Technische Universität München

Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt

Lehrstuhl für Ökophysiologie der Pflanzen

Hydraulic Redistribution – Quantification and mechanisms of the passive water reallocation in Central European tree species

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Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Forstwissenschaften

genehmigten Dissertation.

Vorsitzender: Prof. Dr. Johannes Kollmann

Prüfer der Dissertation:
1. apl. Prof. Dr. Thorsten Grams
2. Prof. Dr. Dr. Hans Pretzsch
3. Prof. Dr. Taryn Bauerle

Die Dissertation wurde am 11. 03. 2020 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 20. 07. 2020 angenommen.

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Abbreviation List

CMN	Common Mycorrhizal Network
CRDS	Cavity Ring Down Spectrometry
DP	Plant in the Dry Pot
HL	Hydraulic Lift
HR	Hydraulic Redistribution
IRMS	Isotope Ratio Mass Spectrometry
K_{act}	Native Hydraulic Conductance
K_{max}	Maximum Hydraulic Conductance
k_s	Hydraulic Conductivity
k_{sa}	Native Hydraulic Conductivity
k_{sm}	Maximum Hydraulic Conductivity
LAT	Laser Ablation Tomography
MO	Micro Organism
MP	Plant in the Moist Pot
PAR	Photosynthetically Active Radiation
PLC	Percent Loss of Conductivity
PPFD	Photosynthetically Active Photon Flux Density
rH	Relative Humidity
SRP	Split Root Plant
SRS	Split Root System
SWC	Soil Water Content
TDR	Time Domain Reflectometry
VPD	Vapor Pressure Deficit
VSMOW	Vienna Standard Mean Ocean Water
ψ	Water potential
ψ_{soil}	Soil Water Potential

Summary

Hydraulic Redistribution (HR) describes the passive flux of water through plants following a moisture gradient. In the soil, plants take up water in layers with high water potentials (ψ) and redistribute it towards layers with low ψ via reverse flow, when transpiration is virtually absent, i.e. usually during nighttime. Water may then be released into dry soils and can also be available to neighbor plants. Several studies have described HR across multiple species among different environmental conditions from hot and dry to rather mesic conditions. However, despite showing that it happened, HR has hardly been quantified, especially for temperate Central European species. Additionally, mechanisms that determine HR and their influence on HR water quantity are not clear. As HR is among the effects suggested to potentially enhance forest stability under predicted future climate conditions, it becomes crucial to estimate the amounts that are redistributed by tree species. Additionally, driving factors of the effect have to be determined. In this thesis, HR mediated by six common tree species in Central Europe was determined, amounts of redistributed water were quantified and HR driving factors assessed under controlled conditions. Beside potential 'external', environmental HR driving factors, a focus was set on possible plant 'internal' factors affecting the amount of HR water. As very few studies have approached root anatomical parameters yet, their influence on HR is basically unknown. The main research questions were:

1. Does HR occur in Central European tree species and how much water is redistributed?
2. Are drought stressed temperate tree species supported with water redistributed by neighboring trees?
3. Is the 'external' environmental factor ψ gradient and are the plant 'internal' factors root hydraulic conductivity (k_s) and root conduit diameter positively correlated with the amount of water Central European tree species redistribute?
4. Are conduit sizes equal throughout a tree's root system and is reverse k_s similar to forward flow in roots?

The questions were tested in controlled environments in a greenhouse and growth chambers, respectively. Saplings were planted in split-root systems (SRS), where one split-root plant (SRP) had its roots equally split between two pots, with an additional tree in each pot. The study plants were chosen to cover a range of different conduit diameters and therefore hydraulic conductivities. Two conifer- (*Picea abies* & *Pseudotsuga menziesii*), two diffuse-porous- (*Acer pseudoplatanus* & *Fagus sylvatica*) and two ring-porous species (*Castanea sativa* & *Quercus robur*) were chosen. Different ψ gradients were established between both pots of the SRS and HR was quantified via deuterium labeling. All species redistributed water within their root systems (0.17 ± 0.02 ml per night) and released fractions into the rhizosphere soil (0.26 ± 0.06 ml per night). Redistribution of water reduced percent loss of conductivity (PLC) of the SRP and contributed between 26 – 60 % to tissue and transpiration water of drought stressed neighbors (1.63 ± 0.31 ml per night). The ψ gradient, conduit diameters, but mostly k_s of root xylem were significantly positively correlated with the amount of redistributed water within SRPs and in the soil. However, for release into the soil, the coefficients of determination were smaller. Therefore, additional parameters as root-soil contact, bark thickness or osmotic potential might be relevant factors for HR water release into the soil. For the amount of HR water taken up by drought stressed neighbors, root characteristics showed significant correlations. Especially root length and number of root tips of the drought stressed tree were significantly positively correlated with HR water uptake. Direct root contact or common mycorrhizal networks (CMN) between the trees may be additional important driving factors. Opposing to the expectation, redistribution of water could be limited by root architecture, as conduits became significantly smaller towards primary roots, potentially reducing reverse flow. In fact, reverse k_s was reduced by c. 40% compared to forward flow in angiosperm species.

Hydraulic redistribution displayed an important water source for all studied species under drought. It could become more prominent in the future when anticipated increased summer drought periods lead to increased ψ gradients in the soil and thus to higher amounts of redistributed water. To establish more stable forests under future climate, trees with high potential for HR (e.g. species that retain high k_s under drought with a deep root system) could be planted and used to increase stand water availability. Drought stressed neighbors substantially benefitted from HR, suggesting that the effect indeed enhances stability of temperate forests in the future.

Zusammenfassung

Hydraulic Redistribution (HR) beschreibt die passive Umverteilung von Wasser durch Pflanzen entlang eines Feuchtegradienten. Pflanzen nehmen Wasser im Boden in Bereichen hohen Wasserpotenzials (ψ) auf und verteilen es in Bereiche niedrigen ψ um. Dies geschieht überwiegend nachts, wenn keine Transpiration stattfindet. Das umverteilte Wasser kann dann in den Rhizosphären-Boden abgegeben und von Nachbarpflanzen aufgenommen werden. Viele Studien haben HR bei unterschiedlichen Pflanzen und unter verschiedenen Klimabedingungen nachgewiesen, sowohl unter sehr trockenen wie auch gemäßigten Verhältnissen. Neben einem generellen Nachweis fehlen aber noch Studien, insbesondere bei mitteleuropäischen Baumarten, welche die Menge des umverteilten Wassers quantifizieren. Hinzu kommt, dass die Mechanismen die HR antreiben und deren Einfluss auf die Menge des umverteilten Wassers weitgehend unbekannt sind. Nachdem HR als ein mögliches Puzzlestück gilt, das künftigen Wäldern unter vorhergesagter erhöhter Sommertrockenheit zusätzliche Stabilität verleihen könnte, ist es immanent wichtig, die Mengen des durch verschiedene Baumarten umverteilten Wassers zu quantifizieren. Außerdem müssen Faktoren, die HR antreiben oder limitieren, erkannt und eingeordnet werden. In der vorliegenden Arbeit wurde HR von sechs häufig in Mitteleuropa anzutreffenden Baumarten untersucht, und die Menge umverteilten Wassers unter kontrollierten Bedingungen quantifiziert. Zudem wurde der Einfluss möglicher treibender Faktoren erfasst: Neben ‚externen', umweltbedingten Faktoren, lag der Fokus auf Pflanzen-‚internen' Mechanismen, insbesondere da bisher kaum Studien zu wurzelanatomischen Aspekten vorliegen. Deren Einfluss auf HR ist dementsprechend unklar. Die wichtigsten Fragestellungen der Arbeit sind:

1. Kommt HR bei mitteleuropäischen Baumarten vor und wie viel Wasser wird umverteilt?
2. Profitieren benachbarte, trockengestresste Bäume vom umverteilten Wasser?
3. Sind sowohl der ‚externe' Faktor ψ-Gradient als auch die ‚internen' Faktoren hydraulische Leitfähigkeit (k_s) und Gefäß-Durchmesser der Wurzel positiv mit der Menge umverteilten Wassers korreliert?
4. Sind die Gefäß-Durchmesser in primären und sekundären Wurzeln gleich? Gibt es einen Unterschied zwischen „normalem", vorwärts-gerichtetem und rückwärts-gerichtetem Wasserfluss in Wurzeln?

Die Experimente wurden unter kontrollierten Bedingungen im Gewächshaus und Klimakammern durchgeführt. Sprösslinge der untersuchten Baumarten wurden in Split-Root Systeme (SRS) gepflanzt, bei denen das Wurzelsystem einer Split-Root Pflanze (SRP) gleichmäßig auf zwei Pflanztöpfe verteilt war. In beiden Töpfen war je ein weiteres Bäumchen gepflanzt. Um ein breites Spektrum an Gefäß-Durchmessern im Xylem und k_s abzudecken, wurden Koniferen (*Picea abies* & *Pseudotsuga menziesii*), zerstreut-porige (*Acer pseudoplatanus* & *Fagus sylvatica*) und ringporige Gehölze (*Castanea sativa* & *Quercus robur*) untersucht. Zwischen den Töpfen wurden unterschiedliche ψ-Gradienten angelegt und HR mittels Deuterium Markierung quantifiziert. Die SRPs aller untersuchten Arten verteilten Wasser über ihr Wurzelsystem um (0,17 ± 0,02 ml pro Nacht) und gaben dieses teilweise in den Rhizosphären-Boden ab (0,26 ± 0,06 ml pro Nacht). Durch HR wurde die Embolie-Bildung in SRPs verringert, und das umverteilte Wasser machte 26-60 % des Wassers in Wurzeln, Stamm und Transpiration der benachbarten Bäume im trockenen Topf aus (1,63 ± 0,31 ml pro Nacht). Sowohl der ψ-Gradient als auch die Gefäß-Durchmesser, aber insbesondere k_s waren signifikant positiv mit der Menge des umverteilten Wassers innerhalb der SRP korreliert. Auch mit der Abgabe von HR Wasser in den Boden waren der ψ-Gradient, die Gefäß-Durchmesser und k_s positiv korreliert. Die Regressionen hatten allerdings ein geringeres Bestimmtheitsmaß als bei der Umverteilung innerhalb des Wurzelsystems. Weitere Faktoren, wie der Wurzel-Boden Kontakt, die Rindendicke oder das osmotische Potenzial der Wurzel, könnten für die Abgabe von HR-Wasser in den Boden relevant sein. Benachbarte, trocken gestresste Pflanzen nahmen umso mehr HR Wasser auf, je länger ihr Wurzelsystem war und je mehr Wurzelspitzen sie hatten. Die Aufnahme könnte maßgeblich vom direkten Kontakt der Wurzeln oder einem gemeinsamen Mykorrhiza-Netzwerk zwischen den benachbarten Bäumchen abhängen. Hydraulic Redistribution könnte, entgegen der ursprünglichen Erwartung, durch die Wurzelanatomie eingeschränkt sein. Die Gefäßdurchmesser in primären Wurzeln waren signifikant kleiner als in sekundären Wurzeln. Bei den Angiospermen war k_s in der Wurzel im Rückwärtsfluss um ca. 40 % niedriger als im Vorwärtsfluss.

Hydraulic Redistribution stellte eine wichtige Wasserquelle für alle untersuchten Arten unter Trockenstress dar. Mit dem prognostizierten Rückgang von Sommerniederschlägen und damit verbundenen erhöhten ψ-Gradienten, ist zu erwarten, dass die Bedeutung von HR in mitteleuropäischen Wäldern zunimmt. Um die Stabilität künftiger Wälder im Klimawandel zu erhöhen, könnten Bäume mit hohem Potenzial für HR (v.a. Bäume mit einem tiefen Wurzelsystem, die unter Trockenheit eine hohe k_s behalten) gepflanzt werden, um die Wasserverfügbarkeit von Waldbeständen zu erhöhen. Nachdem trockengestresste Nachbarbäume stark durch HR profitierten, könnte HR tatsächlich zur Erhöhung der Stabilität von Wald-Ökosystemen der gemäßigten Breiten beitragen.

1. Introduction

1. Temperate forests under global change

It is very likely that above long-term-average warm days and heat waves will increase in Central Europe (Flato et al. 2013). There is constitutive evidence that periodic drought events, especially in summer - during the growing season - will become more frequent (Zimmermann 2011, Flato et al. 2013, Orth et al. 2016). The latest intense dry-years of 2003, 2015 and 2018 demonstrated that temperate forest ecosystems react very sensitive to soil drying and drought (Brinkmann et al. 2016, Dietrich et al. 2018). Especially shallow rooted plants with only few sinker roots, like *Picea abies* (L.) H. Karst. (Kölling 2007) as one of Central Europe's most abundant and economically important tree species (Polley et al. 2014), drastically reduces sap flow (> 90 %, Brinkmann et al. 2016) and growth (Dietrich et al. 2018) under drought conditions. Consequently, trees become more vulnerable to biotic stressors like bark beetle attacks (Netherer et al. 2015, Huang et al. 2019) or other pathogens (Ayres & Lombardero 2000), leading to potential economic losses in the forestry sector (Kirilenko & Sedjo 2007) of estimated up to 50 % of the present value (Hanewinkel et al. 2013). Across Europe alone, forestland value could thus decrease by more than € 190 billion by the year 2100 (Hanewinkel et al. 2013). Currently, foresters try to change tree compositions in forests towards more drought-adapted systems, but tree responses to potential future climate conditions are still uncertain (Spittlehouse & Stewart 2003; Bolte et al. 2009). A promising method seems to be admixture of several different tree species in forests, as this may pool hazards of severe ecological and financial damage due to environmental impacts (Knoke et al. 2008). Mixed stands often react more stable against biotic and abiotic stressors (Metz et al. 2016), reducing (financial) risks, and even might have increased yield than respective monocultures (Morin et al. 2011, Pretzsch 2013). Mechanistic understanding behind this stability is still scarce, as potentially a huge variety of different factors, such as resource competition (Forrester 2014), niche partitioning aboveground (Pretzsch 2014) or belowground (Schmid & Kazda 2002; Bolte & Villanueva 2006) and changes in soil fertility (Rothe & Binkley 2001) or microclimate (Augusto et al. 2002) have an influence on forest ecosystems. One factor that might be increasingly relevant for either monospecific or mixed temperate forest systems, especially under prolonged summer droughts, is enhanced water availability due to hydraulic redistribution (HR) from moist towards dry soil horizons (Pretzsch et al. 2014, Ammer et al. 2018). However, relevance and mechanisms behind HR have not been determined for Central European tree species yet.

2. Water movement from plants to soils: Hydraulic Redistribution

Hydraulic Redistribution is the passive movement of water through plants that follows a spatial water potential (ψ) gradient, e.g. between different soil layers within the rooting zone (Leffler et al. 2005). Roots take up water in moist soil layers with high ψ, redistribute it within their root system towards dry soil layers (with low ψ), and may release it into the rhizosphere when the ψ in the soil is lower than in the roots (Richards & Caldwell 1987). As atmospheric ψ is much lower than in both, soil or plant tissues (Lüttge et al. 2005), HR only occurs when transpiration is virtually absent and stomata are predominantly closed, typically during nighttime (Pereira et al. 2006) or during daytime under very low vapor pressure deficit (VPD) (Espeleta et al. 2004).

Richards & Caldwell (1987) first called the effect hydraulic lift (HL) since they discovered an up-lift of water through deep-seated roots of *Artemisia tridentata* Nutt. in desert regions up into dry shallow soil layers. However, a moisture gradient within the soil and therefore the redistribution of water can also traverse several other directions, e.g. downward, from moist shallow layers towards dry deep layers (‘downward HR’, Burgess et al. 1998) or along a lateral gradient (Smart et al. 2005). In certain ecosystems with regular fog-events and dry soil conditions or under extreme dry conditions in both, shallow and deep soils, water also moves from plant canopies towards the soil (Eller et al. 2013, Schreel et al. 2019; summarized in Prieto et al. 2012). The name ‘HR’ combines all mentioned water reallocation movements from plants towards dry soil layers (Burgess et al. 1998, Prieto et al. 2012).

3. Hydraulic Redistribution in Central Europe

Hydraulic redistribution has been demonstrated across many species and multiple climatic conditions, from dry hot (Richards & Cladwell 1987) to mesic (Dawson 1993) surroundings. It is now seen as a general phenomenon in plant ecology (Quijano & Kumar 2015), potentially occurring in many ecosystems where a ψ gradient is present (Nadezhdina et al. 2010). However, the relevance of HR for different ecosystems and species varies widely. There is indication that HR water quantities are crucial in the water balance of some plants or ecosystems (e.g. Kurz-Besson et al. 2006), while its importance is inferior to others (e.g. Scholz et al. 2010). Although redistribution of water was considered possible in Central European species already in 1727 (Münch 1930), there are only few studies on HR in native or introduced temperate tree species among local habitat conditions (Nadezhdina et al. 2006, 2009, Zapater et al. 2011). Lateral HR in *P. abies* under drought was detected after partial irrigation of the topsoil via sap-flow measurements. Reverse sap-flow, i.e. from irrigated roots towards dry soil layers, could be seen in shallow coarse roots overnight, indicating HR. However, the amount of water was not quantified or put into a mechanistic reference to e.g. soil water content. Additionally, no evidence was found that non-irrigated drought stressed neighbor plants took up HR water (Nadezhdina et al. 2006). After partial irrigation, sap-flow sensors, installed pairwise each on opposite sides in roots of mature *Pseudotsuga menziesii* (Mirbel) Franco trees, revealed water redistribution inside the trees: While sap-flow consequently increased on the irrigated side of the base of the trunk, negative flow was measured on the opposite trunk side. Again, the amount of HR water was not quantified (Nadezhdina et al. 2009). Hydraulic redistribution has also been demonstrated in a mixed species forest in Central Europe. A stable isotope labeling experiment with ^{18}O-enriched water suggested HL of labeled water by *Quercus petrea* Matt. from moist, deep to dry, shallow soil layers. The authors could, however, not quantify the lifted amounts of water or demonstrate an uptake of HR water by shallow rooted neighboring *Fagus sylvatica* L. trees. Nevertheless, the authors suggest that also Central European neighboring tree species may take up HR water under severe drought conditions (Zapater et al. 2011). Thus, for temperate Central European tree species in particular, it is still not clear what amounts of water are redistributed by different tree species and also mechanistic understanding on factors that drive HR by tree species is missing. Further, evidence on a potential uptake and benefit of HR water by neighboring plants in Central European species is missing.

4. Impact of HR water on plants

Plant health and growth can profit from HR: drought sensitive fine roots in dry soils may maintain their lifespan (Bauerle et al. 2008) and the rhizosphere may stay hydrated. Consequently, a sustained root-soil contact (Ryel et al. 2004) preserves accessibility of nutrients in dry soil layers (Caldwell et al. 1998, Querejeta et al. 2003), and eases the use of small rain events' water. Additional available HR water may increase rates of transpiration, stomatal conductance and photosynthesis and therefore directly improve plant carbon-gain and growth (Howard et al. 2009). Even the growing season may be prolonged through HR (Brooks et al. 2002). The increase in carbon gain could be so substantial, that some forest plantations in the temperate zone would become carbon-sinks only because of HR (Domec et al. 2010). Downward HR could reduce water losses due to soil surface water run-off or evaporation and may stimulate root growth in deeper soil layers, creating access to deeper water tables and nutrient reservoirs (Burgess et al. 1998). These nutrients may then become part of the nutrient cycle, increasing the nutrition status of shallow soil layers (Prieto et al. 2012). The effect on groundwater differs depending on HR direction: while downward HR may raise the groundwater-level (Ryel et al. 2004), HL can lower it (Wang et al. 2011). The extent of all these effects depend on the magnitude of redistributed water. As mechanisms that determine HR amounts of Central European trees have not been assessed yet, we still do not know the relevance and extent of possible facilitative HR effects in temperate species. Therefore, we need a mechanistic understanding on plant characteristics and environmental circumstances that may favor potential HR benefits to species (Pretzsch et al. 2014; Ammer et al. 2018).

Hydraulic redistribution may also influence neighboring plants. Plants with only shallow root systems, like grasses or saplings, may take up and use redistributed water in upper soil layers (Brooks et al. 2002). It has been suggested that additional water enhances stomatal conductance (Dawson 1993) and growth (Bogie et al. 2018; Magh et al. 2018) of neighbor plants and even significantly increase survival in some regions (Pereira et al. 2006, Pang et al. 2013). However, effects of HR on plant-plant interactions may differ with species and environments (Prieto et al. 2012). Neighboring plants also compete for available (redistributed) water (Barron-Gafford et al. 2017). Small plants might even grow better without water redistributing bigger neighbor plants, as additional negative effects like shading or nutrient competition could overlap the benefit of HR water (Ludwig et al. 2004). Downward HR may also be a strategy of deep-rooting plants to drain water from shallow rooted plants to deeper soil layers and hence increase drought stress for them (Yu & D'Odorico 2014). Therefore, the actual influence of HR to neighboring plants may vary case dependently. To estimate HR-impact to neighbors for individual trees or ecosystems, the amount of additionally available water needs to be quantified. For temperate Central European species, however, even an uptake of HR water by neighboring trees could not be proven (Nadezhdina et al. 2006; Zapater et al. 2010). Therefore, considerable evidence on HR effects to neighbor plants is still missing.

Hydraulic redistribution likely facilitates mutualism with microorganisms (MO) and fungi (mycorrhiza) (Pereira et al. 2006), as water could be transferred directly between plant and MO/fungus or vice versa (Querejeta et al. 2003). Release of water into the dry soil by hyphae of mycorrhizal fungi may increase solubilization of nutrient substances that can be taken up and enhance plant nutrition status (Querejeta et al. 2003). In addition, direct root-contact or a common mycorrhizal network (CMN) between plants potentially displays a quicker and more effective pathway to transport and exchange HR water than through the soil (Warren et al. 2008, Prieto et al. 2016). Therefore, mycorrhizal abundance and mass as well as length and distribution of the root system could be important for the amounts of HR water that different neighboring plants exchange (Egerton-Warburton et al. 2007). As different species may be colonized by the same mycorrhizal fungi, HR water should be exchanged easily via a CMN (e.g. along a lateral ψ gradient; Nadezhdina et al. 2009; Prieto et al. 2012). Species mixture could hence majorly influence the magnitude of HR water that neighboring plants take up. However, possible differences in the quantity of HR water taken up by neighboring plants between monospecific and mixed species combinations have not been investigated yet.

5. Quantification

Estimated magnitudes of HR range by nearly two orders of magnitude, corresponding to 0.04 – 3.20 mm per night (Neumann & Cardon 2012) across global ecosystems, species and climates. Reported HR amounts equaled between 2 % of total daily water use in Neotropical savanna regions (Scholz et al. 2010) and up to 81 % of tree transpiration in Mediterranean regions (Kurz-Besson et al. 2006). This huge variety exemplifies the need to determine basic principles and mechanisms that influence amounts of redistributed water among controlled conditions, where potential HR driving factors can be varied and their influence studied. Especially for Central European tree species, where even the magnitude of redistributed water is still unclear, driving factors and capability of different species to conduct HR have to be systematically determined to estimate the relevance to local species.

6. External and internal factors that influence Hydraulic Redistribution

Several factors are suggested to have an influence on the extent of HR, and their variability potentially contributes to the huge variation in the studies that quantified HR so far.

Suggested determining factors can roughly be divided into surrounding environmental conditions (plant 'external' factors) and plant specific characteristics (plant 'internal' factors) (Neumann & Cardon 2012).

'External' factors that may have an influence on the magnitude of HR include e.g. groundwater alignment (Ryel et al. 2002) or physical soil properties e.g. particle size (Prieto et al. 2010) or soil hydraulic conductivity (Scholz et al. 2008). Additionally, climatic factors, such as transpiration demand (Smith et al. 1999) might influence the magnitude of HR. All of the mentioned parameters either determine (groundwater, soil properties) or are determined (transpiration) by another potential 'external' factor influencing HR: the ψ in the different soil layers and the resulting moisture gradient (Silvertown et al. 2015). In the shrub *Tamarix ramosissima* Ledeb. under dry conditions, Yu et al. (2013) suggested that the ψ gradient was an important prerequisite and HR driving factor. Between 33 – 45 % of soil depth- and seasonal variation in HR amounts were related to either climatic, soil ψ gradient or VPD variability (Yu et al. 2013). To understand the mechanistic influence of each single parameter, controlled experiments are ideal tools to estimate the relevance of the respective 'external' factors.

Next to site or climate characteristics, also plant specific 'internal' factors might influence the amounts of HR water. An obligate 'internal' factor and HR premise is that plants develop a dimorphic root system (Scholz et al. 2008), spanning soil layers across a moisture gradient. Only then, plants are able to take up or release water in respective layers (Neumann & Cardon 2012). The potential amount of HR water, however, might be influenced by additional 'internal' plant specific characteristics. Firstly, nighttime transpiration (Howard et al. 2009) that is driven by 'external' VPD on the one hand but on the other hand also by the plants capability to keep the stomata closed during the night. Depending on species, nighttime transpiration can reach approx. 1 – 25 % of daytime transpiration (Dawson et al. 2007) and might reduce HR as competitive water sink, accordingly. Secondly, it is suggested that nighttime refilling of water storage organs, e.g. stems, could significantly reduce HR in some species (Hultine et al. 2003), as this again displays an additional competitive sink. Nocturnal storage refilling was estimated to even exceed nighttime transpiration water losses in some species, probably accounting for up to 30 % of daily sap-flow (Yu et al. 2018). Plants could additionally limit the release of water into the rhizosphere or towards other plants by specific root characteristics such as bark thickness (Ryel et al. 2003) or the regulation and number of aquaporins in root cell membranes (Prieto et al. 2012; Li et al. 2014; Maurel et al. 2015). Finally, the 'internal' capacity of a species that regulates the flow of HR water over time might be restricted to the potential magnitude of 'internal' root xylem sap-flow (Quijano & Kumar 2015). This magnitude is determined by root-conduit architecture, or, more accurately, xylem hydraulic conductivity of the roots, as this accounts also for potential drought induced conduit embolisms (Silvertown et al. 2015). In a conducting element, the diameter of the conduits influences the potential conductivity for water the most, as given by the law of Hagen-Poiseuille (Scholz et al. 2013):

$$k_s = \frac{\pi * D^4}{128 * \eta} \qquad \text{(Eqn. 1)}$$

with

k_s: Hydraulic conductivity (in kg MPa^{-1} s^{-1} m^{-1}),

D^4: Diameter of the conduit to the fourth power and

η: Viscosity index of the fluid (water: 1.002×10^{-9} MPa s at 20 °C).

Thus, roots with large conducting elements should have a high water-transport capacity and accordingly be able to redistribute more water than roots with smaller conducting elements. Therefore, e.g. angiosperm species that are known to be diffuse-porous or even ring-porous as classified by stem anatomy, should be able to redistribute more water than conifers with only small-diameter tracheids as conductive elements. However, few studies have determined the diameter of xylem conduits in roots of different species (Koecher et al. 2012, Mrak & Gricar 2016). They indicate that root conduit diameters (i.e. ring-porous, diffuse-porous) might deviate from known conduit structure of the stem xylem, similar to what is already known for twigs (Sperry et al. 2008). As a consequence, also root xylem hydraulic conductivity may be different from known stem conductivity (Eqn. 1; Tyree & Zimmermann 2002). However, as for root anatomy, only few studies actually measured hydraulic conductivity of roots (e.g. Graham & Syvertsen 1984, Fennell & Markhart 1998). Additionally, systematic evidence is needed, if different root orders have an influence on anatomy, e.g. if conducting elements within a species differ between young, primary and older, secondary roots. As water is taken up and released via primary roots, especially their conduit diameters could be an important prerequisite for HR amounts. For HR, also the reverse flow of water through roots – from the stem towards the soil – needs to be considered, as it could be an important limitation. To date it is not known, whether reverse and forward flow in plant tissue might even deviate, as reverse flow of water has never been systematically measured.

2. Objectives of the study

This doctoral thesis aims to clarify basic mechanisms behind the general occurrence of HR in various economically and ecologically important temperate Central European tree species with different 'internal' HR premises (articles I & II). Under controlled conditions, the amounts of water that saplings of different temperate tree species redistribute, release into the rhizosphere and that neighboring saplings take up are calculated (articles II & III). The focus of this thesis is to gain a mechanistic understanding behind different HR quantities. Therefore, 'external' and 'internal' driving factors that determine the magnitude of HR water of different species are discussed (articles II, III & IV). Especially soil moisture status, in a range from rather moderate to severe drought conditions, as 'external' driving factor is intensively studied. Another focus lies on the 'internal' factors root conduit anatomy and xylem hydraulic conductivity. As mentioned, these parameters are hardly studied yet and potentially differ from known stem anatomy and xylem hydraulic conductivity. They could have an important influence on HR amounts for different tree species. Therefore, a systematic analysis of saplings' root conduit architecture and xylem hydraulic conductivity (in forward and reverse direction) is performed (article IV) and their influence on HR (articles II & III) determined. Finally, root length, number of root tips and root xylem hydraulic conductivities of neighboring plants are correlated with the amounts of redistributed water that neighboring saplings receive (article III) as measures of a possible direct exchange of HR water.

The main research questions of the present thesis are:

1. Does HR occur among saplings of Central European tree species and what amounts of water are redistributed (articles I, II & III)?
2. Do saplings of drought stressed neighboring temperate tree species take up and use HR water (article III)?
3. Is the 'external' environmental factor ψ gradient and are the plant 'internal' factors root hydraulic conductivity (k_s) and root conduit diameter positively correlated with the amount of water Central European tree species redistribute (articles II & III)?

4. Might there be limitations for HR due to potential resistance in anatomy and reverse hydraulic conductivity in roots (article IV)?

To quantitatively capture the redistributed water and specifically determine the influence of possible driving factors, the experiments were conducted in controlled environments. Therefore, studies were done with 2 – 4 year old saplings in a greenhouse (article I) or growth chambers (articles II, III & IV), respectively. Studied species were coniferous *P. abies* and *P. menziesii*, diffuse-porous *Acer pseudoplatanus* L. and *F. sylvatica* and ring-porous *Castanea sativa* Mill. and *Quercus robur* L. Plants for all experiments were planted in split-root systems (SRS), where two pots were bolted together and three trees were planted: one with its root system split equally between both pots and one additional plant in each of the pots. Using the SRS, different ψ gradients could be established between both pots, and HR water within plants and towards neighbors sampled separately.

The results of this thesis increase our mechanistic understanding of HR among trees under drought. Determined plant internal and environmental external driving factors of HR may help for a better understanding of Central European tree species' reaction to climate change conditions, especially more frequent summer drought events. Additionally, conclusions and strategies of potential tree species compositions may be drawn to actively use HR as a strategy for increased forest health in view of ongoing climate change.

3. Overview of methods

Detailed descriptions of experimental methods and statistical analyses are given in the respective articles (I, II, III & IV). Here, a summary of the main work done by the author is given.

1. Split-root design

Plants for all experiments were grown in split-root systems (SRS, Fig. **1**). Two pots with a capacity of 10 l each were bolted together with a semicircular notch at the contact point. Three trees were planted in each SRS – with one tree in each pot and a split-root plant (SRP) centrally located above the notch with its root system equally distributed into the two pots. To avoid injury to the roots of the SRP, a small pad of foam-material was placed between the roots and the notch in the pots above the soil surface. Moreover, until plant establishment, each SRP root collar had a soil-filled sleeve made out of foam material to avoid desiccation of the upper roots. The sleeve and the soil within were removed before the respective experiments started. Although root systems of the SRPs were protected as good as possible, the treatment was stressful for the trees and not all plants survived. It has been suggested that HR might also occur through root systems of senescent grasses (Leffler et al. 2005). Therefore, desiccated trees were still studied to clarify the general occurrence of HR (article I). They were, however, excluded in the studies that determined HR driving factors (articles II & III), to exclude potential artifacts due to different plant-health preconditions. For establishment, all trees were well watered and grown in a greenhouse under ambient climate conditions for one half to two years. During plant establishment, SRS were regularly repositioned to minimize potential effects of light and temperature heterogeneities inside the greenhouse chamber.

Using the SRS setup, it was possible to create a moisture gradient between the pots. Irrigation was maintained in one pot (‘moist pot’ with the well watered, “moist plant” ‘MP’), while watering was ceased in the other pot (‘dry pot’ with the drought stressed plant ‘DP’), until the aimed moisture gradient was established. To determine a dependency of HR amount from the moisture gradient (article II), various gradients were established for the species in different SRS. Adjustment of volumetric soil water content (SWC) in the pots was monitored regularly via time domain reflectometry (TDR probes spanning the depth of the pots; i.e.

15 cm; TDR100, Campbell Scientific, Shepshed, UK). Corresponding ψ in the plants was measured pre-dawn, i.e. when ψ was even in all plant tissues and should also be in balance with soil ψ (Bauerle et al. 2006). Water potential was measured in either leaves or twigs of DPs, SRPs and MPs with a Scholander-type pressure bomb (1505D pressure chamber, PMS Instrument Company, Albany, USA).

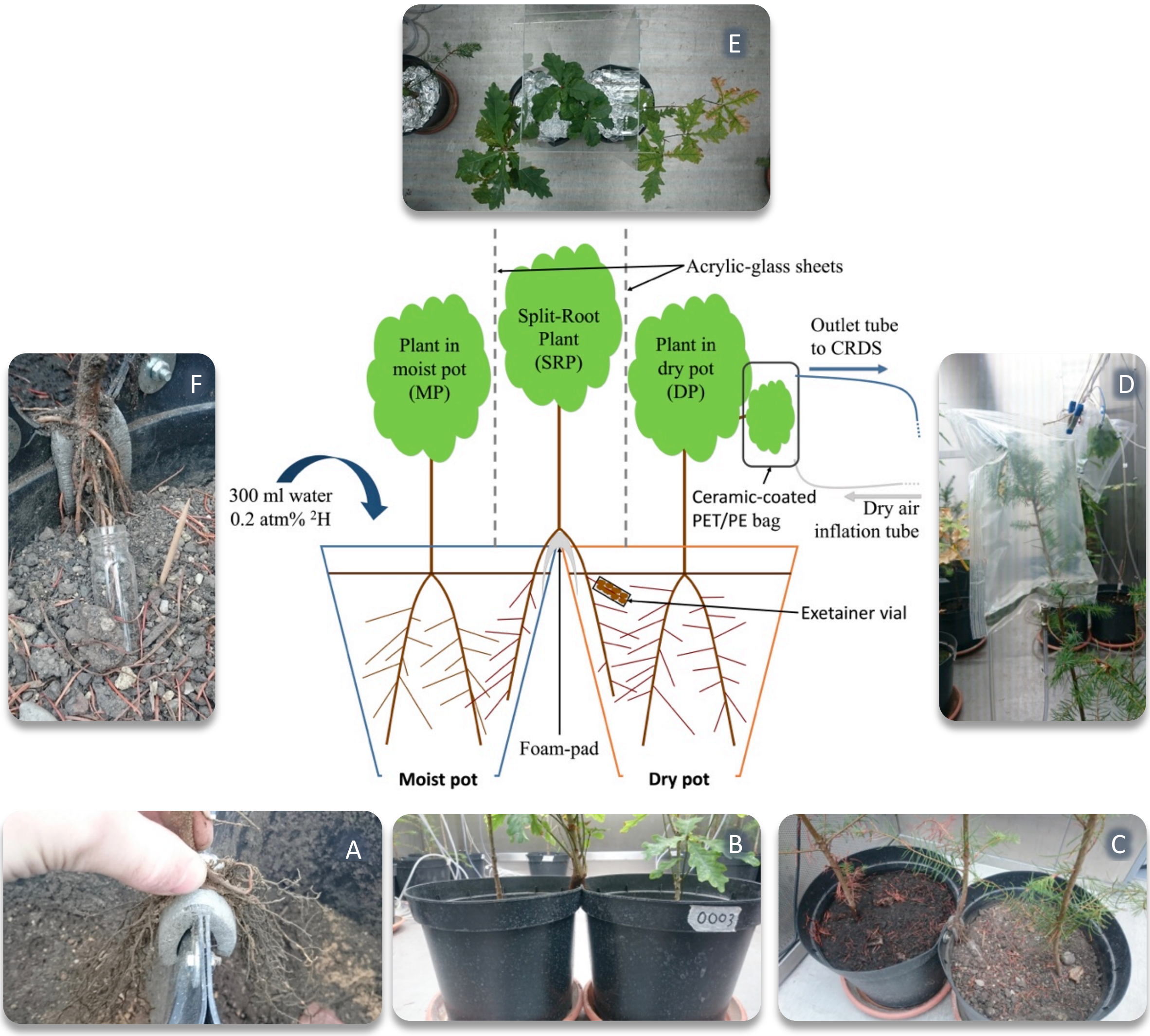

Figure 1: Scheme of the split-root system (SRS) with one tree's (SRP) root system split equally between a 'moist' and a 'dry' pot (A, B). A foam-pad was placed between the roots of the SRP and the pot to minimize injuries (A). Additional plants (MP and DP, respectively) were planted in the moist and dry pot, respectively (B, C). A moisture gradient was established between the pots (C). Foliated twigs of the DP were placed in transparent ceramic-coated PET/PE chambers that were inflated with dry air for DP transpiration analysis (D). Vertical acrylic glass sheets prevented canopy contact between the plants (E). The soils were covered with aluminum foil to reduce

evaporation (E). One root branch of the SRP in the dry pot was inserted into an exetainer vial, including rhizosphere soil to be harvested one night after the experiment started (F).

2. Greenhouse and growth chamber conditions

The experiment in article I was performed in a greenhouse at the “Gewächshauslaborzentrum Dürnast” in Freising, Germany (http://ghl.wzw.tum.de). Day/night mean temperature was 21.2 ± 3.7 / 15.5 ± 1.7 °C (mean ± 1 SD) and relative humidity (rH) at 52.9 ± 13.4 / 70.1 ± 4.5 % (1 SD). Daily global radiation (between 11:00 am and 03:00 pm) was 46.1 ± 20.3 W m^{-2} (1 SD), corresponding to a photosynthetically active photon flux density (PPFD) of c. 96.7 ± 42.7 µmol m^{-2} s^{-1} (1 SD)[1]. Saplings were planted in a luvisol (resulting from loess over Tertiary sediments), mixed with c. 30 % sand to ease soil drying. Soils were retrieved from different forest stands for the different species, each dominated by mature trees of the respective study species, to provide species-specific mycorrhizal inoculum to saplings. However, all soils had a similar, silty texture.

The experiments for articles II, III and IV were conducted in two parallel growth chambers (7.7 m^2 each) at the TUMmesa facility (Technical University of Munich – Model EcoSystem Analyser; http://www.tummesa.de/home). Day/night temperatures were 24.9 ± 0.3 / 15.0 ± 0.1 °C (1 SD) and rH was 60 ± 0 / 89 ± 2 % (1 SD). At canopy level, PPFD reached 434 ± 11 µmol m^{-2} s^{-1} (1 SD). Saplings were planted in a potting soil (mixture of topsoil, compost, turf and lava (20 % organic matter); Wurzer Umwelt GmbH, Eitting, Germany) that was mixed with c. 10 % of soil retrieved from forest stands dominated by mature trees of the study species, again to provide species-specific mycorrhizal inoculum.

3. Labeling approach: Sampling of tissue and in-situ measurements

To trace the potential flow of water from the moist to the dry pot in the experiments (articles I, II & III), soil water in the moist pot was labeled with a stable isotope tracer. Labeling was performed with deuterium (^{2}H) enriched water.

