± 0.07 a	0.71 ± 0.07 a	0.21 ± 0.01 a	0.22 ± 0.01 a
	mix5	8	7	0.14 ± 0.02 a	0.18 ± 0.04 a	0.86 ± 0.09 a	0.79 ± 0.15 a	0.17 ± 0 01 a	0.22 ± 0.01 a
C. betulus	mono	7	7	0.33 ± 0.02 a	0.31 ± 0.01 a	1.28 ± 0.08 a	1.08 ± 0.04 a	0.25 ± 0.01 a	0.28 ± 0.01 a
	mix3	20	18	0.34 ± 0.03 a	0.33 ± 0.04 a	1.25 ± 0.09 a	1.24 ± 0.11 a	0.26 ± 0.01 a	0.26 ± 0.01 a
	mix5	7	7	0.40 ± 0.06 a	0.37 ± 0.03 a	1.59 ± 0.19 a	1.18 ± 0.09 a	0.26 ± 0.03 a	0.32 ± 0.02 a
T. cordata	mono	7	7	0.34 ± 0.02 a	0.37 ± 0.01 a	1.98 ± 0.07 a	1.68 ± 0.07 a	0.17 ± 0.01 a	0.22 ± 0.01 a
	mix3	21	17	0.58 ± 0.04 b	0.45 ± 0.05 b	2.58 ± 0.19 b	1.87 ± 0.15 a	0.22 ± 0.02 ab	0.24 ± 0.01 a
	mix5	8	7	0.61 ± 0.04 b	0.41 ± 0.05 b	2.77 ± 0.24 b	1.81 ± 0.15 a	0.23 ± 0.01 b	0.23 ± 0.02 a
F. sylvatica	mono	7	7	0.28 ± 0.03 b	0.21 ± 0.02 a	1.25 ± 0.10 b	1.00 ± 0.04 b	0.22 ± 0.02 a	0.21 ± 0.02 a
	mix3	20	17	0.19 ± 0.01 a	0.20 ± 0.01 a	0.92 ± 0.05 a	0.82 ± 0.04 a	0.20 ± 0.01 a	0.24 ± 0.01 a
	mix5	8	7	0.21 ± 0.03 ab	0.19 ± 0.02 a	1.12 ± 0.14 ab	0.99 ± 0.10 ab	0.19 ± 0.02 a	0.20 ± 0.02 a

excelsior) was directly linked to large positive deviations in T from the expected value ('overyielding') in the mixtures. The 3-species mixture containing *T. cordata* and *F. excelsior* transpired even more than the 5-species mixtures, in which both species were diluted by others. In monoculture, the saplings of *T. cordata* and *F. excelsior* developed together with *Carpinus betulus* the largest leaf areas (and sapwood areas) of all species within 16 months after planting. *T. cordata* also showed the highest leaf area-specific transpiration rate. Further, the presence of heterospecific neighbors tended to increase the leaf and sapwood areas of these species. Thus, the relevance of selection effects for the water consumption of the mixtures is striking. Complementary water use in the mixtures may also have taken place, but our design did not allow for a quantitative separation between selection and complementarity effects in the sense of Loreau and Hector (2001). In our experiment, complementarity in water uptake is less likely due to the defined volume of the pots, while some stratification of the crowns, and thus complementarity in space occupation, of different species was observed in the mixtures, which may have resulted in enhanced transpiration.

Our findings partly agree with results obtained in other diversity experiments or observational studies, in which mixed stands showed enhanced stand transpiration (Forrester et al. 2010, Kunert et al. 2012). It appears that enhanced stand transpiration was mostly driven by a selection effect when soil water was not limiting (as in the studies of Gebauer et al. 2012 and in this study), while complementarity effects on stand transpiration were visible in the studies of Forrester et al. (2010) and Kunert et al. (2012) with influence of summer drought.

