

Title

Spatio-temporal water uptake patterns of tree saplings are not altered by interspecific interaction in the early stage of a subtropical forest

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Abstract

Complementary resource use has often been claimed to explain positive effects of plant diversity on ecosystem functioning, but the underlying mechanisms of complementarity have rarely been directly quantified in forest systems. The aim of this study was to characterize spatial and temporal water uptake of subtropical tree saplings, to assess the role of interspecific interaction on water uptake patterns and to quantify species niche breadth and overlap. Our experiment comprised two deciduous (*Castanea henryi*, *Quercus serrata*) and two evergreen tree species (*Elaeocarpus decipiens*, *Schima superba*) that were each planted in monoculture and 4-species mixture. We used deuterium-enriched water to trace seasonal water uptake from 5 cm and 20 cm soil depth. *Castanea* exploited predominantly the upper soil (74% of overall tracer uptake across treatments) whereas *Quercus* (50%), *Elaeocarpus* (57%) and *Schima* (62%) tended to use both soil layers more equally. Species identity had an overall significant effect on isotopic enrichment in stem water. There was no effect of species richness: niche breadth and overlap of single species was not affected by interspecific interactions in mixtures. Niche overlap between deciduous species was lowest (74%) whereas the two evergreen species had similar water uptake patterns (91%). According to our results, interspecific competition did not alter water uptake patterns of the studied species during the early phase of forest establishment. Thus, soil water uptake complementarity could only occur through inherent (fundamental) specific differences in water uptake niches based on sapling specialization, while phenotypic adjustments to interspecific interaction or neighbor diversity are less important.

Keywords: BEF-China, deuterium, niche differentiation, resource complementarity, stable isotopes, subtropical broadleaved forest

1. Introduction

Water availability is one of the most important factors for plant productivity and nutrient cycling (Chapin et al. 2011). Moreover, seedling establishment and consequently tree species distribution and community organization are strongly affected by temporal and spatial variation in soil moisture at local and regional scales (Engelbrecht et al. 2007). Competition for belowground resources, including water and rooting space, is recognized as a major factor affecting tree growth and survival in dense seedling and sapling communities (Coomes and Grubb 2000). However, little is known about the spatial and temporal patterns of water uptake by different species and whether water acquisition of individual trees is influenced by the diversity and composition of their local neighborhoods.

Trait divergence (Grime 2006), e.g. interspecific differences in morphological traits, allow tree species to exploit soil water resources differently. Contrasting trait-mediated water exploitation strategies can promote complementary water uptake by hydrological niche differentiation (Silvertown et al. 2015). Generally, complementary resource acquisition has been recognized as important mechanism which can explain higher efficiency in uptake of limiting resources and thus increased biomass production in diverse plant species mixtures (Loreau and Hector 2001, Cardinale et al. 2012) and lower vulnerability to drought because of more complete soil water utilization (Yang et al. 2011). This would also open management options to use complementary mixtures to improve adaptability to more severe and frequent droughts. Spatial complementarity exists when coexisting species use water resources from different soil layers (tap-rooted vs. lateral root system). For example, water uptake from different soil depths promotes species coexistence in savanna ecosystems (Weltzin and McPherson 1997; Jackson et al. 1999; Rossatto et al. 2013) and tropical forests (Jackson et al. 1995; Meinzer et al. 1999; Stratton et al. 2000). Most studies have focused on spatial water partitioning whereas tree species differences in temporal water uptake have often been neglected. Temporal complementarity is a result of seasonal variation, e.g. timing of leaf

flushing and senescence (Williams et al. 1997), or short-term variation, e.g. stomatal control (Oren et al. 1999; Pataki and Oren 2003), in plant activity and water utilization among species. For example, in grassland ecosystems species with maximum root activity early in the year coexist with species that are most active in summer (Fitter 1986; McKane et al. 1990). Thus, according to niche theory spatio-temporal resource partitioning promotes plant species coexistence as different species occupy different resource niches (Tilman 1982).

Niche differentiation can occur due to inherent differences among species, i.e., different fundamental niche: (the niche space occupied by a species under only conspecific competition), or due to phenotypic changes of resource uptake strategies in response to heterospecific neighbors, i.e. differences in realized niche space (the niche space occupied by a species under interspecific competition (von Felten et al. 2009). Indeed, it has been shown that in the presence of interspecific competitors niche breadths and consequently niche overlap among species is smaller than niche breadth and overlap under conspecific competition (Silvertown et al. 1999; Silvertown 2004; von Felten et al. 2009). As diverse plant communities should cover ultimately a larger total niche breadth, belowground resources should be exploited more efficiently by complementary resource use in species mixtures - an important mechanism leading to a positive biodiversity-ecosystem functioning relationship (Vandermeer 1992; Loreau and Hector 2001). Consequently, increasing plant diversity may also lead to enhanced community water uptake in comparison to monocultures, as shown for example in grassland (Verheyen et al. 2008), greenhouse model systems (De Boeck et al. 2006) and a tropical tree plantation (Kunert et al. 2012). Thereby, the hydrological niche of a plant species may depend on the composition or local diversity of the plant community.

Whereas previous studies have often addressed water uptake related to tree species identity (e.g. Xu et al. 2011; Yang et al. 2015), water uptake strategies of coexisting tree species planted at different levels of diversity have been scarcely elucidated. The humid

subtropics represent a global hotspots of woody plant diversity, particularly in Southeast China with similar proportions of evergreen and deciduous tree species (Wang et al. 2007; Bruelheide et al. 2011). But despite the high tree diversity little is known about species coexistence related to spatial and temporal resource partitioning in the humid subtropics (Nie et al. 2011). In particular, the importance of niche differentiation for species coexistence during forest regeneration with tree saplings as the demographic bottleneck is still poorly understood (Rother et al. 2013). Thus, there is a fundamental need to measure directly resource partitioning to evaluate the importance of resource niche differentiation for recruitment success (Clark et al. 1999). Therefore, we focus on tree saplings as trees at early developmental stage are usually most vulnerable to low water availability because of their shallow root system and low non-structural carbohydrate reserves (Niinemets 2010; Anderegg and Anderegg 2013; O'Brien et al. 2014).