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[1] Conversion factor: 4.57 (Sager & Mc Farlane 1997). Note that the value of 4.57 converts W m^{-2} to µmol m^{-2} s^{-1}, assuming that radiation was measured from 400 to 700 nm. However, the pyranometer used in article I measured total solar radiation. As only c. 45% of the energy of solar radiation is actually in the 400 - 700 nm range, the conversion factor was reduced accordingly to 2.1.

The concentration of ^{2}H in the water is expressed in the common delta notation, i.e. relative to an international standard (here: Vienna Standard Mean Ocean Water, VSMOW; Craig 1961) as:

$$\delta^2H = \left(\frac{\frac{^2H}{^1H} sample}{\frac{^2H}{^1H} standard} - 1 \right) * 1000\ ‰ \qquad \text{(Eqn. 2)}$$

with

δ^2H:	Delta value of the sample,
$\frac{^2H}{^1H}\ sample$:	Ratio of ^{2}H to hydrogen in the sample and
$\frac{^2H}{^1H}\ standard$:	Ratio of ^{2}H to hydrogen in VSMOW standard (1/6420).

Before starting the labeling experiments, initial unlabeled samples were collected as reference for samples after labeling. To keep experimental plants largely undisturbed until final harvest, only soil samples were used as unlabeled references. Initial cross-references between unlabeled soil and plant tissues revealed isotopic equality. The labeled water was applied to the soil of the moist pot during daytime, so that ^{2}H water could be well distributed within the soil and in MPs and SRPs (Fig. 1). Application was done very carefully with a small watering can (500 ml), while the soil on the dry pot was covered with foil. To prevent evaporation losses of the labeled water, also the soil on the moist pot was covered with foil after the labeling. Additionally, potential contamination with ^{2}H enriched vapor from labeled soils or plants to non-labeled plants was tested in a pre-experiment but could be excluded (article I). Nevertheless, to prevent any possible canopy-contact between labeled and non-labeled plants, acrylic glass sheets (Fig. **1**) were additionally installed for measurements in articles II & III.

Sampling of soil and plant tissues (leaves, twig-xylem, stem-xylem and roots, respectively) was done either before dawn (articles II & III) or plants were covered in bags (article I) to prevent any transpiration of potentially redistributed labeled water. To collect only xylem water, bark and cambium were quickly removed from samples of twigs and stems with razor blades, while for root samples the rhizosphere soil was carefully removed. All samples in the dry pot were taken before respective samples in the moist, labeled pot to avoid contamination. Stem xylem samples were cut into c. 1 cm long pieces to ease subsequent extraction and all samples were quickly transferred in exetainer vials (Labco, Lampeter, UK) and stored at -18 °C. Depending on the research question, sampling was 1 (article II), 3 (article I) or 7 days (article III) after the labeling. Water from samples was extracted via cryogenic vacuum extraction for two hours (West et al. 2006). Sampled water was analyzed either via an isotope-ratio-mass-spectrometer (IRMS, Isoprime 100, Elementar Analysensysteme GmbH, Langenselbold, Germany) coupled to a multiflow system (222 XL Liquid Handler, Gilson, Middleton, USA) or a cavity-ring-down-spectrometer (CRDS, L2120-i, Picarro, Santa Clara, USA) coupled to a vaporizer module (A0211, Picarro, Santa Clara, USA). Cross measurements of soil, stem- and root xylem samples revealed no statistical differences between both instruments ($P = 0.9$; regression: $R^2 = 0.99$, $P < 0.001$) or putative contamination with organic compounds (West et al. 2010). Measurements were calibrated against two working standards, spanning the majority of measured samples (between -161 ‰ and 133 ‰, for details see articles I, II & III).

To assess HR water in situ in DP transpiration, foliated twigs were placed in ceramic-coated PET/PE chambers connected to a CRDS (L2120-I, Picarro Inc.; see Fig. **1** and article III). To best meet isotopic steady-state conditions, δ^2H of transpiration water was recorded during mid-day. At this time environmental conditions and rate of transpiration were constant. Transpiration δ^2H values were recorded 1 day before harvesting the plants in article III (i.e. 6 days after the labeling).

4. Mixing model calculations

To estimate the fractions of water that were redistributed within plants, towards the (rhizosphere) soil or taken up by neighbors (DP), two-end-member mixing models were calculated. Two possible water sources for the water composition of the respective tissues were assumed. As mentioned, the soil before labeling served as unlabeled source or initial end-member, while the closest tissue that potentially transferred HR water served as labeled source or second end-member. For example, the closest tissue/ source transferring HR water towards DP roots were the roots of the SRP in the dry pot (see Table **1** for specific end-members).

Therefore, mixing models were set up as:

$$HR_{tissue} = \frac{\delta^2 H(tissue) - \delta^2 H(unlabeled\ source)}{\delta^2 H(labeled\ source) - \delta^2 H(unlabeled\ source)} * 100\ (\%) \qquad \text{(Eqn. 3)}$$

with

HR_{tissue}:	Fraction of HR water in the studied tissue potentially receiving HR water,
$\delta^2 H(tissue)$:	Delta value of the studied tissue after labeling,
$\delta^2 H(unlabeled\ source)$:	Delta value of the initial, unlabeled source before labeling and
$\delta^2 H(labeled\ source)$:	Delta value of the second, labeled source after labeling.

Table 1: Labeled and unlabeled end member sources for calculating the fractions of HR water in the studied tissues potentially receiving HR water (1st column). Fractions were calculated according to Eqn. 3. In a second step, fractions were used to calculate absolute amounts of HR water and then related to respective dry root masses according to Eqn. 4 (last column).

Tissue	**Labeled reference**	**Unlabeled reference**	**Related tissue**
SRP $_{\text{root dry}}$	SRP $_{\text{root moist}}$	Bulk soil dry pot $_{\text{before labeling}}$	SRP $_{\text{root dry}}$
SRP $_{\text{rhizosphere}}$	SRP $_{\text{root moist}}$	Bulk soil dry pot $_{\text{before labeling}}$	SRP $_{\text{root dry}}$
Bulk soil dry pot $_{\text{after labeling}}$	SRP $_{\text{root dry}}$	Bulk soil dry pot $_{\text{before labeling}}$	*NA*
DP $_{\text{root}}$	SRP $_{\text{root dry}}$	Bulk soil dry pot $_{\text{before labeling}}$	DP $_{\text{root}}$
DP $_{\text{stem}}$	SRP $_{\text{root dry}}$	Bulk soil dry pot $_{\text{before labeling}}$	DP $_{\text{root}}$
DP $_{\text{transpiration}}$	SRP $_{\text{root dry}}$	Bulk soil dry pot $_{\text{before labeling}}$	DP $_{\text{root}}$

Mixing models were calculated individually for each SRS. Fractions of HR water were then averaged for respective replicates of the same species. To include possible uncertainty errors, mixing models were also calculated with average end-member δ^2H values per species with 'Iso Error' (Philips & Gregg 2001). The approaches were not statistically different from each other and therefore verified the results.

To quantify absolute amounts of redistributed water (in ml), the fractions of HR water were multiplied with the water content in the respective tissues, that were either thoroughly estimated (article I) or carefully assessed at harvest (articles II & III).

Finally, to compare the calculated HR quantities between species, amounts were related to DP and SRP root dry masses, respectively (in ml g^{-1}; see Table **1**):

$$HR_r = \frac{HR_a}{Root\ mass}\ (ml\ g^{-1}) \qquad \text{(Eqn. 4)}$$

with

HR_r: Amount of HR water per DP/ SRP root dry mass and

HR_a : Absolute amount of HR water in tissues (in ml).

5. Root anatomy and length

To assess conduit sizes in the studied tree species, root segments (diameter 1-2 mm and length c. 10 mm; n = 3-9) of additional plant individuals of the same age and grown under the same conditions were sampled. Samples were dehydrated in ethanol: 2 h in 70 %, then 3 h in 90 % and finally overnight in 96 % ethanol. Subsequently, segments were cut with laser ablation tomography (LAT) to get images for anatomical analysis. In brief, a laser beam (Avia 7000, 355 nm pulsed laser) vaporized the root segment as it was incrementally moved into the beam. Simultaneously, images of the laser-illuminated cross section were taken. Imaging was performed at a resolution of 25400 dpi with a Canon T3i camera (Canon Inc. Tokyo, Japan) and 5 × micro lens (MP-E 65 mm) (Chimungu et al. 2015). Pictures of the root cross sections (Fig. **2**) were then analyzed for xylem conduit diameters. All xylem conduits on either three representative sample areas (0.5 mm² each, article II & IV) or on about one-quarter of the total cross-section (article I) were marked manually with GIMP (GNU Image Manipulation Program, Version 2.10.2, The GIMP Team, https://www.gimp.org/). Conduit area was subsequently determined with imageJ (Version 1.47t, Wayne Rasband, National Institutes of Health, Bethesda, USA) (Fig. **2**) and the equivalent circle diameter retrieved (Scholz et al. 2013):

$$D = \sqrt{\frac{4A}{\pi}} \qquad \text{(Eqn. 5)}$$

with

D: Equivalent circle diameter and

A: Conduit area.

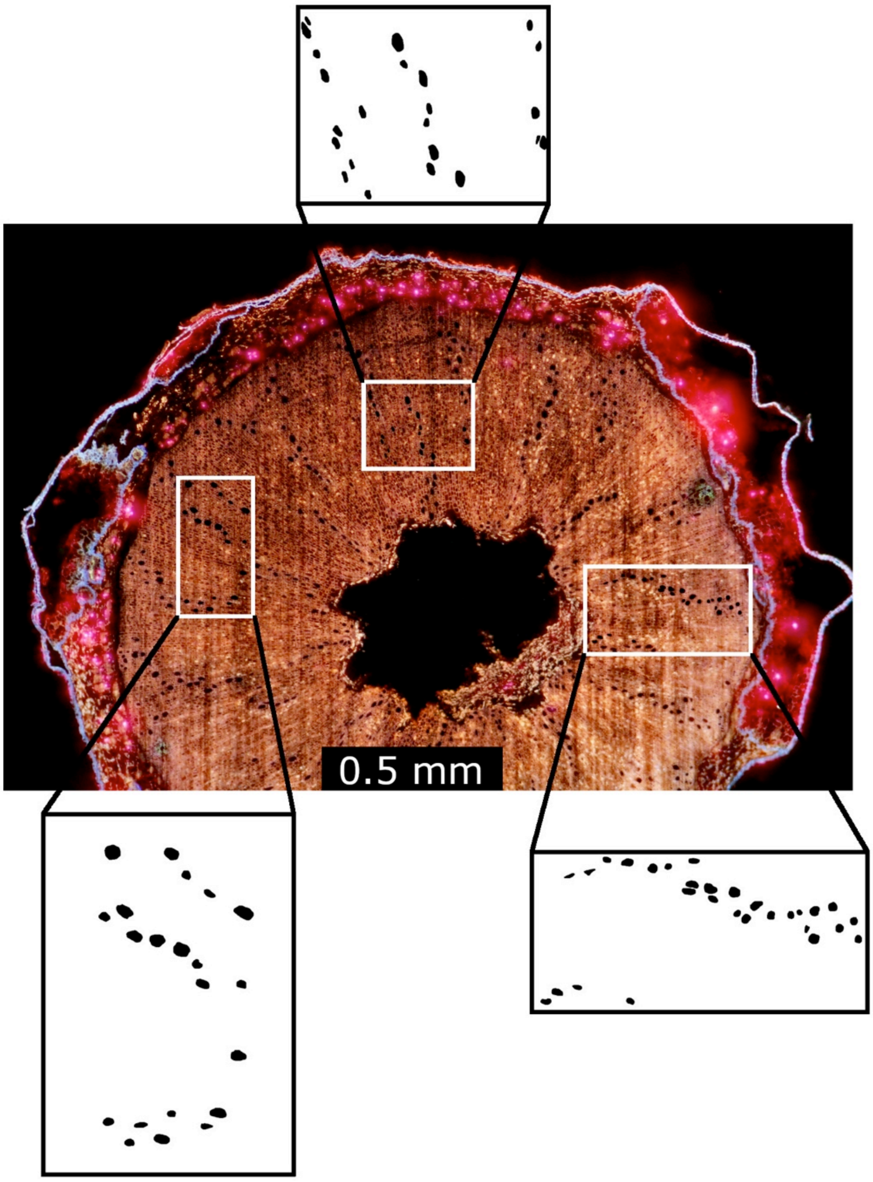

Figure 2: Root cross-section of *Q. robur* cut and photographed via laser ablation tomography. Three analyzed sample areas (0.5 mm² each) are highlighted. Conduits inside the areas were marked manually, conduit area was determined and the equivalent circle diameter calculated.

To assess total root length of saplings, fresh and dry mass of individual root branches (average dry-mass: 0.42 ± 0.05 g, 1 SE, n = 5 per species) were recorded. Root branches were then scanned (1200 dpi, Epson Perfection 4990 Photo, SEIKO Epson CORPORATION, Suwa, Nagano, Japan) and images analyzed with WinRhizo (WinRHIZO Reg 2013e, Regent Instruments Inc., Quebec, Canada) for root length and root tip determination. WinRhizo results were verified by manual estimation of root length and number of root tips. To this end, the root lengths and tips of a subsample of the analyzed scans (*n* = 4 per species) were assessed by hand with

GIMP (GNU Image Manipulation Program) and root lengths and tips subsequently determined with imageJ (Version 1.47t).

Root length and tip correction factors were calculated for each species and used to correct WinRhizo root length/ tip results:

$$RL/Tip_{corr} = \frac{RL/Tip_{WinRhizo}}{RL/Tip_{GIMP}} \qquad \text{(Eqn. 6)}$$

with

RL/Tip_{corr}:	Root length/ Tip correction factor,
$RL_{WinRhizo}$:	Root length/ number of tips determined in WinRhizo and
RL_{GIMP}:	Root length/ number of tips determined by hand.

Root length correction factors ranged between 1.07 and 1.25 for the different species (average: 1.13 ± 0.02, 1 SE) and tip correction factors between 0.47 and 2.53 (average 1.43 ± 0.09, 1 SE). Finally, specific root length (SRL, the ratio of root length to dry mass of the analyzed root branch) was calculated and used to estimate total root length per tree.

6. Hydraulic conductivity of root xylem

For article I, hydraulic conductivity was estimated from root conduit diameters (Scholz et al, 2013) by dividing calculated k_s (Eqn. 1) by the area of the analyzed cross section. For articles II, III and IV, actual (i.e. without removing native embolisms) and maximum hydraulic conductivities were measured and calculated in DPs and SRPs. To this end, hydraulic conductance was measured with a 'xylem embolism meter' (XYL'EM, Bronkhorst France S.A.S., Montigny-Les-Cormeilles, France). Roots (diameter: 2.5 ± 0.1 mm (1 SE), length: 2.7 ± 0.1 cm (1 SE), n = 5 per species) were cut several times under water. The bark was removed on the side that was inserted into the XYL'EM apparatus and the actual hydraulic conductance was assessed (K_{act}, in kg MPa^{-1} s^{-1}). Roots were flushed with degassed, filtered (0.2 µm) water with 10 mM KCl and 1 mM $CaCl_2$ added (Barigah et al. 2013) at approx. 0.007 MPa. After measuring K_{act}, maximum hydraulic conductance (K_{max}, in kg MPa^{-1} s^{-1}) was obtained by flushing the root sample several times at approx. 0.12 MPa for 10 minutes, until conductance did not further increase. Subsequently, the lengths and the conductive areas (A_{cond}) of the root samples were assessed.

Actual and maximum specific xylem hydraulic conductivity (k_{sa} and k_{sm}, respectively, in kg s^{-1} m^{-1} MPa^{-1}) were calculated as:

$$k_{s\,a/m} = \frac{K_{act/max} * Length}{A_{cond}} \qquad \text{(Eqn. 7)}$$

Finally, percent loss of conductivity (PLC) of the respective root tissue and species was calculated as the fraction of k_{sa} from k_{sm}:

$$PLC = \frac{k_{sm} - k_{sa}}{k_{sm}} * 100\ (\%) \qquad \text{(Eqn. 8)}$$

4. Abstracts and contributions to the individual publications

1. Article I: Hydraulic redistribution under moderate drought among English oak, European beech and Norway spruce determined by deuterium isotope labeling in a split-root experiment

Hafner, B. D., Tomasella, M., Häberle, K.-H., Goebel, M., Matyssek, R., & Grams, T. E. E. (2017): *Tree Physiology*, *37*(7), 950–960. doi: 10.1093/treephys/tpx050

Hydraulic redistribution (HR) of soil water through plant roots is a crucial phenomenon improving the water balance of plants and ecosystems. It is mostly described under severe drought, and not yet studied under moderate drought. We tested the potential of HR under moderate drought, hypothesizing that (H1) tree species redistribute soil water in their roots even under moderate drought and that (H2) neighboring plants are supported with water provided by redistributing plants. Trees were planted in split-root systems with one individual (i.e., split-root plant, SRP) having its roots divided between two pots with one additional tree each. Species were 2- to 4-year-old English oak (*Quercus robur* L.), European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst). A gradient in soil water potential (ψ_{soil}) was established between the two pots (−0.55 ± 0.02 MPa and −0.29 ± 0.03 MPa), and HR was observed by labeling with deuterium-enriched water. Irrespective of species identity, 93 % of the SRPs redistributed deuterium enriched water from the moist to the drier side, supporting H1. Eighty-eight percent of the plants in the drier pots were deuterium enriched in their roots, with 61 ± 6 % of the root water originating from SRP roots. Differences in HR among species were related to their root anatomy with diffuse-porous xylem structure in both beech and—opposing the stem structure—oak roots. In spruce, we found exclusively tracheids. We conclude that water can be redistributed within roots of different tree species along a moderate ψ_{soil} gradient, accentuating HR as an important water source for drought-stressed plants, with potential implications for ecohydrological and plant physiological sciences. It remains to be shown to what extent HR occurs under field conditions in Central Europe.

Contributions: I finalized the design of the study and collected, analyzed and interpreted the data and drafted the manuscript. About 25 % of the data were taken from results of my master thesis (Hafner 2015). *Martina Tomasella* helped to design the study and supported me in collecting and interpreting the data. *Karl-Heinz Häberle* originally designed the study and helped to collect the data. *Marc Goebel* helped to run laser ablations for root cross-sections. *Rainer Matyssek* originally designed the study. *Thorsten Grams* helped to finalize the study design and supported me in data collection and interpretation. All co-authors critically revised the manuscript and gave final approval for publication. About 70 % of the work was done by myself.

2. Article II: Water potential gradient, root conduit size and root xylem hydraulic conductivity determine the extent of hydraulic redistribution in temperate trees

Hafner, B. D., Hesse, B. D., Bauerle, T. L. and Grams, T. E. E. (2020): *Functional Ecology*, 00:1–14. doi: 10.1111/1365-2435.13508

1. Hydraulic redistribution (HR) of soil water through plant roots is widely described; however its extent, especially in temperate trees, remains unclear. Here, we quantified HR of five temperate tree species. We hypothesized that both, HR within a plant and into the soil increase with higher water-potential gradients, larger root conduit diameters and root-xylem hydraulic conductivities as HR driving factors.

2. Saplings of conifer (*Picea abies*, *Pseudotsuga menziesii*), diffuse-porous (*Acer pseudoplatanus*) and ring-porous species (*Castanea sativa*, *Quercus robur*) were planted in split-root systems, where one plant had its roots split between two pots with different water-potential gradients (0.23–4.20 MPa). We quantified HR via deuterium labelling.

3. Species redistributed 0.39 ± 0.14 ml of water overnight (0.08 ± 0.01 ml/g root mass). Higher pre-dawn water-potential gradients, hydraulic conductivities and larger conduits significantly increased HR quantity. Hydraulic conductivity was the most important driving factor on HR amounts, within the plants (0.03 ± 0.01 ml/g) and into the soil (0.06 ± 0.01 ml/g).

4. Additional factors as soil-root contact should be considered, especially when calculating water transfer into the soil. Nevertheless, trees maintaining high-xylem hydraulic conductivity showed higher HR amounts, potentially making them valuable ‘silvicultural tools’ to improve plant water status.

Contributions: I designed the study, collected, analyzed and interpreted the data and drafted the manuscript. *Benjamin Hesse* helped collecting and analyzing the data. *Thorsten Grams* provided support for the design of the study and helped interpreting the data. *Taryn Bauerle* gave valuable input in data interpretation. All co-authors critically revised the manuscript and gave final approval for publication. About 85 % of the work was done by myself.

3. Article III: Friendly neighbor: Hydraulic redistribution accounts for one quarter of water used by neighboring drought stressed tree saplings

Hafner, B. D., Hesse, B. D. and Grams, T. E. E. (2020): In Preparation.

Hydraulic redistribution (HR) by tree roots may buffer drought events within individuals for temperate species, however its relevance for neighboring trees remains unclear. Here, we quantified HR to neighboring trees in mono- and mixed species combinations. We hypothesized that the amount of HR water taken up by neighbors positively correlates with their root length, number of root tips and root xylem hydraulic conductivity. Further, we hypothesized that neighbors receive more HR water when in monospecific than in mixed combinations. In a split-root experiment, one sapling redistributed water along its split root system from a moist to a dry pot. Via deuterium labeling, we quantified HR water in roots, stems and transpiration in an additional sapling in the dry pot. Amounts were correlated with its root length, number of tips and hydraulic conductivity of the root xylem. Neighboring saplings of all studied six temperate tree species received HR water. Overall, one quarter of HR water in roots (2.1 ± 0.4 ml), stems (0.8 ± 0.2 ml) and transpiration (1.0 ± 0.3 ml) originated from the split-root tree. In a 3-factor model considering root length, number of tips and root xylem hydraulic conductivity of the sapling in the dry pot, especially root length was significantly positively correlated with the amount of HR water. Trees in mono- and mixed combinations received the same amounts of HR water. Uptake of HR water by drought stressed saplings supports the assumption that HR contributes to increased growth and stability in mixed forests, where some tree species reach moist soil depths by deeper root systems and may redistribute water towards trees without access to this water source. Root proximity in monospecific and mixed species communities should be further investigated as a likely important prerequisite for HR benefit for neighbors.

Contributions: I designed the study, collected, analyzed and interpreted the data and drafted the manuscript. *Benjamin Hesse* helped collecting and analyzing the data. *Thorsten Grams* provided support for the design of the study and helped interpreting the data. All co-authors critically revised the manuscript and gave final approval for submission. About 85 % of the work was done by myself.

4. Article IV: Reverse conductivity for water transport and related anatomy in fine roots of six temperate tree species – a potential limitation for hydraulic redistribution

Hesse, B. D., Hafner, B. D. and Grams, T. E. E. (2019): *Journal of Plant Hydraulics*, 6 (e-001), 1-15. doi: 10.20870/jph.2019.1-8

Hydraulic redistribution (HR), the passive reallocation of water along plant structures following a water potential gradient, is an important mechanism for plant survival under drought. For example, trees with deeper roots reallocate water from deeper moist to shallower, drier soil layers sustaining their upper fine root system. The relevance of HR for temperate forest ecosystems is hardly investigated. Both environmental and tree internal factors limiting the capacity for HR, such as low water potential gradients or root anatomy, respectively, are not well understood. Here we investigate fine root anatomy and related capacity for reverse flow of water of six temperate tree species, i.e. *Acer pseudoplatanus*, *Castanea sativa*, *Fagus sylvatica*, *Picea abies*, *Pseudotsuga menziesii* and *Quercus robur* both in forward and reverse flow direction. Additionally, anatomy of primary and secondary roots was analyzed, to test the hypotheses that root anatomy is similar in primary and secondary roots (H1) and conductivity for forward and reverse flow of water in fine roots is identical (H2). In contrast to the two conifer species, most anatomical parameters, e.g. hydraulic conduit diameter and conduit density, were distinctly different between primary and secondary roots in the angiosperms. Therefore, H1 was not supported for angiosperm trees. The reverse flow of water in fine roots was reduced by approx. 40 % compared to the forward flow in angiosperms, while there was no difference in the conifers. Thus, H2 was confirmed for conifers while there was a significant difference for angiosperms. This reduction may be caused by vessel structure (e.g. tapering or secondary thickening elements), or perforation plate and pit architecture (e.g. width of aperture opening). Because of the reduced conductivity of reverse water flow, the ability of angiosperm trees to redistribute water along their root system might be lower than expected.

Contributions: I designed the study, and helped to collect, analyze and interpret the data. *Benjamin Hesse* collected, analyzed and interpreted the data and drafted the manuscript. *Thorsten Grams* provided support for the design of the study and helped interpreting the data.

All co-authors critically revised the manuscript and gave final approval for publication. About 45 % of the work was done by myself.

5. General discussion

1. Hydraulic redistribution by Central European tree species

Although HR is a passive effect, intuitively suggesting that it should occur in any species under favoring circumstances (Nadezhdina et al. 2010, Prieto et al. 2012), several studies could not detect HR in different species. These studies have in common that they were performed either in extreme (e.g. saline) environments (Zeng et al. 2006, Bazihizina et al. 2017) or on species with root anatomical or functional barriers to prevent HR (Hultine et al. 2003, Espino & Schenk 2009; chapter 5, paragraph 4). Therefore, a general occurrence of HR cannot always be presumed and the first question of this dissertation work was hence to clarify if common Central European tree species showed HR. To estimate HR for a broad spectrum of Central European species, the trees analyzed for this thesis were chosen to cover a range of different conduit diameters. The anatomical prerequisites differed between the species, from conifers with small conduits to ring-porous deciduous species with large vessels in the xylem. Additionally, soil moisture conditions varied between moderately dry (article I) to severe drought conditions (articles II-IV). Independent of the species and premises, all trees showed redistribution of water from moist soils towards dry soils (research question 1). In this thesis, HR has been shown for the first time in *A. pseudoplatanus*, *C. sativa*, *F. sylvatica* and *Q. robur*, increasing the number of Central European tree species known to redistribute soil water. Extreme environments as described above are scarce in temperate regions. Therefore, a frequent occurrence of HR by common Central European tree species can be expected.

2. Quantified amount of HR water and its implications for the plants

The experimental design allowed for calculating the fractions of water that were redistributed. In addition, amounts of water that were redistributed within root systems, released into the (rhizosphere) soil and towards neighboring plants could be estimated (article I) or accurately calculated (articles II & III; research question 1). Across species, HR within the root system amounted to 0.20 ± 0.03 ml per night (estimated from article I) and 0.13 ± 0.03 ml per SRP root and night in article II, respectively. For both studies, the quantity of HR water seemed low, but was enough to decrease PLC of the SRP when compared to the DP (article II). Concurring with this finding, it has also been suggested that HR might decrease embolism formation in roots (Domec et al. 2004, 2006, Prieto & Ryel 2014). Split-root plants also released HR water into the surrounding rhizosphere. Released amounts again seemed small with 0.26 ± 0.06 ml per SRP root and night across species (article II). However, already small amounts of water between root surface and rhizosphere may suffice to maintain root-soil contact (Neumann & Cardon 2012), easing nutrient or water uptake after soil re-watering. Additionally, roots that retain a hydration shell in dry soils may live longer, reducing carbon-costs to the plant (Bauerle et al. 2008). Interestingly, after 7 days, the rhizosphere was only significantly enriched in ^{2}H in *A. pseudoplatanus* ($P < 0.001$), *C. sativa* ($P < 0.05$) and *Q. robur* ($P < 0.01$) (study of article II; data not shown). In addition, the bulk soil was not significantly enriched in ^{2}H after 3 days (article I) and only in one species (*A. pseudoplatanus*, $P < 0.05$) after 7 days (study of article II; data not shown). This indicated that HR water tended to stay in root proximity, where it potentially was frequently taken up again by the SRP during daytime without penetrating far into the soil (Caldwell et al. 1998, Meunier et al. 2017).

For neighbor plants, the HR water seemed very relevant, accounting for 25 ± 2 % of DP tissue and transpiration water in article III (0.30 ± 0.06 ml of labeled HR water per night in DP roots) and up to estimated 61 ± 6 % of DP root water in article I (5.74 ± 0.98 ml of labeled HR water per night; research question 2). For *P. abies* and *F. sylvatica*, uptake of HR water by the DP also happened if the SRP was of another species (article III), demonstrating that HR between different Central European tree species occurs. The amounts of HR water were not different for the respective DPs if in mixture or in monoculture (article III). In addition, there was no difference in the amounts of HR water if the SRP was healthy or not (article I), showing that HR also occurs in desiccated or dead plants. It has been suggested that HR may only stop, once the root system is decayed (Leffler et al. 2005). Therefore, HR displayed a very relevant mechanism for potted saplings in the experiments of this thesis. The results suggest, that HR may play a crucial role also for mature trees of the Central European species analyzed here (Pretzsch et al. 2014; Ammer et al. 2018). However, the actual relevance of HR in temperate Central European forests needs to be tested.

When comparing amounts of labeled HR water in the SRP roots in the dry pot with amounts taken up by the DP roots in article I (Fig 3a), more HR water was found in the DPs than in the SRPs in all species, however only significantly in *P. abies* ($P < 0.01$). For *F. sylvatica* ($P = 0.07$) and *Q. robur* ($P = 0.1$) a distinct trend could be observed. For the species in article III (including unpublished data, Fig. 3b), there were no significant differences between HR water amounts in SRP roots in the dry pot and DP roots, besides in *P. abies*. In *P. abies*, again significantly more HR water was in the DPs than in the SRPs ($P < 0.01$). Therefore, especially *P. abies* DPs seemed to profit substantially from HR water (article III). The ratios were substantially different between the studies (see different scaling of the y-axis in Fig. 3a &b). Compared to HR water amounts in the SRPs, DPs in the SRS of article I took up more HR water than DPs in the SRS of article III. Differences in HR water uptake by DPs between the studies will be discussed in the next paragraph (chapter 5, paragraph 3).

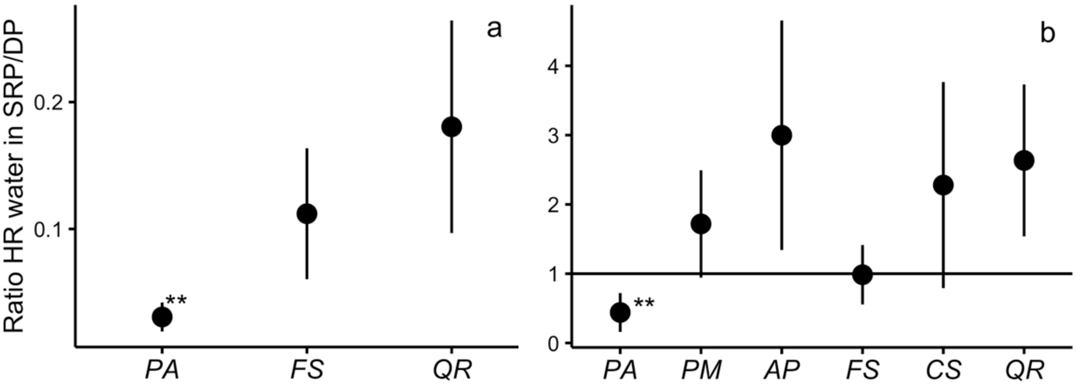

Figure 3: Amounts of labeled HR water found in roots of the SRP in the dry pot divided by the amounts of HR water found in DP roots in article I (**a**) and article III (including unpublished data; **b**). Values higher than 1 indicate more HR water in the SRP than in the DP. Vice versa, values below 1 indicate more HR water in the DP roots compared to the SRP roots. Abbreviations for tree species are: PA (*P. abies*), PM (*P. menziesii*), AP (*A. pseudoplatanus*), FS (*F. sylvatica*), CS (*C. sativa*), QR (*Q. robur*). Asterisks indicate significant differences between HR amounts in SRPs and DPs (** $p < 0.01$). Note the different scaling of the y-axis between **a** & **b**.

3. Amount of HR water in the context of other studies

Several studies quantified amounts of HR water by different species or along different directions (HL, lateral HR, downward HR) as summarized by Neumann & Cardon (2012). Many of these studies estimated HR amounts in the field (e.g. Richards & Cadwell 1987, Caldwell & Richards 1989), while some others studied HR in controlled environments (Hawkins et al. 2009) or simulated quantities in soil–vegetation–atmosphere water transfer models (Jackson et al. 2000, Scholz et al. 2010). Also, environmental conditions varied between those studies from mesic (e.g. Dawson 1993, Emerman & Dawson 1996) towards very arid surroundings (Ryel et al. 2003, Bleby et al. 2010). Finally, also the methods used to determine HR were different. While some quantified HR via sap flow measurements (e.g. Scott et al. 2008, Bleby et al. 2010) others detected changes in soil moisture/ ψ (DaRocha et al. 2004, Domec et al. 2010) or applied stable isotope tracers (Kurz-Besson et al. 2006). Differences in environmental conditions or methodological approaches may be the reason, why reported amounts of HR water vary by two orders of magnitude (Neumann & Cardon 2012). In this dissertation, amounts of HR water were not only quantified, but additionally, driving factors were determined that explain the amplitude of assessed HR values.

When comparing amounts that were redistributed inside the SRPs between articles I & II, quantities were in good agreement (chapter 5, paragraph 2). When studying HR over several days, it is difficult to calculate HR water amounts during a single night using stable isotope mixing models after a labeling event. For this comparison, the amount of HR water from article I was divided by the number of nights (3). However, this can only give an approximation of the actual daily HR water amounts and could slightly over- or underestimate the actual daily HR quantity. That is, because the label signal may accumulate in the studied tissues over time, while trees also use fractions of the redistributed water during the day (Caldwell et al. 1998, Meunier et al. 2017). Therefore, the study from article II is unique as it quantified all HR water during just one night in both, the plant and the rhizosphere. As amounts were still in good agreement, patterns driving HR within plants were potentially similar between articles I & II. Therefore, as the same species were studied, the finding that plant's 'internal' factors are important prerequisites for HR quantities is supported. When comparing HR amounts found in the DP, quantities varied considerably (chapter 5, paragraph 2). Several potentially important 'external' factors might explain this variation. First, different types of soils were used, that potentially influenced root penetration and root-soil contact (Pregitzer et al. 2002, Carminati et al. 2009) and might have had different water hydraulic conductivities (Prieto et al. 2010). Second, as indicated by pre-dawn leaf ψ measurements, drought stress for the DPs was probably less and the moisture gradients were lower in article I than in article III. Combined with different light regimes (chapter 2, paragraph 2), this probably resulted in different transpiration rates and therefore uptake of (HR-) water between the species of the two studies. Finally, also the time that plants could grow in pots before the experiments varied (0.5 years in article I and 1 to 2 years in article III). Therefore, also root length, number of root tips or the degree of mycorrhization might have been different between the studies. These parameters have been shown to significantly influence HR water uptake by neighboring saplings (Warren et al. 2008, Prieto et al. 2016, article III), and therefore might explain why neighboring saplings in article I took up more water than saplings in article III. Studies on uptake of HR water by neighbors among similar species or comparable environmental conditions are scarce, but indicate quantities in the range of HR amounts reported in this thesis (25 ± 2 % in article III and 61 ± 6 % in article I). In neighboring *Acer saccharum* Marshall trees, HR water contributed to 3-60 % of plant tissue water (Dawson 1993), while 0-22 % of the water found in *P. menziesii* saplings originated from HR by mature trees (Schoonmaker et al. 2007). The range of HR water found in these studies is very high and various driving factors may have an impact on the amounts. As mentioned in this chapter, differences in soil types or moisture gradients may have an influence on uptake of HR water. Additionally, the proximity of a neighbor to the redistributing tree and its root system distribution are suspected as influential factors on the amount of HR water taken up by neighbors (Schoonmaker et al. 2007).

4. Environmental conditions and plant characteristics influencing HR

As suggested (Neumann & Cardon 2012, article II), factors that influence HR can be divided into 'external' environmental conditions and plant 'internal' characteristics. Some studies found factors of both categories that may inhibit HR. In saline soils, likely pre-dawn plant ψ is not in equilibrium, but a ψ gradient between roots and leaves is present, promoting night-time water flow from roots to shoots rather than HR towards the soil (Bazihizina et al. 2017). In hyper-arid deserts, no HR could be detected in various perennial desert species. These species only had few roots in dry soil layers (Zeng et al. 2006), indicating that no dimorphic root system, i.e. roots being present in moist and dry soil layers, was established, which is obligate for HR (Scholz et al. 2008). Also other plant 'internal' characteristics may suppress HR: Competitive water sinks in stems (Hultine et al. 2003) or specialized cell structures as warts in root vessels, the structure of perforation plates or vessel tapering (article IV) may reduce or even prevent reverse flow of water (Espino & Schenk 2009). In my thesis I focused on the 'external' factor ψ gradient, indicated as difference between pre-dawn leaf ψ between the MP and the DP. As mentioned (chapter 1, paragraph 6), the ψ gradient combines several potential external factors as transpiration or soil properties. The gradient should therefore be a good measure of 'external' influences on HR quantity. Further, the 'internal' factors root conduit diameter and actual root xylem hydraulic conductivity (k_{sa}) were intensively studied as their role on HR is yet underrepresented in the literature.

a. Factors influencing HR within the SRP

The ψ gradient had a significant influence on the amount of HR water with higher gradients resulting in more water being redistributed within the SRP (article II, research question 3). Gradients in article I were set up to be equal, whereas gradients in article II ranged between 0 - 4 MPa. However, a distinct range in gradients of 0.0 – 0.6 MPa between replicates was still present in article I. By trend also for trees in article I higher gradients resulted in more HR water ($R^2 = 0.1$, $P = 0.05$; Fig. **4**), supporting the results from article II.

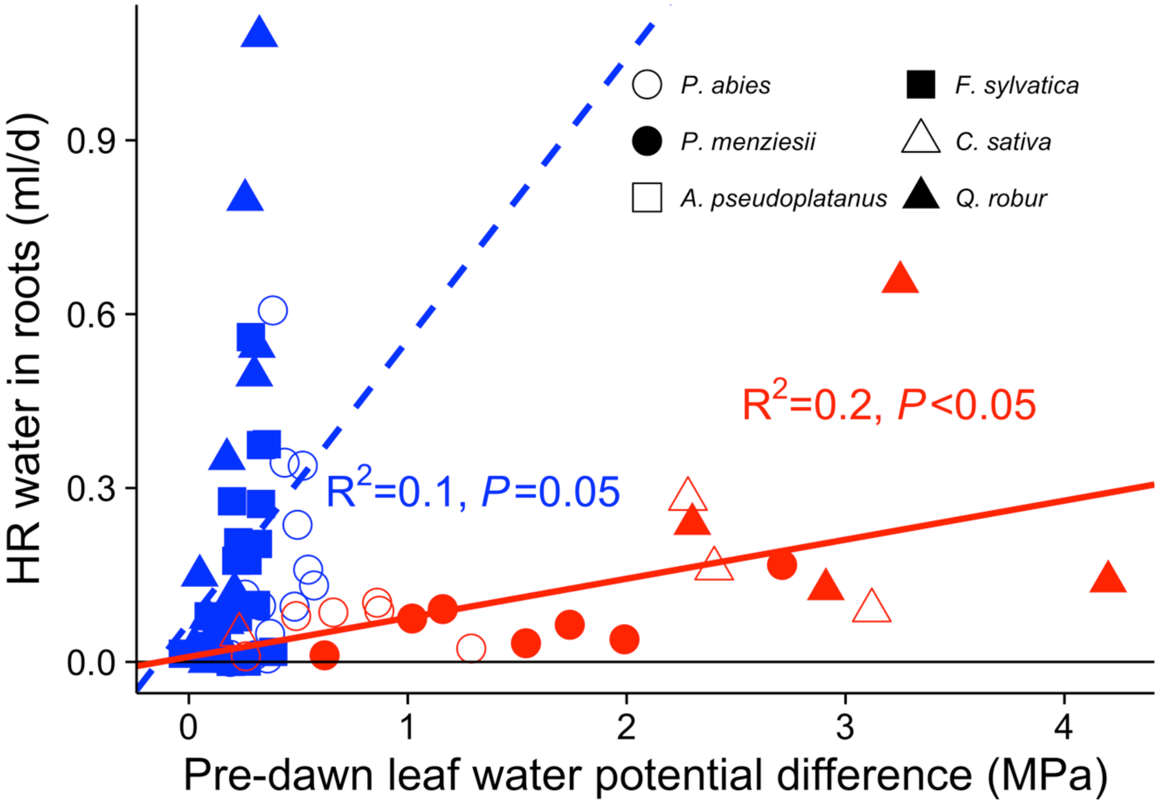

Figure 4: Correlation of water potential (ψ) gradient with the amount of labeled HR water found in the roots of the SRP in the dry pot after one night in article I (**blue**, y = 0.07 + 0.49 x). As reference, also the correlation of article II is given (**red**, y = 0.01 + 0.07 x). Differences in slope and amounts of water may be due to the fact that amounts from study I were divided by the amount of nights that may only give an approximation of the actual amount of HR water (chapter 5, paragraph 3).

