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- TROPICAL SLVICULTURE AND FOREST ECOLOGY -

# TREE WATER UPTAKE PARTITIONING AND WATER USE RATES IN A TEMPERATE MIXED FOREST

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# CHAPTER 1

## INTRODUCTION

# 1. Introduction

## 1.1 Forests: Climate change, biodiversity and ecohydrology

There are a number of main aspects commonly attributed as valuable forest ecosystem services, such as climate stabilization, carbon storage, protection of hydrological function, and conservation of habitats for species biodiversity (Lindner et al. 2010). During this century, the increasing awareness that broad-scale future environmental changes threaten the sustainable provision of these services, has forced foresters to consider climate change in their forest management plans. Global climate change scenarios predict an increase in intensity and frequency of drought events during the vegetation period for large parts of central Europe (Rowell and Jones, 2006; Christensen et al., 2007). Based on IPCC climate change scenarios, summer temperatures in central Europe are expected to increase by about 3.2 - 4.8 °C until the end of the 21$^{st}$ century, coupled with a decrease of precipitation by 21 - 28% (CH2011, 2011). Though in order to adapt managed forests to changing environmental conditions it may be necessary to modify traditional forest management strategies. Thus, to support the inherent adaptive capacity of trees and forest ecosystems (Lindner et al., 2010) the concept of fostering „close-to-nature“ forest structures, already proposed since the ninetieth (Kenk and Guehne, 2001), gained even more importance. The concept of close-to-nature forestry implies a transformation of monocultural stands of narrow tree diameter range into stands composed of several tree species with a broader range of diameter and species which are suitable to prevalent site conditions (Röhrig et al., 2006; LÖWE, 2007). This transformation of even-aged to uneven-aged stands is an issue of changing from a structure that is homogeneous, and relatively well understood to one which is highly variable and with many complex interactions (O'Hara, 2001). This adds to the uncertainty on potential directions and magnitudes of environmental changes and forest responses, which can also not routinely be predicted by modeling approaches with the level of accuracy and precision needed by resource managers (Pilkey and Pilkey-Jarvis, 2007). Thus, it is reasonable to ask whether these silvicultural approaches are sustainable in any case (Gamborg and Larsen, 2003).

A high level of biodiversity is assumed to increase resistance of forest ecosystems against extreme atmospheric conditions and often subsequent forest pests, as well as a high potential of adapting to predicted environmental changes. There are also many other reasons why we may wish to conserve biodiversity, including aesthetic, cultural, and economic. However, it can be assumed that potential impacts and risks are best studied and understood with respect to wood production, which is considered the main indicator for vital forest growth. Though all other

goods and services provided by forests will also be affected by climate change, but much less knowledge is available to quantify functional impacts (Lindner et al., 2010).

Water availability is considered a major control of productivity in forests of central Europe and other regions of the world (Breckle and Walter, 2002; Huxman et al., 2004; Ellenberg and Leuschner, 2010). Therefore an the urgency exists to study possible effects of changing species composition and tree diameter range on the various aspects of the water cycle in diverse broad-leafed deciduous forests while facing more frequent and pronounced desiccation periods during the vegetation period.

## 1.2 The hydrological cycle in forests

The natural water cycle in the soil-vegetation-atmosphere continuum is an important and central process to energy, carbon, and solute balances (Zhang et al., 1999) of forest ecosystems. There are many pathways in forests that water may take in its continuous cycle of input in form of gross precipitation and output as evapotranspiration from vegetation and soil (Fig. 1.1).

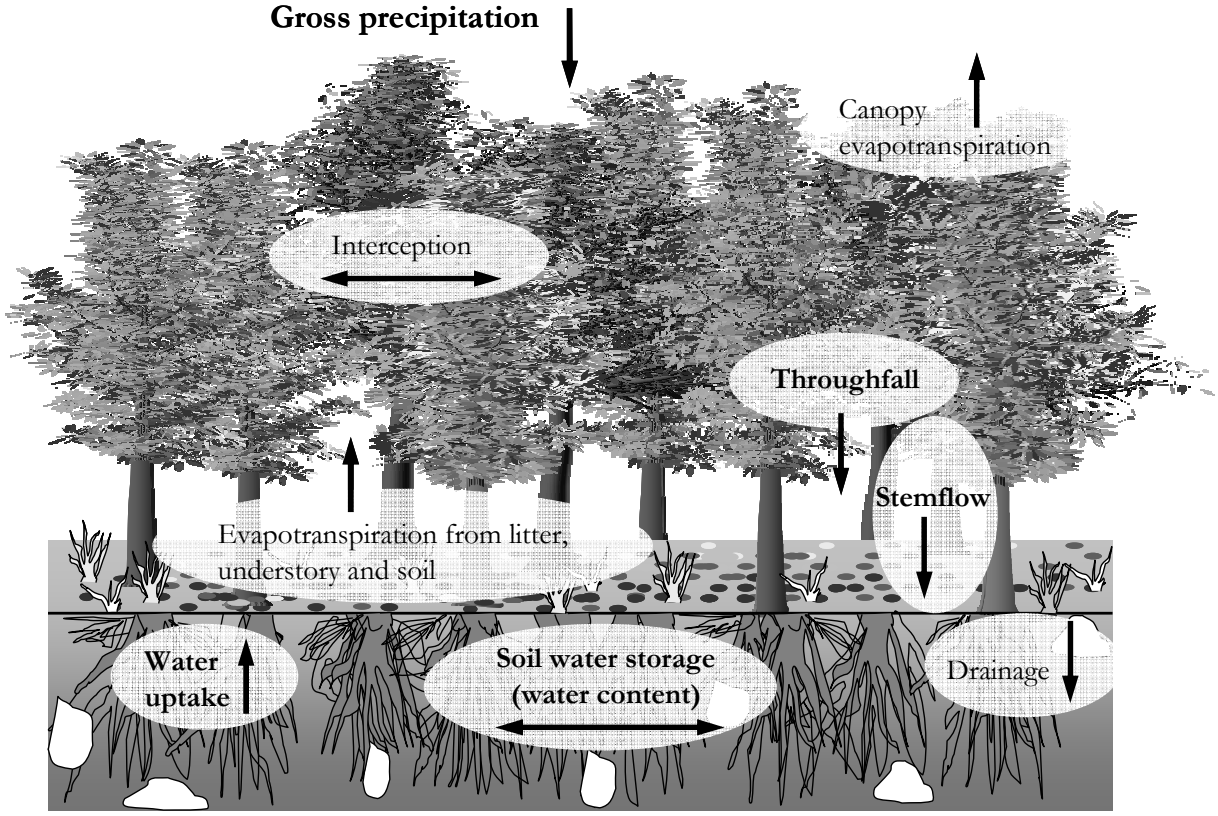


Figure 1.1: The main components of the hydrological cycle in forests. Parameters in the focus of this study (including data from Krämer and Hölscher, 2009) are highlighted in bold.

Some gross precipitation can be intercepted on surfaces of leaves, branches, stems of trees as well as on the herb- and litter layer, from which it evaporates directly back into the atmosphere. Another part passes directly though the canopy or drips from leaves and branches as throughfall or runs down along tree stems as stemflow. The part that actually reaches the forest floor may become surface runoff (mostly on slopes during strong rainfall events), evaporate from the soil or infiltrate the soil. Here it can be stored as soil water in air pockets of the soil matrix or percolate through the rooted soil volume as drainage and recharge groundwater or leave as slope parallel interflow. The part that gets stored in the soil may be taken up by the vegetation for transpiration. The composition of the vegetation may therefore have a significant influence on the rate of water fluxes by controlling rainfall partioning and soil water dynamics in an ecosystem (Krämer and Hölscher, 2009).

The main components of water input (throughfall, stemflow, and interception) in central European forests have been investigated to great extend with focus on beech (*Fagus sylvatica*) and spruce (*Picea abies*) at different ages and variing management intensity (e.g. Mitscherlich and Moll, 1970; Benecke, 1984; Gerke, 1987; Schume et al., 2004; for a detailed overview see Peck, 2004). However, investigations on hydrological aspects of tree species such as lime (*Tilia* sp.), ash (*Fraxinus excelsior*), sycamore (*Acer pseudoplatanus*), and hornbeam (*Carpinus betulus*), their admixture with *Fagus* is relatively scarce (Krämer, 2009). The information to what extend water dynamics in temperate broad-leaved mixed forests, are affected by tree species diversity is limited, but due to the ongoing trend of forest transition from monocultural to mixed species stands such information may be crucial for long-term decision making in forest management (O'Hara, 2001).

Hence, it would be important to know which functional and performance traits are inherent to certain tree species. Functional traits include physiological processes as well as morpho-, and phenological attributes (e.g. leaf, crown, and bark structure, root structure and distribution), which can combine to performance traits (e.g. transpiration rates, stemflow), defining plant performance (e.g. drought tolerance), and individual fitness (Violle et al., 2007). Species specific differences in water use, due to functional and performance traits and how they might combine in close tree neighborhood relationships or forest stands of different species diversity may therefore be of particular interest.

Further, under certain environmental conditions, the degree to which water is available for transpiration and biomass production is governed by a plant's capacity to exploit soil water resources; a property that can be enhanced by complementarity among co-occurring plants.

Resource use complementarity postulates that functional traits enable plants to exploit resources unavailable to others or use the same resource at a different place or time (Vandermeer, 1989). Resource partitioning and the consequently more effective utilization of resources have been suggested as an explanation for the higher productivity observed in many mixed plant communities compared to monospecific stands (Hagger and Ewel, 1997; Hooper et al., 2005). Especially under conditions when water supply is restricted, complementary resource partitioning might be beneficial. In grasslands as well as tree plantations, it has been observed that plant species diversity enhanced transpiration rates (Verheyen et al., 2008; Kunert et al., 2012), and complementarity in respect of water uptake was discussed as an underlying mechanism. Such a strategy may however also lead to a faster decline in available water for diverse communities during drought (van Peer et al., 2004; Verheyen et al., 2008).

### 1.3 Umbrella project and study design

The present study was conducted in the temperate broad-leaved Hainich forest of central Germany within the framework of the interdisciplinary Research Training Group (DFG-Graduiertenkolleg 1086) on 'the role of biodiversity for biogeochemical cycles and biotic interactions in temperate deciduous forests'. The aim of this project is to investigate in several subprojects the effect of different species assemblages in forest stands, on aspects such as productivity, nutrient and water turnover, and biotic interactions among key organism groups (for a more detailed list see Leuschner et al., 2009). The research training group was designed as a three-phased project for nine years of research on this field.

In the first phase of this project, studies were conducted on forest plot level with 12 study plots (Fig. 1.2) of 2500 m² size each, representing a tree species diversity gradient from monospecific *Fagus sylvatica* stands to stands composed of up to 11 broad-leaved tree species (Krämer, 2009), among which *Fagus sylvatica* (beech), *Tilia cordata* and *T. platyphyllos* (lime), *Fraxinus excelsior* (ash), *Carpinus betulus* (hornbeam) and *Acer pseudoplatanus* (sycamore) are the most abundant.

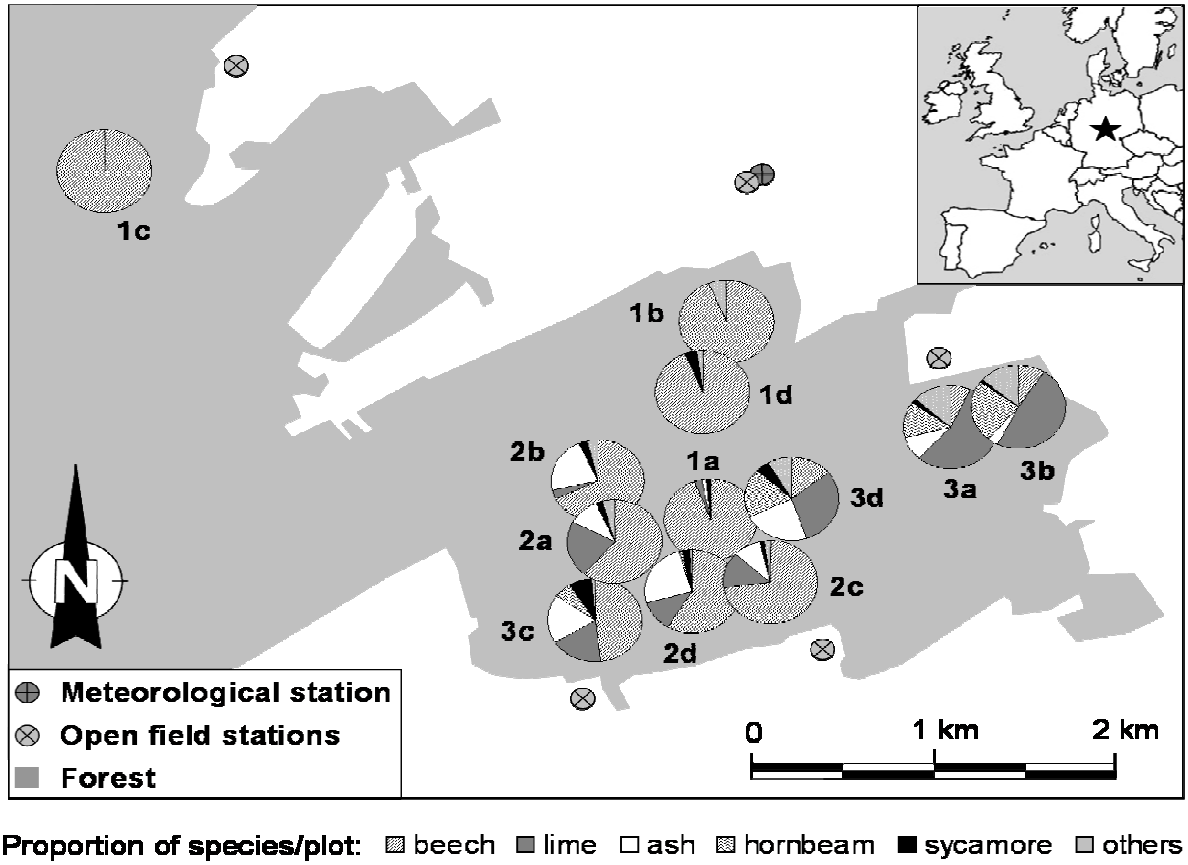


Figure 1.2: Positions and species assemblages of the 12 study plots of the previous research project in the Hainich/Germany (adapted from Krämer and Hölscher, 2009). Pie charts do not illustrate plot size.

Selection criteria for the stands were, comparable pedologic conditions, similar climate conditions (precipitation 600 - 670 mm $yr^{-1}$; annual mean temperature 7.5 - 8.0 °C), comparable stand structure in terms of basal area and tree diameter at breast height (dbh) (Leuschner et al., 2009). Three diversity levels (DL1 - 3) were defined, each represented by four stands. Pure *Fagus* forest (DL1), forest dominated by by *Fagus*, *Tilia* and *Fraxinus* (DL2), and the five species *Fagus*, *Tilia*, *Fraxinus*, *Carpinus* and *Acer* combined (DL3). Previous studies indicate considerable differences in water vapour exchange at the leaf level (Gebauer et al., 2008) as well as whole-tree water use among co-occurring tree species (Köcher et al., 2009). At the stand level, there were indications of enhanced soil water uptake during periods when soil water content declined in mixed stands compared to monospecific beech stands (Krämer & Hölscher, 2010). Important results from precedent observational studies, conducted in the framework of the umbrella project, which are thematically related to the present study, serve as groundwork for the objectives and formulation of hypothesis (e.g. Hölscher et al., 2005, Gebauer et al., 2008; Krämer & Hölscher, 2009; Köcher et al., 2009; Bittner et al., 2010; Krämer and Hölscher, 2009; 2010).

In the first project phase data on volumetric soil water content (%), water potential (ψ), throughfall (mm) and stemflow (mm) was collected on the twelve study plots from summer 2005 to winter 2007. Rainfall partitioning was stated to be influenced by several stand characteristics; tree species diversity represented by the Shannon index (H', ranged from 0.0 to 1.7) was the variable that explained throughfall for different seasons most frequently. In summer 2007 throughfall correlated positively with H', stemflow negatively and interception showed no correlation along *Fagus sylvatica* gradient. These relationships were similar in summer 2005 and autumn 2006, but no or only weak changes of throughfall with tree diversity were observed during other study periods. Influential stand characteristics varied between seasons and years, which was attributed to differing rainfall conditions (intensity and duration). Spatial variability of throughfall within a stand did not change consistently with any stand characteristic (Krämer and Hölscher, 2009).

Overall patterns of soil water dynamics were similar on all study plots. However, during a desiccation period in summer 2006, significant positive relationship between soil water extraction (mm $month^{-1}$) in 0-0.25 m soil depth and tree species diversity (H') of the twelve study plots were observed. At the beginning of this period, soil water was extracted at higher rates in the species rich plots than in the beech-dominated plots. However, later during the desiccation period when atmospheric evaporative demand was higher, only the beech-dominated stands were able to increase soil water extraction. On plots of high tree species diversity, soil water reserves were already low and soil water extraction reduced (Krämer and Hölscher, 2010).

Higher water extraction rates in mixed species plots, at the beginning of the desiccation period, were explained with species specific characteristics such as high maximum water use rate of some species, enhanced exploitation of soil water resources in mixed stands (complementarity effect), and additional water use of the herb layer, which also increased along the tree species diversity gradient. However, the study setup did not permit to draw conclusions on whether traits of certain species or their admixture had an effect on throughfall or the amount of extracted water. Other studies also found differing sap flux densities among the tree species grown at the study site (Hölscher et al., 2005; Gebauer et al., 2008; Köcher et al., 2009). Further, canopy transpiration was found to differ among diverse and less diverse stands in certain years (Gebauer et al., 2012). However, in none of these studies the outcome had been clearly attributed to a biodiversity effect, which was mainly caused by the circumstances that increasing biodiversity was also paralleled by decreasing *Fagus* admixture, and no monocultures of any other species involved were studied.

In order to differentiate between the effects of tree diversity and of species identity, a new experimental design was applied in the second project phase. Therefore it was crucial to establish new plots, in the same study area, and include all relevant tree species (from the first project phase) in monospecific plots and in admixture. We selected 100 groups of three neighbouring trees, hereafter named tree clusters, which contained all possible combinations of the five tree species (*Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Tilia sp.*). All species occurred in single-species clusters (n = 20), as well as in two- and triple-species mixtures (n = 40, each).

Therefore the following hypotheses were formulated to serve as the basis for this dissertation. During summer soil desiccation,

**1)** tree species differ in their vertical soil water uptake patterns;

**2)** in mixed-species clusters there is complementarity in soil water uptake due to niche differentiation in water uptake partitioning; and

**3)** across all trees studied, the depth of soil water uptake scales with tree size.

We further asked, whether stand water use is related to tree diversity and/ or species identity. Our hypothesis was that

**4)** water uptake volume in tree clusters increases with increasing species diversity *and* that

**5)** species specific traits with regard to water uptake are notable in small scale neighborhood relations.

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# Chapter 2

## METHODOLOGY

# 2. Methodology

## 2.1 Overview

The general goal guiding the research presented in this study was to describe the water use and vertical partitioning of soil water uptake of tree groups in a temperate deciduous forest and whether these are affected by species type, diversity, presence or absence of a certain species, tree structural parameters, stand characteristics, and environmental conditions. To this end, data on throughfall, volumetric soil water content, soil water potential, soil and stem xylem samples, tree diameter at breast height (dbh), canopy openness, cluster ground area, soil properties, gross precipitation and global radiation was gathered. This work was conducted within the framework of the 'Research Training Group 1086 – The Role of Biodiversity for Biogeochemical Cycles and Biotic Interactions', second phase of the umbrella research project. The study was conducted in the Hainich forest in Central Germany (Fig. 2.1) close to the village of Weberstedt (51°05'28"N, 10°31'24"E).

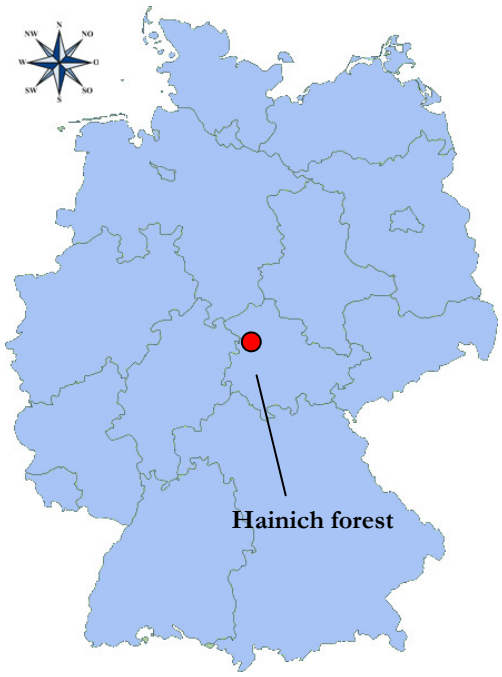


Figure. 2.1: Location of the Hainich forest in Thuringia, Germany (map edited from www.wikipedia.org).

The study sites are located on level terrain in the south-eastern part of the forest area (Fig. 2.2A) in a low mountain range at an elevation of approximately 350 m a.s.l. The park is situated within a subatlantic climate zone with a mean annual precipitation of 544-662 mm (average of 30 years of precipitation records from four climate stations around the national park; DWD, 2008) and a mean temperature of 7.5°C. The geological substrate is Triassic limestone covered by loess, forming nutrient-rich Luvisols with a soil texture characterized by high silt (~ 74%) and low sand (< 5%) content (values for soil depth 0 - 0.3 m, Guckland et al. 2009). The area of the Hainich forest in which our study was conducted has remained free from harvesting or thinning for almost 50 years, due to its use as a military training area since 1964 and its integration into a new national park in 1997 (Mölder et al. 2006). It was estimated that the area has hosted deciduous forest for over 200 years (Mölder et al. 2009).

## 2.2 Cluster selection

In Spring 2008, tree clusters were selected in two mixed forest stands within the Hainich forest area (sub-areas Lindig and Thiemsburg, Fig. 2.2B). All clusters were located in close vicinity to the study plots of the first project phase.

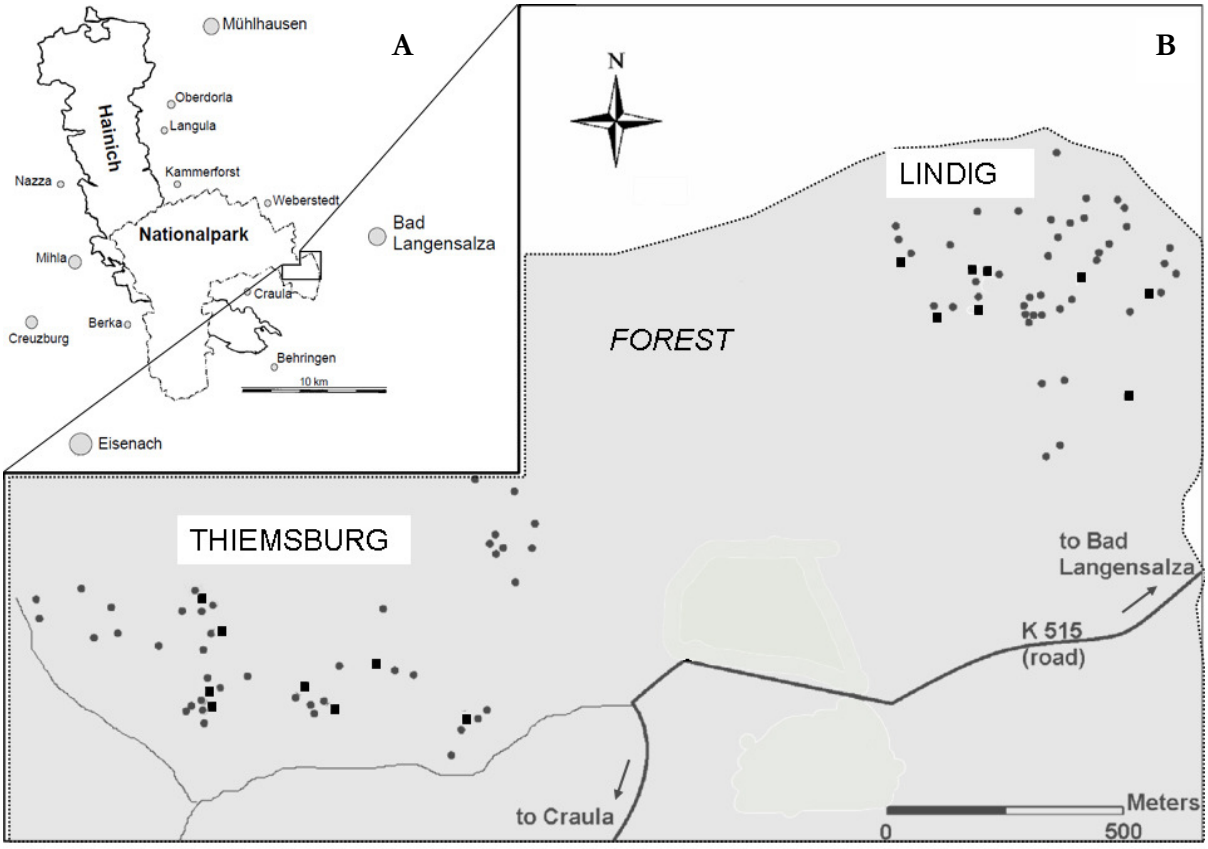


Figure 2.2: Location of the 100 tree clusters in the two forest areas. The grey dots and black rectangles indicate cluster positions. The 16 black rectangles represent intensively measured clusters (Figure based on D. Seidel, 2011).

Each cluster consisted of three co-dominant trees arranged in a triangular shape with their surrounding neighbours (Fig. 2.3). The three cluster trees forming each cluster had an average distance of 7.6 m to each other.

In order to achieve comparability with results from the first phase of the umbrella project, a similar tree species were chosen for the cluster setup. Namely the five species building the diversity levels in the first phase: *Fagus sylvatica* (European beech), *Tilia sp.* (lime), *Fraxinus excelsior* (ash), *Acer pseudoplatanus* (sycamore maple) and *Carpinus betulus* (hornbeam). In this forest, the two *Tilia* species *cordata* and *platyphyllos* often form hybrids, which are phenotypically difficult to

differentiate. Hence, in this study we did not differentiate at the species level and we refer to them as *Tilia* sp.

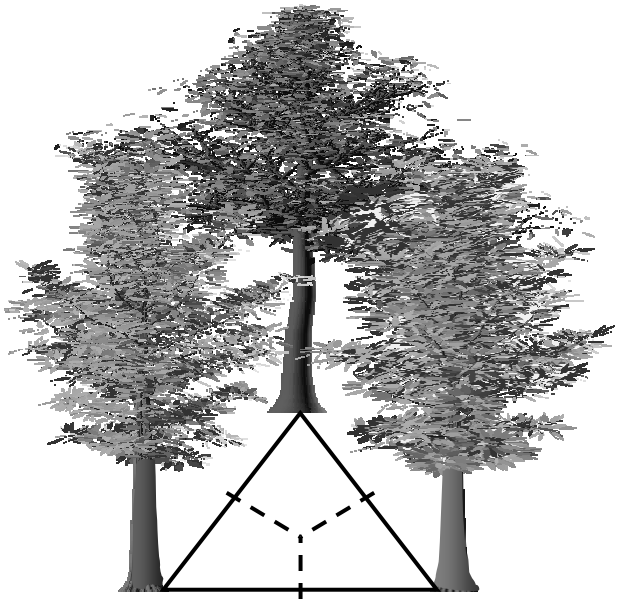

Figure: 2.3: Schematic tree cluster design in the Hainich forest.