To reveal belowground multidimensional resource use, i.e. the spatial and temporal uptake of different nutrients and water, stable isotopes have become a powerful tool as they allow tracing of the flow of elements in biological systems (Adams and Grierson 2001). In particular, stable isotopes allow the direct testing of resource partitioning, while considering root activity, instead of relying on indirect methods such as comparing biomass production or root depth in monoculture and mixture (Bachmann et al. 2015). Because in terrestrial plants no fractionation commonly occurs during water uptake and transport in stem vessels, the isotopic signature of xylem water reflects the isotopic signature of the plant water source, which can be used to infer soil water uptake patterns (Ehleringer and Dawson 1992; Dawson et al. 2002). Often the natural abundance of deuterium (^2H or D) or oxygen-18 (^{18}O) is used to elucidate water uptake from different soil depths or rain events as well as water partitioning among plant individuals or growth forms (Jackson et al. 1995; Meinzer et al. 1999; Meißner et al. 2012; Rossatto et al. 2013, Bertrand et al. 2014). If only weak or no natural isotopic soil gradients exist, application of enriched tracer solutions, e.g. D_2O , by injection into the soil

matrix (Plamboeck et al. 1999) or surface irrigation (Moreira et al. 2000; Sternberg et al. 2002) is best suited to trace water uptake from distinct soil layers. Although the deuterium tracer technique is not aimed at determining absolute plant water use (i.e., it does quantify on its own the total amount of water transpired by the plant), it allows identifying the relative differences in water uptake among species (Schwendenmann et al. 2010). However, most studies that have adopted the tracer injection technique considered only one soil layer and point of time.

Using the tracer injection technique, we examined spatial and seasonal water uptake patterns of four tree species at sapling stage in monoculture (fundamental niche) and mixture (realized niche) in subtropical East China. Based on phenology and natural abundance we selected two deciduous (*Castanea henryi*, *Quercus serrata*) and two evergreen (*Eleaocarpus decipiens*, *Schima superba*) species from the local species pool. We hypothesized that (i) spatial and temporal water uptake patterns of tree saplings are related to species identity and phenology and that (ii) interspecific competition induces adaptive shifts in water uptake patterns of individual species. Finally, we aimed at determining the hydrological resource-based niche breadth, as well as niche overlap between species, to identify those species combinations offering high potential for complementary water resource use.

2. Methods

2.1 Study site

This study was carried out in Jiangxi Province, Southeast China (N29°06.293 E117°55.286). The prevalent climate is subtropical with distinct seasonality: a hot-humid season from April to September and a cool-dry season from October to March (Geißler et al. 2012). Mean annual temperature is 16.7°C and mean annual precipitation is 1821 mm (Yang et al. 2013) most of which (about 80%) occurs between March and September. The region belongs to the subtropical evergreen broadleaved forest belt, one of the most important and diverse

vegetation formations of eastern Asia (Richardson 1990; Wang et al. 2007). The regional mid-subtropical forest is characterized by a high diversity of woody plant species. For example, 159 tree species from 49 families have been identified in a nearby 24 ha plot (Legendre et al. 2009). Evergreen species are dominant in terms of number of individuals but the number of evergreen and deciduous species is similar (Wang et al. 2007; Bruelheide et al. 2011).

2.2 Experimental design

Our experiment was part of a large tree sapling diversity experiment within the BEF-China project (Bruelheide et al. 2014). The experiment was set up on a former agricultural field where rice, rape and vegetables were previously grown. In March 2009, the field with a total area of 7900 m² was ploughed, and four blocks of equal size were established. Each block was divided into 1 m² sized plots separated by 20 cm deep and 20 cm wide trenches. Channels (50 cm deep) were dug around blocks and connected to trenches to allow drainage of excess rainwater. Our experiment used 160 plots in total, with 40 plots randomly distributed in each block. We selected two deciduous species (*Castanea henryi* (Skan) Rehd. et Wils. and *Quercus serrata* Murray) and two evergreen species (*Elaeocarpus decipiens* Hemsley and *Schima superba* Gardn. et Champ.) which were grown from seeds in a tree nursery for 6 to 12 months until saplings had reached a planting size of about 30 cm (Both et al. 2012). Hereafter, genus names are used to refer to the species. The selected species also differed in natural abundance (i.e. number of tree individuals) in the regional subtropical evergreen broadleaved forest with *Quercus* (Fagaceae) and *Schima* (Theaceae) being abundant and *Castanea* (Fagaceae) and *Elaeocarpus* (Elaeocarpaceae) subordinate (Bruelheide et al. 2011). In late March 2009, each plot was planted with 16 tree saplings, establishing model communities of one and four species (Fig. 1). Planting distance was about 25 cm, thus allowing fast formation of below- and aboveground interactions soon after planting. All plots were regularly weeded throughout the experiment.

To test for spatio-temporal differences among species in water uptake, we injected deuterated water (D_2O) into the mineral soil in full factorial combinations of two depths (5 cm and 20 cm) and four dates during the year: in summer (2009) when temperature is highest; in autumn (2009) before leaf shedding in the dry season; in winter (2010) when temperature is low and in spring (2010) during leaf flush (Fig. 2). Mean temperature during the four sampling campaigns was 27.1°C in summer, 12.4°C in autumn, 8.8°C in winter and 21.4°C in spring. The two soil depths for tracer injection were chosen based on the maximum root depth of about 20 cm at the beginning of the experiment. For each of the eight injection treatments, we used a separate plot, replicated four times. Monocultures thus comprised 128 plots (4 species x 4 seasons x 2 soil depths x 4 replicates). Mixtures with equal proportion of each species were established on 32 plots (1 mixture x 4 seasons x 2 soil depths x 4 replicates).

2.3 Tracer application

The tracer solution with an initial δD value of +130,000‰ was prepared with 2% (volume) D_2O (99.9% enriched, Cambridge Isotopes, Andover, USA) and 98% distilled water (with background $\delta D = -8.54$ ‰). To avoid edge effects deuterated water was applied only to the central quadrant containing four target individuals (Fig. 1). Before the tracer application, we measured the basal diameter of the central tree saplings. The tracer solution was injected in a regular grid of 7 x 7 cm, yielding 49 single injections, with a dispenser (Eppendorf Multipette 4780 with Combitips plus 50 mL, Eppendorf, Hamburg, Germany) connected to a 3 mm thick four site-port needle. Each injection hole was pre-drilled with a screwdriver (4 mm in diameter) to its target depth (5 or 20 cm), and received 2 mL of tracer solution (i.e. 98 mL of highly enriched label per soil layer). The deuterated water was slowly released into the soil to avoid spreading and drainage of the tracer along the inner surface of the holes (Hoekstra et al. 2014). By this means, deuterated water could be accurately released in the respective soil layer. The data on enrichment in plant material shows that this label-volume per soil layer was

sufficient to detect water uptake by saplings. The applied amount of water per plot (0.098 L) corresponded to an increase of less than 0.01% in annual precipitation, which was expected to induce no response in plant growth. Furthermore, plots were covered with transparent plastic tarps placed directly on the soil surface to reduce tracer loss by leaching to deeper soil layers during rain events. In addition, soil moisture, expressed as % water per gram dry soil, was determined gravimetrically for the layers 0 - 10 cm and 15 - 25 cm by drying an aliquot of 5 g at 105°C for 24h.