The influence of the ψ gradient was, however, not as significant as the influence of 'internal' conduit diameters and especially k_{sa} (article II, research question 3). Differing amounts of HR water between the replicates of the species were best explained by these 'internal' factors. Thereby, SRPs with larger conduits and higher k_{sa} redistributed more water. While for article I the conduit diameters were used as a surrogate for k_{sm}, actual measured k_{sa} supported the dependency of HR from water conductivity in plants (article II). A trending positive correlation between estimated amount of HR water and conduit diameters (not significantly though) was also found for replicates of article I (Fig. **5**).

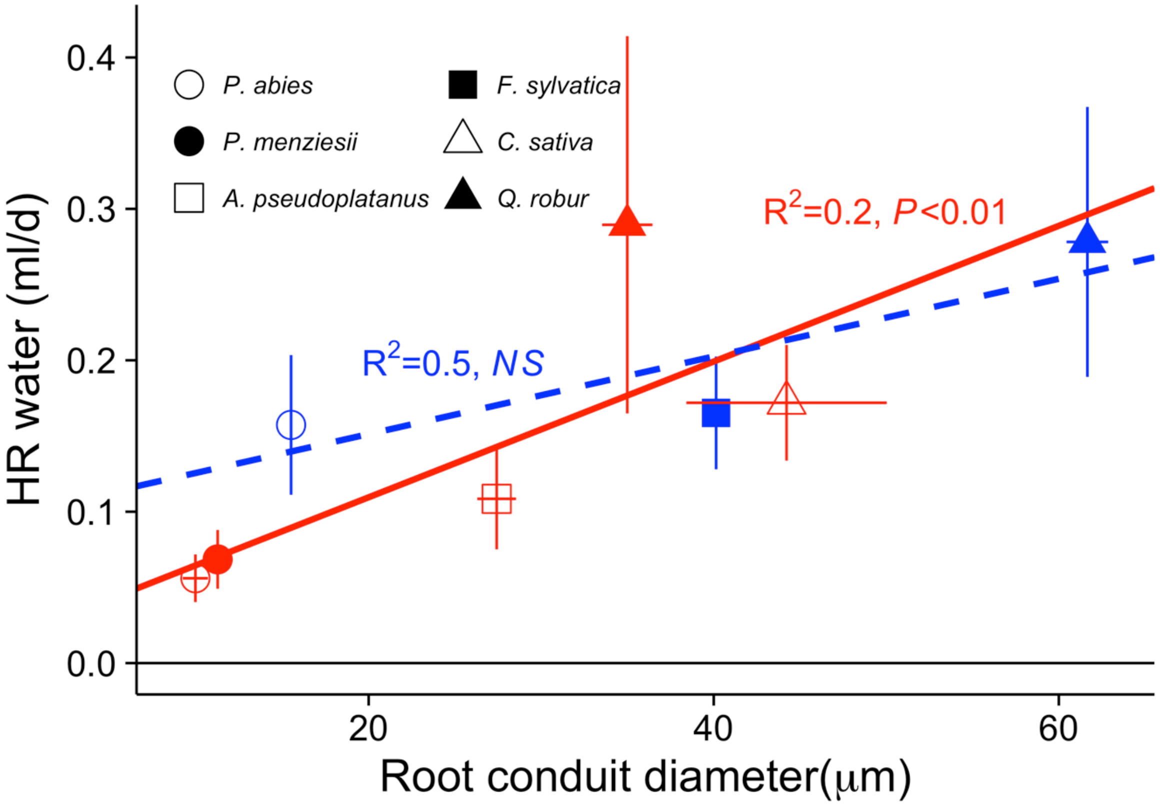

Figure 5: Correlation of mean root conduit diameters with the average amount of HR water per night and species in article I (**blue**, y = 0.10 + 0.003 x). Note that conduit diameters were recalculated as for article I all conduits (i.e. vessels and tracheids) were assessed for the angiosperm species, while in article II only the average vessel diameter was calculated. Therefore, tracheids were excluded for the recalculation of conduit-diameters for *F. sylvatica* and *Q. robur*. As reference, also the correlation of article II is given (**red**, y = 0.02 + 0.004 x).

Another plant 'internal' factor affecting HR water amounts within plants is a potential reduction in reverse water flow compared to forward flow (article IV; research question 4). As reverse flow in angiosperm species was only approx. 40% of forward flow, HR could be significantly reduced. Additionally, xylem conduit diameters varied considerably throughout the roots, with primary roots having significantly smaller diameters (article IV). This could display an additional resistor slowing HR water movement. This study is the first that shows a positive correlation of measured HR water amounts in Central European tree species to measured k_{sa}, that has previously only been predicted in a modeling approach (Quijano & Kumar 2015). As suggested in article II, studies should follow-up that concentrate on whole root system k_{sa} measurements in forward and reverse direction in more detail.

b. Factors influencing release of HR water into the rhizosphere

Amounts of water released into the soil were less well correlated with the assessed internal and external factors than for the redistribution of water within the SRPs (article II). Still, k_{sa} explained the variation best, but additional factors, e.g. at the border between root and soil, will certainly play a role for the release of water. Those might be similar to factors controlling water uptake. For example, diffusion barriers for water in the bark, as embedded suberized cells or the mere bark thickness might limit release and uptake of water (Brunner et al. 2015). Under drought, aquaporin activity along root sections may be down regulated, affecting root k_s (Prieto et al. 2012) and therefore decrease water flow in both directions (Maurel et al. 2015, Li et al. 2014). Additionally, plants are able to adjust osmotic potentials in their roots in order to take up water from soils with low moisture (Ranney et al. 1991, Wang et al. 1995). A low osmotic potential in roots in very dry soils may reduce root ψ to lower values than soil ψ, resulting in a moisture gradient from soil to root. Consequently, the release of water into the soil will be reduced or hindered. Finally, also the contact between roots and soils potentially influences the amounts of water a plant releases into the soil. As soils dry, roots loose contact to soil particles and therefore uptake from or release of water into the soil might be reduced (Carminati et al. 2009). Root-soil contact in drying soils may be dependent on soil type and fine root diameter, root branching or architecture (Pregitzer et al. 2002). Roots may lose contact to soils with a stronger swelling and shrinking behavior (e.g. loamy soils) earlier than to soils that do not form cracks that fast (e.g. soils with a higher sand content; Carminati et al. 2009). However, sandy soils have a coarser texture, and therefore less plant readily available water than loamy soils. Therefore, water movement between root and soil might be greater in loamy soils. By trend, higher amounts of HR water have been found in soils with higher loam and less sand content (Prieto et al. 2010). Additionally, species with thinner fine roots and higher root branching may be able to maintain contact to soil structures longer under drying conditions than species that have grosser fine roots that lose contact earlier (Carminati et al. 2009). A more detailed assessment of soil type influence on HR seems, hence, valuable.

Studies with different soils and plants are needed to estimate the influence of root-soil contact on HR.

c. Factors influencing uptake of HR water by neighbor plants

Root system extent was found to be a significant driving factor in uptake of HR water by neighboring plants (article III). Over all species, root length, number of root tips, but also k_{sa} of the root system of the DP had a significant influence on uptake of HR water. As mentioned in chapter 5, paragraph 2, very little HR water was found in the soils, with almost no HR water in the bulk soil. Therefore, probably most of the HR water was transferred either via direct root contact between species or through a CMN. Previous studies suggest, that a CMN or a direct contact between root systems of neighboring plants may display effective pathways of HR water transfer (Warren et al. 2008, Prieto et al. 2016). The chance that roots of neighboring plants get in contact may increase with root length. Additionally, trees take up most water through their root tips (Häussling et al. 1988), potentially explaining that longer root systems with more tips were found to increase uptake of HR water by the DPs (article III). However, as pointed out in article III, some species seem to avoid contact (Maina et al. 2002, Falik et al. 2003) while others increase root growth when close to neighbors (Armas & Pugnaire 2011). Therefore, also the tendency of a species to make contact to root systems of neighbors should be assessed. Consistently, *P. abies* DP trees showed the highest amounts of HR water in roots and transpiration in articles I & III (also in mixture with *F. sylvatica*). However, none of the studied factors could explain why (tested also for DPs in article I; not shown). Neither did *P. abies* SRS have the highest ψ gradients nor significantly different pre-dawn leaf ψ in the DP compared to other species. Also plant internal factors were not related to this finding, as DP root systems were shorter and root tips less abundant (article III) than in other species. First studies have been performed, suggesting that *Picea mariana* (Mill.) Britt., E. E. Sterns & Poggenburg trees tend to increase root branching when growing close to a neighbor of the same or another species (Paya et al. 2015). However, as concluded in article III, all species should be studied on their tendency to make or avoid contact with their roots to root systems of other plants.

6. Conclusion and outlook

1. Importance of HR for Central European tree species and forests

The results of the present thesis show that HR plays an important role in the water cycles of a variety of Central European species from coniferous over diffuse-porous to ring-porous trees. For both, water redistributing SRPs and drought stressed DPs, drought-mitigating effects of HR water could be demonstrated. Therefore, HR might play a key role for species survival under drought and hence also majorly influence forest ecosystem stability in Central Europe. The surprisingly high benefits of *P. abies* DPs sets hope that this species, already highly affected by drought events, can be sustained in the future on different sites, if it is "supported" via HR. Systematic field studies should now confirm the relevance of HR mechanisms and effects demonstrated in this thesis. They should include monocultures and different mixtures of species on comparable sites to estimate the importance of plant 'internal' driving factors. Additionally, field studies with the same species, but among different climate conditions on various sites should be considered to gain information on the importance of 'external' driving factors. Next to moisture gradients also the soil type could vastly influence HR, with e.g. soils that build cracks faster when drying, leading to an earlier loss of root-soil contact and reducing HR amounts, accordingly.

2. Increasing importance in the face of ongoing climate change?

On the one hand, in a warmer and probably dryer climate, the role of HR for the water balance of trees in Central Europe might increase. Especially in uneven aged or mixed forest systems on sites with deep soil depths, deeper rooting trees may display "water pumps" under drought, lifting water from moist, deep soil layers. This HR water may then be used the following day by shallow rooted neighboring plants or saplings and therefore help to mitigate forecasted increasing drought events. Additionally, the ψ-gradient was determined as one of the main driving factors of HR in this thesis. Increased summer droughts could likely increase moisture gradients between shallow and deep soil layers, thereby enhancing the amount of water species might redistribute. On the other hand, HR itself might be affected by future climate. When the atmosphere gets warmer, it has a higher water holding capacity and therefore the atmospheric water demand might increase during the day and at night. Thus, nighttime transpiration rates might increase, which could lower the amount of HR as a competitive sink for water (Howard et al. 2009, Domec et al. 2012). The actual influence of a dryer climate on HR has to be tested in future controlled studies to assess the impacts of climate change on HR and on Central European species.

3. Potential importance as a tool for silviculture

Foresters are changing Central European forests increasingly into uneven aged, mixed species stands to deal with climate change driven forest diebacks. Here, I could show that also in mixture, redistribution of water from a redistributing (SRP) towards a neighboring (DP) plant occurred (article III). In this approach testing mixed species compositions (i.e. mixed SRS), amounts of HR water were not different as in monospecific SRS. However, the SRS setup could not incorporate different rooting depths between the species in the field. Therefore, species that were proven to be able to conduct HR, but only form a shallow root system in the filed might only profit from HR when in mixture with species that have a deeper root system reaching moist soil layers. HR in the field might therefore be most relevant in mixed or uneven aged forests, where trees vary significantly in rooting depths.

A very clear pattern across the studies summarized in this thesis was the importance of k_{sa} on HR. Species that maintained high root conductivities in dry soils significantly redistributed more water than species with lower k_{sa}. If one dares to extrapolates these findings under controlled conditions to the situation in a forest, a strategic planting of “high performance HR trees”, such as deep rooting ring-porous species with low susceptibility to drought or embolism formation, may contribute to higher forest stabilities in the future. However, more driving mechanisms for water transfer between species than root length and number of tips, have still to be identified. In article I and also in a previous study on a grass (Leffler et al. 2005) the ability of ‘dead’ trees to conduct HR has been described. As long as the root system is not decayed, stumps are still capable to redistribute soil water. Therefore, also new strategies in timber harvesting could be considered. Deep rooted species could be removed first, while their stumps continue for many years to transfer water to shallow rooting trees or saplings of the next generation of the forest. Scaling the amounts of HR water by SRPs, determined in this thesis (0.04 - 0.19 ml per night and g SRP root mass, article II) to mature trees (about 100 kg of root dry mass assumed), total amounts of HR water could reach 4 - 19 l per tree and night (article II). With an average stock of wood of 695 trees ha^{-1} (BWI3, 2014), 2780 – 13205 l per ha and night could be lifted in a forest ecosystem. Therefore, the importance of HR in the water cycle of temperate forests would be substantial, similar as indicated in tropic environments (Lee et al. 2005). However, as the numbers presented here result from saplings under controlled conditions, mixture composition and impact of HR trees to neighbors have to be tested in future field studies. The scale is set by this study and further research on HR in mature stands seems very promising to contribute to solutions for stable future forests.

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Danksagung

An erster Stelle möchte ich meinem Betreuer, **Thorsten Grams**, danken, der immer für mich da war, wenn die Experimentplanung verfeinert werden musste, die Datenanalyse Fokussierung benötigte oder Sätze zu lang wurden. Unsere gemeinsamen Diskussion gingen zudem oft über den wissenschaftlichen Tellerrand hinaus, sodass ich nicht nur wissenschaftlich, sondern auch menschlichen an unseren Treffen gewachsen bin.

Mein Dank geht auch an **Rainer Matyssek**, der mich durch die Anfangsphase meiner Dissertation als Lehrstuhlleiter geführt und währenddessen sehr unterstützt hat. Ihm verdanke ich auch die wertvolle Erfahrung, erste Vorlesungen geben zu dürfen. Dafür, überhaupt erst mein Interesse an HR geweckt zu haben und für viele inspirierende Gespräche möchte ich mich bei **Karl-Heinz Häberle** bedanken. Bei **Manuela Baumgarten** bedanke ich mich für die wertvolle Hilfe bei der Vorbereitung auf die Vorlesungen.

Meine Doktorarbeit wäre ohne die Hilfe unseres Teams am Lehrstuhl für Ökophysiologie der Pflanzen nicht möglich gewesen. An erster Stelle möchte ich mich bei **Peter Kuba** bedanken, ohne den kein einziges Experiment hätte stattfinden können. Unzählige Besuche in diversen Baumärkten und produktives Grübeln und Tüfteln an komplizierten Aufbauten und Mechanismen haben meiner Arbeit eine zusätzliche Komponente gegeben, die ich nicht missen möchte. **Yessica Kölmel** als wohl liebenswerteste Kollegin danke ich für die Messungen meiner Isotopenproben. Die gemeinsame Abenteuer-Reise nach München um ein funktionierendes Pumpenteil zu überprüfen bleibt in ewiger Erinnerung. Auch ihrem Vorgänger, **Christoph Seidler** möchte ich an dieser Stelle für die Messung zahlreicher Proben danken und für die Einführung in den Umgang mit Massenspektrometern und der Cryo-Destillationsanlage. Eine sehr wichtige Stütze war auch **Thomas Feuerbach**, ohne den kein technisches Gerät funktioniert hätte, insbesondere auch die Transpirations-Messanlage. Bei **Roman Meier**, der mir in diversen Funktionen beigestanden hat, möchte ich mich für die gemeinsame Zeit am Massenspektrometer und in TUMmesa bedanken. Außerdem für unzählige Gespräche, die meiner Arbeit oft einen Schubs in die richtige Richtung gegeben haben. Auch **Sepp Heckmeier** war eine wichtige Unterstützung. Wenn es um Probenahmen und Aufräumarbeiten ging, war auf ihn zuverlässig immer Verlass. Für ihre Hilfe möchte ich auch unseren Praktikanten, Bachelor- und Masteranden sowie HiWis danken. Namentlich hervorheben möchte ich **Aline Potreck**: für ihre zuverlässige und saubere Hilfe bei der Aufarbeitung all der angefallenen Proben, von der Wurzel bis zum Blatt und für die besondere Freundschaft, die sich zwischen uns seither entwickelt hat.

Unseren Sekträterinnen, **Karin Beerbaum**, **Helga Brunner** und **Martina Harnisch** möchte ich sehr herzlich danken für das Abwickeln aller bürokratischer Aufwände und für viele Gespräche und teils hitzige Diskussionen in den Kaffeerunden, die jeden Tag am Lehrstuhl lebendig gemacht haben.

Bei meinen Mit-Doktoranden und Post-Docs möchte ich mich besonders bedanken. Doktoranden sind keine Einzelkämpfer, ohne den steten Austausch wäre es sehr schwer die verschiedenen Phasen einer Doktorarbeit zu meistern. Deshalb vielen Dank, **Timo Gebhardt**, **Martina Tomasella** und **Michael Goisser** für Diskussionen, Zuhören, Unterstützung und den ein oder anderen Tritt. Die wichtigste Stütze unter den Doktoranden Kollegen war **Benjamin Hesse**, der mich bei jedem Experiment praktisch und theoretisch zu jeder Tages- und Nachtzeit unterstützt hat. Unsere Zusammenarbeit hat bei „BDHa“ und „BDHe“ denke ich zu einem sehr robusten Selbstbewusstsein und Selbstverständnis geführt.

Auch bei den ausgeschiedenen Kollegen möchte ich mich für ihre vielfältige Unterstützung in der Anfangsphase meiner Arbeit bedanken. So bei **Frank Fleischmann**, **Johanna Lebherz** und **Tina Schmidt**. Auch meinen Kollegen an der Cornell Universität gilt mein Dank, für die Erweiterung meines wissenschaftlichen Horizonts und die Gastfreundschaft während meines Aufenthalts in Ithaca, **Taryn Bauerle** und **Marc Göbel**. Meinem Mentor **Karl Auerswald** möchte ich vor allem dafür danken, mir wichtige Tipps für eine strukturierte Arbeitsweise gegeben zu haben, gerade wenn mehrere Projekte zeitgleich anstanden.

Mein großer Dank geht an die Deutsche Bundesstiftung Umwelt, die meine Dissertation mit ihrem Promotionsstipendium erst ermöglicht und durch Seminare und Veranstaltungen auch inhaltlich erweitert hat. Bei der Graduate School der TUM in Weihenstephan bedanke ich mich für die freundliche Unterstützung und die Finanzierung meines Cornell-Aufenthalts.

Abschließend möchte ich mich bei meiner Familie und Freunden bedanken, die mich über die Jahre begleitet und geprägt haben. Mein größter Dank gilt meinen Eltern und meiner Schwester, die mich während der gesamten Zeit uneingeschränkt immer unterstützt haben.

Appendix

- Article I
- Article II
- Article III (draft)
- Article IV
- Approval letter from Tree Physiology for article I

Tree Physiology 37, 950–960
doi:10.1093/treephys/tpx050

Research paper

Hydraulic redistribution under moderate drought among English oak, European beech and Norway spruce determined by deuterium isotope labeling in a split-root experiment

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Received September 2, 2016; accepted May 2, 2017; published online May 24, 2017; handling Editor Maurizio Mencuccini

Hydraulic redistribution (HR) of soil water through plant roots is a crucial phenomenon improving the water balance of plants and ecosystems. It is mostly described under severe drought, and not yet studied under moderate drought. We tested the potential of HR under moderate drought, hypothesizing that (H1) tree species redistribute soil water in their roots even under moderate drought and that (H2) neighboring plants are supported with water provided by redistributing plants. Trees were planted in split-root systems with one individual (i.e., split-root plant, SRP) having its roots divided between two pots with one additional tree each. Species were 2- to 4-year-old English oak (*Quercus robur* L.), European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst). A gradient in soil water potential (ψ_{soil}) was established between the two pots (−0.55 ± 0.02 MPa and −0.29 ± 0.03 MPa), and HR was observed by labeling with deuterium-enriched water. Irrespective of species identity, 93% of the SRPs redistributed deuterium enriched water from the moist to the drier side, supporting H1. Eighty-eight percent of the plants in the drier pots were deuterium enriched in their roots, with 61 ± 6% of the root water originating from SRP roots. Differences in HR among species were related to their root anatomy with diffuse-porous xylem structure in both beech and—opposing the stem structure—oak roots. In spruce, we found exclusively tracheids. We conclude that water can be redistributed within roots of different tree species along a moderate ψ_{soil} gradient, accentuating HR as an important water source for drought-stressed plants, with potential implications for ecohydrological and plant physiological sciences. It remains to be shown to what extent HR occurs under field conditions in Central Europe.

Keywords: drought, *Fagus sylvatica*, isotope labeling, *Picea abies*, *Quercus robur*, reverse flux of water in roots, soil water potential (ψ_{soil}), stable hydrogen isotopes.

Introduction

Hydraulic redistribution (HR) is the passive movement of water through the roots of a plant following a gradient in the soil water potential (ψ_{soil}, Leffler et al. 2005). Water is taken up by roots present in moist soil locations and released via roots into drier soil locations (Richards and Caldwell 1987). Hydraulic redistribution typically occurs during nighttime in the absence of or at low transpiration upon mostly closed stomata (Pereira et al. 2006). While Richards and Caldwell (1987) first described the effect as 'hydraulic lift', as they discovered an uplift of water from deeper, moist to more shallow, dry soil horizons, the flow path of redistributed water can also be downward ('inverse hydraulic lift', iHL) (Burgess et al. 1998) or lateral (Smart et al. 2005), depending on the direction of the ψ_{soil} gradient. In order to cover all potential directions of water movement, the effect was named HR (Burgess et al. 1998, Prieto et al. 2012).

Plants benefit in many ways from HR, for instance, roots in dry soil depths can be kept alive, their lifespan can be increased

(Bauerle et al. 2008) and consequently, co-located nutrients become accessible (Caldwell et al. 1998, Querejeta et al. 2003). Hydraulic conductance can be maintained and a decline in soil water content (SWC) reduced (Hirota et al. 2004, Meinzer et al. 2004, Domec et al. 2006). Therefore, rates of transpiration, photosynthesis and growth are increased through HR, and growing seasons may be prolonged (Brooks et al. 2002, Howard et al. 2009). In a North American sugar maple (*Acer saccharum* Marsh.) stand, Jackson et al. (2000) assessed an increase in stand-level water consumption by 19–40% when they considered HR in a soil–vegetation–atmosphere water transfer model. Moreover, Brooks et al. (2002) calculated redistributed water to be 28% of total daily transpired water in a Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) stand in Washington, USA. In addition, mutualism with microorganisms and mycorrhizae is likely to benefit from HR as water moves from plant to fungus or vice versa depending on the ψ gradient (Querejeta et al. 2003, Pereira et al. 2006).

Hydraulic redistribution of one plant may influence its neighboring plants. On the one hand, plants with shallow roots, like grasses or seedlings, are able to take up redistributed water in the upper soil (Dawson 1993, Brooks et al. 2002). On the other hand, iHL may be a strategy of deep-rooting plants to drain water into deeper soil horizons where it becomes inaccessible for shallow-rooted plants (Ryel et al. 2003, Yu and D'Odorico 2014). The effectiveness of HR on plant–plant interactions awaits case-dependent clarification (Prieto et al. 2012).

The amount of redistributed water could be species-specific caused by different xylem structures, e.g., conduit diameters in roots (Bauerle et al. 2008). According to the Hagen–Poiseuille law, hydraulic conductivity of a conduit is (approximately) proportional to the fourth power of its radius (Tyree and Zimmermann 2002). Plants with bigger conduit diameters should be able to redistribute more water over time than plants with smaller conduit diameters under otherwise identical conditions. However, density, systematic and size of root conduits are not well known (Koecher et al. 2012).

Authors have predominantly observed HR in very dry regions or during dry seasons on grasses, shrubs and trees (e.g., Caldwell and Richards 1989, Schulze et al. 1998, Meinzer et al. 2004), while in temperate Central Europe, only few studies have addressed HR on native tree species. Nadezhdina et al. (2006, 2009) detected HR in a 26-year-old Norway spruce (*Picea abies* (L.) Karst.) stand and in a 53-year-old Douglas fir (*P. menziesii*) stand in the Czech Republic under dry conditions of about 10 vol% SWC. Zapater et al. (2011) showed that HR occurred in sessile oak (*Quercus petraea* (Matt.) Liebl.) from a 15- to 25-year-old mixed species forest in France under very dry conditions (ψ_{soil} up to −2.0 MPa). However, uptake of redistributed water by neighboring plants could not be demonstrated in any of those studies (Nadezhdina et al. 2006, 2009, Zapater et al. 2011).

Despite the detection of HR in some tree species in temperate Central Europe, it remains an open question whether HR is a general phenomenon, also occurring under moderate drought, and if neighboring plants gain advantage from HR. Moreover, it remains to be shown if potential differences in the amount of redistributed water between different species can be linked to their anatomical root properties.

The present greenhouse experiment focused on three tree species native to Central Europe, i.e., English oak (*Quercus robur* L.), European beech (*Fagus sylvatica* L.) and Norway spruce (*P. abies*). Our aim was (i) to explore if and to what extent the study species show HR even under moderate drought and (ii) to estimate the uptake of released water by neighboring trees. We set up a split-root experiment with a soil moisture gradient between two pots and traced HR from the deuterium-labeled moist soil to the drier soil. We tested the hypotheses that (H1) English oak, European beech and Norway spruce are able to redistribute soil water via reverse flux in their root systems under moderate drought and that (H2) neighboring trees take up water provided by the redistributing trees. To elucidate the impact of species identity on the amount of redistributed water, we additionally analyzed the anatomy of the root xylem to assess the hydraulic conductance of the roots of the three study species.

Materials and methods

Experimental site

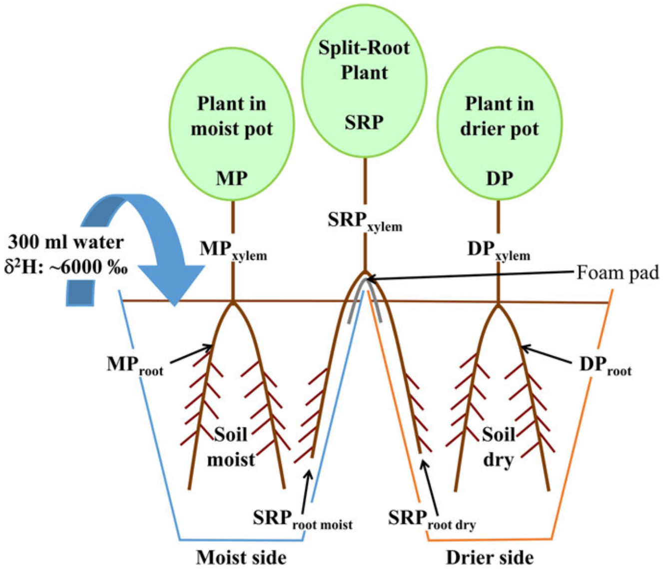
The study was set up in a greenhouse, automatically shaded and aerated during March–August 2014 at the 'Gewächshauslaborzentrum Dürnast', Freising, Germany (N 48°24′15.6″; E 11°41′35.7, 473 m altitude). During the establishment of the soil moisture gradient (July–August 2014), daily mean and daily maximum air temperature was 23 ± 0.5 and 28 ± 1 °C, respectively. Corresponding daily mean and minimum relative air humidity was 58 ± 1 and 43 ± 2%, respectively. Mean and maximum global radiation in the greenhouse during sampling days (19–22 August) was 28 ± 3 and 61 ± 13 W m^{-2}, respectively (for details see Table S1 available as Supplementary Data at *Tree Physiology* Online).

Split-root system setup

In March 2014, 2-year-old English oak and European beech and 4-year-old Norway spruce seedlings were planted in the greenhouse in split-root systems (Figure 1). In spite of having different ages, the mean height of the trees was similar, i.e., 46 ± 1 cm (oak, $n = 41$), 51 ± 1 cm (beech, $n = 52$) and 51 ± 1 cm (spruce, $n = 39$). Two pots (10 l) were screwed together with a semicircular notch at the contact point. Three trees were planted in each split-root system – with one tree in each pot (i.e., moist side and drier side) and the third tree (split-root plant, SRP) above the notch with its root system equally distributed into the two pots (Figure 1). To minimize injury to the roots of the SRP, a foam pad was placed between the roots and the notch in the pots.

In the first 5 months after establishing the split-root systems (March–July), each SRP trunk had a soil-filled collar made out of foam material to avoid desiccation of the upper roots. The collar was removed 4 weeks before the labeling experiment started. The split-root systems were watered manually twice a week with a hose and, between March and August, repositioned three times inside the greenhouse to minimize potential effects of light and temperature heterogeneities.

Three different plant combinations were established, each with the same tree species as SRP and plant in the drier soil (DP). The plant located in the moist soil (MP) was either the same species as DP and SRP or one of the other study species (Table 1), as we originally assumed an impact by the MPs identity on the water redistributed by the SRP. However, the species identity of the MP affected neither HR within the SRP nor redistribution to the DP.



Abbreviation	Denotation
MP	Plant on the moist side of the system
MP_{root}	Root system of the plant on the moist side
MP_{xylem}	Stem xylem of the plant on the moist side
SRP	Split-root plant
$SRP_{root\ moist}$	Root system of the split-root plant on moist side
$SRP_{root\ dry}$	Root system of the split-root plant on drier side
SRP_{xylem}	Stem xylem of the split-root plant
DP	Plant on the drier side of the system
DP_{root}	Root system of the plant on the drier side
DP_{xylem}	Stem xylem of the plant on the drier side

Figure 1. Illustration of split-root system and description of the abbreviations used.

Table 1. Setup of the split-root systems. Species identity is given for trees planted on the moist side (MP), the split-root plant (SRP) and on the drier side (DP).

Split-root system	MP	SRP	DP	*n*
Beech	Beech/oak/spruce	Beech	Beech	17
Oak	Oak/beech	Oak	Oak	14
Spruce	Spruce/beech	Spruce	Spruce	13

The soil used for the split-root system setup was a luvisol, originated from loess over Tertiary sediments, retrieved from three adjacent forest stands (~5 km from Freising) and with similar silty texture. Each stand was dominated by one of the three study species, thus saplings were planted in soils shaped by their own species. About 30% of sand was added to facilitate soil drying. Five grams of controlled-release fertilizer (Osmocote®, ICL Fertilizers Deutschland GmbH, Nordhorn, Germany) were added to each pot when the trees were planted.

Soil water content and water potential

Volumetric SWC was assessed for each pot with a mobile probe (probe length: 14.5 cm; TDR100, Campbell Scientific, Shepshed, UK). Corresponding soil water potential (ψ_{soil}) was retrieved by fitting a soil-specific retention curve between pre-dawn ψ_{leaf} and SWC using pre-dawn ψ_{leaf} as a surrogate for ψ_{soil} (Bauerle et al. 2006). ψ_{leaf} was assessed by means of a Scholander-type pressure chamber on leaves (oak) and twigs (beech and spruce) from additional trees of the same origin and age, potted individually in identical soils, saving the plants in the split-root systems for the deuterium-labeling experiment. The retention curve was compiled before the experiment started (data not shown). Soil water content was measured on Day 2 of the experiment (21 August) and corresponding ψ_{soil} derived.

Establishing a soil moisture gradient, deuterium labeling and sampling

In July 2014, the soil moisture gradient was established between the moist and drier pots of the split-root systems. Over 1 week, the average daily water consumption of the plants was assessed by weighing of pots. Based on the water consumption, the moist side of the split-root system was supplied with water for compensating tree water use while the pot on the drier side was not watered. Soil water content was monitored by TDR every other day with 30% and 15% being targeted in the moist pot and drier pot, respectively. After the soil moisture gradient was established, the deuterium labeling started.

On 19 August at 9:00 a.m. CET (Day 0), 300 ml of deuterium-enriched water (0.11 atom% deuteriumoxide, i.e., 6313‰ δ^2H) was dripped very carefully and homogenously on the soil surface of the MP pot using a small watering can (capacity of 500 ml) to ensure that no labeled water reached the pot of the DP. Afterwards, the soils of the drier and moist sides were each covered with a plastic foil throughout the experiment. Putatively deuterium-enriched water vapor originating from the labeled soils or transpiration of labeled trees did not increase δ^2H in leaves of neighboring plants ($P = 0.71$).

Eleven out of the 44 SRPs (25%) displayed severe signs of shoot mortality during the weeks before the experiment, i.e., absence of green bark tissue and all leaves were brown or shed (for details see Table S2 available as Supplementary Data at *Tree Physiology* Online). As HR is a physical phenomenon and water passively moves through plant tissue by just following a ψ_{soil} gradient, we did not exclude those plants (further referred to as desiccated plants) from the analysis. Indeed, we did not find significant differences in the calculated fraction of redistributed water between desiccated and healthy plants. Our results and conclusion would not change if desiccated plants were excluded from the analysis (see also Results and Discussion sections).

On 19, 20 and 22 August (further referred to as Day 0, Day 1 and Day 3) before sampling at 7:00 a.m. CET, aboveground parts of plants were covered with plastic bags to minimize transpiration and therefore to establish similar hydraulic conditions during sampling. Plastic bags were removed directly after sampling on each day, to reestablish natural conditions.

Prior to labeling on Day 0, from 7:00 to 9:00 a.m. CET, soil samples were taken in all pots with a metal core (diameter of ~1 cm) to the bottom of the pot. All soil samples were put into plastic bags and gently mixed before drawing a representative 10 ml subsample. Pre-labeling samples were taken to determine the natural abundance of deuterium in the soil and were used as reference to check for increases in δ^2H in roots, soils and stem xylem (White et al. 1985). Likewise, soil samples were taken on Day 1 and Day 3.

On Day 3, stem xylem and roots were sampled. Bark and phloem of stem samples were quickly removed with razor blades and the stem xylem was cut into ~1 cm long pieces to facilitate subsequent water extraction. Plants in the drier pots (DPs) were sampled before SRPs and MPs to minimize potential contamination with deuterium label. Sampling of stem xylem lasted from 9:00 a.m. to 12:00 p.m. CET. Subsequent root sampling lasted from 12:00 p.m. to 6:00 p.m. CET and was performed in the same order as stem xylem sampling (DPs before SRPs and MPs). The roots of the SRPs were divided into drier side ($\text{SRP}_{\text{root dry}}$) and moist side ($\text{SRP}_{\text{root moist}}$). The rhizosphere soil was carefully removed, coarse-roots were cut into ~1 cm pieces and a subsample of ~3–4 g (root fresh mass) was taken. All samples were instantly put into airtight Exetainer (Labco, Lampeter, UK) vials and stored at 5 °C until cryogenic water extraction.

Dry mass of fine (<2 mm) and coarse (>2 mm) roots of the $\text{SRP}_{\text{root dry}}$ and $\text{SRP}_{\text{root moist}}$ was assessed after drying at 65 °C for 72 h and total root dry mass was calculated including the roots sampled for water extraction.

Water extraction and analysis

Water was extracted by cryogenic vacuum distillation for 2 h (West et al. 2006). Extracted plant water of roots and xylem of the DPs and SRPs was analyzed for its δ^2H with an isotope-ratio-mass-spectrometer (IRMS) coupled with a multiflow system (Isoprime, Elementar, Langenselbold, Germany). Measurements were performed against two laboratory standards with δ^2H of −161.21‰ and +124.36‰, respectively. Measurement precision for the two standards was higher than ±2.5‰. In the case of water extracted from soils and highly deuterium-enriched root and stem xylem samples of MPs and SRPs, δ^2H was assessed with a cavity ring-down spectrometer (CRDS, Picarro, Santa Clara, CA, USA). We used the same laboratory standards as for the IRMS and analysis precision was higher than ±2.1‰. Since the CRDS may be affected by organic contaminants in the water extract (West et al. 2010), comprehensive correlations between the CRDS and the IRMS were performed, revealing no differences in the δ^2H of soils and highly enriched stem xylem or root samples (data not shown).

Calculation of water redistribution within SRP

The fraction of redistributed water in the $\text{SRP}_{\text{root dry}}$ was calculated using a two-end-member mixing model. To this end, δ^2H of water in $\text{SRP}_{\text{root dry}}$ on Day 3 was assumed to result from two water sources: (i) reverse flux of water from the $\text{SRP}_{\text{root moist}}$ and (ii) water taken up directly by the roots from the soil of the drier pot:

$$f(\text{SRP}_{\text{root moist}}) = \frac{\delta^2\text{H}(\text{SRP}_{\text{root dry}}) - \delta^2\text{H}(\text{soil dry})}{\delta^2\text{H}(\text{SRP}_{\text{root moist}}) - \delta^2\text{H}(\text{soil dry})} \times 100(\%) \quad (1)$$

with $f(\text{SRP}_{\text{root moist}})$ being the fraction of redistributed water in the $\text{SRP}_{\text{root dry}}$.

Calculation of water redistribution from $\text{SRP}_{\text{root dry}}$ to DP

Similarly, the fraction of water in DP_{root} originating from the $\text{SRP}_{\text{root dry}}$ was calculated from two sources: (i) the water provided by the $\text{SRP}_{\text{root dry}}$ to the drier side and (ii) the soil water in the drier pot:

$$f(\text{SRP}_{\text{root dry}}) = \frac{\delta^2\text{H}(\text{DP}_{\text{root}}) - \delta^2\text{H}(\text{soil dry})}{\delta^2\text{H}(\text{SRP}_{\text{root dry}}) - \delta^2\text{H}(\text{soil dry})} \times 100(\%) \quad (2)$$

Root anatomy

Roots (diameters of ~1 mm) were sampled to assess conduit sizes in the studied tree species. The samples were dehydrated with ethanol; 2 h in 70%, subsequently 3 h in 90% and finally overnight in 96% ethanol. Three roots of each species were cut by laser ablation tomography (Clabby 2014), while pictures of the ablated root slices were continuously photographed (resolution of 25,400 dpi). Afterwards ablated pictures of each species were analyzed for the size of their xylem conduits. On about one-quarter of the total cross-section, the conduits were hand-selected and marked on an extra image layer with gimp 2.8.16 (GNU Image Manipulation Program, The GIMP Team). Conduit area was obtained with ImageJ 1.47t (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) and the equivalent circle diameter of each conduit was retrieved according to Scholz et al. (2013). Hydraulic conductivity per square meter of the root cross-section was calculated based on the Hagen–Poiseuille law (modified according to Scholz et al. 2013, see Eq. (3)), assuming all visible vessels and tracheids to be conductive:

$$K_h = \frac{\sum_{i=1}^{n} \frac{\pi \times D^4}{128\eta}}{A} \tag{3}$$

where K_h is the hydraulic conductivity (in kg MPa^{-1} s^{-1} m^{-1}), D the diameter of the conduit, η the viscosity index of water (1.002×10^{-9} MPa s at 20 °C) and A the area of the analyzed cross-section.