Cluster selection was based on a predetermined combination of tree species comprising all possible neighborhood combinations of the five tree species. This resulted in five different single-species, ten double-species and ten triple-species cluster combinations, with each combination being replicated four times (twice replicated in each sub-area, Thiemsburg and Lindig). Average distance between the clusters of each area was 124 m at Thiemsburg and 112 m at Lindig.

From the 100 clusters, a subset of 16 clusters was selected containing the species *Fagus sylvatica*, *Tilia sp.* and *Fraxinus excelsior* both in monoculture and in triple-species clusters, with each type being represented by four replicates (two replicates per sub-area, Thiemsburg and Lindig). The selected clusters were used to monitor soil water content in the subsoil, to increase the temporal resolution of soil water content measurements and to conduct throughfall measurements (Fig. 2.2B).

## 2.3 Field set up and instrumentation

### *2.3.1 Hydrological measurements*

The most important component of the field set up on the tree clusters was the installation of sensor access tubes in July 2008 (Fig. 2.4) for measurements of volumetric soil water content ($\theta$ in $m^3\ m^{-3}$) and throughfall collectors on the 16 intensive clusters. Those were equipped with an overall of 72 PVC access tubes (4 replications per cluster; fig. 2.5), enabling measurement of $\theta$ with a portable FDR sensor (Frequency Domain Reflectometry; Diviner 2000, Sentek Pty Ltd. Stepney, Australia). Access tubes were installed to a maximum depth of 0.7 m in which sensor readings were taken at depth intervals of 0.1 m.

Figure 2.4: Installation of PVC access tubes for volumetric soil water content measurements on the 16 clusters.

On some clusters, it was not possible to install all access tubes to the full extent, as heterogeneously weathered limestone debris occurred already at shallow depths and obstructed the installation. The FDR sensor had already been soil- and depth-specifically calibrated for the local soil conditions in the field (Krämer and Hölscher, 2010).

In addition, 72 sets of tensiometers were installed on the intensive clusters in 0.1, 0.3 and 0.5 m soil depth. Throughfall was monitored on the 16 clusters with rainfall collectors consisting of a plastic bottle screwed to a funnel with an opening of 10.5 cm in diameter. The bottle was housed in a plastic tube attached to a metal rod at a height of 1 m. To reduce evaporation from the rain gauge, a table tennis ball was placed in the funnel.

On all 100 clusters measurements of soil volumetric water content ($\theta$ in $m^3\ m^{-3}$) were conducted at four points with a mobile TDR probe (Time Domain Reflectometry probe CS616, Campbell Scientific) at a depth of 0 - 0.3 m. A site specific calibration for the TDR probes was established by correlating 72 FDR readings at different soil water contents with corresponding TDR readings in the direct vicinity of the FDR sensors (Fig. 2.5).

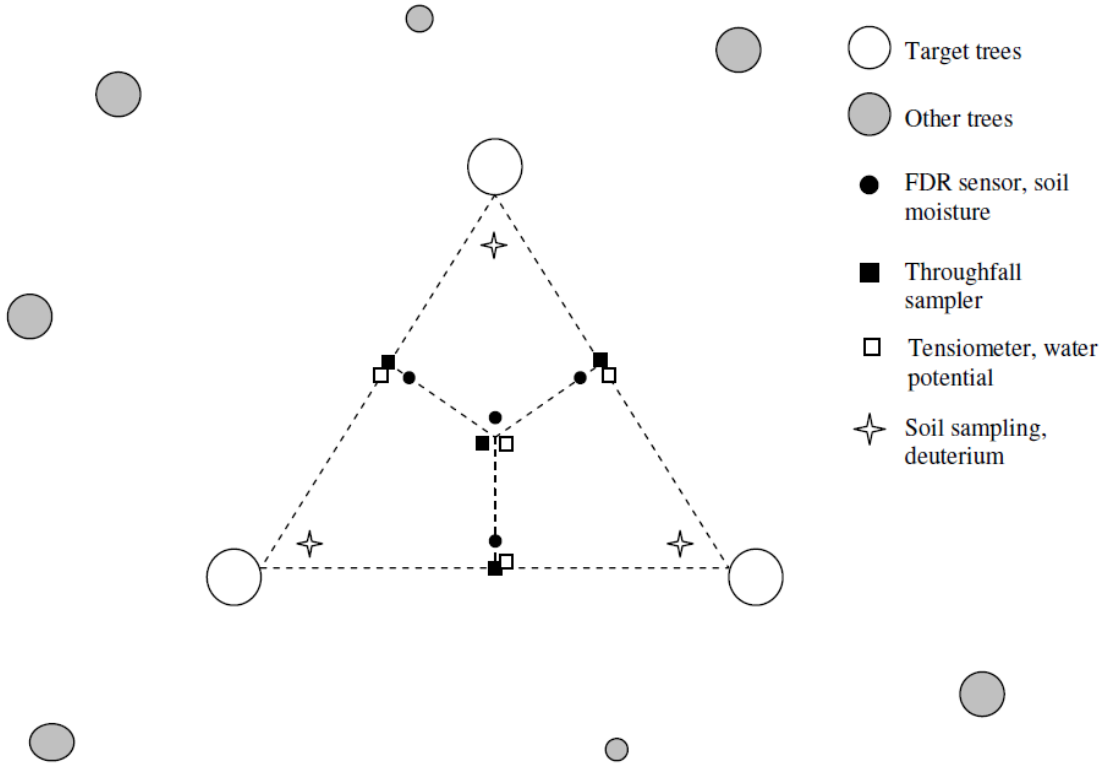


Figure 2.5: Schematic study plot design (tree cluster) with measurement locations.

Additional instrumentation consisted of an automated weather station (Meteomedia, Germany) close to the nearby town Weberstedt on an open area, 2-3 km northwest of our study area at an altitude of 270 m above sea level. The station provided data on air temperature (C°), gross precipitation (mm), global radiation (MJ $m^{-2}$ $day^{-1}$) and wind speed (m $s^{-1}$).

### *2.3.2 Natural abundance of water stable isotopes*

To assess profiles of relative water uptake for each of the observed species we determined the natural abundance of the stable isotopes $^{2}H$ = Deuterium, D and $^{18}O$.

Isotopic fractionation of water isotopes is predominantly associated with phase changes from solid to liquid to vapour and *vice versa* called equilibrium fractionation, and to the diffusion processes of water vapour, the kinetic fractionation (Cappa et al., 2003). When in the liquid phase, the heavier (or stable) isotopes are bound more tightly than the lighter isotopes and thus are not so easily released into the vapour phase. During prolonged desiccation of soil water without frequent water input by precipitation, an isotopic gradient will develop in the soil profile.

According to several studies, fractionation of O and H isotopes follow the same patterns during the evaporation processes (Wershaw et al., 1966; Thorburn and Walker, 1993). Both water isotopes in precipitation, ocean, lake, soil and ground water are therefore closely related, as demonstrated by the global meteoric water line (GMWL) formulated by Craig (1961).

Thus, it is assumed that the naturally occurring vertical gradients of $\delta^{2}H$ and $\delta^{18}O$ in soil water provide similar information about plant water uptake depth from soils (Plamboeck et al., 1999). It is further assumed that the isotopic composition of water is unchanged by root uptake and during transport through the stem xylem in most plants (Wershaw et al., 1966; White et al., 1984), with the exception of some coastal wetland species (Lin et al., 1993) and certain woody xerophytes, which fractionate $^{2}H$ but not $^{18}O$ during root water uptake (Ellsworth et al., 2007). Still, it can be assumed that $\delta^{2}H$ and $\delta^{18}O$ can be used similarly in water uptake studies for a wide range of woody plant species where samples of different possible water sources are obtained together with plant (xylem) water samples.

### *2.3.3 Soil and stem sampling for stable isotope analysis*

Samples from soil and trees of the 16 clusters were taken once in a summer desiccation period on 25 and 26 August in 2009. Soil samples were taken at depth intervals of 0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.5 and 0.5-0.7 m under the crown area inside the clusters. Each sample consisted of a mixture of two adjacent soil cores taken at the same depth. Xylem tissue samples were taken from the outer 6 cm of the stem at three points at breast height from each individual tree with a corer. The bark was removed after sampling to avoid contamination of xylem water with phloem water. Soil samples were taken with an auger at depth intervals of 0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.5 and 0.5-0.7 m directly under the crown area inside the clusters; tissue samples of each tree were taken simultaneously to soil samples. All samples were stored in 40 ml glass tubes (ND24), closed with a Teflon coated lid, sealed with paraffin film, and then kept frozen until water extraction to reduce subsequent evaporation from the samples. Extraction of water from plant

and soil samples was conducted via cryogenic vacuum extraction adapting a description of Ehleringer and Osmond (1989) and West et al. (2006).

### *2.3.4 Sample water extraction*

The apparatus used for the water extraction consisted of five independent u-shaped glass units (extraction unit, Fig. 2.6), attached to a main glass vacuum manifold. Each unit could be isolated from the main manifold by a glass-rubber stopper valve. The entire main vacuum line was connected to a cooling trap and a vacuum pump. Prior to water extraction, frozen sample material (15 g soil and 4 g stem), was filled into glass tubes (ND24, 40 ml) and equipped with glass wool directly above the sample to prevent any soil particles from traveling through the extraction unit. Care was required to ensure that wool filaments did not interfere with the insulating rubber O-ring sitting between the sample glass tube and the extraction unit as this could compromise the vacuum seal. To extract water from a sample, a separate extraction unit was equipped with a glass tube containing the material and an identical empty collecting tube. Afterwards the tube containing the material was immersed into liquid nitrogen for about 15 minutes, to ensure that all water in the sample is still frozen. This is crucial, as water in liquid state could be sucked out of the extraction unit when vacuum is established. Afterwards, connection to the main vacuum manifold was reestablished and the entire extraction unit was pumped down to a pressure of 0.01 mbar. After establishing vacuum in the extraction unit, the connection valve between main manifold and extraction unit was closed and the Dewar vessel, containing the liquid nitrogen was replaced by a heating lamp. Air temperature close to the heating lamp was ~115°C. Care has to be taken, that all condensation water on the outside of the material tube was removed with a blow-dryer beforehand, as this could cause damage to the heating lamp in the long run. The Dewar was then placed under the collecting tube to freeze all water emanating from the sample, traveling along the temperature gradient and into the collecting tube. Periodic addition of liquid nitrogen was required to keep the tube adequately cooled.

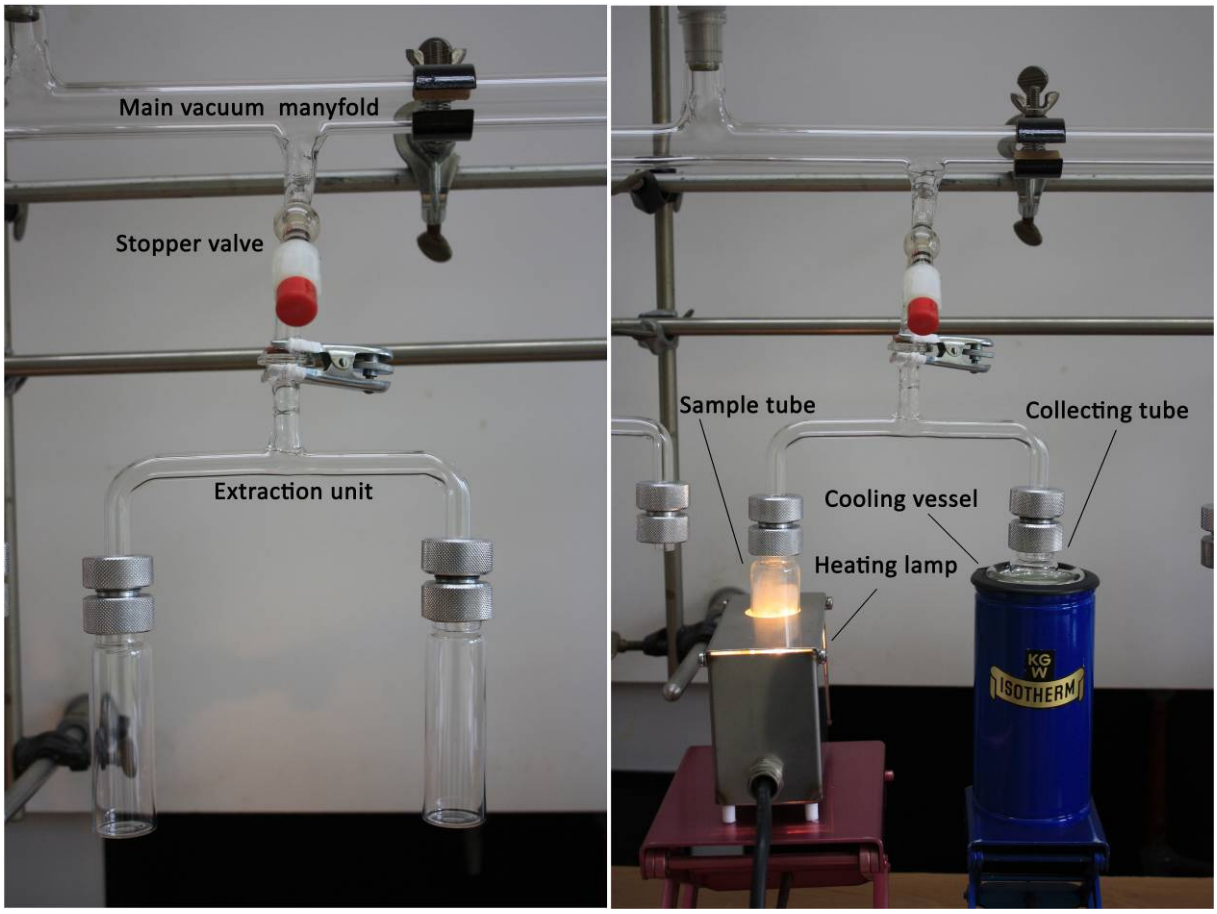


Figure 2.6: Distillation unit for cryogenic vacuum extraction of water from sample material..

The collection tube was than sealed and allowed to thaw. Extracted water was stored in 1.5 ml or 0.6 ml storage vials, dependent on the volume of extracted water, and was retained in a fridge until isotopic analyses. Before and after the distillation process, all glass tubes were weighted empty and with material.

In order to test if the extraction process was complete and no water was retained in the sample, the material was stored in paper bags after the distillation, weighted and oven-dried at 110° C for 72 hours. After drying samples were weighted again to ensure all water was extracted during the vacuum distillation.

### *2.3.5 Stable isotope analysis*

The analysis of extracted water was carried out at the Center for Stable Isotope Research and Analysis (Georg-August-Universität Göttingen, Germany). Measurements of the hydrogen and oxygen isotopic composition ($\delta^{2}H$ and $\delta^{18}O$. were conducted by injecting the water into a high

temperature conversion elemental analyzer (TC/EA, Thermo Electron Corporation, Bremen, Germany) coupled via a Con-Flo III interface to a Delta V Plus isotope ratio mass spectrometer (Thermo Electron Corporation) with reversed sample flow (Gehre et al., 2004). Isotope ratios were expressed as per mill deviations to the internationally accepted Vienna Standard Mean Ocean Water (VSMOW, $R_{Standard}$) (Gonfiantini, 1978) with a measurement precision of ±2.0 ‰ for $\delta^2H$ and ±0.2 ‰ for $\delta^{18}O$.

### *2.3.6 Extraction time test*

In a study by West et al. (2006) they tested different extraction times to soil samples differing in texture and stem samples from different species. They propose a minimum extraction time of 60 to 75 minutes for stem samples in general and about 40 minutes for a clay soil. They found that once the minimum extraction time was reached, only a very small amount of water remained in the sample and another 3 hours of extraction would be necessary for its recovery. However, this additional extraction time appeared to be unnecessary for the purpose of gaining an unfractionated sample, with respect to the current state of analytical precision (West et al., 2006).

As clay content in the present soil can be up to 45% in deep soil layers an extraction time series was conducted to estimate the appropriate extraction time for soil and stem samples. Thus four different extraction times (60, 90, 180 and 240 minutes) were tested in order to estimate the extraction time needed to gain unfractionated samples and to optimize working time.

For this experiment soil samples from the depth intervals 0.3-0.5 and 0.5-0.7 m were used. According to the study of West et al. (2006) samples with high clay content need longer extraction times to receive an unfractionated sample (40 minutes), therefore only the two deeper soil intervals were included in this experiment, as clay content increased with soil depth on the clusters. Stem samples of each tree species (*Fagus sylvatica*, *Tilia* sp. and *Fraxinus excelsior*) were tested as well. For stem samples of varying wood density, West et al. (2006) proposed extraction times between 60 to 75 minutes. Five soil samples (5 g each) and stem samples (1 cm per core) were then extracted for 60, 90, 180 and 240 minutes and analyzed for their isotopic composition of $\delta^2H$ and $\delta^{18}O$. After extraction for 90, 180 and 240 minutes, $\delta^2H$ and $\delta^{18}O$ (‰) extracted water samples had similar isotopic compositions (-57.0±0.5 ‰ and -10.1±0.05 ‰). The isotope composition from the 240 min. extraction was set as equilibrium value and used as reference for the other samples. Water extracted from soil and stem samples for 60 minutes differed notably from the equilibrium: -5.5 ‰ $\delta^2H$; -0.83 ‰ $\delta^{18}O$ for soil and -7.0 ‰ $\delta^2H$; -0.97 ‰ $\delta^{18}O$ for stem samples, respectively (Fig. 2.7 A and B).

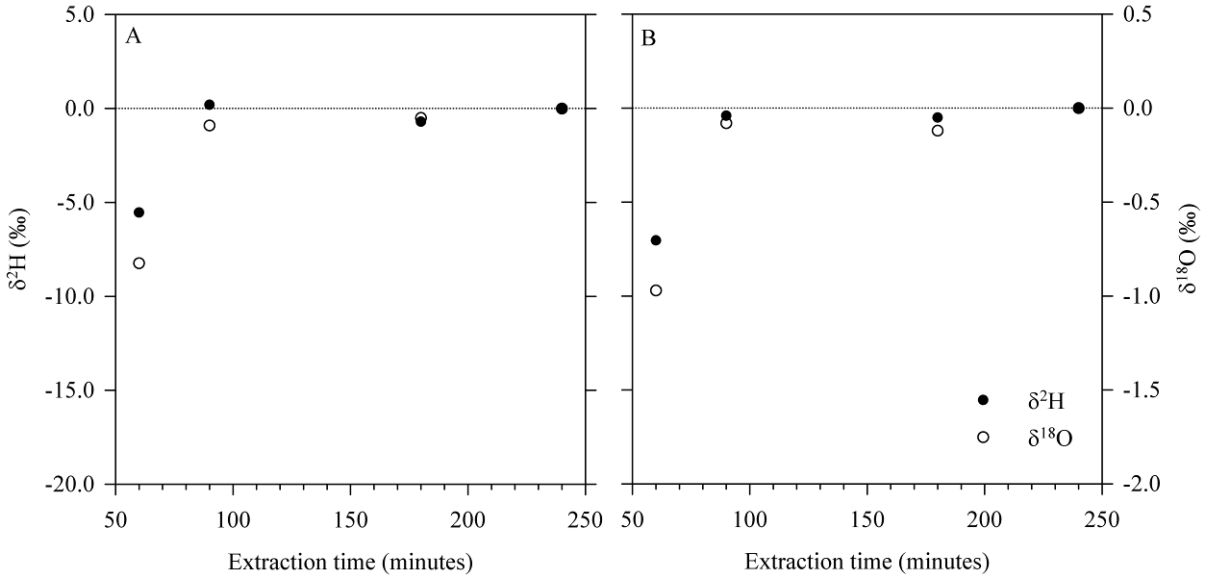


Figure: 2.7: Isotopic composition (δ‰) of $^{2}H$ (filled circles) and $^{18}O$ (open circles) of water extracted from soil (A; 0.5-0.7 m soil depth) and stem (B; *Fraxinus excelsior*) samples in cryogenic vacuum distillation using different extraction times.

Results are shown for soil samples from 0.5-0.7 m soil depth and *Fraxinus excelsior* stem samples only as deviations from the equilibrium values were similar among soil depth intervals and among the observed tree species. Based on the extraction time test, 90 minutes extraction intervals appeared to be sufficient to receive unfractionated water samples.

### *2.3.7 Stable isotope mixing model*

In order to identify the depth of water uptake for a given tree, it is necessary to identify the source of the isotopic composition found in the tree xylem, such as soil water at multiple depths, groundwater or stream water, depending on which sources are available. The isotopic composition of stem water is therefore compared with that of potential water sources in order to identify where both match best, based on direct inference (e.g., Jackson et al., 1996; Brunel et al., 1991, 1995; Mora and Jahren, 2003; Schwendenmann et al., 2010). The main assumption of this approach is that roots obtain soil water from one soil depth at any given point in time, which does not always adequately reflect the high heterogeneity of water sources that may be available for a tree. Furthermore, this visual method precludes the possibility of assessing proportional contributions of multiple water sources by quantitative means and can not reveal subtle differences in water uptake patterns (Asbjornsen et al., 2007). Therefore we used a "multiple source mass-balance mixing model" (Isosource; Phillips and Gregg, 2003) that calculates the

relative contribution of each soil depth interval to stem water in order identify each trees' water uptake depth. The underlying assumption is that the isotopic signature of the plant water is a mixture of the signatures found in the soil.

Hence, with five soil depth intervals there are five potential water sources that could contribute to the mixture in the stem xylem. In this case the model is basically a mathematical underdetermined system of two equations in five unknowns (Phillips and Gregg, 2003). With one isotope system ($\delta^2$H or $\delta^{18}$O) and five sources, the following system of mass balance equations can be solved to determine the proportions ($f_A$ - $f_E$) of soil water isotopic signatures ($\delta_A$ - $\delta_E$) which coincide with the observed signature in xylem water ($\delta$M):

$$\begin{aligned} \delta M &= f_A\delta_A + f_B\delta_B + f_C\delta_C + f_D\delta_D + f_E\delta_E \\ 1 &= f_A + f_B + f_C + f_D + f_E \end{aligned} \qquad (1)$$

It calculates the range of all feasible source contributions where the number of sources is greater than one (n + 1). In this model the fractional contribution of each potential source (soil interval) is iteratively increased in small steps (e.g. 1%) and an isotopic mass-balance is performed at each increment to determine if the result compares well with the isotopic composition of the mixture (xylem water). Each time a feasible combination produces the isotopic value of the mixture within a given uncertainty or "mass balance tolerance", e.g. ±0.1‰ (Phillips and Gregg, 2003), those combinations are stored. At the end of the calculation, one obtains an output file containing the frequency of the number of times a fractional contribution for a given source produces a feasible combination. Also the maximum, minimum, mean, 1-, 50- and 99%ile are given.

The fractional increment used in the model calculations was set to 1% for all calculations and the tolerance level to 0.5‰ ($\delta^2$H) and 0.02‰ ($\delta^{18}$O). The applied levels appeared appropriate as they were even smaller compared to the average analysis error during mass spectrometry for both isotopes. Still, sensitivity analyses were conducted with varying mass balance tolerances for $\delta^2$H (1‰ and 0.1‰) and $\delta^{18}$O (0.05‰ and 0.01‰), respectively. Changing tolerance levels had no significant effect on the model output.

The mixing model output gives a range of feasible source contributions of a given soil depth interval to the respective mixture (xylem water). For further statistical analyses the mean of all source contribution estimates (mean model outcome) for a given source was used.

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# Chapter 3

## WATER UPTAKE BY TREES USING WATER STABLE ISOTOPES - ASSESSING THE EFFECTS OF SOIL WATER, CLAY AND CARBONATES ON $\delta^2H$ AND $\delta^{18}O$ SIGNATURES IN SOIL WATER

# 3. Water uptake by trees using water stable isotopes - assessing the effects of soil water, clay and carbonates on $\delta^2H$ and $\delta^{18}O$ signatures in soil water

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**Abstract.** Stable isotopes of oxygen and hydrogen are often used to determine plant water uptake depths. We investigated whether and to what extend soil moisture, clay content, and soil calcium carbonate influences the water isotopic composition. In the laboratory, dried soil samples varying in clay content were rewetted with different amounts of water of known isotopic composition. Further, we removed soil carbonate from a subset of samples prior to rewetting. Water was extracted from samples via cryogenic vacuum extraction and analysed with a mass spectrometer. The isotopic composition of extracted soil water was similarly depleted in both $^{18}O$ and $^{2}H$ with decreasing soil moisture and increasing clay content. Soil carbonate changed the $\delta^{18}O$ composition while $\delta^{2}H$ was little affected. We recommend that studies of plant water uptake from soil based on isotope analyses are treated with caution, particularly in cases where only one isotope is studied and for clay and carbonate rich soils with low water content.

Key words: isotopes, water, soil, clay, carbonate

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## 3.1 Introduction

The natural abundance of the stable isotopes of $\delta^2H$ (deuterium) and $\delta^{18}O$ are widely used to investigate ecological and hydrological processes, such as plant-water relations. Using the "spatial isotope fingerprint of water" (West et al. 2006) in plant physiological studies has significantly improved our understanding of ecological processes since its first emergence in the 1970s.

Several studies investigated the water sources of plant species in a wide range of environments differing in climate, soil type and vegetation type (Dawson et al. 1991, Ehleringer et al. 1991, Flanagan et al. 1992, Dawson et al. 1993a, Dawson 1993b, Jackson et al. 1995, Jackson et al. 1999, Meinzer et al. 1999). Water stable isotopes can provide useful information on resource complementarity or competition among individuals of a population, especially in studies on plant species diversity effects or neighborhood relationships (Schwendenmann et al. 2010).

According to several studies, fractionation of O and H isotopes follow the same patterns during the evaporation processes (Wershaw et al. 1966, Thorburn and Walker 1993). Thus, it is assumed that the naturally occurring vertical gradients of $\delta^2H$ and $\delta^{18}O$ in soil water can provide similar information about plant water uptake depth from soils (Plamboeck et al. 1999). It is further assumed that the isotopic composition of water is unchanged by root uptake and during transport through the stem xylem in most plants (Wershaw et al. 1966, White et al. 1984), with the exception of some coastal wetland species (Lin and Sternberg 1993) and certain woody xerophytes (Ellsworth and Williams 2007), which fractionate $^2H$ but not $^{18}O$ during root water uptake. Still, it can be assumed that $\delta^2H$ and $\delta^{18}O$ can be used similarly in water uptake studies for a wide range of woody plant species where samples of different possible water sources are obtained together with plant (xylem) water samples.

Previous studies suggest that physicochemical soil properties may lead to fractionation of hydrogen and/or oxygen in soil water. For example, isotopic exchange reactions with chemical carbonate compounds and water can occur at ambient temperatures in the soil (Zeebe 2009). Thus, exchange reactions with soil carbonate originating from limestone parent rock could be an additional source for oxygen isotope variation in meteoric water additional to fractionation by evaporation (Gat 1996). In particular, as carbonate has formed by deposition of marine organisms or precipitated inorganically from aqueous solutions at equilibrium conditions and incorporated the ambient water isotope composition (Clayton 1960), which was most likely different from the prevalent composition at the time of exchange reaction.

Chemically reactive clay particles may react with bulk water and create pools of energetically differing water with varying isotope compositions (Oerter et al. 2012). Further, the formation of hydration spheres around cations in aqueous solutions fractionates oxygen isotopes of water (Sofer and Gat 1972). The potential for clay particles to create measurable isotopic fractionation effects would be most evident in soils with high cation exchange capacity (CEC) (Oerter et al. 2012). Laboratory experiments revealed preferential adsorption of $^{18}O$ isotopologues within hydrations shells around the clay/ion complex (Oerter et al. 2012) which indicates a potentially high impact on measurements of plant-soil water fluxes using $^{18}O$.