2.4 Plant sampling and analysis of δD in stem water

To determine seasonal variation of natural background deuterium levels we took stem samples from four randomly selected outer individuals per species in the monocultures (one individual per species and block) one day prior tracer application. Six days after tracer injection, we harvested one randomly selected target individual within the center of each plot from the monospecific plots and all four target trees in the mixtures. Stem samples were taken from harvested plants, sealed in glass vials (Exetainer; Labco, High Wycombe, UK) and kept frozen at -20°C until water extraction by cryogenic vacuum distillation (West et al. 2006; Orlowski et al. 2013). In short, vials with frozen stem samples were heated in a water bath (80°C), and connected to a vacuum pump (ca. $4 \cdot 10^{-2}$ mbar) through a liquid nitrogen water trap for 2h. At the end of the extraction, the frozen water samples were let to melt, transferred to glass vials and kept frozen until isotope analysis. Stem water was analyzed for deuterium using a high-temperature elemental analyzer (TC/EA; Finnigan MAT, Bremen, Germany) coupled to a Delta^{plus}XP isotope ratio mass spectrometer via a ConFlo III interface (Finnigan MAT; Werner et al. 1999). Deuterium content of stem water is reported in δ notation calculated as:

$$\delta D (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}}) - 1 \quad (1)$$

where R is the ratio of heavy (D) to light isotope (H) in the sample (R_{sample}) and in the standard (R_{standard}), respectively. V-SMOW (Vienna Standard Mean Ocean Water) was used as standard with a D/H ratio of 155.95×10^{-6} .

Note that to avoid evaporation and thus fractionation while peeling the branches, we decided not to remove phloem before stem water extraction. As a result of fractionation during evaporation phloem is normally slightly more enriched in deuterium than xylem. However, such differences in isotopic enrichment are irrelevant in our approach as we used a highly enriched tracer solution to resolve patterns of water uptake. Furthermore, water is continuously exchanged between phloem and xylem (e. g, Sevanto et al. 2011) and thus even if the phloem had been removed, contamination of xylem water by phloem water cannot be avoided prior to sampling even if the sample are later peeled of the living bark. However, because phloem is quantitatively very limited compared to xylem our results should not have been noticeably affected by including phloem tissue in stem water extraction. Excess δD (δD_{excess}) was calculated as the difference between δD values measured in stem water of labeled ($\delta D_{\text{labeled}}$) and the mean of δD values ($\delta D_{\text{natural}}$) of unlabeled plants ($n=4$) for each species and season (Eq. 2).

$$\delta D_{\text{excess}} = \delta D_{\text{labeled}} - \overline{\delta D}_{\text{natural}} \quad (2)$$

We set δD_{excess} to zero if $\delta D_{\text{labeled}}$ was smaller than mean $\delta D_{\text{natural}}$ due to no or very little D uptake, which occurred only in winter in the deciduous species. In addition, we harvested in each season 20 individuals per species (10 individuals from monocultures and 10 individuals from mixtures) and determined dry weight of roots, stem and leaves for the calculation of species-specific allometries across treatments. Measurements of tree basal diameter and rooting depth were taken from the harvested tree saplings. We calculated the model coefficients intercept (A) and slope (B) for total tree biomass as

$$\ln(biomass) = A + B(\ln D) \quad (3)$$

relying on basal stem diameter (D) as single predictor for tree biomass (Chave et al. 2001). Based on determined root-shoot ratios, we used species-specific allometries to calculate root biomass for the target tree saplings harvested for stem water extraction.

2.5 Niche breadth and niche overlap

Similar to McKane et al. (2002), proportional tracer uptake (p) in each treatment (i) was calculated based on the species' overall tracer uptake across the eight treatments (4 seasons x 2 soil depths, Eq. 4). Treatment proportions to overall tracer uptake were calculated based on the mean of two to four replicated plants in each treatment.

$$p_i = \frac{\delta D_{excess\ i}}{\sum_{i=1}^8 \delta D_{excess\ i}} \quad (4)$$

Niche breadth was calculated as Levins' normalized B_n (Levins 1968) which is an index for the uniformity of resource use (Eq. 5).

$$B_n = \frac{1}{8 \sum_{i=1}^8 p_i^2} \quad (5)$$

In our case maximum niche breadth ($B_n = 1$) is reached if a species shows equal uptake of deuterium from both labeled soil depths in all seasons. That is, proportional tracer uptake (p) contributes equally to a species' total tracer uptake. In contrast, niche breadth is lowest if the species uses one resource combination exclusively ($B_n = 1/8$), e.g. D is taken up solely in summer from 5 cm soil depth.

Niche overlap between pairs of species was calculated as proportional similarity (PS, Eq. 6) based on Parrish and Bazzaz (1976) and Schoener (1970):

$$PS = 100\% - 0.5 \sum_{i=1}^8 |p_{1i} - p_{2i}| \quad (6)$$

where p_1 and p_2 are proportions (in %) of deuterium uptake from one out of eight possible treatments (2 soil depths x 4 seasons) for species 1 and species 2, respectively. PS ranges from 0% to 100%, the latter happens when D uptake patterns of two species are identical in space and time leading to full niche overlap. If the two species take up injected deuterium exclusively from different soil depths or seasons, respectively, and thus have no spatial and temporal overlap, PS would be 0%.

2.6 Statistical analyses

Analyses were performed using R 3.1.3 (R Core Team 2015). First, we tested for effects of species identity and season (fixed factors with four levels, respectively) on natural δD values in stem water using mixed-effects models (package 'nlme', Pinheiro and Bates 2000). Plot nested within block was defined as random effect. Post-hoc Tukey tests (package 'multcomp', Hothorn et al. 2008) were applied for multiple comparison of species identity and seasons, respectively. Due to limited degrees of freedom, in a separate model a contrast for functional group (i.e. evergreen vs. deciduous) was included instead of species identity. Second, we tested for treatment effects on δD_{excess} in stem water and proportional water uptake by fitting mixed-effects models including species richness (factor with 2 levels), species identity, soil depth (a factor with 2 levels), season and all two-way interaction terms as fixed effects, respectively. Root biomass was included as covariate to adjust indirectly for differences in plant size before assessing treatment effects. In a separate model, we additionally tested for the effect of phenological group instead of species identity on isotopic enrichment in stem water. δD_{excess} and proportional data were square-root transformed to meet assumptions of normality and homoscedasticity.

3. Results

3.1 Natural background δD in stem water

Natural δD values of stem water exhibited large seasonal variation (Fig. 3a-d). Across species stem water was most depleted in summer ($-71 \pm 1.4\text{‰}$; mean \pm SE) and autumn ($-50 \pm 1.8\text{‰}$), and was relatively more enriched in spring ($-25 \pm 0.6\text{‰}$) and winter ($-19 \pm 1.1\text{‰}$). Species differed significantly in natural δD in all seasons with the exception of winter. Plant functional group (i.e. evergreen vs. deciduous) had a significant effect on natural δD ($F = 19.74$, $p < 0.01$). Compared to deciduous species, evergreens were more enriched in spring ($+3.5\text{‰}$, $F = 15.51$, $p < 0.01$), summer ($+6.9\text{‰}$, $F = 11.33$, $p < 0.01$) and autumn ($+8.9\text{‰}$, $F = 10.02$, $p = 0.01$) but not in winter (-1.5‰ , $F = 0.54$, $p = 0.47$).