Apart from the significant though minor diversity effect on water consumption, species identity was the main factor responsible for the considerable variation in T among the different species combinations. Matching results were obtained with sap flux measurements in a mature stand of the same species in the Hainich forest (Gebauer et al. 2012) and in a boreal tree plantation (Grossiord et al. 2013). Such

Table 5. Three traits related to hydraulics and water consumption (sapwood area-specific hydraulic conductivity of the stem, k_s; stomatal conductance of sun leaves, g_s; carbon isotope ratio of sun leaf mass, $\delta^{13}C$) of plants of the five species either growing in monoculture (mono) or in 5-species mixture (mix5) in the moist and dry treatments (means ± SE of n replicate pots). Different small letters indicate significant (p < 0.05) differences between the plants in monoculture or mixture of a species.

Species	Diversity level	Sample size [n]		k_s [kg m⁻¹ MPa⁻¹ s⁻¹]		g_s [mmol m⁻² s⁻¹]		$\delta^{13}C$ [‰]	
		Moist	Dry	Moist	Dry	Moist	Dry	Moist	Dry
F. excelsior	mono	6	6	1.70 ± 0.47 a	1.73 ± 0.12 a	221.6 ± 20.1 a	167.3 ± 13.7 a	−27.49 ± 0.18 a	−25.61 ± 0.32 a
	mix5	6	6	2.86 ± 0.36 a	2.18 ± 0.44 a	286.3 ± 28.1 a	212.5 ± 8.4 b	−28.67 ± 0.59 b	−26.37 ± 0.30 b
A. pseudopl	mono	6	6	0.99 ± 0.18 a	0.77 ± 0.11 a	270.6 ± 22.1 a	238.5 ± 30.0 a	−27.59 ± 0.38 a	−26.74 ± 0.31 a
	mix5	6	6	1.11 ± 0.22 a	0.67 ± 0.12 a	284.8 ± 34.7 a	171.6 ± 12.2 a	−27.53 ± 0.28 a	−26.79 ± 0.26 a
C. betulus	mono	6	6	1.51 ± 0.17 b	0.69 ± 0.09 a	197.5 ± 12.6 a	84.6 ± 11.3 a	−29.19 ± 0.16 a	−27.91 ± 0.18 a
	mix5	6	6	0.99 ± 0.11 a	1.06 ± 0.11 b	206.3 ± 27.8 a	105.2 ± 10.9 a	−29.31 ± 0.25 a	−28.54 ± 0.24 a
T. cordata	mono	6	6	2.03 ± 0.26 a	2.28 ± 0.37 a	241.3 ± 18.6 a	179.8 ± 37.2 a	−29.25 ± 0.54 a	−28.60 ± 0.35 a
	mix5	6	6	1.79 ± 0.33 a	1.38 ± 0.15 a	252.3 ± 30.2 a	150.4 ± 26.9 a	−29.36 ± 0.50 a	−28.14 ± 0.24 a
F. sylvatica	mono	6	6	2.27 ± 0.25 a	1.67 ± 0.18 a	228.8 ± 29.2 a	140.8 ± 15.3 a	−28.49 ± 0.41 a	−27.82 ± 0.17 a
	mix5	6	6	1.95 ± 0.22 a	1.54 ± 0.24 a	278.4 ± 18.6 a	156.0 ± 11.9 a	−28.89 ± 0.29 a	−28.52 ± 0.15 b

species effects on stand transpiration are not surprising given the well-known species differences in transpiration rate among temperate tree species (Lyr et al. 1992).

Does drought enhance positive diversity effects?