Statistical analyses

With a one-sided two-sample *T*-test we checked for increases in leaf δ^2H of unlabeled trees due to water vapor evaporation or transpiration of labeled soils or plants. A two-sided two-sample *T*-test was used to check (i) for initial differences in the isotopic signature of the soil between the moist and the drier pot prior to labeling, (ii) for differences in root mass of $\text{SRP}_{\text{root dry}}$ and $\text{SRP}_{\text{root moist}}$, (iii) for effects of the species identity of MPs on the fractions of redistributed water within SRPs, and (iv) to confirm that δ^2H of unlabeled soil water corresponded to δ^2H of unlabeled root and stem xylem samples (White et al. 1985). We performed one-sided two-sample *T*-tests to check if δ^2H values in root, soil and stem xylem were significantly increased when compared with the soil reference δ^2H values on Day 0. One-way ANOVA with post-hoc tests (Tukey HSD test) were used to compare δ^2H of roots and stem xylem of the three plant combinations. Levels of statistical significance were <0.05, <0.01 and <0.001 and indicated with *, ** and ***, respectively. Means are generally shown with ±1 standard error (SE). All statistical analyses were performed with R version 3.1.3 (R Development Core Team 2015).

Results

Soil water content and ψ_{soil}

Soil water content (SWC) and corresponding ψ_{soil} were twice as high in the moist pots compared with the drier pots ($P < 0.001$, Table 2). Averaged SWC at the moist side amounted to 32.6 ± 0.6 vol% and to a ψ_{soil} of −0.29 ± 0.03 MPa across all split-root systems. In comparison, SWC at the drier side and corresponding ψ_{soil} was significantly lower, with a mean of 17.0 ± 0.2 vol% and −0.55 ± 0.02 MPa across all split-root systems, respectively. Comparing species, the SWC and corresponding ψ_{soil} in the drier pot of spruce split-root systems were about 2 vol% and 0.2 MPa lower than in beech ($P < 0.01$) and oak ($P < 0.001$, Table 2).

Dry mass of fine-roots

The fine-root mass of spruce $\text{SRP}_{\text{root dry}}$ exceeded the corresponding $\text{SRP}_{\text{root dry}}$ fine-root mass of both, beech ($P < 0.05$) and oak ($P < 0.001$). In the moist pot, the fine-root mass of spruce $\text{SRP}_{\text{root moist}}$ tended to be higher compared with beech ($P = 0.056$) and was significantly higher than in oak ($P < 0.05$, Table 3).

Table 2. Volumetric SWC and corresponding water potential (ψ_{soil}) (means ± 1 SE). SWC was measured on Day 2 of the experiment. SWC and ψ_{soil} are significantly higher on the moist side than on the drier side for all plant combinations. Letters indicate statistical differences across species.

	SWC (vol%)		*P*	ψ_{soil} (MPa)		*P*
	Moist	Drier		Moist	Drier	
Beech	32.3 ± 1.1^a	17.3 ± 0.3^a	<0.001	-0.29 ± 0.01^a	-0.52 ± 0.02^a	<0.001
Oak	33.4 ± 0.8^a	17.8 ± 0.4^a	<0.001	-0.29 ± 0.01^a	-0.47 ± 0.02^a	<0.001
Spruce	32.2 ± 1.0^a	15.8 ± 0.3^b	<0.001	-0.28 ± 0.01^a	-0.67 ± 0.03^b	<0.001
Mean	32.6 ± 0.6	17.0 ± 0.2	<0.001	-0.29 ± 0.03	-0.55 ± 0.02	<0.001

Table 3. Fine-root and total root mass of the SRPs (means ± 1 SE). Letters indicate statistical differences across species. Note that there was only one desiccated SRP of spruce.

	Beech	Oak	Spruce	Mean
SRP total root mass (g)				
Drier side	4.09 ± 0.72^a	3.72 ± 0.75^a	3.76 ± 0.50^a	3.97 ± 0.39
Moist side	3.40 ± 0.87^a	5.13 ± 1.21^a	5.36 ± 1.15^a	4.48 ± 0.61
Desiccated drier side	1.49 ± 0.19^a	0.89 ± 0.30^a	1.26^a	1.20 ± 0.18
Desiccated moist side	0.79 ± 0.40^a	1.02 ± 0.35^a	0.38^a	0.82 ± 0.24
SRP fine root mass (g)				
Drier side	1.32 ± 0.28^a	0.58 ± 0.21^a	2.24 ± 0.42^b	1.45 ± 0.22
Moist side	1.75 ± 0.46^{ab}	1.17 ± 0.38^a	3.59 ± 0.78^b	2.14 ± 0.35
Desiccated drier side	0.05 ± 0.02^a	0.01 ± 0.01^a	0.26^b	0.05 ± 0.02
Desiccated moist side	0.06 ± 0.02^{ab}	0.00 ± 0.00^a	0.15^b	0.05 ± 0.02

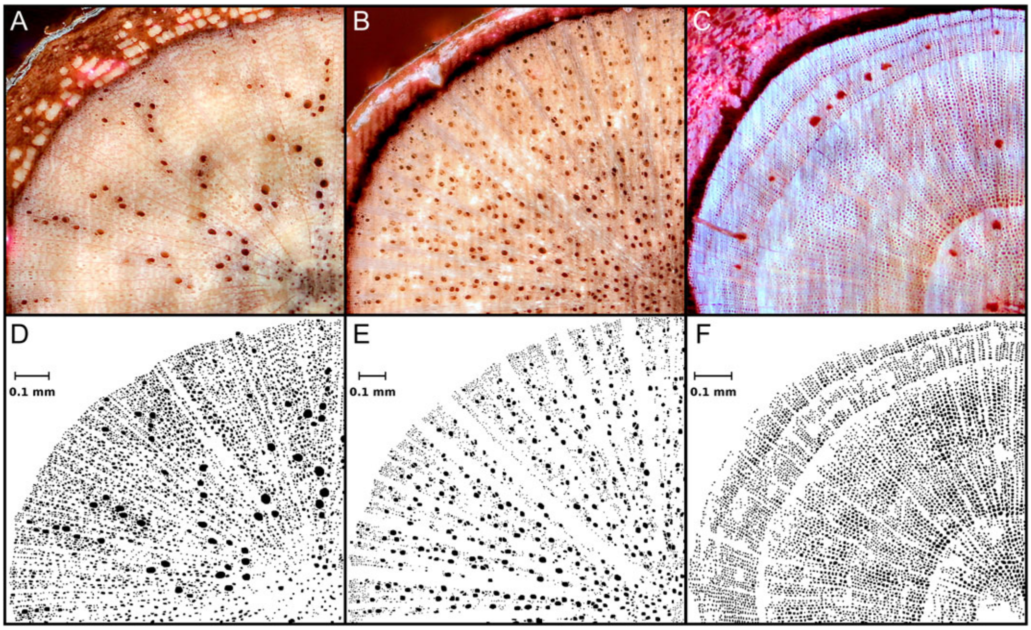


Figure 2. Cross-sections of oak (A), beech (B) and spruce (C) roots and the different distribution of xylem vessels and tracheids in black and white contrast (D, E and F, respectively).

Analysis of xylem conduits in the roots

The commonly assumed diffuse-porous structure in beech stems (Schütt et al. 2006) was reflected in beech roots, whereas the characteristic ring-porous distribution of conduits in oak stems did not match with the anatomy found in oak roots. As assumed, spruce roots had exclusively tracheids (Figure 2). Maximum root conduit diameters were 93.7 ± 7.1 μm (oak, $n = 3$), 75.4 ± 2.1 μm (beech, $n = 3$) and 44.4 ± 6.0 μm (spruce, $n = 3$), respectively, and were significantly smaller in spruce compared with beech ($P < 0.05$) and oak ($P < 0.01$). The maximum conduit size of oak was similar to beech ($P = 0.12$, Table 4). The estimated conductivity of spruce (1.95 ± 0.19 kg $\text{MPa}^{-1}\,\text{s}^{-1}\,\text{m}^{-1}$) tended to be lower compared with beech (8.48 ± 1.38 kg $\text{MPa}^{-1}\,\text{s}^{-1}\,\text{m}^{-1}$, however this was not significant, $P = 0.1$) and was significantly lower than in oak (11.42 ± 2.85 kg $\text{MPa}^{-1}\,\text{s}^{-1}\,\text{m}^{-1}$, $P < 0.05$). The difference between beech and oak was not significant ($P = 0.5$, Table 4).

Table 4. Calculated hydraulic conductivity, maximum and mean conduit diameter (means ± 1 SE) of the roots of the three study species. Letters indicate statistical differences across species.

	Hydraulic conductivity (kg $\text{MPa}^{-1}\,\text{s}^{-1}\,\text{m}^{-1}$)	D_{max} (μm)	D_{mean} (μm)
Beech	8.48 ± 1.38^{ab}	75.4 ± 2.1^{a}	16.8 ± 0.2^{a}
Oak	11.42 ± 2.85^{a}	93.7 ± 7.1^{a}	16.7 ± 0.1^{a}
Spruce	1.95 ± 0.19^{b}	44.4 ± 6.0^{b}	15.5 ± 0.0^{b}

δ^2H in soils

On Day 0, prior to labeling, the δ^2H in the soil water of the moist and drier side were identical, and unaffected by species identity (Table 5). Upon deuterium labeling, δ^2H in the soil on the moist side was evidently deuterium-enriched across all species (972 ± 72 and 916 ± 47‰ on Day 1 and 3, respectively). Water extracted from drier soils did not increase in δ^2H, varying between −60 ± 1 and −63 ± 1‰ across all species (Table 5).

δ^2H in MP, SRP and DP

Prior to labeling, δ^2H of root water corresponded to that of surrounding soils (data not shown). MPs were significantly deuterium-enriched in their stem xylem water on Day 3 (mean: 763 ± 45‰; $P < 0.001$ across all species) when compared with Day 0. In the SRPs of all species, water in the SRP$_{\text{root moist}}$ was significantly deuterium-enriched on Day 3 compared with Day 0 (mean δ^2H of 413 ± 33‰, Figure 3). There was no difference between species or between desiccated (337 ± 58‰) and healthy plants (438 ± 39‰). The overall mean δ^2H of the stem xylem water in the SRPs of all species was 327 ± 44‰ on Day 3 (no difference between the species). We detected a significant difference ($P < 0.001$) when measuring the desiccated plants (mean of −19 ± 7‰) in comparison with the healthy plants (mean of 443 ± 43‰).

Irrespective of species identity, more than 90% of SRP$_{\text{root dry}}$ displayed significantly enriched deuterium concentrations on Day 3 compared with Day 0 ($P < 0.001$ for all observed species) with δ^2H of −36 ± 6, −42 ± 5 and −46 ± 4‰ in oak, beech and spruce, respectively (Figure 3). There was no significant difference between desiccated (mean over all species of −37 ± 6‰) and healthy plants (mean over all species of −43 ± 3‰) for any of the species

Table 5. Soil δ^2H during the experiment (means ± 1 SE). There were no statistical differences between the split-root systems of beech, oak and spruce.

	Beech		Oak		Spruce		Mean	
	Moist	Drier	Moist	Drier	Moist	Drier	Moist	Drier
δ^2H Day 0 (‰)	−60 ± 1	−60 ± 1	−60 ± 1	−60 ± 2	−60 ± 2	−61 ± 1	−60 ± 1	−60 ± 1
δ^2H Day 1 (‰)	900 ± 115	−61 ± 1	938 ± 91	−63 ± 1	1115 ± 159	−62 ± 2	972 ± 72	−62 ± 1
δ^2H Day 3 (‰)	881 ± 66	−61 ± 1	940 ± 81	−60 ± 1	943 ± 114	−62 ± 1	916 ± 47	−61 ± 1

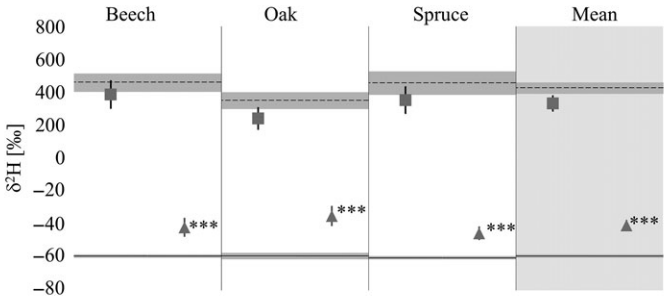


Figure 3. δ^2H of the SRPs (and overall means ± 1 SE) on Day 3 after labeling. The solid line (in gray bar) indicates the mean δ^2H (±1 SE) of the drier soil on Day 0, while the dotted line (in gray bar) represents the mean δ^2H (±1 SE) of the $SRP_{root\ moist}$ on Day 3. Both lines are given for orientation as they represent the two end-members of the linear mixing model in Eq. (1). The symbols indicate stem xylem (SRP_{xylem}, squares) and root water ($SRP_{root\ dry}$, triangles) on the drier side of the SRPs. Significant increases of δ^2H in comparison with Day 0 are given for $SRP_{root\ dry}$ by *** ($P < 0.001$). Note the different scaling of the *Y*-axis above and below 0.

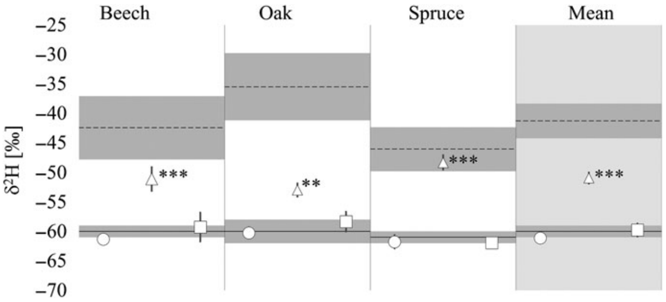


Figure 4. δ^2H values of the DP (and overall means ± 1 SE) on Day 3 after labeling. The solid line (in gray bar) indicates the mean δ^2H (±1 SE) of the drier soil on Day 0, while the dotted line (in gray bar) represents the mean δ^2H (±1 SE) of the $SRP_{root\ dry}$ on Day 3. Both lines are given for orientation as they represent the two end-members of the linear mixing model in Eq. (2). The symbols indicate soil water (circles) of the drier soil, root water (DP_{root}, triangles) and stem xylem water (DP_{xylem}, squares) of the DP. If no error bar is visible, SE was smaller than the symbol size. Significant increases of δ^2H in comparison with Day 0 are given for DP_{root} by ** ($P < 0.01$) and *** ($P < 0.001$).

(see Table S2 available as Supplementary Data at *Tree Physiology* Online).

The DP_{root} also showed deuterium enrichment when compared with Day 0. δ^2H values were −53 ± 1, −51 ± 2 and −48 ± 1‰ for oak, beech and spruce, respectively, and therefore significantly higher than before labeling ($P < 0.01$ for oak and $P < 0.001$ for beech and spruce, Figure 4). The increase in δ^2H of DP_{root} was present in 79% of oak, 88% of beech and in all DPs of spruce. However in DP_{xylem}, the deuterium label was not found in any species on Day 3, as δ^2H was not significantly increased above natural abundance on Day 0 (mean δ^2H of −60 ± 1‰ over all species, Figure 4).

Fractions of redistributed water in $SRP_{root\ dry}$ and into DP_{root}

Averaged over all observed species ($n = 44$), 5 ± 1% of the water in $SRP_{root\ dry}$ originated from $SRP_{root\ moist}$ (Eq. (1), Figure 5A). The fraction of redistributed water within oak trees (8 ± 2%) was similar to beech (4 ± 1%, $P = 0.1$) and was significantly higher than in spruce (3 ± 1%, $P < 0.05$). There was no significant difference between desiccated (7 ± 1%) and healthy plants (4 ± 1%). Regarding all observed DPs of all species ($n = 44$), we calculated that 61 ± 6% of the water extracted from DP_{root} originated from $SRP_{root\ dry}$ (Eq. (2), Figure 5B). More specifically, in spruce the contribution of water from $SRP_{root\ dry}$ to the water in DP_{root} was highest (82 ± 8%), while contribution was smaller in oak (46 ± 10%, $P < 0.05$) and tended to be smaller in beech trees (59 ± 10%, however this was not significant). The water taken up by the DP_{root} from the $SRP_{root\ dry}$ did not differ significantly in desiccated (50 ± 6%) versus healthy plants (65 ± 6%).

Discussion

Our results support both of our hypotheses, the redistribution of soil water under moderate drought via reverse flux within root systems of English oak, European beech and Norway spruce and the uptake of redistributed water by neighboring plants. The occurrence and importance of HR under very dry conditions have been demonstrated (e.g., ψ_{soil} of −2.0 MPa, Nadezhdina et al. 2006, 2009, Zapater et al. 2011). In addition, our results now stress the importance of HR for temperate Central European tree species also under moderate drought.

Hydraulic redistribution within the root system of split-root plants

Previous studies tracing HR by isotope labeling found plant roots to redistribute ~3% (Prieto and Ryel 2014) and 17% (Leffler et al. 2005, Hawkins et al. 2009) of applied labeled water. In

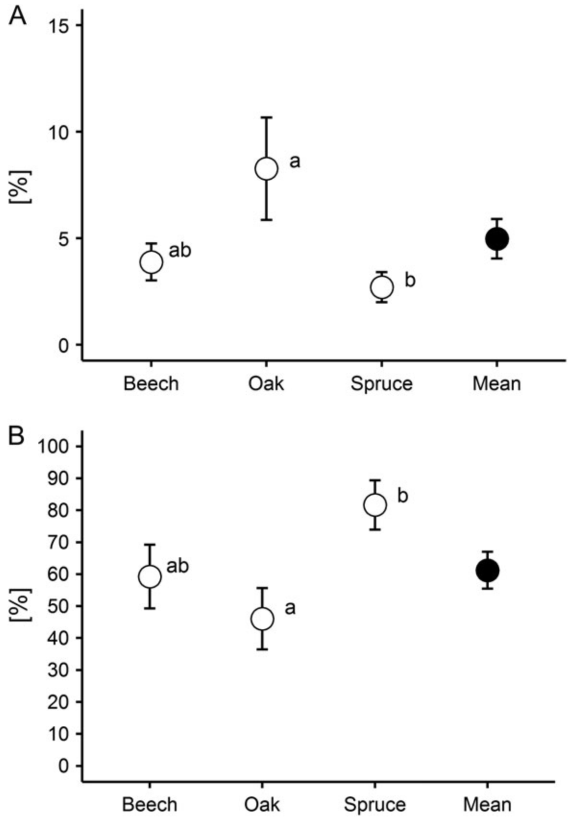


Figure 5. (A) Fraction of water (±1 SE) redistributed by the SRP from the roots in the moist pot ($SRP_{root\ moist}$) to the roots in the drier pot ($SRP_{root\ dry}$). (B) Fraction of water (±1 SE) taken up by the roots of the plant in the drier soil (DP_{root}) released by $SRP_{root\ dry}$. Letters indicate statistical differences between species. Note the different scaling between (A) and (B).

the present study, 3–8% of water taken up by the SRP in the moist pot was redistributed to the $SRP_{root\ dry}$. The fraction of redistributed water within the SRP was different for the three study species, being higher in oak than in spruce and intermediate in beech (Figure 5A).

In stems, ring-porous oak has the largest conduit diameter in comparison with diffuse-porous beech and spruce (tracheids only, Schütt et al. 2006). Based on the Hagen–Poiseuille law (Tyree and Zimmermann 2002), oak should be able to redistribute more water per unit of time than beech and spruce under otherwise identical conditions. As for all our study species ψ_{soil} was well above thresholds known to cause vessel embolisms (Cochard 1992, Cochard et al. 1992, 1999), we assumed that all conduits were fully conductive. Hence, the magnitude of HR within a single root mostly depends on its transport capacity and the driving force, i.e., the diameter of (water conducting) conduits and the water potential gradient, respectively (Warren et al. 2007). Indeed, spruce roots had significantly lower conduit diameters and hydraulic conductivities than oak, however, not significantly different from beech (Figure 2 and Table 4). Oak roots did not show the expected ring-porous conduit structure but were similar to diffuse-porous beech in size of conduits and hydraulic conductivity. The absence of ring-porous conduits in roots was reported for other stem ring-porous trees, such as *Fraxinus excelsior* (L.), *Castanea sativa* (Mill.) and *Q. petraea* (Koecher et al. 2012, Mrak and Gricar 2016). However, as the anatomy of root xylem was found to be very variable even in the same species and depending on environmental factors such as water limitation (Bauerle et al. 2011, Koecher et al. 2012), clarification about root anatomy is generally needed. Nevertheless, the fraction of redistributed water within the SRP appears to be positively correlated with the conduit size in the roots.

The potential for HR of the SRPs showing shoot desiccation was not different from the root systems of healthy plants, although total root mass and fine-root content were significantly lower in the desiccated compared with healthy plants (Table 3). The overall root system of the desiccated SRPs seemed to still be alive (no black tissue beyond the bark or other signs of decline) in most SRPs. Actually, 91% of the desiccated SRPs redistributed water within their root system towards the drier soil, similar to the 93% of the healthy SRPs. In addition, the fraction of redistributed water was similar between desiccated and healthy trees, stressing that HR is a passive process of water moving through plant roots driven by a ψ_{soil} gradient. Thus, as exclusion of desiccated plants did not affect any result presented here, we kept them included. Likewise, HR of senescing or dead plants to dry soil has been reported previously as long as their root system was not decayed (Leffler et al. 2005, Warren et al. 2008).

Redistributed water in the DP_{root}

We did not find deuterium label in the soil of the drier pots, either on Day 1 or on Day 3. The ψ_{soil} in the drier pot might not have been low enough (hardly exceeding −0.5 MPa) to force the water out of the roots. To some extent, plants are able to limit reverse water flux out of their roots by aquaporins (Prieto et al. 2012, Li et al. 2014) or may create other barriers, e.g., by increasing the suberization of the exodermis and endodermis in the roots (Bauerle et al. 2008). However, the deuterium-enriched water might have only penetrated into the rhizospheric soil directly surrounding water-releasing roots. As not only the rhizospheric soil water was sampled, but the whole soil profile of the pot, redistributed, labeled water in the rhizosphere soils might have been diluted by the surrounding bulk soil water and, thus, was not detected in the analysis. Likewise, soil sampling might have missed heterogenically distributed label in the drier soil, whereas roots of the DP might have reached these spots as labeled water was detected in DP_{root}. In addition, a putative direct flux between tree roots through ectomycorrhizal fungi may be a relevant mechanism (Querejeta et al. 2003, Egerton-Warburton et al. 2007, Plamboeck et al. 2007). As soils were taken from forest stands dominated by the study species, respective mycorrhizae were abundant and frequently observed

Table 6. Estimated amount of redistributed water in $SRP_{root\ dry}$ and DP_{root} (means ± 1 SE). Root water was estimated as: root mass × 3.5 (Sharpe and Mason 1992) and multiplied with the fraction of redistributed water, i.e., $f(SRP_{root\ moist})$ for the $SRP_{root\ dry}$ and $f(SRP_{root\ dry})$ for the DP_{root}, respectively.

	$SRP_{root\ dry}$ (ml)	DP_{root} (ml)
Beech	0.5 ± 0.1	14.0 ± 5.5
Oak	0.8 ± 0.3	15.0 ± 5.0
Spruce	0.5 ± 0.1	25.9 ± 4.8
Mean	0.6 ± 0.1	18.3 ± 3.8

in each pot during root sampling. Although there was no difference in total root mass (Table 3), the distribution of fine- and coarse-roots varied between the species. Spruce had a significantly higher fine-root mass than oak. Therefore, spruce SRPs may have had the most abundant mycorrhizal contact to the root systems of the DPs, explaining the higher fractions of redistributed water in spruce DPs (Figure 5B) and why we did not observe redistributed water in few DPs of oak and beech. In fact, for spruce, we observed root systems of SRPs and DPs to be strongly attached to each other and sometimes even obviously connected through a common mycorrhizal network. We did not assess root morphological parameters such as fine-root surface area or specific root length that may affect HR and hence the amount of redistributed water taken up by the DP (Eissenstat 1992, Warren et al. 2007). Those morphological parameters should be addressed in more detail in future studies.

We roughly estimated the amount of redistributed water in the $SRP_{root\ dry}$ and DPs. To this end we made the assumptions that (i) total root mass of the DPs was similar to the SRPs and (ii) root water content was 350% of the dry root mass (Sharpe and Mason 1992, see Table 6 for details of the calculations). Over all species, we found 0.6 ± 0.1 ml of redistributed water in the $SRP_{root\ dry}$ and 18.3 ± 3.8 ml in the DP_{root} (Table 6). The total amount of the water redistributed during the three experimental days may be higher as some of the water is likely to be used by transpiration and thus was not recovered in plants. Nevertheless, the fraction of redistributed water found in the root systems of the DPs was 61 ± 6% of their total root water. Similarly, Dawson (1993) found that neighboring plants used up to 60% of HR water lifted by sugar maple (*A. saccharum*). Over all study species, more than half of the plant water in the DP_{root} originated from HR of the SRP. Hence, this transfer of water by the SRP represents an effective mechanism of water exchange between plants, stressing the relevance and importance of HR for trees in drought-stressed forest ecosystems.

Conclusion

Hydraulic redistribution represents an important mechanism in plant responses to dry soil conditions as it improves the water balance of individual plants and whole stands, leading to increased growth and higher survivorship, especially of plant roots. We found HR of soil water in temperate Central European tree species, i.e., English oak, European beech and Norway spruce, even under moderate drought conditions. Neighboring trees growing in the drier soils made use of the water released from redistributing trees (SRPs). However, our findings result from a pot experiment, which subjects the study to certain restrictions, e.g., root growth is bound to pot size and root distribution might be potentially unfavorable (Passioura 1988, Poorter et al. 2012). As a next step, the occurrence of HR under moderate drought should be tested under field conditions to prove and quantify this facilitative effect in Central European forests. In addition, species-specific differences in the potential of HR due to different anatomical properties of roots should be addressed more carefully. Dead or senescent plants seem to provide capacities for redistributing water to living plants that should not be underestimated and should be further studied on given forest sites.

Supplementary Data

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

Acknowledgments

We thank two anonymous reviewers for their valuable comments on an earlier version of this manuscript. We would like to thank Christof Seidler and Dr Rudi Schäufele for the IRMS and CRDS water measurements, and Benjamin Hall and Asheesh Lanba of Lasers for Innovative Solutions (L4iS) for conducting laser ablation tomography.

Conflict of interest

None declared.

Funding

German Federal Environmental Foundation (to B.D.H.); Technische Universität München International Graduate School of Science and Engineering; Bavarian State Ministry for Nutrition, Agriculture and Forestry; Bavarian State Ministry of the Environment and Consumer Protection; German Research Foundation (grant no. MA 1763/7-1).

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Received: 28 June 2019 | Accepted: 1 December 2019

DOI: 10.1111/1365-2435.13508

RESEARCH ARTICLE

Functional Ecology BRITISH ECOLOGICAL SOCIETY

Water potential gradient, root conduit size and root xylem hydraulic conductivity determine the extent of hydraulic redistribution in temperate trees

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Funding information
Europamöbel Umweltstiftung; Hartig Stiftung; German Federal Environmental Foundation

Handling Editor: Emma Sayer

Abstract

1. Hydraulic redistribution (HR) of soil water through plant roots is widely described; however its extent, especially in temperate trees, remains unclear. Here, we quantified HR of five temperate tree species. We hypothesized that both, HR within a plant and into the soil increase with higher water-potential gradients, larger root conduit diameters and root-xylem hydraulic conductivities as HR driving factors.
2. Saplings of conifer (*Picea abies*, *Pseudotsuga menziesii*), diffuse-porous (*Acer pseudoplatanus*) and ring-porous species (*Castanea sativa*, *Quercus robur*) were planted in split-root systems, where one plant had its roots split between two pots with different water-potential gradients (0.23–4.20 MPa). We quantified HR via deuterium labelling.
3. Species redistributed 0.39 ± 0.14 ml of water overnight (0.08 ± 0.01 ml/g root mass). Higher pre-dawn water-potential gradients, hydraulic conductivities and larger conduits significantly increased HR quantity. Hydraulic conductivity was the most important driving factor on HR amounts, within the plants (0.03 ± 0.01 ml/g) and into the soil (0.06 ± 0.01 ml/g).
4. Additional factors as soil-root contact should be considered, especially when calculating water transfer into the soil. Nevertheless, trees maintaining high-xylem hydraulic conductivity showed higher HR amounts, potentially making them valuable 'silvicultural tools' to improve plant water status.

KEYWORDS
diffuse-porous temperate trees, drought, hydraulic redistribution driving factors, hydraulic redistribution quantity, ring-porous temperate trees, split-root experiment, stable water isotope labelling (^{2}H/deuterium), temperate conifer trees

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1 | INTRODUCTION

Hydraulic redistribution (HR) describes the passive flux of water through plants and their roots, for example from moist to dry soil layers. Roots that span soil layers across a soil water-potential (Ψ) gradient, take-up, redistribute and release water overnight, when transpiration is virtually absent. Initially described for (semi-) arid regions (e.g. Burgess, Adams, Turner, & Ong, 1998; Caldwell & Richards, 1989; Richards & Caldwell, 1987), HR has also been demonstrated in mesic environments (Dawson, 1993) and is now considered a general phenomenon occurring across different climates and ecosystems (e.g. Quijano & Kumar, 2015; Sardans & Peñuelas, 2014).

Hydraulic redistribution of soil water can facilitate plant health and growth in multiple ways. For example, roots in dry soil layers may stay alive, maintaining root life span (Bauerle, Richards, Smart, & Eissenstat, 2008) and functionality (Scholz et al., 2008) in suboptimal growing conditions. If water is released into the rhizosphere (Emerman & Dawson, 1996), roots may retain soil contact (Ryel, Leffler, Peek, Ivans, & Caldwell, 2004), and consequently, access to nutrients (Caldwell, Dawson, & Richards, 1998; Querejeta, Egerton-Warburton, & Allen, 2003). Additionally, higher rates of transpiration, photosynthesis and hence growth can occur as a result of HR, prolonging plant growth throughout a season (Brooks, Meinzer, Coulombe, & Gregg, 2002; Howard, Van Iersel, Richards, & Donovan, 2009). However, a critical point remains under debate; what factors play the greatest role in determining the magnitude of redistributed water? For temperate tree species in particular, it is currently unclear which driving factors influence the amount of water that is hydraulically redistributed the most, both, within the root system and into the rhizosphere soil. Therefore, we have yet to understand which plant and environmental circumstances must be given that facilitative HR effects occur (Ammer et al., 2018; Pretzsch et al., 2014).

While a number of suggested factors drive HR, including 'external' environmental and 'internal' plant factors (Neumann & Cardon, 2012), a mechanistic understanding of the role each factor plays in determining the quantity of HR is still lacking. Reported 'external' factors include soil texture, soil Ψ gradients and atmospheric vapour pressure deficit (VPD), while plant-driven 'internal' factors affecting HR include xylem vessel conductivity (Quijano & Kumar, 2015), rates of (night-time) transpiration (Dawson et al., 2007; Howard et al., 2009) and water refilling of plant storage tissues (Yu et al., 2018). In addition, root characteristics such as bark thickness (Bauerle et al., 2008) or the regulation of aquaporins (Li, Santoni, & Maurel, 2014; Prieto, Armas, & Pugnaire, 2012) might affect the amount of redistributed water. Potential seasonal shifts in 'external' and 'internal' factors could also help to explain seasonal shifts in the magnitude of HR (Hultine, Scott, Cable, Goodrich, & Williams, 2004; Priyadarshini et al., 2016). However, a large variability in the quantification of HR (Neumann & Cardon, 2012) exemplifies the need to tease apart the aforementioned 'internal' and 'external' factors. In this study, we aimed to quantify both, the amounts of water that saplings of five common temperate tree species redistributed within their root system and the amounts of HR water these saplings released into the rhizosphere soil. We hypothesized that more water would be redistributed following an increasing Ψ gradient between dry and moist root branches of the same plant ('external' driving factor for HR). Furthermore, we hypothesized that the amount of redistributed water would increase with increasing root conduit diameter and, potentially more accurate, with increasing 'actual' (unflushed) xylem hydraulic conductivity of the roots, as it accounts for potential native embolisms in the conduits due to drought conditions ('internal' driving factors). We chose two conifer-, one diffuse-porous and two ring-porous, species to cover a range of root conduit diameter sizes and, therefore, potential root-xylem hydraulic conductivities. Trees were planted in split-root systems that enabled us to create and control different Ψ gradients between dry and moist roots for each species and to quantify HR through deuterium labelling.

2 | MATERIALS AND METHODS

2.1 | Plants and growth conditions

In December 2015, trees were planted into split-root systems (Figure 1), where two pots (10 L each) were bolted together with one centrally located tree's (split-root plant, SRP) root system split equally between a 'dry' and a 'moist' pot (Figure 1). Additional 'reference' trees for water potential and hydraulic conductivity contrasts were planted in each of the pots (plant in the dry pot, 'DP' and plant in the moist pot, 'MP', respectively, Figure 1). Therefore, each complete split-root system was composed of three trees of the same species. Replicate systems were composed of 2- to 4-year-old saplings of *Picea abies* (L. Karst.), *Pseudotsuga menziesii* ((Mirb.) Franco, planted in December 2016), *Acer pseudoplatanus* (L.), *Quercus robur* (L.) and *Castanea sativa* (Mill.). A soil-filled foam-sleeve was placed around the root collar of the SRP to prevent root desiccation after planting. The sleeve and the soil within were removed several weeks before the experiment. We aimed for seven replicate systems per species; however, as the split-root setup displayed a stressful environment for some SRPs, not all plants survived and therefore the number of replicate systems deviated between the species (Table 1). Over all species, the height of the studied SRPs ranged from 50 to 95 cm and root biomass in the 'dry' pot from 3.0 to 18.8 g. While most species were very similar in size, *C. sativa* trees had larger root systems (Table 2). We used a potting soil (mixture of topsoil, compost, turf and lava (20% organic matter); Wurzer Umwelt GmbH, Eitting, Germany), mixed with 10% of soil retrieved from forest-stands dominated by the respective species to provide species-specific mycorrhizal inoculum. All trees were well-watered and grown in a greenhouse in Freising, Germany (48°23′57.98″N, 11°43′00.99″E) under ambient climate conditions until July 2017 to ensure establishment.

The experiment was conducted in two 7.7 m^2 growth chambers at the 'TUMmesa' research facility (Technical University of Munich – Model EcoSystem Analyser; http://www.tummesa.de/home) to

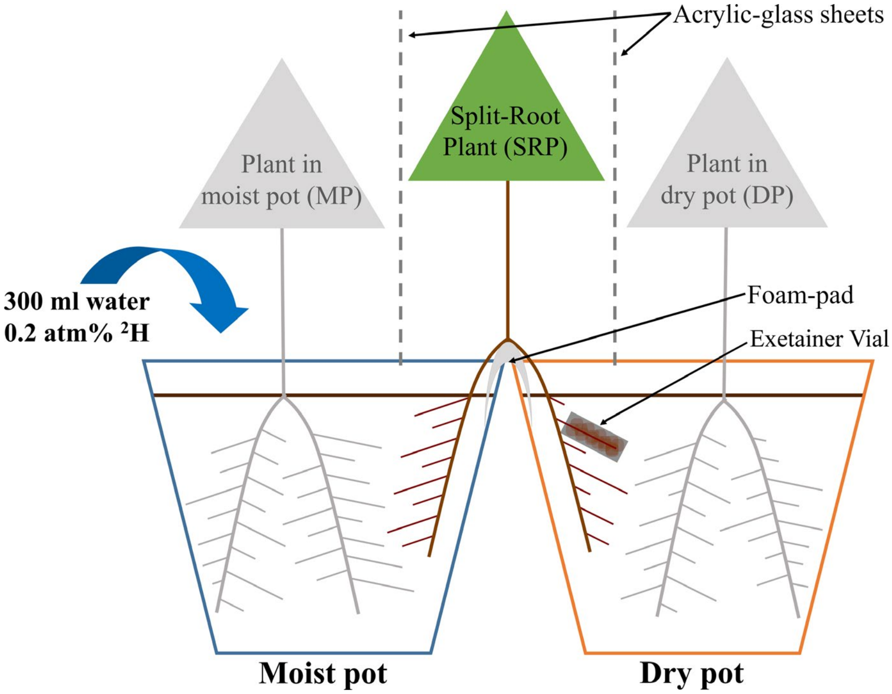

FIGURE 1 Scheme of the split-root system. Split-root systems consisted of one centrally located tree's (split-root plant, SRP) root system split equally between a 'moist' and a 'dry' pot. A foam-pad was placed between the roots of the SRP and the pot edges to minimize injury to the roots. Additional reference trees were planted completely within each of the pots ('MP' and 'DP', respectively). Acrylic-glass sheets were placed vertically on the pots, to prevent canopy contact between the plants. One root branch of the SRP in the dry pot was inserted into an exetainer vial, including rhizosphere soil. The root and the soil were harvested in separate exetainer vials upon labelling

TABLE 1 Intersecting set of analysed split-root systems and respective driving factors soil water content (SWC), pre-dawn water potential (Ψ_{PD}) and actual root-xylem hydraulic conductivity (k_{sa})

	Isotope analysis	SWC	Ψ_{PD}	k_{sa}
	n			
Picea abies	7	7	6	3
Pseudotsuga menziesii	7	7	7	5
Acer pseudoplatanus	3	3	0	2
Quercus robur	4	4	4	2
Castanea sativa	6	6	4	4

provide strictly controlled environmental conditions. Plants were acclimated to growth chamber conditions for at least 3 weeks before the experiment started. Day/night hours were maintained at 15/9 hr with corresponding temperatures of 25/15°C. Relative humidity (rH) was 89 ± 0% (1 *SE*) during the night to limit potential night-time transpiration and decreased to 60 ± 0% (1 *SE*) during the day. During the day, the mean photosynthetically active photon flux density at canopy level was 305 ± 4 µmol m^{-2} s^{-1} (1 *SE*) with a plateau of 434 ± 0 µmol m^{-2} s^{-1} (1 *SE*) that lasted for 7 hr. Temperature and light were gradually increased and decreased during morning and evening hours respectively.

2.2 | Soil water content and leaf water potentials

Volumetric soil water content (SWC) was recorded in both pots when the experiment started with a TDR probe spanning the depth of the pots (i.e. 15 cm; TDR100, Campbell Scientific). Additionally, we measured pre-dawn water potential (Ψ_{PD}) in leaves of most of the DP, MP and SRP (Table 1) with a Scholander-type pressure bomb (1505D pressure chamber, PMS Instrument Company). *A. pseudoplatanus* trees were excluded from Ψ_{PD} measurements due to the heavy exudation of milky sap from the petioles. At pre-dawn, the whole sapling water potential can be assumed to be in equilibrium, that is between leaves and roots. The moisture gradient within the SRP between the roots in the moist and dry pots was calculated as the difference in Ψ_{PD} between the SRP and the DP and will be referred to as 'Ψ_{PD} difference' in the following paragraphs.