3.2 Excess δD in stem water

Tracer application was successful and led to excess δD values in stem water that ranged from 0‰ to 1138‰ with a mean of $195 \pm 13\text{‰}$ across all treatments. Across diversity levels and seasons δD_{excess} in stem water was generally higher when deuterated water was injected at 5 cm ($242 \pm 20\text{‰}$) than at 20 cm soil depth ($147 \pm 15\text{‰}$). Tracer enrichment of stem water was highest in summer ($338 \pm 21\text{‰}$) and significantly lower in autumn ($285 \pm 31\text{‰}$), spring ($141 \pm 13\text{‰}$) and winter ($27 \pm 5\text{‰}$). As shown by the mixed effects model the fixed factors species identity, soil depth and season but not species richness had an overall significant effect on δD_{excess} (Table 1). Significant two-way interaction terms (species identity x soil depth, species identity x season, soil depth x season) indicated the dependence of δD_{excess} on the level of several factors. For example, the effect of soil depth on stem water enrichment varied from one species to another which led to the strong interaction between species identity and soil depth. In this respect, *Castanea* was isotopically more enriched than *Quercus* when uptake of deuterated water from 5 cm soil depth was considered. However, the opposite pattern was found if the tracer was injected at 20 cm. In summer, autumn and winter enrichment of stem

water was significantly influenced by species identity when the tracer solution was applied at 5 cm soil depth (Fig. 3e-h). In particular, the maximum enrichment difference was observed between the two deciduous species, with stem water being significantly more enriched in *Castanea* than in *Quercus*. For the evergreen species, δD_{excess} tended to be higher in *Schima* than in *Elaeocarpus* when tracer uptake from 5 cm soil depth is considered. Although not always significant these relative differences in species-specific enrichment remained rather constant in spring, summer and autumn. Considering tracer uptake from 20 cm soil depth (Fig. 3i-l), species differed in tracer enrichment only in winter.

In a subsequent analysis including tree phenological group instead of species identity δD_{excess} was significantly affected ($F = 23.92$, $p < 0.001$) by phenology with higher enrichment in evergreen ($209 \pm 18\text{‰}$) than in deciduous species ($179 \pm 18\text{‰}$). Plot species richness did not affect δD_{excess} ($F = 1.03$, $p = 0.31$) whereas soil depth ($F = 31.20$, $p < 0.001$) and season ($F = 105.74$, $p < 0.001$) influenced significantly D uptake. Lowest values of δD_{excess} were observed for both evergreen and deciduous species in winter with evergreen species being more enriched than deciduous species.

3.3 Proportional water uptake from soil depth and season

Proportional D uptake (as percentages from overall D uptake) was not influenced by species richness but was affected by species identity, soil depth and season (Table 1). When taking into account the proportional tracer uptake from the eight treatments (2 soil depths x 4 seasons) across the two species richness levels, *Castanea* exploited predominantly the upper soil (74%) whereas *Quercus* (50%), *Elaeocarpus* (57%) and *Schima* (62%) tended to use both soil layers more equally (Table 2). In summer, *Quercus* was the only species that preferably exploited the deep soil layer with deep soil water contributing 23% to overall tracer uptake (sum of excess δD across all treatments) compared to 15% taken up from shallow soil water resources in this season. Species also differed slightly in seasonal uptake patterns. For

example, *Castanea* acquired 48% of its overall tracer uptake in summer (32% from 5 cm and 16% from 20 cm soil depth) whereas *Quercus* utilized only 38% (15% from 5 cm and 23% from 20 cm soil depth). In contrast, the two evergreen species *Elaeocarpus* and *Schima* followed a similar pattern with 41% of tracer taken up in summer, respectively. In winter, tracer uptake of evergreen species was detectable and contributed 9% in *Elaeocarpus* and 4% in *Schima* to overall tracer uptake. In contrast, negligible proportions (rounding to zero) were traced for deciduous species during the leafless period.

3.4 Niche breadth and niche overlap

Niche breadth averaged across the four species was slightly smaller in the 4-species mixture (0.62) than in monoculture (0.68). However, the reduction in niche breadth was not significant even though all species tended to reduce their niche breadth by up to 16.7% (for *Elaeocarpus*; other species: *Quercus*: 12.9%, *Schima*: 1.2%, *Castanea*: 0.4%) when grown under interspecific competition (Fig. 4a).

Average niche overlap in monoculture ($79.4 \pm 2.4\%$) for all species combinations ($n = 6$) did not differ from niche overlap calculated for species growing in mixture ($80.0 \pm 1.9\%$; Fig. 4b). Calculated across the two levels of species richness highest niche overlap was found between the two evergreen species *Elaeocarpus* and *Schima* (91.1%, Table 3). The second highest niche overlap was calculated for the species pair *Castanea/Schima* (86.7%). In contrast, the two deciduous species *Castanea* and *Quercus* showed the smallest overlap in niche space (74.3%). The species combinations *Quercus/Schima*, *Castanea/Elaeocarpus* and *Quercus/Elaeocarpus* had a similar niche overlap with 78.5%, 79.8% and 80.8%, respectively.

4. Discussion

Our study is one of the few that directly investigated spatio-temporal water resource uptake complementarity of subtropical tree saplings during early forest establishment. Isotopic enrichment of stem water and proportional values of tracer water uptake differed significantly among species revealing specific water sourcing strategies (i.e. fundamental niche), thus confirming our first hypothesis. Moreover, we tested the effect of interspecific interactions on spatial and seasonal water uptake patterns, and whether species growing in mixture increase water uptake complementarity (hypothesis 2). Contrary to our expectation, interspecific interactions or plot species diversity had no effect on spatio-temporal water uptake patterns of individual species with niche breadth of individual species and niche overlap among species being independent from plot species richness. This implies that tree saplings of the planted species cannot or have not yet adjusted their water uptake strategies when growing with heterospecific neighbors (i.e. realized niche).

4.1 Natural levels of deuterium

Large seasonal variation in natural deuterium levels of stem water was observed, with tree saplings being the most depleted in summer. This is in accordance with the temporal isotopic precipitation pattern modeled for our field site coordinates (Bowen and Revenaugh 2003, www.waterisotopes.org). The seasonal isotopic fluctuation in rainfall is a result of shifts in the general weather situation triggered by the oscillation of the Intertropical Convergence Zone (ITCZ). In summer, moist pacific monsoon air masses transport deuterium depleted precipitation to Southeast China. In contrast, winter precipitation mainly originates from proximate evaporative sources like the Yellow Sea and has therefore higher δD values (Araguás-Araguás et al. 1998). All tree saplings studied reflected the seasonal isotopic pattern in rain water as they relied on water from recent rainfall events. However, based on natural water isotopes Nie et al. (2011) found that subtropical woody species differ in seasonal use of rain water and deep water sources.

Thus, this uniform isotopic pattern across species may change with succession as soon some species get access to groundwater which is isotopically different from rainwater (Ehleringer and Dawson 1992).