By means of the natural abundance of water isotopes to assess the vertical water uptake patterns of *Fagus sylvatica*, *Tilia* sp. and *Fraxinus excelsior* during a summer desiccation period in a temperate forest, we encountered a notable difference between the isotopic composition of $\delta^2H$ and $\delta^{18}O$ in soil compared to xylem water samples. Depending on whether $^2H$ or $^{18}O$ was used the uptake depth differed, irrespective of tree species.

Isotopic deviations of extracted soil water from the input water were previously reported in particular for clayey soils (Walker et al. 1994, Hsieh et al. 1998, Koeniger et al. 2011). To our knowledge, only very few studies (Brunel et al. 1991, 1995; Kukowski et al. 2012) on plant water uptake depth were conducted on soils originating from limestone parent rock, which analyzed both isotopes.

The aim of our study was to test which physical and chemical soil properties may influence the isotopic composition of extracted soil water. To this end, we examined the effect of soil water and clay content, as well as soil carbonate presence on the isotopic composition of extracted soil water in a laboratory experiment.

## 3.2 Methods

### *3.2.1 Study site description*

Soil and plant material was collected from several research sites within the Hainich National Park close to the village of Weberstedt (51°05'28"N, 10°31'24"E) in Central Germany. Sites were located on a low mountain range at an elevation of approximately 350 m a.s.l., situated within a subatlantic climate zone. Mean annual precipitation is 544-662 mm (average of 30 years precipitation records from four climate stations around the national park; DWD, 2008) with frequent soil desiccation periods occurring during summer months. The geological substrate of

the area is Triassic (shell-) limestone overlain by loess, which forms nutrient-rich Luvisols. Clay content ranged from 17% in the surface soil to 45% at 0.7 m depth (Tab. A1). Soil clay minerals are base-rich vermiculites in 0-0.3 m and smectites from 0.3-0.7 m soil depth (Butz-Braun 2001). Soil pH increases with soil depth, apparently influenced by the limestone parent rock underneath (Butz-Braun 2001, Guckland 2009).

### *3.2.2 Sample preparation*

During summer 2009, increment cores were taken at 1.3 m from 48 trees (n = 48 samples). Soil samples were taken at five depth intervals (0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.5 and 0.5-0.7 m) below the respective trees (n = 240) with an auger. Mean gravimetric water content of the samples was around 24% (17 - 32%). Water was extracted from all samples using the cryogenic vacuum extraction method (Ehleringer et al. 1989, West et al. 2006) at ~115°C and 0.01 mbar. Prior to sample extraction, we tested the effect of different extraction times (60, 90, 180 and 240 minutes) on the isotopic composition of water extracts to determine the time required to obtain unfractionated water samples (West et al. 2006) from soil and stem. According to our tests, an extraction time of 90 minutes is required to obtain unfractionated water samples from soil and plant material, which is substantially longer than the times recorded in a similar experiment for clayey soil and stem samples by West et al. (2006).

After cryogenic extraction, samples were oven-dried at 110° C for 72 hours and stored for further analysis. In 2012, some of these samples were used to study the effects of soil physical and chemical properties on the isotopic composition of soil water. A total of 25 samples was then randomly selected with each sample being remoistened with water of known isotopic composition, extracted and oven-dried again in order to treat all samples equally before the onset of the experiment.

Fifteen of the 25 samples (5 g soil per sample) were again moistened with the same water to three different extents: 0.6 ml (low water input), 1.5 ml (medium water input) and approximately 3 ml (saturating to water holding capacity; high water input) (n = 5 per group). The isotopic composition of the water used for the rewetting experiment was -56.0±1.8 ‰ ($\delta^2H$) and -9.5±0.2 ‰ ($\delta^{18}O$). Rewetting of soil samples with different amounts of water produced soil water potentials of approximately -2.5 MPa, -0.6 MPa and -0.08 MPa, respectively. Water potentials were calculated from water contents, using the Rosetta DLL (Dynamik Linked

Library) program by Schaap et al. (2001), implemented in the HYDRUS-1D model (Simunek et al., 2008).

From each of the remaining 10 samples, we separated two subsamples (n = 5 per group with 5 g soil per subsample). One subsample was remoistened with 1.0 ml water of known isotopic composition (control sample; -56.2±0.4 ‰ $\delta^2H$ and -9.4±0.03 ‰ $\delta^{18}O$) and the other was treated with hydrochloric acid (10 % HCl to remove carbonates) and washed until neutral pH was reached. Afterwards, the soil was dried to remove all water from the sample and remoistened with the same water as that used in the control sample. Water potential of remoistened samples was about -0.8 MPa. Sample vials from both laboratory experiments were sealed with paraffin tape and kept in the refrigerator to avoid water evaporation and left for 72 hours. Water was later extracted from the soil samples as described above.

Extracted water from both experiments was weighted to determine the water recovery rate. In addition, remaining sample material was also weighted, dried and weighted again for the same purpose. The water recovery rate during extraction of remoistened samples was always near 100%, according to subsequent tests of drying and re-weighing.

Measurements of $\delta^2H$ and $\delta^{18}O$ isotopic composition were conducted on a high temperature conversion elemental analyzer (TC/EA, Thermo Electron Corporation, Bremen, Germany) coupled to a Delta V Plus isotope ratio mass spectrometer (Thermo Electron Corporation) with reversed sample flow (Gehre et al. 2004) giving a measurement precision of ± 2 ‰ for $\delta^2H$ and ± 0.2 ‰ for $\delta^{18}O$, respectively.

### *3.2.3 Statistical analysis*

A paired t-test was applied to test for differences among treatments (low, medium and high water input; HCl and control). Statistical tests were considered significant at $p \leq 0.01$. All statistical analyses were done in R version 2.15.1 (R Core Team, 2012).

## 3.3. Results

### *3.3.1 Soil and xylem water isotopic composition from the field study*

The isotopic composition of soil water extracted from soil samples acquired at different depths during the soil desiccation period in 2009 ranged from -35.0 to -80.0 ‰ ($\delta^2H$) and -5.2 to -11.5 ‰ ($\delta^{18}O$) (Fig. A3.1). Due to a strong evaporative effect on the water's isotopic composition, an isotopic gradient developed in concert with increasing soil depth (Fig. 3.1). As soil and stem samples were taken simultaneously, the isotopic composition of xylem water was expected to reflect the signatures of soil water at the depth of soil water uptake. However, comparing the signatures of either $\delta^2H$ or $\delta^{18}O$ soil and xylem water with a simple graphical interference approach indicates a distinct difference between both isotopes (Fig. 3.1).

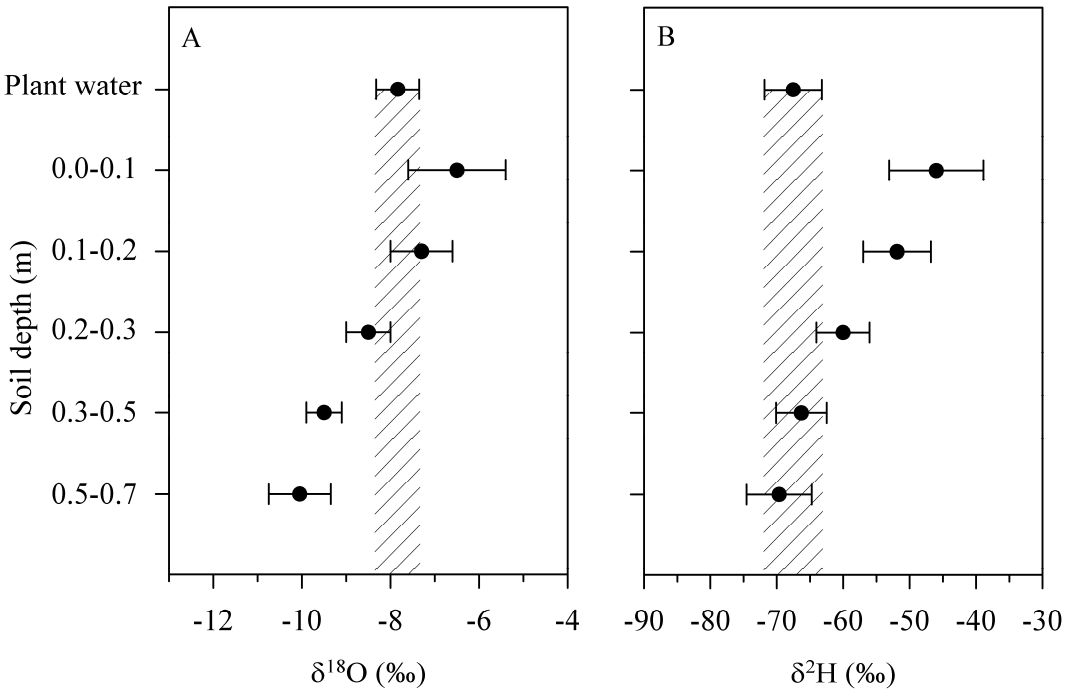


Figure 3.1: Average stable isotope composition of $\delta^{18}O$ and $\delta^2H$ (A and B, respectively) in soil and xylem samples. Values are means ± standard deviation (n = 48). The striped areas indicate the depth of water uptake for a given element as indicated by graphical interference.

Though a graphical "best match" approach is not suitable to estimate water uptake depth as plants often withdraw water from more than one distinct soil depth; as such it is here only used to illustrate the bias found between $\delta^2H$ or $\delta^{18}O$.

### *3.3.2 Effect of soil moisture on soil water isotopic composition*

Extracted water from soil samples with low and medium input water (0.6 and 1.5 ml, respectively) showed a significant deviation ($p < 0.01$) from the input water. The $\delta^2H$ and $\delta^{18}O$ of low water input samples was -68.9±7.2 ‰ and -11.2±1.5 ‰, respectively. The isotopic composition of medium water input samples was 62.7±4.1 ‰ ($\delta^2H$) and -10.3±0.9 ‰ ($\delta^{18}O$). In contrast, water from samples with high water input (3.0 ml) had an isotopic composition similar (-56.6±3.0 ‰ ($\delta^2H$) and -9.2±0.2 ‰ ($\delta^{18}O$)) to that of the input water (Fig. 3.2).

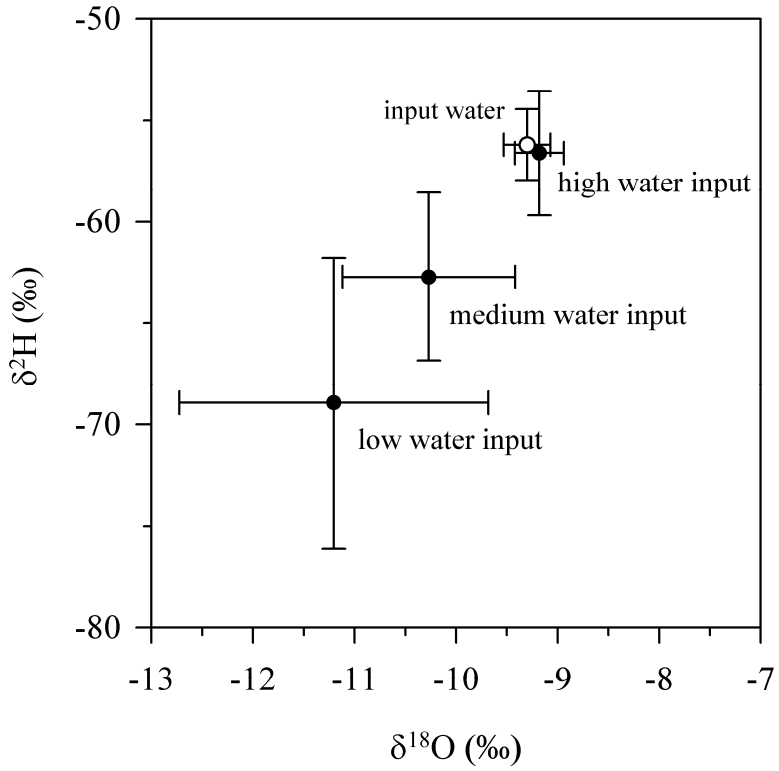


Figure 3.3: Isotopic composition of extracted soil water (filled circles) in relation to input water (open circle). Samples were treated with different quantities of input water, (low = 0.6 ml; medium = 1.5 ml; high = 3.0 ml). Values are means ± standard deviation (n = 15).

### *3.3.3 Effect of clay content on soil water isotopic composition*

The isotopic composition of both isotopes showed a significant negative relationship with clay content at low water content (Fig. 3.3, A1 and A2). In contrast, no significant correlation was found for samples rewetted with a medium (Fig. 3.3, B1 and B2) and high (data not shown) amount of input water. Hence, the effect of clay on the isotopic composition of extracted water after rewetting strongly depends on the amount of water used for rewetting.

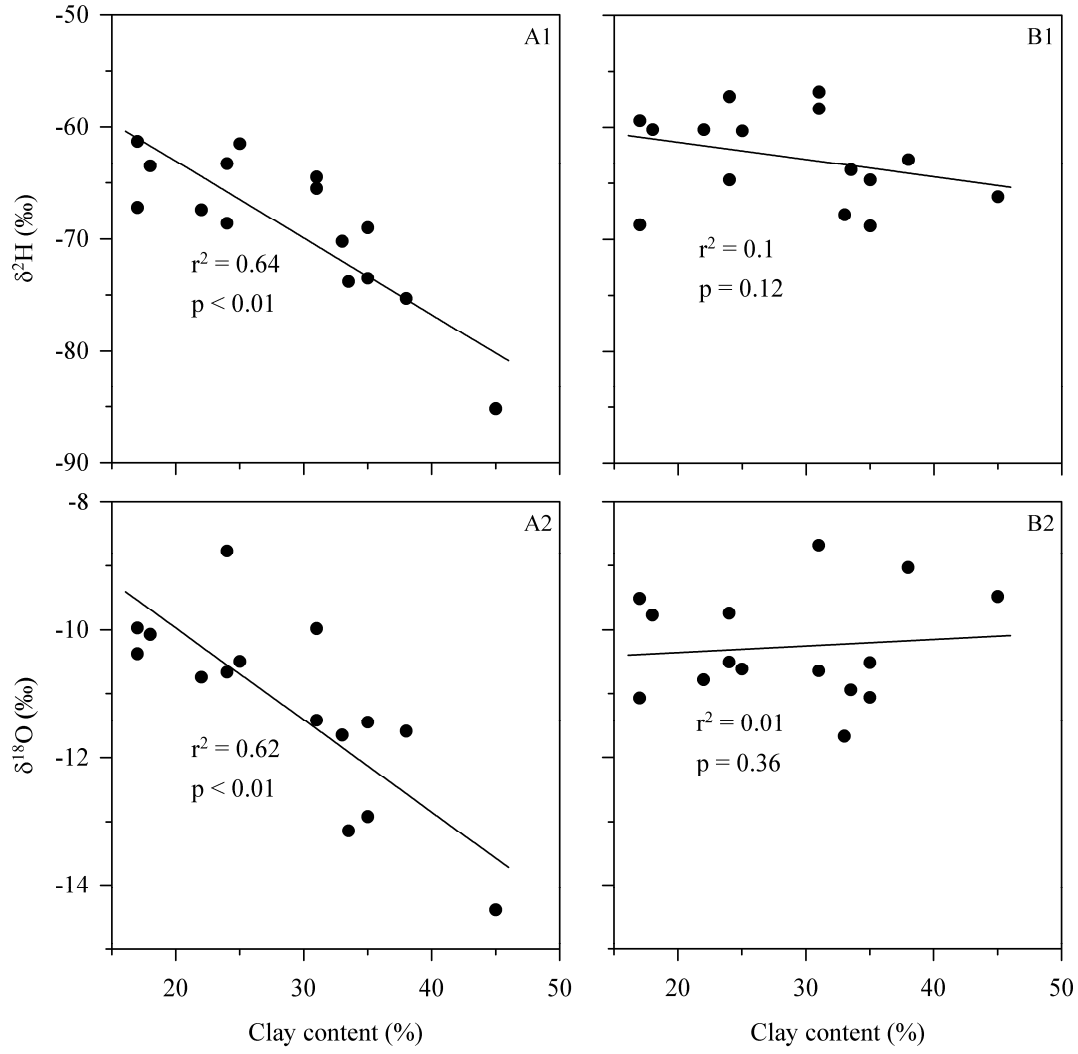


Figure 3.3: Effect of clay content (%) in sample soil on the isotopic composition of extracted soil water. Data shown for samples with low (A1 and A2) and medium (B1 and B2) input water, respectively.

### *3.3.4 Effect of soil calcium carbonate on soil water isotopic composition*

Control samples with a water input of 1 ml were depleted in $\delta^2H$ and $\delta^{18}O$, as was expected from the experiment with differing quantities of input water. However, samples that were additionally treated with hydrochloric acid (HCl) were significantly enriched in $\delta^{18}O$ ($p < 0.01$) compared to control samples (Fig. 3.4), but they did not differ significantly with respect to input water. The effect of HCl treatment on the average $\delta^2H$ value was not significant (Tab. 3.1).

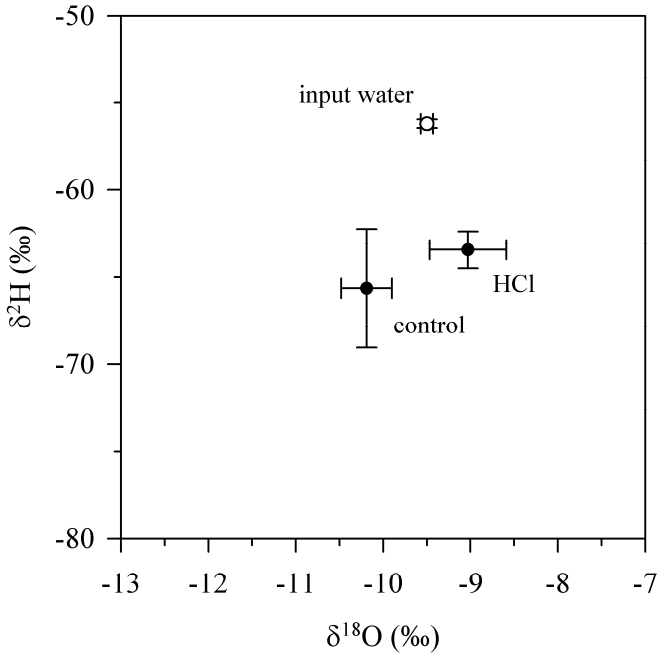


Figure 3.4: Isotopic composition ($\delta^2H$ and $\delta^{18}O$) of extracted soil water from control samples and samples pre-treated with hydrochloric acid (HCl), in relation to original input water. Values are means ± standard deviation.

Table 3.1: Comparison of mean isotopic deviation between input water and extracted water among the treatments. Significant differences between mean values (paired t-test) are marked with an asterisk ($p < 0.01$).

| **Treatments** | **Mean isotopic deviation (‰)** | |
|---|---|---|
| | **$\delta^{18}O$** | **$\delta^2H$** |
| Input water - control | -0.70* | -6.40* |
| Input water - HCl | 0.40 | -4.24* |
| Control - HCl | -1.31* | -2.20 |

## 3.4 Discussion

We found that the isotopic composition of $\delta^2H$ or $\delta^{18}O$ between soil and xylem water resulted in different soil water uptake depth by trees depending on which of the isotopes is considered (Fig. 3.1). Using graphical interference to derive plant water uptake depth is a

simplistic approach assuming that water is withdrawn from only a single discreet soil layer. However, it is more likely that plant xylem water is a mixture of water from several soil depths. Multiple source mixing models (Phillips and Gregg 2001, 2003; Moore and Semmens 2008; Parnell et al. 2010) account for water uptake from more than one discrete soil layer and weigh the importance of certain layers for water uptake by incorporating e.g. soil water potentials into the calculation. Such models can also account for a certain bias in the isotopic composition between $\delta^2H$ and $\delta^{18}O$. However, due to the magnitude of the bias presented here and its systematic nature across all samples, even mixing models would not yield reliable results.

The isotopic differences were independent of the observed tree species (*Fagus*, *Tilia*, *Fraxinus*). Thus it seems unlikely that our findings are the result of a discrimination against $^2H$ in plant roots as described by Lin and Sternberg (1993) and Ellsworth and Williams (2007). Results similar to ours were also reported in a study by Brunel et al. (1991), where the isotopic composition of xylem water samples did not match with those of soil samples in a $\delta^2H$-$\delta^{18}O$ plot (xylem δ‰ values arranged notably above the best fit regression line of soil samples). However, this discrepancy was relatively small and did not appear in all observations during the study, and further investigation into the difference was not pursued by the authors (Brunel et al. 1991). A considerable difference between $\delta^2H$ and $\delta^{18}O$ isotopic composition in soil and xylem water was also observed by Li et al. (2007), who investigated the water uptake of *Larix sibirica* in a semiarid forest region. The authors concluded that an independent application of both isotopes did not yield a consistent estimate of soil water uptake depth for several of their measurements (Li et al. 2007). The discrepancy was however explained as an artifact of either the analytical procedure (cryogenic vacuum extraction and analysis with a Finnigan MAT252 isotope ratio mass spectrometer) or related to natural processes, which could not be explained with the available data (Li et al. 2007). A visual survey of graphs from other dual water isotope studies (Midwood et al. 1998, Eggemeyer et al. 2008, Rossatto et al. 2011) suggests that comparable differences due to isotopic shifts of $\delta^2H$ and $\delta^{18}O$ may also be found in other datasets. However, such inconsistencies in studies of plant water uptake often remain undiscussed, since they rarely have significant impacts on the results if soil depths are pooled to larger soil depth intervals (e.g. upper and lower soil profile).

It has previously been shown that high clay content in soil samples may hinder complete water extraction (West et al. 2006) but also affect the isotopic composition of extracted water, especially if soil water content is low (Walker et al. 1994, Hsieh et al. 1998, Koeniger et al. 2011). Our findings corroborate both effects, since we observed that the deviation of the signature of

input water from extracted water increases with both decreasing soil water content (Fig. 3.2) and increasing clay content (at low soil water contents) (Fig. 3.3). This is also in line with the results of Savin and Epstein (1970), who showed that adsorbed and interlayer water of clays can exchange with atmospheric water at ambient temperatures. However, these findings cannot explain the discrepancy between $\delta^2H$ and $\delta^{18}O$ isotopic signatures in our study (Fig. 3.1), as all observed processes affect both $\delta^2H$ and $\delta^{18}O$. The possibility that hygroscopic water stored in the interstices between clay particles exchanged with ambient soil water, causing a so called "memory effect" (Koeniger et al. 2011) is unlikely as samples were already processed in vacuum extraction before rewetting, and we repeatedly added water of the same isotopic composition during our experiments. Moreover, an exchange between hygroscopic water and soil water would probably affect both $\delta^2H$ and $\delta^{18}O$ (Araguás-Araguás et al. 1995).

However, our study revealed that the presence of carbonates significantly alters the isotopic composition of $\delta^{18}O$ of added water, whereas the shift of $\delta^2H$ between added and extracted water is not affected (Fig. 3.4). Water from soil samples with carbonate were more depleted in $\delta^{18}O$ compared to samples where carbonate has been removed before rewetting (~ 1.3 ‰). This implies that, at least for our samples, different mechanisms exist that affect the isotopic composition of water extracted from soil. The $\delta^{18}O$ is presumably altered by exchange of oxygen with carbonates in soil, which could serve as an explanation for the difference between $^{18}O$ and $^2H$ shown in figure 3.1. The observed increase of isotopic deviation with increasing clay content also supports this finding, as clay and carbonate content in this area increase with soil depth (Butz-Braun 2001, Guckland 2009).

Although soil carbonate content can serve as an explanation for the isotopic shift in our samples, it is still possible that other processes are responsible for the alteration of oxygen isotopic composition of input water in the other studies mentioned. The isotopic composition of hydrogen may also be altered by water exchange with soil particles; this could either be exchangeable hydrogen on the clay surface or exchangeable O-bonded hydrogen in the organic matter. As isotopic depletion in our samples was correlated with clay (and therefore co-correlated with soil depth), we assume that the latter process only plays a minor role in our soils, as organic matter content decreased with increasing soil depth. We therefore assume that hydrogen exchange with clay minerals is responsible for the observed shifts in our laboratory experiments.

The assumption of isotopic exchange independently for H and O is in agreement with the available information on soil properties in the aforementioned studies where differences for O

and H isotopic composition were found. In the study of Brunel et al. (1991) the soil had a 1 m thick clay horizon above a calcium carbonate layer, Midwood et al. (1998) described theirs as (partly) clayey or loamy, and Li et al. (2007) studied a Podsol with presumably high organic matter content (dislocation of humic compounds through leaching).

Although our study revealed that clay content has a significant effect on the isotopic composition of extracted water after rewetting, the difference between $\delta^2H$ and $\delta^{18}O$ of extracted soil water and xylem water (Fig. 3.1) could also indicate that the water pool extracted by cryogenic vacuum techniques is different from the water pool available for root uptake. Based on the data shown in figure 3.1, this means that roots may have taken up water that has been subjected to $\delta^2H$ but not $\delta^{18}O$ exchange; while for cryogenic vacuum extraction, both effects are relevant. If this holds true, we can conclude that the bias between $\delta^2H$ and $\delta^{18}O$ may be caused by oxygen exchange between soil water and carbonates, and that $\delta^2H$ fractionation plays only a minor role at best (presumably because cryogenic vacuum extraction and root uptake are equally affected by $\delta^2H$ exchange).

The treatment of soil samples in our experiments can certainly be seen as artificial and would probably not occur under natural circumstances. Therefore, it is debatable whether oven-drying and rewetting soil samples is representative for naturally dried soil regarding the influence on the isotopic composition of recovered water. This approach can be justified for the sake of treating all our samples equally and in order to test only effects of soil physicochemical properties.

Although our data suggest that shifts of $\delta^2H$ and $\delta^{18}O$ in soil water can be caused by soil related processes, there is also the possibility that isotopic shifts may occur during transport through roots or xylem due to metabolic processes. Increasing evidence for a probable post-photosynthetic exchange of oxygen and hydrogen between carbohydrates and xylem water during cellulose formation in the tree trunk has been already found in several experiments (Yakir et al. 1990, Hill et al. 1995, Farquhar et al. 1998) and has been discussed in some modelling approaches (Roden et al. 2000, Waterhouse et al. 2002).

## 3.5 Conclusion

We conclude that soil physical properties (e.g. clay content) and/or chemical properties (e.g. carbonate content) can lead to isotopic fractionation, especially with low soil water content. Special care should be therefore taken when water stable isotope composition will be analyzed in soils with high clay and/or soil calcium carbonate content. It should be noted that the use of only one element ($\delta^2H$ or $\delta^{18}O$) in plant water uptake studies might generate errors that pass unnoticed and may result in incorrect evaluation of the soil water uptake depth. It is therefore recommended that further parameters are investigated with regard to the cause of isotope fractionation and/or exchange in soils and plants to establish a sound methodology for investigating plant water uptake from different soils. It is also recommended for future studies that ecohydrological data, such as high-resolution measurements of soil water potentials, or tree sapflow data should be obtained alongside stable isotope data to constrain possible sources for tree water uptake. Further experimental inquiry should be also conducted on possible fractionation processes in the tree xylem.

## 3.6 Acknowledgements

This study was conducted within the framework of the Research Training Group "Graduiertenkolleg 1086: The role of biodiversity for biogeochemical cycles and biotic interactions in temperate deciduous forests", funded by the DFG (German Research Foundation). We thank Christina Langenbruch and Anja Guckland for providing data on soil properties.