The net diversity effect on stand-level water consumption was slightly smaller in the dry than in the moist treatment (T increased on average by ~8% from the monocultures to the mixtures compared to an 11%-increase in the moist treatment). Even though the mixtures depleted the soil moisture reserves on average more rigorously than the monocultures, we did not find the expected stronger 'overyielding' in the dry as compared to the moist treatment. While *C. betulus*, *F. excelsior* and *T. cordata* indeed tended to slightly increase their leaf and/or sapwood areas in the mixtures compared to the monocultures, overyielding in T was the exception under dry conditions and occurred markedly only in the *Acer–Carpinus–Fraxinus* mixture and the 5-species mixture. Our results do not support hypothesis 2) because the assumed positive effect of diversity on water consumption was generally not higher under dry conditions. A possible explanation could be that in agreement with Forrester et al. (2013) canopy interactions including complementarity for space and light use were less important under dry conditions. This is indicated by generally smaller leaf areas in comparison to the moist treatment. On the other hand, we found some indication that a net diversity effect may be larger under dry than under moist conditions in certain species constellations (e.g. in the *Acer–Carpinus–Fraxinus* mixture). This observation together with the findings of Forrester et al. (2010) and Kunert et al. (2012) suggest that positive effects of diversity on water consumption occur under water-limited conditions but that species identity and thus functional traits and their differentiation between species are the important drivers of such an effect. The dominant influence of species identity on T is indeed not only effective under moist but also under dry conditions. The species effects were closely related to the presence of *F. excelsior* and *T. cordata* in the mixtures; both species maintained relatively large leaf areas and/or high leaf conductance under dry conditions in the experiment. This matches the sap flux data of Gebauer et al. (2012) in the mature forest, where *T. cordata* had a particularly high water consumption early in a dry period leading to drier soil and reduced transpiration in the mixed stand later in summer. The coupling between higher productivity and greater resource depletion, which ultimately feeds back on stand vitality, can be viewed as manifestation of the 'tragedy of the commons' hypothesis with respect to water consumption. Similar negative effects of diversity have also been observed in other hydrological studies (Forrester et al. 2010, Kunert et al. 2012). In our experiment, we avoided soil water over-exploitation in pots with higher water consumption by carefully adding water to achieve similar soil moisture conditions across all species combinations. Such a design favors water spending assemblages to a certain degree, but it is inevitable when a homogenous drought regime shall be established across all species combinations in the dry treatment.

By considering the reduction in stand-level transpiration from the moist to the dry treatment, we tested the third hypothesis that more diverse stands reduce leaf area-specific transpiration (T_{LA}) on average to a smaller extent than monospecific stands, because less sensitive species may achieve larger leaf areas in mixtures. Our results do not support this hypothesis by showing that the 5-species mixture reduced T_{LA} more rigorously in both relative (–34%) and absolute terms (–197 ml m^{-2} d^{-1}) than the monocultures (mean of five species: –27%, –152 ml m^{-2} d^{-1}) and 3-species mixtures (mean of five combinations: –28%, –152 ml m^{-2} d^{-1}). The five different 3-species mixtures differed substantially in their relative transpiration reduction (–19 to –37%) with largest reduction found in the mixtures that contained *T. cordata* and *F. sylvatica*, the two species with largest transpiration reduction in monoculture (besides *C. betulus*). Thus, species identity largely determined the extent of transpiration reduction in the tree assemblages. *T. cordata* with high water consumption in moist soil and apparent drought-sensitive stomatal regulation was most likely the main cause of the relatively rigid down-regulation of water loss in mixtures containing this species, while 3-species mixtures with *Acer pseudoplatanus* (which reduced T_{LA} only minimally) represented species combinations with relatively small transpiration reduction. Corresponding to the T_{LA} reduction, leaf conductance showed a higher average reduction upon drought in the 5-species mixture (–38%) than in the monocultures (–30%). Our results on species differences in transpiration reduction do principally match observations on the drought sensitivity of the five species obtained from ecophysiological measurements in adult trees (Köcher et al. 2009). When applied to short time horizons (weeks to months), the diversity–community resistance hypothesis (Yachi and Loreau 1999) is not supported by our data. Over longer time spans (decades to centuries), species-richer forests might well be more resistant to drought, if the species sample includes trees with better drought adaptation that may replace more drought-sensitive species. On the other hand, higher diversity could also lead to the opposite effect, higher susceptibility of the community to drought, if water consumption is enhanced by species diversity (Grossiord et al. 2014b).