4.2 Interspecific differences in spatio-temporal water uptake patterns

Our results show clear indications for spatial partitioning of water resources among tree species, even for trees at sapling stage. This provides additional information to previously published studies that have revealed spatial water partitioning for example in Hawaiian dry forests (Stratton et al. 2000) and neotropical rainforests (Meinzer et al. 1999; Moreira et al. 2000; Stahl et al. 2013) but focused on older trees. Thus, we provide further evidence for the importance of water uptake complementarity at tree sapling stage during forest establishment. In our tracer experiment the differences in shallow to deep soil water uptake among species might be primarily the result of differential root depth and architecture. Root depth data (Table A.1, Appendix A) for the tree species studied are in accordance with the identified spatial water uptake pattern revealed by our tracer approach. Whereas shallow-rooted *Castanea* used predominantly the upper soil for water uptake, tap-rooted *Quercus* explored also deeper soil layers at the sapling stage. The two deciduous species therefore adopted distinct water sourcing strategies mediated by contrasting root systems. In general, a shallow root system would be beneficial for capturing light rainfall events, which occur especially in autumn and winter in the study area, whereas species with tap roots may still have access to deeper soil water resources (Stratton et al. 2000). Especially in autumn when precipitation is low (Yang et al. 2015), the topsoil layer can quickly desiccate whereas deep layers may retain sufficient soil moisture for deep rooting species (Table A.2, Appendix A). We expect that differences in rooting depth of the two deciduous species become even more pronounced with time as *Quercus* showed fast root extension into deeper soil layers (Table A.1, Appendix A). Similarly, the evergreen species differed significantly in rooting depth that might explain why

the shallow-rooting species *Schima* tended to be more enriched than *Elaeocarpus* when tracer uptake from 5 cm soil depth is considered.

For the species studied, we observed significant differences in seasonal tracer uptake in summer and winter. Interspecific differences in temporal water uptake patterns can be caused by species-specific timing of leaf flush, leaf senescence and maintenance of physiological activity in winter (Stratton et al. 2000; Zhang et al. 2013). In spring, leaf flush was rather synchronized among species but contrary to our expectations evergreen species did not show a higher fraction of tracer uptake than deciduous species. Because *Elaeocarpus* and *Schima* shed a high proportion of their older leaves at the beginning of the vegetation period (Yang et al. 2005), transpiration was mainly based on present year's leaves, i.e. similar to deciduous species. In contrast to *Quercus*, *Castanea* relied most heavily on water uptake in summer. Besides the observed seasonal dynamics in water uptake, smaller temporal scales such as daily and hourly dynamics in the stomatal control of transpiration (Kulmatiski and Beard 2013; Kröber and Bruehlheide 2014) might be also relevant to explain this pattern. In autumn, proportional tracer uptake did not differ between species but high stem water enrichment was still detectable. This indicates that evergreen and deciduous tree saplings still relied intensively on soil water resources as late as beginning of November. In winter, unlike deciduous trees which shed leaves and thus stopped transpiring, evergreen species continued to use soil water resources, albeit to a smaller extent, because of ongoing physiological activity and transpiration. Thus, in agreement with other studies, unsynchronized phenology patterns may foster water resource partitioning (Meinzer et al. 1999; Stratton et al. 2000). Given these differences in spatial and temporal water uptake there is evidence from the same experimental site that belowground complementarity can also promote nitrogen acquisition in tree species mixtures (Lang et al. 2014). Together with niche separation in canopy space, belowground complementarity may thus explain higher growth rates found in some species combinations by Lang et al. (2012).

4.3 Interspecific interaction, niche breadth and overlap

Contrary to our expectations, interspecific competition between tree saplings did not affect δD_{excess} , niche breadth of single species or niche overlap among species. Indeed, we found no indication that the species-specific patterns in soil water exploitation was affected by interspecific interactions or plot diversity. This supports previous findings confirming relatively low species plasticity in water uptake depth in response to tree diversity in a temperate tree plantation (Grossiord et al. 2014a). Following Silvertown (2004), we anticipated that the competitive manipulation (intraspecific and interspecific competition) would cause hydrological niche shifts and consequently reduced niche overlap among species in the 4-species mixture. There are several possible explanations why patterns of water uptake were not affected by the competition treatment in our study, although we cannot distinguish between them with the available data. First, water availability might not have been a strong limiting factor for tree growth for most of the year and was rather similar between monocultures and species mixtures (Table A.2, Appendix A). Therefore, low competition for water irrespective of the diversity treatment may also have prevented adaptive changes in water uptake patterns. However, the similar tracer uptake pattern (Fig. 3) in summer – when water is abundant – and autumn – when water is relatively scarce – suggests that water availability is not the only cause of the lack of a competition effect. Second, belowground interspecific interactions between tree saplings might have not sufficiently developed yet despite the low planting distance. It has been shown in grassland experiments that plant species allocate less biomass to belowground organs when grown in mixture (Bessler et al. 2009). It can thus be suggested that belowground responses to changing diversity may have lagged behind aboveground interactions. According to this assumption, belowground competition for water and space were probably too small to induce modification in root depth and uptake activity in the 4-species mixture. Thus, no significant shifts in spatio-temporal water uptake patterns, niche breadth or niche overlap was detectable with the applied tracer

approach. However, there is evidence that niche shifts could occur at rather early stages but also in mature forest stands. For example, in a temperate tree diversity experiment, Lei et al. (2012) showed that competition in 6-year old mixtures might occur belowground even earlier than aboveground, with fine root growth of dominant species benefitting from being mixed with inferior species. By using a stable isotope approach Meißner et al. (2012) observed that *Fraxinus excelsior* responded to interspecific competition by extending its vertical zone of water uptake in mature mixed stands compared to single species clusters. Complementary use of soil water might be also an explanation for the positive relationship between tree diversity and stand transpiration found in a tropical tree plantation (Kunert et al. 2012). However, similar to our study tree diversity did not affect water uptake patterns in this neotropical plantation (Schwendenmann et al. 2015). Given these examples, it is possible that competition-induced shifts in root architectural or physiological traits may later trigger detectable niche shifts in our young experimental tree plantation. Similar to observations in grassland experiments (Allan et al. 2013) biodiversity effects may strengthen during forest development. For example, the phase of self-thinning in dense tree sapling communities is a good candidate for the onset of niche differentiation related to drought sensitivity and might be an important driver of species composition in mature forest stands (Engelbrecht et al. 2007). Third, it is possible that facilitative effects rather than competitive effects drive the interspecific interactions in the studied mixture. In that case, no niche differentiation would be expected to take place. While our data do not allow to identify such facilitative processes any further, hydraulic lift (e.g., Horton and Hart 1998) would theoretically be a good candidate to explain facilitative effects regarding water resources. However, due to the young age of the plant and the limited difference in rooting depth, hydraulic lift might at most be limited.