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## 3.8 Appendix

Table A3.1: Soil texture of sampling depths. Values are means ± standard deviation (based on C. Langenbruch, personal communication).

| Soil depth (m) | Soil texture (%) | | |
|---|---|---|---|
| | Clay | Sand | Silt |
| 0-0.1 | 20 ± 4 | 3 ± 1 | 77 ± 4 |
| 0.1-0.2 | 20 ± 5 | 3 ± 1 | 77 ± 6 |
| 0.2-0.3 | 29 ± 8 | 3 ± 0 | 68 ± 8 |
| 0.3-0.5 | 34 ± 3 | 2 ± 1 | 64 ± 3 |
| 0.5-0.7 | 39 ± 8 | 3 ± 0 | 58 ±8 |

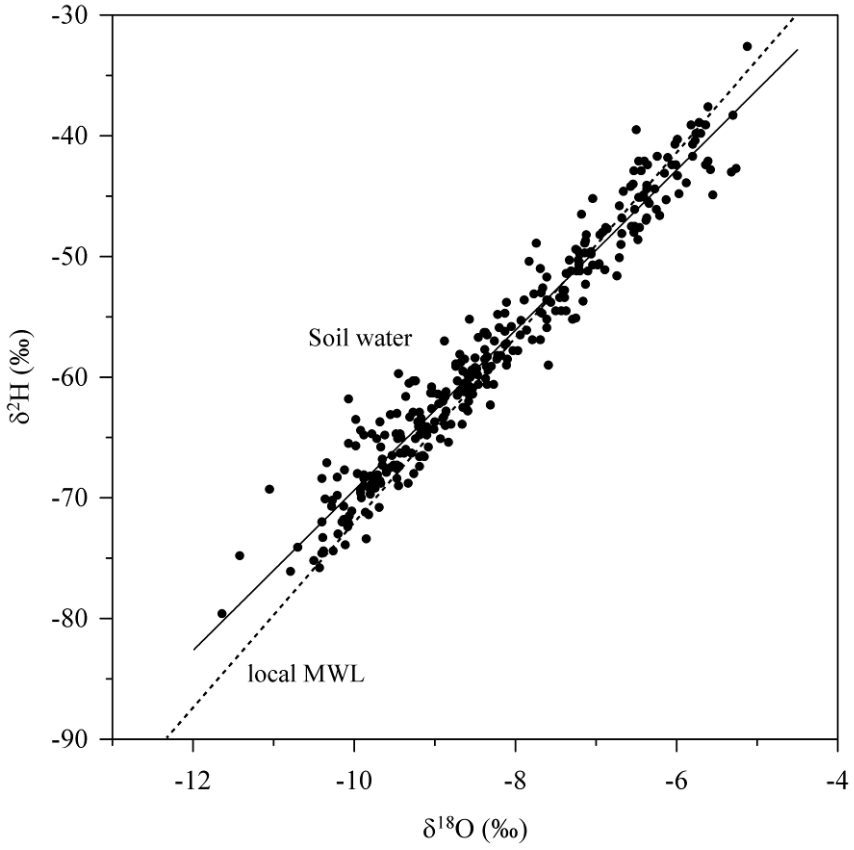


Figure A3.1: Relationship between $\delta^2H$ and $\delta^{18}O$ (‰) of soil water samples in relation to the local meteoric water line (local MWL), based on data from the International Atomic Energy Agency (IAEA) archives.

# Chapter 4

## PARTITIONING OF SOIL WATER AMONG CANOPY TREES DURING A SOIL DESICCATION PERIOD IN A TEMPERATE MIXED FOREST

# 4. Partitioning of soil water among canopy trees during a soil desiccation period in a temperate mixed forest

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**Abstract.** Complementary resource use is considered an important mechanism in the study of biodiversity effects. Here we explore how species identity, species mixture and tree size influence the vertical partitioning of soil water among canopy trees during a soil desiccation period. In the Hainich forest, Germany, the species Fagus sylvatica, Tilia sp. and Fraxinus excelsior were studied in single- and three-species mixed clusters, each consisting of three co-dominant trees situated within a larger mixed forest stand. Vertical soil water uptake depth was assessed by analyzing the hydrogen stable isotope composition (deuterium, $\delta^2H$) of water from depth intervals throughout the soil profile and in tree xylem water. For single species clusters, a mixing model suggested that Fagus distinctively drew water from soil depths of 0.3-0.5 m, Tilia from 0.3-0.5 m and 0.5-0.7 m and Fraxinus mainly used water from 0.5-0.7 m. In mixed clusters, the uptake patterns of Fagus and Tilia were similar to those of the single-species clusters (mainly uptake form 0.3-0.5 m), but Fraxinus showed a different uptake pattern. Fraxinus in mixture had a somewhat homogenously distributed uptake over the soil depths 0.2-0.7 m. For single species clusters, there was no correlation between main soil water uptake depth and tree diameter, irrespective of variations in tree size. In contrast, for mixed clusters there was a significant decrease in the main uptake depth with increasing tree size ($P < 0.001$, $R^2_{adj} = 0.73$), irrespective of species mix. In consequence, soil water partitioning was strongest where species were mixed and tree size varied. We further analyzed whether single and mixed-species clusters differed in the level of water uptake, e.g. due to complementarity, but our soil water budgeting did not indicate any such differences. A possible explanation might be that the volume of water used is predominantly governed by properties at the stand level, such as aerodynamic roughness, rather than by processes acting at the meter scale between neighbouring trees. With respect to application, we assume that the upcoming close-to-nature forestry approach for the area, which fosters mixed stands of heterogonous diameters, may result in enhanced complementarity in soil water uptake among canopy trees.

*Keywords: biodiversity, complementarity, deuterium, ecohydrology, Hainich, water uptake*

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## 4.1 Introduction

Water availability is considered a major control of productivity in forests of central Europe and other regions of the world (Breckle and Walter, 2002; Huxman et al., 2004; Ellenberg and Leuschner, 2010). Under certain environmental conditions, the degree to which water is available for transpiration and production is governed by a plant's capacity to exploit soil water resources; a property that can be enhanced by complementarity among co-occurring plants. Resource use complementarity postulates that functional traits enable plants to exploit resources unavailable to others or use the same resource at a different place or time (Vandermeer 1989). Resource partitioning and the consequently more effective utilization of resources have been suggested as an explanation for the higher productivity observed in many mixed plant communities compared to monospecific stands (Hagger and Ewel, 1997; Hooper et al., 2005).

One approach to studying plant water acquisition patterns and complementary water use is the assessment of water stable isotopes such as deuterium in soil and plants (Ehleringer and Dawson, 1992). Since roots do not fractionate water during uptake, the deuterium signatures in the plant water reflect the uptake-weighted average of $\delta^2H$ of potential water sources (Ehleringer and Dawson, 1992). A comparison of plant water $\delta^2H$ with that of soil water from different soil depths can reveal the actual soil water source depth for any plant.

By using such an approach in a diverse Panamanian old-growth forest, considerable spatial soil water partitioning among co-existing trees was documented (Jackson et al., 1995; Meinzer et al., 1999). Within and among species, water uptake depth was strongly related to tree size, with smaller trees preferentially tapping deeper sources of soil water than larger trees; species-specific characteristics were however difficult to disentangle (Meinzer et al., 1999). Species-specific soil water uptake patterns were found in a study on Indonesian cacao agroforests. Cacao trees mainly took up water from upper soil layers, whereas the associated *Gliricidia* shade trees acquired soil water mainly from deeper soil layers (Schwendenmann et al., 2010); however, the shade trees tapping deeper water sources were considerably higher and had a greater diameter than that of the cacao trees.

In grasslands as well as tree plantations, it has been observed that plant species diversity enhanced transpiration rates (Verheyen et al., 2008; Kunert et al., 2012), and complementarity in respect of water uptake was discussed as an underlying mechanism. Such a strategy may however also lead to a faster decline in available water for diverse communities during drought (van Peer et al., 2004; Verheyen et al., 2008). There is little information on complementarity in relation to water uptake in temperate broad-leaved mixed forests, but such information is becoming more

relevant as there is an ongoing trend in silviculture towards more naturalness or *close-to-nature* forestry (O'Hara, 2001; LÖWE, 2007). Close to nature forestry implies a transformation of monocultural stands of narrow tree diameter range into stands composed of several tree species with a broader range of diameter. Since global climate change scenarios predict an increase in intensity and frequency of drought events during the vegetation period for large parts of central Europe (Rowell and Jones, 2006; Christensen et al., 2007; IPCC, 2007), there is an increasing urgency to study possible effects of changing species composition and tree diameter range on soil water use.

The present study was conducted in the temperate broad-leaved Hainich forest of central Germany. Previous studies from the region indicate considerable differences in water vapour exchange at the leaf level (Gebauer et al., 2008) as well as whole-tree water use among co-occurring tree species (Köcher et al., 2009). At the stand level, there were indications of enhanced soil water uptake during periods when soil water content declined in mixed stands compared to monospecific beech stands (Krämer and Hölscher, 2010). In this study, we focused on groups of neighbouring trees (tree clusters), as neighborhood was suggested to be highly important in diversity studies (Potvin and Dutilleul, 2009), and particularly important for the assessment of complementarity. Our hypotheses were therefore as follows: During summer soil desiccation, 1) tree species differ in vertical soil water uptake patterns; 2) in mixed-species clusters there is complementarity in soil water uptake; and 3) across all trees studied, the depth of soil water uptake scales with tree size. We further asked whether differences in water uptake volumes occur among the differently composed tree clusters.

## 4.2 Methods

### *4.2.1 Study area*

This study was conducted in the Hainich forest in northern Thuringia, central Germany, an area rich in tree species. The study plots are located in the south-eastern part of the forest area on a low mountain range at an elevation of approximately 350 m (a.s.l.). The geological substrate is Triassic limestone covered by loess, forming nutrient-rich Luvisols (Guckland et al., 2009). The climate is subatlantic with a mean annual temperature of 7.5° C and a long term mean precipitation of approximately 590 mm (1973-2004, Deutscher Wetterdienst, Offenbach,

Germany). For the last 40 years, the forest has remained almost free of harvesting or thinning due to its use as a military training area since 1964 and its integration into a new national park in 1997 (Mölder et al., 2006). The forest stands in which our study clusters are located may contain deciduous forest over 200 years old (Mölder et al., 2009). The dominant tree species are *Fagus sylvatica* (L.), *Fraxinus excelsior* (L.), *Tilia cordata* (Mill). and *Tilia platyphyllos* (Scop.). The two *Tilia* species often form hybrids, which are phenologically difficult to differentiate. Hence, in this study we did not differentiate between the species and refer to them as *Tilia sp.*

### *4.2.2 Tree clusters*

In two mixed forest stands within the Hainich (Lindig and Thiemsburg, approx. 2 km apart) the species *Fagus sylvatica, Tilia sp.* and *Fraxinus excelsior* were studied in single and three-species mixed clusters. Clusters consisted of three co-dominant trees and each cluster type was replicated four times resulting in a total of 16 clusters (8 in each area). The average distance between the clusters of each area was 124 m at Thiemsburg and 112 m at Lindig. There were no significant differences among cluster types with respect to tree height, but the monospecific *Fagus* clusters showed significantly larger stem diameter at breast height (dbh) than the monospecific *Fraxinus* clusters (Tab. 4.1).

Table 4.1: Characteristics of single and mixed species tree clusters. Values are means ± sd (n=4), similar letters specify no significant difference between cluster types. The Shannon biodiversity index (H') refers to a 20 m radius around the center of each cluster.

| | | **Cluster** | | | |
|---|---|---|---|---|---|
| | | ***Fagus*** | ***Tilia*** | ***Fraxinus*** | **Mixed** |
| Tree dbh | (cm) | 54.4 ± 12.0 a | 47.1 ± 14.3 ab | 30.2 ± 3.7 b | 40.5 ± 9.4 ab |
| Tree height | (m) | 28.6 ± 2.8 a | 27.9 ± 2.5 a | 29.9 ± 1.1 a | 28.4 ± 2.2 a |
| Cluster area | ($m^2$) | 33.3 ± 14.6 a | 27.1 ± 18.8 a | 7.8 ± 1.6 a | 21.7 ± 21.4 a |
| Shannon H' | | 0.8 ± 0.3 a | 1.0 ± 0.1 a | 1.2 ± 0.3 a | 1.2 ± 0.2 a |

A Shannon-Wiener diversity index (H') of trees within a 20 m radius of the clusters did not reveal any significant differences among cluster types. Soil clay content and bulk density increased with increasing soil depth comparably within all clusters (Tab. A4.1).

### *4.2.3 Soil moisture measurements*

Soil volumetric water content ($\theta$ in $m^3\ m^{-3}$) was measured with a portable FDR probe (Frequency Domain Reflectometry; Diviner 2000, Sentek Pty Ltd., Stepney, Australia). Four PVC access tubes were installed on each cluster (Fig. 4.1) to a maximum depth of 0.7 m and readings were taken at depth intervals of 0.1 m at an average distance of 3.4 ± 1.5 m from the clustered trees. In some clusters, it was not possible to install all access tubes to the full extent, as heterogeneously weathered limestone debris occurred already at shallow depths and obstructed the installation. The FDR sensor was depth-specifically calibrated for the local soil conditions (Krämer and Hölscher, 2010). Data on soil water content were collected weekly from 30 April to 31 October 2009.

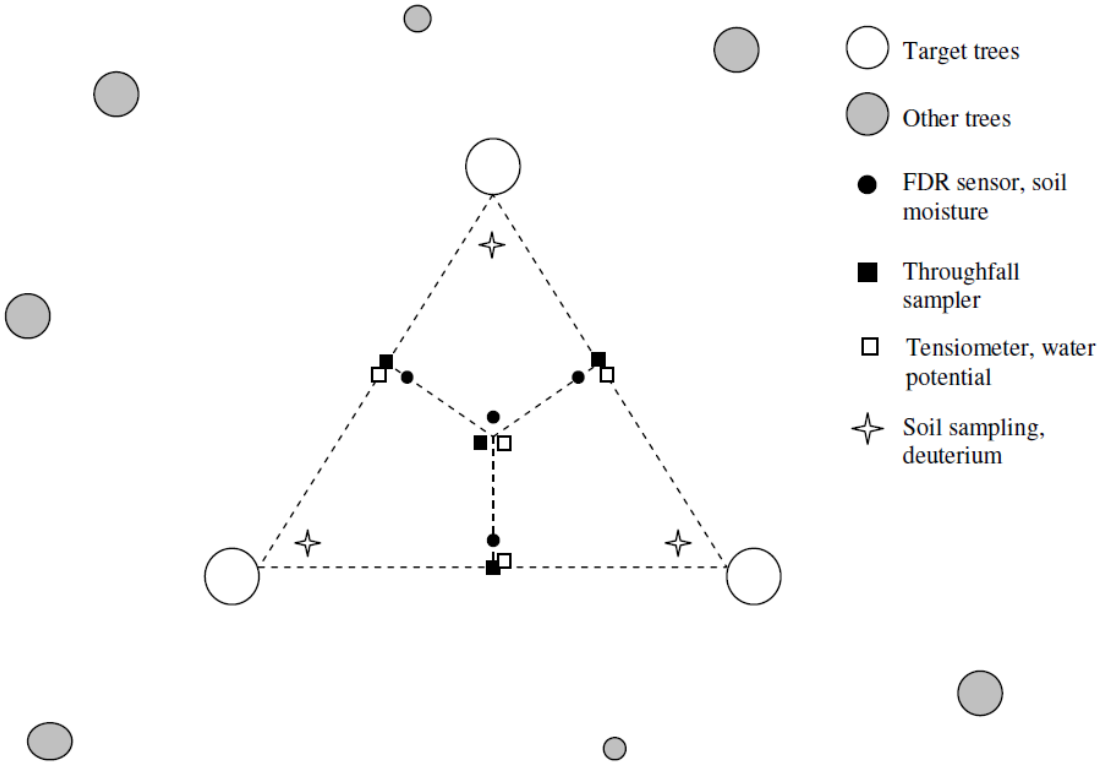


Figure 4.1: Schematic study plot design (tree cluster) with measurement locations.

Soil water potentials ($\psi$ in hPa) were measured with tensiometers (T1-UMS, Umwelt Monitoring Systeme, Germany) at depths of 0.1, 0.3 and 0.5 m. The tensiometers used had a measurement limit of -700 hPa, which resulted in them drying-out during prolonged desiccation periods. To compensate for this effect we used the Rosetta DLL (Dynamik Linked Library) program by

Schaap et al. (2001), implemented in the HYDRUS-1D model (Simunek et al., 2008), to transform measured volumetric soil water contents into soil water potentials. Measured water potentials from tensiometers were used as inputs for the model calculation, as were soil bulk density, sand, silt and clay content (Tab. A4.1). Calculated values were used when water potentials fell below the minimum measurement threshold of the tensiometers.

### *4.2.4 Soil water uptake depth*

To assess profiles of relative water uptake for each of the observed species we determined the natural abundance of the stable isotope $^{2}H$ (deuterium). Samples from soil and trees of the 16 clusters were taken once during a summer desiccation period on 25 and 26 August in 2009 (Fig. 4.2).

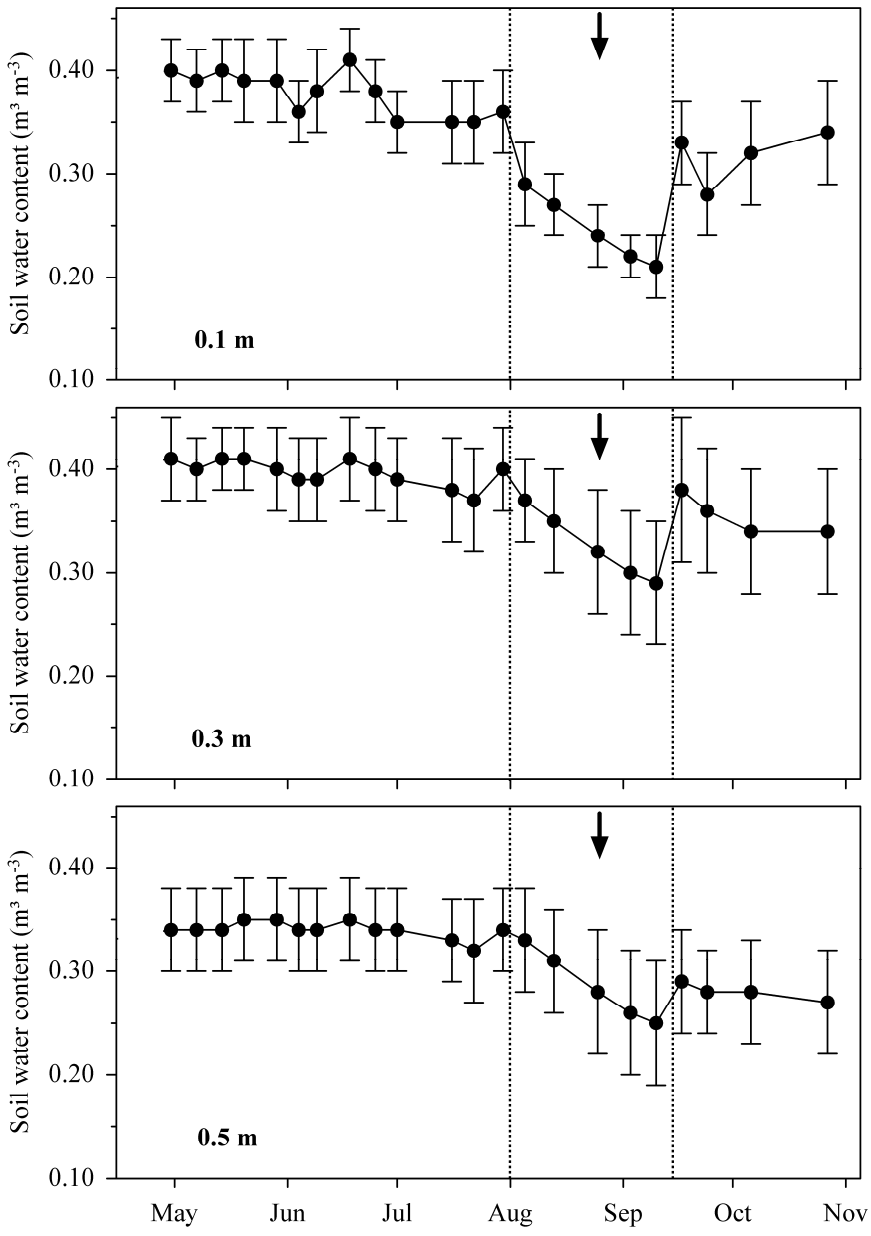


Figure 4.2: Volumetric soil water content at 0.1, 0.3 and 0.5 m soil depth for the tree clusters during the study period in 2009. Values are means ± sd (n = 16). Dotted lines indicate the timeframe for which soil water budgeting was conducted, arrows mark the time when soil and tree samples were taken for deuterium analysis.

Soil samples were taken at depth intervals of 0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.5 m and 0.5-0.7 m under the crown area inside the clusters. Each sample consisted of a mixture of two adjacent soil cores taken at the same depth. Xylem tissue samples were taken from the outer 6 cm of the stem at three points at breast height from each individual tree with an increment borer. The bark was removed after sampling to avoid contamination of xylem water with phloem water. All samples were stored in 40 ml glass bottles, closed with a Teflon coated lid, sealed with Parafilm, and then kept frozen until water extraction to reduce subsequent evaporation from the samples (Ehleringer et al., 2000). Extraction of water from plant and soil samples was conducted via cryogenic vacuum extraction according to Ehleringer and Osmond (1989). The applied extraction time was 90 minutes for soil and stem samples (West et al., 2006).

The analysis of extracted water was carried out at the Center for Stable Isotope Research and Analysis (KOSI, Georg-August-University Göttingen, Germany). Measurements of the hydrogen isotopic composition were conducted by injecting the water into a high temperature conversion elemental analyzer (TC/EA, Thermo Electron Corporation, Bremen, Germany) coupled via a Con-Flo III interface to a Delta V Plus isotope ratio mass spectrometer (Thermo Electron Corporation) (Gehre et al., 2004). Isotope ratios were expressed as per mill deviations to the internationally accepted Vienna Standard Mean Ocean Water (VSMOW, $R_{Standard}$) (Gonfiantini, 1978) with a measurement precision of ± 2 ‰ for $\delta^2H$.

In order to identify the depth of water uptake for plants, many studies utilized the direct inference method, by comparing the plant signatures with the isotopic gradients in the soil profiles and assuming that plants are obtaining water mainly from one soil depth. However, this visual method precludes the possibility of assessing proportional contributions of multiple water sources by quantitative means (Asbjornsen et al., 2007). Therefore we used a mixing model (Isosource, Phillips and Gregg, 2003) that calculates the relative contribution of each soil depth to stem water in order to assess the soil depth each tree used as a potential water source. The underlying assumption is that the isotopic signature of the plant water is a mixture of the signatures found in the soil. In their study, Asbjornsen et al. (2007) showed that this model can reveal subtle differences in water uptake patterns that are not apparent through visual assessment alone. The fractional increment used in our model calculations was set to 1% and the tolerance to 0.5 ‰. It has to be noted that the mixing model outcome showed a range of feasible source contributions for a given soil layer. For statistical analyses the mean of all feasible source contribution estimates (mean model outcome) for a given soil layer was used. In order to relate

tree dbh to main soil water uptake depth we also plotted the isotope signature ($\delta^2H$) of each trees' main water uptake depth against its respective dbh.

### *4.2.5 Soil water budgeting*

In order to determine the daily water uptake per tree cluster (mm $day^{-1}$) during the soil desiccation period in 2009 (30 July to 09 October 2009), soil water budgeting was conducted at 0-0.7 m soil depth for each cluster (Eq. 4.1). Average throughfall on all clusters during that period was 8.1 mm. Soil water storage was calculated for each cluster from soil water content ($m^3$ $m^{-3}$) multiplied by the depth of each soil layer (0.1 m).

$$Wu = \frac{(Tf + Sf) - \Delta S}{\Delta t} \tag{4.1}$$

Variables included in the budgeting equation were throughfall (*Tf*), stemflow (Sf), change in soil water storage between two successional measurements ($\Delta S$) and the elapsed time between two successional measurements ($\Delta t$).Runoff and deep drainage can be neglected in our case, due to the level terrain and low soil water content during the desiccation period. According to a modeling study by Bittner et al. (2010), for these forests stands drainage can be considered zero during summer months and particularly so during dry spells.

Stemflow was estimated from data for the same forest area taken from the study of Krämer and Hölscher (2009). For each cluster tree, we used the available data on stemflow in relation to dbh and rainfall intensity to calculate total inflow of stemflow per cluster and rainfall event. However, its quantity was of comparatively little importance (0-3 % of gross precipitation), even for *Fagus*. We measured throughfall at four positions on each cluster. The throughfall gauges consisted of a plastic bottle screwed to a funnel with an opening of 10.5 cm in diameter. The bottle was housed in a plastic tube attached to a metal rod at a height of one meter. To reduce evaporation from the rain gauge, a table tennis ball was placed in the funnel. Gauges were emptied weekly from 30 April to 31 October 2009.

### *4.2.6 Data analysis*

For every sampling date, mean values and standard deviation of rainfall, soil water content and soil water tension were calculated for each cluster (n = 4). Before analysis, parameters were tested for normality using the Shapiro-Wilks test. We applied a linear mixed effect model to

identify effects of tree species and soil depth on fractional water uptake. An ANOVA was applied on the model outcome for variance analysis followed by a post-hoc HSD-test for pair wise comparison and correlation analysis. The model output suggested that the explanatory variables soil depth and the soil depth by species interaction had significant effects in the single species clusters as well as in the mixture ($p < 0.01$). For species difference in terms of amounts of daily water uptake and throughfall, ANOVA and HSD-test analysis were conducted, too. All analyses were carried out using R, version 2.11.1 (R Development Core Team, 2010).

## 4.3 Results

### *4.3.1 Soil water uptake depth*

At the time of sampling, the soil water isotopic signature for $\delta^2H$ showed a decline in the soil profile from the topsoil to 0.5 m, levelling off at 0.5-0.7 m (Fig. 4.3A), most likely due to the isotope fractionation caused by evaporation. Isotopic gradients of $\delta^2H$ in the soil profiles were comparable among the different species in single-species and in mixed clusters (Fig. A4.1). Figure A4.1 shows that stem water $\delta^2H$ matched soil water $\delta^2H$ in deeper layers 0.3-0.5 m and 0.5-0.7 m depending on species and mixture. Soil water potentials ($\Psi$) in the clusters increased from an average of ~ -1200 hPa in 0-0.1 m to ~ -600 hPa in 0.1-0.2 m, followed by a mellower increase to ~ -230 hPa in 0.5-0.7 m soil depth (Fig. 4.3B).

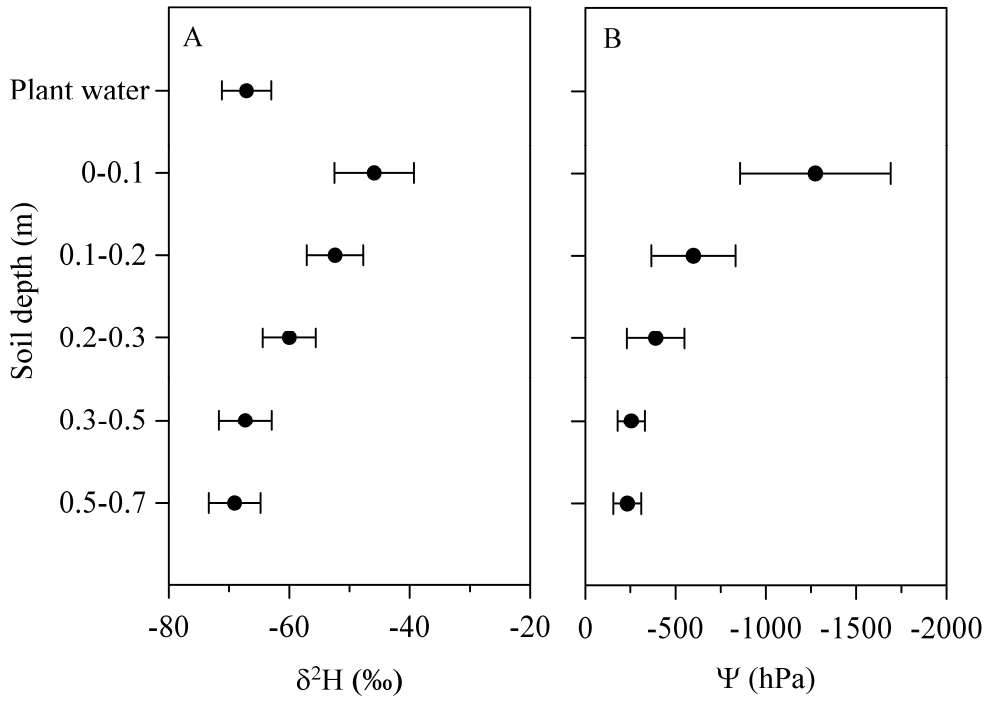


Figure 4.3: Isotopic signatures, $\delta^2H$ (‰) of plant and soil water of the tree clusters (A) and corresponding soil water potentials (B). Values are means ± sd (n = 16).