2.3 | Experimental setup

Because of limited space in the growth chambers, the experiment was conducted in four campaigns from July to September 2017. All replicates of a maximum of two species were studied in parallel in one campaign (timing of the experiment did not influence the amounts of HR water, $p = .2$). Replicates of each species were equally split between both growth chambers. First, we initiated different soil moisture gradients between replicates of the two pots. Irrigation was limited to different extents from the 'dry' pot, resulting in SWCs ranging from 6.6 to 19.9 vol%. The 'moist' pot was well-watered, with SWCs ranging from 11.0 to 41.7 vol% (Table 3). In order to capture all HR water of a root, without losing any amount to the bulk soil or neighbouring trees, c. 1 week before the experiment, we carefully excavated a single root branch of the SRP (average 0.28 ± 0.06 g (1 *SE*) dry mass, Table 2, c. 9 cm length). This root branch with its attached rhizosphere soil (average dry mass of 5.3 ± 0.2 g (1 *SE*), Table 2) was put into an exetainer vial (Labco) and buried back

TABLE 2 Basic tree characteristics of the studied split-root plants (SRP; means ± 1 *SE*)

		SRP dry pot					
	Tree height (cm)	Root biomass (g)	Root biomass exetainer (g)	Root water content (ml)	Root water exetainer (ml)	Mass rhizosphere exetainer (g)	Water rhizosphere exetainer (ml)
Picea abies	57 ± 2^{ab}	4.6 ± 0.9^{a}	0.16 ± 0.02^{a}	7.7 ± 1.7^{ab}	0.27 ± 0.04^{ab}	4.5 ± 0.3^{a}	1.0 ± 0.1^{a}
Pseudotsuga menziesii	67 ± 1^{a}	3.0 ± 0.7^{a}	0.11 ± 0.01^{a}	4.6 ± 1.0^{a}	0.16 ± 0.01^{ab}	6.4 ± 0.4^{b}	0.8 ± 0.1^{a}
Acer pseudoplatanus	66 ± 3^{ab}	5.8 ± 1.1^{ab}	0.31 ± 0.05^{ab}	6.4 ± 0.9^{ab}	0.35 ± 0.03^{ab}	5.0 ± 0.4^{ab}	1.1 ± 0.2^{ab}
Quercus robur	67 ± 3^{a}	3.8 ± 1.0^{a}	0.14 ± 0.04^{a}	3.7 ± 1.0^{a}	0.14 ± 0.04^{a}	5.6 ± 0.3^{ab}	0.8 ± 0.1^{a}
Castanea sativa	49 ± 7^{b}	18.8 ± 4.0^{b}	0.68 ± 0.22^{b}	20.5 ± 5.2^{b}	0.67 ± 0.19^{b}	4.8 ± 0.4^{a}	1.6 ± 0.2^{b}

Note: Different letters indicate significant differences between the species per parameter.

TABLE 3 Soil water content (SWC) in the moist and dry pots of the split-root systems when the experiment started (means ± 1 *SE*)

	SWC moist pot (vol%)	SWC dry pot (vol%)	SWC difference (vol%)
Picea abies	29.6 ± 4.2^{a}	12.2 ± 1.6^{***a}	17.5 ± 3.7^{a}
Pseudotsuga menziesii	20.1 ± 1.4^{a}	8.5 ± 0.4^{***a}	11.6 ± 1.5^{a}
Acer pseudoplatanus	25.4 ± 5.2^{a}	10.3 ± 0.8^{*a}	15.1 ± 5.0^{a}
Quercus robur	22.3 ± 4.1^{a}	10.6 ± 0.5^{*a}	11.7 ± 3.6^{a}
Castanea sativa	21.7 ± 3.9^{a}	9.0 ± 0.5^{***a}	12.7 ± 3.7^{a}

Note: Letters indicate significant differences between the species; asterisks give significant differences between the moist and dry pot (*< 0.05, ***< 0.001).

into the dry pot soil (Figure 1). We took care that only entire root branches that were vital in their appearance and therefore representative for the whole root system were buried in the vials. Before labelling, we sampled bulk soil with a metal core (diameter c. 1 cm), placed the soil into a plastic bag, gently mixed the soil and transferred a subsample into an exetainer vial. These soil samples served as reference for soil and root samples after the labelling (Hafner et al., 2017). Additional xylem sap samples, extracted prior to labelling from branches with the bark removed, confirmed isotopic equality between unlabelled soil and plant xylem sap (p = .3, xylem sap and respective soil measured in nine pots; data not shown).

On the labelling day, 300 ml of deuterium-enriched water (0.2 atom-%) was carefully added to the soil of the moist pot. During this time, the dry pot was covered with aluminium foil to prevent contamination. Then, the moist soil was covered with foil and acrylic-glass sheets were placed vertically on the pots, to prevent canopy contact between the plants (Figure 1). Deuterium labelling was performed at midday to ensure the optimal uptake of label by the moist pot's SRP roots before any potential HR was initiated. Subsequent soil sampling took place as described above before dawn on the following day, minimizing the chance that redistributed water in the dry pot was taken up again by the SRP for transpiration. In parallel, we harvested the single root branch of the SRP in the dry pot, removed it from the buried exetainer vial—leaving the rhizosphere soil—and quickly put it into a separate exetainer vial. Both vials were sealed and all isotope samples were stored at −18°C until further processing. All samples on the dry side were taken before the soil samples on the moist, labelled side to avoid contamination. The water was extracted by cryogenic vacuum distillation for 2 hr (West, Patrickson, & Ehleringer, 2006) and mass difference revealed sample water content. Additionally, we determined the dry mass for all soil samples to calculate the relative water content (in mass%), revealing no difference between bulk and rhizosphere soil (p = .3, data not shown).

All water samples were analysed for their δ^2H with an isotope-ratio mass-spectrometer (IRMS, Isoprime 100, Elementar Analysensysteme GmbH) coupled to a multiflow system (222 XL Liquid Handler, Gilson) or a cavity ring-down spectrometer (CRDS, L2120-i, Picarro) coupled to a vaporizer module (A0211, Picarro). Cross measurements of soil and root-xylem samples revealed no statistical differences between both instruments (p = .9; regression: R^2 = .99, p < .001) or putative contamination with organic compounds (West, Goldsmith, Brooks, & Dawson, 2010). Measurement precision was determined against two laboratory standards ('heavy': δ^2H of 133.3 ± 1.7 ‰ [1 *SD*] and 'light': δ^2H of −159.4 ± 1.9 ‰ [1 *SD*]) and was better than ±0.8 ‰ (1 *SE*) for the IRMS and ±1.9 ‰ (1 *SE*) for the CRDS respectively.

2.4 | Assessment of root characteristics

We recorded the fresh and dry mass of the harvested root systems of the SRPs in the dry pot, after the experiment ended to calculate root water content (Table 2). Roots were separated into root mass inside the exetainer vials and root mass of the remaining root system. Additional root samples (length c. 1 cm, diameter 1.9 ± 0.2 mm (1 *SE*), n = 3–9 per species) of parallel plant individuals, not used for the labelling experiment but of the same age and grown under the same environmental conditions, were dried in ethanol (Hafner et al., 2017) and subsequently cut with laser ablation tomography (Chimungu, Brown, & Lynch, 2014). Pictures of the root slices photographed with a resolution of 25,400 dpi were analysed for xylem conduit diameters. All xylem conduits on three representative sample areas (0.5 mm^2 each) of each cross section were marked by hand with GIMP (GNU Image Manipulation Program, Version 2.10.2, The GIMP Team, https://www.gimp.org/) and conduit area was determined with ImageJ (Version 1.47t, Wayne Rasband, National Institutes of Health; Figure S1). Following Scholz, Klepsch, Karimi, and Jansen (2013), the equivalent circle diameter was calculated.

Finally, we calculated the 'actual' and maximum root-xylem hydraulic conductivity for the SRP and the neighbour plants in the dry pots. To this end, we measured hydraulic conductance with a 'xylem embolism meter' (XYL'EM, Bronkhorst France S.A.S.). Roots of experimental SRPs plus additional SRPs of the same age and grown under the same environmental conditions (Table 1, $n = 5$ per species) were cut several times under water. Resulting root parts had a diameter of 2.6 ± 0.7 mm (1 *SE*) and a length of 2.7 ± 0.6 cm (1 *SE*). Subsequently, the bark was removed on the side that was inserted into the XYL'EM apparatus. First, the 'actual' (unflushed) hydraulic conductance was measured (K_{act}, in kg $\mathrm{MPa}^{-1}\ \mathrm{s}^{-1}$) at c. 0.007 MPa with degassed, filtered (0.2 µm) water with 10 mM KCl and 1 mM CaCl_2 added (Barigah et al., 2013). After the measurement of K_{act}, we obtained maximum hydraulic conductance (K_{max}, in kg $\mathrm{MPa}^{-1}\ \mathrm{s}^{-1}$) by flushing the sample several times at c. 0.12 MPa for 10 min, until no further increase in conductance occurred. Subsequently, the length was measured and the conductive area (A_{cond}) was assessed by analysing a picture of each cross section photographed under a stereomicroscope with ImageJ. Actual and maximum specific xylem hydraulic conductivity (k_{sa} and k_{sm} in kg $\mathrm{s}^{-1}\ \mathrm{m}^{-1}\ \mathrm{MPa}^{-1}$) were then calculated as:

$$k_{\mathrm{s\,a/m}} = \frac{K_{\mathrm{act/max}} \times \mathrm{length}}{A_{\mathrm{cond}}}. \tag{1}$$

The fraction of k_{sa} from k_{sm} revealed the percent loss of conductivity (PLC) of the respective species:

$$\mathrm{PLC} = \frac{k_{\mathrm{sm}} - k_{\mathrm{sa}}}{k_{\mathrm{sm}}} \times 100\,(\%). \tag{2}$$

2.5 | Mixing model calculations

The relative fractions (in %) and absolute amounts (in ml) of the redistributed labelled water (further referred to as 'HR water') were calculated as a mixture of two end-members for each single split-root system. We assumed the isotopic composition of the water in the SRP roots to be a mixture of soil water retrieved from the soil in the dry pot and HR water from the moist pot:

$$\mathrm{HR}_{\mathrm{SRP}} = \frac{\delta^2H\left(\mathrm{SRP}_{\mathrm{root\,dry\_L}}\right) - \delta^2H\left(\mathrm{soil}_{\mathrm{dry\_BL}}\right)}{\delta^2H\left(\mathrm{soil}_{\mathrm{moist\_L}}\right) - \delta^2H\left(\mathrm{soil}_{\mathrm{dry\_BL}}\right)} \times 100\,(\%), \tag{3}$$

with $\mathrm{HR}_{\mathrm{SRP}}$: Fraction of HR water in the SRP root in the dry pot, δ^2H ($\mathrm{SRP}_{\mathrm{root\ dry\_L}}$): Delta value of the SRP root in the dry pot upon labelling, δ^2H ($\mathrm{soil}_{\mathrm{moist\_L}}$): Delta value of the soil in the moist pot upon labelling and δ^2H ($\mathrm{soil}_{\mathrm{dry\_BL}}$): Delta value of the soil in the dry pot before labelling.

Correspondingly, the HR water in each rhizosphere soil of the SRP root in the dry pot was calculated as:

$$\mathrm{HR}_{\mathrm{rhizosphere}} = \frac{\delta^2H\left(\mathrm{SRP}_{\mathrm{rhizosphere\_L}}\right) - \delta^2H\left(\mathrm{soil}_{\mathrm{dry\_BL}}\right)}{\delta^2H\left(\mathrm{soil}_{\mathrm{moist\_L}}\right) - \delta^2H\left(\mathrm{soil}_{\mathrm{dry\_BL}}\right)} \times 100\,(\%), \tag{4}$$

with $\mathrm{HR}_{\mathrm{rhizospere}}$: Fraction of HR water in the rhizosphere soil of the SRP root in the dry pot and δ^2H ($\mathrm{SRP}_{\mathrm{rhizosphere\_L}}$): Delta value of the rhizosphere soil of the SRP root in the dry pot upon labelling.

We calculated the absolute amount of HR water in the single root systems (in ml, $\mathrm{HR}_{\mathrm{a\,SRP}}$) by multiplying each corresponding relative fraction of HR water (Equation 3) with the respective water content of the SRP root system in the dry pot (Table 2). We first calculated the amount of HR water in each exetainer vial ($\mathrm{HR}_{\mathrm{a\,exetainer}}$) for rhizosphere soils and SRP roots, by multiplying the relative fraction of HR water (Equations 3 and 4) with the water content of the sample ($\mathrm{wc}_{\mathrm{sample}}$) in the exetainer vial (Table 2):

$$\mathrm{HR}_{\mathrm{a\,exetainer\,rhizosphere/SRP}} = \mathrm{HR}_{\mathrm{rhizosphere/SRP}} \times \mathrm{wc}_{\mathrm{sample\,rhizosphere/SRP}}\ (\mathrm{ml}). \tag{5}$$

We then calculated the ratio of HR water in the exetainer vial between rhizosphere soil and SRP root ($R_{\mathrm{rhizosphere/SRP}}$):

$$R_{\mathrm{rhizosphere/SRP}} = \frac{\mathrm{HR}_{\mathrm{a\,exetainer\,rhizosphere}}}{\mathrm{HR}_{\mathrm{a\,exetainer\,SRP}}}. \tag{6}$$

By multiplying the ratio with the absolute amount of HR water in the SRP root ($\mathrm{HR}_{\mathrm{a\,SRP}}$), and assuming this ratio was consistent for the whole root system of the SRP, we calculated the absolute amount of HR water in the rhizosphere ($\mathrm{HR}_{\mathrm{a\,rhizosphere}}$) per pot:

$$\mathrm{HR}_{\mathrm{a\,rhizosphere}} = R_{\mathrm{rhizosphere/SRP}} \times \mathrm{HR}_{\mathrm{a\,SRP}}\ (\mathrm{ml}). \tag{7}$$

Finally, we added the absolute amount of HR water in the SRP root to the absolute amount of HR water in the rhizosphere to calculate total HR of one plant during one night and then averaged the amounts for each species. To put the redistributed amount of water into a comparable context, we also calculated the ratio between HR water in the roots and rhizosphere and the dry root mass of the SRP in the dry pot (Table 2) to get the amount of HR water per root mass (HR_r):

$$\mathrm{HR}_{r\,\mathrm{SRP/rhizosphere}} = \frac{\mathrm{HR}_{\mathrm{a\,SRP/rhizosphere}}}{\mathrm{root\ mass}_{\mathrm{SRP}_{\mathrm{dry}}}}\ (\mathrm{ml/g}). \tag{8}$$

To verify our results on the fractions of redistributed water, we also ran the calculations with a mixing model accounting for uncertainty errors when calculating with average isotope values ('Iso Error', Phillips & Gregg, 2001). Applying the model on the average δ^2H values of the respective end-members per tree species gave the same values as our calculations.

2.6 | Statistics

All isotope data were checked for significant ($p < .05$) increases of δ^2H upon labelling. The pot identities served as random factor nested over the respective growth chamber identities in a linear mixed effect model (R package NLME, version 3.1-137) where the δ^2H values of the different samples (soil, root) were tested individually for increases (day and species as independent variables). Increase of δ^2H upon labelling and differences between the species were revealed with the lsmeans post-hoc test (R package LSMEANS, version 2.27-62). Residuals of the model data were checked for normal distribution (shapiro.test) and the data, where necessary, were transformed to meet residual normal distribution. Model data were checked for variance homogeneity (Levene test; R package CAR, version 2.1-2). The same model and tests were applied to check for the differences between the species in the amount of HR water and driving factors (Ψ_{PD}, conduit diameters, $k_{s\ a/m}$). Correlations between driving factors and mixing model outputs were performed via a multiple factor linear regression on split-root systems, where respective data were completely available (Table 1, i.e. amount of HR water and measured driving factors Ψ_{PD} difference and k_{sa}). Additional single factor linear models were calculated for each determining factor to gain slope, intercept, R^2 and p-value of the correlation. Finally, as we did not measure root anatomy of the SRPs that were analysed for their amount of HR water, the average mixing model output was correlated with the average conduit diameter per species. Mean values are shown ±1 *SE*. All statistical analyses were performed with R version 3.3.1 (R Development Core Team, 2018) in RStudio version 1.1.447 (RStudio Team, 2015).

3 | RESULTS

3.1 | Soil water content and leaf water potentials

For all species, SWC in the dry pot (mean: 10.1 ± 0.5 vol%) was significantly lower than in the moist pot (mean: 23.8 ± 1.7 vol%) and not different between the species (Table 3). Also, the SWC difference between the moist and dry pot (mean of 13.8 ± 1.5 vol%) did not differ across species (Table 3). This difference in soil moisture between the moist and dry pot resulted in a mean Ψ_{PD} difference between the SRP and the DP of 1.71 ± 0.24 MPa, with the maximum difference found in *Q. robur* (Table 4). For all species, a range of Ψ_{PD} differences, and therefore in the 'external' HR driving factor, of at least 1.03 MPa per species was established between the moist and dry pot (Table 4). Pre-dawn Ψ of the SRP was not different to Ψ_{PD} of the MP ($p > .8$), while the DP showed a significantly lower Ψ_{PD} than MP or SRP ($p < .001$, each, Table S1).

3.2 | Conduit diameters and root hydraulic conductivities

The potential 'internal' HR driving factors root conduit diameter and k_{sa}, were largest and highest in stem ring-porous *C. sativa* and *Q. robur* (average over both species: 40.53 ± 0.84 μm and 0.55 ± 0.06 kg s^{-1} m^{-1} MPa^{-1}, respectively). Stem diffuse-porous *A. pseudoplatanus* had a smaller diameter but similar k_{sa} (Table 5), while the conifers—limited to tracheids—showed the smallest diameter and lowest k_{sa} (mean: 10.61 ± 0.10 μm and 0.30 ± 0.03 kg s^{-1} m^{-1} MPa^{-1}, respectively). All deciduous species formed embolisms (mean PLC in the SRPs: 64 ± 4%) in the roots growing in the dry pots, whereas no difference between k_{sa} and k_{sm} was present in the conifers (Table 5). Percent loss of conductivity was significantly higher in stem ring-porous *C. sativa* and *Q. robur* than in *A. pseudoplatanus*.

3.3 | Isotopic enrichment upon labelling

Upon labelling, we found a significant ^{2}H enrichment in the soil of the moist pot across all species (Table 6) with an average value of 1,835 ± 203 ‰. The roots of the SRP in the dry pot were significantly ^{2}H enriched upon labelling in *C. sativa*, *P. menziesii* and *Q. robur* trees ($p < .001$, each), whereas, despite several samples being clearly enriched (i.e. δ^2H after labelling was at least 10 ‰ higher than before labelling in seven of 10 samples), average δ^2H values in *A. pseudoplatanus* ($p = .08$) tended to be and *P. abies* ($p = .2$) were not significantly increased above the unlabelled reference (Table 6).

TABLE 4 Pre-dawn water potential (Ψ_{PD}) differences between split-root plants and plants grown in the dry pots when the experiment started (means ± 1 *SE* and min and max values respectively)

	Ψ_{PD} difference (MPa)	Min difference (MPa)	Max difference (MPa)	Difference range (max–min) (MPa)
Picea abies	0.74 ± 0.15^a	0.26	1.29	1.03
Pseudotsuga menziesii	1.54 ± 0.26^a	0.62	2.71	2.09
Acer pseudoplatanus	NA	NA	NA	NA
Quercus robur	3.17 ± 0.40^b	2.30	4.20	1.90
Castanea sativa	2.01 ± 0.62^{ab}	0.23	3.12	2.89

Note: Min and Max differences in Ψ_{PD} were set to differ by at least 1 MPa per species to generate higher and lower 'external' driving factors for each species. The range in Ψ_{PD} differences per species is given in the last column. Letters indicate significant differences between the species. Note that *A. pseudoplatanus* trees were excluded from the measurements due to heavy milky sap exudation from petioles.

TABLE 5 Conduit diameters and hydraulic conductivity (k_s; separated into 'actual' k_{sa}, i.e. considering drought-induced xylem embolism and maximum k_{sm}, i.e. full conductivity without embolism) of the split-root plants in the dry pot (means ± 1 *SE*)

	Conduit diameter		k_{sa}	k_{sm}
	n (conduit diameter)	µm	kg s^{-1} m^{-1} MPa^{-1}	
Picea abies	6	9.96 ± 0.73^{a}	0.27 ± 0.02^{a}	0.27 ± 0.02^{a}
Pseudotsuga menziesii	3	11.25 ± 0.43^{a}	0.34 ± 0.08^{ab}	0.34 ± 0.08^{a}
Acer pseudoplatanus	9	27.42 ± 1.12^{b}	0.61 ± 0.10^{bc}	1.18 ± 0.18^{b}
Quercus robur	9	34.99 ± 1.45^{bc}	0.64 ± 0.09^{c}	2.01 ± 0.21^{***b}
Castanea sativa	9	44.22 ± 5.81^{c}	0.46 ± 0.08^{abc}	2.28 ± 0.48^{***b}

Note: Letters indicate significant differences between the species; asterisks give significant differences between k_{sa} and k_{sm} (*** < 0.001).

TABLE 6 Deuterium isotope signals (δ^2H) of soils and roots (roots of split-root plants in the dry pot) of the split-root system (means ± 1 *SE*)

	Before labelling	After labelling	Before labelling	After labelling		
	Soil moist	Soil moist	Soil dry	Soil dry	Rhizosphere soil dry	SRP root dry
	δ^2H (‰)					
Picea abies	−61 ± 1^{a}	1,846 ± 271^{***a}	−55 ± 2^{a}	−55 ± 3^{a}	−40 ± 2^{***a}	−43 ± 3^{a}
Pseudotsuga menziesii	−61 ± 1^{a}	2,033 ± 386^{***a}	−53 ± 2^{a}	−53 ± 3^{a}	−42 ± 2^{***ab}	−19 ± 11^{***ab}
Acer pseudoplatanus	−66 ± 3^{a}	1,224 ± 402^{***a}	−61 ± 2^{a}	−61 ± 2^{a}	−49 ± 2$^{\circ ab}$	−40 ± 9$^{\circ a}$
Quercus robur	−59 ± 3^{a}	893 ± 154^{***a}	−58 ± 2^{a}	−56 ± 2^{a}	−48 ± 2^{ab}	18 ± 30^{***b}
Castanea sativa	−68 ± 0^{a}	2,524 ± 591^{***a}	−67 ± 2^{a}	−66 ± 4^{a}	−58 ± 4$^{\circ b}$	−43 ± 4^{***a}

Note: Letters indicate significant differences between the species; asterisks give significant increases above reference values after the labelling (° ≤ 0.1, *** < 0.001).

TABLE 7 Hydraulically redistributed water in roots, rhizosphere soils and combined total amount of the split-root plants (means ± 1 *SE*) for the whole root system (in ml) and per root dry mass (in ml/g)

	SRP root		Rhizosphere		Total	
	ml	ml/g	ml	ml/g	ml	ml/g
Picea abies	0.06 ± 0.02^{a}	0.01 ± 0.00^{a}	0.29 ± 0.13^{a}	0.06 ± 0.02^{a}	0.35 ± 0.13^{a}	0.07 ± 0.02^{ab}
Pseudotsuga menziesii	0.07 ± 0.02^{a}	0.03 ± 0.01^{a}	0.13 ± 0.04^{a}	0.05 ± 0.01^{a}	0.19 ± 0.04^{a}	0.08 ± 0.02^{ab}
Acer pseudoplatanus	0.11 ± 0.03^{ab}	0.02 ± 0.01^{ab}	0.20 ± 0.05^{a}	0.04 ± 0.01^{a}	0.31 ± 0.02^{a}	0.06 ± 0.02^{ab}
Quercus robur	0.29 ± 0.12^{b}	0.08 ± 0.03^{b}	0.27 ± 0.10^{a}	0.11 ± 0.06^{a}	0.56 ± 0.15^{a}	0.19 ± 0.07^{a}
Castanea sativa	0.17 ± 0.04^{ab}	0.01 ± 0.00^{a}	0.42 ± 0.24^{a}	0.02 ± 0.01^{a}	0.59 ± 0.23^{a}	0.04 ± 0.01^{b}

Note: Letters indicate significant differences between the species.

The rhizosphere soil in the dry pot was significantly enriched in *P. abies* ($p < .001$) and *P. menziesii* ($p < .01$), whereas the δ^2H tended to be significantly enriched in *A. pseudoplatanus* ($p = .09$) and *C. sativa* ($p = .08$). No enrichment was detected in *Q. robur* ($p = .16$, again despite two of four samples being clearly enriched). The bulk soil in the dry pot did not increase in δ^2H after one night (Table 6).

3.4 | Amounts of HR water in the SRP roots and in the rhizosphere soil

After one night, the root systems of stem ring-porous *C. sativa* and *Q. robur* redistributed more water (average over both species of 0.22 ± 0.05 ml) than stem diffuse-porous *A. pseudoplatanus* (0.11 ± 0.03 ml, not significant though) and significantly more than the conifer species (average of *P. abies* and *P. menziesii* of 0.06 ± 0.01 ml, $p < .05$, Figure S2). All species released similar amounts of HR water into the rhizosphere soil as they redistributed within their respective root systems (Table 7). When comparing the combined roots and rhizosphere amounts of HR water by the SRP, total HR was not different between the species. Total HR of stem ring-porous *C. sativa* and *Q. robur* trees (0.59 ± 0.23 ml and 0.56 ± 0.15 ml respectively, Table 7) tended to have the highest average values ($p = .08$ vs. conifers, see Figure S2). The amount of HR water in the root system per root dry mass was significantly higher in *Q. robur* trees (0.08 ± 0.03 ml/g) than in the conifers and *C. sativa* (Table 7). Per root dry mass, species did not deviate in the amount of released water into the rhizosphere (average: 0.06 ± 0.01 ml/g), but for total HR water by root dry mass, *Q. robur* redistributed more than *C. sativa* ($p < .01$), while the other species were in between (Table 7).

3.5 | Dependence of HR on 'external' and 'internal' factors

In a single factor regression, we found a positive correlation in both the amount of HR water in the SRP roots and the total HR water in the dry pot when a Ψ_{PD} difference existed between the pots in our systems, indicating that higher Ψ_{PD} differences resulted in higher amounts of redistributed water across the observed species (Figure 2a). Regression and significance level were higher for HR water in the roots ($R^2 = .2$, $p < .05$) than for total HR water ($R^2 = .1$, $p = .07$). The same pattern was true for the amount of water that was redistributed per root dry mass. The regression was the same for root water only ($R^2 = .2$, $p < .05$), while for total HR water no significant correlation was found ($R^2 = .1$, $p = .16$; Figure 2b). Although not significant, a distinct trend in the correlation between the amount of HR water and Ψ_{PD} difference was also observed on the single species level. However, the range of respective Ψ_{PD} differences and the number of replicates per species were too few for a significant regression (e.g. *P. menziesii* that had a relatively wide range and seven replicates: $R^2 = .3$, $p = .10$ for the amount of HR water in the SRP roots vs. the Ψ_{PD} difference).

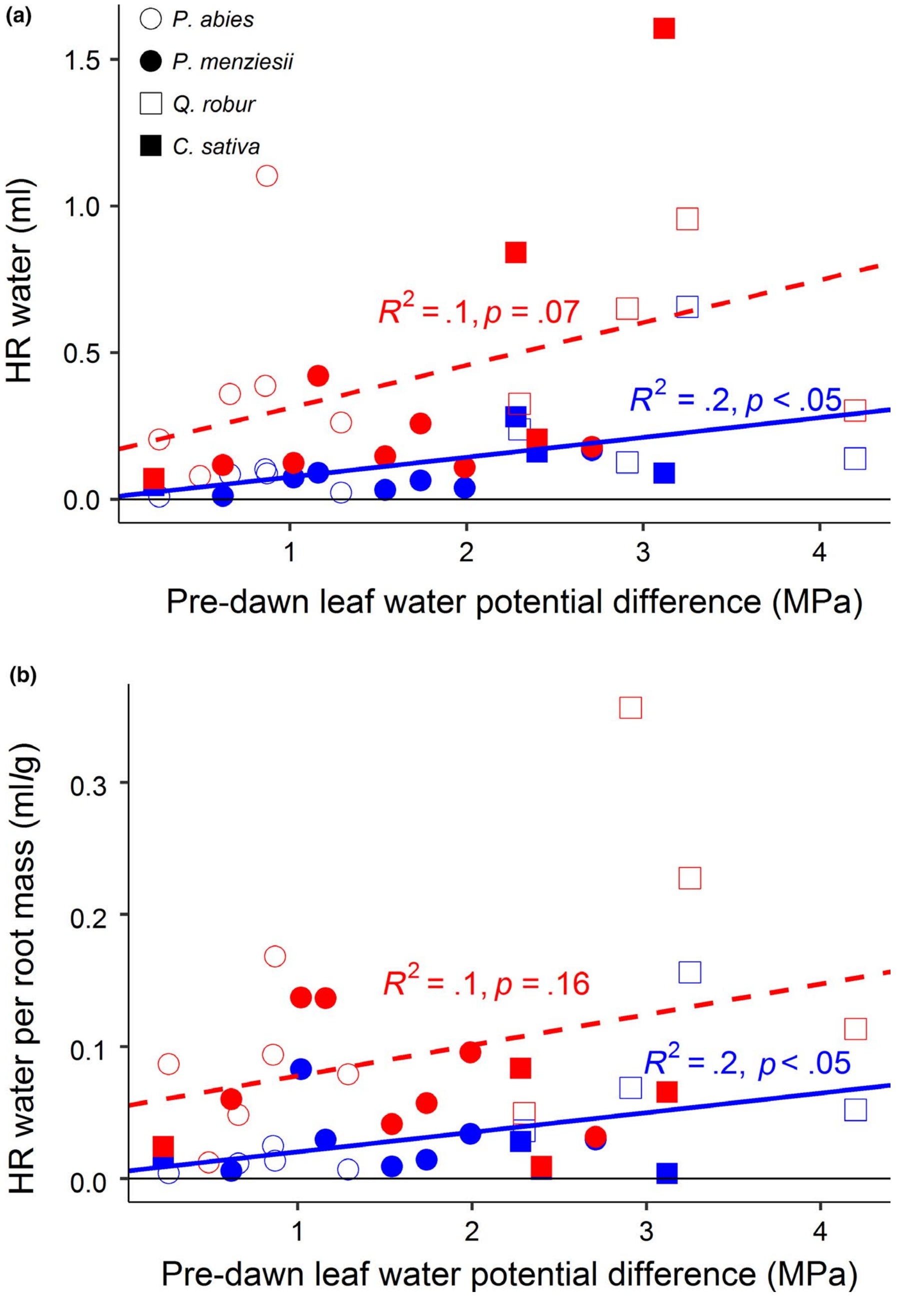

FIGURE 2 Correlation of pre-dawn water potential (Ψ_{PD}) difference with the amount of HR water (a: whole root system and b: per root dry mass; blue: amount in the roots, red: total amount). Note that *Acer pseudoplatanus* trees were excluded from the measurements due to heavy milky sap exudation. For number of replicates per species, see Table 1

With increasing conduit size, the amount of HR water increased for both, HR water found in SRP roots and total HR water (Figure 3a). The correlation ($R^2 = .2$, $p < .01$) for HR water found in SRP roots was slightly better compared to the total amount of HR water ($R^2 = .1$, $p < .05$). However, we did not find a correlation between either the root or total amount of HR water per root dry mass and the conduit size (Figure 3b). A strong positive correlation was found for both, HR water within the SRP and total HR water, when correlated with k_{sa} of the respective root system ($R^2 = .4$, $p < .01$ for the SRP root and $R^2 = .3$, $p < .05$ for total HR, respectively, Figure 4a). A positive relationship was also found between the amounts of water that were redistributed per root dry mass; regression and significance level were the same for HR within the root system, while the R^2 was slightly lower but still significant for total HR ($R^2 = .2$, $p < .05$, Figure 4b). On a single species level, respective k_{sa} values were too narrow and replicates were too few to determine significant correlations (for *C. sativa* a positive correlation with $R^2 = .3$ was found, though not significant).

When combining Ψ_{PD} difference and k_{sa} in a multifactor model, a positive correlation for the HR water inside the root system was found, both for the HR water in the whole root system ($R^2 = .5$, $p < .05$) and per root dry mass ($R^2 = .4$, $p < .05$). Within the model, k_{sa} had a significant influence on the outcome ($p < .05$), while the influence of Ψ_{PD} difference was not significant. A positive trend between HR amounts and the combined driving factors was also found for both, total HR water across the whole root system and total HR water per root dry mass ($R^2 = .2$, $p = .1$, each). Within the model, k_{sa} had a significant impact on the total amount of HR water per root dry mass ($p < .05$). A positive trend ($p = .07$) of k_{sa} on total HR amounts across the whole root system was also detected. The impact of Ψ_{PD} difference on total HR amounts was again not

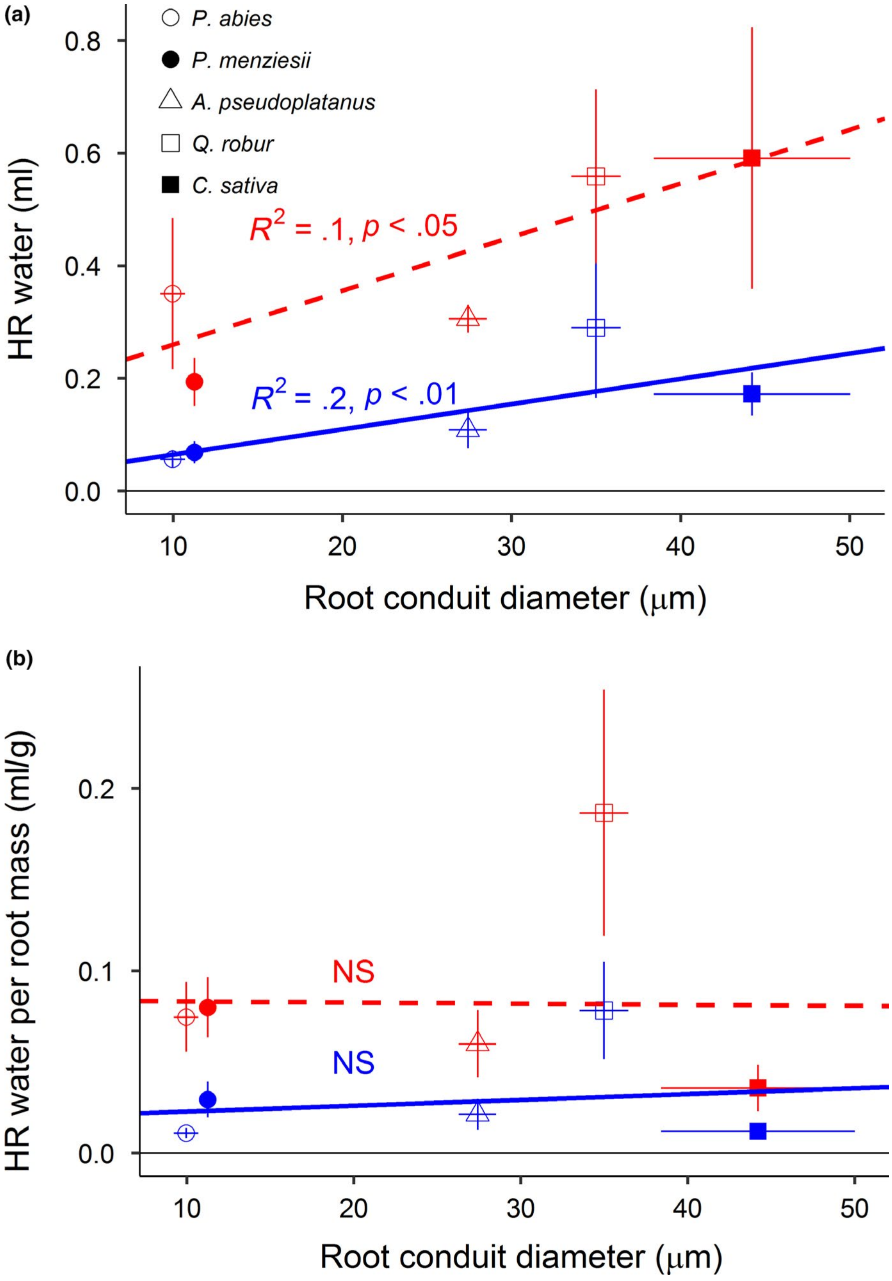

FIGURE 3 Correlation of mean root conduit diameters with the average amount of HR water (a: whole root system and b: per root dry mass; blue: amount in the roots, red: total amount)

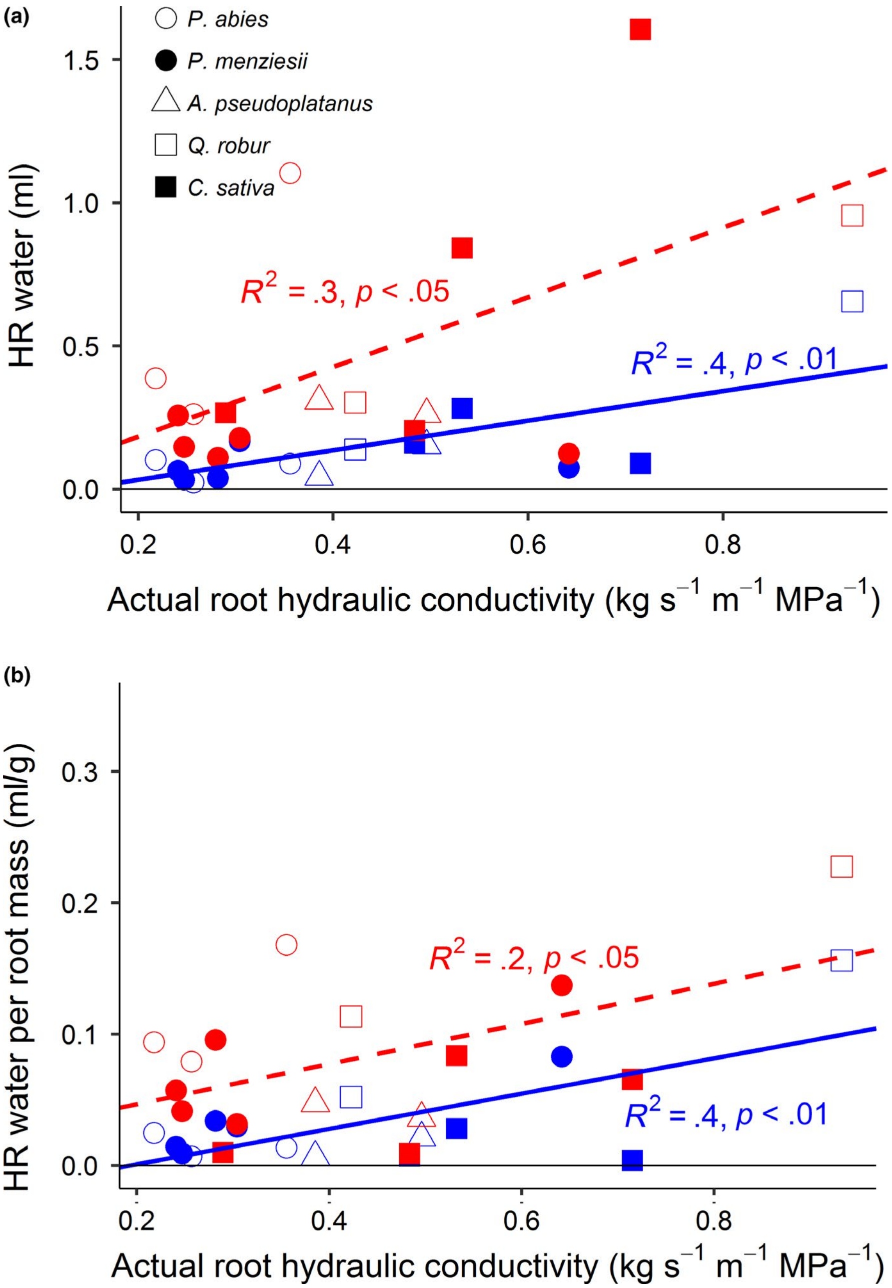

FIGURE 4 Correlation of 'actual' (unflushed) root hydraulic conductivity (i.e. conductivity considering drought-induced xylem embolism; k_{sa}) with the amount of HR water (a: whole root system and b: per root dry mass; blue: amount in the roots, red: total amount). For number of replicates per species, see Table 1

significant. Therefore, most of the variation in HR by the plants in our systems was explained by the variation of the 'internal' driving factor k_{sa}.