4.4 Consequences for subtropical forest management

Knowledge on species-specific water uptake patterns and potential complementary effects could improve forest restoration and plantation management. Considering the characterized hydrologic niches for the few species studied here, our results suggest that reforestation with *Castanea henryi* and *Quercus serrata* would lead to the most complementary water uptake at sapling stage as these species showed the lowest niche overlap. However, species interactions are likely to be subject of temporal and spatial variability during forest succession (Forrester 2014). Our study emphasizes the need of thorough information of species-specific resource use during forest development to identify the most beneficial species combinations. Especially in Eastern China where a large increase in forest plantation area is observed (FAO 2010) knowledge on functional interactions between species related to water resource use may help to improve drought resistance of forest stands. Nevertheless, the relationship between tree species diversity and forest resistance to drought is likely to be forest type-dependent (Grossiord et al. 2014b). We recommend that tree species selection for forest restoration should be based on profound information on multidimensional resource use and traits important for plant survival under drought to increase forest resistance and thereby maintaining ecosystem functioning under environmental stress (Kelty 2006; Zhou et al. 2013; O'Brien et al. 2014). In this respect, tree species selection may also influence tree water use at the stand level, and hence has to be considered in watershed management (Dierick and Hölscher 2009; Kunert et al. 2010). Further experimental investigations are therefore needed to elucidate how species diversity and composition may affect the water budget at ecosystem scale during forest development.

5. Conclusions

Our study provides an important step forward to evaluate the relevance of resource uptake complementarity as an important mechanism for tree diversity effects on ecosystem functioning by direct quantification of niche differentiation with respect to water uptake. Our

results suggest that at this early stage of forest development, soil water complementarity could only occur through differences in the fundamental water uptake niches, but yet not due to phenotypic adjustments in response to neighbor diversity. Thus, we found evidence that species identity rather than interspecific interactions or neighbor tree diversity controls water uptake patterns during sapling establishment. We propose that future work should account for multidimensional resource uptake by including multiple resources to reveal the role of tree diversity for forest performance during forest establishment.

Acknowledgements

We highly appreciate the support of the BEF-China research group during establishment and planting of the experiment. Peter Kühn and Thomas Scholten kindly provided climate data. We thank Annika Ackermann from the Grassland Sciences group (Prof. Nina Buchmann) at ETH Zurich for stable isotope analyses. Financial support was provided by the German Research Foundation (DFG FOR 891/1) with a grant to MSL (SCHE 695/2-1). YS was funded by SSSTC JRP (project IZL CZ3 123883).

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Tables

Table 1 Results of the mixed-effects models for treatment effects on δD_{excess} in plant stem water and proportional D uptake (as percentage of each species' overall D uptake across all 8 treatments), respectively.

Fixed effect:	δD_{excess} (‰)				Proportional D uptake		
	DFn	DFd	F	p	DFd	F	p
Root biomass (RBM)	1	59	5.8	0.019	59	3.8	0.057
Species richness (SR)	1	139	1.0	0.319	139	0.9	0.348
Species identity (SI)	3	59	10.2	<0.001	59	5.5	0.002
Soil depth (SD)	1	139	34.2	<0.001	139	31.7	<0.001
Season (S)	3	139	118.7	<0.001	139	120.8	<0.001
SR x SI	3	139	0.8	0.515	139	0.0	0.994
SR x SD	1	139	0.2	0.632	139	0.1	0.764
SR x S	3	139	2.1	0.098	139	2.3	0.080
SI x SD	3	59	7.7	<0.001	59	6.6	0.001
SI x S	9	59	4.8	<0.001	59	5.2	<0.001
SD x S	3	139	9.1	<0.001	139	9.0	<0.001

Numerator degrees of freedom (DFn), denominator degrees of freedom (DFd), F-values and p-values are shown.

797 **Table 2** Percentage values for uptake of deuterated water from 5 cm and 20 cm in each season across levels of species richness.

Species	Spring		Summer		Autumn		Winter	
	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm
<i>Castanea</i>	14.8±1.9 a	2.9±1.0 a	32.0±1.6 c	16.4±2.6 a	27.0±6.5 a	6.9±2.5 a	0.0±0.0 a	0.0±0.0 a
<i>Quercus</i>	17.1±3.3 a	6.8±2.0 a	15.2±1.0 a	22.8±3.6 a	18.1±3.6 a	20.0±7.0 a	0.0±0.0 a	0.0±0.0 a
<i>Elaeocarpus</i>	9.9±2.0 a	5.8±1.5 a	21.1±2.9 ab	20.3±3.8 a	22.6±2.0 a	11.5±4.3 a	3.4±0.8 c	5.4±1.2 c
<i>Schima</i>	9.7±1.3 a	6.4±1.2 a	24.4±2.0 bc	16.4±2.0 a	26.4±6.4 a	12.7±5.5 a	1.5±0.4 b	2.5±0.9 b

798 Values are means ± standard errors (n = 5 to 8). The sum of all values in each row equals 100% which is defined as overall tracer uptake across all 8

799 treatments. Different letters indicate significant differences (p <0.05) between species based on post-hoc Tukey`s test.

800 **Table 3** Niche overlap (proportional similarity) between species calculated across levels of
801 species richness.

	<i>Castanea</i>	<i>Quercus</i>	<i>Elaeocarpus</i>	<i>Schima</i>
<i>Castanea</i>	100%			
<i>Quercus</i>	74.3%	100%		
<i>Elaeocarpus</i>	79.8%	80.8%	100%	
<i>Schima</i>	86.7%	78.5%	91.1%	100%

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803

Figure captions

Fig. 1. Experimental layout showing the planting scheme of tree communities established as monocultures and 4-species mixtures. Different shading of circles represents the four tree species: *Castanea henryi* (black), *Quercus serrata* (dark grey), *Elaeocarpus decipiens* (light grey) and *Schima superba* (white). Each plot contained 16 tree individuals. Planting distance was about 25 cm. Deuterated water was injected in 49 single equally-spaced injection points (7 x 7) covering the inner square (dashed line) in each plot.

Fig. 2. Seasonal pattern of monthly mean temperature (black line) and monthly sum of precipitation (grey bars) during the study period from June 2009 to June 2010. Vertical arrows represent the date of deuterium tracer application in summer (end of August 2009), autumn (November 2009), winter (end of February 2010) and spring (end of April 2010). In September, October, November and December precipitation was less than 100 mm.