The patterns shown in figure 4.3 resulted in a strong relationship between soil water $\delta^2H$ and $\Psi$; $\delta^2H$ decreased with increasing $\Psi$ (Fig. 4.4).

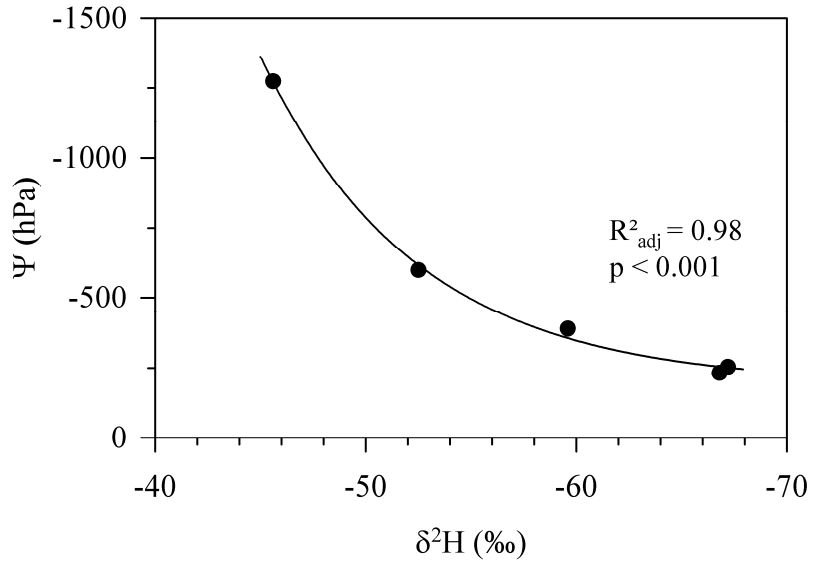


Figure 4.4: Relationship between soil water potential and soil water $\delta^2H$ for the data shown in figure 4.3.

The mixing model indicated that in single-species clusters, *Fagus* obtained water mainly from 0.3-0.5 m, and that *Tilia* utilized the soil depth range of 0.3-0.5 m and 0.5-0.7 m to a similar extent. *Fraxinus* largely drew water from 0.5-0.7 m soil depth, which differed significantly from the other species (Fig. 4.5A).

In mixed clusters, *Fagus* and *Tilia* mainly took water from 0.3-0.5 m. *Fraxinus* showed a wider range of water uptake by also tapping water sources from 0.2-0.3 m depth but taking a lower fraction from 0.5-0.7 m soil depth (Fig. 4.5B). The significant difference in water uptake for *Fraxinus* compared to the other species was a lower uptake fraction from 0.3-0.5 m.

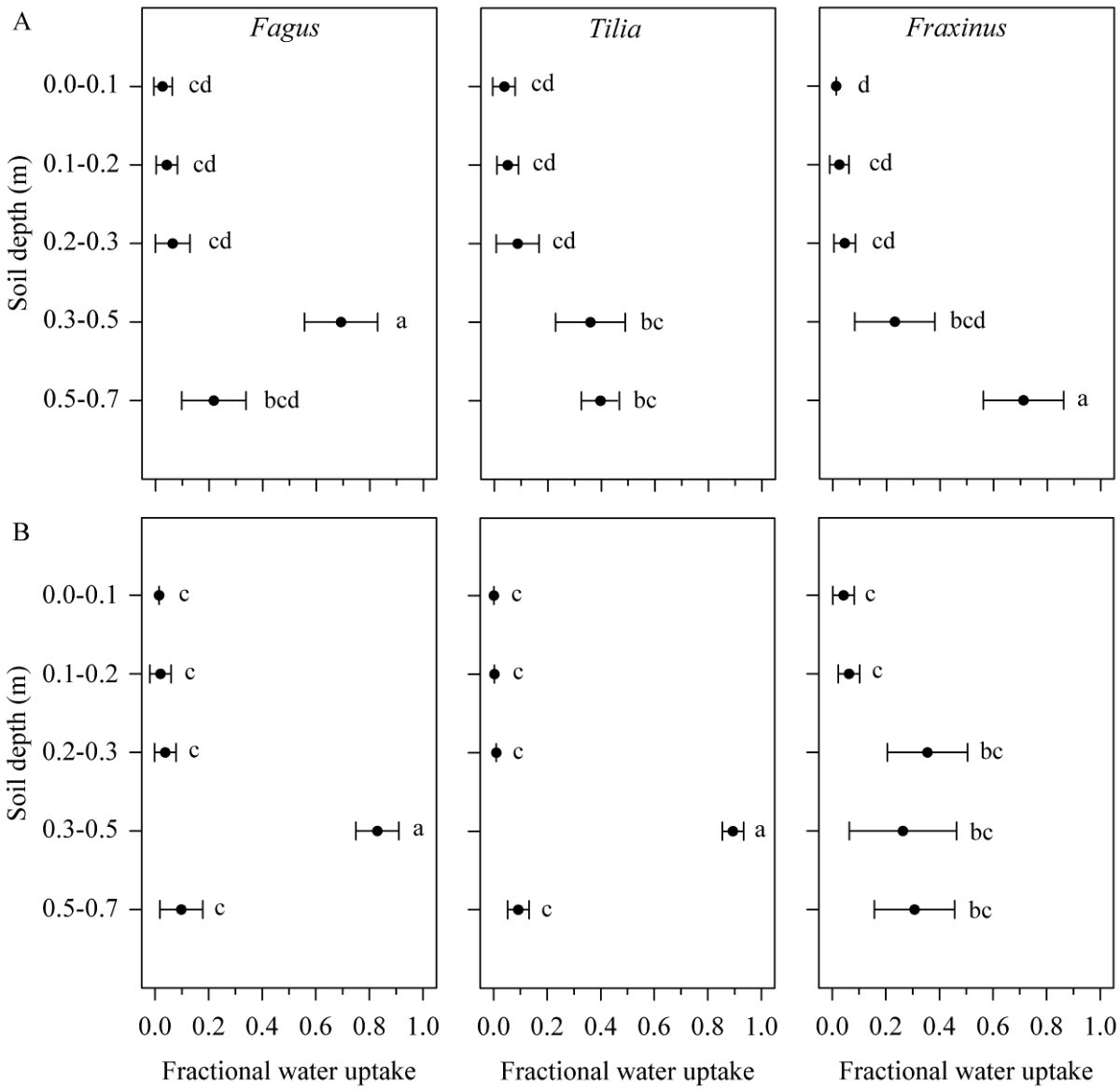


Figure 4.5: Proportional $\delta^2H$ source contribution of the observed soil depth to the mixture (xylem water), expressed as fractional water uptake. Shown are the results for trees in single-species clusters (A) and mixed clusters (B). Values are means ± sd (n = 12 for single and n = 4 for mixed clusters), different letters specify significant differences among species (ANOVA).

A comparison of fractional water uptake between species in single and mixed species clusters showed significant differences at the depth intervals 0.3-0.5 m and 0.5-0.7 m for *Tilia* and at 0.5-0.7 m for *Fraxinus* while the pattern of *Fagus* showed no such difference. In single species clusters compared to mixed clusters, *Tilia* drew significantly less water from 0.3-0.5 m depth and more from 0.5-0.7 m, and *Fraxinus* drew less water from 0.5-0.7 m in admixture with other species.

In the single-species clusters, there was no clear relation between tree diameter and deuterium signature of the main soil water uptake depths despite a considerable diameter range (38.1-72.2 cm for *Fagus*, 23.6-70.3 cm for *Tilia* and 22.3-38.6 cm for *Fraxinus*) (Fig. 4.6A-C). In contrast, in the mixed clusters (dbh ranging from 24.0-56.1 and no species dominating a certain diameter range) $\delta^2H$ of the main uptake depth increased significantly with increasing dbh ($p < 0.001$; $R^2_{adj} = 0.73$; Fig. 4.6D).

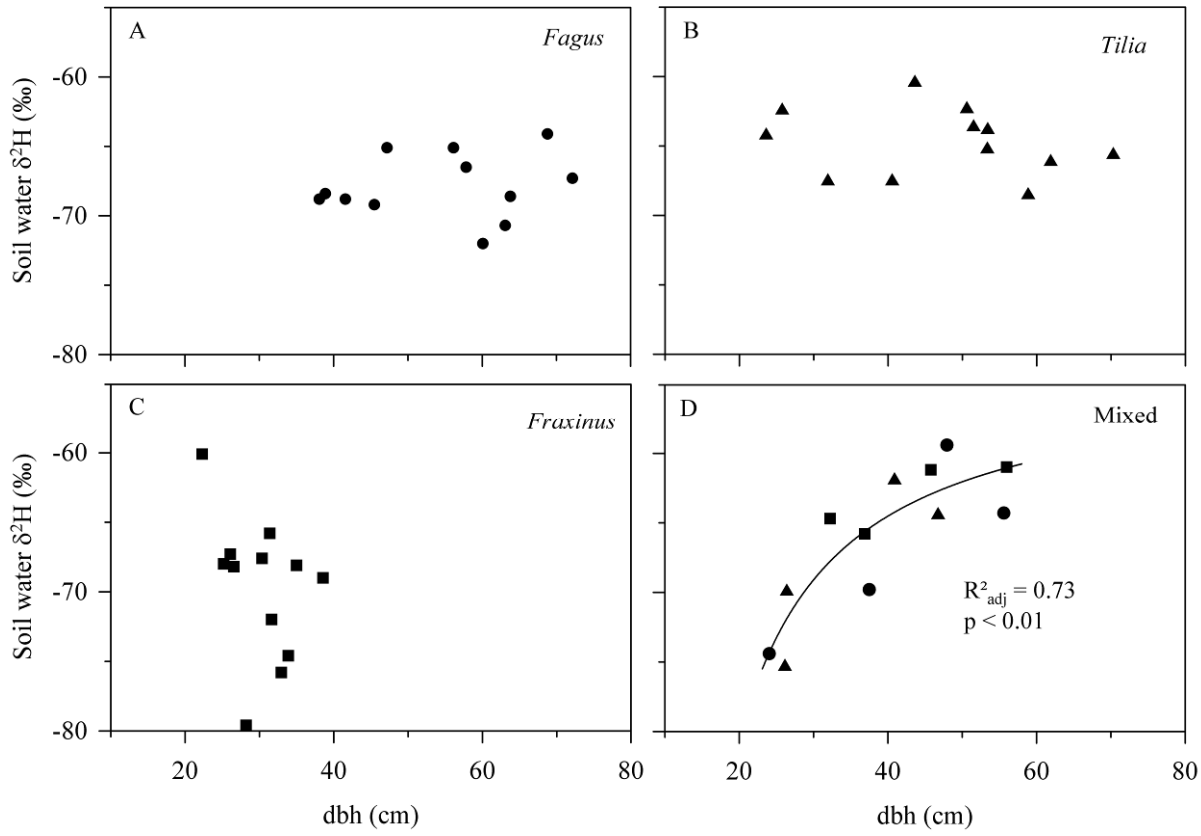


Figure 4.6: Soil water $\delta^2H$ of the main water uptake depth per tree in relation to diameter at breast height (dbh) on single and mixed species tree clusters.

This suggests that in the mixed clusters, trees with large dbh obtained water mainly from 0.3 m and above, whereas trees with a smaller dbh mainly tapped the soil layers below 0.3 m.

### *4.3.2 Volume of soil water uptake*

The computed average daily water uptake during the soil desiccation period (from 30 July 2009 to 10 September 2009) for the single-species clusters was $2.6 \pm 0.4$ mm $d^{-1}$ for *Fagus*,

2.9 ± 0.5 mm $d^{-1}$ for *Tilia*, 3.0 ± 0.5 mm $d^{-1}$ for *Fraxinus*; for the mixed species clusters it was 2.8 ± 0.4 mm $d^{-1}$. There were no significant differences in average daily water uptake between the four cluster types (Fig. 4.7).

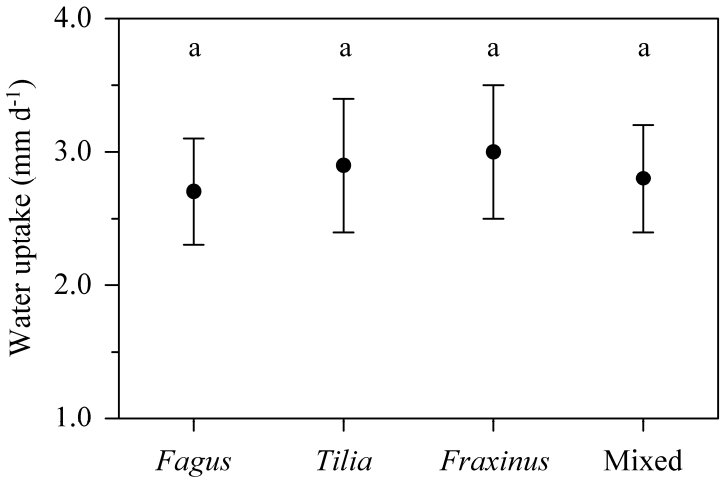


Figure 4.7: Estimated water uptake (mm $d^{-1}$) from 0 to 0.7 m soil depth during desiccation period from 30 July to 09 October 2009. Measured on single and mixed species clusters composed of *Fagus*, *Tilia* and *Fraxinus*. Values are means ± sd (n = 4), similar letters specify no significant difference between species.

## 4.4 Discussion

### *4.4.1 Water uptake depth*

The samples for the deuterium analysis were taken during a summer period when soil water content declined, as it frequently occurs in the region as e.g. documented in Hölscher et al. (2003) and Krämer and Hölscher (2010). At the time of sampling, a gradient in soil water $\delta^2H$ signatures had established that accordingly allowed for a differentiation of soil depth. The lowest $\delta^2H$ signatures (more negative) were found at greater soil depths, where the soil water potential was also highest (less negative). Due to the water potential gradient it can be assumed that with increasing soil depth water extraction became easier for the trees. A study on fine root distribution conducted in 12 nearby study plots and on the same tree species found that fine root biomass decreased markedly with soil depth with ~ 64-77 % being located in the upper 0.2 m of the soil profile, independent of tree species or species mixture (Meinen et al., 2009a). Therefore,

we can assume a comparable root distribution for our tree clusters and species, with a higher fine root allocation in shallow layers and less in the deeper ones.

The comparison of xylem and soil water $\delta^2H$ values indicated differences in water uptake patterns of the three studied species of canopy trees growing in single species clusters. It revealed a significantly higher water uptake at the depth interval of 0.3-0.5 m for *Fagus* clusters and at 0.5-0.7 m also a higher uptake for *Fraxinus* clusters, compared to the other species respectively (Fig. 4.5A). From such data, one may be expected to find water uptake complementarity where these species occur in mixtures, due to differing main water uptake depths. In mixed clusters, *Fraxinus* showed a distinctly lower water uptake from 0.3-0.5 m soil depth and again a higher uptake from 0.5-0.7 m compared to the other two species (Fig. 4.5B). The assumed complementarity from the single cluster observation was not confirmed in the mixture, as *Fagus* and *Tilia* appear to draw water from the exact same depth (0.3-0.5 m) and *Fraxinus* shows a different water uptake pattern.

A comparison between single and mixed clusters showed that *Tilia* and *Fraxinus* seemed to have a markedly different water uptake pattern when growing in a mixture with other species, while the uptake pattern of *Fagus* remained independent of admixture. *Fagus* is usually considered a highly competitive species in central European forests, which is mainly attributed to the well developed ability of mature trees to cast shade that constricts the development of many other tree species, and considering its own offspring are shade tolerant (Leuschner and Ellenberg, 2010). Moreover, in the rhizosphere, *Fagus* was documented to be a strong competitor (Leuschner et al., 2001; Rewald and Leuschner, 2009; Meinen et al., 2009a, b). Thus, it is possible that competition with *Fagus* in mixed clusters led to changes in uptake patterns of *Tilia* and *Fraxinus*.

Complementarity in soil water uptake among species was mainly related to the difference in soil water uptake depth between *Fraxinus* and the other two species, and it was observed in both single- and in mixed species clusters. The water uptake of *Fraxinus* differed by one and two soil depth intervals in the single and mixed species clusters respectively. However, in both situations it only withdrew approximately 70% of its water from the given depths, which suggests that the water uptake pattern of *Fraxinus* was flexible, but that the share of soil water uptake from soil depths with little interference from other species was similar in both situations. Interestingly, *Fraxinus* rarely occurs in central European forests as a mature tree in single-species stands while it is frequently found in mixed forests with neighbors belonging to other tree species.In the mixed clusters we found a strong relationship between $\delta^2H$ signature of the main water uptake depth and dbh ($R^2_{adj} = 0.73$, $p < 0.001$). Trees with larger dbh obtained water predominantly from the topsoil, whereas trees with smaller dbh mainly tapped the soil layers below. A similar

pattern with smaller trees tapping at deeper sources of water than larger ones was found by Meinzer et al. (1999) in a tropical old-growth forest in Panama. The authors suggested that large trees have a more extended horizontal root system, allowing for partial compensation of the reduced water content in upper soil layers with a more extensively explored horizontal soil area. Such an explanation would fit with the finding of Lang et al. (2010) in our study area who found that dbh is positively correlated with root distance from the tree. A higher soil nutrient content is also often found in the upper soil layers, making it beneficial for trees with large dbh to utilize these soil regions, as their demand on nutrients is higher when compared to smaller trees.

Meinzer et al. (1999) further mention that diurnal stem water storage capacity increases exponentially with stem size, which might serve as a buffer for peak demand of water uptake. In contrast, in an Indonesian cacao agroforest, Schwendenmann et al. (2010) found that *Gliricidia* shade trees, which had larger dbh than cacao trees, used deeper water sources. Here it has to be taken into account that *Gliricidia* had about double the height of the cacao trees, whereas our trees were very homogenous in height.

In our data set, tree size did however show no effect on the vertical distribution of soil water uptake of trees when located in the single species clusters, despite the similar diameter range compared to the mixed clusters. In addition, in the mixed clusters, there was no particular species dominating in particular tree size (see Fig. 4.6D). Thus, the relation seems to be independent of species identity, but conditional on the presence of other species as neighbors. No further comparison with the Meinzer et al. (1999) study is possible however, as there was no differentiation between trees with con-specific neighbors or different-species neighbors. The reasons behind the strong relation between tree diameter to main soil water uptake depth in mixed clusters and its absence in single-species clusters in our study is ambiguous. It may be influenced by the plasticity in soil water uptake depths of *Tilia* and *Fraxinus* growing in single or mixed species clusters as observed in combination with differing dbh sizes.

### *4.4.2 Volume of soil water uptake*

Complementarity in soil water uptake may also lead to enhanced soil water use. For example, in a Panamanian tree plantation tree transpiration rates increased with increasing tree species diversity, which by way of statistical analysis, Loreau and Hector (2001) suggest to be significant biodiversity effects mainly based on species complementarity (Kunert et al., 2012). There were also indications in the Hainich forest that mixed stands used more water for transpiration in the beginning of a drought period than monospecific beech stands (Krämer and Hölscher, 2010; Bittner et al., 2010). Despite the observation of complementarity in soil water uptake depth in

the present study, we did not observe statistically significant differences in the volume of water uptake between single- and mixed-species clusters. A possible explanation is that evapotranspiration rates, which have an affect on the volume of water uptake in trees, are controlled by stand structure and can vary with changes in e.g. aerodynamic roughness. These processes however act at a much larger scale than tree clusters and therefore might cover effects of complementarity.

All our study clusters were embedded in two larger mixed forest stands and thus possible differences between single- and mixed species stands may not have been detected. It may also be argued that the volume of water extracted in the single- and mixed species clusters is the same, but that the expense of water uptake may be different, e.g. leading to altered water use efficiency.

Our study also has implications with respect to forest management in close-to-nature forestry, which is an upcoming practice for temperate broad-leaved forest management. It shows that this practice leads to stands of a wider diameter distribution and it may also enhance tree species diversity. Our data would suggest that a wide diameter range in a single species stand would not lead to a variation in soil water uptake depth. Species mixture hints to complementarity, but our data did not suggest that complementarity leads to increased water consumption. In summary, our results imply that soil water partitioning among canopy trees is strongest where species are mixed *and* tree size varies.

## 4.5 Acknowledgements

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## 4.7 Appendix

Table A4.1: Soil texture and soil bulk density of single and mixed species tree clusters. Values are means ± sd (n = 4)

| | **Soil depth (m)** | **Cluster** | | | |
|---|---|---|---|---|---|
| | | ***Fagus*** | ***Tilia*** | ***Fraxinus*** | **Mixed** |
| Soil texture (sand/silt/clay) | 0-0.1 | 2/ 74/ 24 | 3/ 73/ 25 | 3/ 68/ 29 | 3/ 75/ 23 |
| | 0.1-0.2 | 3/ 75/ 22 | 2/ 74/ 24 | 2/ 69/ 29 | 3/ 75/ 23 |
| | 0.2-0.3 | 3/ 71/ 26 | 3/ 75/ 23 | 2/ 72/ 26 | 2/ 71/ 27 |
| | 0.3-0.4 | 4/ 68/ 28 | 3/ 67/ 30 | 2/ 67/ 31 | 3/ 68/ 29 |
| | 0.4-0.6 | 2/ 59/ 39 | 2/ 58/ 40 | 2/ 56/ 42 | 2/ 54/ 44 |
| | 0.6-0.8 | 2/ 59/ 39 | 2/ 58/ 40 | 2/ 56/ 42 | 2/ 54/ 44 |
| Bulk density ($g\ cm^{-3}$) | 0-0.1 | 1.1 ± 0.2 | 1.1 ± 0.2 | 1.0 ± 0.1 | 1.1 ± 0.1 |
| | 0.1-0.2 | 1.3 ± 0.1 | 1.2 ± 0.1 | 1.3 ± 0.1 | 1.3 ± 0.0 |
| | 0.2-0.3 | 1.4 ± 0.1 | 1.4 ± 0.1 | 1.4 ± 0.1 | 1.5 ± 0.1 |
| | 0.3-0.4 | 1.4 ± 0.1 | 1.4 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 |
| | 0.4-0.6 | 1.5 ± 0.1 | 1.5 ± 0.1 | 1.4 ± 0.0 | 1.5 ± 0.1 |
| | 0.6-0.8 | 1.5 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 |

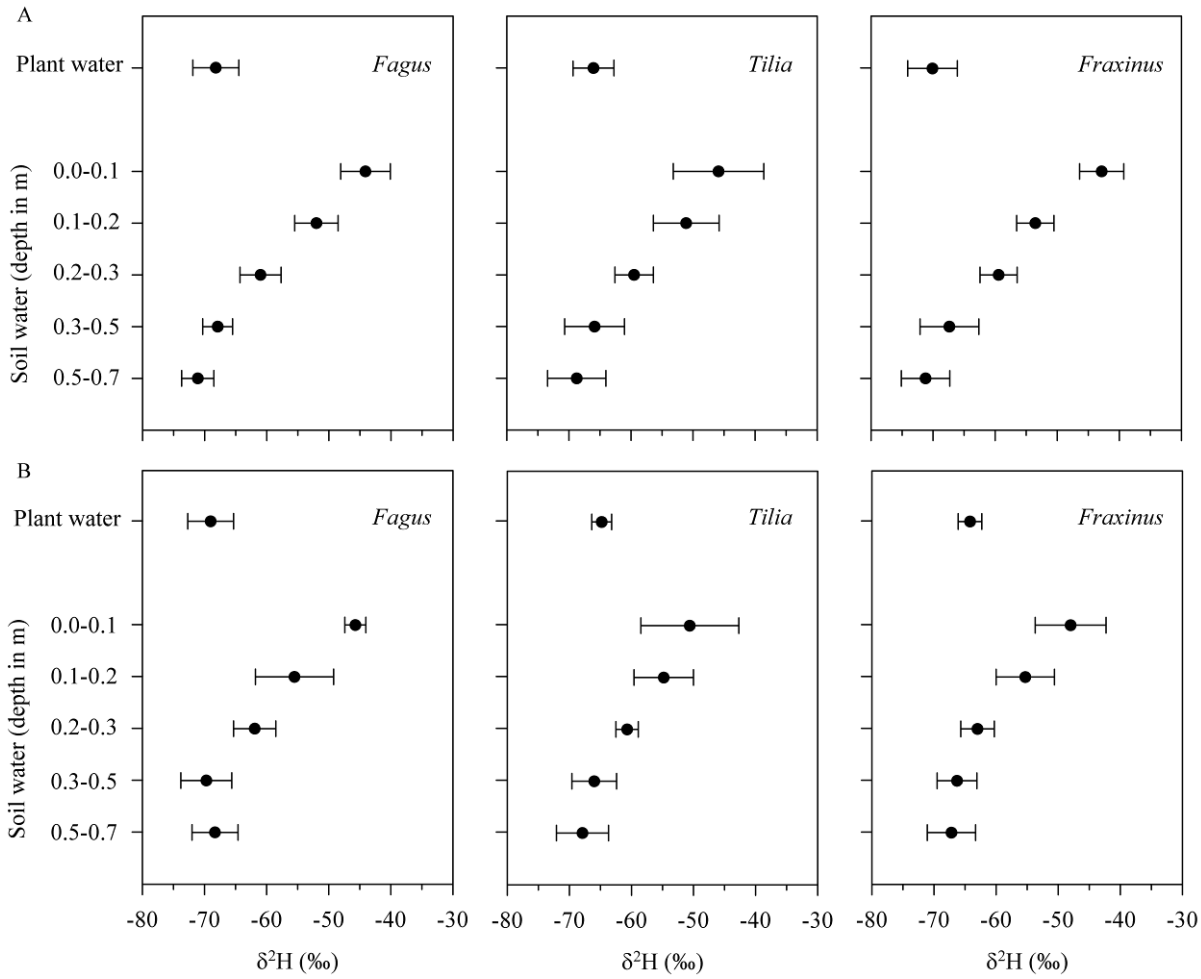


Figure A4.1: Plant and soil water $\delta^2H$ values for single- and mixed-species clusters (A and B respectively). Values are means ± sd (n = 12 for A, n = 4 for B).