4 | DISCUSSION

On average, within a single night, saplings of five temperate tree species redistributed 0.39 ± 0.14 ml (0.08 ± 0.01 ml/g root dry mass) of water, with 0.13 ± 0.03 ml (0.03 ± 0.01 ml/g) of HR water held within the roots, and 0.26 ± 0.06 ml (0.06 ± 0.01 ml/g) released into the soil. These amounts represent the minimum quantities of water transferred via HR over one night. The actual amounts of transferred water might be larger, as unlabelled water still present in the roots of the SRP in the dry pot was redistributed first and could not be detected with our approach. We found significant evidence to support our hypotheses that plants redistribute more water with increasing Ψ_{PD} differences, with larger root conduit diameters and higher root-xylem hydraulic conductivity. The influence of k_{sa} was greater than that of the Ψ_{PD} difference or root conduit diameters and, therefore presented the main driver for variation in water redistribution over one night within our systems.

We note an analogy to Ohm's law in electricity, where a 'tension' (here: Ψ_{PD} difference) and 'resistors' (here: conduit diameter, xylem hydraulic conductivity) define the extent of the 'current' (here: HR).

By establishing a range of Ψ_{PD} differences between the roots in the moist and dry pots of the split-root systems, we created different 'external' HR driving 'tensions'. We found a positive correlation between the Ψ_{PD} differences and the amount of HR water across all species, supporting our hypothesis. While perhaps intuitive that a moisture difference is an important prerequisite and driving factor for HR (Caldwell et al., 1998; Yu, Feng, Si, Xi, & Li, 2013), this study shows that the magnitude of external 'tensions' relates to how much water is redistributed within a root system. As the amount of HR water increased with the increase in the Ψ_{PD} difference, HR and its benefits to trees might increase in those regions where more frequent summer drought events are forecasted (Flato et al., 2013; Orth, Zscheischler, & Seneviratne, 2016).

There was a positive correlation between xylem conduit size as k_{sa} and the amount of HR water. In light of the 'resistor' concept, species with smaller xylem conduits showed lower conductance for HR than species with larger conduits and higher k_{sa}. Root conduit anatomy (Hafner et al., 2017) and xylem hydraulic conductivity (Quijano & Kumar, 2015) reflected the magnitude of the internal 'resistor' of different species for HR, confirming our hypothesis that HR increases with increasing root conduit diameter and root-xylem hydraulic conductivity. Because the deciduous species of our study had embolism formation in their roots by the end of the experiment (Table 5; Figure 5), the correlation between the amount of HR water and k_{sa} as surrogate for conductivity was more accurate and resulted in better correlations than the regression with xylem conduit diameter.

There was a lack of a correlation for total HR water, as additional factors may influence water efflux into the soil. For example, depending on species and respective root bark thickness, suberized cells in the periderm may serve as a barrier for the water flow into the soil (Brunner, Herzog, Dawes, Arend, & Sperisen, 2015). Moreover, water transport could be limited by the regulation and number of aquaporins in the root cell membranes (Maurel et al., 2015). Additionally, for the angiosperm species, the maximum vessel length (not shown) was higher than the length of the segment we analysed. Therefore, total root conductivity of angiosperm species could deviate from the values estimated here, although we found a positive correlation between k_{sa} and root conduit diameters ($R^2 = .3$, $p < .001$; not shown). Moreover, within the root's conduit system, warts inside the vessel, pit aperture, vessel tapering or the architecture of perforation plates could additionally affect the amount of water being moved (Hesse, Hafner, & Grams, 2019). There is also indication that finer roots have a different water transport capability than larger roots (Dawson, 1997; Hesse et al., 2019). Hence, additional experiments on the hydraulic conductivity of whole root systems should be considered. Finally, poor root–soil contact in dry soils (Carminati, Vetterlein, Weller, Vogel, & Oswald, 2009) may prevent the movement of HR water into the rhizosphere (Ryel et al., 2004). At similar soil water potentials, species-specific root branching or number of tips may affect root–soil contact differently (Pregitzer et al., 2002). Therefore, the Ψ_{PD} presented in this study cannot easily be translated into soil Ψ but rather represent the Ψ experienced by the roots, including overall loss of root–soil contact. As k_{sa} is a parameter that combines root architecture (conduit diameters, representing maximum xylem hydraulic conductivity) with environmental conditions (Ψ gradient, reflected through PLC in the roots), it proved to be a robust driving factor with a strong influence on HR over the one night frame used in our systems.

4.1 | Potential of the 'exetainer-setup'

We recognize that placing root branches together with their rhizosphere soil directly into exetainer vials was an uncommon approach and, therefore, subjected to potential bias. There was no significant difference between the water content of the bulk soil and the rhizosphere soil inside the exetainer vials, suggesting that we did not influence the amount of HR due to altered soil moisture

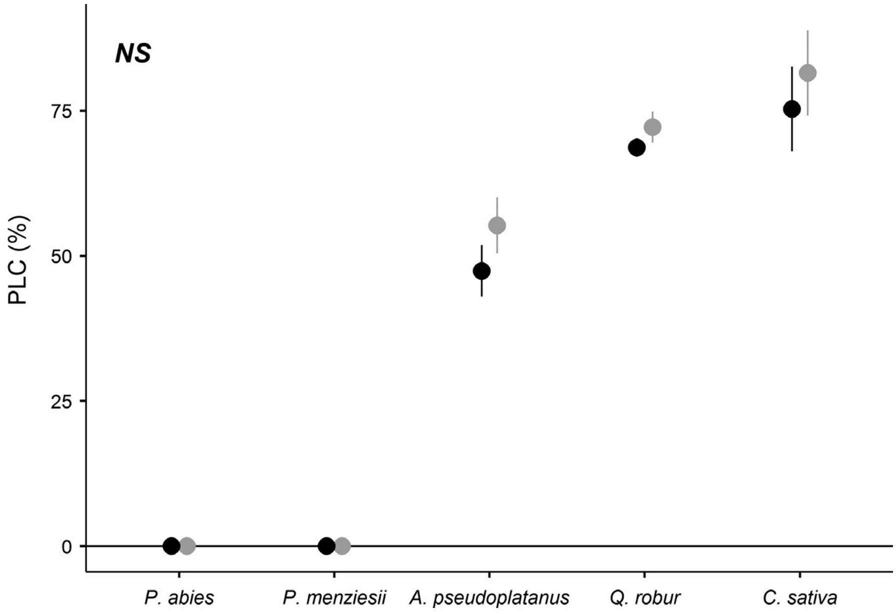

FIGURE 5 Percent loss of conductivity (PLC) assessed for the five study species in the split-root plant (SRP, black) and the reference plant with its root system only in the dry pot (DP, grey). The conifers did not show embolism while the deciduous trees showed average PLC of 64 ± 4% (1 *SE*; SRP) and 70 ± 4% (1 *SE*; DP; not significantly different, though)

conditions. Although the root systems were treated with great care, we cannot exclude that root–soil contact was affected. Thus, the difference in Ψ_{PD} might not necessarily translate into the gradient experienced by the 'exetainer' root branches. Additionally, Ψ_{PD} difference was calculated between SRP and DP and not within the root system of the SRP. The difference experienced by the SRP could therefore slightly deviate, potentially explaining why the regression with Ψ_{PD} difference was weak compared to k_{sa}. Moreover, the presented amounts of HR water in the rhizosphere soil represent minimum amounts, as more water could potentially be released in an 'undisturbed' root–soil system. Effectively, the exetainer-setup proved very beneficial, as it allowed us to obtain all water redistributed by a single root branch in one night. The minimal chance for water evaporation from the vial and the set-up ensured that no water penetrated further into the bulk soil or was taken up by neighbouring plants.

4.2 | Benefits to the SRP

Plants may maintain transpiration and 'safe' water potentials even with only parts of their conductive area (Dietrich, Hoch, Kahmen, & Körner, 2018), and only a portion of their root system hydrated, potentially explaining why we did not find a difference in Ψ_{PD} between the SRP and MP. Furthermore, redistributing water within their root system and releasing it into the soil can be beneficial to the plant (Prieto et al., 2012; Ryel et al., 2004). In our system, the additional water held inside the roots due to HR proved to have a positive impact. When we compared PLC of the SRP to their neighbouring plants with roots only in the dry pots, PLC was always lower in the SRP (Figure 5). This is in line with several studies reporting fewer embolisms to occur or embolisms likely to be repaired through HR (Domec et al., 2006; Domec, Warren, Meinzer, Brooks, & Coulombe, 2004; Prieto & Ryel, 2014). In addition to the benefit of maintaining well-hydrated roots via HR, these roots can also live longer, thereby reducing carbon-costs to the plant (Bauerle et al., 2008).

4.3 | Conclusions

The amount of water, the temperate tree species in our study redistributed through their root systems towards dry soil during one night was significantly dependent on an external driving 'tension', that is Ψ_{PD} difference and on internal 'resistors', that is root conduit diameter and especially k_{sa}. The amount of HR water, that is, 0.08 ± 0.01 ml/g root dry mass appears rather small. However, if one scales this number to mature forest trees with dry root masses of c. 100 kg, HR amounts could be in the range of 4–20 L per tree per day. Daily transpiration in 'typical' Central European forests may reach 30 L per tree per day (Larcher, 1994). Therefore, HR would account for c. 10%–70% of total daily transpiration. The amounts presented here result from a strictly controlled environment, therefore we emphasize this approximation should be treated with care. However, if applicable, HR would contribute substantially to the water cycle in temperate forests, as already indicated for tropical regions (Lee, Oliveira, Dawson, & Fung, 2005). With anticipated precipitation shifts in the future, HR could become more relevant in temperate forests facing increasing drought periods and thus greater soil-moisture gradients. Tree species that retained higher root k_{sa} under the drought conditions in our experiment clearly had a higher 'internal' potential for HR, predestinating them for selective planting if HR is to be used as a 'silvicultural tool' to improve plant water status in future forests.

ACKNOWLEDGEMENTS

The authors thank Yessica Kölmel and Rudi Schäufele for the isotope measurements and Benjamin Hall and Asheesh Lanba of Lasers for Innovative Solutions (L4iS) for conducting laser ablation tomography. For growth chamber maintenance, experimental set-up help, sampling and water extractions, we thank Thomas Feuerbach, Josef Heckmair, Peter Kuba, Roman Meier, Aline Potreck and Tommi Schieder. The study was supported by the 'Europamöbel Umweltstiftung' and the 'Hartig Stiftung'. B.D.Ha. and B.D.He. were funded by a doctoral scholarship from the German Federal Environmental Foundation (DBU).

AUTHORS' CONTRIBUTIONS

B.D.Ha. and T.E.E.G. designed the study. B.D.Ha. and B.D.He. collected and analysed the data. B.D.Ha., T.L.B. and T.E.E.G. interpreted the data. B.D.Ha. drafted the manuscript. All the authors critically revised the manuscript and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.tmpg4f4v3 (Hafner, Hesse, Bauerle, & Grams, 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Hafner BD, Hesse BD, Bauerle TL, Grams TEE. Water potential gradient, root conduit size and root xylem hydraulic conductivity determine the extent of hydraulic redistribution in temperate trees. *Funct Ecol*. 2020;00:1–14. https://doi.org/10.1111/1365-2435.13508

Title: Friendly neighbor: Hydraulic redistribution accounts for one quarter of water used by neighboring drought stressed tree saplings

Running title: Hydraulic redistribution uptake by neighbors

Keywords: temperate conifer trees, drought, temperate diffuse porous trees, temperate ring porous trees, split-root experiment, stable water isotope labeling (^{2}H/deuterium), hydraulic redistribution to neighbors, root characteristics, mixed species forests

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Abstract

1. Hydraulic redistribution (HR) by tree roots may buffer drought events within individuals for temperate species, however its relevance for neighboring trees remains unclear. Here, we quantified HR to neighboring trees in mono- and mixed species combinations. We hypothesized that the amount of HR water taken up by neighbors positively correlates with their root length, number of root tips and root xylem hydraulic conductivity. Further, we hypothesized that neighbors receive more HR water when in monospecific than in mixed combinations.
2. In a split-root experiment, one sapling redistributed water along its split root system from a moist to a dry pot. Via deuterium labeling, we quantified HR water in roots, stems and transpiration in an additional sapling in the dry pot. Amounts were correlated with its root length, number of tips and hydraulic conductivity of the root xylem.
3. Neighboring saplings of all studied six temperate tree species received HR water. Overall, one quarter of HR water in roots (2.1 ± 0.4 ml), stems (0.8 ± 0.2 ml) and transpiration (1.0 ± 0.3 ml) originated from the split-root tree. In a 3-factor model considering root length, number of tips and root xylem hydraulic conductivity of the sapling in the dry pot, especially root length was significantly positively correlated with the amount of HR water. Trees in mono- and mixed combinations received the same amounts of HR water.
4. Uptake of HR water by drought stressed saplings supports the assumption that HR contributes to increased growth and stability in mixed forests, where some tree species reach moist soil depths by deeper root systems and may redistribute water towards trees without access to this water source. Root proximity in monospecific and mixed species communities should be further investigated as a likely important prerequisite for HR benefit for neighbors.

Introduction

Hydraulic redistribution (HR) is the passive reallocation of water through roots and other tissues of plants along a water potential (ψ) gradient. In the soil, roots redistribute water overnight from moist to dry layers. The water can subsequently be released into the dry rhizosphere soil (Emerman & Dawson, 1996, Hafner et al. 2020).

Under drought, HR may facilitate plant growing conditions, by e.g. easing rhizosphere soil re-wetting or even prolonging the growing season (Brooks et al. 2002, Howard et al. 2009). A beneficial impact of HR has been suggested for neighboring plants: Under drought, neighboring trees may take up HR water released by plants with access to moist soil layers (Brooks et al. 2002, Hafner et al. 2017). The additional water was hypothesized to maintain higher stomatal conductance and growth (Dawson 1993, Magh et al. 2018), with up to nine times higher biomass production in dry regions (Bogie et al. 2018) and to potentially improve survival (Pang et al. 2013) of neighboring plants. It is suggested that HR, amongst other factors, increases growth and stability (Knoke et al. 2008, Pretzsch et al. 2010, Morin et al. 2011) of mixed species forests compared to monocultures (Goldstein et al. 2008, Pretzsch et al. 2014, Ammer et al. 2018). With anticipated reductions of forest vitality (Breda et al. 2006, Allen et al. 2010) under prolonged summer droughts (Flato et al. 2013, Orth et al. 2016), HR might become a relevant mechanism in temperate forests. However, to estimate the relevance of HR water for neighbors it is necessary to quantify the amount of HR water neighbors receive and use for tissue rehydration and transpiration. An important prerequisite for the quantification could be the mechanism, how HR water becomes available and is taken up by neighboring plants. Rather than moving through the soil matrix, probably a major fraction is transported directly from plant to plant or via a common mycorrhizal network (CMN, Warren et al. 2008, Prieto et al. 2016). Abundance of mycorrhiza, length or the number of tips of the root system might therefore be crucial for the amount of HR water neighboring plants receive (Egerton-Warburton et al. 2007). Hence, species composition may play a role in HR water amounts, where neighbors that are colonized by the same mycorrhizae as redistributing plants potentially take up more HR water (Egerton-Warburton et al. 2007). Therefore, neighbors in monospecific combinations can be expected to receive more HR water than plants in mixed neighborhoods. Another factor influencing the amount of received HR water could be root hydraulic conductivity (k_s). There is evidence that roots retaining higher k_s under drought redistribute more water (Quijano & Kumar 2015, Hafner et al. 2020) and are also able to take up water faster after re-wetting (North & Nobel 1998) than roots with lower k_s. Therefore, higher k_s in drought stressed neighboring plants could also increase the amount of HR water they take up. Here, we quantify how much HR water drought stressed neighboring saplings of six common temperate tree species received from a redistributing tree in a growth chamber experiment. We hypothesized that the amount of received HR water increases with more favorable root characteristics of drought stressed saplings, i.e. higher root length, number of root tips and k_s. Additionally, we hypothesized that flux of HR water to neighbor trees is higher within individuals of the same species than across species.

Material & Methods

Plant material and establishment

Saplings of *Acer pseudoplatanus* (L.), *Fagus sylvatica* (L.), *Castanea sativa* (Mill.), *Quercus robur* (L.), *Picea abies* ((L.) Karst.) and *Pseudotsuga menziesii* ((Mirb.) Franco), between two – four year old, were planted in December 2015 (*P. menziesii* in December 2016) into split-root systems (SRS, Fig. **1**). The potting soil was a mixture of topsoil, compost, turf and lava (including 20 % organic matter, Wurzer Umwelt GmbH, Eitting, Germany). We admixed soil (10 %) taken from native forest-stands dominated by mature trees of the study species, to provide species-specific mycorrhizal inoculum to the SRS. Each SRS (Table **1**) consisted of three trees with one central split-root plant ('SRP') that had its roots equally split between two pots (10 l each), that contained one additional tree each (Fig. **1**, Hafner et al. 2020). Eight different types of SRS were set up, either as monospecific or *P. abies* / *F. sylvatica* mixed SRS (Table **1**). Trees were established in the pots until July 2017 in a greenhouse in Freising, Germany (48°23'57.98'' N, 11°43'00.99'' E).

Experimental setup

We conducted the experiment from July to September 2017 at the TUMmesa facility (Technical University of Munich – Model EcoSystem Analyser; http://www.tummesa.de/home) in two parallel growth chambers (7.7 m² each). We established 15 hrs of daytime and 9 hrs of nighttime. Temperature, photosynthetic photon flux density (PPFD) and relative humidity (rH) were incrementally increased or decreased during morning and evening hours. The corresponding temperatures were 24.9 ± 0.3 / 15.0 ± 0.1 °C (1 SD), rH was 60.0 ± 0.3 / 88.9 ± 2.4 % (1 SD) and PPFD at canopy level reached 434.2 ± 10.7 µmol m^{-2} s^{-1} (1 SD) for 7 hrs during mid-day.

Once the plants were inside the growth cambers, we established a soil moisture gradient between the two pots by withholding irrigation from one pot ('dry pot' with the 'DP') while keeping the other pot well-watered ('moist pot' with the 'MP', Fig. **1**). Volumetric soil water content (SWC) was assessed via TDR measurements at the start of the experiment (15 cm probe; TDR100, Campbell Scientific, Shepshed, UK). We also measured water potentials in leaves pre-dawn (ψ_{leaf}) (Table **2**) using a pressure bomb (1505D pressure chamber, PMS Instrument, Albany, USA). As *A. pseudoplatanus* trees exuded a lot of milky sap from the petioles, we discarded them from ψ_{leaf} measurements.

^{2}H labeling and sampling

Prior to ^{2}H labeling, we sampled initial reference bulk soil from both dry and moist pot using a metal core (diameter of 1 cm) and transferred a subsample into an exetainer vial (Labco, Lampeter, UK). We then carefully labeled the soil of the moist pot with 300 ml of deuterated water (0.2 atom-% ^2H_2O), while the soil in the neighboring dry pot was covered with aluminum foil to prevent contamination. Afterwards, also the soil in the moist pot was covered with foil to minimize evaporation, and acrylic-glass sheets were installed, preventing canopy contact between plants (Fig. **1**). The experiment ended 7 days after the deuterium labeling. Xylem and roots of DPs and SRPs were sampled, DPs before highly ^{2}H enriched SRPs to avoid contamination. Before dawn, all trees were cut at the root collar and a c. 5 cm long subsample of the lower stem xylem, with the bark removed, was taken. Representative subsamples of the root systems of the DPs and SRPs each were collected, quickly but carefully freed from rhizosphere soil, and transferred into exetainer vials. All samples were stored at -18°C until water was extracted by cryogenic vacuum distillation for 2 hours (West et al. 2006). We used an isotope-ratio-mass-spectrometer (IRMS, Isoprime 100, Elementar Analysensysteme GmbH, Langenselbold, Germany) coupled to a multiflow system (222 XL Liquid Handler, Gilson, Middleton, USA) and a cavity-ring-down-spectrometer (CRDS, L2120-i, Picarro Inc., Santa Clara, USA) coupled to a vaporizer module (A0211, Picarro Inc., Santa Clara, USA) to analyze the samples for their δ^2H. Sample cross measurements between both instruments showed no statistical differences ($P = 0.9$; regression: $R^2 = 0.99$, $P < 0.001$) or possible organic compound contamination of the CRDS (West et al. 2010). We used two working standards (“heavy”: δ^2H of 133.3 ± 1.7 ‰ (1 SD) and “light”: δ^2H of -159.4 ± 1.9 ‰ (1 SD)) to determine measurement precision that was better than ± 0.8 ‰ (1 SE, IRMS) and ± 1.9 ‰ (1 SE, CRDS), respectively.

Assessment of leaf transpiration

Between one and five DP replicates per type of SRS were assessed for δ^2H of leaf transpiration (Table **3**). About half of total DP leaf mass per sapling was placed in transparent 3 L plastic bag chambers (ceramic-coated PET/PE mix of approx. 115 µm thickness, Long Life for Art, Eichstetten, Germany; Fig. **1**) and sealed at the bottom with Terostat-IX (Henkel AG & Co. KGaA, Düsseldorf, Germany). Chambers were continuously flushed with dry air with a flow rate of 1 L/min and outlet air was connected to a CRDS (L2120-I, Picarro Inc.) via a 16-Port Distribution Manifold (A0311, Picarro Inc., Santa Clara, USA), switching every 10 min between the different chambers. Vapor and isotope concentrations stabilized after c. 5 min, and the last 2 min of each interval were averaged. To best meet isotopic steady-state conditions, δ^2H of transpiration water was recorded during mid-day, when environmental conditions and rate of transpiration were constant. δ^2H values were calibrated against two working standards and corrections for vapor concentration offsets were performed (see supplements).

Using a two end-member mixing model, we calculated the δ^{2}H of the transpired water as the difference to the vapor concentration and isotope signal of an empty reference chamber:

$$\delta^2 H_T = \frac{(\delta^2 H_C * ppm_C - \delta^2 H_{EC} * ppm_{EC})}{(ppm_C - ppm_{EC})} \qquad \text{(Eqn. 1)}$$

with

$\delta^2 H_T$: δ^{2}H of the DP transpiration,

$\delta^2 H_C$: δ^{2}H in the foliated chamber,

ppm_C: Volumetric water vapor concentration in the foliated chamber,

$\delta^2 H_{EC}$: δ^{2}H in the empty reference chamber and

ppm_{EC}: Volumetric water vapor concentration in the empty reference chamber.

Similarly, the vapor concentration of the transpired water (ppm_T) was calculated as difference between the vapor concentration of the foliated chamber and of the empty chamber:

$$ppm_T = ppm_C - ppm_{EC} \qquad \text{(Eqn. 2)}$$

From ppm_T we calculated the absolute humidity (aH_T, in ml/m^3) of the transpired water:

$$aH_T = \frac{ppm_T * M * \rho * 0.1}{R * T} \qquad \text{(Eqn. 3)}$$

with

M: Molar mass of water (18.02 g/mol),

ρ Atmospheric pressure (in mbar)

R Molar gas constant (8.3144598 J/mol K) and

T Temperature (in K).

By interpolating linearly across measurement steps (each chamber was measured 13 times per day), we calculated the amount of daily transpired water (T, in ml) of the plants by:

$$T = \sum_0^i \Delta t * Q * aH_T * \frac{total\ leaf\ area}{leaf\ area\ in\ chamber} \qquad \text{(Eqn. 4)}$$

with

Δt: Time difference between measurement steps and

Q: Flow rate (1 l/min).

For upscaling to whole-tree transpiration, leaf area (inside and outside the chamber) was assessed. A conversion factor of 3.2 (*P. abies*, Homolová et al. 2012) and 2.32 (*P. menziesii*, Barker 1968) was used to calculate total needle area from projected area for the conifers (Table **4**).

Root characteristics

Fresh and dry mass of DP root branches, DP rootstocks and DP stems were recorded. Individual root segments (*n* = 5 per type of SRS; average mass: 0.42 ± 0.05 g (1 SE)) were scanned (1200 dpi, Epson Perfection 4990 Photo) and images analyzed with WinRhizo (WinRHIZO Reg 2013e, Regent Instruments Inc., Quebec, Canada) for determination of root length and number of tips. Specific root length (SRL, the ratio of root length to dry mass of the root) was calculated and used to estimate total root length per tree (Table **4**). Total number of root tips was linearly scaled from the fraction of the analyzed root segment to the total root system (Table **4**).

Finally, we measured native k_s of the DPs with a 'xylem embolism meter' (XYL'EM, Bronkhorst France S.A.S., Montigny-Les-Cormeilles, France). DP roots (*n* = 5 per species, *n* = 8 for *P. abies*) were cut several times under water and their barks gently peeled on the sides that were inserted into the XYL'EM (measured pieces had a diameter of 2.5 ± 0.1 mm (1 SE) and a length of 2.7 ± 0.1 cm (1 SE)). Hydraulic conductance was measured (K_{act}, in kg MPa^{-1} s^{-1}) at approx. 0.007 MPa (i.e. without removing native embolisms) using degassed, filtered (0.2 µm) water, additionally containing 10 mM KCl and 1 mM $CaCl_2$ (Barigah et al. 2013). Length and conductive area (A_{cond}) of the root sample were measured and k_s (in kg s^{-1} m^{-1} MPa^{-1}) was calculated as:

$$k_s = \frac{K_{act} * Length}{A_{cond}} \qquad \text{(Eqn. 5)}$$

Model calculations

We calculated the relative fractions (in %) and absolute amounts (in ml) of HR water in roots, stems and transpiration for each DP using two end-member mixing models. For the DP root, we assumed the δ^2H in the root water to be a mixture of soil water in the dry pot and redistributed labeled water released by the SRP:

$$HR_{DP\_root} = \frac{\delta^2H(DP_{root}) - \delta^2H(Soil_{dry\_BL})}{\delta^2H(SRP_{root}) - \delta^2H(Soil_{dry\_BL})} * 100\ (\%) \qquad \text{(Eqn. 6)}$$

with

HR_{DP_root}: Fraction of HR water in the DP root,

$\delta^2H(DP_{root})$: δ^2H of the DP root 7 days after labeling,

$\delta^2H(Soil_{dry\_BL})$: δ^2H of the soil in the dry pot before labeling and

$\delta^2H(SRP_{root})$: δ^2H of the SRP root in the dry pot 7 days after labeling.

Similarly, we calculated the isotopic composition of water in the DP stem as:

$$HR_{DP\_stem} = \frac{\delta^2H(DP_{stem}) - \delta^2H(Soil_{dry\_BL})}{\delta^2H(SRP_{root}) - \delta^2H(Soil_{dry\_BL})} * 100\ (\%) \qquad \text{(Eqn. 7)}$$

with

HR_{DP_stem}: Fraction of HR water in the DP stem and

$\delta^2H(DP_{stem})$: δ^2H of the DP stem 7 days after labeling.

We used the transpiration δ^2H of day 6 (i.e. 1 day before harvesting the plants) to calculate the fraction of labeled HR water in DP transpiration:

$$HR_{DP\_transpiration} = \frac{\delta^2H(DP_{transpiration}) - \delta^2H(Soil_{dry\_BL})}{\delta^2H(SRP_{root}) - \delta^2H(Soil_{dry\_BL})} * 100\ (\%) \qquad \text{(Eqn. 8)}$$

with

$HR_{DP\_transpiration}$: Fraction of HR water in the DP transpiration and

$\delta^2H(DP_{transpiration})$: δ^2H of the DP transpiration on day 6 after labeling.

The respective fractions of HR water were multiplied with the amounts of water in DP roots, DP stems and total DP transpiration of day 6 (Eqn. 4) to gain the absolute amounts of HR water per tree. As root masses significantly differed between species (Table **4**), we divided the absolute amounts of HR water by the dry root mass of each DP to compare HR amounts between species.

We also ran the mixing model calculations (Eqn. 6-8) using the average δ^2H values per type of SRS with a mixing model accounting for uncertainty errors (‘Iso Error’, Philips & Gregg 2001), giving the same results as our single-value based calculations.

Statistics

The isotope data were checked for significant ($P < 0.05$) increases in δ^2H after labeling. We used the pots as random factors nested over respective growth chamber identities in a linear mixed effect model (R package nlme, version 3.1-137). We tested the δ^2H values of the samples (SRP root, DP root, DP stem and DP transpiration) individually for increases (using day and type of SRS as independent variables). Increases in and differences between the δ^2H values of the types of SRS were revealed with the lsmeans post-hoc test (R package lsmeans, version 2.27-62). Model data were checked for residual normal distribution (shapiro.test) and variance homogeneity (leveneTest; R package car, version 2.1-2). Where necessary, values were transformed to meet residual normal distribution. Differences between the types of SRS in the amounts and fractions of HR water and between plant or soil characteristics (biomass, root length, leaf area, ψ_{leaf}, SWC, k_s) were checked using the same model and tests. We performed a 3-factor linear model to determine a potential correlation between mixing model outputs and root characteristic parameters (root length, number of tips, k_s). Mean values are given ± 1 standard error (1 SE). All statistical analyses were performed with R version 3.3.1 (R Development Core Team, 2018) in RStudio version 1.1.447 (RStudio Team, 2015).

Results

Plant and soil characteristics

Volumetric soil water content in the dry pot ranged between 8.5 ± 0.4 vol% (*P. menziesii*) and 16.8 ± 2.5 vol% (*P. abies mix*, Table **2**) and was significantly lower for all types of SRS than in the moist pot (average in the moist pot: 28.7 ± 1.4 vol%). Pre-dawn ψ_{leaf} of the DP ranged from -0.8 ± 0.1 MPa in *P. abies* to significantly lower -2.8 ± 0.7 and -3.5 ± 0.4 MPa in C. sativa and *Q. robur*, respectively (Table **2**). The root systems of *C. sativa* DP trees were significantly heavier (27.7 ± 4.4 g) and longer (149 ± 25 m) than the systems of all other species (average of other species: 5.5 ± 0.6 g and 48 ± 6 m, respectively, Table **4**). Accordingly, the stems of *C. sativa* had the highest biomass (14.4 ± 1.9 g), while stems of *P. abies* trees (1.6 ± 0.1 g in *mono* and 1.2 ± 0.1 g in *mix SRS*) had a significantly lower biomass than all other species (Table **4**). Root hydraulic conductivity (k_s) was highest in *A. pseudoplatanus* and *F. sylvatica mix* (0.64 ± 0.08 kg s^{-1} m^{-1} MPa^{-1} and 0.64 ± 0.02 kg s^{-1} m^{-1} MPa^{-1}, respectively) and lowest in *P. abies mono*, *P. menziesii* and *C. sativa* (0.28 ± 0.07 kg s^{-1} m^{-1} MPa^{-1}, 0.29 ± 0.03 kg s^{-1} m^{-1} MPa^{-1} and 0.33 ± 0.08 kg s^{-1} m^{-1} MPa^{-1}, respectively, Table **4**). Root systems of *Q. robur* had the highest number of root tips (1850 ± 424), while *P. menziesii* trees had the lowest number of tips (287 ± 63). Total leaf area was highest in *C. sativa* (1648 ± 261 cm^2) and *Q. robur* (993 ± 173 cm^2), while all other species had significantly smaller leaf areas (from 89 ± 18 cm^2 in *P. abies mix* to 389 ± 101 cm^2 in *A. pseudoplatanus*, Table **4**).

Enrichment in ^2H upon labeling

Before labeling, δ^2H was at -59 ± 1 ‰ across all SRS. Upon labeling, δ^2H of the SRP roots in the dry pots were significantly increased in all types of SRS ranging from -14 ± 7 ‰ in *C. sativa* to 168 ± 68 ‰ in *P. menziesii* (average value of 54 ± 14 ‰, Table **3**), confirming a translocation of labeled water within the SRP from the labeled, moist to the dry pot. The DP roots showed significantly increased values in all types of SRS except for *F. sylvatica mix* (-51 ± 3 ‰) and *Q. robur* (-46 ± 3 ‰) with the same tendency ($P = 0.05$). In the stems, all DP were significantly enriched in δ^2H with the exception of again *F. sylvatica mix* (-42 ± 4 ‰, $P = 0.07$) and *Q. robur* (-51 ± 2 ‰). Finally, the transpired water was significantly enriched in *P. abies (mono & mix)*, *P. menziesii*, *F. sylvatica mix* and *Q. robur* (Table **3**); by trend also in *F. sylvatica mono* (-51 ± 1 ‰, $P = 0.1$), while no enrichment was found in *A. pseudoplatanus* and *C. sativa* (-58 ± 1 ‰ and -69 ‰, respectively, Table **3**).

Amounts of HR water in roots, stems and transpiration of DP in monospecific SRS

Seven days after labeling, the DP roots growing in monospecific SRS contained on average 2.26 ± 0.48 ml (25 ± 4 %) of HR water (see blue bars in Fig. **2**). The absolute amount was highest in *P. abies* trees (5.07 ± 1.82 ml) and significantly higher than in *Q. robur* and *F. sylvatica* with the lowest amounts of 0.43 ± 0.22 ml and 0.72 ± 0.36 ml, respectively (Table **5**). The results were consistent also for the fractions of HR water in total root water, with highest fractions in *P. abies* (50 ± 13 %) and lowest in *Q. robur* roots (9 ± 3 %, blue numbers in Fig. **2**). In DP stems, we found 0.79 ± 0.19 ml (fraction of 17 ± 3 %) of HR water over all species (green bars in Fig. **2**). The absolute amount was significantly higher in *C. sativa* (3.18 ± 0.50 ml) than in all other species (Table **5**). The relative fractions were highest in *P. abies* and *C. sativa* (30 ± 8 % and 34 ± 5 %, respectively), while the lowest fractions were found in *P. menziesii* and *Q. robur* (8 ± 2 % and 4 ± 1 %, respectively; green numbers in Fig. **2**).

In total daily transpiration, HR water amounted to 1.02 ± 0.34 ml (fraction of 22 ± 6 %) over all plants with two individuals almost entirely transpiring HR water (see data points close to the 1:1 line, Fig. **3**). *C. sativa* did not transpire HR water and also the fraction in *A. pseudoplatanus* was very low (4 ± 0.4 %). However, both species had the lowest daily transpiration of about 1.3 ml (Fig. **3**).

In a 3-factor regression, we found significant correlations of root length, number of tips and k_s with absolute amounts of HR water in roots ($R^2 = 0.8$, $P < 0.001$) and stems ($R^2 = 0.7$, $P < 0.01$), but only by trend in transpiration ($R^2 = 0.7$, $P = 0.1$; Table **6**). Within the model, root length and the interaction root length and number of tips were significantly positively correlated with the amount of HR water in roots and stems (Table **6**). In the roots, k_s had a (trending) negative effect on the quantity of labeled HR water, either as single factor ($P = 0.1$) or in interaction with root length ($P < 0.01$), number of tips ($P = 0.05$) or both ($P = 0.08$; Table **6**).

Amounts of HR water in mixed SRS

In the mixed SRS, DP roots of *P. abies* contained 2.51 ± 0.75 ml (i.e. 45 ± 11 % of 7.30 ± 3.00 ml total root water) of HR water redistributed by *F. sylvatica* SRPs. Conversely, DP roots of *F. sylvatica* received 0.20 ± 0.07 ml (i.e. 6 ± 2 % of 3.14 ± 0.92 ml total root water, Fig. **4**) from *P. abies* SRPs. In the DP stems we found 0.27 ± 0.10 ml (29 ± 11 % of 0.93 ± 0.04 ml total stem water) of redistributed water in *P. abies*, whereas 1.46 ± 0.64 ml (47 ± 20 % of 3.11 ± 0.20 ml total stem water) of the water in *F. sylvatica* DPs originated from the other species (Fig. **4**). The transpired water on day 6 contained 0.66 ± 0.19 ml (30 ± 9 % of 2.66 ± 0.55 ml total transpired water) of HR water in *P. abies* DPs and 1.43 ± 1.14 ml (48 ± 25 % of 2.48 ± 1.16 ml total transpired water) in *F. sylvatica* DPs in mixture (Fig. **4**). Divided by DP dry root mass, there was no difference for both species in the amounts of HR water in roots and transpiration whether the neighbor was the same or another species (Table **5**). For *P. abies*, the received amount of water per dry DP root mass in the stems was not different if the SRP was also *P. abies* or *F. sylvatica*, while the amount in stems per dry DP root mass was significantly higher in mixture than in the monospecific SRS for *F. sylvatica* DPs (Table **5**).

Discussion

Hydraulic redistribution displayed an important process for the water supply of the drought stressed saplings of all tested temperate tree species. On average, one quarter of the water in the DPs originated from HR. Root length was the best predictor of variations in HR water amounts, supporting our first hypothesis, that more favorable root characteristics increase HR amounts. Among *F. sylvatica* and *P. abies*, it was insignificant whether the SRP was of the same or another species. Therefore, our second hypothesis that DP in monospecific SRS take up higher amounts of HR water than in mixed SRS was not supported by our findings.

Amounts of HR water taken up by DPs in monospecific SRS

We found HR water in the DPs of all types of SRS, confirming the general occurrence of the effect in our study species (Hafner et al. 2017). The contribution of HR water was different for the species: In *Q. robur* and *F. sylvatica*, we hardly found HR water in roots or stems. These species used the HR water directly in their transpiration, as the amounts of daily transpired HR water were high (20.7 ± 1.7 % in *Q. robur* and 31.1 ± 25.9 % in *F. sylvatica*, respectively). Conversely, *A.*

pseudoplatanus and *C. sativa* hardly transpired but largely kept the HR water within their roots and stems. For these species, HR water played a bigger role for refilling of internal water storages (Yu et al. 2018) as transpiration rates were rather low. Within the 3-factor model, the amount of HR water taken up by neighboring DPs was especially dependent on their root length and the interaction of root length and number of tips. Concurring with our expectations, longer root systems with more tips lead to higher amounts of HR water in roots and stems. It has been reported that HR water amounts increase with higher root densities (Aanderud & Richards 2009). Instead of mere root length or number of tips, the actual contact between root systems could be essential here. Distribution and proximity of roots has been suggested as an important driving factor for HR and HR between species, with higher root contact increasing HR amounts (Hultine et al. 2003, Schoonmaker et al. 2007, Scholz et al. 2008). Some species seem to avoid contact with other roots, to reduce (self-) competition (Maina et al. 2002, Falik et al. 2003), while others show increased growth when root systems are in close vicinity with their neighbors (Armas & Pugnaire 2011). We detected a negative correlation of amounts of HR water in roots with root k_s in our model. Potentially, labeled HR water was transported faster from roots to stems and then transpired in plants with higher k_s, reducing the labeling signal. There was no significant correlation of root length, number of tips or root k_s with HR amounts in DP transpiration. For HR water in transpiration, potentially additional driving factors have to be considered that also influence plant stomatal conductance, e.g. vapor pressure deficit or temperature (Lange et al. 1971, Will et al. 2013). Interestingly, coniferous *P. abies* trees, where root length or number of tips were not significantly different to the other species, showed the highest amounts of HR water in roots and transpiration (Table **5**). Also, SRPs of *P. abies* did not redistribute more water from the moist to the dry pot than the other species (Hafner et al. 2020). Rather, the potential amounts of water redistributed from moist to dry soils by coniferous *P. abies* SRPs with low k_s and conduit diameters is expected to be low compared to the other study species (Hafner et al. 2020). It has, however, been shown that *Picea* trees tend to increase root branching when growing with neighbors (Paya et al. 2015). Therefore, the high amounts of HR water in *P. abies* DPs might be explained by closer root proximity of *P. abies* trees to its neighbors. However, no clear hierarchical pattern or mechanistic background on root proximity is known yet (Armas & Pugnaire 2011).