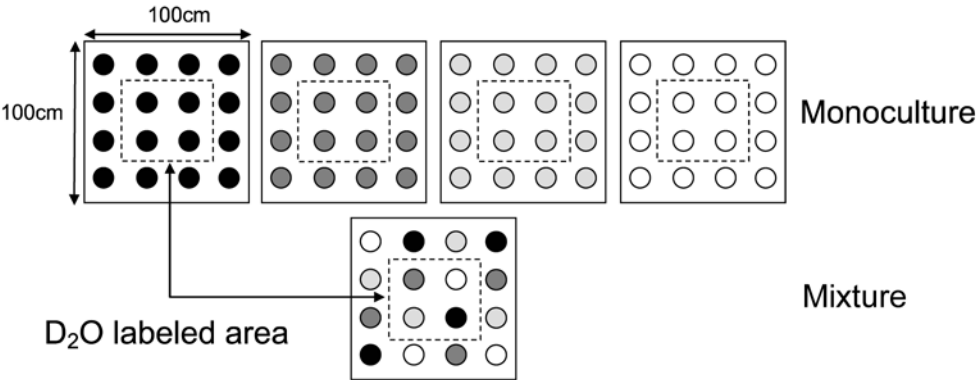
Fig. 3. Natural δD and excess δD of stem water. Natural δD (‰) of stem water of *Castanea* (black) *Quercus* (dark gray), *Elaeocarpus* (light gray) and *Schima* (white) in each season (a-d) and δD_{excess} (‰) in stem water across levels of species richness 6 days after tracer application at 5 cm (e-h) and 20 cm (i-l) soil depth, respectively. Error bars are standard errors of the mean. Different letters indicate significant differences between species in each season based on post hoc Tukey's test ($p < 0.05$); for clarity, letters are given only in those panels with significant differences. Number of replicates was $n = 4$ for natural δD and $n = 5 - 8$ for δD_{excess} .

Fig. 4. Niche breadth and niche overlap. Boxplots of (a) niche breadth and (b) niche overlap in monoculture and 4-species mixture. Overlaid data points represent calculated niche

829 breadths of individual species ($n = 4$) and niche overlap for all species combinations ($n = 6$),
830 respectively.

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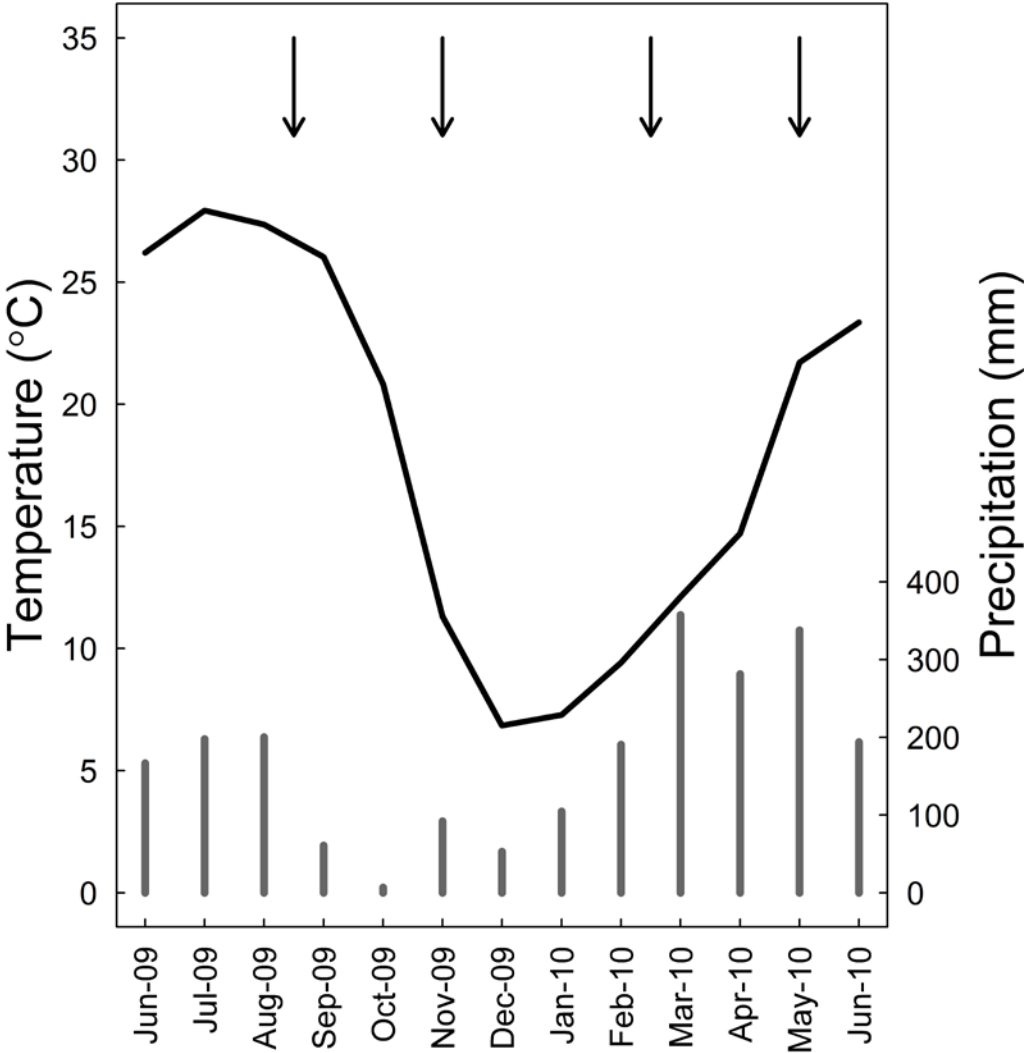
832 **Fig. 1.**