# Chapter 5

## DIVERSITY DID NOT INFLUENCE SOIL WATER USE OF TREE CLUSTERS IN A MIXED TEMPERATE FOREST

# 5. Diversity did not influence soil water use of tree clusters in a mixed temperate forest

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**Abstract.** Compared to monocultures, diverse ecosystems are often expected to show more comprehensive resource use. However, with respect to diversity - soil water use relationships in forests, very little information is available. We analyzed soil water uptake in 100 tree clusters differing in tree species diversity and species composition in the Hainich forest in Central Germany. The clusters contained all possible combinations of five broad-leaved tree species in one-, two- and triple-species clusters (three diversity levels), replicated fourfold (20 1-species, 40 2-species and 40 3-species clusters). We estimated soil water uptake during a summer dry period in 0 - 0.3 m soil depth, based on throughfall and soil moisture measurements and a simple budgeting approach. Throughout the whole vegetation period in 2009, soil water uptake was additionally determined at a higher temporal resolution and also for a greater part of the soil profile (0 - 0.7 m) on a subset of 16 intensive clusters. During the dry spell, mean soil water uptake was 1.9 ± 0.1 mm $day^{-1}$ in 0 - 0.3 m (100 clusters) and 3.0 ± 0.5 mm $day^{-1}$ in 0 - 0.7 m soil depth (16 clusters), respectively. Besides a slightly higher water use of *Fraxinus* clusters we could not detect any effects of species identity or diversity on cluster water use. We discuss that water use may indeed be a conservative process, that differences in tree species specific traits may be compensated for by other factors such as herb layer coverage and tree spatial arrangement, and that diversity driven differences in water use may arise only at a larger scale. We further conclude that with respect to stand water use "tree diversity" alone is not an appropriate simplification of the complex network of interactions between species traits, stand properties and environmental conditions that have varying influence on stand water use, both in space and time.

*Key words: Acer pseudoplatanus, Carpinus betulus, Fagus sylvatica, Fraxinus excelsior, Tilia sp., resource use, soil water, forest hydrology*

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## 5.1 Introduction

Little information is available on the relationship between tree diversity and stand water use in temperate forests; but water use is most likely related to productivity in forest stands (Law et al., 2002). For grasslands, an increase in productivity with species diversity has been widely recorded (e.g. Hector et al., 1999). Evidence for a positive relationship between productivity and tree species diversity in forests is accumulating, indicated by a modelling exercise of competitive interactions of randomly chosen species (Tilman et al., 1997). From a forest succession model dealing with "real" species, the conclusion was derived that "*tree diversity strongly influences primary productivity in European temperate forests across a wide range of sites with different climates through a strong complementarity effect*" (Morin et al., 2011). Similar findings are also supported by some field studies: a positive relationship between tree species diversity and productivity was indicated in early successional and disturbed sclerophilous and conifer forests before canopy closure (Vilà et al., 2005). In a Panamanian experimental plantation, mixed-species plots yielded on average 30 - 58% higher summed tree basal area compared to monocultures after 5 years (Potvin and Gotelli, 2008). On 12,000 permanent forest plots in Canada, a strong positive effect of biodiversity on tree productivity (controlled for environmental conditions) was obtained (Paquette and Messier, 2011). Another large scale study in Sweden, across 400,000 $km^2$ found approximately 50% higher biomass productivity comparing one and five species plots (Gamfeldt et al., 2013). Also a large scale cross-European modelling study indicated that tree wood productivity was positively related species richness (Vilà et al., 2013). owever, mainly due to the longer life cycle of trees and possible changes in biodiversity-productivity relationships with tree age experimental approaches in forests remain complicated (Pretzsch and Schütze, 2009). Pretzsch (2005) reported that productivity of mixtures of Norway spruce (*Picea abies*) and European beech (*Fagus sylvatica*) trees may differ from the respective monocultures by -20 to 10%, dependent on site conditions. In addition climatic variables influenced wood production in varying direction and magnitude dependent on forest type (Vilà et al., 2013). Even a weak negative relationship between tree species diversity and above-ground biomass was found on several sites across Central European forests (Szwagrzyk and Gazda, 2007) and also in at our study site (Jacob et al., 2010).

In grasslands, it has been observed that plant species diversity enhances transpiration rates (Verheyen et al., 2008). In addition, in a experimental tree plantation in Panama, transpiration increased with increasing tree species diversity (Kunert et al., 2012). In both studies,

complementarity of water uptake was discussed as an underlying mechanism (Verheyen et al., 2008; Kunert et al., 2012). This would imply water resource partitioning and, consequently, more effective utilization of water resources (Hagger and Ewel, 1997; Hooper et al., 2005). Consequently, biodiversity rich stands may be more susceptible to drought events since they extract water "more efficiently" than less diverse stands. This coherence has already been demonstrated for grasslands (van Peer et al., 2004; Verheyen et al., 2008).

It is important to study if a water use – diversity relationship also exists for forests, since there is an ongoing trend in Central European silviculture towards more naturalness or close-to-nature forestry (O'Hara, 2001) which implies a transformation of monocultural stands of narrow tree diameter range into stands composed of several tree species with a broader range of diameters. In addition to improving ecological, commercial and recreational purposes of forests, it is believed that this forest transformation might increase the resilience of forests to extreme climatic conditions (LÖWE, 2007). Climatic extremes are predicted to occur more frequently for large parts of central Europe (Rowell and Jones, 2006; Christensen et al., 2007). Now if the results from grasslands are valid for forests too, the anticipated effect of forest restructuring might not be achieved.

First studies on the relationship between tree species diversity and forest water use were carried out in the broad-leaved Hainich forest in Germany: here increased water extraction from the top soil during a summer drought in diverse plots compared to *Fagus sylvatica* dominated plots was observed (Krämer and Hölscher, 2010). Canopy transpiration was also found to differ among diverse and less diverse stands in certain years (Gebauer et al., 2012). However, none of the outcomes could be clearly attributed to a biodiversity effect, as increasing biodiversity was paralleled by decreasing *Fagus* admixture, and no monocultures of any other species involved were studied. In order to differentiate between the effects of tree diversity and of species identity, we applied a new experimental design in the same study area, where all observed tree species occur in monospecific study plots and in admixture. We selected 100 groups of three neighbouring trees, hereafter named tree clusters, which contained all possible combinations of five tree species (*Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Tilia sp.*). All species occurred in single-species clusters (n = 20), as well as in two- and triple-species mixtures (n = 40, each). We asked whether stand water use is related to tree diversity. Our hypothesis was that water uptake in tree clusters increases with increasing species diversity.

## 5.2 Methods

### *5.2.1 Study area*

The study was conducted in the deciduous Hainich forest in Central Germany close to the village of Weberstedt (51°05'28"N, 10°31'24"E). The forest has remained free from harvesting or thinning for almost 50 years, and it was estimated that the area has hosted deciduous forest for over 200 years (Mölder et al., 2006, 2009). The study sites are located on level terrain in the south-eastern part of the forest area (Fig. 5.1A) at an elevation of approximately 350 m (a.s.l.). The park receives a mean annual precipitation of 544 - 662 mm (average of 30 years of precipitation records from four climate stations around the national park; DWD, 2008) and a mean temperature of 7.5°C. Soil texture is characterized by high clay content of ~ 25% at a soil depth of 0 - 0.3 m and 33 - 41% at 0.4 - 0.6 m, respectively (Guckland et al., 2009). Limestone already occurred at shallow soil depths (0.6 - 1.0 m) limiting the rooted soil volumes. Stand fine root biomass in the area decreased exponentially with soil depth with 63 - 77% being concentrated in the upper 20 cm (Meinen et al. 2009).

In 2008, tree clusters were selected in two mixed forest stands within the Hainich forest area (sub-areas Lindig and Thiemsburg, Fig. 5.1B). All clusters were located in close vicinity to the study plots of Krämer and Hölscher (2009, 2010). Each cluster consisted of three co-dominant trees arranged in a triangular shape with their surrounding neighbours. Observed tree species on these clusters were *Acer pseudoplatanus* (sycamore maple), *Carpinus betulus* (hornbeam), *Fagus sylvatica* (European beech), *Fraxinus excelsior* (ash) and *Tilia sp.* (lime). In this forest, the two *Tilia* species *cordata* and *platyphyllos* often form hybrids, which are phenotypically difficult to differentiate. Hence we did not differentiate at the species level and we refer to them as *Tilia* sp.. Cluster selection was based on a predetermined combination of tree species comprising all possible neighbourhood combinations of the five tree species. This resulted in five different single-species, ten double-species and ten triple-species cluster combinations, with each combination being replicated four times (twice replicated in each sub-area, Thiemsburg and Lindig). In the two species combinations it was assured that not one species dominated the mixture in all four replicates.

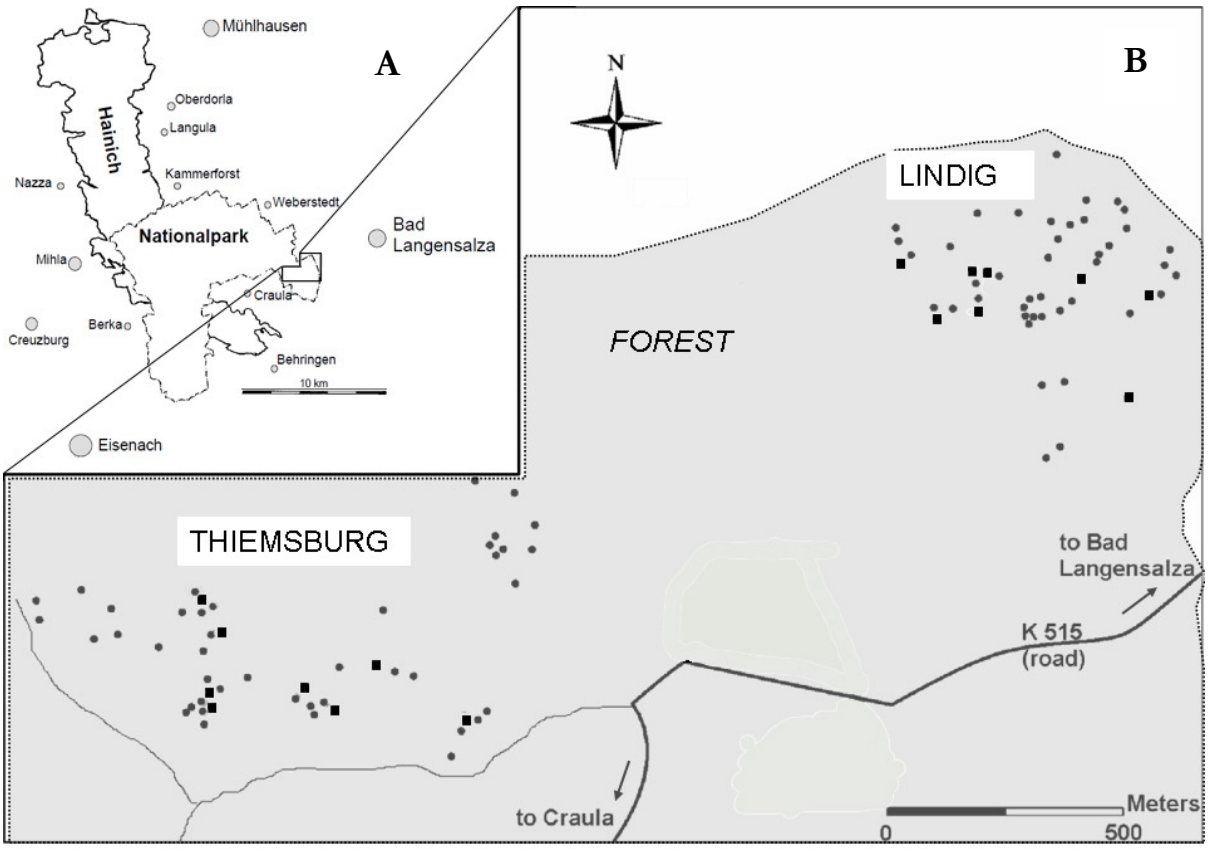


Figure 5.1: Location of the 100 tree clusters in the two forest areas. The grey dots and black rectangles indicate cluster positions. The 16 black rectangles represent intensively measured clusters (Figure based on D. Seidel, 2011).

From the 100 clusters, we selected a subset of 16 clusters containing the species *Fagus sylvatica*, *Tilia sp.* and *Fraxinus excelsior* in monoculture and in triple-species clusters. The selected clusters were used to monitor soil water content in the subsoil, to increase the temporal resolution of soil water content measurements and to conduct throughfall measurements (Fig. 5.1B).

Since the clusters of the two forest sub-areas were statistically not different with regard to soil properties and tree structural characteristics, they were pooled in the subsequent analysis. Soil and stand structural characteristics, such as soil bulk density (g $cm^{-3}$), clay content (%), tree diameter at breast height (dbh in m), cluster ground area ($m^2$) and openness (%), were also not significantly different among diversity levels (Tab. 5.1).

Table 5.1: Soil properties (0-0.3 m soil depth) and structural characteristics of the 1- to 3-species tree clusters (means ± sd). Similar letters indicate no significant differences between the three diversity levels ($p \leq 0.05$, ANOVA and Tukey's HSD or Kruskal-Wallis-Test (canopy openness)).

| **Cluster characteristics** | **Diversity level** | | | | | |
|---|---|---|---|---|---|---|
| | **1-species (n=20)** | | **2-species (n=40)** | | **3-species (n=40)** | |
| Canopy openness (%) | 10.7 ± 5.6 | a | 9.6 ± 5.6 | a | 9.0 ± 2.5 | a |
| Diameter at breast height (m) | 0.43 ± 0.11 | a | 0.43 ± 0.08 | a | 0.45 ± 0.07 | a |
| Cluster area ($m^2$) | 25.2 ± 17.9 | a | 23.3 ± 13.4 | a | 23.8 ± 15.1 | a |
| Soil bulk density (g $cm^{-3}$) | 1.18 ± 0.08 | a | 1.21 ± 0.09 | a | 1.19 ± 0.09 | a |
| Soil clay content (%) | 28 ± 4 | a | 27 ± 5 | a | 28 ± 7 | a |

### *5.2.2 Meteorological data, soil water content and throughfall measurements*

Data on air temperature (C°), gross precipitation (*Pg*, mm), global radiation (MJ $m^{-2}$ $day^{-1}$) and wind speed (m $s^{-1}$) were recorded hourly at the meteorological station Weberstedt (Meteomedia, Germany), 2 - 3 km northwest of our study area at an altitude of 270 m a.s.l. On all 100 clusters we conducted measurements of soil volumetric water content ($\theta$ in $m^3$ $m^{-3}$) at four points with a TDR probe (CS616, Campbell Scientific) at a depth of 0 - 0.3 m. Water content was assessed monthly throughout the vegetation period in 2009 (30 April to 31 October) and on four occasions during a dry spell in summer (30 July, 10 and 24 August, 1 September).

The 16 intensive clusters were equipped with PVC access tubes, enabling measurement of $\theta$ with a portable FDR sensor (Diviner 2000, Sentek Pty Ltd. Stepney, Australia) in addition to the TDR measurements. Access tubes were installed to a maximum depth of 0.7 m in which sensor readings were taken at depth intervals of 0.1 m. Volumetric soil water content was measured weekly throughout the vegetation period. The FDR sensor had already been soil- and depth-specifically calibrated for the local soil conditions in the field (Krämer and Hölscher 2010). By correlating 72 FDR readings at different soil water contents with corresponding TDR readings in the direct vicinity of the FDR, we established a site specific calibration for the TDR probes.

Throughfall was monitored weekly throughout the whole vegetation period on the 16 clusters with rainfall collectors consisting of a plastic bottle screwed to a funnel attached to a metal rod at a height of 1 m. To reduce evaporation from the rain gauge, a table tennis ball was placed in the funnel. The instrumental setup within a tree cluster is shown in figure 5.2.

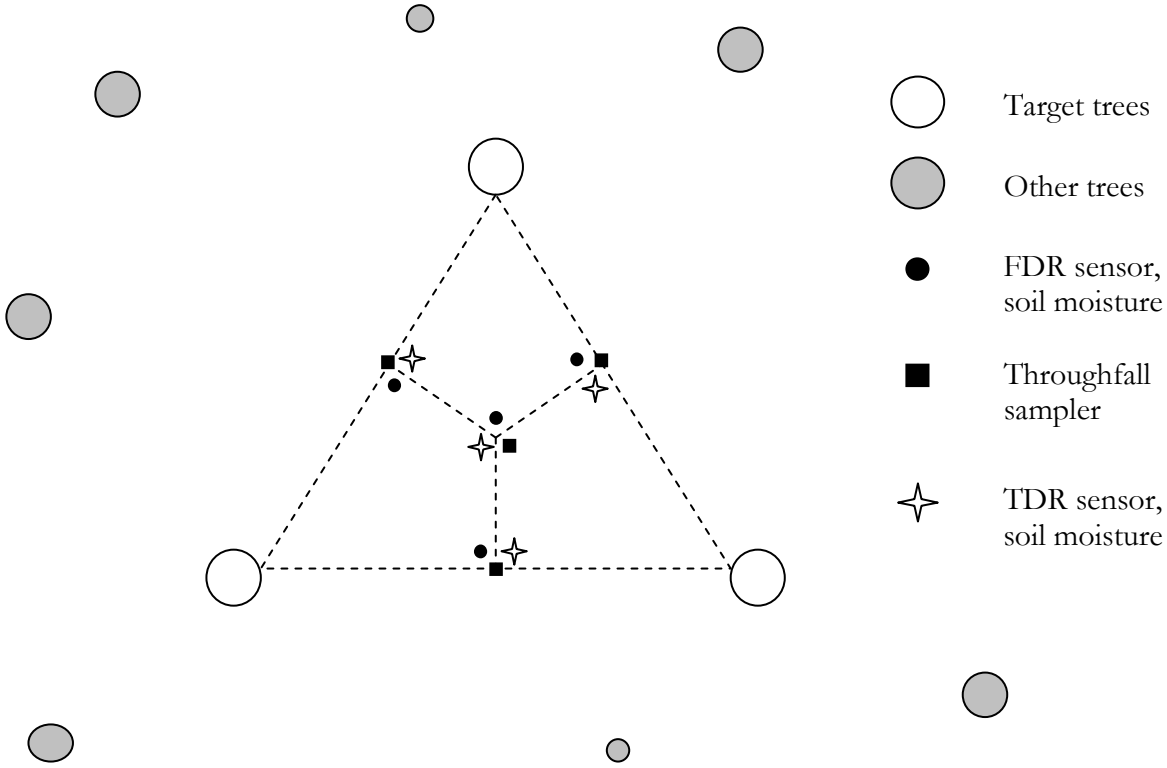


Figure 5.2: Schematic study plot design (tree cluster) with locations of FDR, TDR sensors and throughfall samplers.

### *5.2.3 Soil water budgeting*

Daily water uptake *Wu* (mm day$^{-1}$) between two consecutive measurements of soil water content was calculated by Eq. 5.1:

$$Wu = \frac{(Tf + Sf) - \Delta S}{\Delta t} \tag{5.1}$$

where *Tf* = throughfall (mm), *Sf* = stemflow (mm), $\Delta S$ = change in soil water storage between two successional measurements (mm) and $\Delta t$ = the elapsed time between the two successional measurements (days). $\Delta S$ (mm) was calculated for each cluster from $\theta$ ($m^3\ m^{-3}$), measured by TDR sensors, multiplied by the depth of the soil layer in which $\theta$ was measured und converted to mm.

*Tf* was either measured directly (16 cluster subset) or calculated from an established relationship with average cluster dbh (*Tf* = 81.7 – 0.2 * dbh) for the remaining clusters. *Sf* for each rainfall event during our study period was estimated from findings of an earlier study in the same area using 50 stemflow collectors on all five tree species during two successive years (Krämer and Hölscher, 2009). The magnitude of *Sf* in the Hainich forest in general is usually relatively low (~ 0.4 to 6.3% of *Pg*), varying more between seasons than between plots of differing tree species diversity/*Fagus* admixture. It was highest on *Fagus* trees of large dbh and during high rainfall events but even then stemflow was lower compared to other *Fagus* dominated forests (Krämer and Hölscher, 2009). We quantified intensity and duration of single rainfall events from hourly data on gross precipitation automatically recorded at the nearby weather station. We than calculated *Sf* for given rainfall intensities for each of our cluster trees dependent on tree species and dbh based on raw data from the study of Krämer and Hölscher (2009). For *Fagus* and *Carpinus Sf* was calculated as 1% of gross precipitation for trees with dbh > 10 and < 30 cm; for trees > 30 cm *Sf* was 3% at rainfall intensities > 2.0 and < 6.0 mm hour$^{-1}$. For *Acer*, *Fraxinus* and *Tilia* 0.5% of *Pg* was added to the water budget for trees with dbh > 30 cm, at rainfall intensities > 4 mm hour$^{-1}$. All incoming water (*Tf* and *Sf*) is regarded to infiltrate the soil, hence evaporation from understory vegetation and litter layer do enter the budget as root water uptake.

Water use of all 100 clusters was only calculated during the dry spell for the soil layer in which the TDR was inserted (0.3 m in depth; hereafter referred to as $Wu_{30d}$). With regard to the 16 cluster subsets on which water content was additionally measured down to 0.7 m by FDR

sensors, $Wu$ was determined for all 0.1 m wide subsections of soil according to equation 1 and then summed to yield $Wu_{70}$. Average water use measured on the 16 clusters during the dry spell is referred to as $Wu_{70d}$. $Wu_{70}$ was determined on several occasions during the vegetation period only where trees were fully in leaf. Drainage or surface runoff could be neglected here (Bittner et al., 2010 and pers. communication with the author). Also the soil parameters (high residual water content and low saturated hydraulic conductivity in the subsoil) lead to very slow water movement rates (Bittner et al., 2010) from which we gain further confidence in our no-drainage assumption.

### *5.2.4 Statistical analysis*

All statistical analyses were done with R version 3.0.0 (R Core Team, 2012). We fitted linear mixed effect models (LME, lme4 package) using maximum likelihood estimation (ML) to determine the influence of the 25 possible species combinations, the three diversity levels or absence/presence of the five species (set as fixed effects respectively). For the analysis of $Wu_{30d}$ the sub-areas (Tiemsburg/ Lindig) served as random effect. We included the covariates cluster area and cluster dbh and, in case of the three diversity level model, also their interactions, since they are likely to influence cluster water use.

Another LME was used to judge the influence of the four possible species combinations (*Fagus*, *Tilia*, *Fraxinus* and their mixture), dbh and area on $Wu_{70}$ with the date of measurement (n = 11) as random effect. To ensure homoscedasticity, $Wu_{70}$ was log-transformed here. $Wu_{70}$ was further modelled as a smoothing function of radiation and the factor species combination with generalized additive models (GAM, mgcv package) employing thin plate regression splines. The model was supplied with weights ($Wu_{70}^{-1}$) to ensure homoscedasticity. Model comparison and the assessment of the significance of the smoothers and the factor species combination within a model were done with F-tests.

Residuals of all models were visually checked for homoscedasticity and normality by boxplots, residuals against fitted values plots and qq-plots. Non-significant effects ($p > 0.05$) in LMEs were discarded from the full model stepwise by comparing models with the same random effects structure fitted with ML estimation. To this end, likelihood ratio tests were used, since both, *t–statistic* provided by "anova (LME)" and *F-statistic* provided by "summary (LME)" are only approximate (Zuur et al., 2009). Differences between species combinations or diversity levels, whenever significant in the mixed model, were further investigated using Tukey-HSD post-hoc tests (glht, multcomp package).

The relationship between *Tf* and dbh was determined using a linear regression model. We further used Spearman's rank correlation analysis to relate selected stand structural variables and $Wu_{30d}$ on all 100 clusters. All statistical tests were considered significant where $p \leq 0.01$ and marginally significant where $p \leq 0.05$.

## 5.3 Results

### *5.3.1 Meteorological conditions*

Rainfall in 2009 totalled 773 mm, which was higher than the long term average rainfall measured at four stations around the park (544 - 662 mm $year^{-1}$). This is mainly attributed to two heavy storms in July. The rather wet July was followed by an August of below-average rainfall, the month on which we mainly focused our study. Here, the average maximum and minimum air temperatures were about 25°C and 12°C respectively. The global radiation average was 17.5 MJ $m^{-2}$ $day^{-1}$. During the dry spell, *Pg* was about 9 mm per week.

### *5.3.2 Soil water content*

Throughout May and June 2009, volumetric soil water content averaged over the 16 intensively studied clusters was continuously high at around 0.40 $m^3$ $m^{-3}$ (Fig. 5.3). Two storms at the end of July were not found to notably increase soil water content. Thus, we assume that drainage or overland flow could have possibly occurred here and therefore we did not include these occasions in the calculations of $Wu_{70}$. In a following period of low rainfall, soil water content decreased continuously from the end of July through to the beginning of September. For this dry spell, soil water budgeting was conducted on both the 16 intensive and the 100 cluster groups (see Fig. 5.1) yielding $Wu_{70d}$ and $Wu_{30d}$.

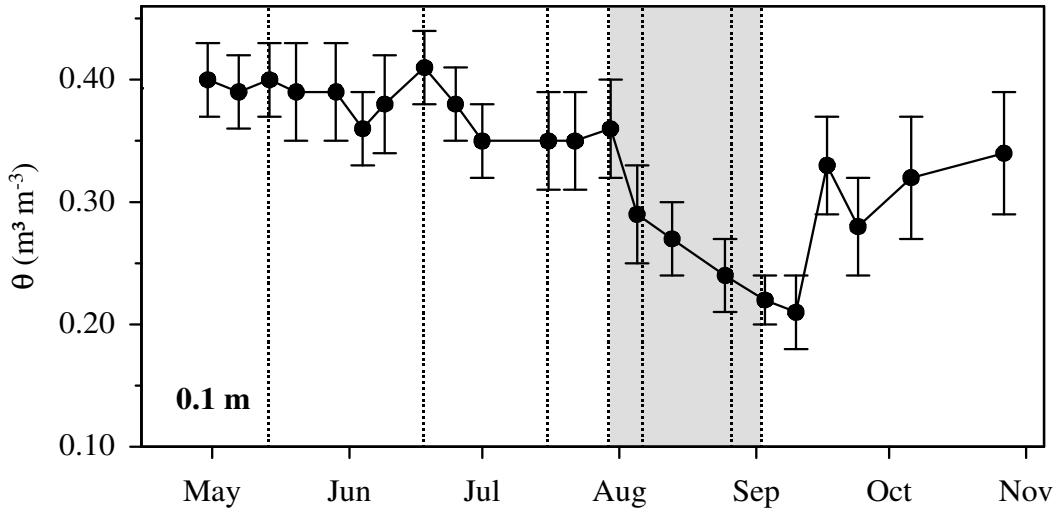


Figure 5.3: Average volumetric soil water content (FDR sensor) at 0.1 m soil depth during the study period in 2009. Values are means ± sd (n = 16 clusters). Dotted lines indicate the occasions where $\theta$ was measured on all 100 clusters with TDR's; the shaded area represents the dry spell (three subsequent measurement intervals) for which $Wu_{30d}$ und $Wu_{70d}$ were determined.

### *5.3.3 Soil water budget – 16 clusters*

Average $Wu_{70}$ (n = 16 clusters) calculated for all occasions within the vegetation period (trees fully in leaves) ranged overall between 0.8 and 4.0 mm day$^{-1}$ (*Fagus* 1.2 - 4.0; *Tilia* 0.9 - 3.8; *Fraxinus* 1.3 - 3.9 and Mix 0.8 - 3.1 mm day$^{-1}$) and was closely related to average daily global radiation (*Rad)* during these occasions (Fig. 5.4).

However, modelling $Wu_{70}$ as a smoothing function of *Rad* and species combination (levels: *Fagus*, *Tilia*, *Fraxinus* and Mix) with a GAM revealed a highly significant smoothing term ($F = 16.21$, $p < 0.001$) but no effect of the factor species combination ($F = 2.38$, $p = 0.07$). A model with a species specific smoothing term was not significantly different from a model with one smoothing term for all species.