Mixed combinations

In contrary to our hypothesis, neighbor saplings grown in monospecific SRS did not receive more HR water than DPs in mixed SRS, at least for the tested combinations of *F. sylvatica* and *P. abies*. Preconditions for HR such as SWC, root mass or leaf area were not different between saplings in monospecific and mixed SRS, allowing for direct comparison between the two. With the exception of HR water in stems of *F. sylvatica mono* vs *F. sylvatica* mix, where more HR water was found in DPs of the mixed SRS, we did not find differences in amounts of HR water between monospecific and mixed SRS. We did not quantify mycorrhization or determine mycorrhizal morphotypes, however frequently observed invested root tips of both, *P. abies* and *F. sylvatica* during harvest. It has been described that *P. abies* and *F. sylvatica* are partially colonized by the same mycorrhizae (Trappe 1962), therefore potentially allowing for a HR-pathway via a CMN. However, the influence of species mixture on either benefit or competition on HR water seems to vary widely with involved species, as water can be provided to or drained from neighbors (Prieto et al. 2012) and might even change from facilitation to competition across a growing season (Priyadarshini et al. 2016, Muler et al. 2018). Based on our results, *P. abies* and *F. sylvatica* both profit from HR in mixture with each other, indicating a predominant facilitative interaction between these two species. Occurrence and seasonality of HR in a grown mixed-species forest of *P. abies* and *F. sylvatica* should be further investigated to estimate the relevance of water redistribution for both species in mixture under field conditions.

Conclusions

All observed species benefited from HR, either by refilling water storages or for transpiration, irrespective of growth in mixture or a monospecific SRS. Therefore, HR could be an important process in temperate forests increasingly facing periods of water limitations under ongoing climate change. Rooting patterns, especially root proximity between same and different species need to be investigated as close proximity and even direct contact might have an important influence on the amount of HR water uptake by neighbor trees. Our results further support the assumption that HR between different species contributes to increased growth and stability in mixed forests, in particular among species with different rooting depths (Pretzsch et al. 2014; Ammer et al. 2018). The actual relevance of HR in temperate forests in either mixture or monoculture needs to be tested to determine to what extent the considerable benefit, found in this study, also translates into the field.

Author contributions

BDHa and TEEG designed the study. BDHa and BDHe collected and analyzed the data. BDHa and TEEG interpreted the data. BDHa drafted the manuscript. All authors critically revised the manuscript and gave final approval for publication.

Acknowledgements

The authors thank Yessica Kölmel and Rudi Schäufele for the isotope measurements and Christine Stumpp and Eckart Priesack for loaning the CRDS system for transpiration measurements. For growth chamber maintenance, experimental set-up help, sampling and water extractions, we thank Thomas Feuerbach, Josef Heckmair, Peter Kuba, Roman Meier, Aline Potreck and Tommi Schieder. The study was supported by the “Europamöbel Umweltstiftung” and the “Hartig Stiftung”. BDHa and BDHe were funded by a doctoral scholarship from the German Federal Environmental Foundation (DBU).

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Tables

Table 1: Types of split-root systems (SRS) analyzed in this study. The SRS consisted of one tree in a moist pot (MP), one tree in a dry pot (DP) and one tree with its root system split between both pots (SRP; see also Fig. 1). In total eight types of SRS were set up: six monospecific SRS and two mixed SRS with mixtures of *P. abies* and *F. sylvatica* trees.

	n	**MP**	**SRP**	**DP**
P. abies mono	7	*P. abies*	*P. abies*	*P. abies*
P. abies mix	7	*F. sylvatica*	*F. sylvatica*	*P. abies*
P. menziesii	7	*P. menziesii*	*P. menziesii*	*P. menziesii*
A. pseudoplatanus	7	*A. pseudoplatanus*	*A. pseudoplatanus*	*A. pseudoplatanus*
F. sylvatica mono	7	*F. sylvatica*	*F. sylvatica*	*F. sylvatica*
F. sylvatica mix	5	*P. abies*	*P. abies*	*F. sylvatica*
C. sativa	7	*C. sativa*	*C. sativa*	*C. sativa*
Q. robur	7	*Q. robur*	*Q. robur*	*Q. robur*

Table 2: Volumetric soil water content (SWC) in dry pots and measured pre-dawn leaf water potentials (ψ_{leaf}) of plants in the dry pots (DP). Letters indicate significant ($P < 0.05$) differences between the types of SRS. Note that ψ_{leaf} of *A. pseudoplatanus* trees could not be assessed due to heavy milky sap exudation.

	SWC (vol-%)	**ψ_{leaf} (MPa)**
P. abies mono	11.2 ± 1.8 ^{ab}	-0.9 ± 0.3 ^{a}
P. abies mix	16.8 ± 2.5 ^{a}	-0.8 ± 0.1 ^{a}
P. menziesii	8.5 ± 0.4 ^{b}	-2.0 ± 0.3 ^{ab}
A. pseudoplatanus	11.1 ± 0.6 ^{ab}	*NA*
F. sylvatica mono	11.9 ± 1.1 ^{ab}	-1.6 ± 0.5 ^{ab}
F. sylvatica mix	16.7 ± 2.6 ^{a}	-0.9 ± 0.1 ^{a}
C. sativa	9.0 ± 0.4 ^{b}	-2.8 ± 0.7 ^{bc}
Q. robur	11.2 ± 0.7 ^{ab}	-3.5 ± 0.4 ^{c}

Table 3: Initial δ^2H (‰) of soil water before labeling and of roots of the split-root plant (SRP) in the dry pot and of roots, stems and transpiration of the plant in the dry pot (DP) at day 7. Letters indicate significant ($P < 0.05$) differences between the types of SRS, and asterisks give significant enrichment in ^{2}H above initial soil water at day 7 (° ≤ 0.1, * < 0.05, ** < 0.01, *** < 0.001).

	Before Labeling	**SRP root**	**DP root**	**DP stem**	***n* transpiration**	**DP transpiration**
P. abies mono	-57 ± 1 ^{ab}	21 ± 35 *** ^{a}	-40 ± 3 *** ^{ac}	-46 ± 2 * ^{ab}	3	-42 ± 2 ** ^{ab}
P. abies mix	-57 ± 2 ^{ab}	4 ± 18 *** ^{a}	-41 ± 1 *** ^{ac}	-47 ± 3 * ^{ab}	5	-44 ± 2 ** ^{ab}
P. menziesii	-53 ± 2 ^{a}	168 ± 68 *** ^{a}	-28 ± 8 *** ^{a}	-40 ± 5 * ^{a}	4	-28 ± 3 *** ^{b}
A. pseudoplatanus	-63 ± 1 ^{ab}	86 ± 46 *** ^{a}	-40 ± 9 *** ^{abc}	-55 ± 2 * ^{b}	3	-58 ± 1 ^{ac}
F. sylvatica mono	-60 ± 1 ^{ab}	48 ± 29 *** ^{a}	-43 ± 3 *** ^{abc}	-50 ± 3 * ^{ab}	3	-51 ± 1 ° ^{ab}
F. sylvatica mix	-55 ± 2 ^{ab}	33 ± 45 ** ^{a}	-51 ± 3 ^{bc}	-42 ± 4 ° ^{ab}	4	-41 ± 2 ** ^{b}
C. sativa	-66 ± 2 ^{b}	-14 ± 7 *** ^{a}	-55 ± 1 *** ^{b}	-50 ± 1 *** ^{ab}	1	-69 ^{c}
Q. robur	-57 ± 2 ^{ab}	76 ± 17 *** ^{a}	-46 ± 3 ° ^{abc}	-51 ± 2 ^{ab}	3	-26 ± 1 *** ^{b}

Table 4: Characteristics of plants in the dry pots (DP), i.e. root and stem biomass, total root length, native hydraulic conductivity (k_s) and whole-tree leaf area. Letters indicate significant ($P < 0.05$) differences between the different types of split-root systems.

	Root biomass (g)	**Stem biomass (g)**	**Root length (m)**	**Root tips**	**k_s (kg s^{-1} m^{-1} MPa^{-1})**	**Leaf area (cm^2)**
P. abies mono	7.0 ± 1.1 ^{a}	1.6 ± 0.1 ^{a}	46 ± 12 ^{a}	828 ± 165 ^{abc}	0.28 ± 0.07 ^{a}	299 ± 56 ^{a}
P. abies mix	2.8 ± 0.8 ^{a}	1.2 ± 0.1 ^{a}	42 ± 23 ^{a}	478 ± 151 ^{abc}	0.34 ± 0.04 ^{ab}	89 ± 18 ^{a}
P. menziesii	5.6 ± 0.8 ^{a}	3.8 ± 0.1 ^{b}	59 ± 13 ^{ab}	287 ± 63 ^{a}	0.29 ± 0.03 ^{a}	300 ± 45 ^{a}
A. pseudoplatanus	8.1 ± 1.3 ^{a}	4.4 ± 0.2 ^{b}	59 ± 17 ^{ab}	1176 ± 373 ^{abc}	0.64 ± 0.08 ^{b}	389 ± 101 ^{a}
F. sylvatica mono	5.0 ± 2.3 ^{a}	3.0 ± 0.7 ^{b}	62 ± 24 ^{ab}	1493 ± 460 ^{bc}	0.49 ^{ab}	255 ± 45 ^{a}
F. sylvatica mix	2.4 ± 0.7 ^{a}	3.6 ± 0.2 ^{b}	40 ± 19 ^{ab}	1377 ± 544 ^{abc}	0.64 ± 0.02 ^{b}	146 ± 44 ^{a}
C. sativa	27.7 ± 4.4 ^{b}	14.4 ± 1.9 ^{c}	149 ± 25 ^{b}	453 ± 74 ^{ab}	0.33 ± 0.08 ^{a}	1648 ± 261 ^{b}
Q. robur	4.8 ± 0.8 ^{a}	4.1 ± 0.3 ^{b}	25 ± 6 ^{a}	1850 ± 424 ^{c}	0.57 ± 0.06 ^{ab}	993 ± 173 ^{b}

Table 5: Amounts of redistributed water in roots, stems and transpiration (total and per DP root dry mass) in the different types of SRS. Letters indicate significant differences between the types of SRS.

	Root		**Stem**		**Transpiration**	
	ml	ml g^{-1}	ml	ml g^{-1}	ml	ml g^{-1}
P. abies mono	5.07 ± 1.82 ^{a}	0.63 ± 0.22 ^{ab}	0.46 ± 0.12 ^{ac}	0.07 ± 0.03 ^{a}	2.68 ± 1.39 ^{a}	0.30 ± 0.18 ^{a}
P. abies mix	2.51 ± 0.75 ^{ab}	0.75 ± 0.22 ^{a}	0.27 ± 0.10 ^{ac}	0.10 ± 0.04 ^{a}	0.66 ± 0.19 ^{a}	0.39 ± 0.22 ^{a}
P. menziesii	1.05 ± 0.31 ^{abc}	0.19 ± 0.04 ^{abc}	0.21 ± 0.06 ^{ac}	0.05 ± 0.02 ^{a}	0.85 ± 0.31 ^{a}	0.13 ± 0.00 ^{a}
A. pseudoplatanus	2.93 ± 1.31 ^{abc}	0.39 ± 0.18 ^{abc}	0.44 ± 0.23 ^{ac}	0.05 ± 0.02 ^{a}	0.06 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}
F. sylvatica mono	0.72 ± 0.36 ^{bc}	0.24 ± 0.09 ^{abc}	0.35 ± 0.20 ^{ac}	0.11 ± 0.05 ^{a}	0.93 ± 0.86 ^{a}	1.60 ± 1.56 ^{a}
F. sylvatica mix	0.20 ± 0.07 ^{c}	0.08 ± 0.02 ^{bc}	1.46 ± 0.64 ^{ab}	0.85 ± 0.46 ^{b}	1.43 ± 1.14 ^{a}	0.44 ± 0.25 ^{a}
C. sativa	3.38 ± 1.14 ^{abc}	0.14 ± 0.05 ^{bc}	3.18 ± 0.50 ^{b}	0.13 ± 0.03 ^{a}	0.00 ^{a}	0.00 ^{a}
Q. robur	0.43 ± 0.22 ^{bc}	0.08 ± 0.03 ^{c}	0.09 ± 0.02 ^{c}	0.02 ± 0.01 ^{a}	1.00 ± 0.45 ^{a}	0.28 ± 0.19 ^{a}

Table 6: Output parameters of a 3-factor linear model for regression between amounts of HR water found in roots, stems and transpiration of DPs with root length, number of tips and hydraulic conductivity (k_s) of the DPs. The linear model includes interactions between the independent variables. Coefficients of determination and *P* values are each given for the whole models for roots, stems and transpiration. Significant correlations are highlighted in bolt.

	HR water in Root			HR water in Stems			HR water in Transpiration		
	Estimate	*t value*	*P value*	*Estimate*	*t value*	*P value*	*Estimate*	*t value*	*P value*
Root length	0.0555	4.81	**< 0.001**	0.0299	5.61	**<0.001**	-0.0041	-0.31	0.8
Tips	0.0010	1.20	0.3	0.0004	1.06	0.3	-0.0009	-1.29	0.3
k_s	-3.9140	-1.81	0.1	0.2531	0.25	0.8	-2.3250	-1.66	0.2
Root length x Tips	0.0001	2.55	**< 0.05**	0.0000	2.31	**<0.05**	-0.0001	-2.84	0.07
Root length x k_s	-0.3741	-3.95	**< 0.01**	-0.0156	-0.36	0.7	-0.3090	-0.36	0.7
Tips x k_s	-0.0177	-2.21	0.05	0.0040	1.09	0.3	-0.0072	-1.88	0.2
Root length x Tips x k_s	-0.0005	-1.95	0.08	0.0002	1.49	0.2	-0.0006	-1.94	0.1
Adjusted R^2			0.81			0.70			0.72
P value			**< 0.001**			**< 0.01**			0.1

Figures

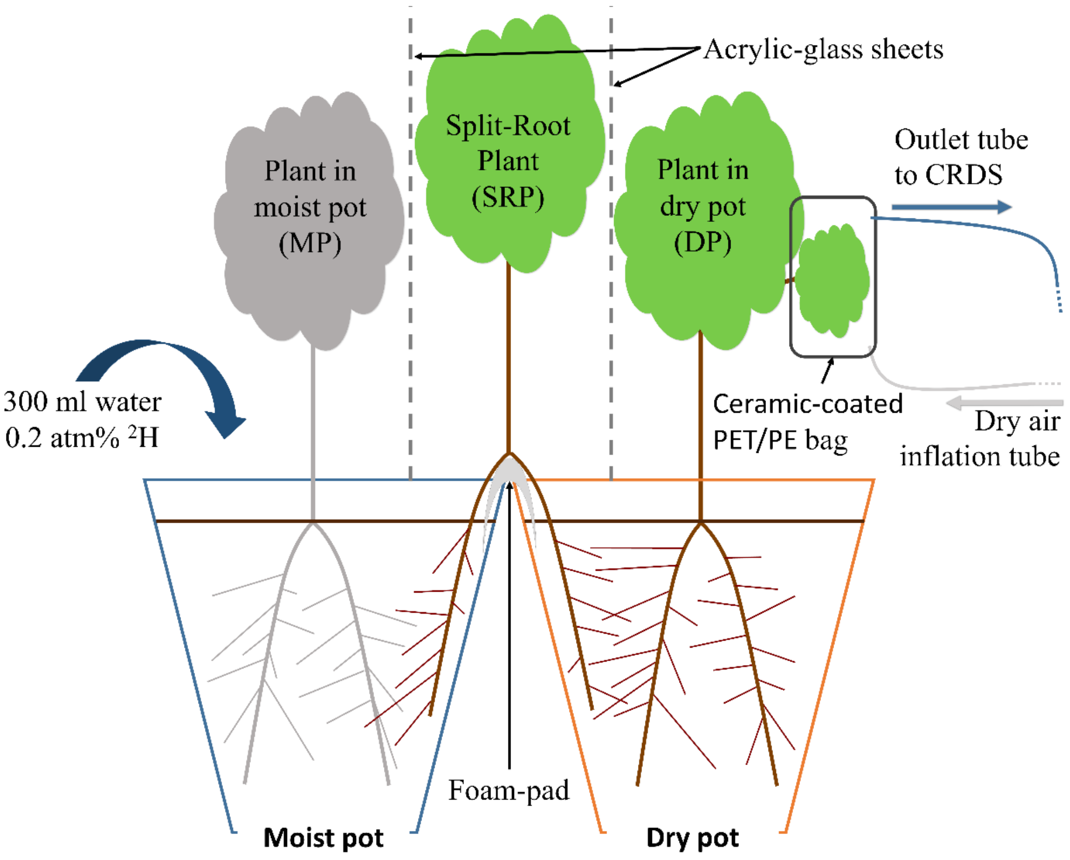

Figure 1: Scheme of the split-root systems (SRS) with one tree's (split-root plant, SRP) root system split equally between a 'moist' and 'dry' pot. A foam-pad was placed between the roots of the SRP and the pot to prevent injuries. Additional trees were planted in both pots ('MP' and 'DP', respectively). Vertical acrylic-glass sheets prevented canopy contact between the plants. Foliated twigs of the DP were placed in transparent ceramic-coated PET/PE chambers that were inflated with dry air. Outlet air went via PVC tubes to a cavity-ring-down-spectrometer (CRDS) for ^{2}H isotopic analysis.

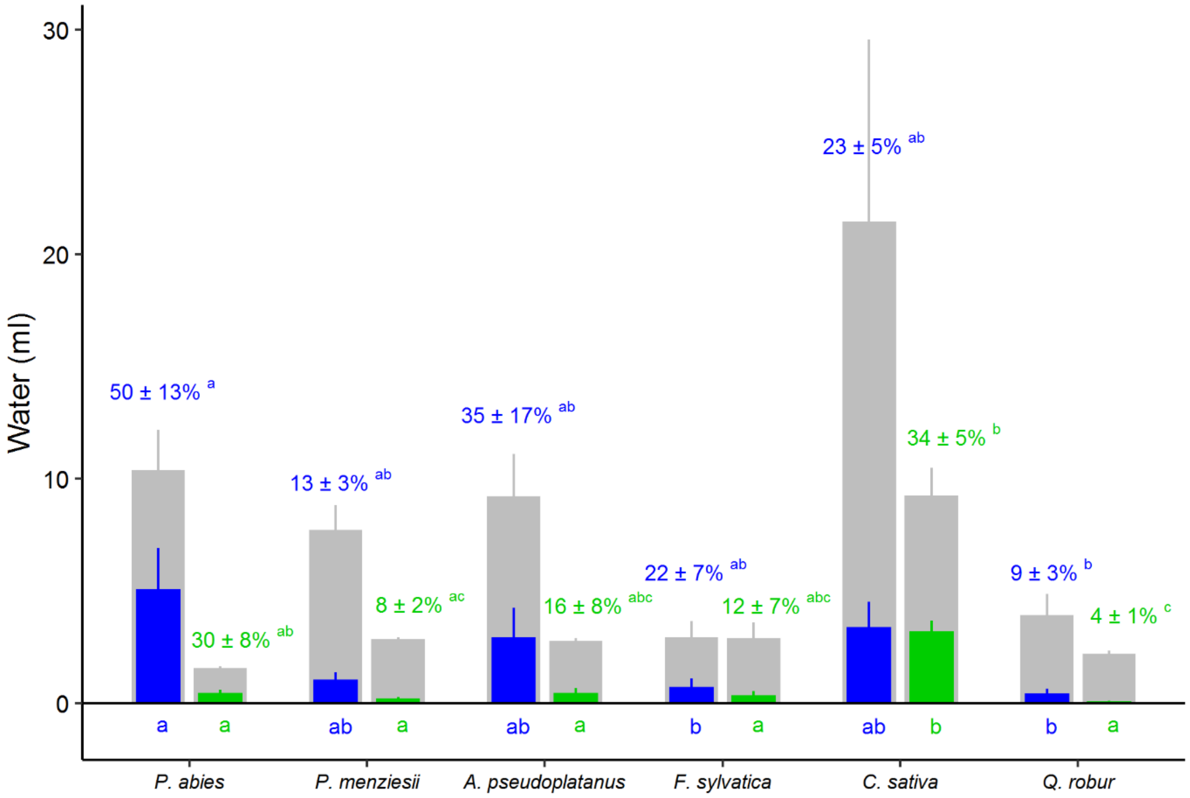

Figure 2: Amount (in ml, bars) of HR water in roots (blue) and stems (green) in DP of monospecific split-root systems. Grey bars give total water of tissues with relative fraction of HR water given above bars. All values are means ± 1 SE.

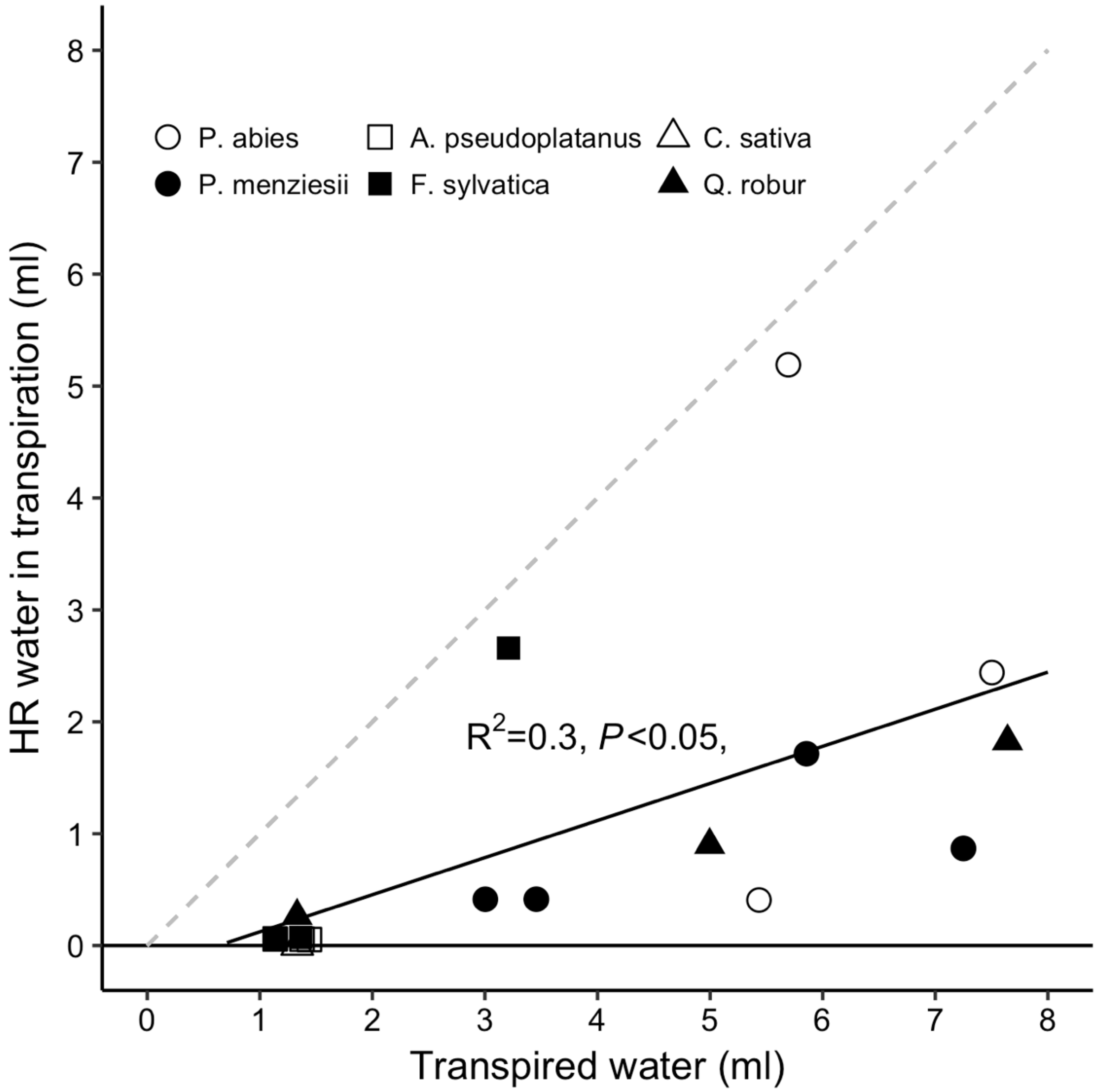

Figure 3: Amount of transpired HR water versus total daily transpired water of the DP in monospecific split-root systems. The dotted line indicates equality.

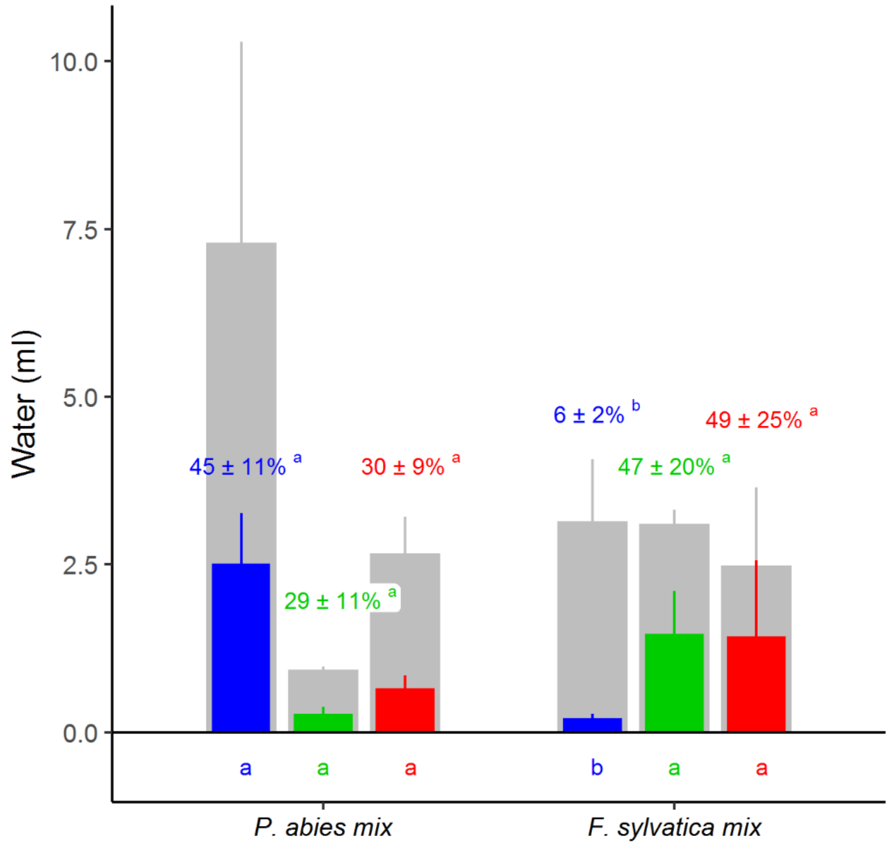

Figure 4: Amount (in ml, bars) of HR water in roots (blue), stems (green) and transpiration (red) in DPs of mixed split-root-systems. Grey bars give total water in tissues with the relative fraction of HR water given above bars. All values are means ± 1 SE.

Supplemental Material to *Friendly neighbor: Hydraulic redistribution accounts for one quarter of water used by neighboring drought stressed tree saplings* by Benjamin D. Hafner, Benjamin D. Hesse and Thorsten E. E. Grams.

Calibration and transformation of the CRDS transpiration data

To calibrate the CRDS we used two laboratory standards (kept in bags in a refrigerator at a constant temperature of 10 °C) spanning the range of the transpiration values (-65 ‰ and 77 ‰, respectively). Standards were measured twice a day. As the CRDS measured the vapor phase above the liquid standards, measured standard values (liquid) were transferred to vapor values via equilibrium-fractionation-equations (taken from Majoube, 1971; Friedman & O'Neil, 1977; Kakiuchi & Matsuo, 1979 and Horita & Wesolowski, 1979). Additionally the δ^2H signal drifted with the vapor concentration ("ppm-drift"). Therefore, we ran a linear regression (in a range from 6000 – 30000 ppm; $P < 0.001$, $R^2 = 0.9$, slope: 0.0019) with water of a known δ^2H signature and referred all measured values to a vapor concentration of 12000 ppm. Finally, an offset of the vapor concentration was corrected by fitting a linear regression between known concentrations produced by a dew point generator (Heinz Walz GmbH, Effeltrich, Germany) and corresponding measured concentrations by the CRDS ($P < 0.001$, $R^2 = 0.9$, $y = 1.1 x + 472$).

After the corrections, unlabeled transpiration and soil δ^2H values showed an offset of about 10-20 ‰. The specific offset was added to the end-members of each replicate system (i.e. day 7 δ^2H values of the split-root plant's root in the dry pot and day 0 soil δ^2H values) to calculate the respective mixing model (Eqn. 7).

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Journal of Plant Hydraulics

Journal of Plant Hydraulics 6: e-001

Reverse conductivity for water transport and related anatomy in fine roots of six temperate tree species – a potential limitation for hydraulic redistribution

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Date of submission: 8 May 2019
Date of publication: 9 September 2019

Key words

Climate change, drought stress, water flow, Sycamore maple (*Acer pseudoplatanus*), Sweet chestnut (*Castanea sativa*), Norway spruce (*Picea abies*), Douglas fir (*Pseudotsuga menziesii*), English oak (*Quercus robur*), European beech *(Fagus sylvatica)*

Abstract

Hydraulic redistribution (HR), the passive reallocation of water along plant structures following a water potential gradient, is an important mechanism for plant survival under drought. For example, trees with deeper roots reallocate water from deeper moist to shallower, drier soil layers sustaining their upper fine root system. The relevance of HR for temperate forest ecosystems is hardly investigated. Both environmental and tree internal factors limiting the capacity for HR, such as low water potential gradients or root anatomy, respectively, are not well understood. Here we investigate fine root anatomy and related capacity for reverse flow of water of six temperate tree species, i.e. *Acer pseudoplatanus, Castanea sativa, Fagus sylvatica*, *Picea abies, Pseudotsuga menziesii* and *Quercus robur* both in forward and reverse flow direction. Additionally, anatomy of primary and secondary roots was analyzed, to test the hypotheses that root anatomy is similar in primary and secondary roots (H1) and conductivity for forward and reverse flow of water in fine roots is identical (H2). In contrast to the two conifer species, most anatomical parameters, e.g. hydraulic conduit diameter and conduit density, were distinctly different between primary and secondary roots in the angiosperms. Therefore, H1 was not supported for angiosperm trees. The reverse flow of water in fine roots was reduced by approx. 40 % compared to the forward flow in angiosperms, while there was no difference in the conifers. Thus, H2 was confirmed for conifers while there was a significant difference for angiosperms. This reduction may be caused by vessel structure (e.g. tapering or secondary thickening elements), or perforation plate and pit architecture (e.g. width of aperture opening). Because of the reduced conductivity of reverse water flow, the ability of angiosperm trees to redistribute water along their root system might be lower than expected.

Introduction

Prolonged and intense drought periods are one of the consequences of ongoing climate change (IPCC 2007, 2014) as predicted by most climate change scenarios (Burke *et al.*, 2006). Central Europe experienced extreme droughts during summers 2003, 2015 and 2018 (Ionita *et al.*, 2017; Hänsel *et al.*, 2019). The ramifications of such extreme events were drastic for temperate European (Ciais *et al.*, 2005; Leuzinger *et al.*, 2005) and North American forests (Asner *et al.*, 2016; Hartmann *et al.*, 2018). Long and severe drought periods often lead to a severe shortage of water for plants which may result in hydraulic failure, the collapse of the water conducting system, often resulting in tree death (Brodribb and Cochard, 2009; Urli *et al.*, 2013). In particular trees as long-living organisms possess versatile strategies against drought such as hydraulic (Tomasella *et al.*, 2017a) or photosynthetic acclimation (Watkinson *et al.*, 2003). Another strategy is hydraulic redistribution (HR) of water from moist (e.g. deeper) to drier soil layers via the root system (Neumann and Cardon, 2012; Prieto *et al.*, 2012), a phenomenon hardly explored for temperate forests (Emerman and Dawson, 1996). If deeper roots have access to more water-saturated soil layers, the complete root system will equilibrate at high water potentials, in particular during the night when stomata close. The water potential gradient within the soil is the driving factor for the redistribution of water towards the soil layers with lower water potential along the root. A wide range of plant species, including conifers and angiosperm trees, are able to redistribute water (e.g. Prieto *et al.*, 2012; Hafner *et al.*, 2017), while the amount of redistributed water can strongly vary (from 0.04 to 4 mm water per day) even within one ecosystem (Neumann and Cardon, 2012). Nevertheless, on average HR water can represent up to 15 % of water used for transpiration (Neumann and Cardon, 2012) or up to 80% of root water (Hafner *et al.*, 2017) and therefore play an important role for the water balance of plants. Several empirical studies report a suit of factors that define the amount of HR and consequently are used in model predictions (Neumann and Cardon, 2012 and citations within). Among these are ‘external’ factors, such as the water potential gradient (Ryel *et al.*, 2004) or root-soil contact and ‘internal’ driving factors, such as root conduit anatomy (Hafner *et al.*, 2017). However, fine root anatomy and related hydraulic conductivities are hardly studied. Especially conductivity measurements of roots are scarce and in the reverse direction, i.e. from the rootstock to the fine roots as it is the case of HR, unreported.

In a conducting element, the diameter of the pipes is the most confining factor for the amount of transported water, as given by the law of Hagen-Poiseuille. Thus, roots with high water transport capacity should also redistribute more water. During HR water flows against the “usual” direction; nevertheless, we expect the conductivity of fine roots for water to be independent of its direction. To this end, we investigated the fine root systems of six temperate tree species, i.e. Norway spruce (*Picea abies* L. Karst), European beech (*Fagus sylvatica* L.), sycamore maple (*Acer pseudoplatanus* L.), sweet chestnut (*Castanea sativa* Mill.), English oak (*Quercus robur* L.) and Douglas fir (*Pseudotsuga menziesii* Mirb. Franco). We chose these species not only for their importance in forestry and natural forests stands, but also for their differences in anatomy. As spruce and Douglas fir are conifers, they have only tracheids as conductive elements. Among the four angiosperms, two species were stem-diffuse porous (beech and maple) and two had a ring porous stem anatomy (chestnut and oak). Hence, we examined a broad spectrum in conduit sizes and wood traits, which are controlled by genetic and environmental factors (Schreiber *et al.*, 2015). To test whether root order has an influence on anatomy, we compared primary roots with older, secondary roots, hypothesizing that fine root anatomy is similar in primary and secondary roots (H1). Closely related to anatomy is the root conductivity for water. As the reverse flow of water in roots could be an important limitations for HR, we compared the conductivity of forward and reverse flow in secondary roots of the six study tree species. Based on the analogy between roots and pipes and the law of Hagen-Poiseuille we hypothesized that there is no difference between forward and reverse water flow in fine roots (H2).

Materials and Methods

Plant material

The study was done on two – four years old saplings of two conifers (*P. abies* and *P. menziesii*) and four angiosperms (*A. pseudoplatanus*, *C. sativa*, *F. sylvatica* and *Q. robur*). Plants were potted two years before the start of the experiment in potting soil (90 %, mixture of topsoil, compost, turf and lava (20 % organic matter; Wurzer Umwelt GmbH, Eitting, Germany) mixed with soil taken from respective native stands (10 %), except for Douglas fir which was potted the year before the experiment. During these two years, plants were kept in a greenhouse under near ambient climate conditions in Freising, Germany (48°23’57.98’’ N, 11°43’00.99’’ E).

Fine root anatomy in laser ablated cross-sections

Five individuals of each species were harvested prior of the experiment for assessments on fine root anatomy. Root systems were carefully freed of the soil and one sample of approx. 1 cm length was taken. The sample preparation and drying in different concentrations of ethanol (first 70 %, 95 % and finally 99 %) followed the protocol of Hafner *et al.*

(2017). Primary roots (diameter 0.23 ± 0.14 mm), with only primary growth, secondary roots (diameter 1.46 ± 0.50 mm) and stem segments (diameter 3.89 ± 0.70 mm) were cut by laser ablation tomography (Chimungu *et al.*, 2014) with cross-sections photographed continuously (resolution: 25400 dpi, figure 1). The complete cross-section was analyzed for primary roots, whereas for secondary roots three areas of interest (AOI, each area 0.5 mm^2) of a square shape were selected randomly and representatively in each cross-section and analyzed for their xylem conduit size and distribution. In each AOI, the xylem conduits were marked by hand on an extra layer using GIMP (version: 2.8.16, GNU Image Manipulation Program, The GIMP Team, https://www.gimp.org). The conduits were then analyzed with ImageJ (Version 1.47t, Wayne Rasband, National Institutes of Health, Bethesda, USA) for the area (C_A, in µm^2) of each xylem element. Calculations and analyses of the pictures followed Scholz *et al.* (2013) and Zanne *et al.* (2010). From the conduit area, the equivalent circle diameter (D, in µm) was calculated:

$$D=\sqrt{\frac{4C_A}{\pi}} \tag{1}$$

Via the equivalent circle diameter, the hydraulic diameter (D_H in µm) was calculated following Tyree and Zimmermann (2002), (equation 2). The hydraulic diameter is the weighted diameter of vessels that contribute to the overall conductivity.

$$D_H=\left(\frac{\sum D^4}{N}\right)^{0.25} \tag{2}$$

Conduit density (C_D, in mm^{-2}) was calculated by dividing the number of conduits by the respective AOI. The vessel lumen fraction (F, unitless) shows the proportion of an area covered by vessel lumen (see equation 3). The higher F is, the lower would be the support tissue fraction, and it can be used as an indicator for mechanical strength and hydraulic conductivity (Jacobsen *et al.*, 2005, Preston *et al.*, 2006). It is calculated as:

$$F=C_D*C_A \tag{3}$$

The vessel composition index as introduced by Zanne et al. (2010, S, in mm^4) indicates how resistant a plant is against cavitation. Low values indicate a higher resistance against drought and frost induced cavitation, but also a less efficient water transport (Zanne *et al.*, 2010).

$$S=\frac{C_A}{C_D} \tag{4}$$

For the angiosperms, the vessel grouping index, V_G, (Carlquist, 2001) was assessed additionally. The V_G gives an idea about the number of solitary versus grouped vessels (see equation 5). The total number of vessels ($N_{vessels}$) is divided by the number of vessel groups ($N_{groupings}$), where solitary vessels also count as a group. A V_G of one indicates that only solitary vessel are present.

$$V_G=\frac{N_{vessels}}{N_{groupings}} \tag{5}$$

Conductance measurements and conductivity calculations

Five plants per species were harvested between July and September 2017. The whole root system was quickly and carefully cleared from the soil and one subsample, after the first branching and without side roots, was cut under water. We chose roots with a similar diameter (average diameter was 2.6 ± 0.7 mm) to the secondary roots used for anatomical measurements (average diameter 1.46 ± 0.50 mm). The sample was then cut several times under water, until it reached about the double of the desired length. Next, the sample was cut in half and randomly one piece was used for assessment of the forward conductivity and the remaining piece for the reverse conductivity. To measure the hydraulic conductance (Cochard *et al.*, 2013), the xylem embolism meter (XYL'EM, BRONKHORST France S.A.S., Montigny-Les-Cormeilles, France) was used. The bark was removed on the side that was inserted into the XYL'EM apparatus and from each side of every sample several thin cuts were made and preserved for the assessment of the conductive area. Every conductance measurement was made at approx. 7 kPa and with degassed, filtered (0.2 µm) water with 10 mM KCl and 1 mM $CaCl_2$ added (Barigah *et al.*, 2013). This happened to avoid clogging and the formation of microbubbles within the xylem elements. Salts were added to mimic the ion concentration of natural xylem water, to avoid swelling of cell walls. After each measurement, every sample was flushed several times at approx. 0.1 MPa for 10 minutes and measured until there was no further increase in measured conductance (K_{max}, kg MPa^{-1} s^{-1}, for details see Tomasella *et al.* (2017b)). Subsequently, the length (L [m], mean over all species: 0.027 ± 0.006 m) of each sample was measured with a caliper for conductivity calculations. The thin sections, cut from each sample before the conductance measurements, were photographed using a stereo-microscope and analyzed for the conductive area (A_{cond} in m^2) using the software ImageJ 1.47t. From A_{cond} the diameter (D_{cond} in mm) for every sample was calculated. There was no difference between the six species in D_{cond} (average diameter was 2.6 ± 0.7 mm) and no difference between the distal and proximal diameter of each segment (P = 0.97). Maximum specific hydraulic conductivity for both forward ($k_{s\_max\_f}$, kg s^{-1} m^{-1} MPa^{-1}) and reverse conductivity ($k_{s\_max\_r}$, kg s^{-1} m^{-1} MPa^{-1}) was calculated as:

$$k_{s_{max}f/r} = \frac{K_{max} * L}{A_{cond}} \qquad (6)$$

Forward and reverse hydraulic conductivity of stems ($k_{s\_max\_stem\_f}$ and $k_{s\_max\_stem\_r}$, respectively) was assessed on additional plants of maple and oak. Two consecutive pieces of the stems were measured following the same procedure as for the roots.