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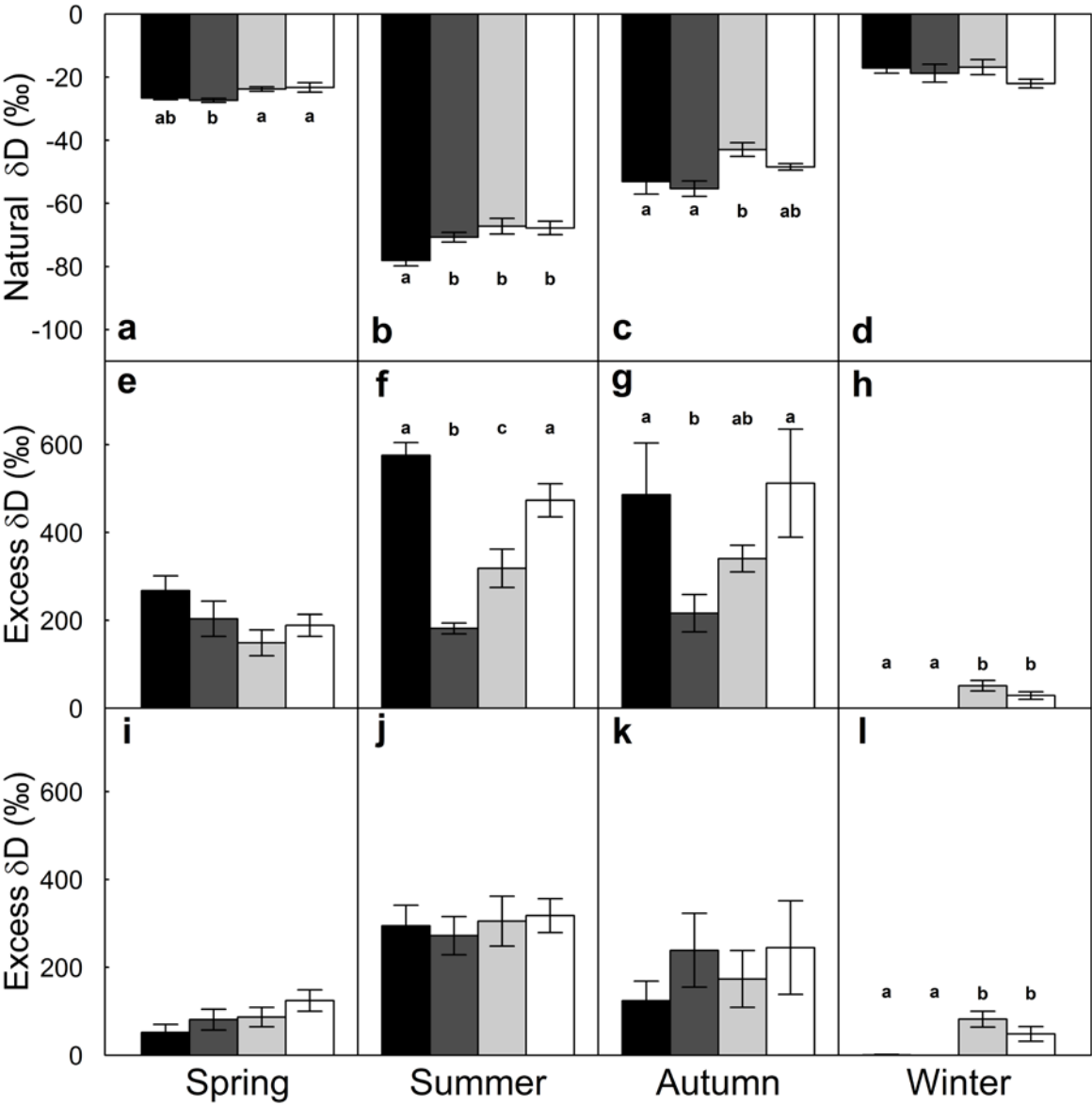
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835 Fig. 2.

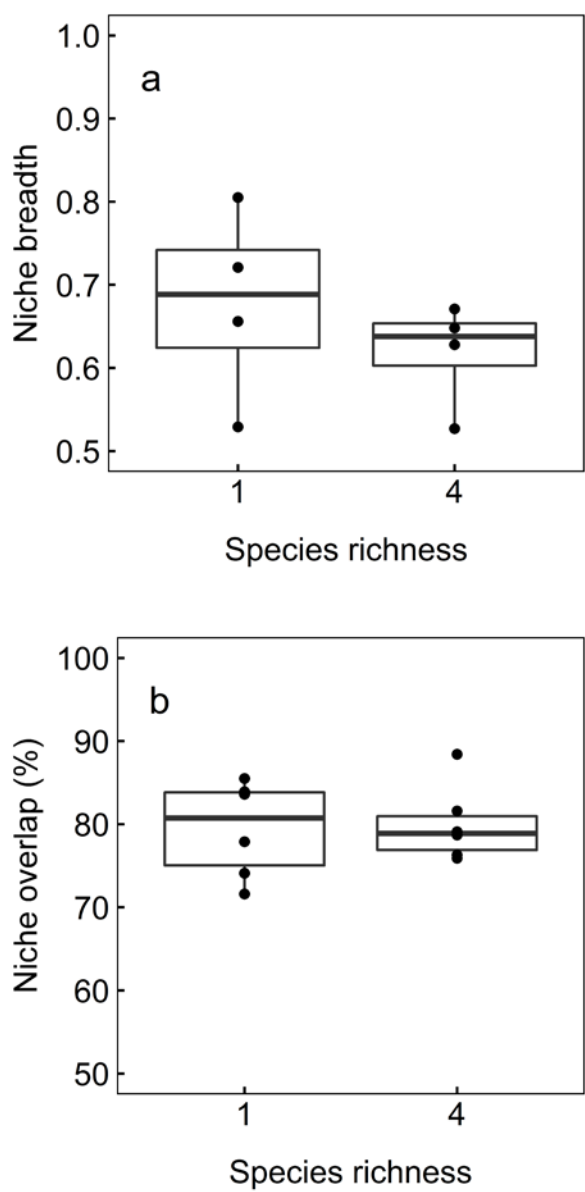


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841 **Fig. 4.**



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Appendix A

Table A.1. Mean root depth in cm (\pm SE) for the four species studied in each season.

Species	Summer 2009	Autumn 2009	Winter 2010	Spring 2010
<i>Castanea</i>	12.6 \pm 0.8 a	13.5 \pm 0.9 a	15.2 \pm 0.7 a	17.0 \pm 1.3 a
<i>Quercus</i>	19.1 \pm 1.3 b	25.8 \pm 1.6 c	29.6 \pm 2.0 c	31.6 \pm 2.7 c
<i>Elaeocarpus</i>	19.1 \pm 0.8 b	21.9 \pm 1.0 c	20.9 \pm 1.1 b	24.1 \pm 1.2 b
<i>Schima</i>	14.4 \pm 0.8 a	17.6 \pm 0.9 b	18.1 \pm 0.7 ab	18.9 \pm 0.7 ab

Different letters indicate significant differences ($p < 0.05$) in root depth among species in each season revealed by post hoc Tukey`s test (n = 20).

Table A.2. Gravimetric soil water content (% water per gram dry soil) prior tracer application at 5 cm and 20 cm soil depth in each season.

	Spring		Summer		Autumn		Winter	
Species	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm
<i>Castanea</i>	37.4±0.6 a	21.8±0.4 a	22.6±0.8 ab	18.1±0.4 a	11.8±0.8 a	16.6±1.0 a	36.8±1.0 a	25.6±1.7 a
<i>Quercus</i>	36.4±0.6 a	21.9±1.1 a	21.9±0.5 ab	18.7±0.8 a	11.4±0.7 a	15.1±0.8 a	31.2±1.6 a	19.3±2.0 a
<i>Elaeocarpus</i>	35.5±0.6 a	21.2±0.4 a	19.7±0.8 a	22.4±3.1 a	6.8±0.7 a	12.3±0.9 a	36.6±3.3 a	28.5±3.8 a
<i>Schima</i>	36.9±1.3 a	22.0±1.0 a	23.6±0.9 b	19.6±0.4 a	12.1±2.3 a	14.5±1.6 a	38.2±3.4 a	28.4±2.2 a
Mixture	35.5±0.9 a	22.6±0.8 a	21.7±1.2 ab	17.8±0.7 a	12.1±2.8 a	15.1±0.8 a	33.4±0.8 a	22.8±1.6 a

Values are means ± standard errors (n = 6, for winter: n = 2 - 6). Different letters indicate significant differences (p < 0.05) in gravimetric soil water content revealed by post hoc Tukey`s test between tree communities in each season.