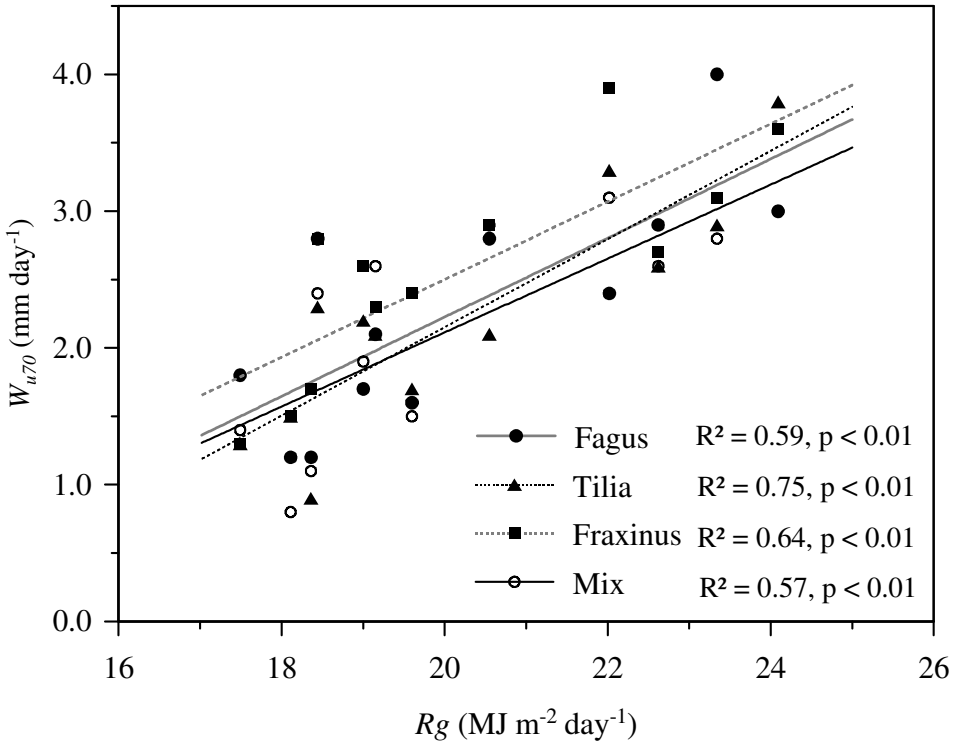


Figure 5.4: Average $Wu_{70}$ as a function of daily global radiation (*Rg*) for 4 different species combinations (n = 4 per species combination). Shown are data of 11 measurement occasions from June to mid September 2009 when trees were fully foliated and linear regression models between average $Wu_{70}$ and radiation.

Comparing a full MLE (explanatory variables: species combination, dbh and area and all two-way interactions) with MLEs with selectively dropped two-way interactions indicated no significant two-way interactions. Dropping species, dbh or area selectively from a new full MLE fitted without interaction terms indicated no effect of either one of these variables on $Wu_{70}$. However, having a full model with species as only explanatory variable and comparing it to a model including only random effects revealed that species had slight effects on $Wu_{70}$ (L.ratio = 9.07, p = 0.03). Tukey HSD post-hoc tests showed, that monospecific *Fraxinus* clusters had higher water use (average of 11 measurement occasions = 0.35 mm day$^{-1}$) compared to the mixed species clusters (t = -2.67, p = 0.04). Neither *Fraxinus* nor mixed clusters where any different from the other species combinations.

The measured throughfall component of $Wu_{70}$ was not related to the species composition or diversity throughout the measurement occasions in the year of 2009. The same result was found during the dry spell (30 July 2009 – 1 September 2009), where average *Tf* was low (32 ± 9.5 mm) ranging between 62 to 80% of *Pg*. *Tf* during the dry spell however declined with increasing

average dbh of each cluster (Fig. 5.5). Therefore we used this relationship to calculate *Tf* for all 100 clusters here. Estimated *Sf* input on the clusters was 0.3 ± 0.25 mm during the whole desiccation period and played therefore only a marginal role.

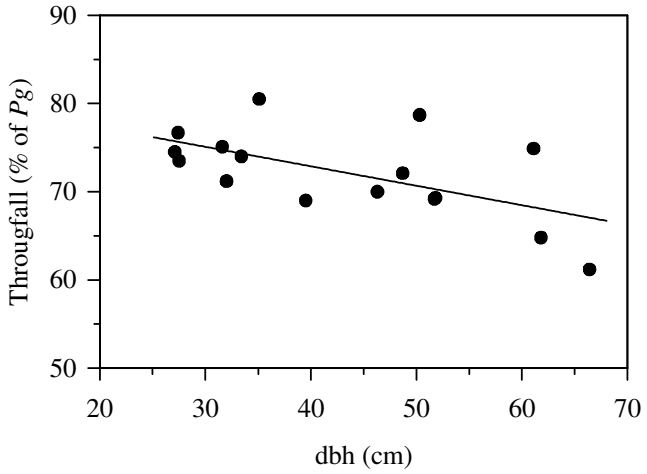


Figure 5.5: Relationship between average troughfall (% of gross precipitation) and average cluster dbh during the soil desiccation period (30 July to 1 September 2009). The equation reads $Tf = 81.7 - 0.2$ dbh.

### *5.3.4 Soil water budget – 100 clusters*

The 25 possible species combinations, cluster dbh and cluster area had no influence on $Wu_{30d}$. A LME with species combination as the only explanatory variable was not different from a model with only random effects (L.ratio = 3.96, p = 0.14; Fig. 5.6).

Likewise, testing for presence or absence of the five species resulted in no effect on daily water uptake. A similar picture was found when $Wu_{30d}$ of the 100 tree clusters was grouped according to diversity levels (Fig. 5.7): LMEs showed no significant main or interaction effect of the explanatory variables on $Wu_{30d}$. As there were three subsequent measurement intervals throughout the dry spell (Fig. 5.3), we also calculated water uptake for each interval. Mean water uptake across all clusters was 2.2 ± 0.7 for 30 July – 6 August; 1.9 ± 0.2 for 6 – 24 August, and 1.6 ± 0.7 mm $day^{-1}$ for 24 August – 1 September. Again, no significant differences between tree species combinations or diversity levels were found employing an LME with date of measurement as a random effect.

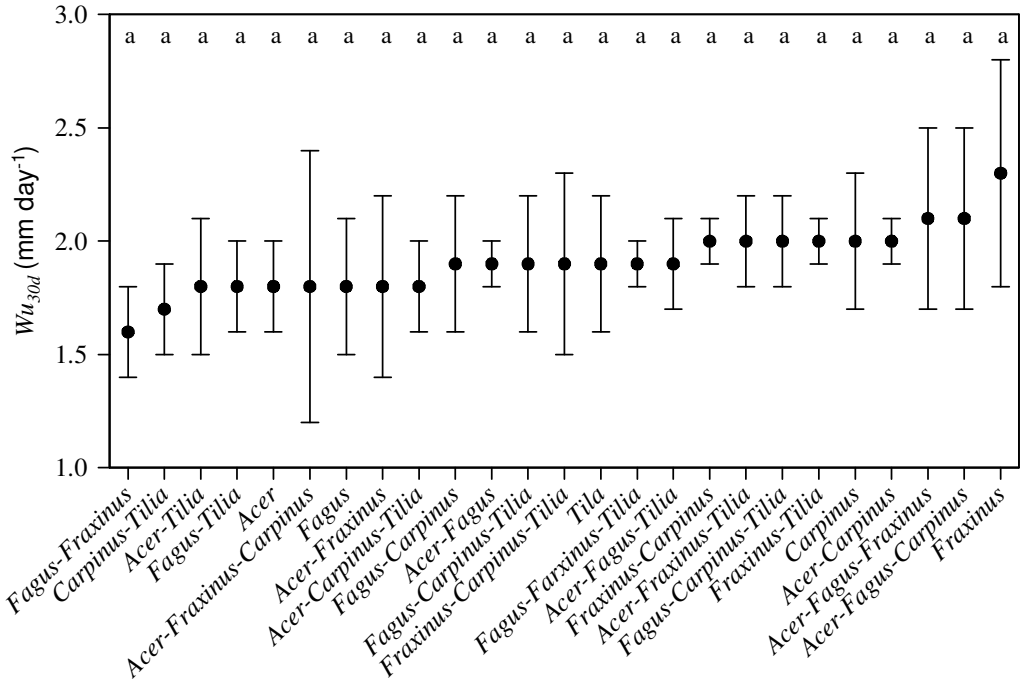


Figure 5.6: $Wu_{30d}$ for all possible species combinations of *Fagus*, *Tilia*, *Fraxinus*, *Acer* and *Carpinus* during the soil desiccation period from 30 July to 1 September 2009. Values are means ± sd (n = 4), same letters specify no significant difference between species (LME and Tukey's HSD).

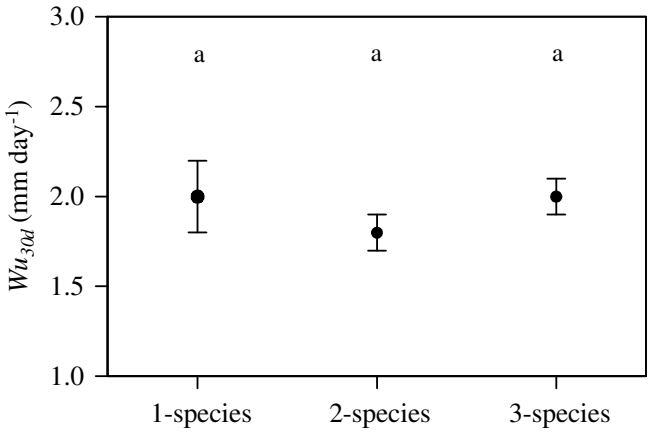


Figure 5.7: $Wu_{30d}$ grouped for the three diversity levels during the soil desiccation period from 30 July to 1 September 2009. Values are means ± sd (1-species: n = 20; 2-, 3-species: n = 40); same letters specify no significant difference between species (LME and Tukey's HSD).

Further correlation tests between $Wu_{30d}$ and selected stand structural variables from all 100 clusters showed only a slight correlation between bulk density and water use (Tab. 5.2). Still, certain stand characteristics correlated with cluster area. Average dbh as well as canopy openness

increased with increasing ground area of the clusters ($r = 0.39$, $p < 0.01$ and $r = 0.25$, $p = 0.01$, respectively).

Table 5.2: Relationship between $Wu_{30d}$ during soil desiccation period from 30 July to 1 September 2009 and selected stand structural variables on the clusters. All 100 clusters were included in the analysis (Spearman's correlation analysis).

| **Variable** | **$r^2$** | **p** |
|---|---|---|
| Canopy openness (%) | -0.18 | 0.08 |
| Bulk density (g $cm^{-3}$) | -0.21 | 0.04 |
| Clay content (%) | 0.17 | 0.09 |
| Cluster area ($m^2$) | -0.18 | 0.08 |
| Mean dbh of cluster trees (cm) | -0.18 | 0.08 |

## 5.4 Discussion

### *5.4.1 The approach*

The temporal frequency of measurement in hydrological studies is often very high considering that data can be logged automatically at almost any desired rate. At the same time, it is barely possible to establish a similar level of measurement replication on a broader spatial scale due to restrictive costs for instrumentation or logistical issues. As a result, the number of spatial replicates is often disproportionate to the frequency of sampling and it is questionable whether such data can be spatially representative. With our 100 cluster approach and 400 measurement points overall, we tried to compensate for the lack of spatial resolution at the cost of a finer temporal resolution. However, a subset of 16 intensive clusters, for which data was gathered more frequently, served to support the 100 cluster approach. During cluster selection, care was taken to ensure clusters were as homogenous as possible in terms of ground area, soil physical properties, tree height, dbh, and terrain inclination. As such, it was also not a randomized selection. Moreover, there is still uncertainty around how one can account for stemflow values in water budget calculations, as there is no understanding on how stemflow water distributes through the soil. In our approach, measurement devices were arranged along the median line between each tree pair and in the cluster centre, which made it possible for stemflow water not to be measured where the distance to the next respective stem was too far. However, as we concentrated our measurements on a period of soil water desiccation with low rainfall, the water budget was only very marginally affected by stemflow anyway.

An analysis of the relative fine root contribution at 0 - 0.2 m also showed that belowground cluster space was not exclusively occupied by roots of tree species forming the respective cluster but also by neighbouring trees outside the cluster (Jacob et al., 2012, supplemental data). However, across all clusters the target tree species contributed 84.2 ± 10.2 % to the standing fine root biomass. Single species clusters of *Carpinus* and 3-species clusters including *Fagus* and *Carpinus* appeared to be more affected by root space occupation of non-cluster tees compared to other species. In addition, the fine root biomass on 2- and 3-species clusters was not always homogenously distributed among the cluster forming tree species. As such, the identification of possible species identity effects on soil water uptake was further complicated.

We nonetheless assume that our high number of spatial replicates, which is quite unusual in ecohydrological studies, represents a special advantage of this design over others and that it may

be very helpful in unravelling possible effects of species composition and diversity. Additionally, the strong relationship between cluster water use and global radiation gave us confidence in the data.

### *5.4.2 Throughfall and stemflow*

Throughfall as the main input of water to the system under consideration was not related to species identity in the 16 clusters nor did the mixed clusters differ from the monocultures. In addition, stand structural parameters only explained *Tf* during some measurement occasions (e.g. average cluster dbh explained *Tf* during the dry spell). This finding may have several reasons: first of all, we set up our experiment to test for effects of differing diversity levels or species combinations. Thus, clusters were selected to minimize variations in ground area, tree size and tree age etc. and a lack of correlation between tree or stand structural variables and *Tf* was expected. Secondly, *Tf* is not only driven by tree architecture (leaf inclination, nature of the bark, branch angle) but also by stand structural characteristics such as stand height, crown length, and canopy roughness (Krämer and Hölscher, 2009). Consequently, it is expected that these parameters influence rainfall partitioning at a much larger scale than on the rather small tree clusters. All our study clusters were embedded in a larger mixed forest stand and possible differences between single- and mixed species stands could only have been detected at a larger scale. However, respective large scale monocultures of all tree species are not likely to be found in unmanaged mixed forests of advanced age. Thirdly, climatic conditions such as rainfall intensity and duration, wind and relative humidity which affect *Tf* (Crockford and Richardson, 2000) might additionally work unequally on diverging species. Therefore it depends very much on the nature of the respective rainfall event or the season under consideration if a diversity or species identity effect is detectable (Krämer and Hölscher, 2009). Fourthly, 3D laser scans on the clusters showed that canopy space exploration, which is highly influential on throughfall, was not influenced by species diversity (Seidel et al., in review). However, denser canopy crowns were found where *Fagus* was present, which might also partly explain why Krämer and Hölscher (2009) found decreasing *Tf* with increasing proportion of *Fagus* trees present for some of their measurement occasions. However, none of the relationships between *Tf* and tree diversity, proportions of tree species present or stand characteristics established by them at our research site were stable during different seasons or over years. Indeed, *Tf* correlated with tree diversity only for half of the seasons for which data were gathered.

Hence, we conclude that a clear relationship between *Tf* and tree diversity and *Tf* and species identity or other parameters could not be found at our site. This implies that the relationship found for dbh and *Tf* during the dry spell should only be taken as an aid to transfer *Tf* measured in a certain period from the 16 clusters to the 100 cluster approach and not as a general rule for the given stand. The second input to our system, stemflow, is of small magnitude compared to the water input to the soil via throughfall and, as our focus was on the dry spell during which precipitation was generally low, $Wu_{30d}$ was only marginally influenced by *Sf*. In summary, the water inputs to the soil were not driven by tree diversity or species identity in our study.

### *5.4.3 Soil water uptake*

Measured $Wu_{70d}$ on the 16 clusters ranged from about 2.6 to 3.5 mm day$^{-1}$ and was higher compared to values obtained for the plots with differing diversity levels at our research site based on sap flux estimates for the years 2005 and 2006 (1.1 to 2.5 mm day$^{-1}$; Gebauer et al., 2012). However, in contrast to our method, sap flux studies do not account for understory transpiration and evaporation from the topsoil and, in this case, only trees with dbh above 10 cm were included (Gebauer et al., 2012). In addition, a species specific calibration for *Fraxinus* (Herbst et al., 2007) was not applied, which leads to an underestimation of water use by *Fraxinus* trees and thus to an overall lower water use of plots with strong *Fraxinus* presence.

Calculated amounts of daily soil water uptake for the whole period from 30 July to 1 September agree well with model calculation for the adjacent plots of differing diversity levels in Hainich forest (Bittner et al., 2010). We also found positive relationships between the calculated volume of daily water uptake of the 16 clusters throughout the season and the average daily global radiation during the respective measurement intervals (Fig. 5.4), giving us further confidence in the applied water uptake calculation.

Our data did not indicate an influence of species diversity (1-, 2-, and 3-species), nor of species composition on $Wu_{30d}$ of the 100 clusters during the dry spell. Further, cluster dbh and area or the presence of any certain species had no effect on water use. Recognizing that the input of water (*Tf, Sf*) was alike for all diversity levels, water uptake by roots per unit soil volume must also have been similar. However, this result is in contrast to findings obtained in monocultures and 2-, 3- and 5-species mixtures in a Panamanian tree plantation (Kunert et al., 2012) and in advanced forest plots of two species and their mixture (Schume et al., 2004). We also tested for possible effects of the wider neighborhood on calculated water uptake on the clusters. Thus, Shannon biodiversity index was determined for a 20 m radius surrounding the center point of

each cluster (Seidel et al., in review) and correlated with water uptake. As no significant relationship was found (data not shown) we are confident that the ascertained findings remain similar even on a wider spatial resolution.

However, in figure 5.6 it can be seen, that water use of *Fraxinus* monocultures during the dry spell was at the upper end of the range of water use rates measured. Also the analysis of the 16 clusters that were monitored intensively in time showed, that the water use of *Fraxinus* was about 0.35 mm $day^{-1}$ higher compared to the mixture (marginally significant), but not significantly different from *Fagus* and *Tilia* clusters. But since the degrees of freedom used in the calculation of Tukey tests can only be approximated for LMEs (see also Bates 2006) and given the fact that the differences found are only marginally below our significance level of $p = 0.05$ this statement should be interpreted with care. Since we did not find any other indication for a diverging water uptake of mixtures compared to monocultures we suppose that the difference between *Fraxinus* and mixed clusters is based on *Fraxinus* properties rather than on specific properties of the mixture. Indeed, *Fraxinus* differs in many characteristics from other tree species. Herbst et al. (2007) mention a considerably higher magnitude of sap flux densities of *Fraxinus*, compared to diffuse-porous species with calibrated sap-flux sensors. Also a higher transpiration per unit leaf area of *Fraxinus* was reported for our area (Hölscher et al., 2003). But the detected effect could also be a result of water use of the undergrowth in *Fraxinus* clusters.

It is somewhat remarkable that the water use of the monospecific plots did only differ marginally from one another (slightly higher water use of *Fraxinus* clusters), since many authors found strongly differing hydraulic parameters and sap flux densities for the trees grown at our site (Hölscher et al., 2005; Gebauer et al., 2008; Köcher et al., 2009). Moreover, trees in our 16 clusters were shown to take up water from different soil depths when tree species were mixed and varied in dbh (Meißner et al., 2012), despite the lack of vertical fine root stratification among the species under consideration from the Hainich forest (Meinen et al., 2009b). These findings lead to the assumption that if a species-dependent water use of trees as supported by physiological measurements does exist, the spatial arrangement of different species might override such an effect (in particular belowground) and yield similar water uptake per unit soil volume among the monospecific plots and the diversity levels of the clusters. This balancing effect could not be found in the Panamanian plantation (Kunert et al., 2012), since this plantation was newly established (7 years old) and arranged in regular planting schemes.

The same would be valid if, in contrast to species identity, simple size effects of trees governed their water use ("functional convergence") which means that large trees should use more water than smaller ones, irrespective of species identity (Meinzer et al, 2005). This also implies that large trees, having a higher water use per individual, must occupy more ground area compared to smaller ones if the water uptake per unit soil volume is not affected by the size of cluster trees. In our clusters we found a positive correlation between average cluster dbh and cluster area, which could indicate spatial arrangements of trees according to their water use requirements for a given dbh. Likewise, there was a positive correlation between canopy openness and cluster area, which could additionally lead to higher amounts of throughfall input. Krämer and Hölscher (2009) found a relationship between canopy gap fraction and *Tf* ($r = 0.74$) in one out of three seasons for which gap fraction was determined. In our short measurement period however this could not be found.

Furthermore, it was stated that the understory in forests can effectively buffer differences in tree canopy transpiration (Roberts, 1983). Since both cover and species richness of the herb layer increased with tree diversity in our clusters (Vockenhuber et al., 2011), it is likely that some sort of feedback between herb and tree layer exists. Still there is much uncertainty in the estimation of the contribution of understory (evapo-) transpiration to the overall cluster water use because the density of herb layer cover varies during the vegetation period and, under prolonged desiccation, herb layer cover is diminished, because most herbaceous plants are drought sensitive. Moreover, the thickness of the litter layer was negatively related to tree species diversity/ decreasing *Fagus* abundance in our area (Mölder et al., 2008). A thick litter layer would intercept much of the throughfall but prevent water from evaporating from the soil and suppress competition for water by the undergrowth. A closed herb layer on the other hand would intercept rainfall as well, but it would also transpire water taken up from the soil.

Nevertheless, the effects of (evapo-) transpiration differences between different trees of a cluster and among trees and the cluster understory might cancel each other out. In basic terms, in mature forests with less human interference, trees with differing demands for resources as well as the herb layer of the understory might "arrange" themselves according to resource availability. Stand transpiration may therefore be more extensively controlled by other stand structural variables, such as it is not by stand species composition or species diversity in our case. This is in line with conclusions drawn by Roberts (1983), who states that forest transpiration is a rather "*conservative process*" with little variation of transpiration among (differently composed) stands.

In addition, one might also argue that besides a mere tree diversity effect, interactions between tree diversity and certain environmental conditions (e.g. rainfall intensity and duration, evaporative demand, soil water availability, etc.) are crucial. That would explain why relationships between species composition/diversity and throughfall seem to be dependent on prevalent rainfall and weather conditions (Krämer and Hölscher, 2009) and canopy transpiration only differed among diverse and less diverse stands in certain years (Gebauer et al., 2012). This is further supported by the fact that diversity effects on soil water extraction only occurred in certain periods (Krämer and Hölscher, 2010). These findings indicate that it is not only that there is no "magic effect" of biodiversity per se (Hector et al., 2000) (the characteristics of underlying species determine whether tree diversity matters or not), but that it also seems that an ecosystem needs to be subject to specific environmental conditions under which tree diversity can accomplish importance.

Furthermore, more than one characteristic or trait of a species can influence a single ecosystem process (such as water use). These traits may additionally be linked or may counteract each other: The variability of drought sensitivity (high to low: *F. sylvatica* > *A. pseudoplatanus* > *T. cordata* > *C. betulus* > *F. excelsior*) and water consumption (high to low: *F. sylvatica* > *A. pseudoplatanus* > *C. betulus* > *T. cordata* > *F. excelsior*) among tree species in Hainich (Hölscher et al., 2005; Köcher et al., 2009) reveals an almost similar behaviour of species in both parameters. It still however depends very much on the severity and duration of a given drought event, if a given species uses much water because it's a big water consumer or because it is very drought tolerant. In addition, the volume of soil water extraction of a stand is strongly dependent on the percentage mixture of drought tolerant and high water using trees, because both act on stand transpiration in differing ways under certain soil water availability. These complex relationships between traits within one species and in a mixture as well as between traits and environmental conditions were discussed in a simplified modelling exercise of water use in artificial stands of *Fagus*, *Tilia* and *Fraxinus* (Bittner et al., 2010): *Fraxinus* was parameterized to have half of the transpiration of *Fagus* under wet soil conditions (based on findings with uncalibrated sap flux sensors (Gebauer et al., 2012)). However, *Fraxinus* was also set up to maintain high transpiration at much drier soil conditions compared to *Fagus*. It was observed that at times of high potential transpiration rates accompanied by soil water depletion, modelled *Fraxinus* monocultures maintained higher water uptake rates compared to times with low evaporative demand and sufficient soil water supply. Modelled *Fagus* monocultures showed the opposite behaviour: Transpiration in wet years was higher compared to dry years, despite the lower evaporative demand during these times, since it was more sensitive to declining soil water availability. Thus

the differences in soil water uptake between modelled *Fagus* and *Fraxinus* monocultures were lower in the dry years than in the wet years. The authors conclude further that, depending on the mixture and the climatic conditions, drought tolerant species may even exert damage to drought sensitive species depending on the severity of the drought. We have confidence that no pronounced water stress occurred during the dry spell in 2009 since there was no drop in water uptake during periods of high evaporative demand (Fig. 5.4) and water uptake from the top soil layer continued throughout the whole dry spell. Therefore we believe that not "drought tolerance" but "maximum water use rate under wet soil conditions" of the trees was the trait influencing measured soil water uptake by trees here. It remains questionable whether we could have detected an influence of tree tree diversity on water uptake under more severe drought since Krämer and Hölscher (2010) found that differences in soil water extraction rates of diverse and *Fagus* dominated stands in our area disappeared as soil drought advanced.

In summary, we did not find differences in water uptake among single species clusters besides a marginally higher water use of *Fraxinus* clusters or among tree clusters of differing diversity levels throughout the vegetation period of 2009. We discuss that water use may indeed be a conservative process, that differences in tree species specific traits do not necessarily translate to neighbourhoods or stand level and that they can be compensated for by one another or by stand parameters such as herb layer and tree spatial arrangement. Furthermore, species identity or diversity effects on stand water use may only arise under certain environmental conditions. Thus, considering effects of tree tree diversity on stand water use exclusively may not be an appropriate simplification of the complex network of interactions between species traits, stand properties and environmental conditions that have varying influence on stand water use, both in space and time.

## 5.5 Acknowledgements

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# Chapter 6

## SYNTHESIS

# 6. Synthesis

Recently the establishment of mixed and broad-leaved forest is promoted and forest transformation reduces monocultural spruce stands in central Europe. Forest agencies and enterprises foster species which are better suited to given site conditions (Röhrig et al., 2006). The aim of such efforts is to achieve supposedly higher stability against storms and diseases, and economical assurances. Especially with regard to projected climate change scenarios, there is a need to investigate the effect of tree species diversity and certain species admixtures of potential natural occurring tree species on tree water use in forest areas.

The main objective of this study was to investigate the role of tree species and their mixture on relative and absolute water uptake in the temperate mixed Hainich forest. The selected target species, *Fagus sylvatica, Tilia* sp.*, Fraxinus excelsior, Acer pseudoplatanus, and Carpinus betulus* belong to the potential natural vegetation of a beech forest community (Ellenberg and Leuschner, 2010). This study set out to investigate possible effects of different species and their mixture, as well as species diversity on water uptake in small tree clusters during a soil desiccation period.

This thesis consists of three parts:

**1.)** One aim was to identify the vertical partitioning soil water uptake among *Fagus sylvatica*, *Tilia* sp. and *Fraxinus excelsior* in mono and mixed clusters by analysing the natural abundance of water stable isotopes ($^{2}H$ and $^{18}O$). However, beforehand it was tested how well the application of $\delta^{2}H$ and $\delta^{18}O$ is suitable to stratify tree water uptake depth under the given soil physical and chemical conditions and what restrictions might exist with regard to soil water, clay and soil calcium carbonate content.

**2.)** Based on findings from the first study, the natural abundance of $^{2}H$ was than used to identify the depth of soil water uptake of the individual tree species and their mixture, during a summer soil desiccation period. It was hypothesized that soil water uptake is different among species, resulting in uptake complementarity in the species mixture, due to niche occupation. Secondly, it was assumed that the uptake depth scales with tree size. Thus, the isotopic composition of xylem and soil water samples was analyzed and compared to identify the partitioning of soil water uptake and tree size effects, respectively.