Does species mixing influence the water consumption and hydraulics of target trees?

We found some evidence in support of hypothesis 4) that heterospecific neighbors with higher or lower water consumption than a target species seem to influence the water flux regulation of trees of that species through alterations in plant leaf area, leaf conductance and the stem hydraulic system. We observed the most consistent species-specific response to heterospecific neighbors in the mixtures for *T. cordata* that significantly increased its plant leaf area (from 0.34 to 0.61 m^2), sapwood area (from 1.98 to 2.77 cm^2) and Huber value (from 0.17 to 0.23 m^2 cm^{-2}) from plants grown in monoculture to those grown in the 5-species mixture (moist treatment); sapwood area-specific conductivity (k_s) and leaf conductance (g_s) were not altered in this species. A different response to heterospecific neighbors was observed in *F. excelsior*, which increased g_s (mirrored in significantly reduced δ¹³C values) and tended to increase k_s in the 5-species mixture compared to monoculture; this species did not modify its leaf and sapwood area in a consistent manner.

Due to their relatively high transpiration rates, *T. cordata* and also *F. excelsior* must have profited from the presence of neighbors with lower water consumption such as *C. betulus* and *A. pseudoplatanus*. Most likely, *T. cordata* responded to an improved plant water status with extension of its leaf area which required a larger sapwood area. *F. excelsior* may also have profited from neighbors with more conservative water use because it increased leaf and xylem conductance and consumed more water, which probably was associated with higher carbon gain. In contrast to *T. cordata*, leaf area showed no consistent increase in *F. excelsior*. A species that may have suffered from heterospecific neighbors compared to monoculture growth in terms of leaf area and hydraulic properties enhancing water consumption was *F. sylvatica*. In the moist treatment, beech plants in 5-species mixture had a smaller leaf area and sapwood area than plants in monoculture which fits to the increase in leaf area and/or leaf conductance in *T. cordata* and *F. excelsior* in the same mixture, pointing to asymmetric competition in favor of the latter species.

Our findings about neighborhood effects on the physiology of target species in mixtures match dendrochronological results obtained in the Hainich mixed forest where a target species (*F. sylvatica*) profited in its mean growth rate and susceptibility to drought events from the presence of certain heterospecific neighbors in comparison to growth with conspecific neighbors (Mölder et al. 2011, Mölder and Leuschner 2014).

Conclusions

A main result of our experiment is that diversity effects on stand transpiration are relatively weak in these assemblages and transgressive overyielding in terms of water consumption occurs only as an exception. Several results suggest that species identity effects, i.e. inherent differences in the species' water consumption and drought tolerance, are more important drivers than complementary water use and facilitation. Moreover, the diversity effect on water consumption was not principally different between conditions of ample and limited water supply. Rather, diversity effects tended to be more influential under favorable than unfavorable moisture conditions, which contrasts with several earlier findings (Vilà et al. 2007, Pretzsch et al. 2013, Río et al. 2013). The observed significant modification in morphology, physiology and water consumption in response to specific neighborhood conditions is a clear sign that neighbor effects are an important structuring force in mixed stands and deserve more attention.

No doubt, pot experiments with saplings lack the realism of observational studies in old-growth forests along diversity gradients and do not allow conclusions on the performance of larger trees as they may be possible from diversity experiments with planted trees. However, a main strength of our study is the relatively precise measurement of transpiration and the good control of soil moisture, which is often not possible in drought experiments in the field. In addition, plant morphology and physiology could be recorded in a more comprehensive way than is possible in large-scale tree diversity experiments. Our experiment has the additional advantage that the findings can be compared to results obtained from comparative hydrological measurements in

an old-growth forest varying in tree diversity. The three main findings (only small diversity effect on water consumption; greater importance of species identity effects; importance and direction of neighbor effects on water consumption) agree with the field results, which is support for our main conclusions.

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Supplementary material (available online as Appendix oik-02367 at <www.oikosjournal.org/appendix/oik-02367>). Appendix 1.