Statistics

The data were statistically analyzed using R (version 3.5.2, R Development Core Team, 2008) in RStudio (version 1.1.442, RStudio Team, 2015). A linear mixed effect model (lme function of the package: nlme, version: 3.1-137) was used to test for differences in the conductivity and anatomy parameters. For every model, the residuals were tested for normality (shapiro test of the package: stats, version: 3.5.2) and for homogeneity of variances (levene test of the package: car, version 3.0-2). For the conductivity, the plant species, the conductivity direction (forward vs. reverse, n = 5 for both directions and each species) and their interaction were used as fixed factors and the plant pot as a random factor. For the anatomy, plant species, root orders (primary vs. secondary roots, n = 5 for both orders and each species) and their interaction were used as fixed factors and the root system from which the samples were taken as the random factor, for all examined anatomy parameters. If the lme showed any significances, we used a post-hoc test (emmeans function with Tukey correction of the package: emmeans, version: 1.3.1) to test for differences between the single groups. Data in text and tables is given as the mean ± 1 SD.

Results

Anatomy of primary and secondary roots

For the two conifers, within and between, there was no difference in the hydraulic diameter (D_H) between primary and secondary roots (P = 0.86, overall mean: 10.9 ± 2.1 µm, figure 2). For all angiosperms, primary roots had significantly smaller D_H compared to secondary roots (P < 0.05, figure 2). Among angiosperms, D_H in primary roots did not differ (P = 0.47, overall mean 19.4 ± 4.9 µm) (figure 2). For secondary roots, angiosperms showed significant differences between the species (P < 0.01, figure 2), with chestnut (47.0 ± 14.0 µm) having the largest D_H, followed by oak (37.1 ± 3.2 µm) and maple (29.9 ± 2.7 µm) and beech (29.2 ± 3.5 µm) having the smallest D_H. Overall, angiosperms showed a significantly larger D_H than conifers for both root orders (P < 0.001, figure 2).

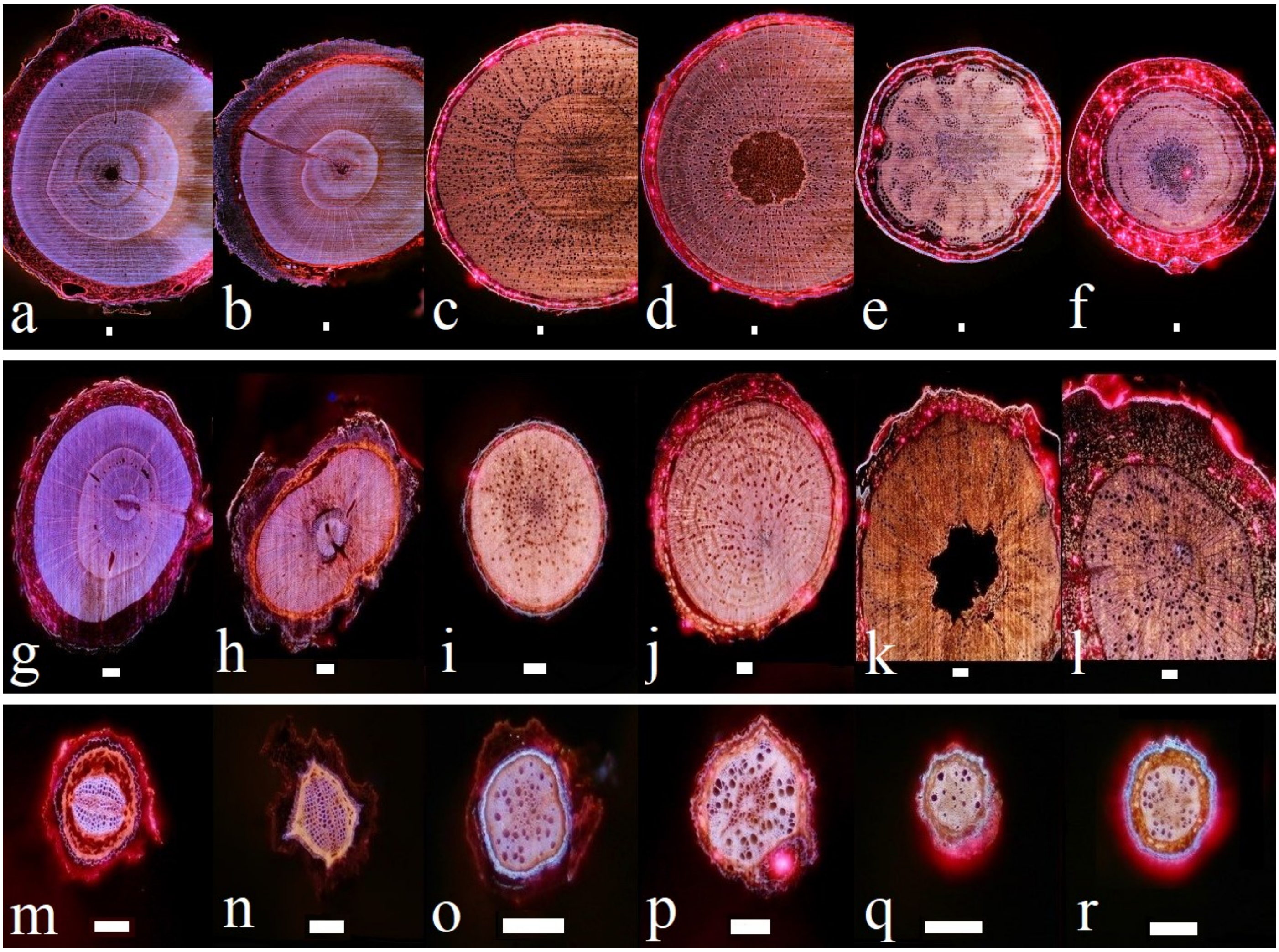


Figure 1: Examples of laser ablated cross-sections.
Laser ablated cross-sections of stem segments (upper row), secondary (middle row) and primary (lower row) roots of spruce (a, g & m), Douglas fir (b, h & n), beech (c, i & o), maple (d, j & p), oak (e, k & q) and chestnut (f, l & r). Bars represent 1000 µm for a-f and 100 µm for g-r.

The same pattern was found for the conduit diameter (D, table 1). No differences in D were found for conifers, neither between root orders nor between the two species (table 1). Secondary roots in angiosperms showed on average 1.9 times larger D than primary roots (table 1). While no differences in D were found in primary roots between the angiosperm species ($P > 0.05$, table 1), in the secondary roots, chestnut showed significant larger D, than oak, being in between, and beech and maple showing the smallest D (table 1).

Conduit density (C_D) of conifers was 17 times in primary and 32 times in secondary roots higher than of angiosperms ($P < 0.001$, table 1). While spruce tended to a higher C_D than Douglas fir, especially in primary roots, there was no intraspecific difference between primary and secondary roots within both species (table 1). For angiosperms, C_D was on average about five times higher in primary roots compared to secondary roots ($P < 0.05$, table1), however, no differences were found between the four species in each root order, respectively (table 1).

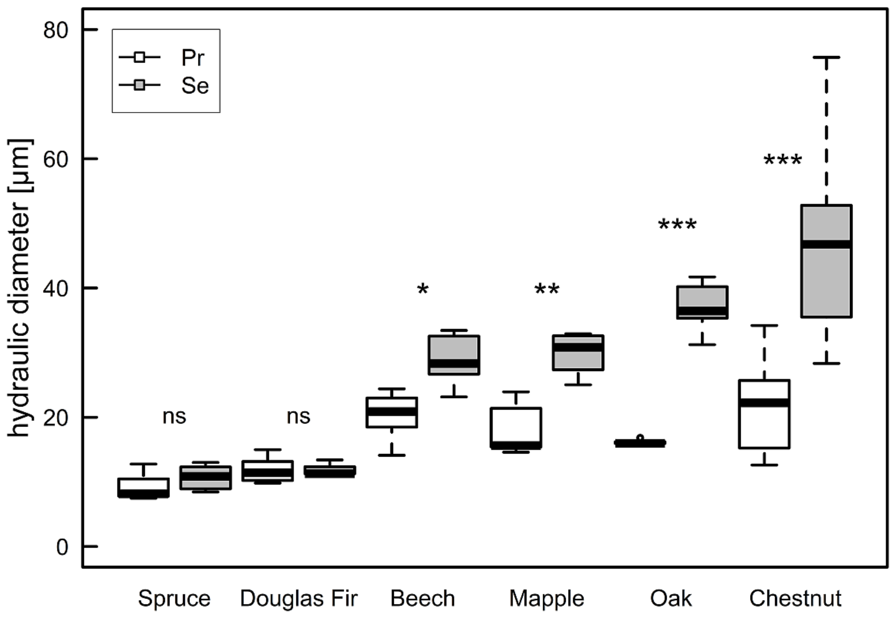
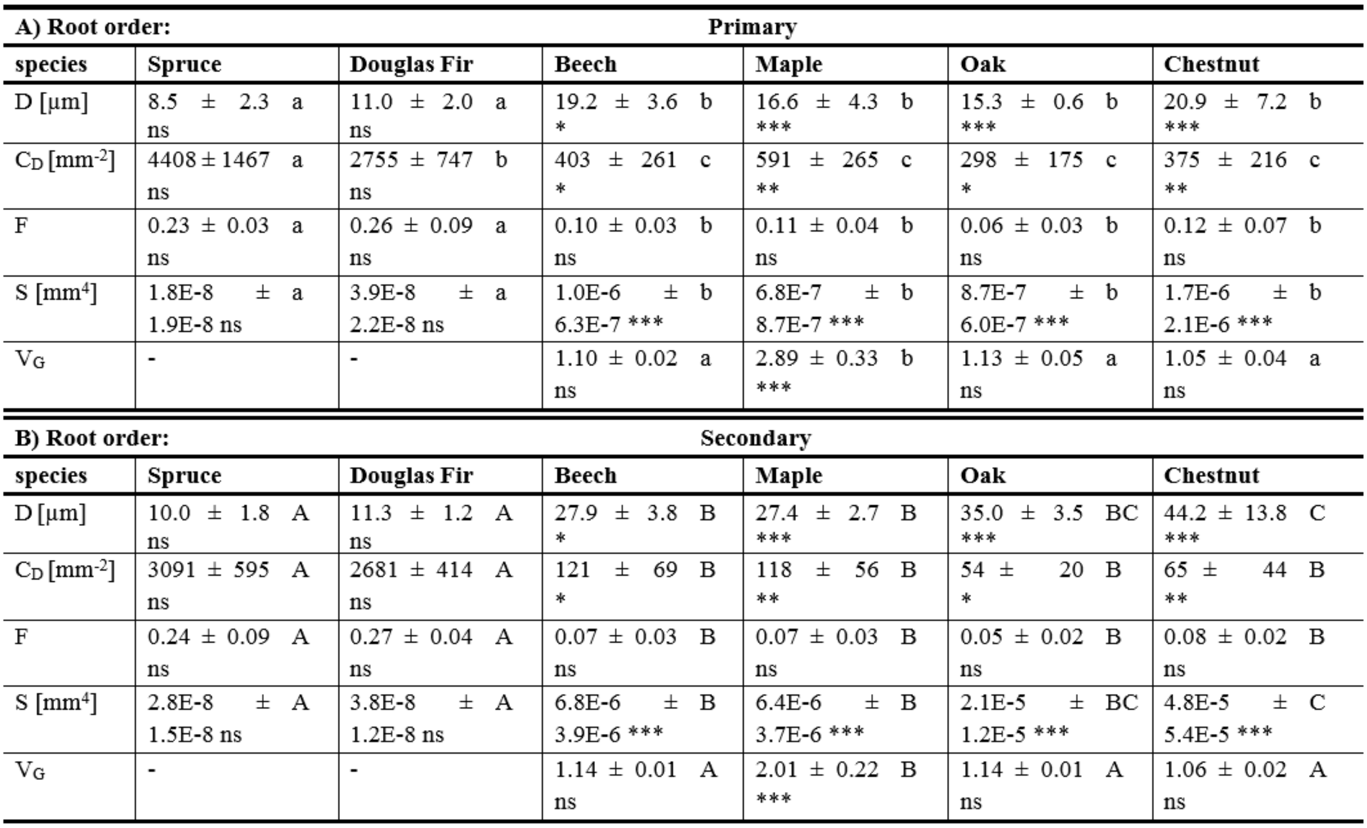


Figure 2: Mean hydraulic diameter per species
Hydraulic diameter (D_H) in primary roots (white) and secondary roots (grey); asterisks indicate significant differences within one species between primary and secondary roots (*** < 0.001, ** < 0.01, * < 0.05, ns > 0.05).

For the vessel lumen fraction (F) and the vessel composition index (S) a similar pattern was found. For both indices, no differences were found in primary roots within the four angiosperm and the two conifers, respectively (table 1). However, conifers tended to have double the amount of vessel lumen (F) (0.25 ± 0.07) than angiosperms (0.10 ± 0.05). In primary roots on the other hand, conifers (3.05E-8 ± 2.13E-8 mm^4) had an almost sixty times lower vessel composition index (S) than angiosperms (1.76E-6 ± 1.21E-6 mm^4). Overall, F for angiosperms was similar in primary roots (0.10 ± 0.05) compared to secondary roots (0.07 ± 0.03, table 1) and the same for conifers (0.25 ± 0.07 vs. 0.25 ± 0.07). For angiosperms, the vessel composition index (S) of secondary roots (2.06E-5 ± 3.12E-5 mm^4) was on average about 19 times higher compared to primary roots (1.10E-6 ± 1.22E-6 mm^4) (P <0.001, table 1), in particular in oak. Conversely, conifers showed very similar S in both root orders, but 37 times lower S in primary and 623 times lower S in secondary roots compared to angiosperms (table 1). Despite the high C_D in primary roots, only maple showed significant vessel grouping (V_G in table 1). In primary roots of maple, V_G was more prominent than in secondary roots (2.89 ± 0.33 to 2.01 ± 0.22, respectively). The three other angiosperms did not show any vessel grouping (table 1), neither in primary roots (1.09 ± 0.05) nor in secondary roots (1.11 ± 0.04).

Root hydraulic conductivity

The diameter of the root pieces used for forward (2.35 ± 0.95 mm) and reverse conductivity (2.36 ± 1.00 mm) did not show any significant difference. Both the flow direction and the plant species had a significant influence on the hydraulic conductivity (figure 3a). In forward direction, the species with the highest conductivity ($k_{s\_max\_f}$) was chestnut (2.39 ± 1.19 kg s^{-1} m^{-1} MPa^{-1}), followed by oak (2.05 ± 0.43 kg s^{-1} m^{-1} MPa^{-1}) and beech/ maple with similar values (1.35 ± 0.24 kg s^{-1} m^{-1} MPa^{-1} and 1.21 ± 0.29 kg s^{-1} m^{-1} MPa^{-1}, respectively). Douglas fir and spruce had relatively low and similar values (0.32 ± 0.12 kg s^{-1} m^{-1} MPa^{-1} and 0.28 ± 0.09 kg s^{-1} m^{-1} MPa^{-1}, respectively). The same pattern was found for the reverse direction ($k_{s\_max\_r}$, figure 3a). For the two conifers there was no difference between the forward and the reverse conductivity, whereas the four angiosperms showed a highly significant difference (figure 3a). The reverse conductivity was reduced by 39 ± 11 % compared to the forward conductivity in angiosperms, irrespective of species (figure 3b). Conversely, hydraulic conductivity in stems ($k_{s\_max\_stem\_f}$ and $k_{s\_max\_stem\_r}$) of maple and oak was not different between forward and reverse direction (P = 0.30, figure 3c). Concurring with the results in the roots, oak had a higher conductivity in stems than maple in both directions (P < 0.001, figure 3c).



A) Root order:			Primary			
species	Spruce	Douglas Fir	Beech	Maple	Oak	Chestnut
D [µm]	8.5 ± 2.3 a ns	11.0 ± 2.0 a ns	19.2 ± 3.6 b *	16.6 ± 4.3 b ***	15.3 ± 0.6 b ***	20.9 ± 7.2 b ***
C_D [mm^{-2}]	4408 ± 1467 a ns	2755 ± 747 b ns	403 ± 261 c *	591 ± 265 c **	298 ± 175 c *	375 ± 216 c **
F	0.23 ± 0.03 a ns	0.26 ± 0.09 a ns	0.10 ± 0.03 b ns	0.11 ± 0.04 b ns	0.06 ± 0.03 b ns	0.12 ± 0.07 b ns
S [mm^4]	1.8E-8 ± a 1.9E-8 ns	3.9E-8 ± a 2.2E-8 ns	1.0E-6 ± b 6.3E-7 ***	6.8E-7 ± b 8.7E-7 ***	8.7E-7 ± b 6.0E-7 ***	1.7E-6 ± b 2.1E-6 ***
V_G	-	-	1.10 ± 0.02 a ns	2.89 ± 0.33 b ***	1.13 ± 0.05 a ns	1.05 ± 0.04 a ns

B) Root order:			Secondary			
species	Spruce	Douglas Fir	Beech	Maple	Oak	Chestnut
D [µm]	10.0 ± 1.8 A ns	11.3 ± 1.2 A ns	27.9 ± 3.8 B *	27.4 ± 2.7 B ***	35.0 ± 3.5 BC ***	44.2 ± 13.8 C ***
C_D [mm^{-2}]	3091 ± 595 A ns	2681 ± 414 A ns	121 ± 69 B *	118 ± 56 B **	54 ± 20 B *	65 ± 44 B **
F	0.24 ± 0.09 A ns	0.27 ± 0.04 A ns	0.07 ± 0.03 B ns	0.07 ± 0.03 B ns	0.05 ± 0.02 B ns	0.08 ± 0.02 B ns
S [mm^4]	2.8E-8 ± A 1.5E-8 ns	3.8E-8 ± A 1.2E-8 ns	6.8E-6 ± B 3.9E-6 ***	6.4E-6 ± B 3.7E-6 ***	2.1E-5 ± BC 1.2E-5 ***	4.8E-5 ± C 5.4E-5 ***
V_G	-	-	1.14 ± 0.01 A ns	2.01 ± 0.22 B ***	1.14 ± 0.01 A ns	1.06 ± 0.02 A ns

Table 1: Anatomical parameters

Anatomy parameters conduit diameter (D), conduit density (C_D), vessel lumen fraction (F), vessel composition index (S) and vessel grouping index (V_G) in primary (A) and secondary roots (B). Asterisks indicate significant differences within species between primary and secondary roots with *** < 0.001, ** < 0.01, * < 0.05 and ns > 0.05. Different letters indicate significant differences between species with small letters for primary roots and capital letters for secondary roots (values are given as the mean ± 1 SD).

Discussion

In this study, reverse conductivity in fine roots was systematically tested for the first time. Opposing to H1, differences in several anatomical parameters (e.g. D_H, C_D, S) were found between young, primary and older, secondary roots. Furthermore, we found differences in the magnitude between forward and reverse hydraulic conductivity in fine roots of angiosperms, contradicting H2. Reverse conductivity was reduced by about 40 % compared to the forward conductivity.

Root anatomy of primary and secondary roots

Roots with predominantly primary growth, showed different xylem anatomical structures compared to older, secondary roots. Additionally, primary roots of rather unrelated species (e.g. angiosperm genera) were anatomically rather similar. Conversely, for older, secondary roots, clear differences between species were found in D, D_H and S. For the two conifers, the conduit size was consistent for both root orders, which is contradictory to findings along the stem to branches, where conduit size is decreasing (McCulloh and Sperry, 2005; Woodruff *et al.*, 2008). Therefore we accept H1 for the two conifer species. Additionally, anatomy of fine roots in conifers seems to be very similar to anatomy of twigs in many parameters (D and C_D, Tomasella *et al.*, 2017a). For the four angiosperm species, on the other hand, we found distinct differences between the two root orders, showing that with increasing age, roots seem to change their xylem anatomy. Especially the increase in diameter and the decrease in density of water conducting conduits is obvious. We therefore reject H1 for the four angiosperm species.

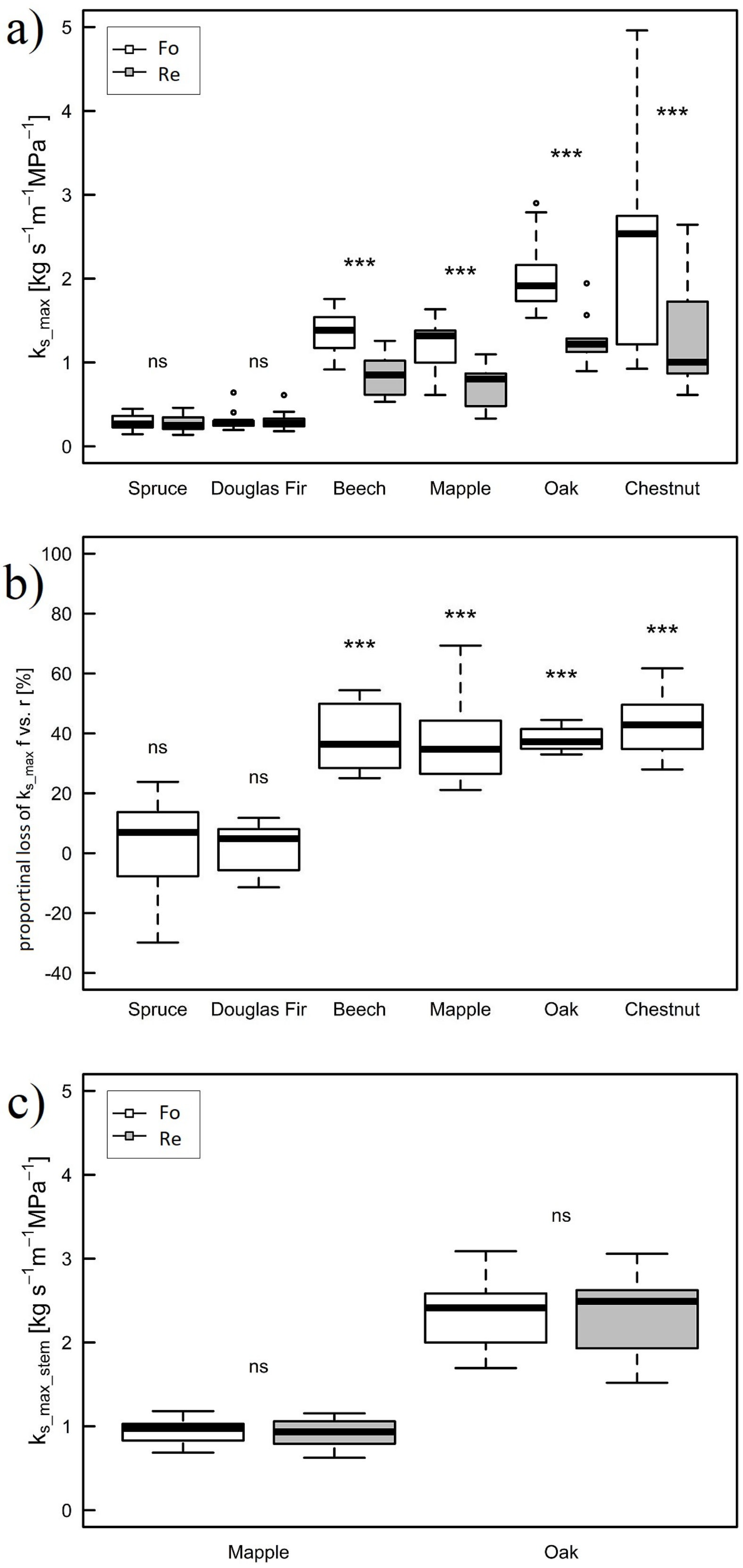


Figure 3: Maximum specific hydraulic conductivity and loss of conductivity.
Maximum specific hydraulic forward (white) and reverse (grey) conductivity of roots (k_{s_max}, a), proportional loss of conductivity of the reverse direction compared to forward direction in roots (proportional loss of k_{s_max} f vs. r, b) and hydraulic maximum specific forward (white) and reverse (grey) conductivity of stems ($k_{s\_max\_stem}$, c). Asterisks indicate significant differences within one species between forward and reverse conductivity (3a & c). In 3b asterisks indicate significant differences to zero (*** < 0.001, ns > 0.05).

Whereas conifers seem to be very conservative in their anatomy, angiosperms tend to be more plastic. Over the four angiosperms, the two stem ring porous species (chestnut and oak) showed bigger vessels in the roots than the two stem diffuse-porous species (maple and beech), which is consistent with anatomical measurements of the stem (McCulloh et al. 2010). However, vessel distribution in all analyzed fine roots seemed more diffuse-porous, i.e. even distribution of similar-sized conduits over the whole year-ring, including chestnut and oak, which is in conflict with their vessel arrangement in stems (Barbaroux and Bréda, 2002; Fonti and Garcia-Gontález, 2004). This may be related to the longer growth period of roots, depending on water availability and soil temperature (Tryon and Chapin, 1983) with vessels being formed over the whole root growing season unlike stem growth peaking in early summer (McDougal, 1916; Alvarez-Uria and Körner, 2007). Vessel density also supports the diffuse porous anatomy of roots, as no differences were found among angiosperm species, neither in the primary nor in the secondary roots. Typical vessel densities in stems for ring-porous species are at 52 ± 17 mm^{-2} (for 3-year-old oak from Steppe and Lemeur, 2007) and for diffuse-porous species at 872 ± 113 mm^{-2} (beech twigs, Tomasella *et al.*, 2017a). In the roots the four angiosperm species showed values in between with on average 417 mm^{-2} in primary and 78 mm^{-2} in secondary roots despite their consistent diffuse-porous distribution of vessels. The higher vessel density in primary roots emphasizes the importance of vessel structures for the water transport in angiosperms (Sperry, 2003). At the same time the high density of vessels is at the expense of mechanical strength, which is more important for stems and coarse roots (Preston *et al.*, 2006), as they need to withstand harsh compressive stress during storms and snow load (James *et al.*, 2006). Vessel lumen fraction (F) in stems of angiosperms is around 0.136 (Zanne *et al.*, 2010) and similar to those in fine roots (0.083 for both ages). Therefore, roots seem to build xylem with a similar amount of conductive area as stems. Otherwise, the vessel composition index (S) for angiosperms in primary roots is about 90 times and in secondary roots 5 times lower compared to aboveground values (9.6E-5 mm^4 from Zanne *et al.* (2010)). Hence, the conductive area in angiosperm roots is composed of more but smaller conduits than in stems (Zanne *et al.*, 2010). According to the vessel composition index, roots seem to follow a more conservative strategy, reducing the risk of embolisms at the cost of a lower water transport (Zanne *et al.*, 2010), with primary roots being even more conservative than secondary roots. However, as roots face very different environmental conditions than stems/twigs, the vessel composition index might not be a useful tool to predict cavitation resistance in roots, especially as our measured species are all mesophilous with similar cavitation resistances (P_{50} of -2.2 MPa for *Acer pseudoplatanus* (Lens *et al.*, 2011), -4.74 MPa for *Quercus robur* (Lobo *et al.*, 2018) and -3.4 MPa for *Fagus sylvatica* (Tomasella *et al.*, 2017a)). This could be an important issue under drought stress conditions, as hydraulic failure in the water up-taking organ would consequently kill the plant (Jackson *et al.*, 2000). Therefore, the higher safety margin against embolisms in roots may be one strategy of plants to avoid hydraulic failure (Delzon and Cochard, 2014). Overall, stem anatomy is not a reliable proxy for root anatomy, especially for fine roots of angiosperms.

Reverse vs. forward hydraulic conductivity in roots

Direction of water flow in roots, i.e. forward or reverse, did not affect conductivity in conifer roots, yet for all angiosperms a 40 % reduction in the reverse compared to forward conductivity was found. Looking at the conductive system, the xylem, the main difference between the two groups are the existence of vessels, which are only in angiosperms (Lüttge *et al.*, 2005). Hence, the anatomy/structure of vessel elements in angiosperm wood most likely plays a decisive role in the reduction of conductivity of reverse water flow. To our knowledge, only very few studies have dealt with vessel anatomy in detail. Deducted from this knowledge we identified four putative reasons for the conductivity reduction in reverse direction (figure 4).

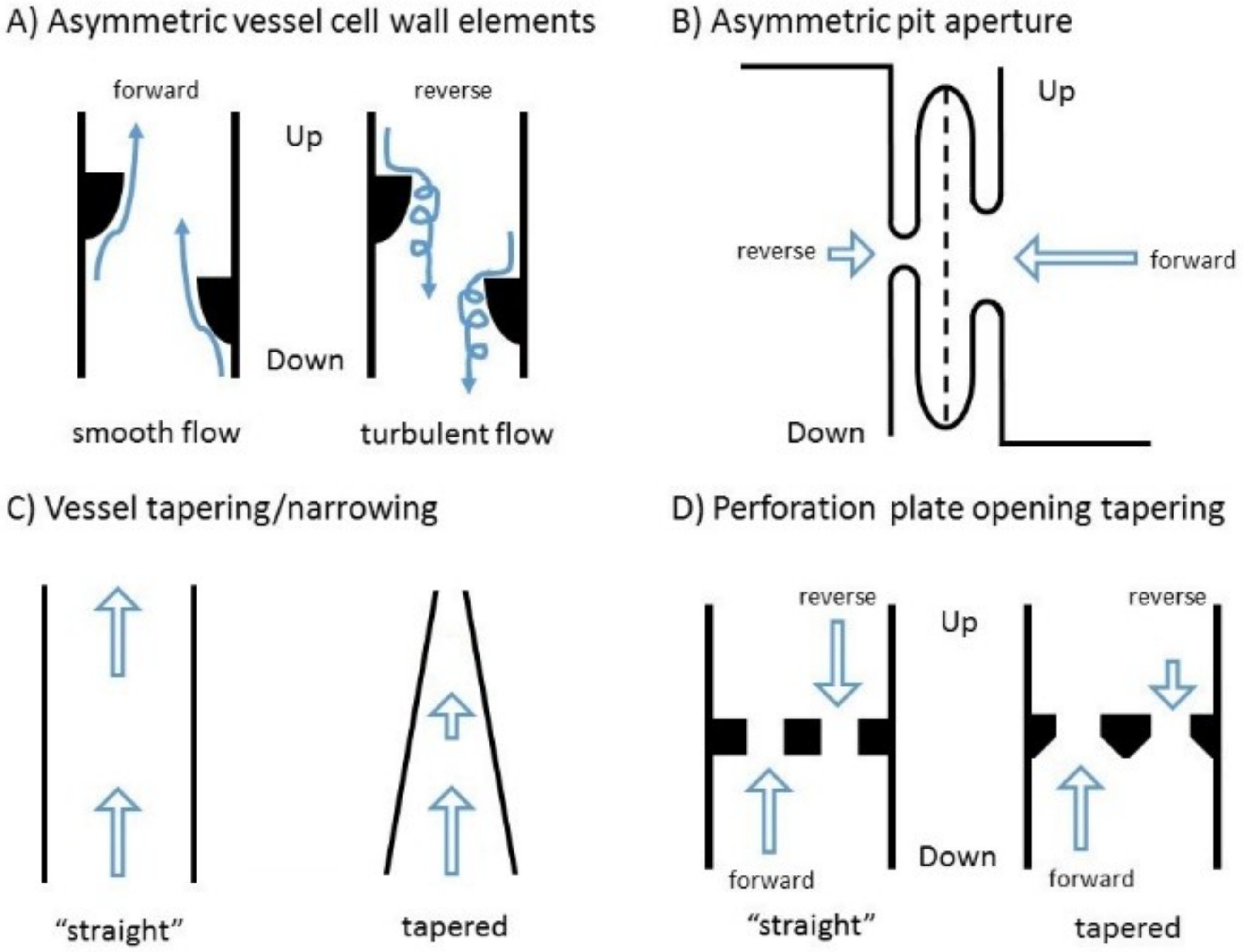


Figure 4: Schematic figures for possible anatomical causes of reverse conductivity reduction
Schematic figures for possible anatomical causes of reverse conductivity reduction: A) asymmetric vessel cell wall elements (e.g. warts), B) Asymmetric pit aperture, C) tapering/narrowing of vessels and D) tapering of perforation plate openings (Arrows indicate flow direction and the length is indicating the magnitude of the conductivity).

a) Asymmetric vessel warts

Independent of the type of thickening, vessels have elements pointing into the lumen (e.g. warts, Bailey, 1944; Ohtani *et al.*, 1983, Jansen *et al.*, 1998), possibly causing some disturbances in the conductivity. If these elements were asymmetric, they could cause higher resistance and turbulences for one flow direction (figure 4 a). Such disturbances in the conductivity would then hinder the reverse flow (Karino *et al.*, 1987). However, this would mean that the manner of secondary thickening of vessels and especially its remnants are different between root and stem xylem, as no difference was found in the conductivity for the stem xylem.

b) Asymmetric pit aperture

Pits and the connectivity between vessel elements are supposed to play an important role in safety and resistance for the hydraulic system of plants (Choat *et al.*, 2008). If the pits opening would be smaller on one side, the conductivity could be reduced (figure 4 B). If the smaller opening would be consistently at the vessel side closer to the root tip, this could result in a reduction in the reverse conductivity (Steven Jansen, personal communication, figure 4 b). But as no reduction in the reverse conductivity was found in the stem segments, this would indicate, that pits of the stems are built differently from the pits in roots. Up to this point however, most studies about pits were made in aboveground organs (Choat *et al.*, 2008 and citations within). However, it appears unlikely that the pits themselves are responsible for the reduction of reverse conductivity. First, pits in angiosperms are well studied (e.g. Choat *et al.*, 2008) and so far there are no indications that they would favor one direction, as they have to "seal" either vessel in case of cavitation. Second, as the vessel grouping index (V_G) in the four angiosperm roots was diverse, with only maple showing a considerable amount of vessel grouping, it appears unlikely that reverse conductivity reduction is related to pits anatomy. Plants with a high vessel grouping index would be considered to have more connecting pits between the vessels than plants with a low vessel grouping index. Nevertheless, we saw no differences in the amount of conductivity reduction between maple with high and the other three angiosperms with lower V_G.

c) Vessel tapering

Another reason for the reduced conductivity along a conduit could be tapering towards one end (Petit et al. 2008). Vessel tapering is known to occur in stems and branches (Anfodillo *et al.*, 2006; Petit *et al.*, 2010) and is assumed to happen in roots (Enquist, 2003). As given by the law of Hagen-Poiseuille, the conductivity is dependent on the fourth power of the vessel radius (Sperry *et al.*, 2006). Tapering of vessels towards the root tip would limit reverse flow compared to forward flow (Walander and Prasassarakich, 1976; Rubenstein *et al.*, 2011). However, the reduction of reverse conductivity was only found in angiosperms, but tapering is known to happen in conifers too. Nevertheless, the degree of tapering may be related to the size of conduits and xylem dimension and may therefore be neglectable in tracheids and large diameter stems but not for vessels and small roots/twigs respectively.

d) Perforation/end plates

Perforation plates, i.e. the remnants of cell walls between connected vessels (Christman and Sperry, 2010), increase the resistance for water flow. While there are different forms, scalariform perforation plates are common in angiosperms (Ellerby and Ennos, 1998). If the openings in these plates would taper to one side (e.g. conical shaped), the resistance for one direction would increase (figure 4 d, Walander and Prasassarakich, 1976; Rubenstein *et al.*, 2011). Again, this would imply a different structure and/or building process of root xylem compared to stem xylem, as no reduction of conductivity was found in stems.

As conifers showed no difference between the conductivity directions, H2 was accepted for them. For angiosperms, H2 was rejected, as a reduction of 40 % was found in the reverse conductivity compared to the forward conductivity. The four points mentioned above are based on the physical principles of resistance and turbulent flow, although flow rates under HR are relatively low compared to maximum forward flow and therefore the impact of turbulences might not be very big. None of them has been examined so far and will hopefully stimulate future work. However, reverse root conductivity seems to limit internal HR in angiosperm trees stronger than assumed (Neumann and Cardon, 2012). Transfer of water into the soil might be additionally limited as the conductance of the whole root system is additionally limited by other mechanisms such as forcing of water through the symplasm by the casparian stripe during water uptake or root suberization. The reduction in reverse conductivity in roots can also be interpreted from an evolutionary point of view. As HR is a passive phenomenon, trees reducing the loss of water into the soil to some extent may benefit during drought events and therefore may be favored during selection processes. As conifers only have tracheids, which conduct smaller amounts of water compared to vessels, the losses might be bearable anyway.

Conclusions

Wood structure of conifer was very similar between primary and secondary roots but showed distinct differences to angiosperms. While primary roots were similar among angiosperms, secondary roots show clear anatomical differences between species. Fine root anatomy of angiosperms cannot be predicted by stem anatomy, as angiosperm fine roots tended to build more but smaller and generally diffuse-porous conduits.

The reduction in reverse conductivity in angiosperm roots sheds new light on the potential of HR in general. Especially under drought scenarios, when embolism already reduces conductivity, the amount of redistributed water might be lower than expected and the reduced root conductivity in the reverse direction in angiosperm roots should be considered. Whether this reduction is due to the anatomical structure of vessels should be tested e.g. by assessing reverse conductivity in vesselless angiosperms (Hacke *et al.*, 2007). In general, more detailed information about vessel anatomy (e.g. structure of cell walls and perforation plates) will help to understand the process of water transport and its limitations in plant roots.

Acknowledgements

We thank Roman Meier, Peter Kuba and Thomas Feuerbach for the maintenance of the climate chambers and help during the setup of the experiment. We thank Dr. Marc Göbel (Cornell University) and Benjamin Hall and Dr. Asheesh

Lanba of Lasers for Innovative Solutions (L4iS) for conducting laser ablation tomography. In addition, we want to thank Dr. Steven Jansen and Dr. Taryn Bauerle for stimulating discussions about the results of this study.

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