**3.)** The third study focused on the effect of tree species common in this forest ecosystem, different species combination, species diversity levels, stand structural and environmental variables on the volume of daily soil water uptake in small tree groups (clusters). Observations

concentrate in particular, on a summer soil desiccation period when species functional traits or species diversity were supposed to be most important, due to shortage of water resources. The hypothesis was that water uptake in tree clusters increases with increasing species diversity.

The study design with 100 clusters and 25 different tree species combinations was a consequential follow-up to the plot design used in previous studies of the umbrella project in the Hainich forest. The main purpose of this study design was to enable a separation between diversity and species effects, with the possibility to further include tree structural variables into the analysis. The aim was also to increase the degree of spatial resolution, which however came at the cost of a finer temporal resolution. Therefore the subset of 16 intensive clusters, for which data was gathered more frequently, served to support the 100 cluster approach. In order to accomplish a focus on a larger number of species, in single and mixed neighborhood combinations, the trade-off between spatial and timely resolution could be justified.

### 6.1 Impact of soil water, clay and carbonate content on stable isotope experiments

During a series of laboratory experiments on the effect of soil water, clay and carbonate content on the soil water isotopic composition it was discovered that all three factors have a significant impact. Differences in water and clay content significantly altered the isotopic composition of both $\delta^2H$ and $\delta^{18}O$. Water extracted from soil samples was depleted in both isotopes with the effect being strongest at low water and high clay content. Further, the presence of carbonates in the soil significantly affected the isotopic composition of $\delta^{18}O$ whereas $\delta^2H$ was not affected.

The findings from the first study imply that the application of water stable isotopes (natural abundance) can be constrained when carbonate content in the soil is high and estimation of tree water uptake depth based on $\delta^{18}O$ analysis could be incorrect under such conditions. When carbonate content is high the analysis of $\delta^2H$ appears to be preferable above $\delta^{18}O$. However, in general it can be advised that both $\delta^2H$ and $\delta^{18}O$ should always be analyzed together.

Although the data from this study suggest that shifts of $\delta^2H$ and $\delta^{18}O$ in soil water can be caused by soil related processes, there is also the possibility that isotopic shifts may occur during transport through roots or stem xylem of trees due to metabolic processes. Increasing evidence for a probable post-photosynthetic exchange of oxygen and hydrogen between carbohydrates and xylem water during cellulose formation in the tree trunk has already been found in several experiments (Yakir et al., 1990; Hill et al., 1995; Farquhar et al., 1998) and is discussed in some

modeling approaches (Roden et al., 2000; Waterhouse et al., 2002). Possible alteration of $\delta^2H$ or $\delta^{18}O$ signatures in xylem water of trees might therefore take place during the cellulose and/or lignin synthesis, where an exchange with xylem water could occur (Barbour et al., 2001). In a recent study this was specifically discussed for $^{18}O$ in tree rings of *Fagus sylvatica* (Offermann et al., 2011). It was stated that an exchange might occur during tree ring formation or starch remobilization, with the effect of $\delta^{18}O$ being depleted in the tree phloem, which *vice versa* could imply an isotopic enrichment of $\delta^{18}O$ in the xylem water. It is also possible that xylem water isotopic signatures in large trees can be influenced by water removed from storage in their voluminous non-hydroactive xylem (Meinzer et al., 1999). The isotopic composition of stored water would be expected to reflect that of previous precipitation under moist conditions rather than that of water near the soil surface enriched via evaporative fractionation.

Also, in this study, observations were only conducted on a small part of existing soil physicochemical properties, thus it might be possible that there are still other factors with the potential to alter the composition of $\delta^2H$ and/ or $\delta^{18}O$ in soil water (e.g. exchangeable O-bonded hydrogen in soils with high organic matter content). Hence, there might still be more unidentified factors in plant-soil-water relations that could affect the isotopic composition of water stable isotopes and thereby hinder the estimation of plant water uptake depth via $\delta^2H$ and $\delta^{18}O$ natural abundance.

### 6.2 Depth of water uptake and soil water partitioning

By analyzing isotope composition of $^2H$ ($\delta$‰) in stem xylem and soil water showed that *Fagus sylvatica*, *Tilia* sp. and *Fraxinus excelsior* had different patterns of relative water uptake depth in monospecific tree groups, varying in the lower soil depth intervals (0.3-0.5 and 0.5-0.7 m). This pattern was partially different in mixed groups; *Tilia* and *Fraxinus* showed different patterns, whereas *Fagus* was similar. *Fraxinus* showed a relative water uptake that was distributed over a wider range of soil depth intervals compared to the other species in the mixture. This could indicate a complemental water resource use due to a higher flexibility of *Fraxinus*. In mixed clusters the isotopic composition of the trees' main water uptake depth was also positively correlated with dbh (less negative δ‰ with increasing dbh), irrespective of species identity. In connection with a strong isotopic gradient in the soil profile (more negative δ‰ with increasing soil depth), it implies that larger trees withdrew more water from shallower soil depth in the mixed clusters than smaller trees. However, neither single, nor mixed species clusters varied in absolute water uptake (mm $day^{-1}$) during the soil desiccation period.

This also leads to the conclusion that with respect to soil water partitioning, species diversity effects alone may probably be covered by combinations of complex interactions between species traits, stand properties and environmental conditions that have varying influence on stand water use, both in space and time. Still species mixture and stand properties combined obviously caused a complementarity effect, altering the vertical partitioning of tree water uptake depth, which was not found on the single species clusters. Thus it can be assumed that water uptake complementarity was not just caused by one species or certain dbh variation but was a result of a variation in both species mixture and diameter variation.

As a result of the method test in study one of this thesis, only $^{2}H$ was used to identify depth of soil water uptake in the following. Still, for the sake of completion, vertical partitioning of water uptake was analyzed on the basis of the $\delta^{18}O$ isotopic composition in the isotope mixing model. Additionally, the isotopic composition of main water uptake depth of each target tree was compared to its respective diameter (dbh). Although $\delta^{2}H$ and $\delta^{18}O$ were indicating different water uptake depths when analyzed independently by direct inference and the model calculation, the relationship of tree water uptake depth and dbh was similar (Fig. 6.1).

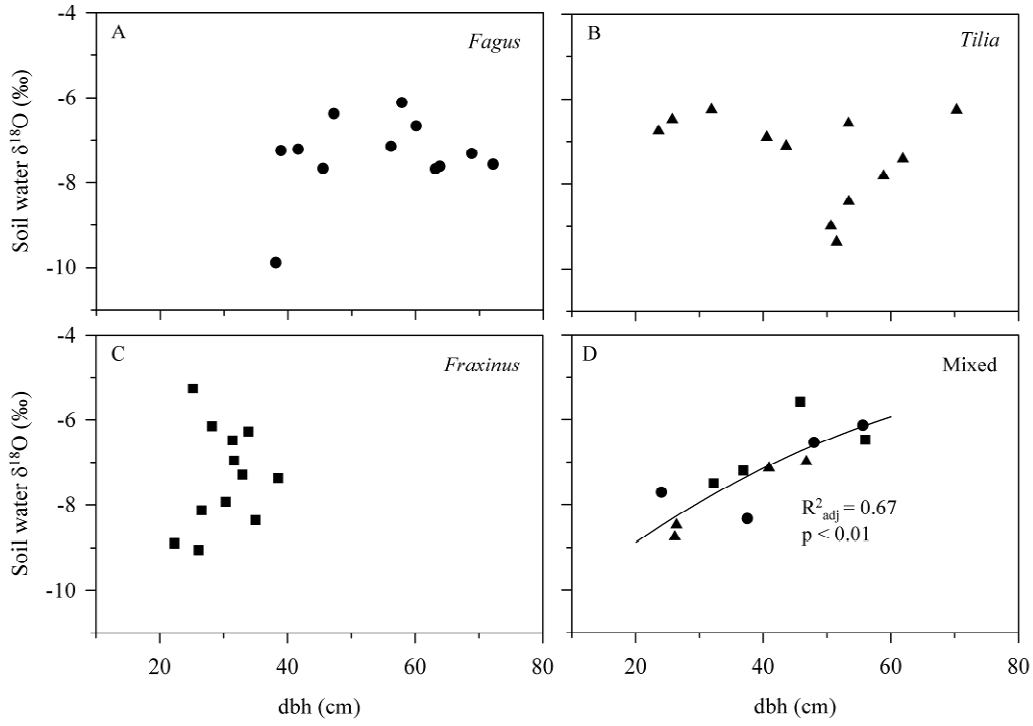



Figure 6.1: Soil water $\delta^{18}O$ of the main water uptake depth per tree in relation to diameter at breast height (dbh) on single and mixed species tree clusters.

On the mixed clusters, trees with larger dbh withdrew water from shallower soil depth compared to trees with smaller dbh, irrespective of tree species. Likewise, no such effect could be observed on the monospecific clusters. The relationship between tree dbh and $\delta^{18}O$ isotopic composition is comparable to results illustrated in chapter four for $\delta^{2}H$ (Fig. 4.6). A direct comparison, of the relationships between tree diameter and isotopic composition of main water uptake for $\delta^{18}O$ and $\delta^{2}H$ also shows the difference in water uptake depths, as indicated by the two isotopes (Fig. 6.2). Due to the isotopic gradient in the soil a more negative isotope signature corresponds with a deeper soil depth.

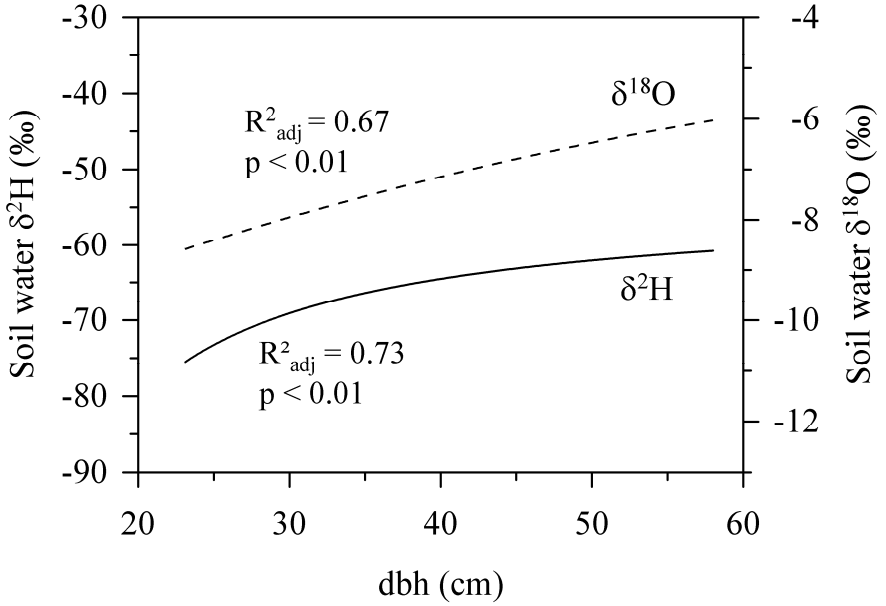



Figure 6.2: Regression line of soil water $\delta^{18}O$ and $\delta^{2}H$ isotopic composition of the main water uptake depth per tree in relation to diameter at breast height (dbh) on mixed species tree clusters.

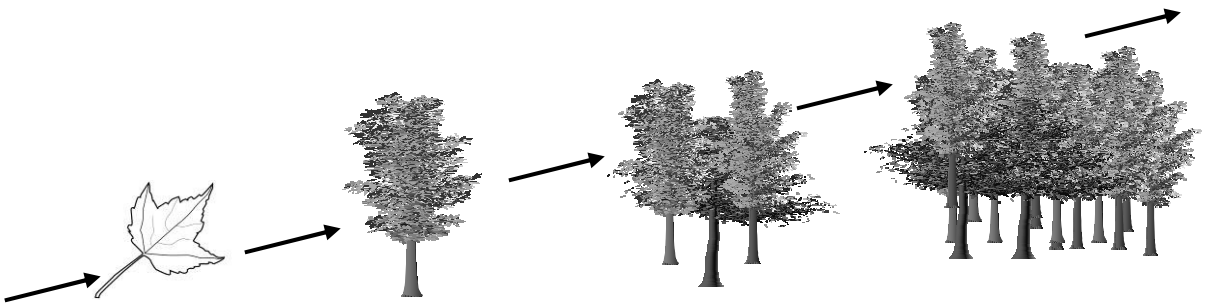
### 6.3 Volume of soil water uptake

In this study no differences in water uptake among certain tree species, different species combinations of species or diversity levels were found during the soil desiccation period. Throughfall as the main input of water to the system was also not related to species identity on the 16 clusters nor did the mixed clusters differ from the monocultures. However, calculated average water uptake (mm $day^{-1}$) on the 16 clusters was closely related to average daily global radiation throughout the vegetation period. Though, said relationship (linear regression) did neither differ in slope nor in intercept among the three monocultures of *Fagus*, *Tilia* and *Fraxinus* or in the mixture. Likewise, testing for presence or absence of the five observed species resulted in no effect on daily water uptake, either.

As conclusion, water input and water use is predominantly governed by stand structural characteristics (e.g. dbh, height, canopy openness, cluster area) and climatic conditions (e.g. global radiation, rainfall intensity and duration), species differences that potentially might influence water evapotranspiration are assumed to be of less importance or might actually even cancel each other out (e.g. litter and herb/ understory layer). In mature forests with less human interference, trees with differing demands for resources might have arranged themselves according to the resources available and thus creating relatively balanced tree neighborhood relationships. Still, at an early successional stage of forest growth species traits, their composition and species diversity may have a stronger impact, but at least during a late successional stage it seems to be of minor importance.

### 6.4 Scaling hydrological processes with regard to species traits and diversity

Hence, a multitude of parameters have the potential to affect the hydrological cycle in forests, and can serve as main controls for water fluxes in plant-water-atmosphere interactions at different scales (Fig. 6.3), some of which might only be of significance on a certain spatial and timely scale, some might actually even each other out or combine.



| **Leaf level** | **Tree level** | **Cluster level (tree group)** | **Stand level (50 x 50 m plot)** |
|---|---|---|---|
| - Stomatal conductance<br>- Leaf boundary layer conductance<br>- Leaf roughness<br>- Leaf inclination<br>- Leaf water status | - Leaf area<br>- Crown shape (leaf distribution)<br>- Height<br>- Tree diameter<br>- Stem water storage capacity<br>- Bark roughness<br>- Root conductivity, biomass/ distribution | - Neighbourhood<br>- Tree distance<br>- Leaf area index<br>- Openness<br>- Undergrowth<br>- Litter layer<br>- Competition<br>- Facilitation | - Atmospheric boundary layer<br>- Canopy roughness<br>- Surface inclination<br>- Soil type |

Figure 6.3: Conceptual model of traits with effect on plant-water-atmosphere interactions from leaf to whole trees to tree clusters (groups of three trees) to forest stands, with emphasis on the hydrological main controls on water fluxes that act at the respective scale.

In this section of the spatial scale, individual traits play an important role, such as species different functional (morpho-physio-phenological) traits that affect species performance and performance traits that contribute directly to fitness of an individual (Violle et al., 2007) or a group. Though, traits that influence water input or output at leaf or whole tree level might not necessarily translate to tree group level (cluster). On the other hand, species diversity could maintain a mechanistic influence at stand level, under specific environmental conditions which might be of less importance on a smaller spatial scale. Some species or stand traits may also be correlated or linked by feedback mechanisms. Those might add or even each other out at different scales.

The importance of species diversity for forest stand productivity still appears uncertain and is probably of less importance compared to prevalent site conditions or the age of a forest stand (Pretzsch, 2005) and thus it seems to be impossible to completely separate the impact of species diversity from other biotic and environmental factors (Vila et al., 2005; 2013). It can also be assumed that productivity is related to water use in a forest stand (Law et al., 2002), but as with productivity and also independent from productivity there are other factors that can affect tree water use, such as species functional and performance traits or stand structural components. It is therefore highly questionable whether functional effects of tree species diversity can be addressed to influence water dynamics or plant-water relations in a forest per se. This said, species diversity might play a functional role together with e.g. stand structural components but it is very difficult to predict which effects and to what magnitude they occur under changing environmental conditions and at different spatial scales. Thus, results from this study imply that, in particular at small neighborhood scale, structural characteristics together with spatial arrangements can cover effects of species diversity on soil water uptake (e.g. compensation by feedback mechanisms), which might be visible during certain environmental conditions and at a different spatial scale.

Since 2001, a multitude of studies have been conducted in the Hainich forest with focus on the five main tree species (*Fagus sylvatica, Tilia* sp.*, Fraxinus excelsior, Acer pseudoplatanus, and Carpinus betulus*), their mixture and biodiversity in general. Many of the findings are directly or indirectly related to the water dynamics of this forest ecosystem with various effects on soil water budget at different timely and spatial scales. In order to give an overview of said studies, parameters observed and their results, a selection of studies from the first and second project phase of the umbrella project and other related studies were compiled in table 6.1.

Studies are listed according to the scale on which any given parameter was observed; beginning at leaf level as the smallest unit, followed by tree level, neighborhood relation observed in tree clusters (tree groups) and forest stand level. Tree species diversity effects (SDE) as well as the effects that can be attributed to certain species traits (SE) were separated if the respective study allowed for such a classification. Species diversity in tree groups ranged from one to triple species composition. On stand level diversity was indicated using the Shannon diversity index or diversity levels I – III (one, triple and five species composition).

This study compilation shows that the majority of effects on components of the forest hydrological cycle can be attributed to SE instead of SDE. Moreover, in most cases when diversity was found to influence certain parameters of the water budget, it was paralleled by a *Fagus sylvatica* gradient and therefore a clear separation between diversity and species effect may not be possible. This implies that functional or performance traits measured at individual tree level were more important than biodiversity. The tree species that differed most obviously in their functional traits (e.g. leaf, crown, and bark structure, leaf chemistry) and performance traits (e.g. transpiration rates, stemflow) were *Fagus sylvatica* and *Fraxinus excelsior* and the latter showing lower drought sensitivity.

Table 6.1: Compilation of studies conducted in the umbrella project, measured parameters, such as throughfall (Tf), canopy and understory evapotranspiration (ET), and evaporation from the soil ($E_{soil}$), results and relevance for the hydrological cycle of the forest area.

| Scale | Parameter | Year of Observation | SDE | SE | Result | Conclusion | Reference | |
|---|---|---|---|---|---|---|---|---|
| Leaf level | Stomatal conductance | 2001 | | x | Highest in *Fraxinus* and comparable low in *Acer*, *Tilia*, *Carpinus* | Higher transpiration per unit leaf area under optimal light/water conditions of *Fraxinus* | Hölscher et al., 2005 | |
| Tree level | Stemflow | 2007 | | x | High to low: *Fagus*, *Carpinus* > *Acer*, *Tilia*, *Fraxinus*<br>Stemflow amount highly dependent on $P_{gros}$ | *Fagus* and *Carpinus* may channel water to their root system | Krämer and Hölscher, 2009 | |
| Tree level | Drought sensitivity | 2006 | | x | Drought sensitivity high to low:<br>*Fagus* > *Acer* > *Tilia* > *Carpinus* > *Fraxinus* | Stand water stress is sensitive to species composition and abundance<br>*Fraxinus* may increase water stress for drought sensitive species | Hölscher et al., 2005;<br>Köcher et al., 2009 | * |
| Tree level | Root axial hydraulic conductivity | 2008 | | x | Conductivity high to low:<br>*Fagus* > *Acer*, *Tilia*, *Carpinus* > *Fraxinus* | Root water uptake is not only controlled by root biomass and rooting patterns | Köcher et al., 2012 | |
| Tree level | Transpiration | 2009 | | x | $T_{act}/T_{pot}$ high to low (high evaporative demand, wet soil)<br>*Fagus*, *Tilia* > *Fraxinus*<br>*Fraxinus* did not reduce transpiration under dry soil | Contribution of *Fraxinus* to stand transpiration is low at high water availability and high at low water availability | Bittner et al., 2012a | |
| Tree group | Litter quality | 2005 - 2007 | | x | Lower decomposition rate of *Fagus* litter compared to other species | Increasing *Fagus* abundance decreases $E_{soil}$ and ET of understory vegetation and may also decrease Tf infiltration | Jacob et al., 2010a | |
| Tree group | Fine root biomass topsoil | 2008 | | x | Fine root biomass in monoculture high to low:<br>*Fraxinus*, *Fagus* > *Carpinus*, *Tilia*, *Acer*<br>*Fraxinus* roots overrepresented in mixtures | *Fraxinus* may benefit from small amounts of rainfall reaching only the topsoil during drought | Jacob et al., 2012 | |
| Tree group | Crown space occupation | 2008/ 2009 | | x | Independent of tree species diversity;<br>Increased with *Fagus* presence | *Fagus* presence decreases Tf and $ET_{pot}$ of the understory | Seidel et al., in review | |
| Tree group | Herb layer cover and species richness | 2009 | x | | Increased both with tree species diversity | Tree species diversity increases understory ET | Vockenhuber et al., 2011 | ** |
| Tree group | Volume of water uptake | 2009 | | | Independent of tree species diversity and species identity (during desiccation period) | Species traits effects on water uptake must be compensated for by one another or by other stand parameters (herb layer, tree spatial arrangement) | Meißner et al., in review | |
| Tree group | Vertical soil water uptake distribution | 2009 | x | | Size (dbh) dependent water uptake depth in mixtures | Complementarity in water uptake depth when species were mixed and dbh varied | Meißner et al.,2012 | |
| Stand level | Herb layer biomass | 2005 - 2007 | x | | Increased with tree species diversity (Shannon Index) | Tree species diversity increases understory ET | Mölder et al., 2008a; b | ** |
| Stand level | Litter production | 2005 | | | No correlation with tree species diversity (Shannon Index) | Similar LAI among stands | Guckland et al., 2009 | |
| Stand level | Litter layer thickness | 2005 - 2007 | x | | Negative correlation with tree species diversity (Shannon Index) | Increasing *Fagus* abundance decreases $E_{soil}$ and ET of understory vegetation and may also decrease Tf infiltration | Mölder et al., 2008a; b | ** |
| Stand level | Wood biomass productivity | 2005 - 2007 | | x | No to negative correlation with diversity (Shannon index); *Fagus* and *Fraxinus* > *Tilia*, *Carpinus* and *Acer* | No (or slightly negative) effect of diversity on productivity coupled to stand water use | Jacob et al, 2010b | |
| Stand level | Leaf area index, leaf biomass productivity | 2005 - 2007 | | x | No correlation with tree species diversity (Shannon Index); productivity: *Tilia*, *Carpinus* and *Acer* > *Fagus* and *Fraxinus* | Effect of LAI on canopy and understory evapotranspiration and soil evaporation is similar; No effect of diversity on productivity coupled to water use | Jacob et al, 2010b | |
| Stand level | Foliation period | 2005 | | x | *Acer*, *Carpinus*, *Fraxinus*: 5 months; *Tilia*: 5.5 months; *Fagus*: 6 months | Increasing average foliage duration of a stand increases annual T and decreases Tf and ET from understory | Bittner et al., 2010 | |
| Stand level | Fine root biomass and distribution | 2005/ 2006 | | | No difference among species and tree species diversity levels (0-40 cm) | No complementarity in vertical rooting patterns | Meinen et al., 2009a | |
| Stand level | Throughfall | 2005 - 2007 | x | | Positive correlation with tree species diversity (Shannon Index) in summer 2005, autumn 2006, and summer 2007 | Increased water input (Tf) dependent on climatic conditions; no clear effect of tree species diversity | Krämer and Hölscher, 2009 | ** |
| Stand level | Soil water extraction | 2006 | x | | Positive correlation with tree species diversity (Shannon Index) at the onset of a desiccation period (0.0-0.25 m soil depth) | Species trait or mixture effect on soil water uptake lead to faster exhaustion of soil water storage | Krämer and Hölscher, 2010 | ** |
| Stand level (3 of 12 plots) | Canopy transpiration | 2005/ 2006 | x | | Increased with tree species diversity (3 levels) at ample water availability (2005); no differences during soil desiccation period (2006) | Effect of species diversity (or identity) on canopy transpiration depends on water availability | Gebauer et al., 2012 | * |
| Stand level | Stand water budget (model approach) | 2005 - 2007 | | | Difference in annual (E+T+I) were larger among different years as among tree species diversity levels | Differences in soil water uptake between modeled *Fagus* and *Fraxinus* monocultures were lower in the dry years than in the wet years | Bittner et al., 2010 | * |
| Stand level | Stand water budget (hypothetical species composition simulation) | 2003 and 2005 - 2007 | | x | T of *Fraxinus* monocultures increased with decreasing $P_{gros}$<br>T of *Fagus* monocultures decreased with decreasing $P_{gros}$<br>T of *Tilia* monocultures were similar at all levels of $P_{gros}$ | Differences in soil water uptake between modeled *Fagus* and *Fraxinus* monocultures were lower in the dry years than in the wet years | Bittner et al., 2010 | * |

* species specific calibration for ring-porous trees (*Fraxinus*), as proposed by Herbst et al. (2007) was not applied, which leads to an underestimation of water use by *Fraxinus* trees.
** effect is probably caused by variation in *Fagus* abundance.

However, at the given successional stage of the forest stand, a general effect of species diversity on the water cycle at neighborhood or stand level can not be assumed and is more dependent on prevalent environmental condition. Thus, corroborating with Roberts (1983), that forest transpiration is a rather "conservative process" with little variation of transpiration among (differently composed) stands.

If considering this accumulation of results with respect to the context of ongoing forest transition in central European forestry and projected climate change scenarios, a crucial finding (species effect or performance trait) appears to be the difference in drought tolerance among the observed tree species. Diffuse-porous *Fagus* were more drought sensitive compared to ring-porous *Fraxinus* trees (Hölscher, 2005; Köcher et al., 2009). It indicates that *Fraxinus* could be more compatible compared to *Fagus* considering more pronounced dry periods in the long run and might generally result in increased dominance of ring-porous species, which are not necessary climax species (e.g. *Fraxinus* and *Quercus*) (Meinzer et al., 2013).

Above-ground biomass productivity in this mature temperate deciduous forest was found to be relatively constant for different levels of tree species diversity and over subsequent years but species differences were found in terms of leaf and stem wood productivity, under sufficient water availability (Jacobs et al., 2010). *Fagus* and *Fraxinus* exceeded co-occurring species in stem wood productivity but showed less leaf biomass productivity. As such, productivity is much more controlled by environmental and edaphic factors as well as species traits than dependent on tree species diversity (Jacobs et al., 2010). Thus, in less water limited areas biomass productivity might increase in the medium term, due to temperature increase and higher air $CO_2$ content (Lindner et al., 2010), irrespective of tree species. In the long run under continuing temperature increase, net primary production might depend more on drought resistance of tree species.

Though, when considering water use in the forest in terms of groundwater recharge, species diversity and species mixture seem to have only little effect, at least at the given successional stage of the Hainich. As to that the findings presented in this dissertation were that complemental water resource use among mixed tree species was only found with regard to relative water uptake depth and was also related to dbh. However, there was no hint that a more efficient water use might be related to an exploitation of soil water resources in a mixed forest during soil desiccation. It appears that biodiversity alone is probably not an appropriate measure to estimate the performance of a forest ecosystem, without also considering its tree species traits at certain successional stages and under given environmental conditions. Thus it would be important to get a more detailed insight into the mechanistic effect of species traits on water dynamics in a forest under varying environmental conditions and at different successional stages.

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# DECLARATION OF HONOR

I hereby declare that I am the sole author of this dissertation entitled 'Tree water uptake partitioning and water use rates in a temperate mixed forest' and that all references and data sources used have been acknowledged as such. I further declare that this work has never been submitted in any form as part of other dissertation procedures.

Göttingen, June 2013 _____

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